ClearLLab 10C Panels

IVD ANTIBODY COMBINATIONS FOR LEUKEMIA / LYMPHOMA* ANALYSIS

CASEBOOK

EVERY
event matters.

* Non-Hodgkin Lymphoma only

BECKMAN COULTER
Life Sciences

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INTRODUCTION

This casebook has been designed to assist in the analysis of flow cytometric immunophenotyping data generated using Beckman Coulter's ClearLLab 10C Panels IVD marked reagent for Leukemia and Lymphoma analysis on the Beckman Coulter Navios and Navios EX flow cytometers.

Sample cases with characteristic findings typical of various lymphoid and myeloid neoplasms are included, as are cases from patients with clinical and/or laboratory findings that suggest an underlying neoplastic process, but in which no immunophenotypic abnormality is identified. Specimen types include peripheral whole blood, bone marrow, and lymph nodes.

Each case includes a clinical vignette that describes the patient demographics and clinical history, case-specific listmode data files for reanalysis by the user of this casebook, ClearLLab 10C specific analysis protocols to be used with the listmode data, and a report showing the analysis with provided protocols. Each report includes analysis notes that highlight the immunophenotypic findings as well as potential pitfalls.

NOTE: Casebook examples are provided for illustrative purposes only, and not all categories of hematolymphoid neoplasms may be represented, nor are all possible immunophenotypic variants described or demonstrated.

BACKGROUND

Flow cytometric immunophenotyping evaluates the presence and absence of specific antigens for each individual cell present in the specimen. When taken together, these results generate an immunophenotypic profile for each cell which is either consistent with an expected population (i.e. normal) or inconsistent with an expected population (i.e. aberrant) in that sample type. When evaluating samples from patients with suspected hematolymphoid malignancies, several steps are involved[1]:

- Assessment of all cell populations in the sample.
- Assignment of each cell population to either "normal" or "aberrant".
- Detailed characterization of the aberrant population according to the presence or absence of antigens as well as increased or decreased intensity of staining by fluorochrome-labeled antibodies.
- Interpretation of the aberrant immunophenotype, incorporating where available additional information such as clinical history, histology, cytology, immunohistochemistry, and genotyping studies such as in situ hybridization, karyotyping, and molecular diagnostics.

CONSENSUS RECOMMENDATIONS FOR IMMUNOPHENOTYPING

Consensus recommendations for flow cytometric immunophenotyping of samples from patients with known or suspected hematolymphoid malignancies have emerged over the last two decades, and several guidelines have been published in the scientific literature.

Flow cytometric immunophenotyping has been included in the WHO classification of Tumors of Haematopoetic and Lymphoid Tissues since 2008[2].

Medical indications and flow cytometry assay validation including pre-analytic, analytic, and post-analytic details of testing are addressed in the 2006 Bethesda International Consensus Conference recommendations[3,4,5] and the ICSH/ICCS practice guidelines for cell-based fluorescence assays[6,7,8].

ClearLLab 10C PANELS INTENDED USE

The ClearLLab 10C Panels are intended for in vitro diagnostic use for qualitative identification of cell populations by multiparameter immunophenotyping on the Navios and Navios EX flow cytometers. These reagents are used as an aid in the differential diagnosis of hematologically abnormal patients having or suspected of having the following hematopoietic neoplasms: chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). The reagents can be used with peripheral whole blood (collected in K_2EDTA, Acid Citrate Dextrose (ACD) or Heparin), bone marrow (collected in K_2EDTA, Acid Citrate Dextrose (ACD) or Heparin) and lymph node specimens. Interpretation of the results should be confirmed by a pathologist or equivalent professional in conjunction with other clinical and laboratory findings.

These reagents provide multiparameter, qualitative results for the surface antigens listed below:

ClearLLab 10C Panels		Blue Laser					Red Laser			Violet Laser	
PN	**Tube**	**FITC**	**PE**	**ECD**	**PC5.5**	**PC7**	**APC**	**APC-A700**	**APC-A750**	**PB**	**KRO**
B96805	B Cell Tube	Kappa	Lambda	CD10	CD5	CD200	CD34	CD38	CD20	CD19	CD45
B96806	T Cell Tube	TCRγδ	CD4	CD2	CD56	CD5	CD34	CD7	CD8	CD3	CD45
B96807	M1 Cell Tube	CD16	CD7	CD10	CD13	CD64	CD34	CD14	HLA-DR	CD11b	CD45
B96808	M2 Cell Tube	CD15	CD123	CD117	CD13	CD33	CD34	CD38	HLA-DR	CD19	CD45

ClearLLab COMPENSATION KIT

	Blue Laser					Red Laser			Violet Laser	
PN	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700	APC-A750	PB	KRO
B74074	CD4	CD4	CD3	CD4	CD4	CD4	CD4	CD4	CD4	CD8

The above reagent is provided in a standardized format to be used with reagents for sample preparation and cytometer set-up, along with software for data acquisition and analysis. ClearLLab 10C Panels meet recommendations for standardization as outlined by the Bethesda guidelines[2].

Additional information regarding ClearLLab 10C Panels is available at **beckman.com/ClearLLab**.

CASE SELECTION AND INTERPRETATION

The data presented in this case book was generated following the procedure detailed within the ClearLLab 10C Panel Instructions For Use (IFU) available at **beckman.com**.

Representative cases were selected from clinical trial data and were reviewed, annotated, and interpreted by:

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We wish to thank our Principal Investigators & clinical trial sites for their contribution to the clinical trial and to the development of this casebook:

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Fort Myers, FL USA

Analysis Protocols:
Download the ClearLLab 10C analysis protocol.

Analysis:
Download case specific Kaluza C analysis files.

RELATED DOCUMENTS

- ClearLLab 10C Application System Guide, PN C24688
- Kaluza C Flow Cytometry Software Instructions For Use, PN C10993
- Navios Flow Cytometer Instructions For Use, PN A96247
- Navios EX Flow Cytometer Instructions For Use, PN B73084AB
- ClearLLab 10C Panels Instructions For Use, PN C00197
- ClearLLab Compensation Beads Instructions For Use, PN C00201
- ClearLLab Compensation Kit Instructions For Use, PN B74074
- ClearLLab Control Cells Instructions For use, PN B99884
- ClearLLab Control Cells QC Analysis Protocols Download Addendum, PN C31984

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1. Flow Cytometric Immunophenotyping for Hematologic Neoplasms. F.E. Craig, K.A. Foon. Blood. 2008; 111; 3941-3967.
2. Swerdlow SH, Campo E, Harris NL, Jaffe EA, Pileri SA, Stain H, Thiele J, & Vardiman JW (eds) (2008) WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon
3. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Swerdlow SH, et al. Blood. 2016;127:2375-90.
4. The 2016 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia. Arber DA, et al. Blood 2016 127:2391-2405.
5. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, Oldaker T, Shenkin M, Stone E, Wallace P. Cytometry B Clin Cytom. 2007;72 Suppl 1:S14-22
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7. 2006 Bethesda International Consensus Conference on Flow Cytometric Immunophenotyping of Hematolymphoid Neoplasia. Stetler-Stevenson M, Davis B, Wood B, Braylan R. Cytometry B Clin Cytom. 2007;72 Suppl 1:S3
8. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part III - analytical issues. Tanqri S, Vall H, Kaplan D, Hoffman B, Purvis N, Porwit A, Hunsberger B, Shankey TV; ICSH/ICCS Working Group. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):291-308. doi: 10.1002/cyto.b.21106
9. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part IV - postanalytic considerations. Barnett D, Louzao R, Gambell P, De J, Oldaker T, Hanson CA; ICSH/ICCS Working Group. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):309-14. doi: 10.1002/cyto.b.21107
10. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part V - assay performance criteria. Wood B, Jevremovic D, Béné MC, Yan M, Jacobs P, Litwin V; ICSH/ICCS Working Group. Cytometry B Clin Cytom. 2013 Sep-Oct;84(5):315-23. doi: 10.1002/cyto.b.2110

CASES

The following Color Precedence Gating is applied to the cases:

- Lymphocytes (Gate Ly)/NK cells: red
- CD19+ B cells: orange
- CD3+ T cells: aqua
- Monocytes (Gate Mo): green
- Granulocytes (Gate Gr): blue
- CD45dim: purple
- Additional Aberrant populations: teal
- CD45 negative population: gray

NO IMMUNOPHENOTYPIC ABNORMALITY

Flow cytometry is a means of characterizing leukocyte populations. It can aid in the differential diagnosis of hematologically abnormal patients having, or suspected of having hematopoietic neoplasia including chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). Crucial to the identification of aberrant populations in these clinical situations is familiarity with normal cell populations present in whole blood, bone marrow and lymph node tissue samples. The following are examples of normal samples stained with ClearLLab 10C panels.

PERIPHERAL WHOLE BLOOD

Case #1: Normal Whole Blood

Clinical Vignette

This 65 year old male presents with thrombocytopenia. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

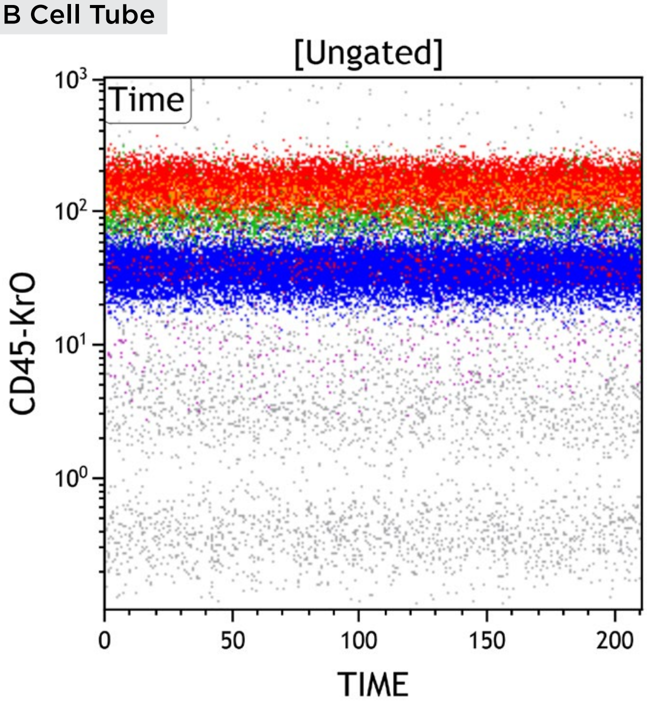

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

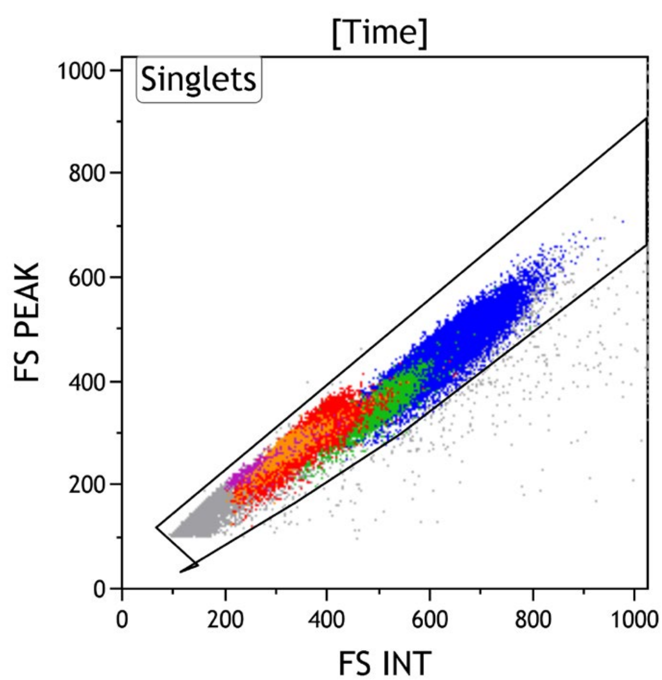

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

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B Cell Tube

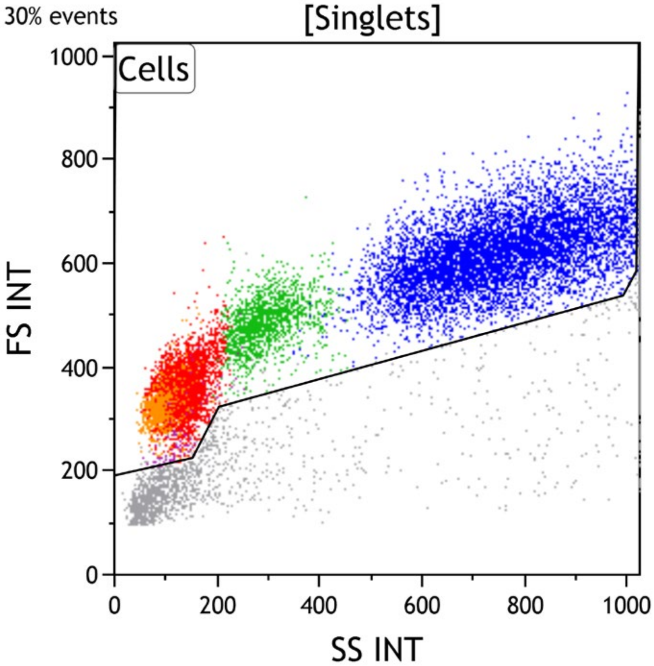


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

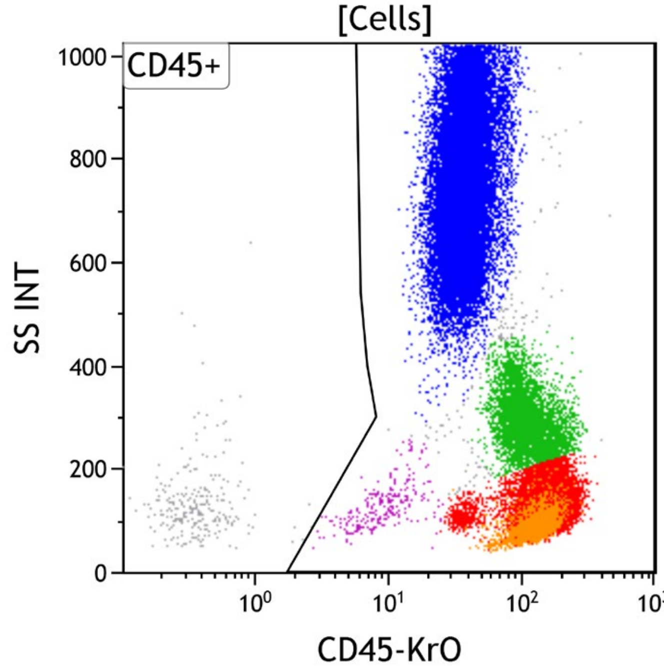


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

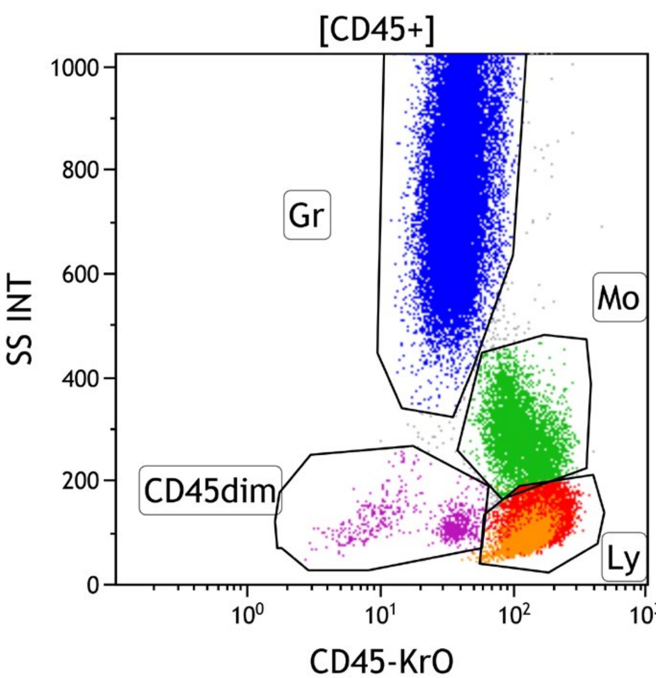


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

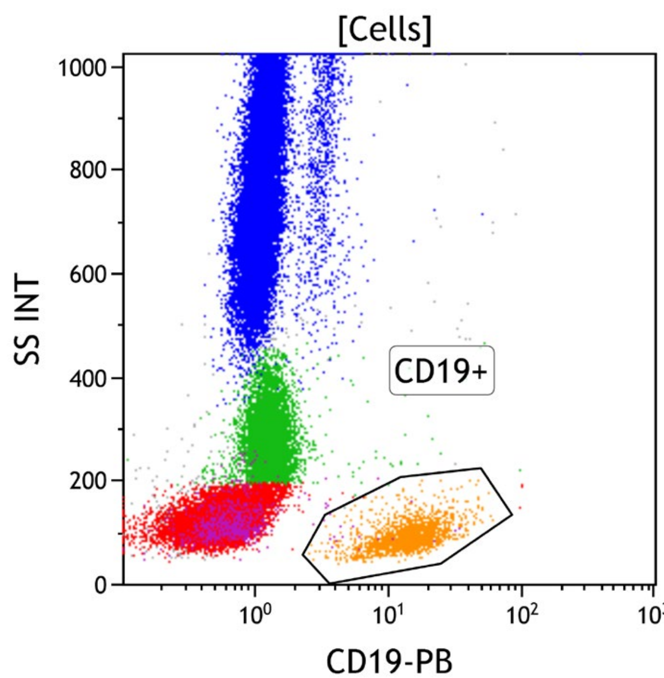


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B Cell Tube

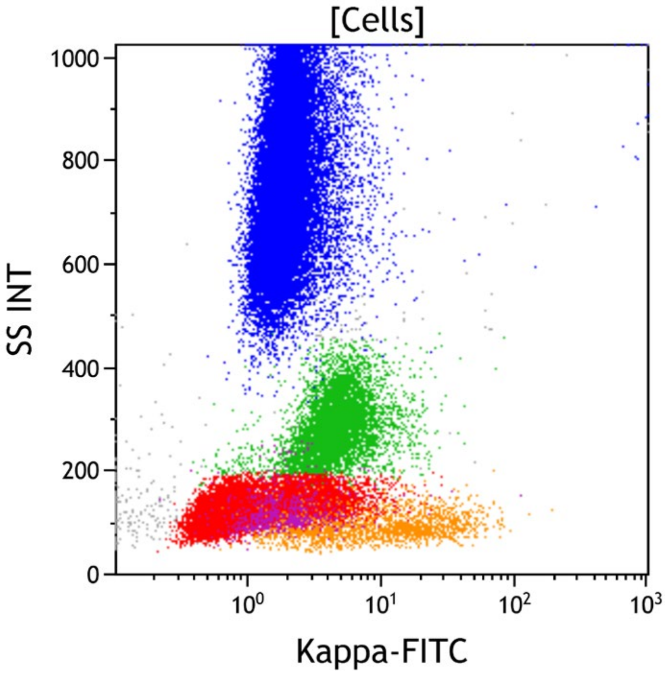


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot.

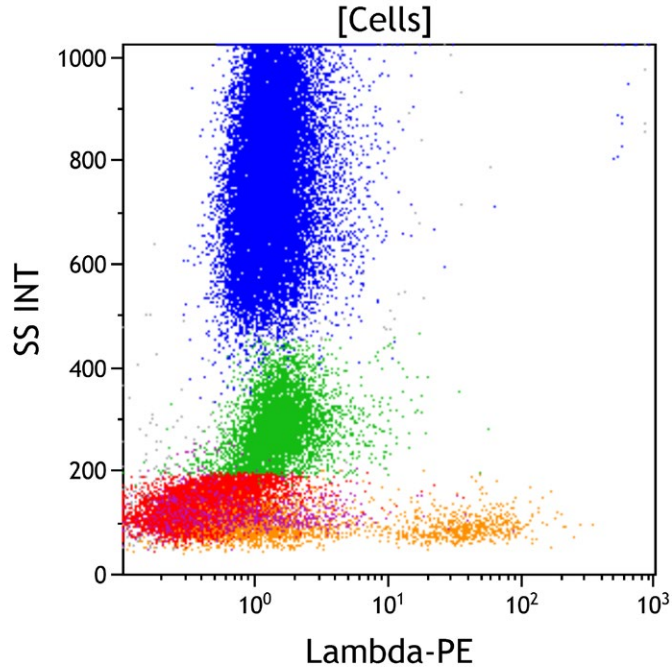


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot.

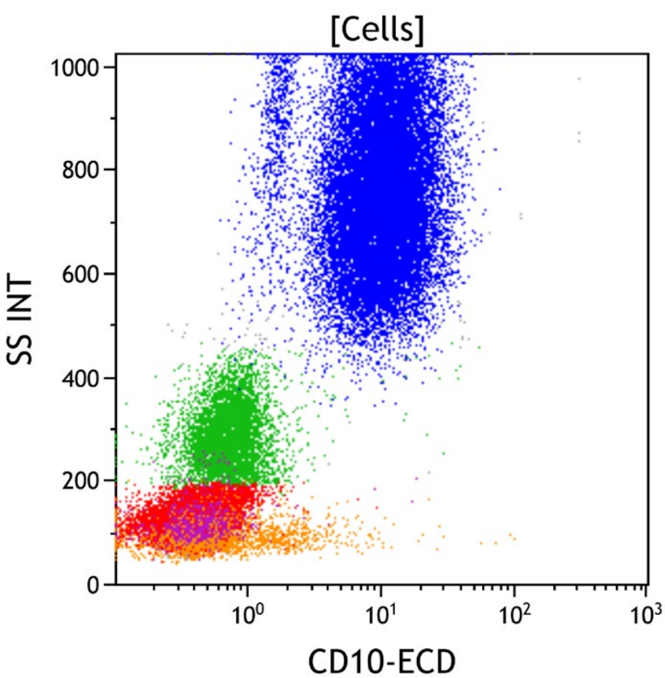


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

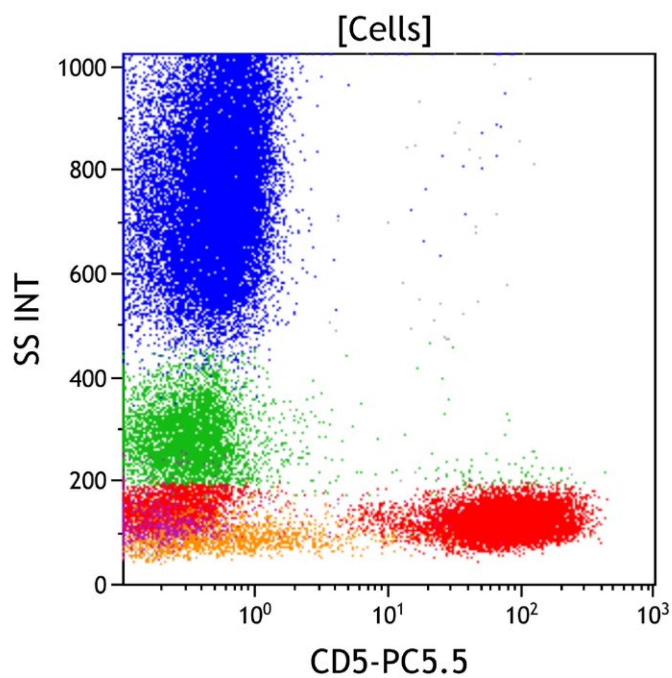


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red, lower right), as well as dimly in a subset of mature B cells (orange). These lymphoid cells typically have low side scatter.

B Cell Tube

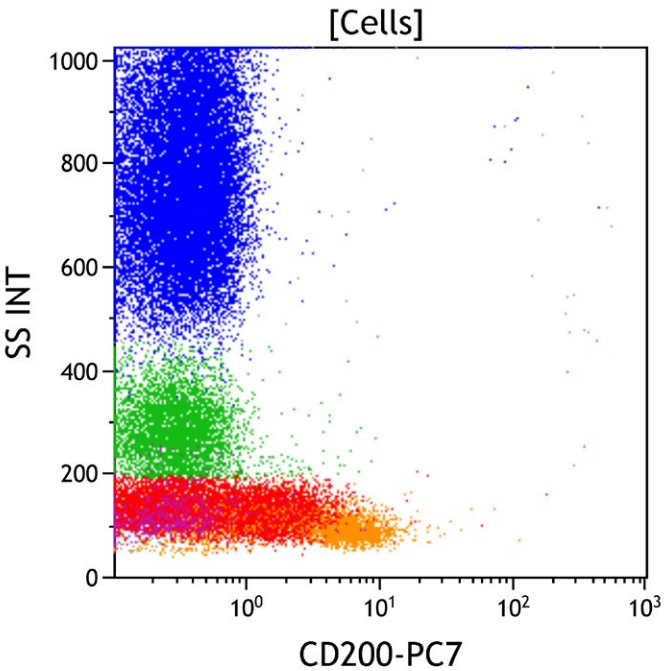


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

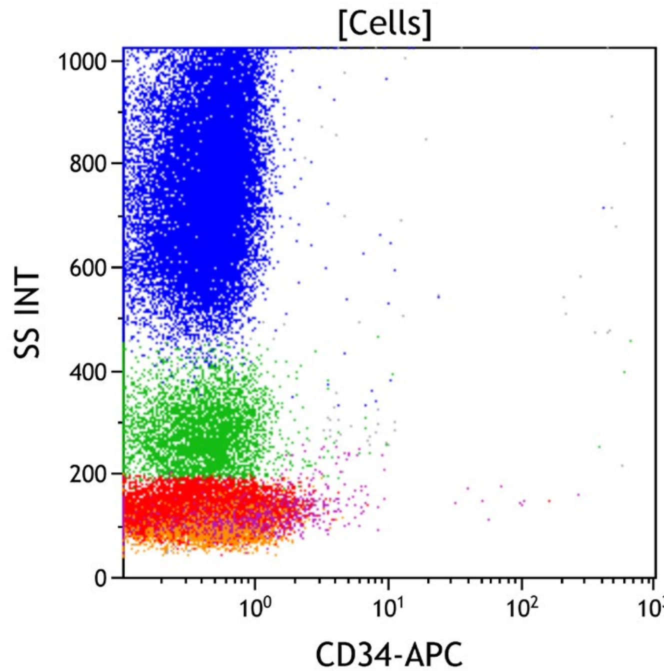


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

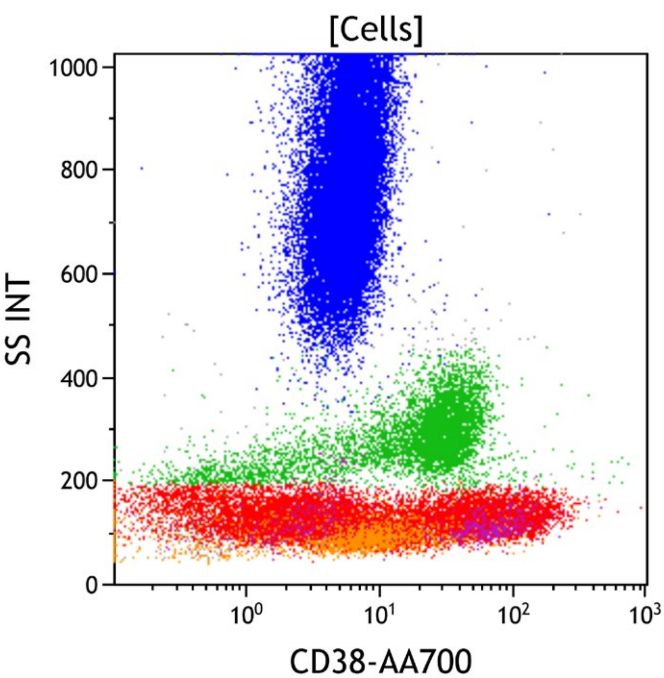


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes.

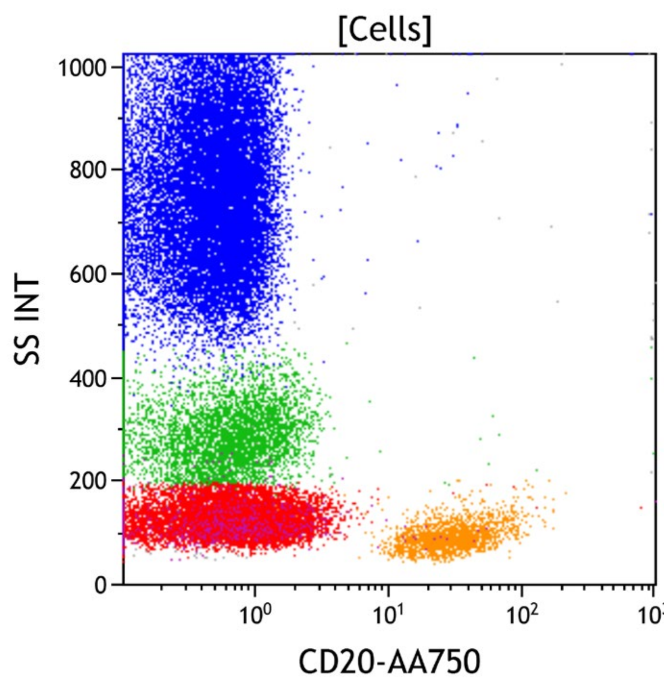


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.

B Cell Tube

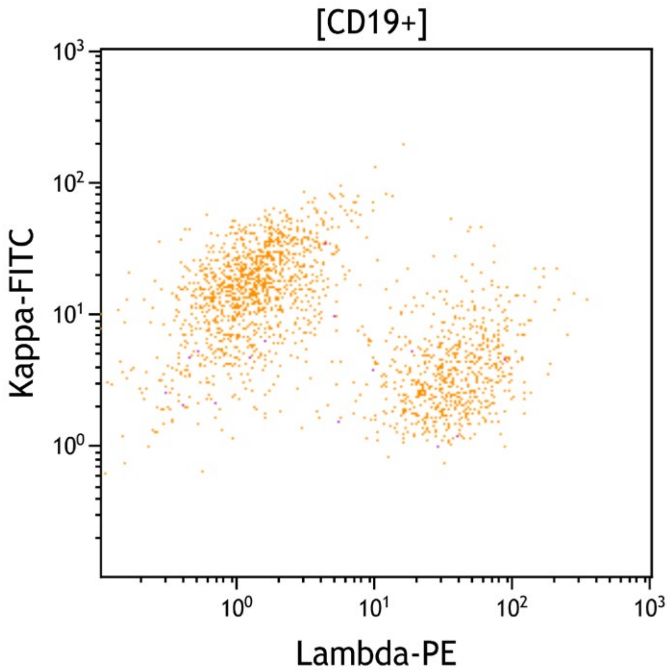


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

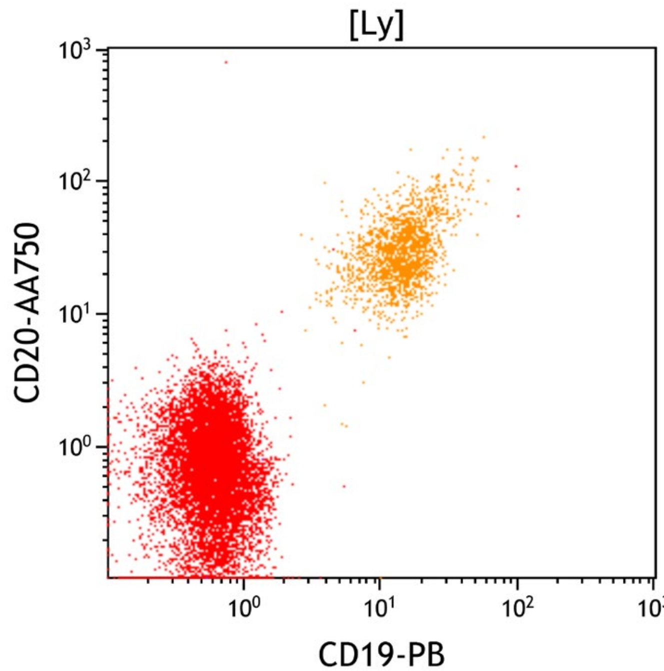


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression.

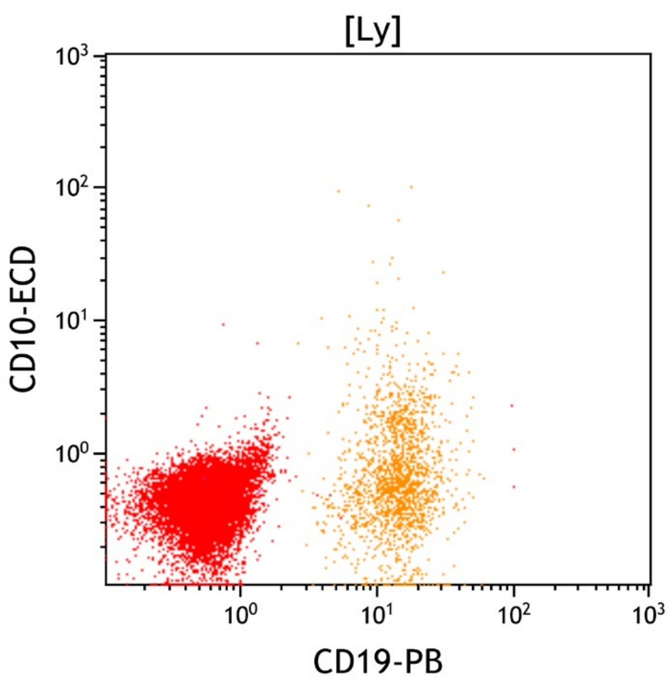


Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates.

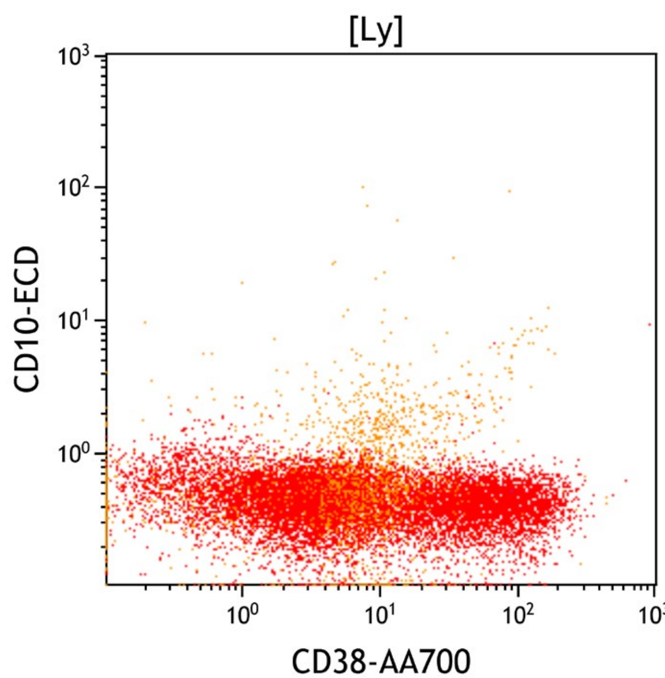


Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state.

B Cell Tube

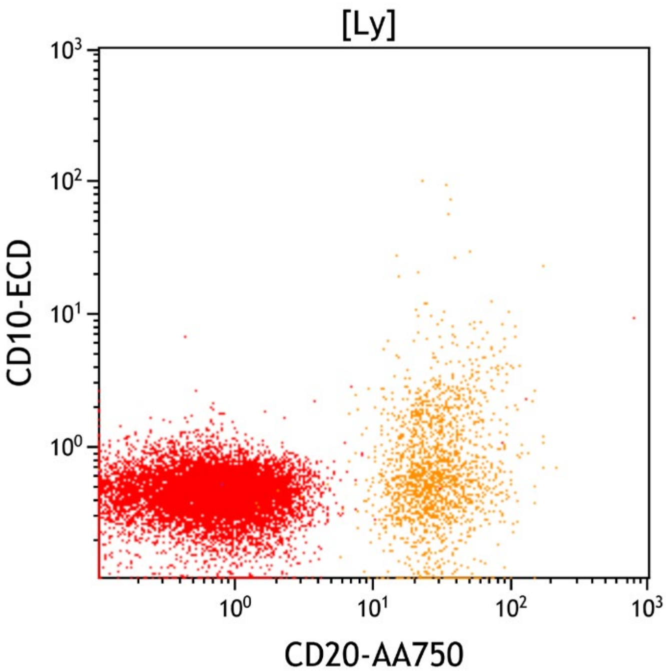


Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20 without CD10.

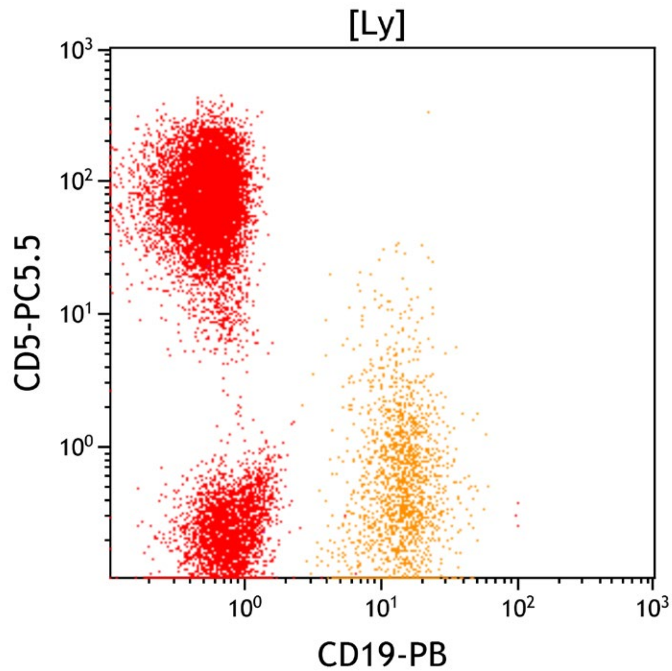


Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper left), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

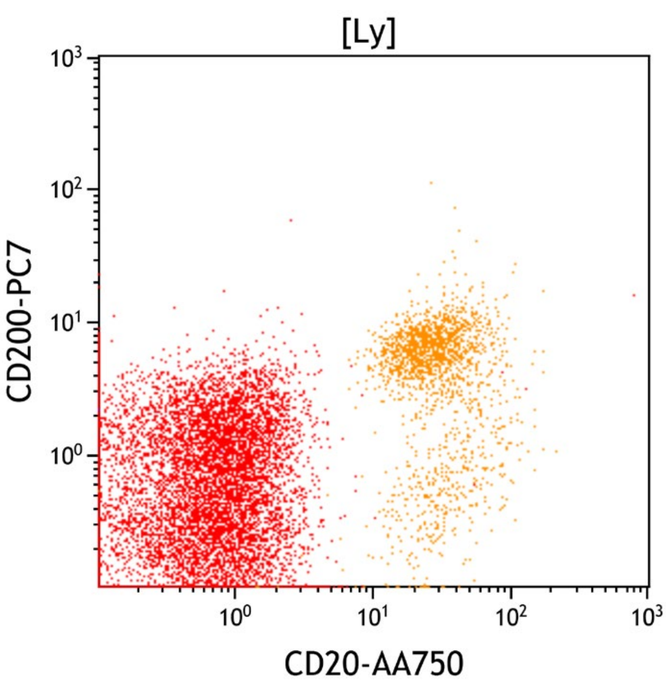


Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

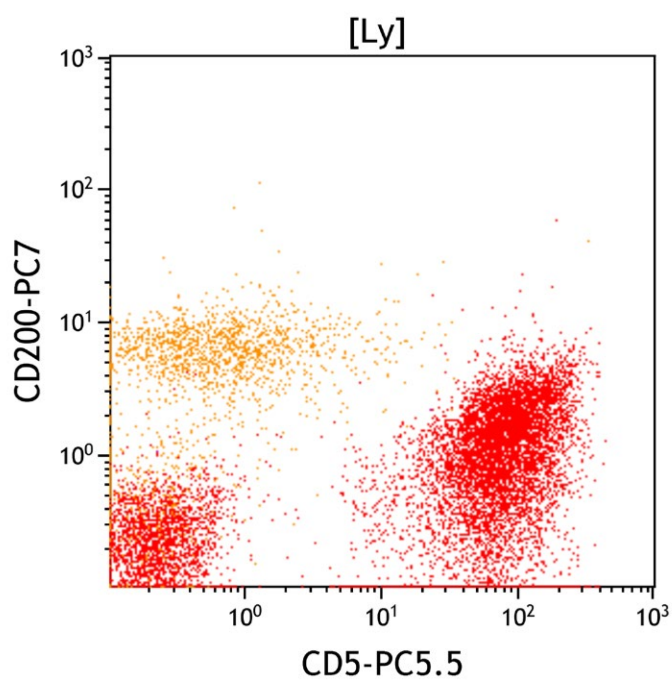


Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

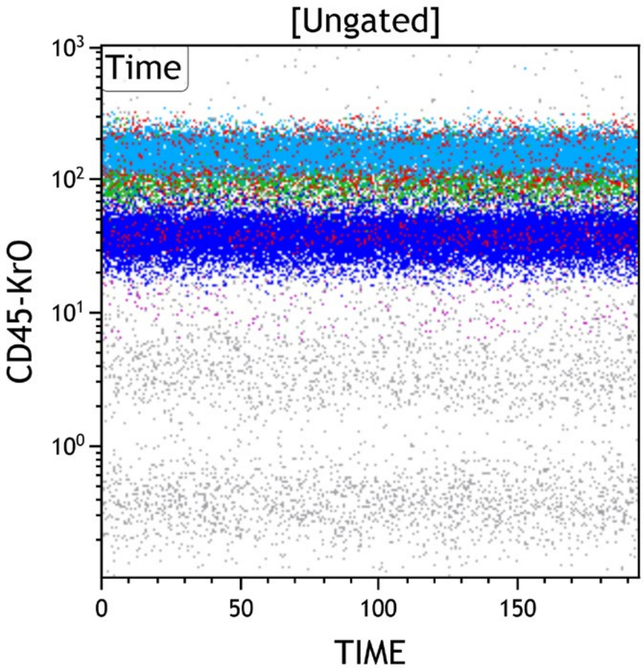


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

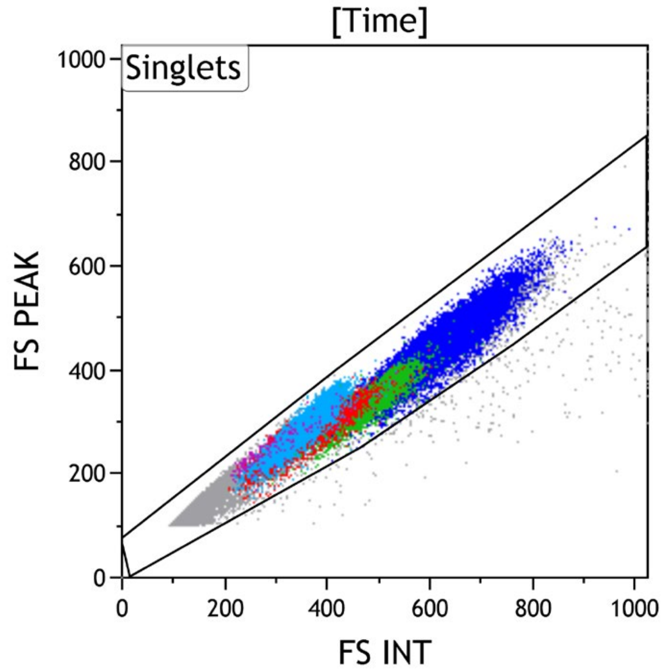


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

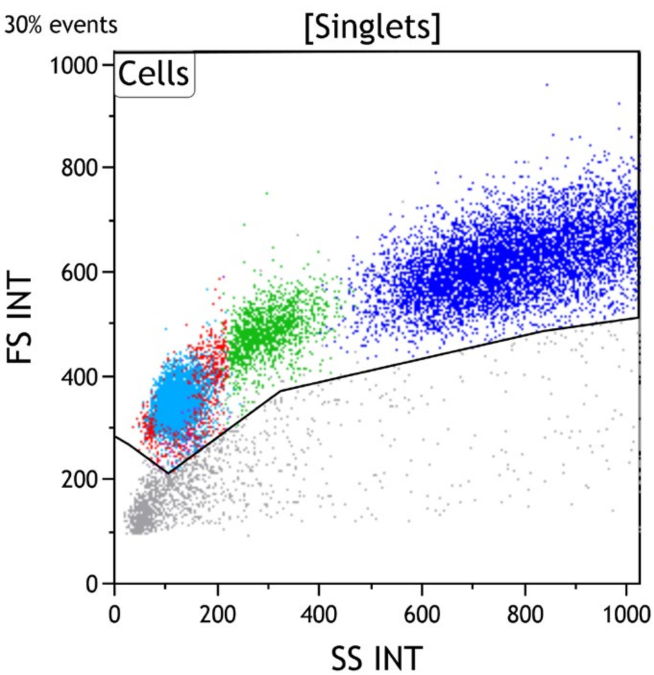


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

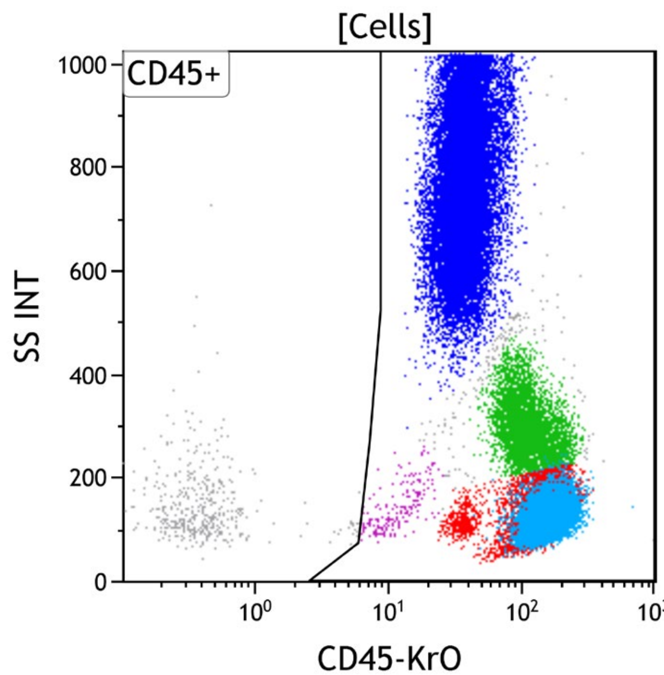
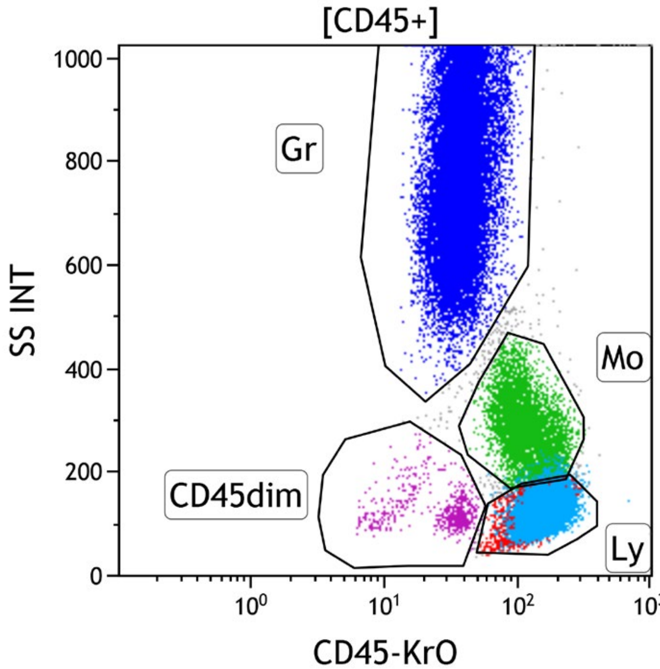


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube



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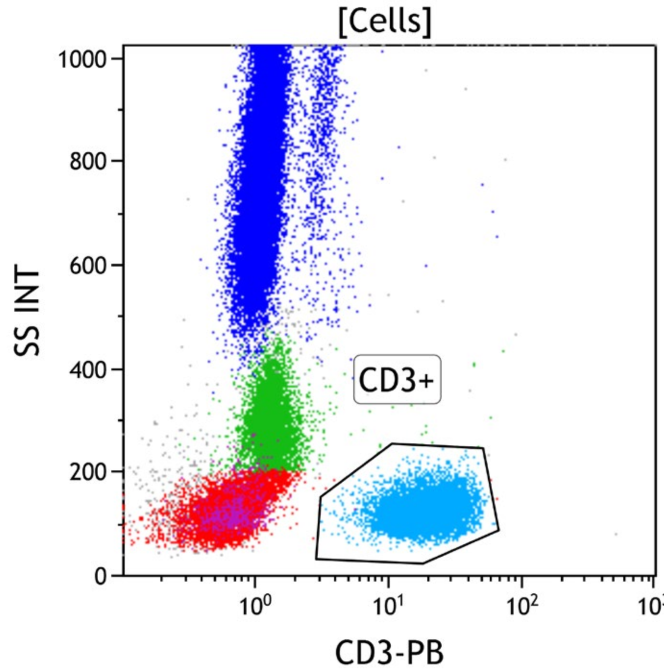

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

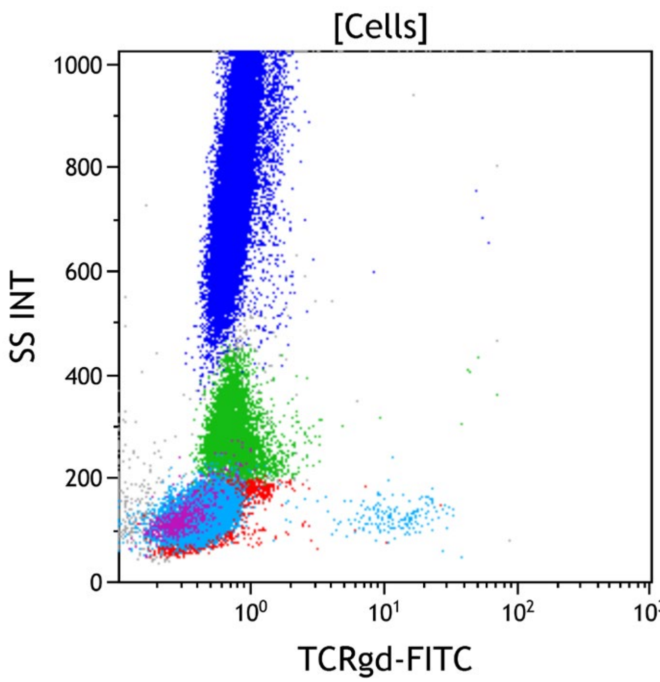

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

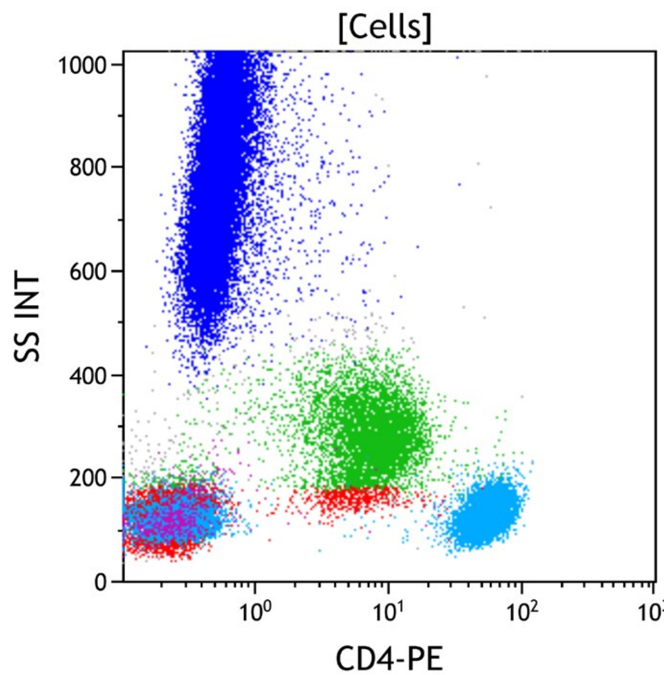

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells.

T Cell Tube

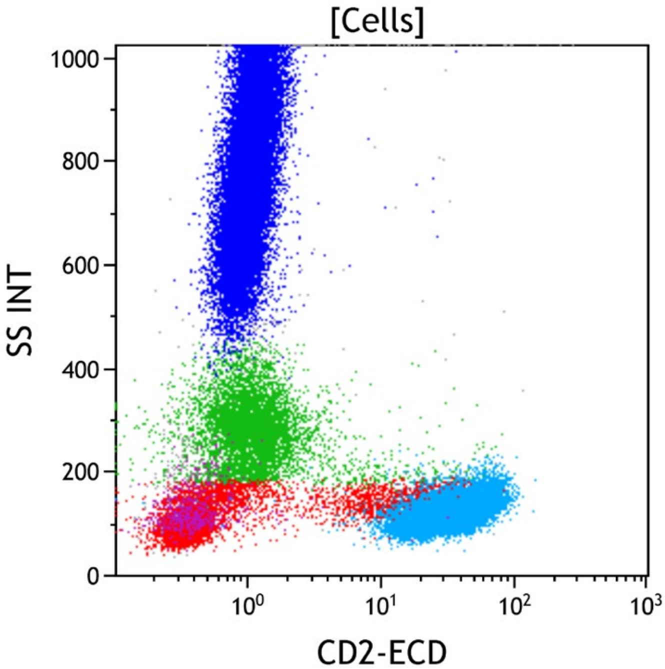

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

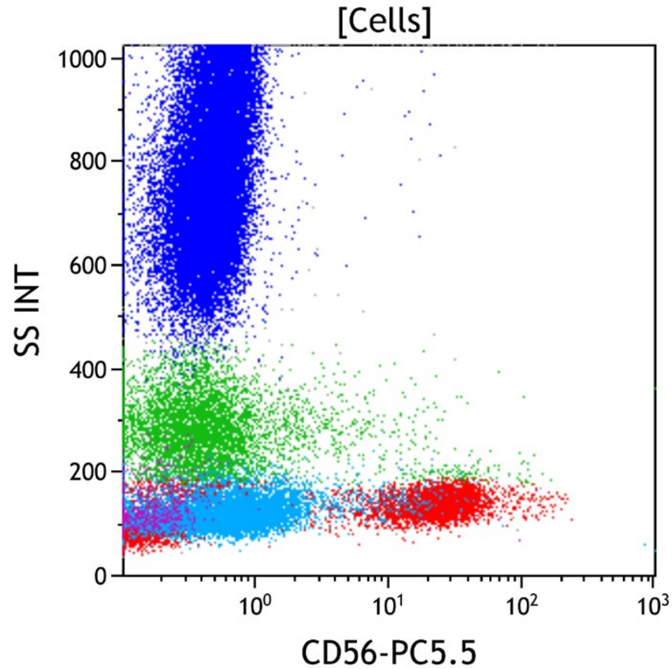

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

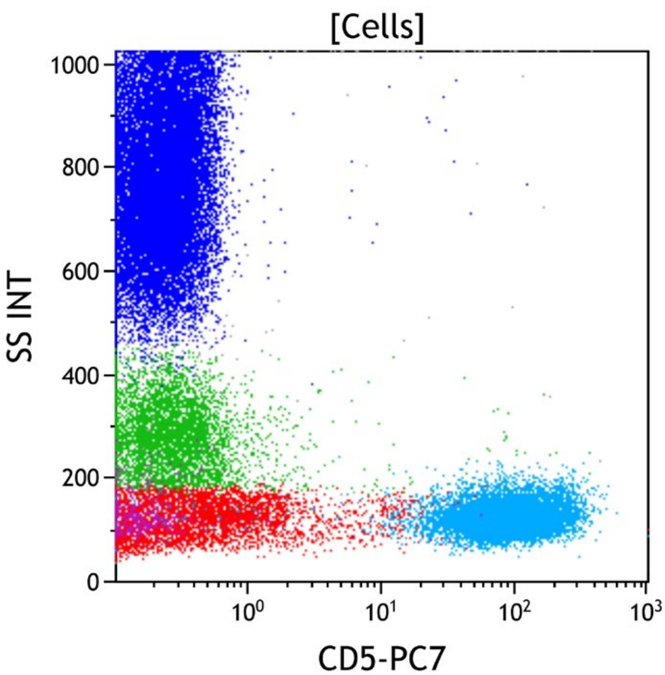

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

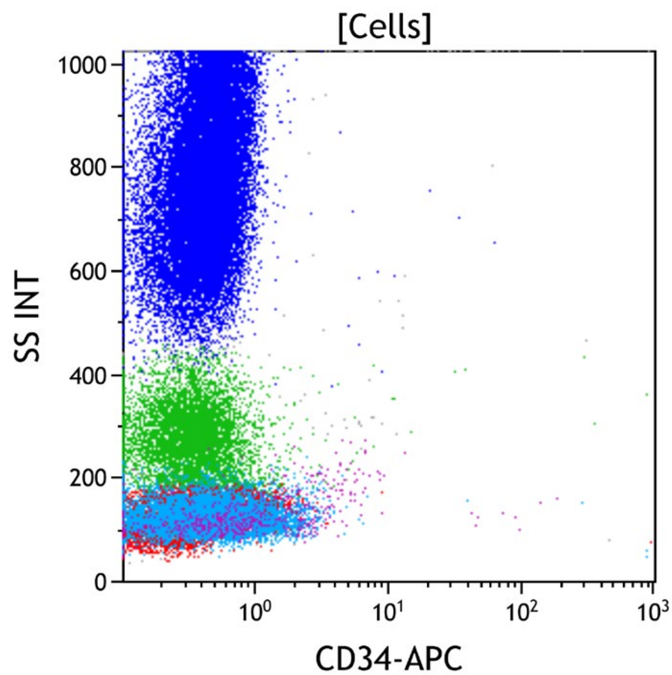

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

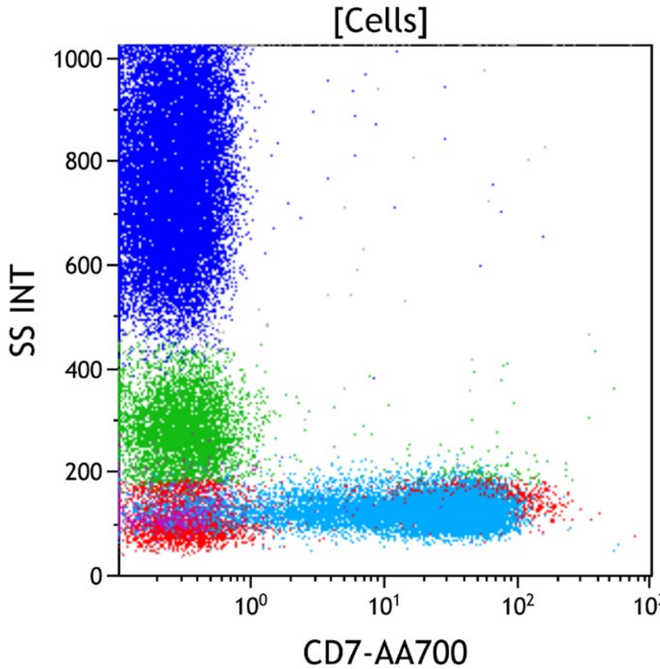

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

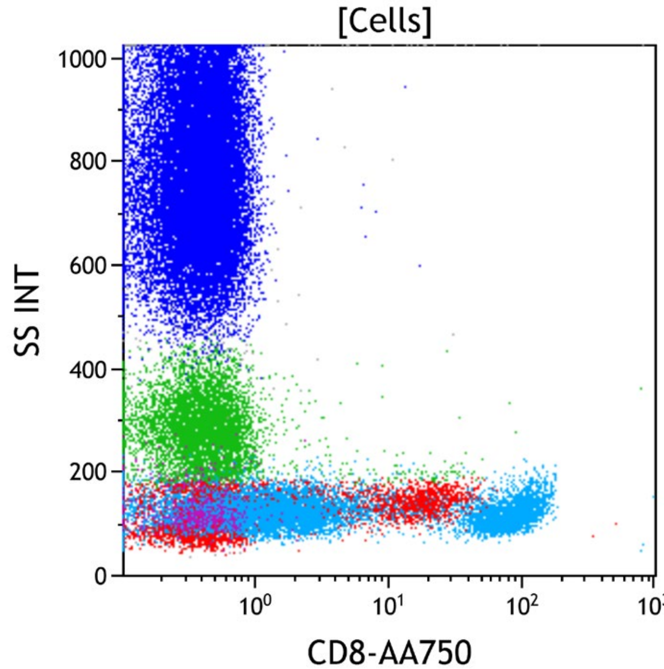

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

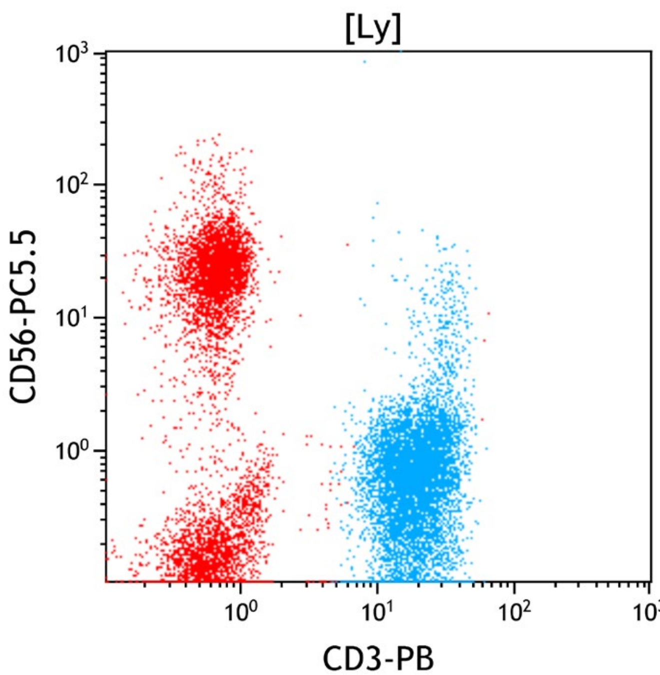

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

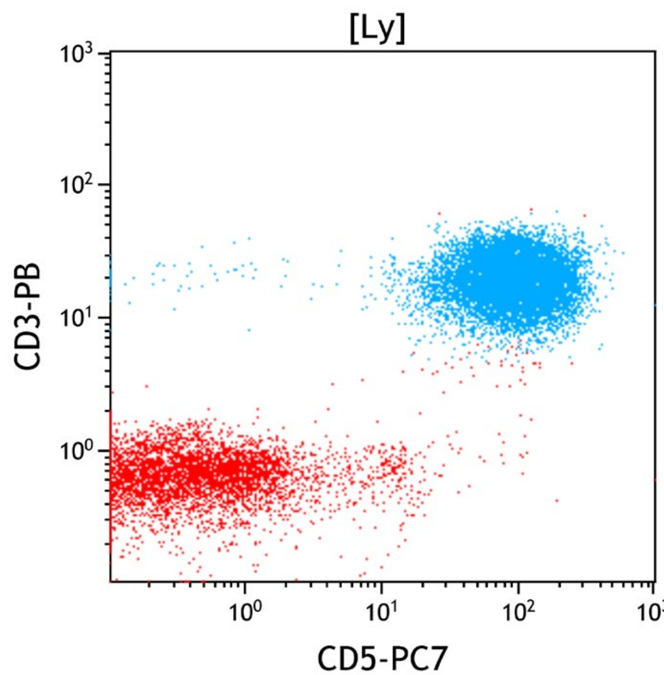

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

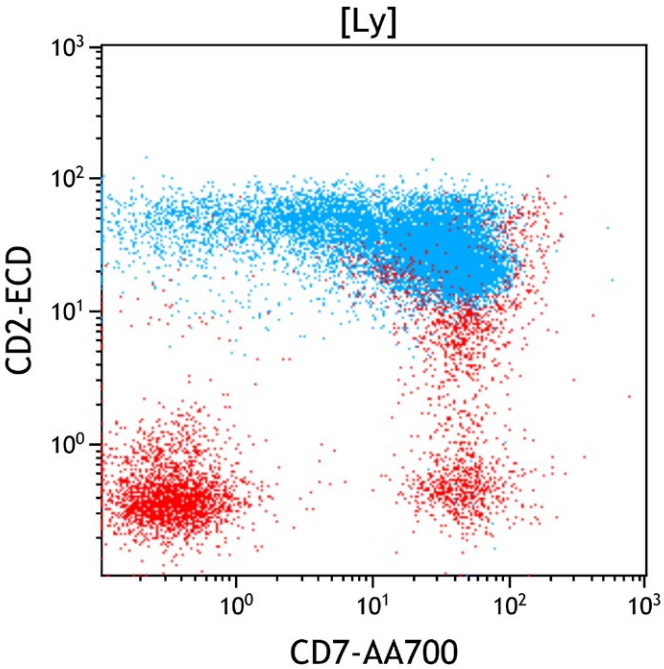

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

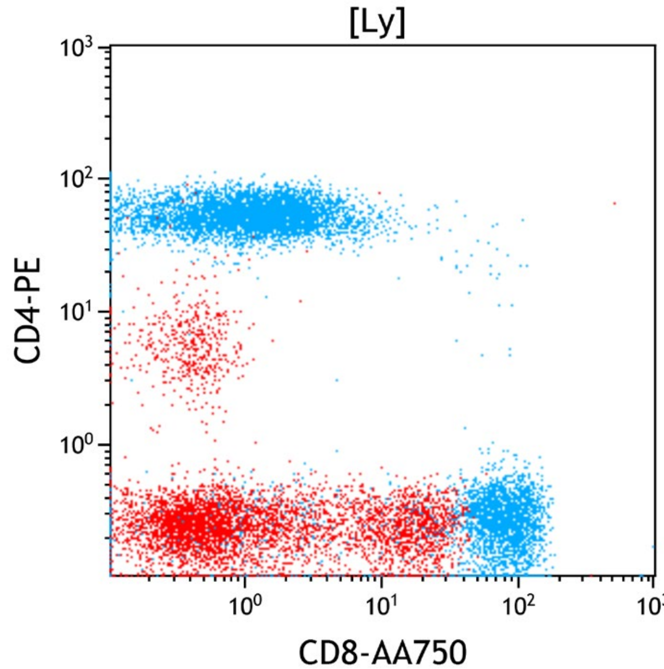

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

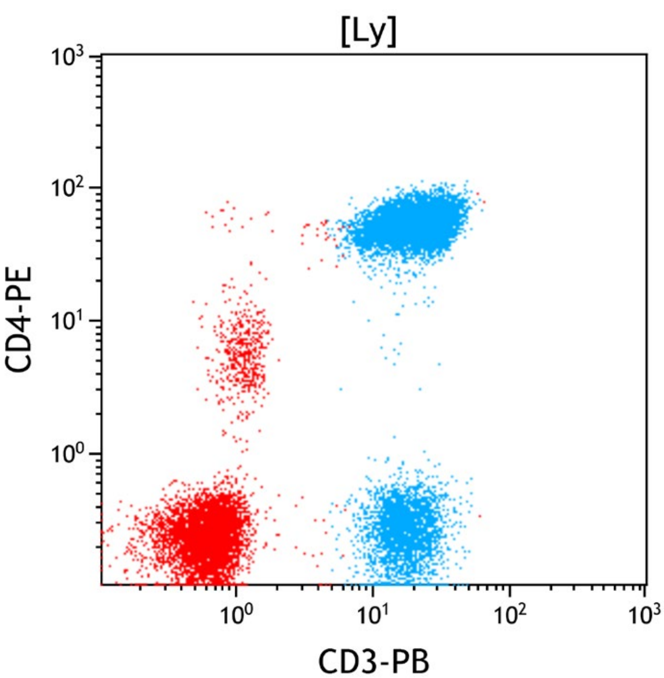

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

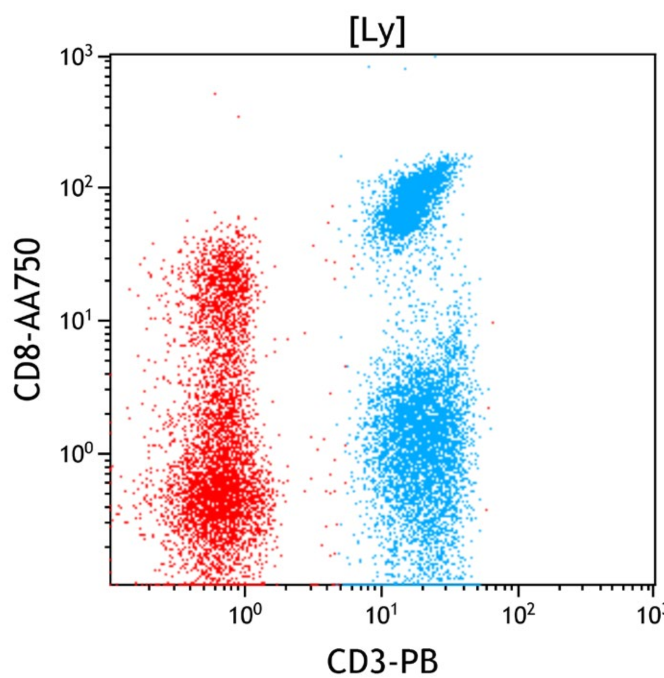

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

T Cell Tube

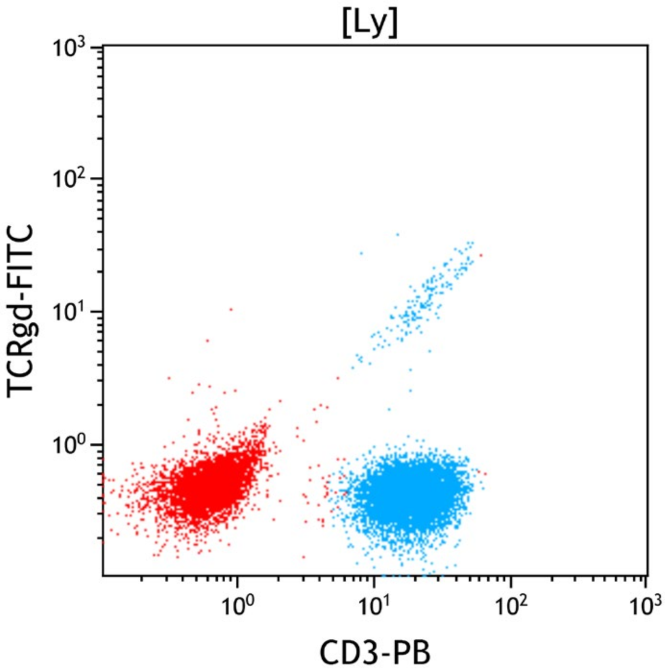

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

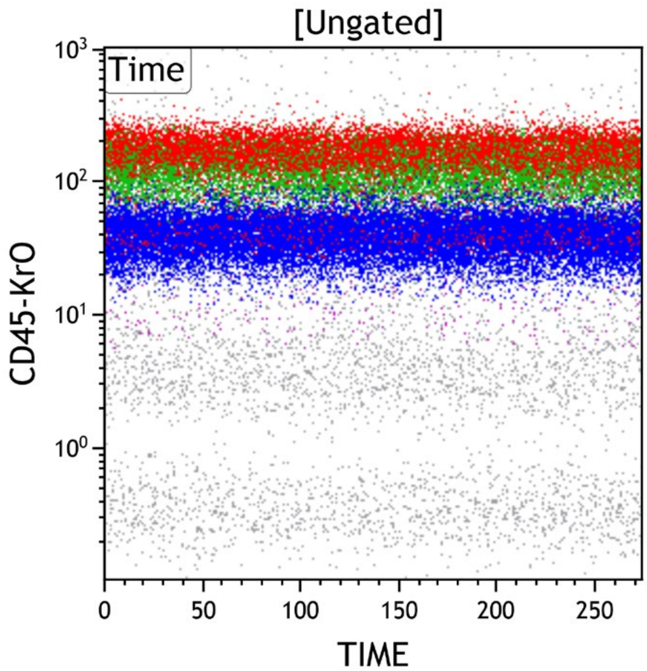

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

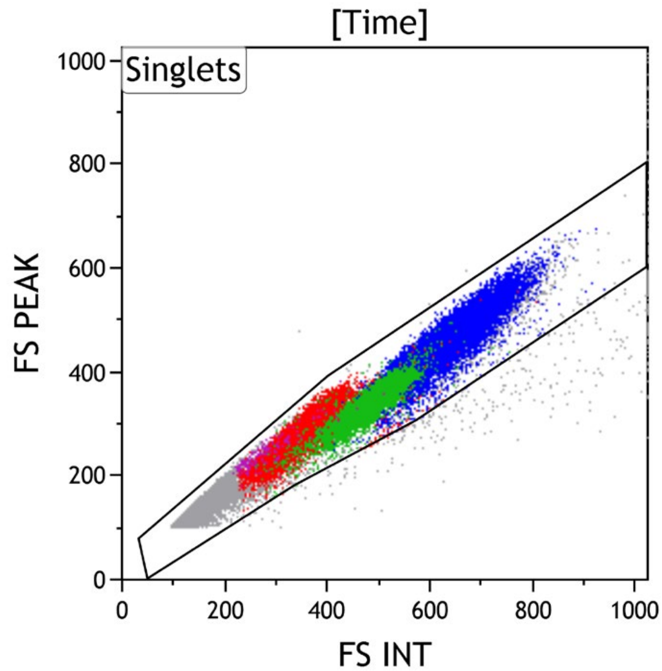

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

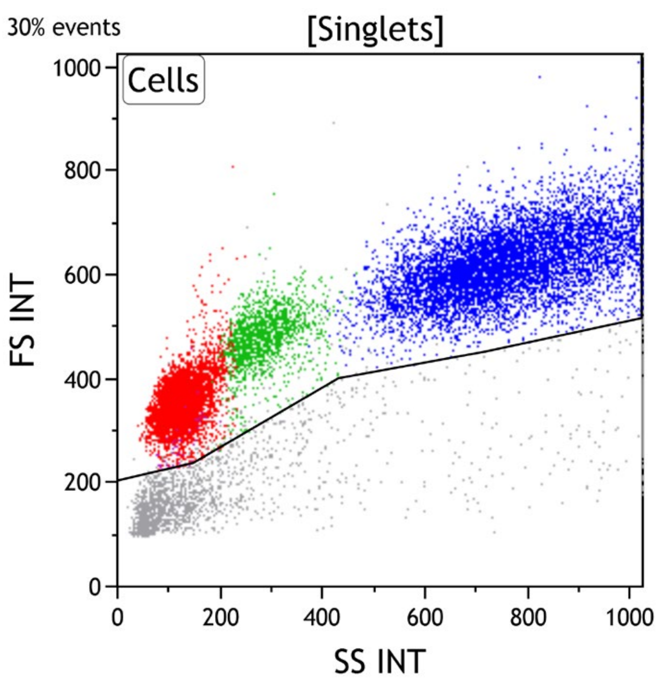

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

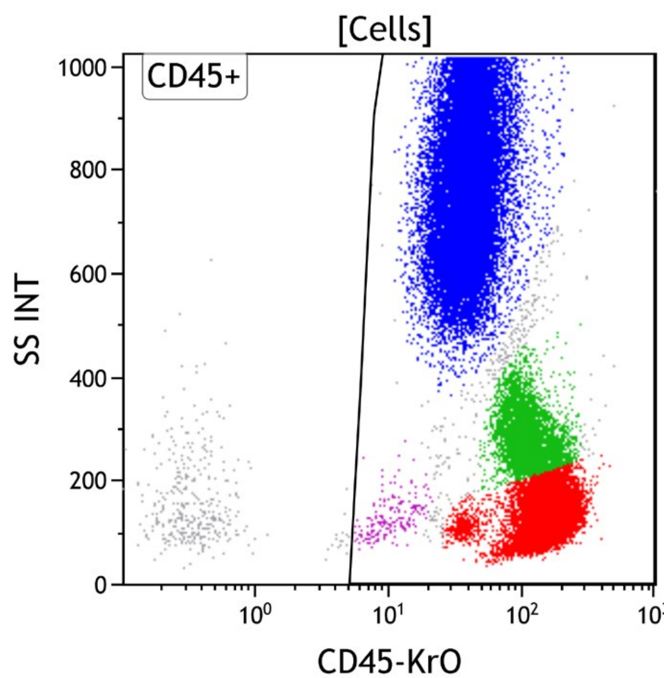

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

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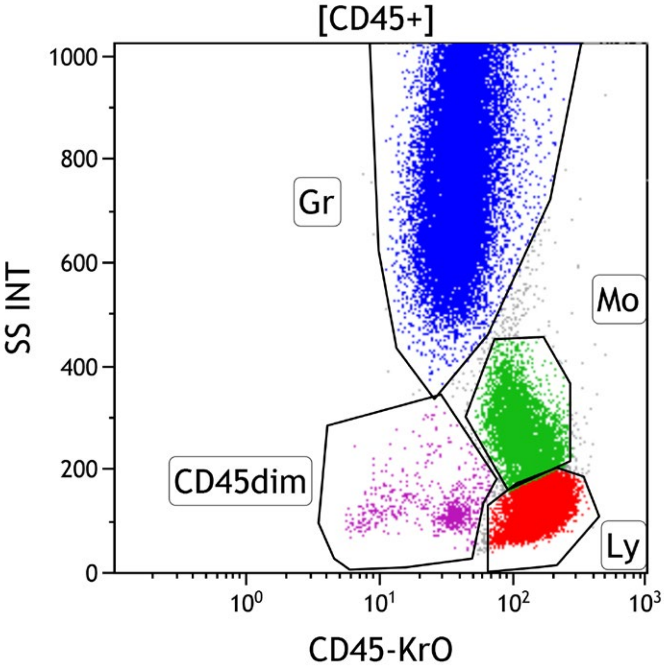

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

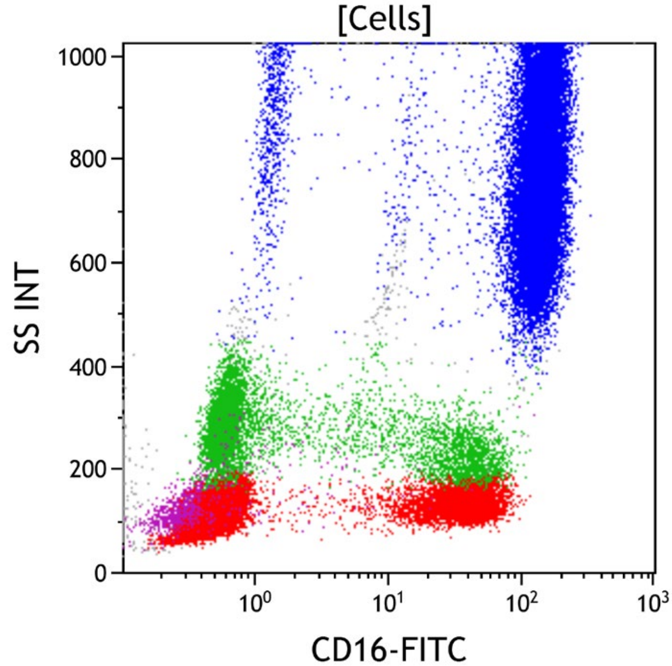

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

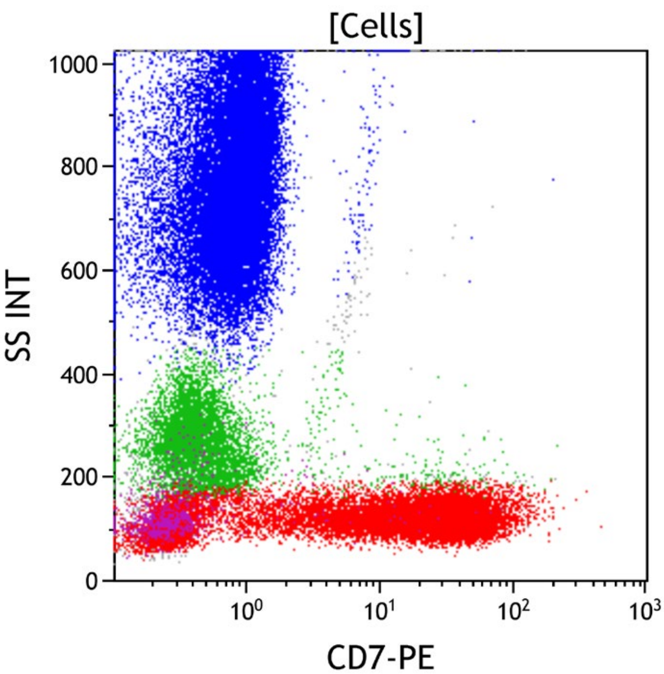

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red, lower right). It is also expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

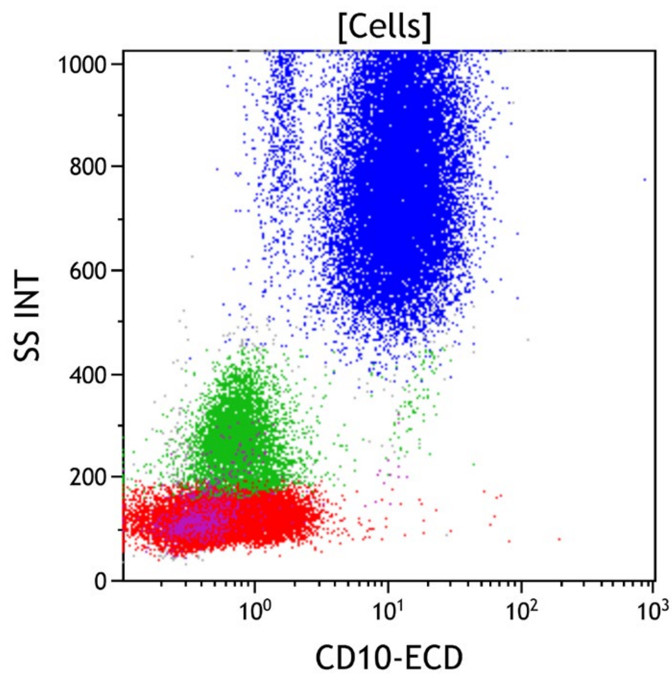

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

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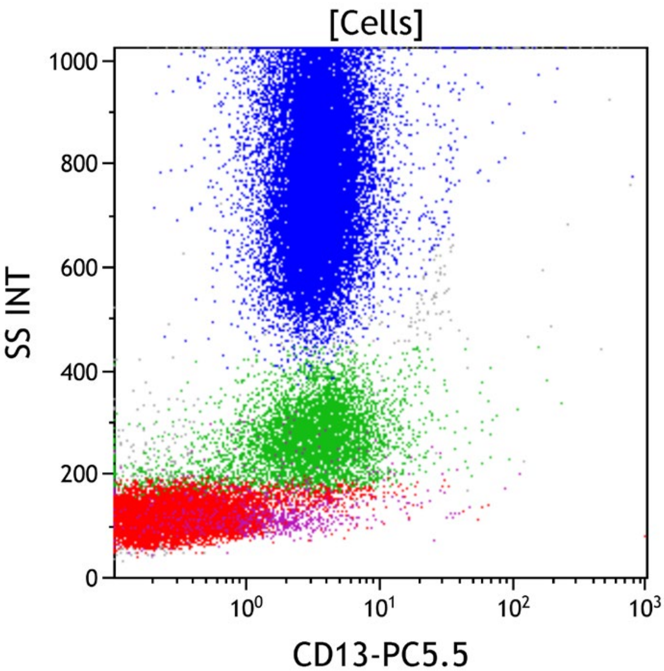

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), mature monocytes (green), and myeloid progenitors (purple).

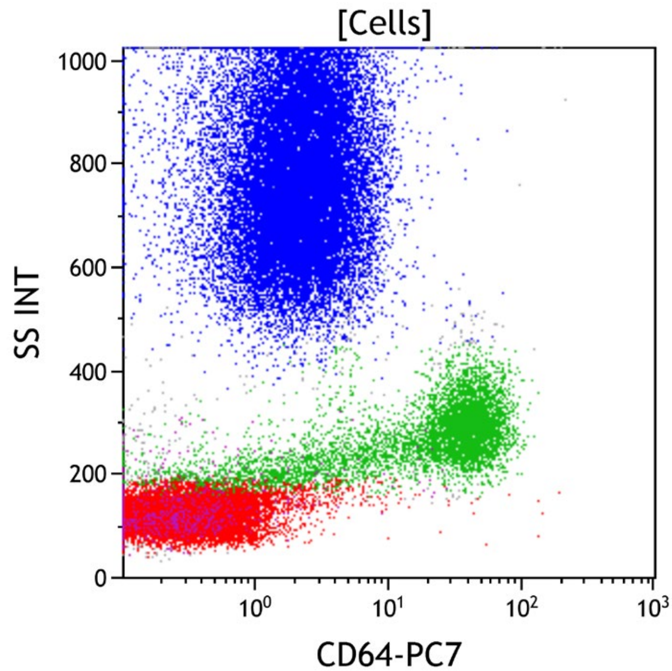

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature monocytes (green). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphocytes (red) or most CD34 positive progenitors. Activated mature monocytes express CD64 at lower level and have lower side scatter.

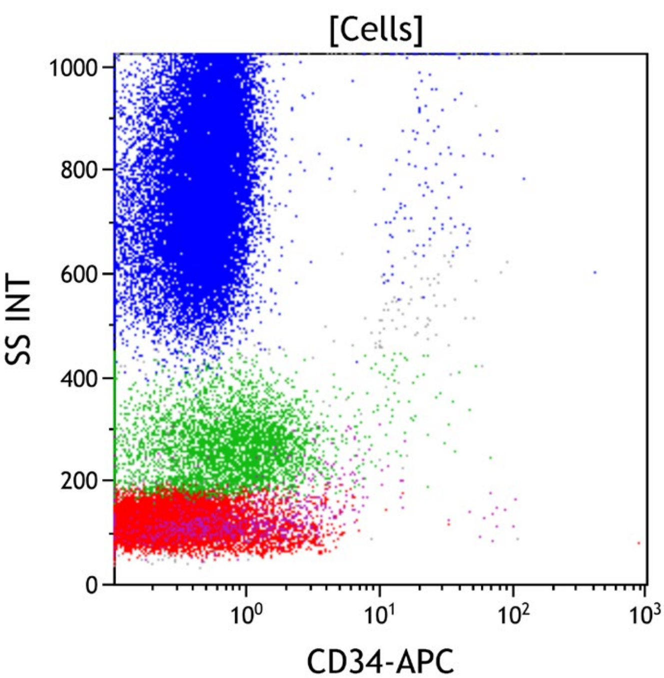

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

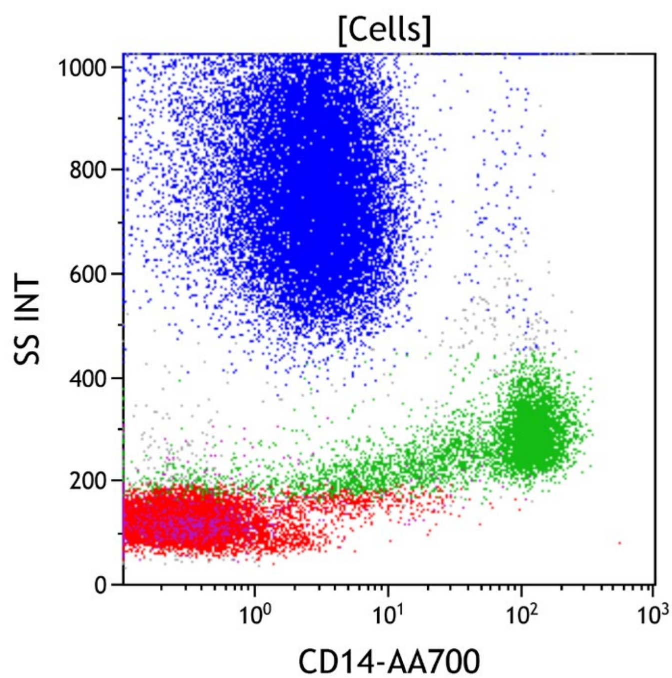

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a low level on mature granulocytes (blue). Activated mature monocytes express CD14 at a lower level and have lower side scatter.

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M1 Cell Tube

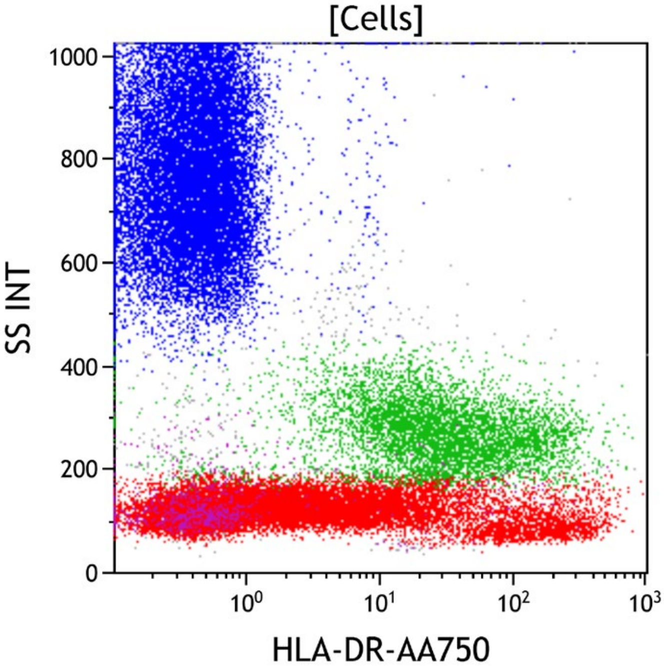


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells, and activated T cells.

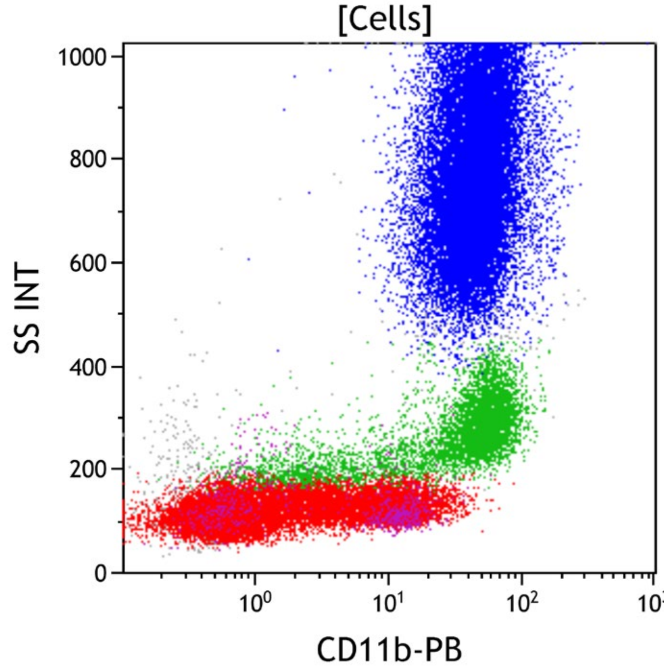


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (blue) and on monocytes (green). CD11b is also expressed on NK cells and basophils.

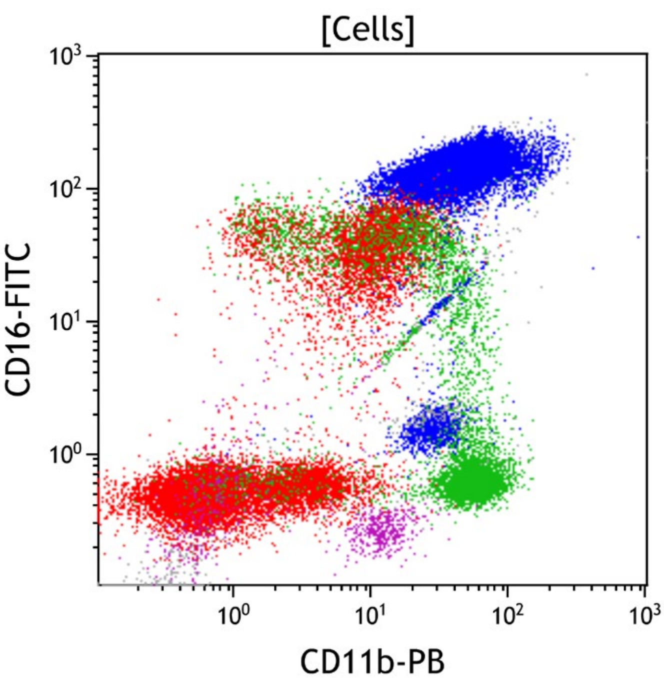


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). Activated mature monocytes express CD16 at a variable level and are CD11b positive.

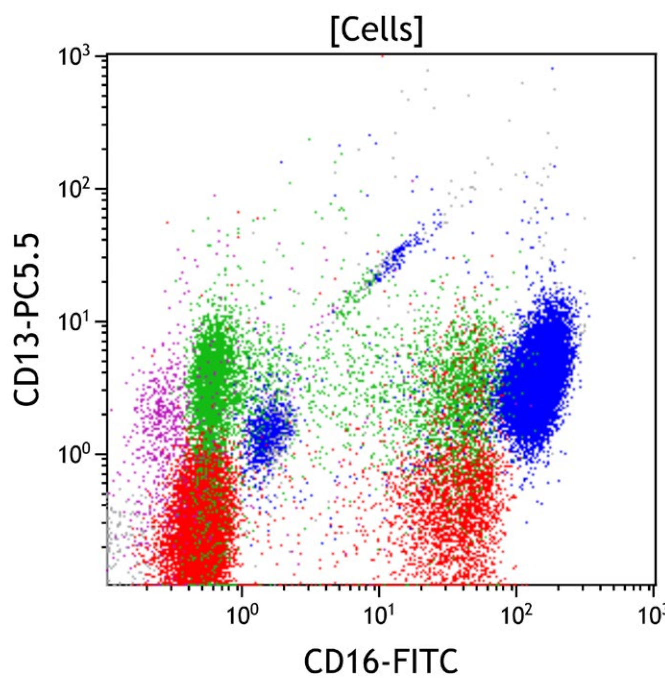


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right).

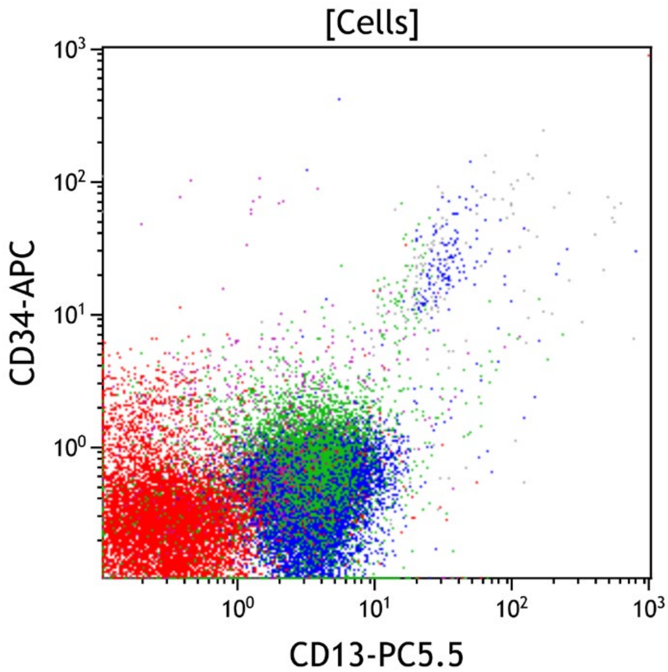


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34.

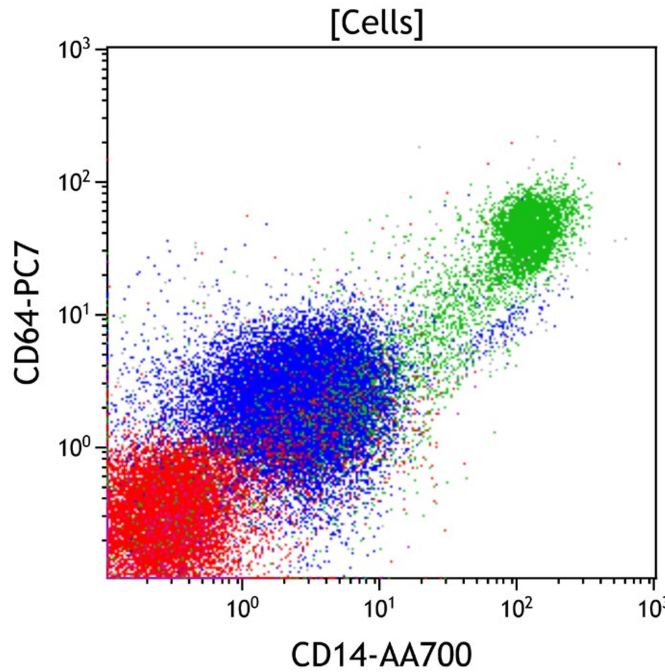


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes (blue). Activated mature monocytes express CD14 and CD64 at a variably lower level.

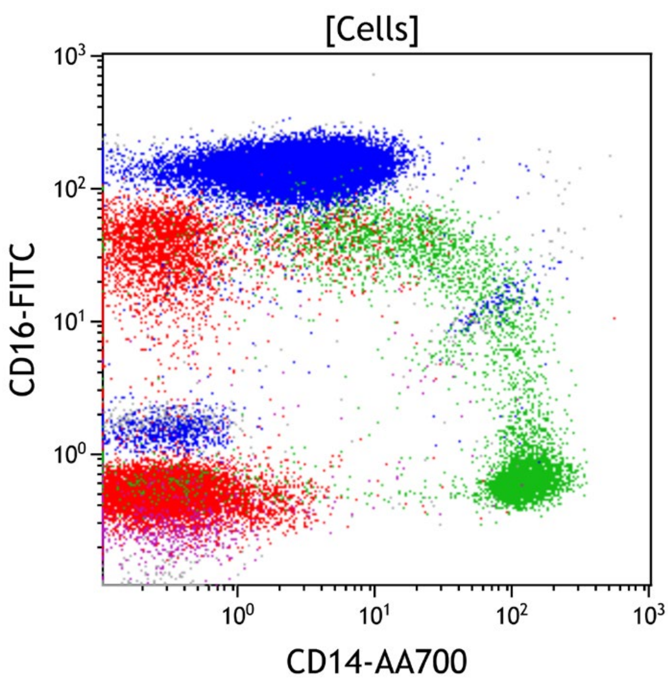


Figure 19. This CD14 vs CD16 dot plot shows all viable cells. CD14 is expressed at a high level on monocytes (green) and a lower level on granulocytes (blue, upper left). CD16 is expressed on granulocytes and a subset of NK cells (red, middle left). Activated mature monocytes express CD14 and CD16 at a variably lower level.

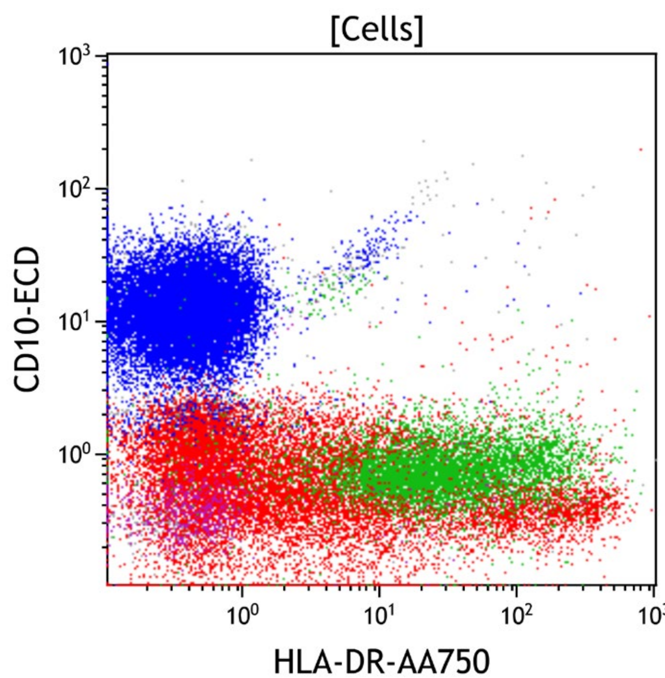


Figure 20. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (Green), B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue).

M1 Cell Tube

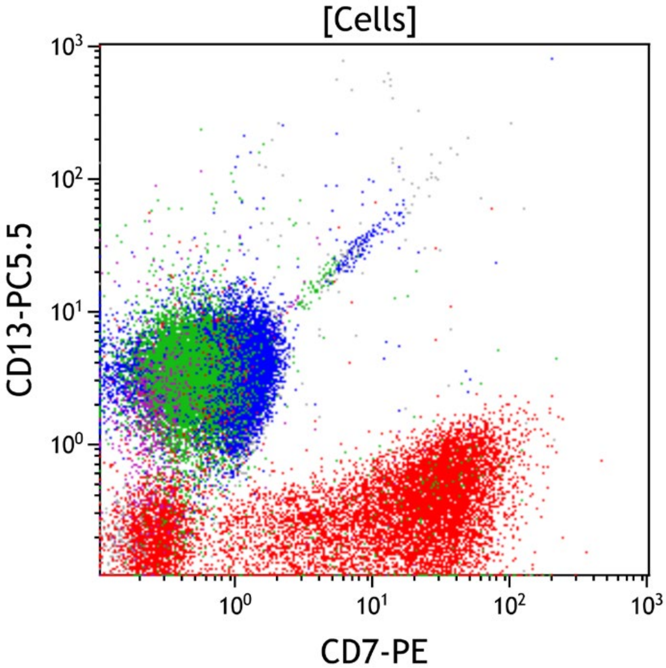


Figure 21. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

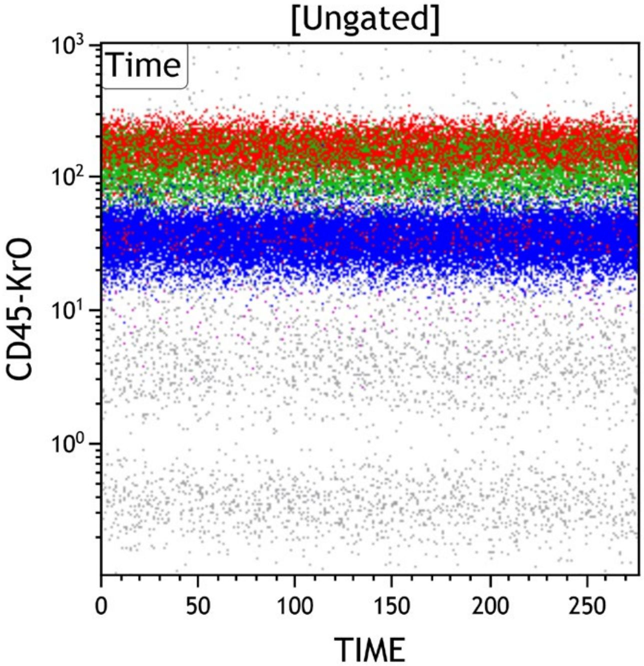
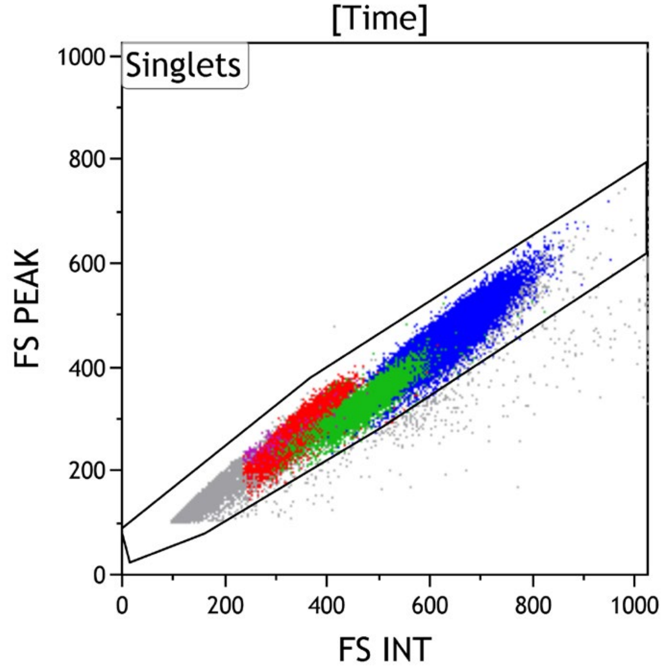


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.



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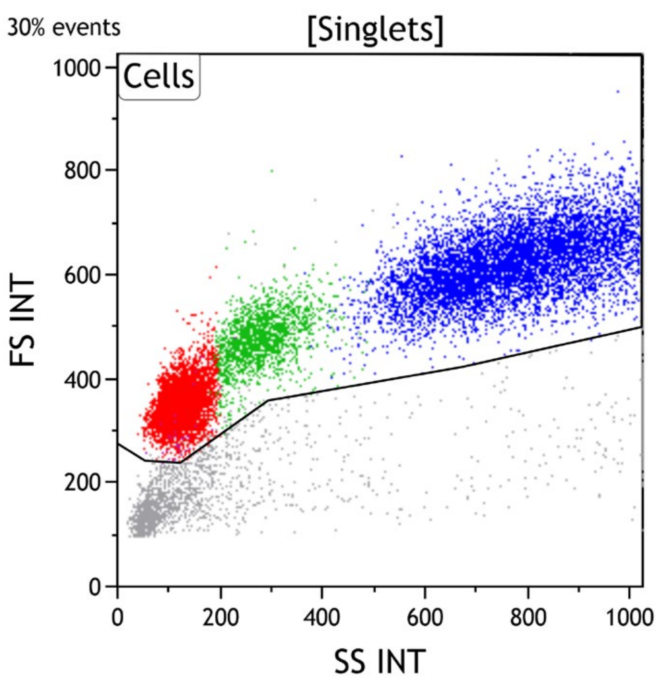


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

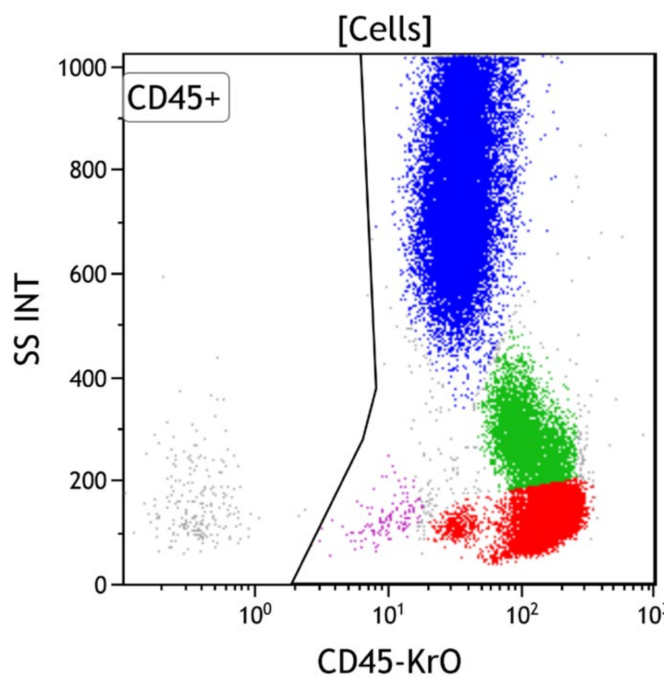
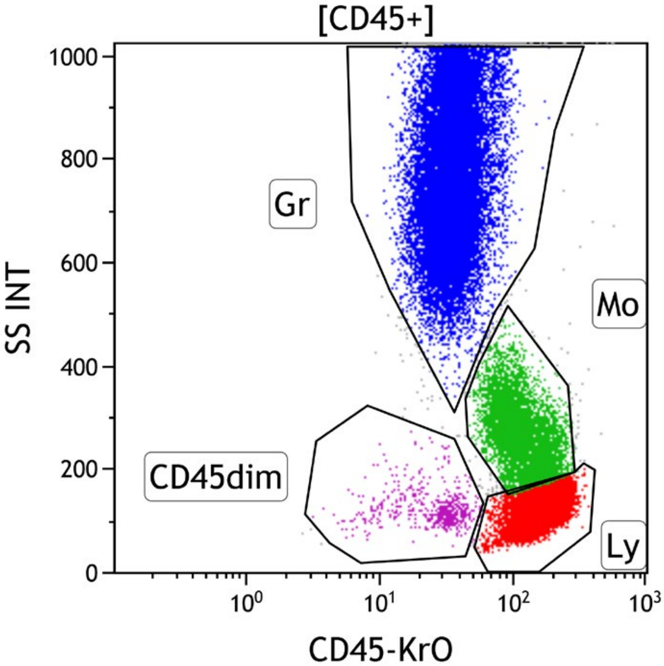


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.



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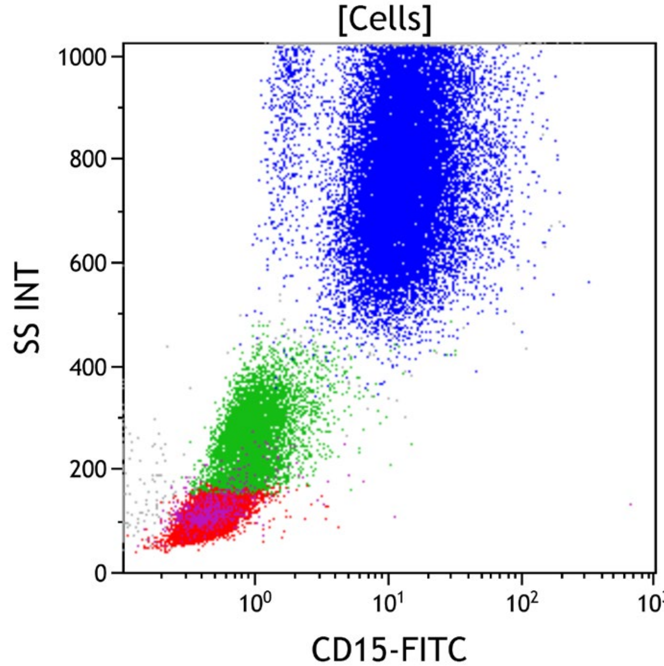

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes (green).

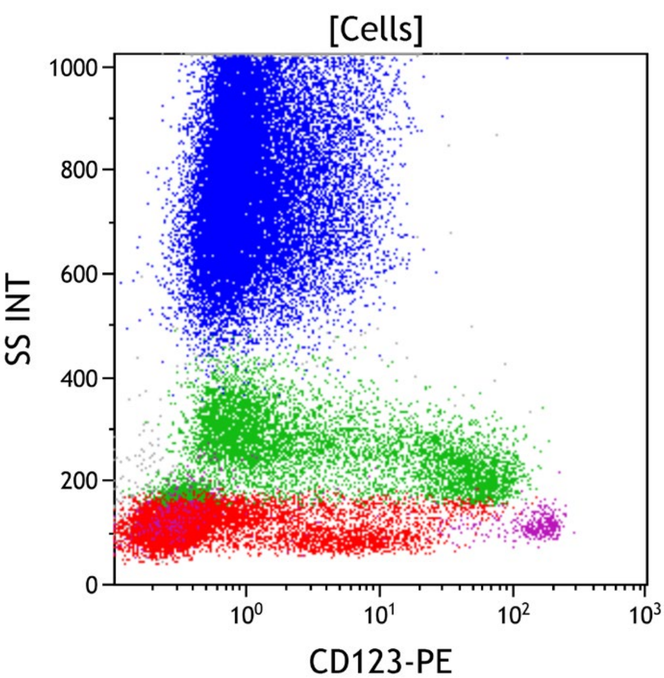

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

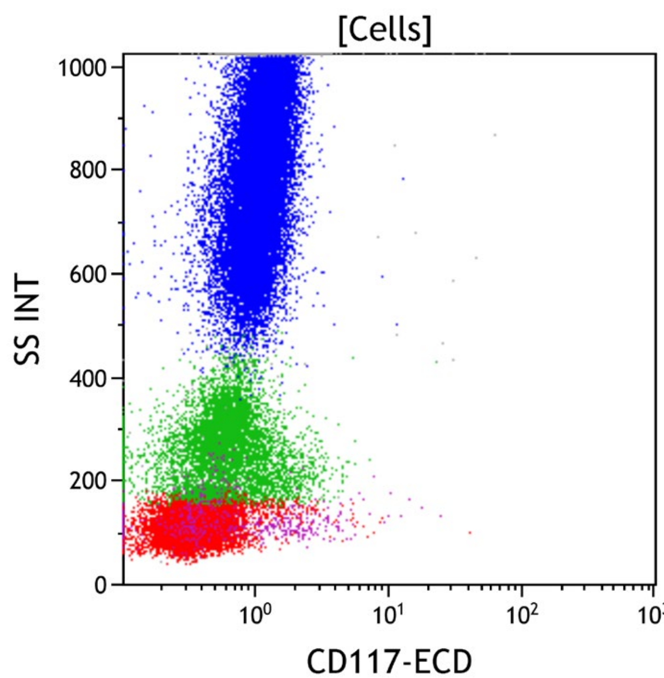

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Mature granulocytes, monocytes, and lymphocytes are negative for CD117.

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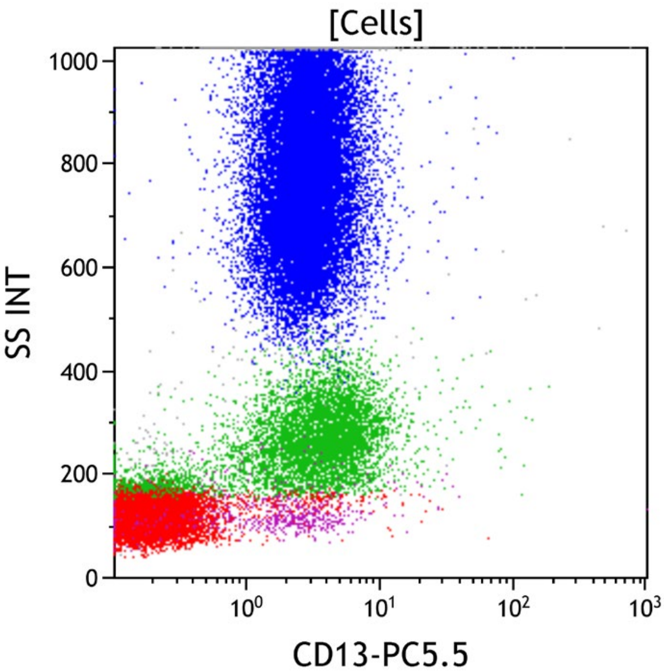

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and mature monocytes (green) and variably on myeloid progenitors.

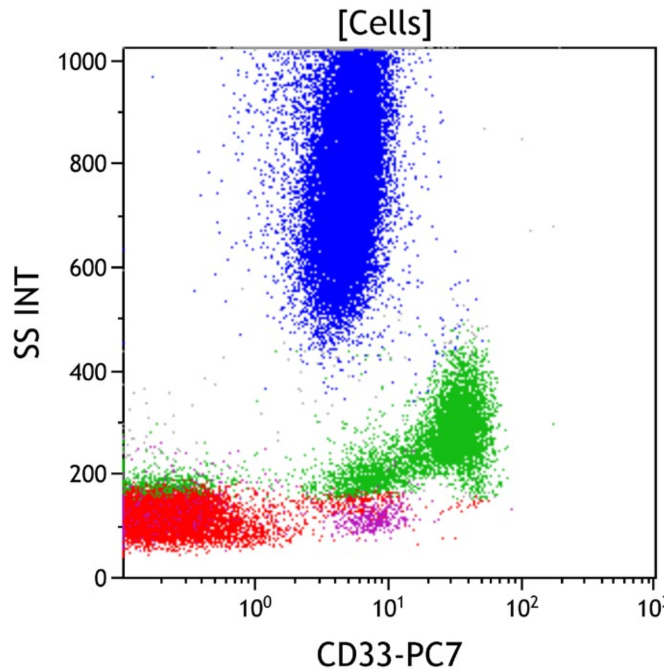

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors.

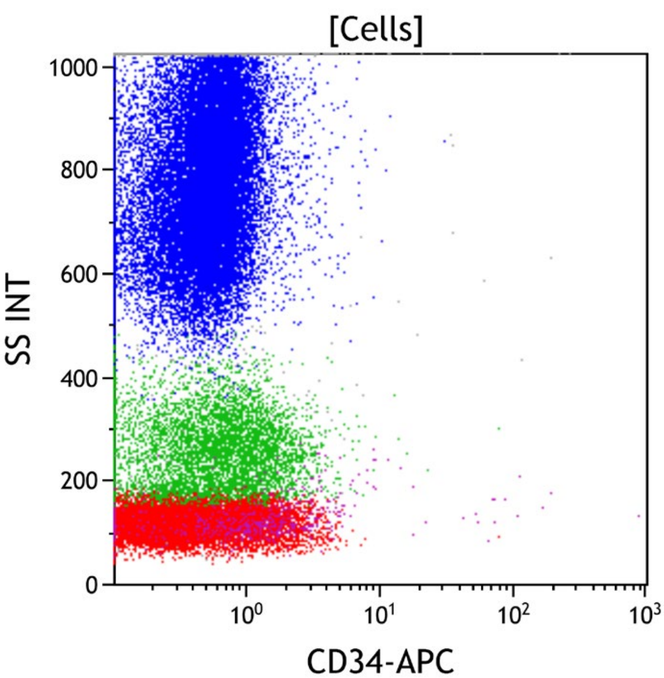

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

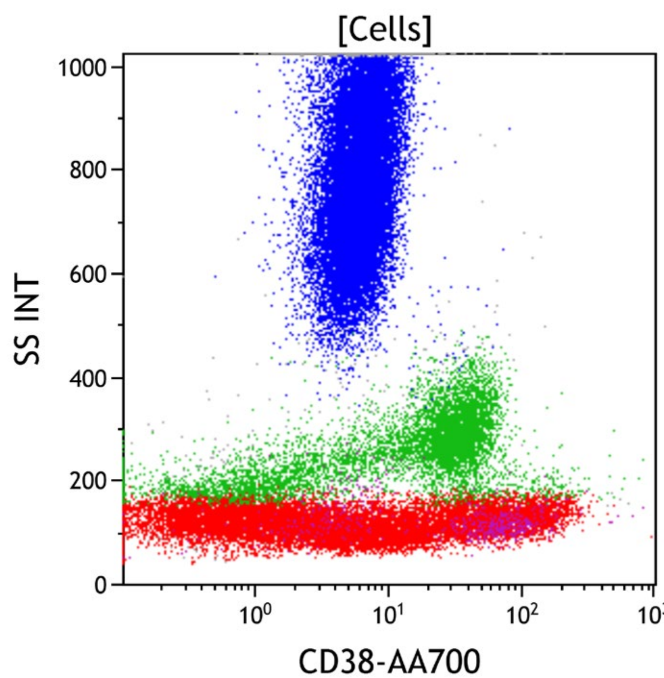

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red).

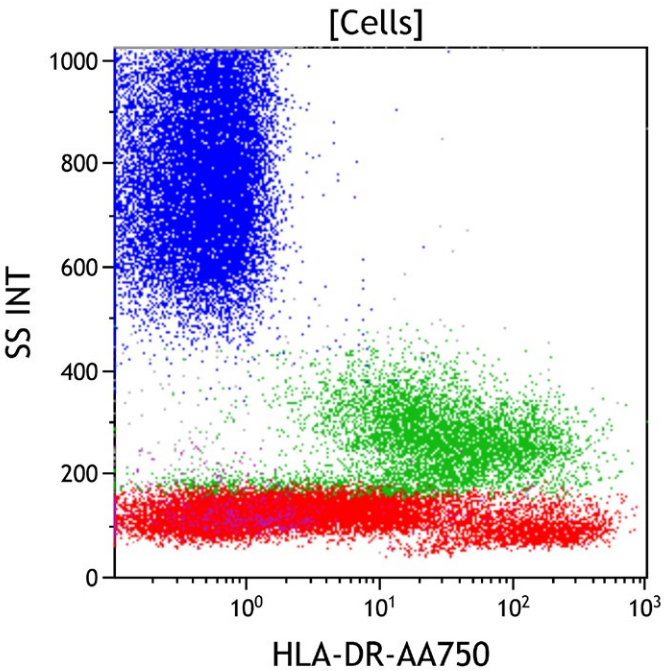

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red, lower right), and activated T cells.

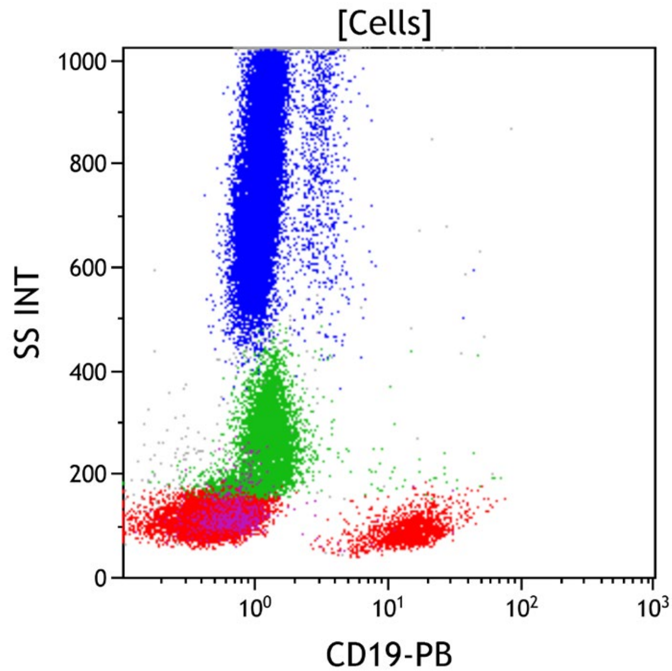

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on immature and mature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter.

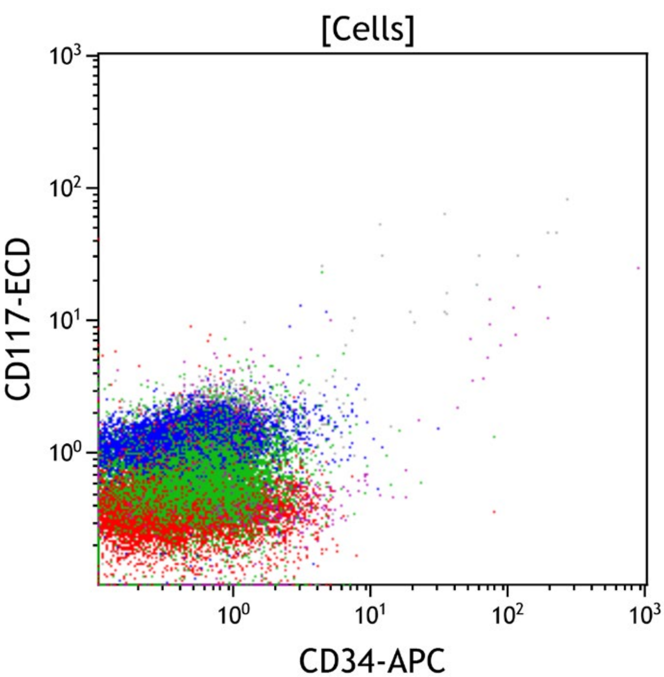

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34 and CD117. CD34 and CD117 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

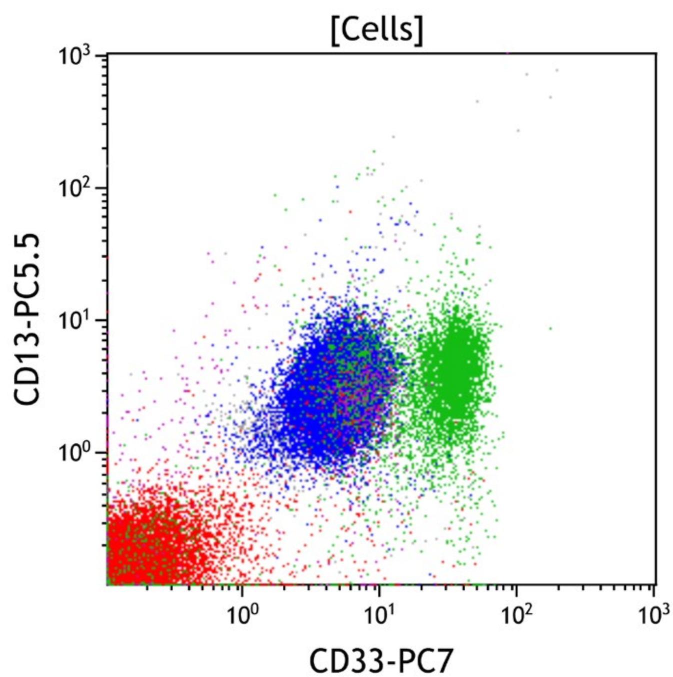

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes (red) do not express either CD13 or CD33.

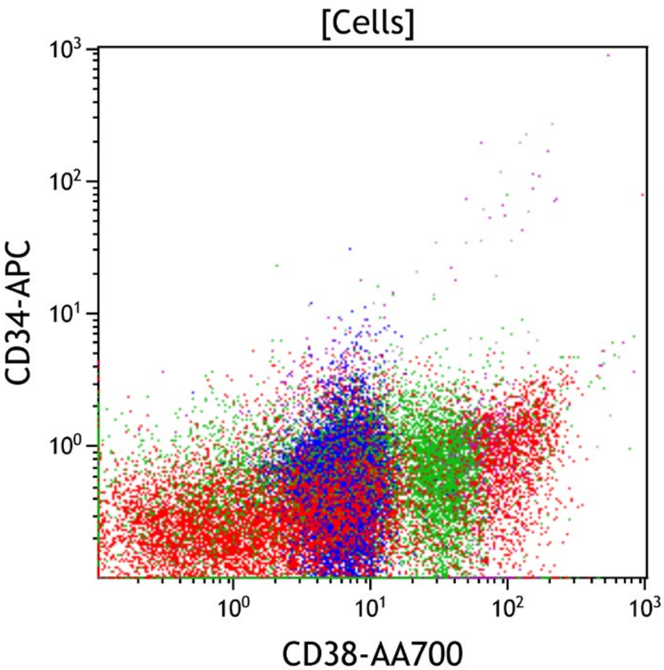

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is an activation marker. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes, monocytes, and lymphocytes are negative for CD34 with variable CD38. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

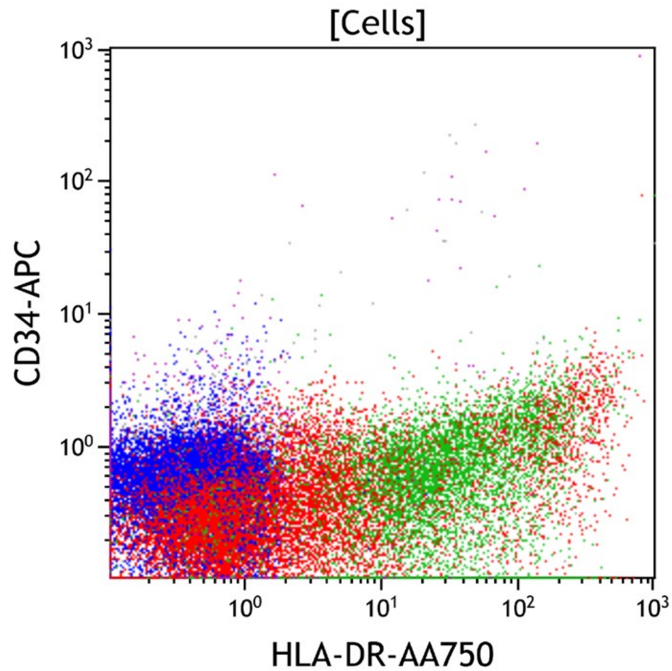

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

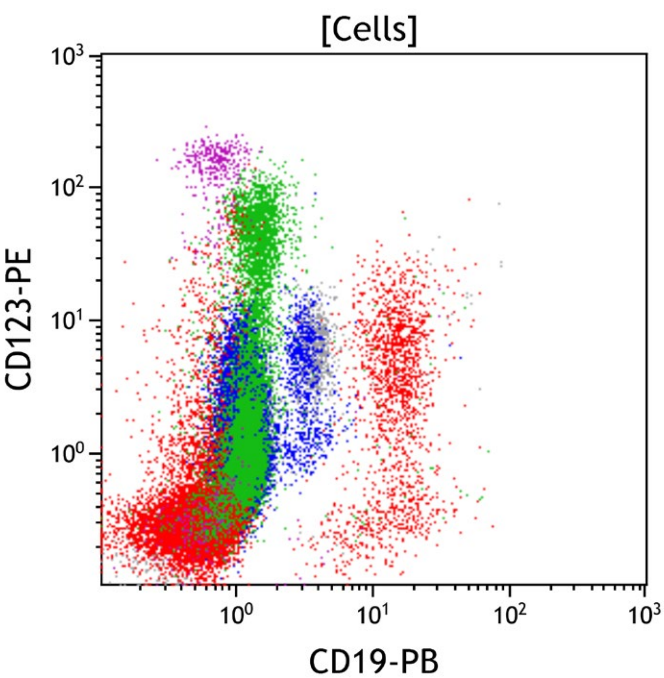

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red, right) normally do not express significant CD123. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

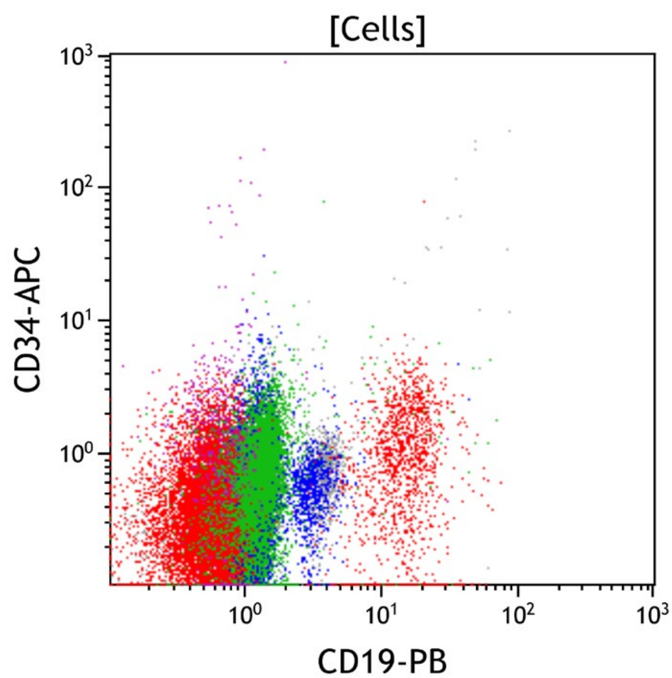

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white cells in peripheral blood.

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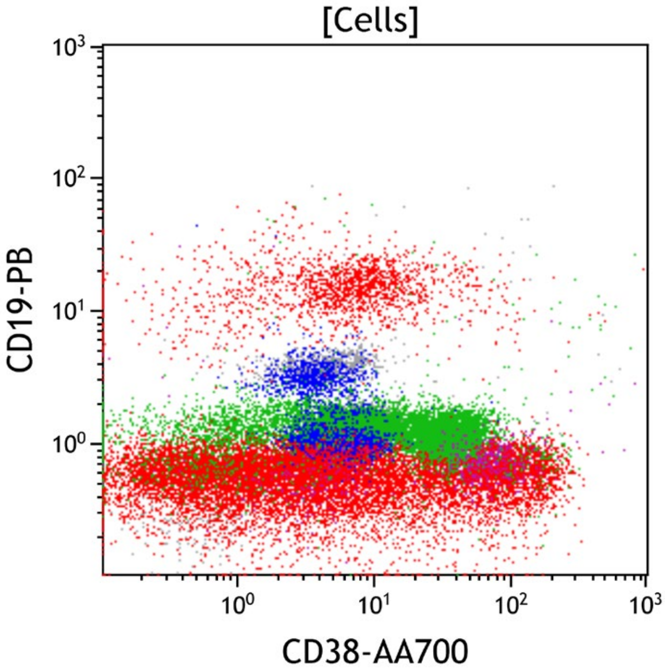

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells show intermediate expression of CD38 (red, upper). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

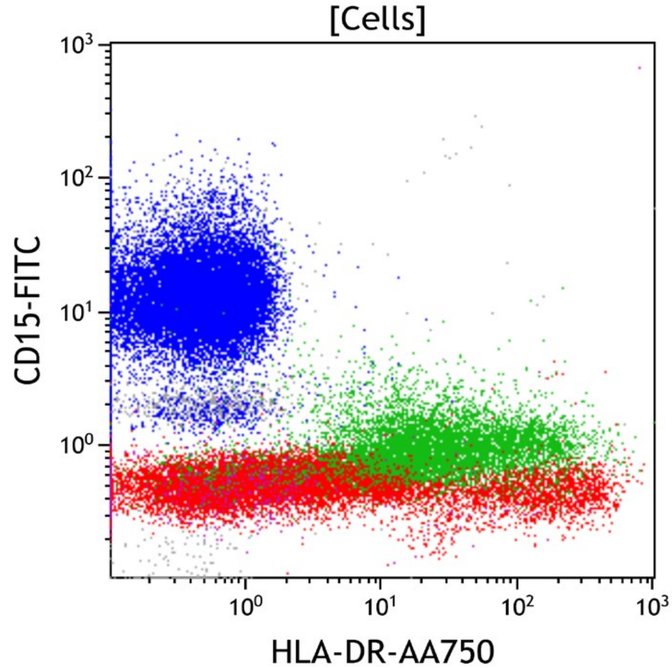

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Mature granulocytes do not express HLA-DR (blue).

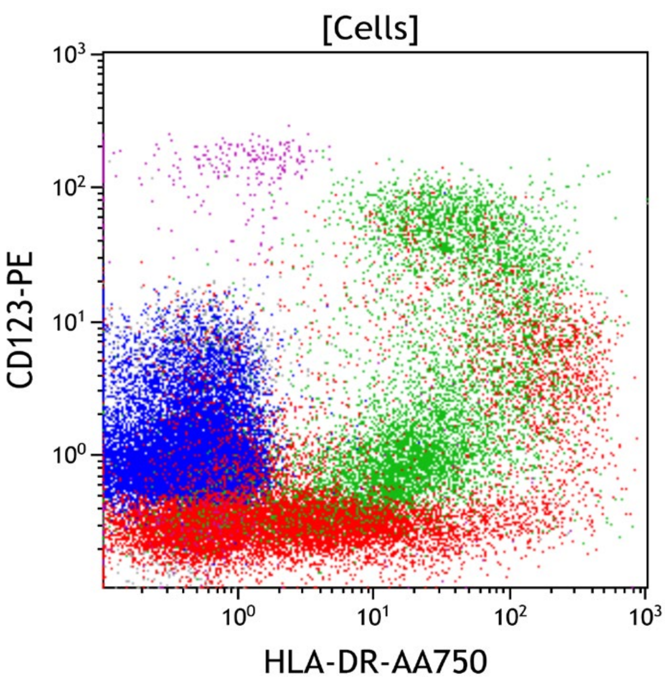

Figure 23. This HLA-DR vs CD123 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Mature granulocytes do not express HLA-DR (blue). CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors.

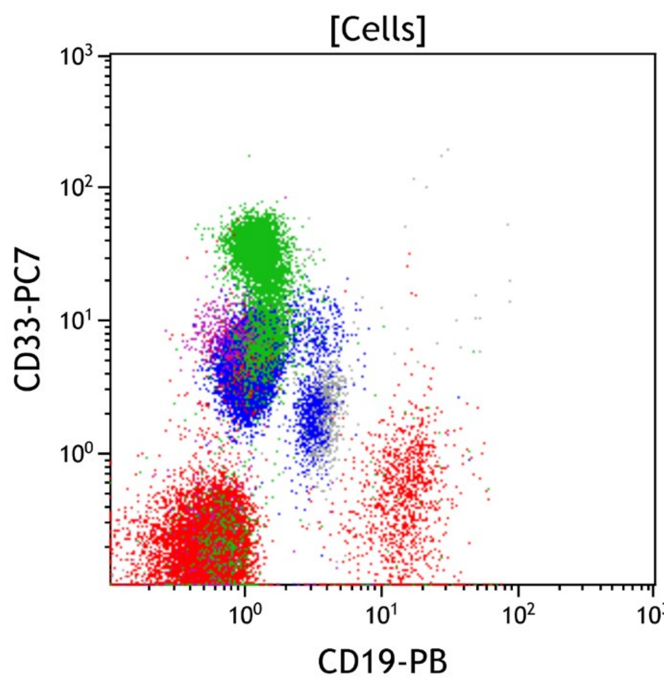

Figure 24. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red, lower right). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations in this case. No white cell abnormality is identified by morphology. Note that correlation with clinical, morphologic, and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay.

Case #2: Normal Bone Marrow

Clinical Vignette

This 37 year old male presents with anemia. A bone Marrow sample is submitted for Flow Cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

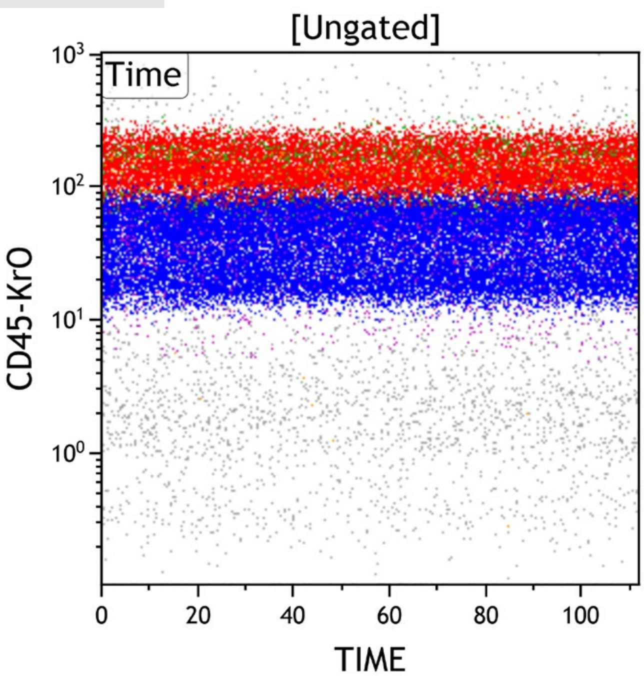

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

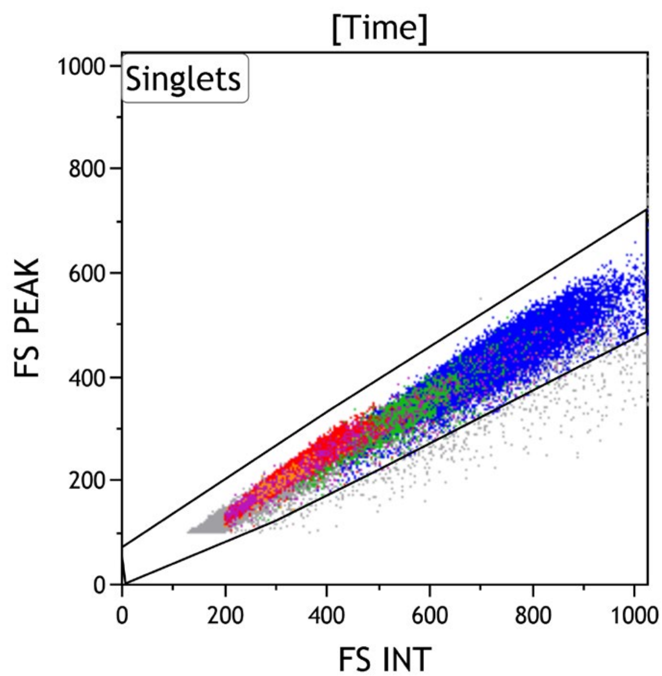

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

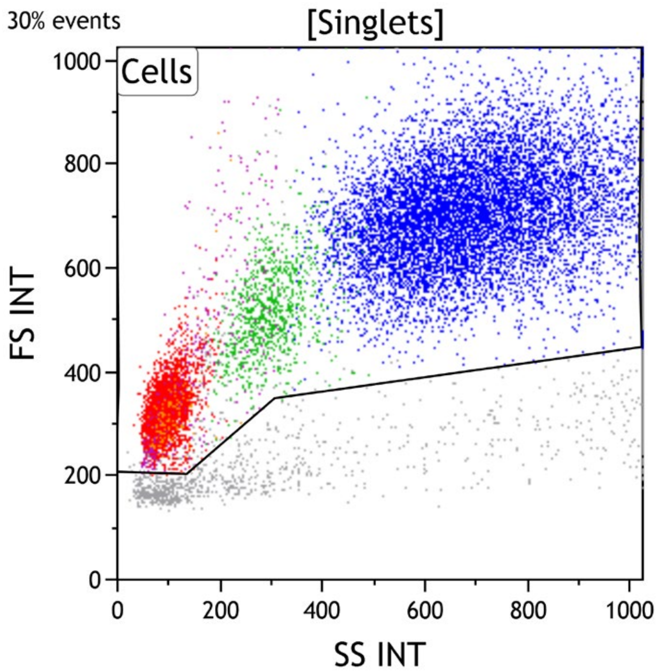

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

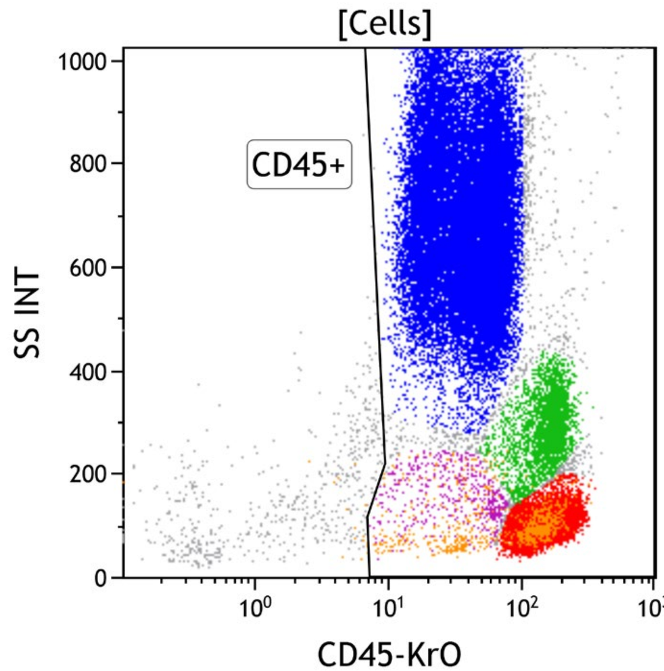

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

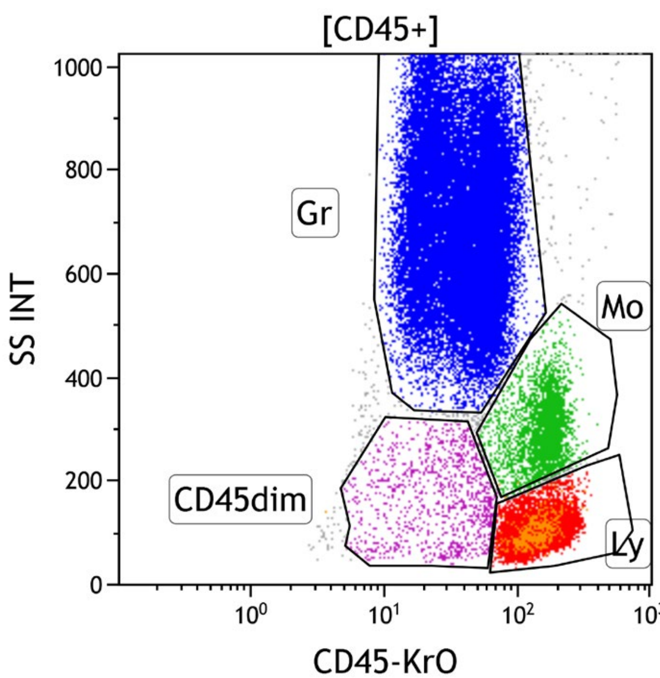

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

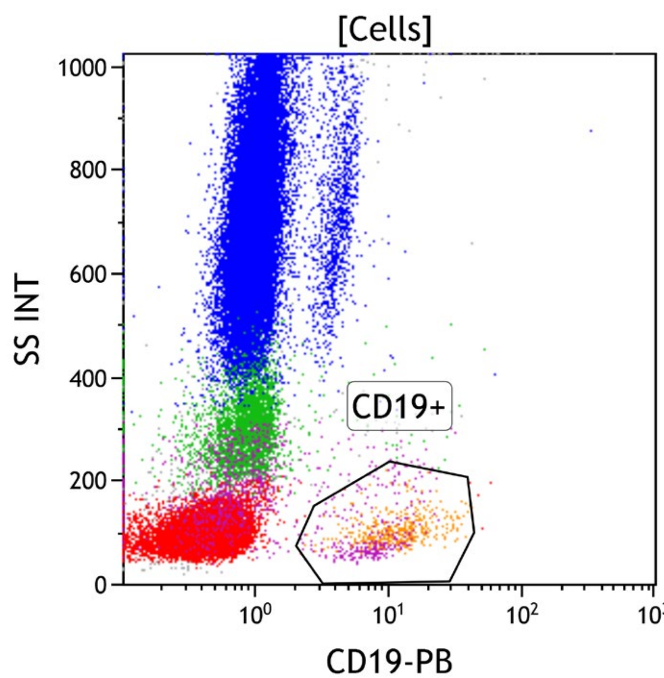

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature (orange) and immature B cells (purple), as well as most plasma cells. These cells typically have low to moderate side scatter.

B Cell Tube

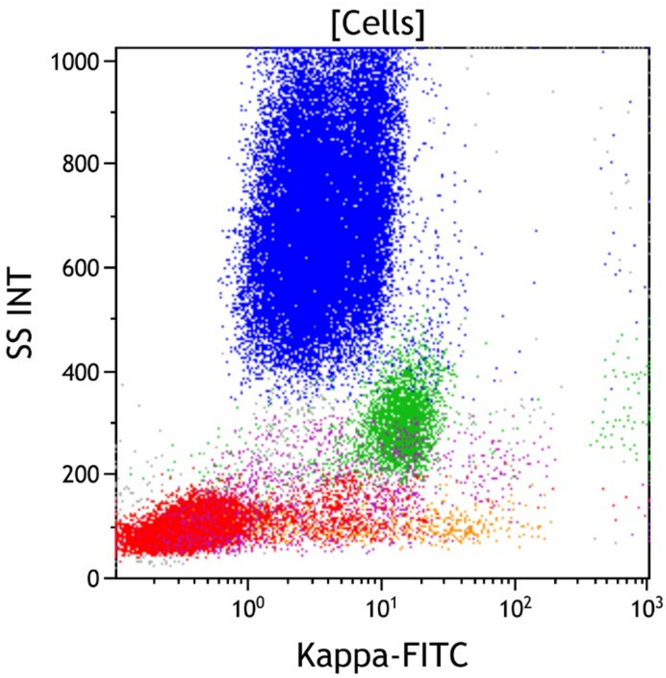

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot.

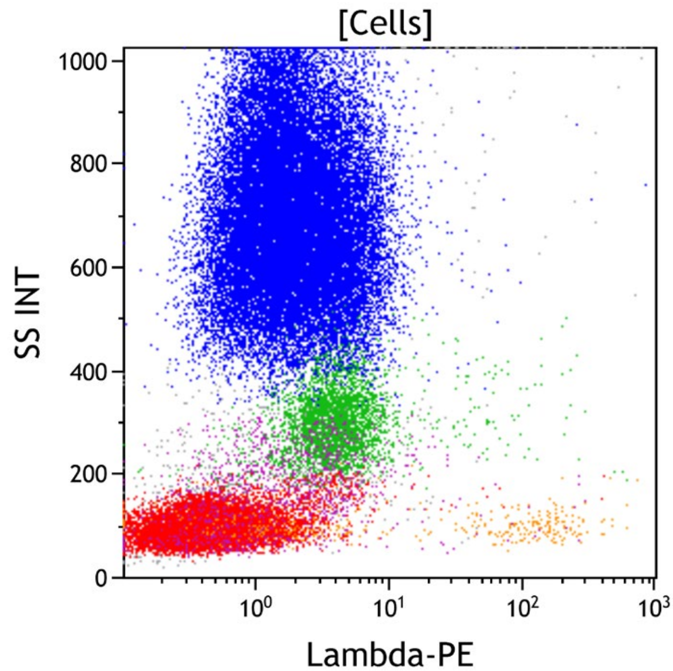

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot.

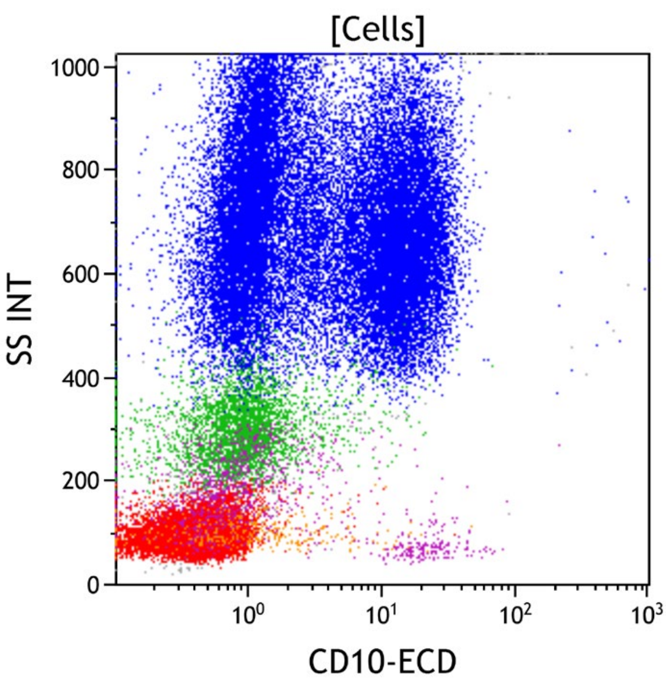

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

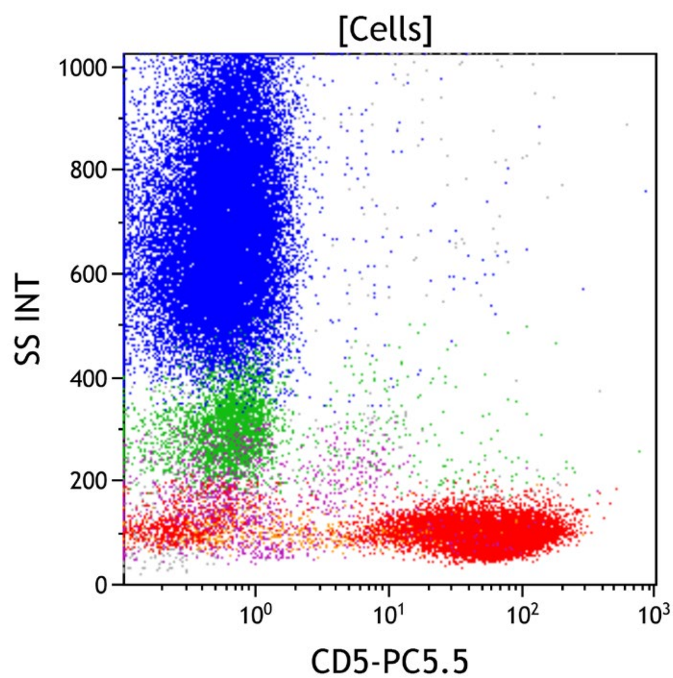

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter.

Table of Contents > No Immunophenotypic Abnormality > Case #2: Normal Bone Marrow

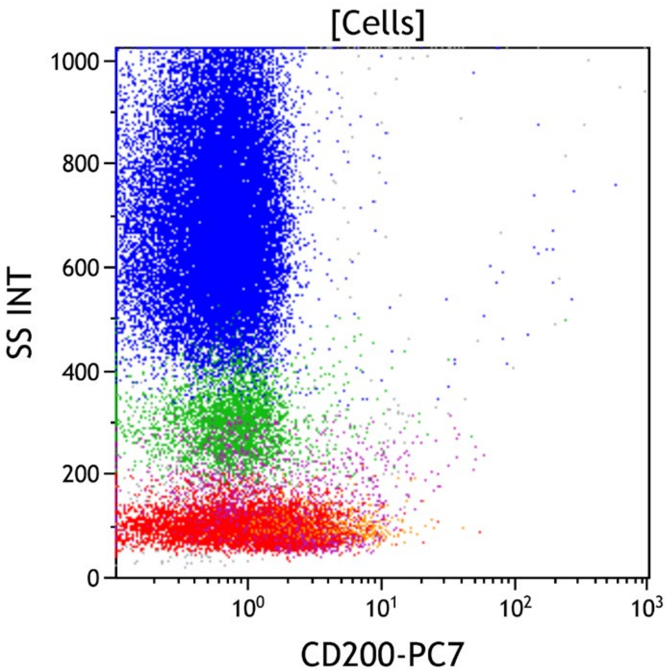

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

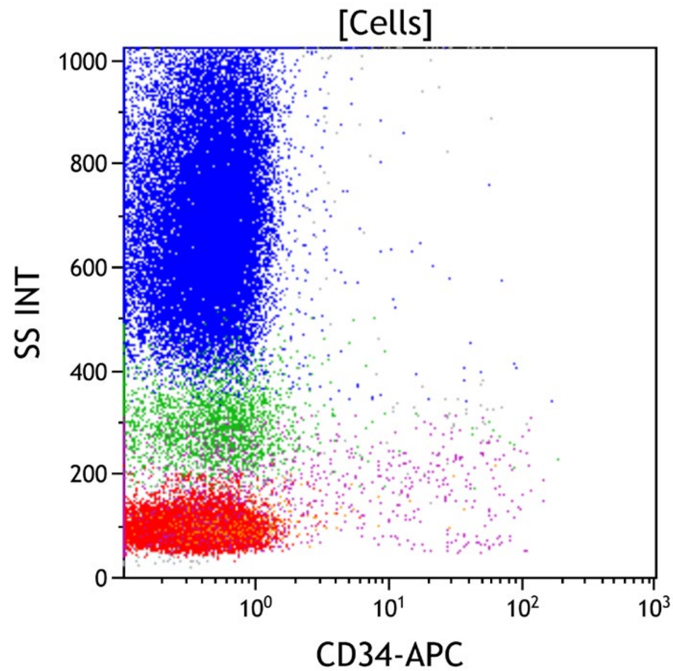

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

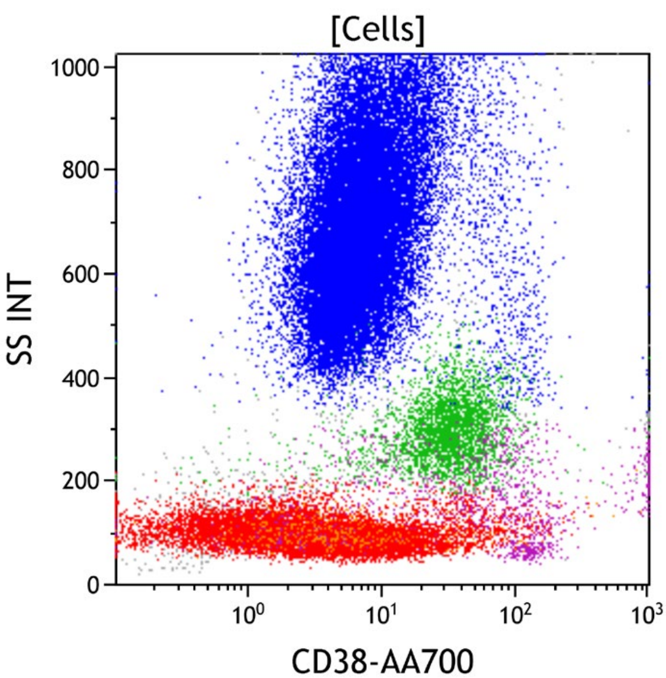

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level.

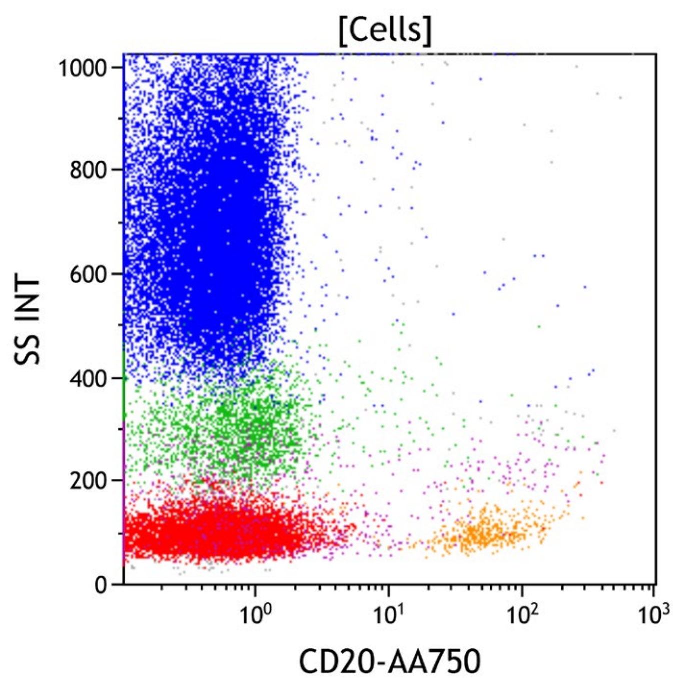

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.

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B Cell Tube

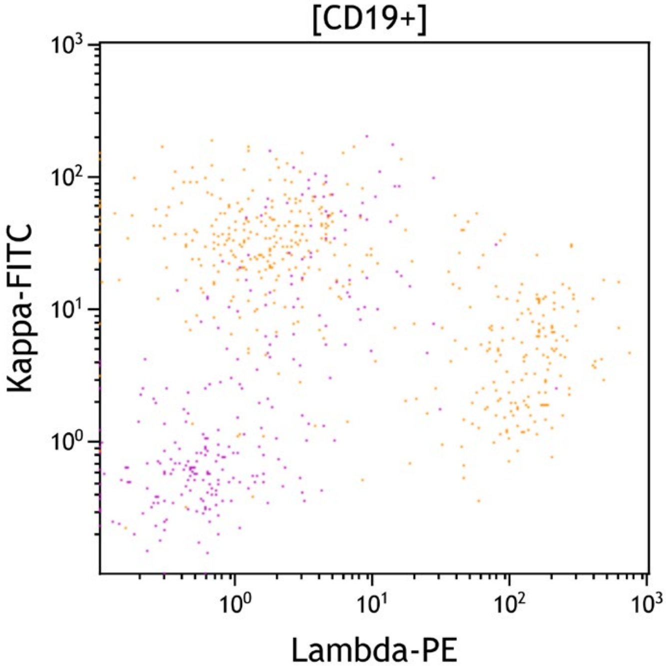

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

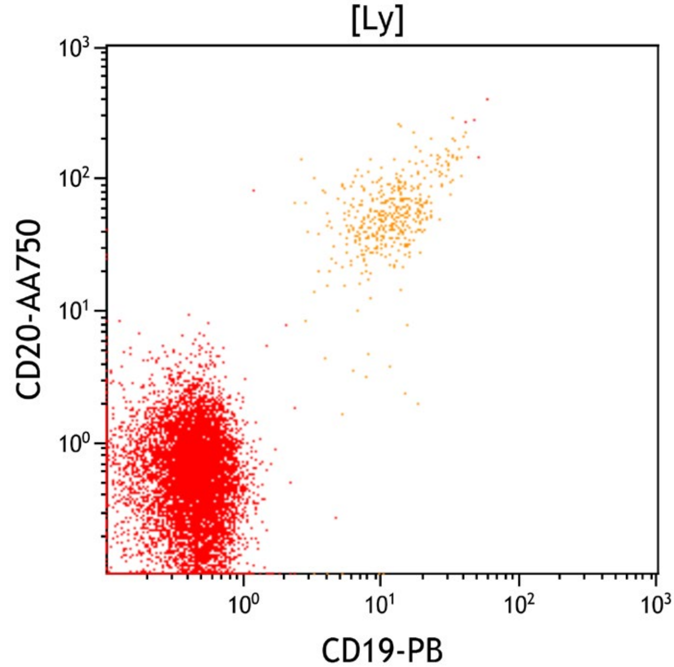

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

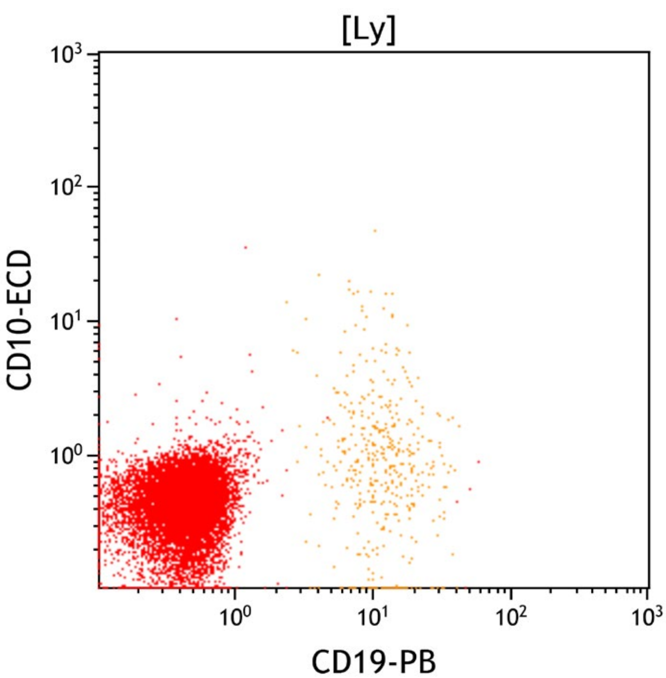

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

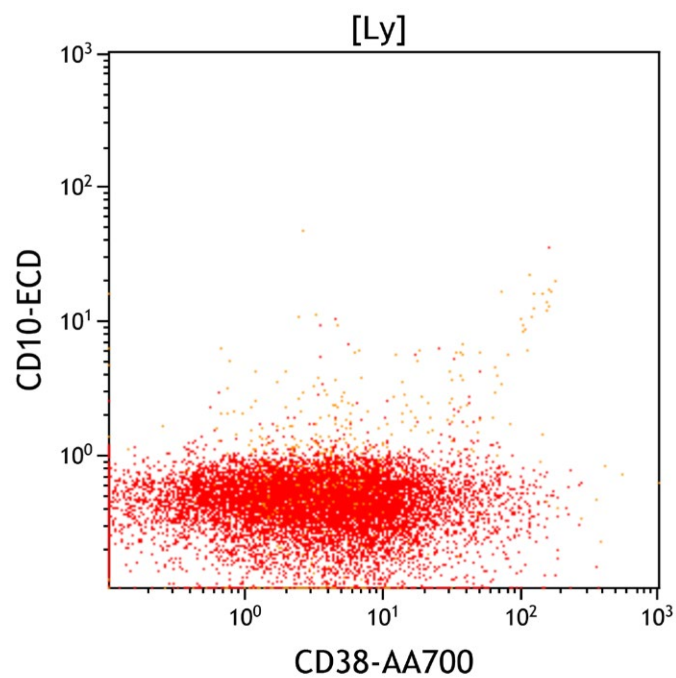

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

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B Cell Tube

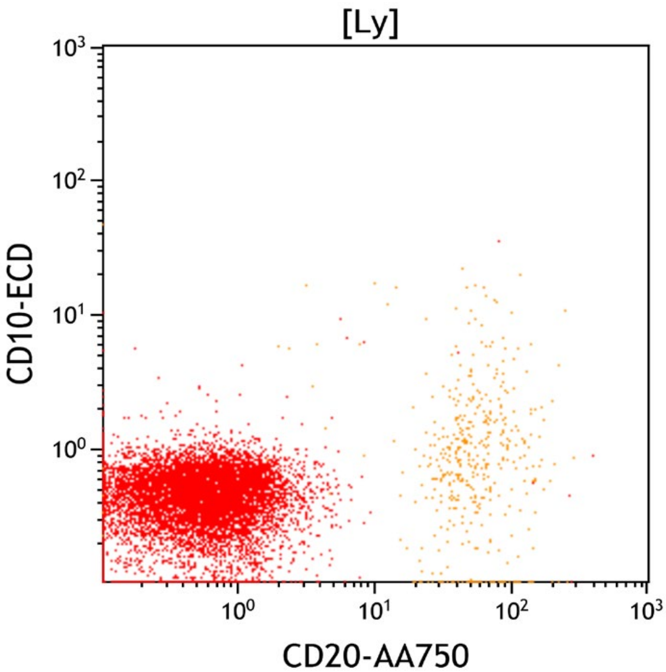

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

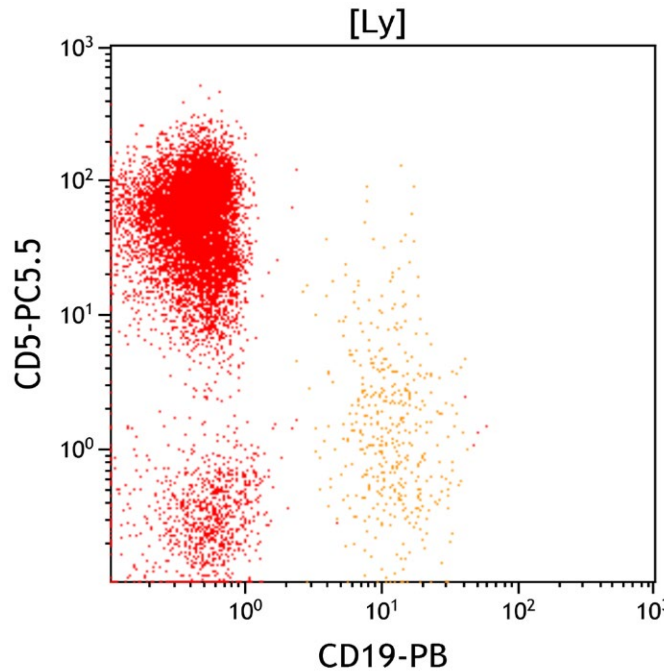

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

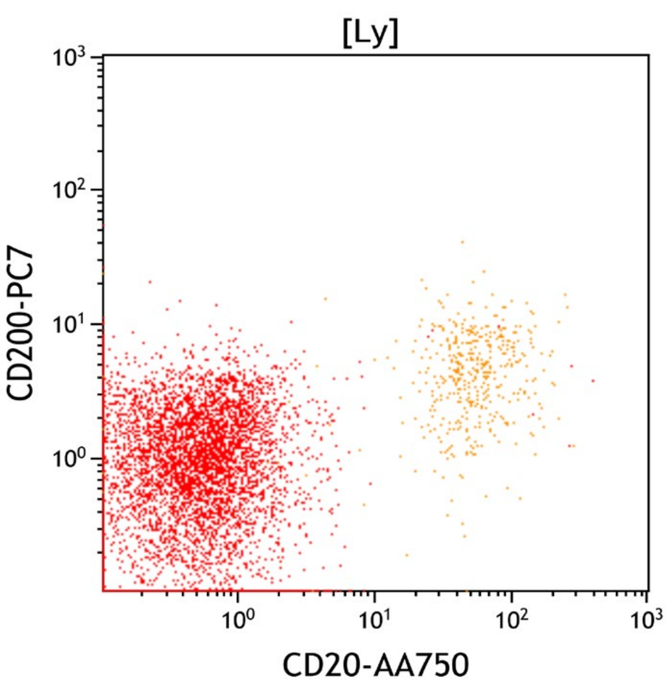

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

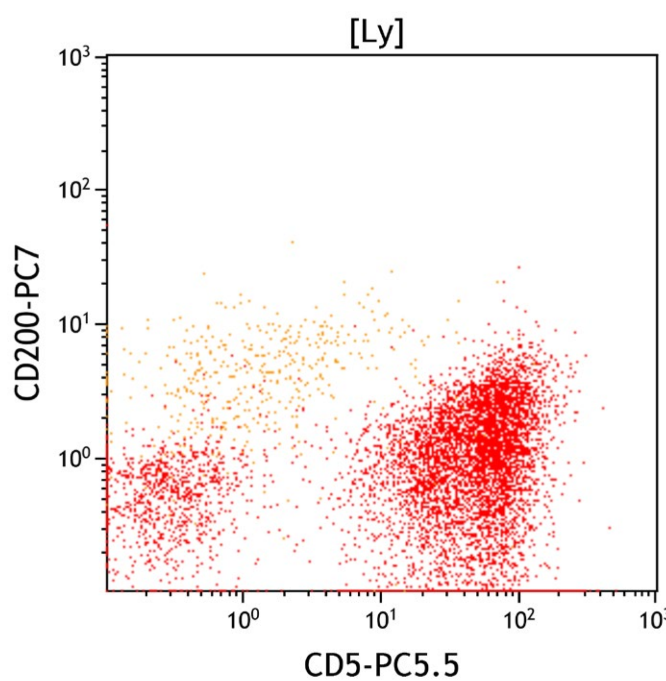

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

Table of Contents > No Immunophenotypic Abnormality > Case #2: Normal Bone Marrow

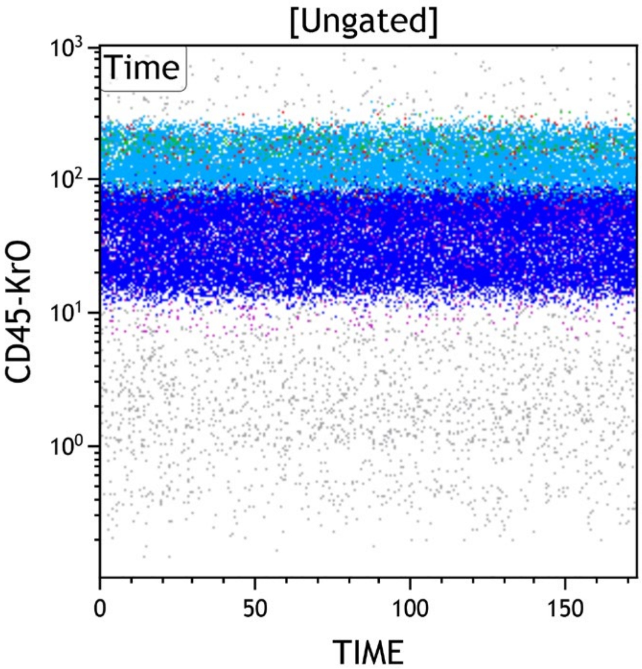

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

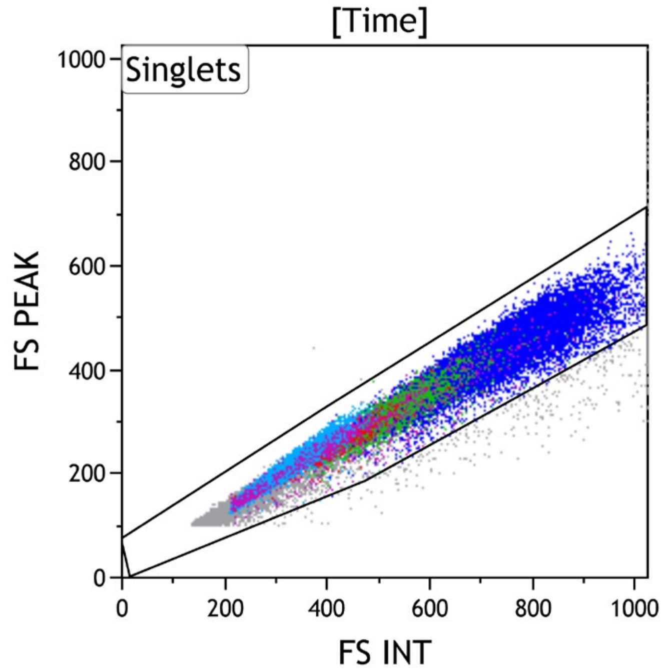

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

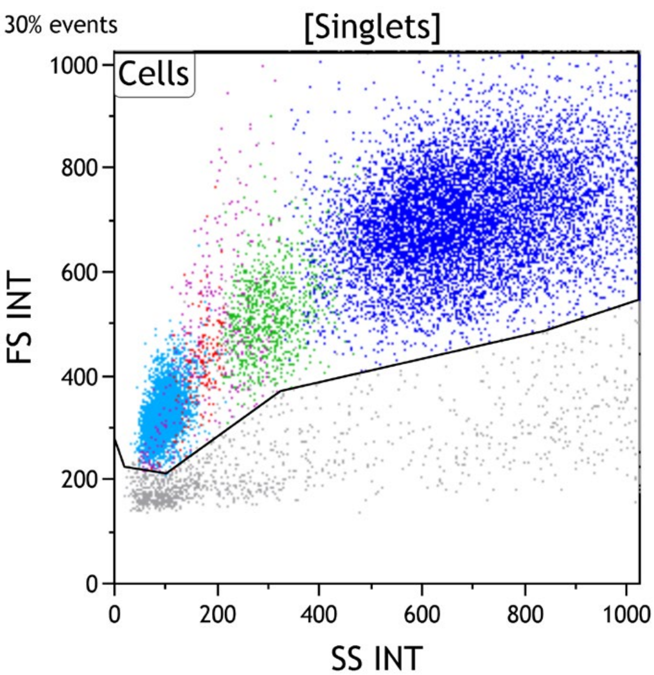

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

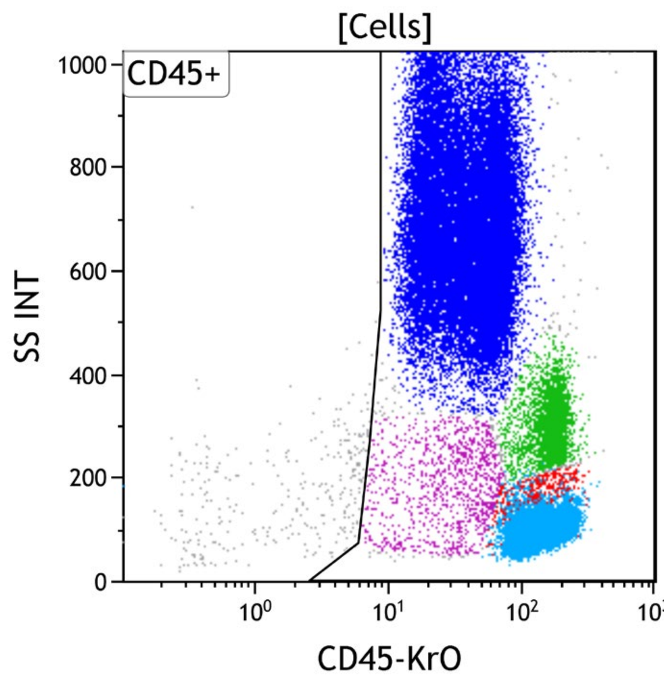

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray) usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

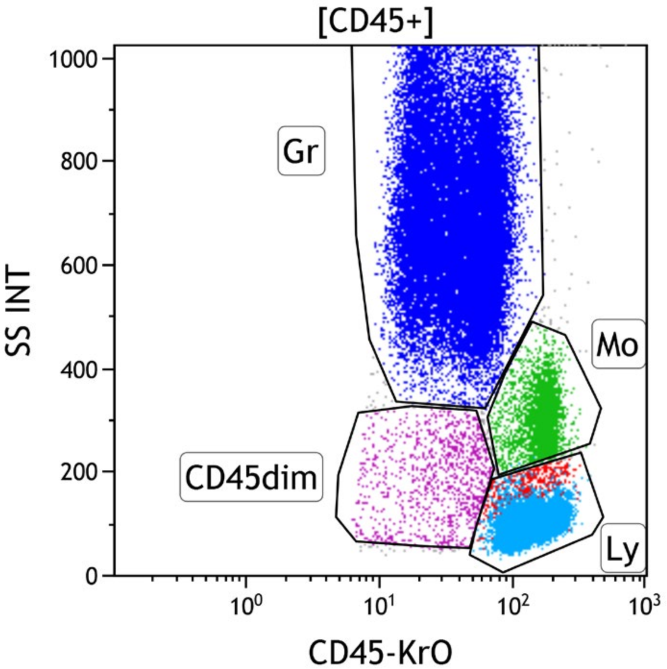

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

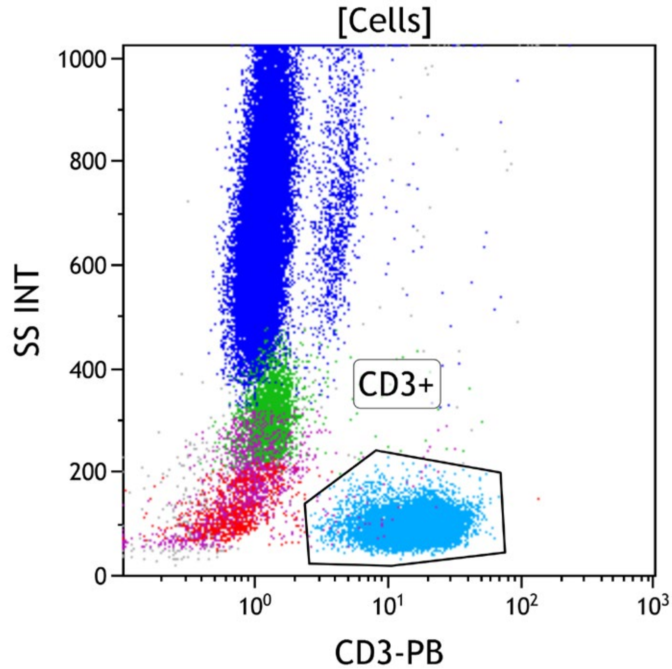

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

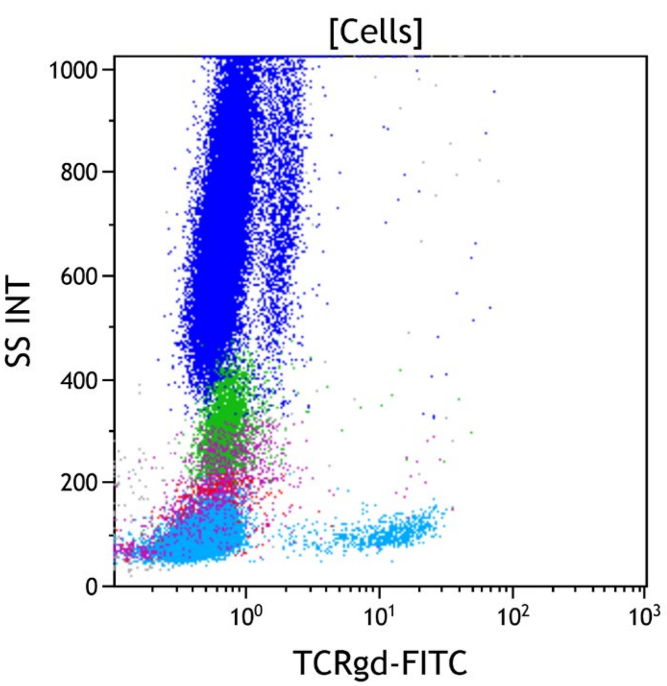

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

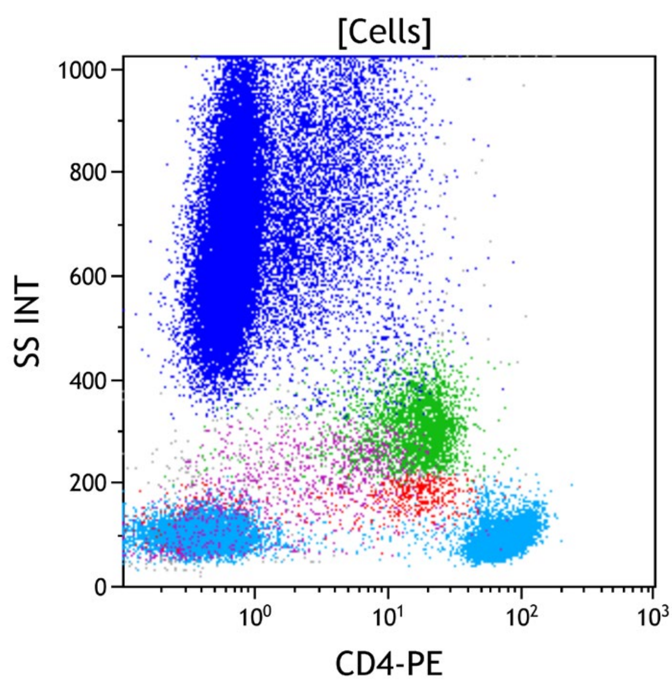

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

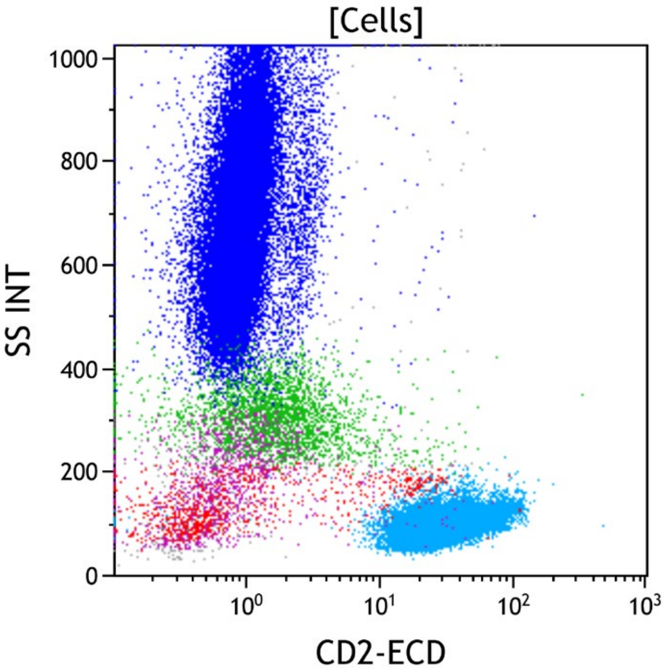

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

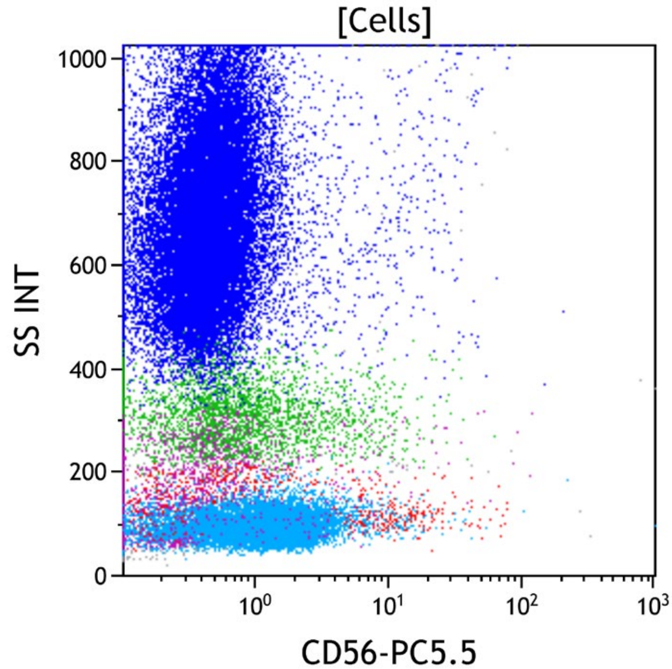

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

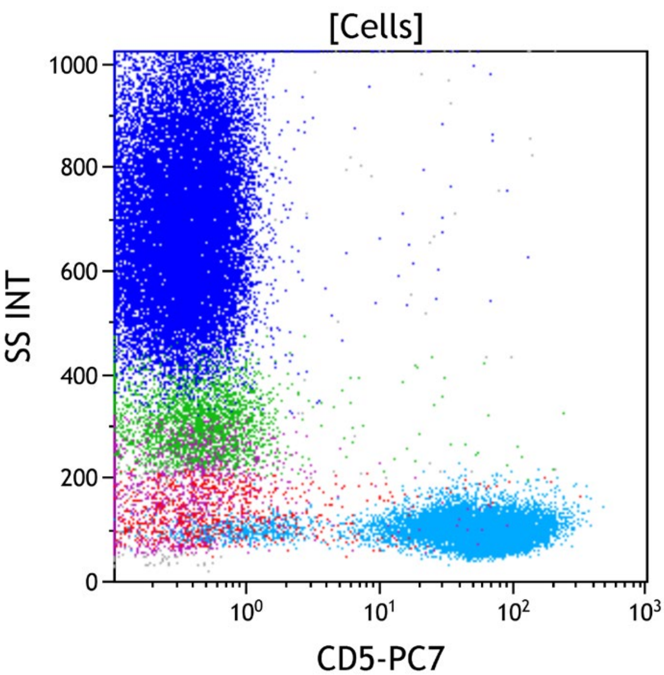

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

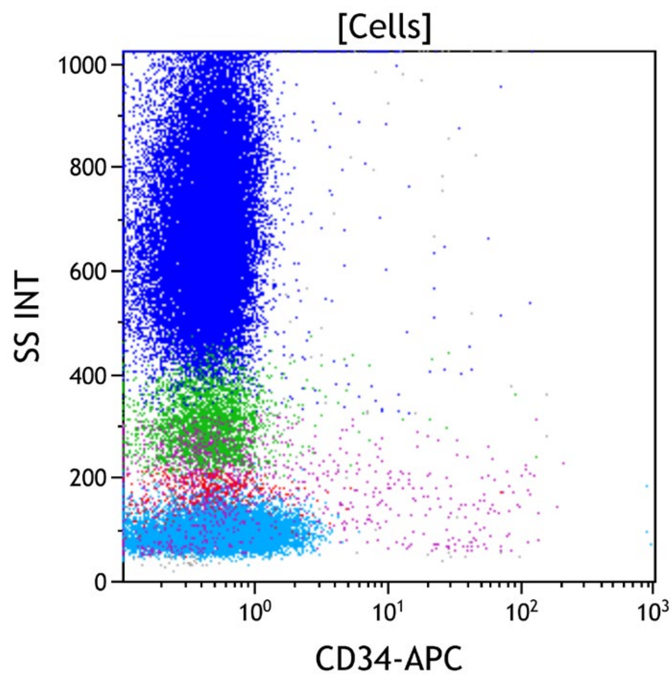

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

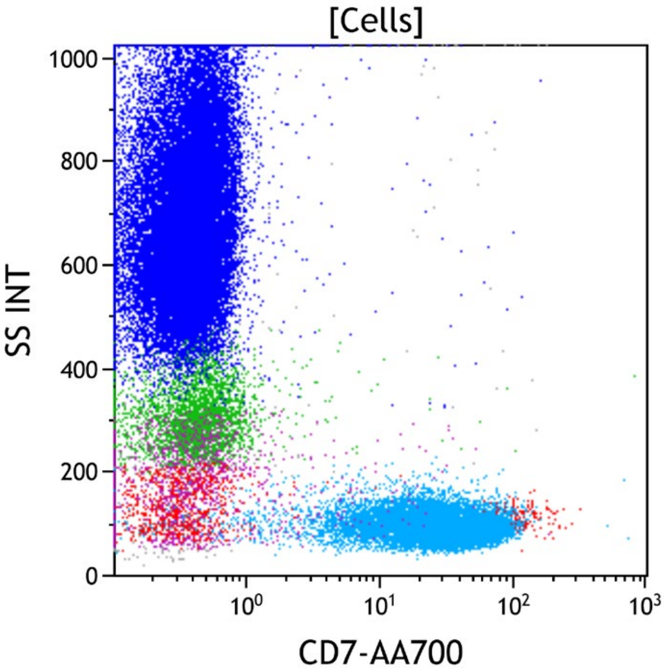

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

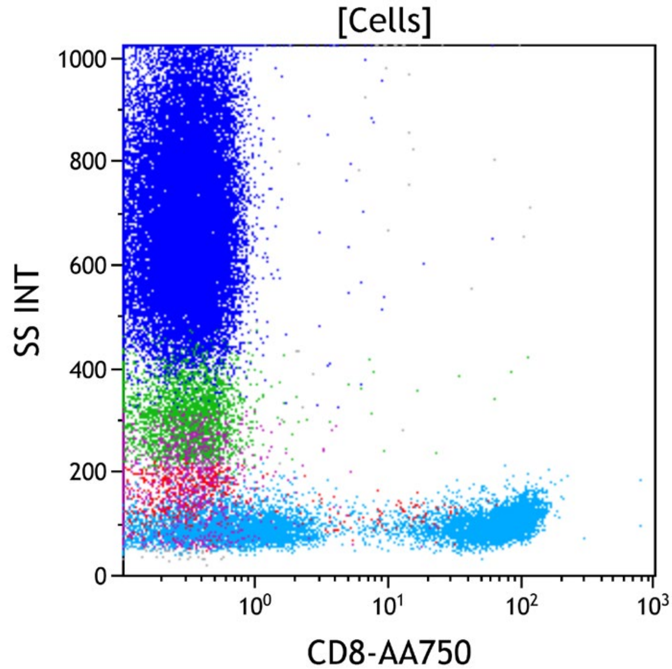

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

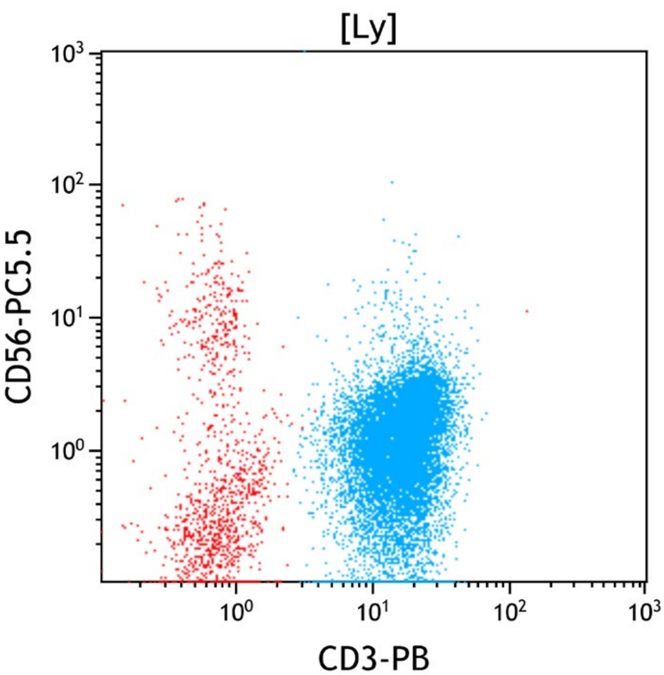

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

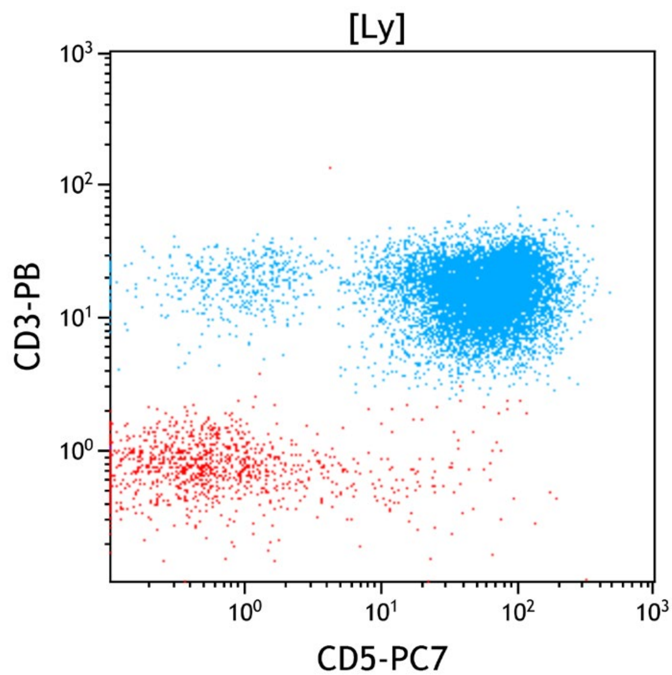

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

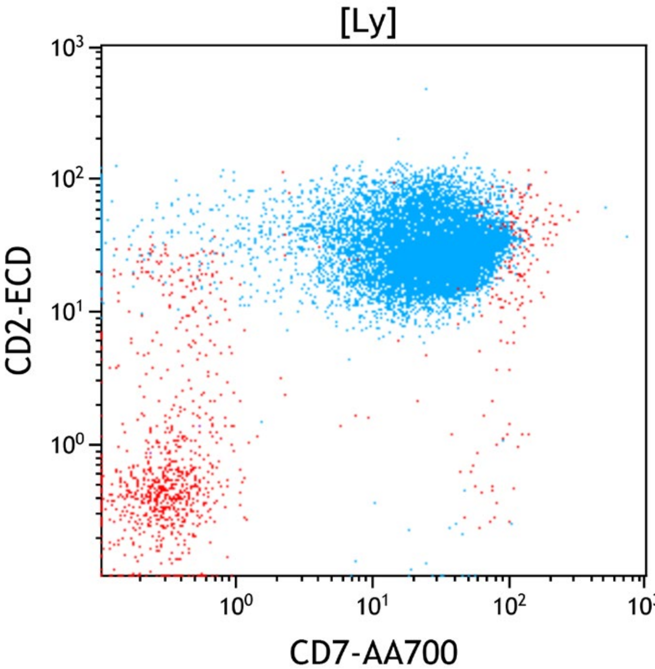

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

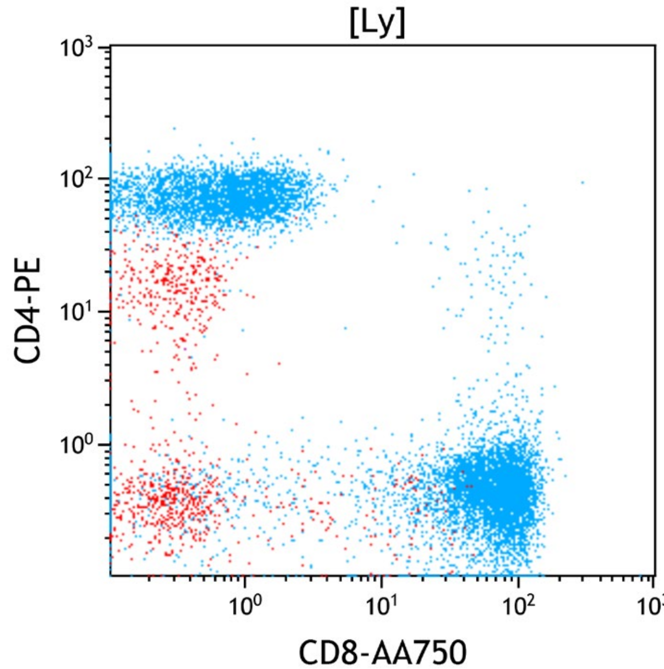

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

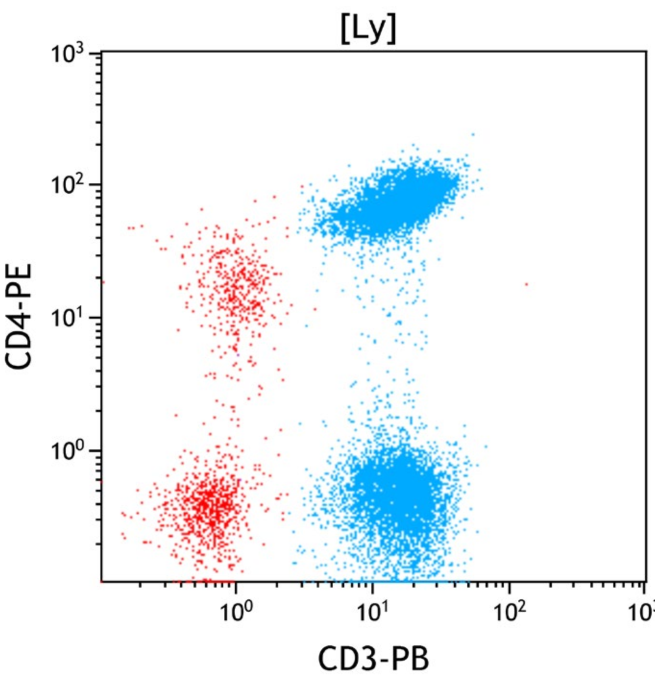

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

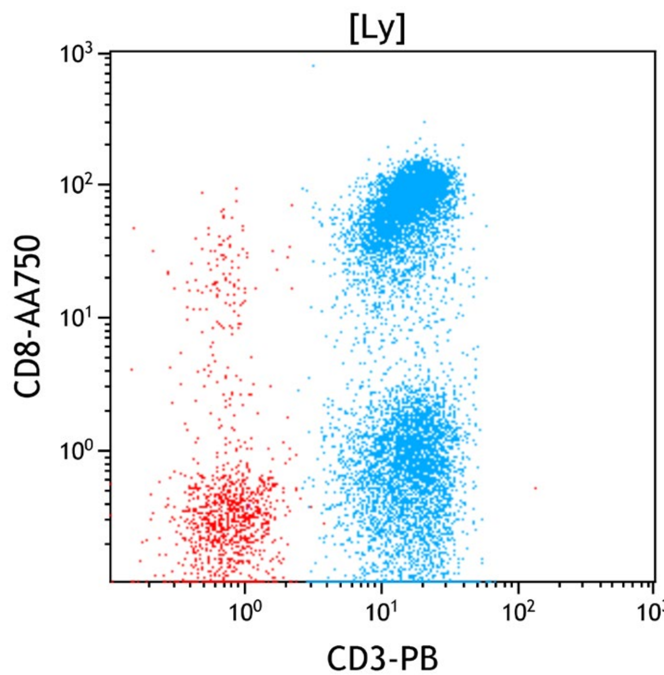

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

T Cell Tube

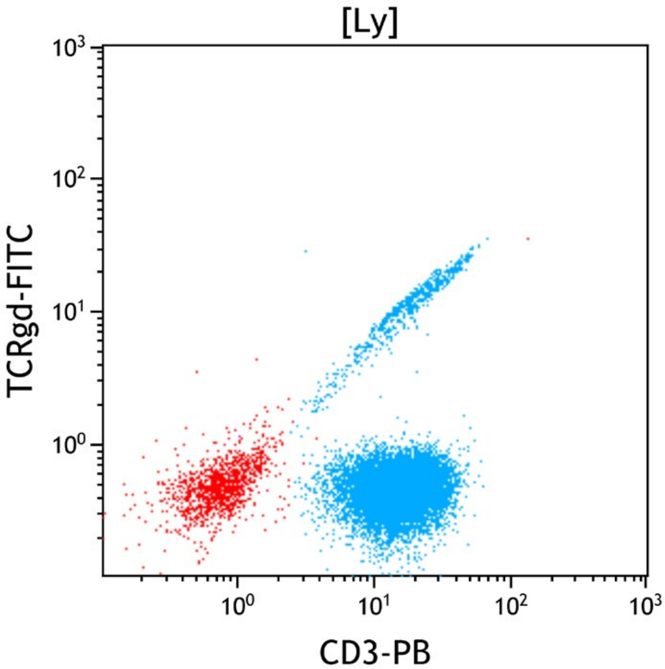

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

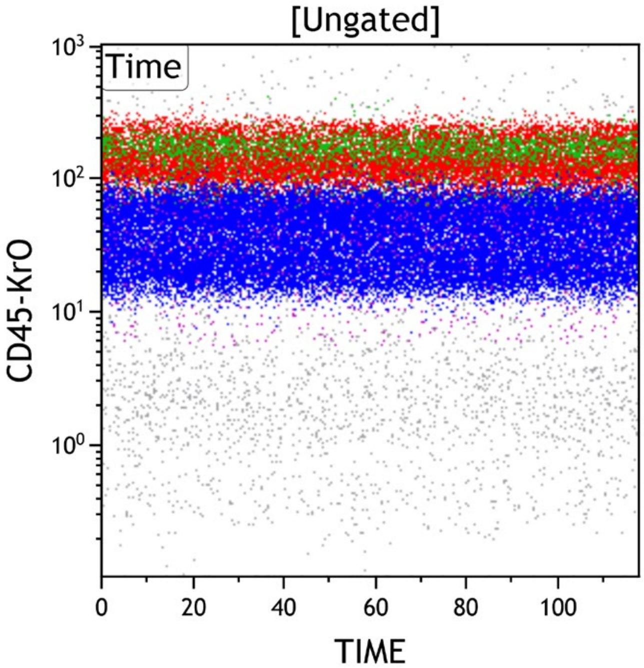

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

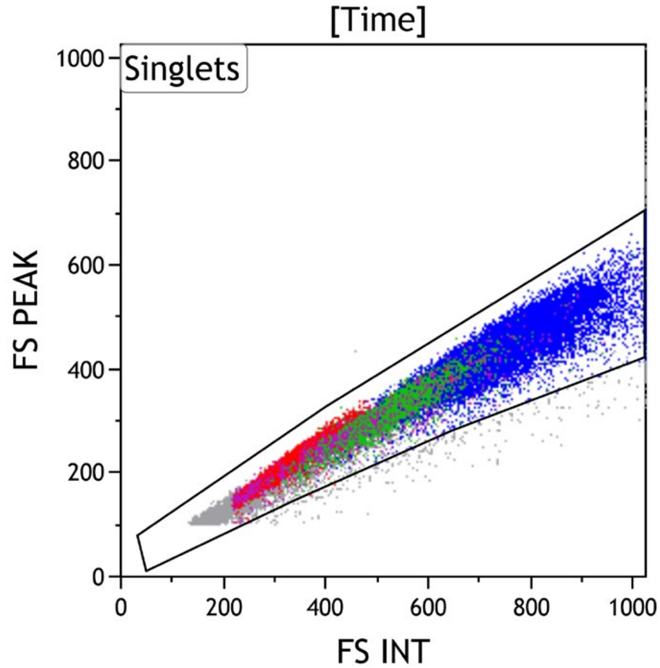

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

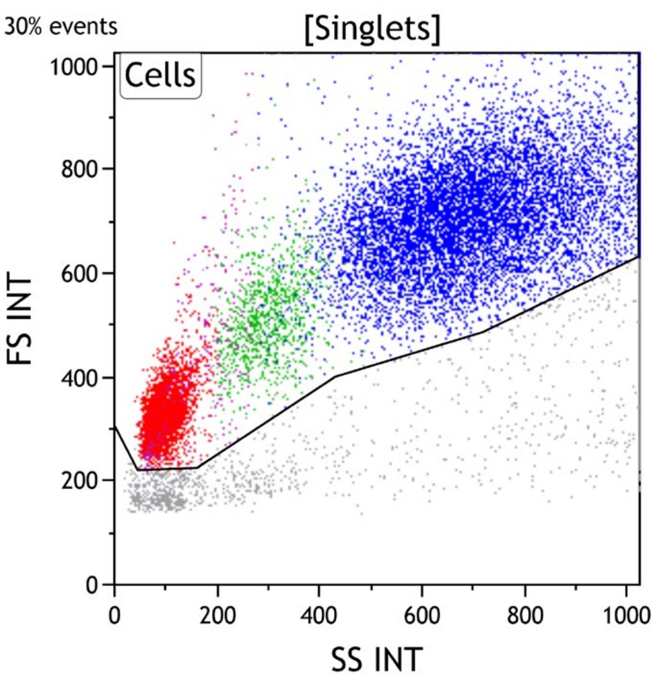

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

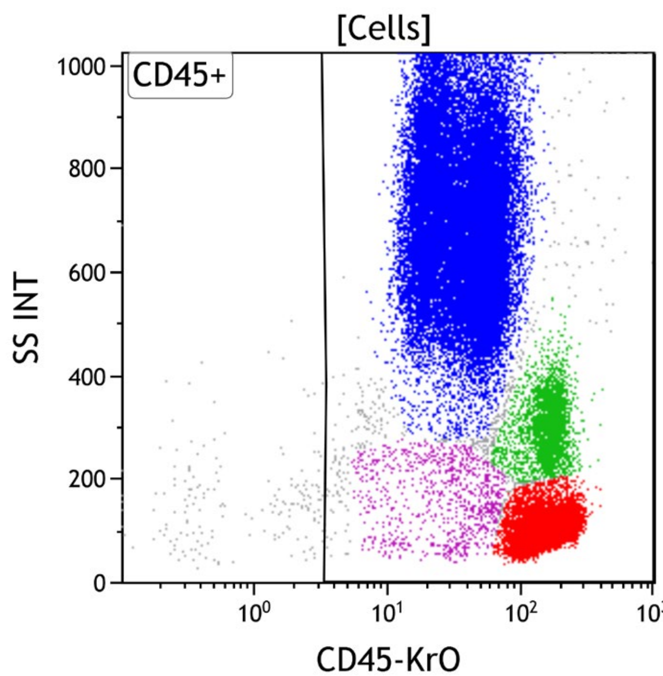

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray) usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

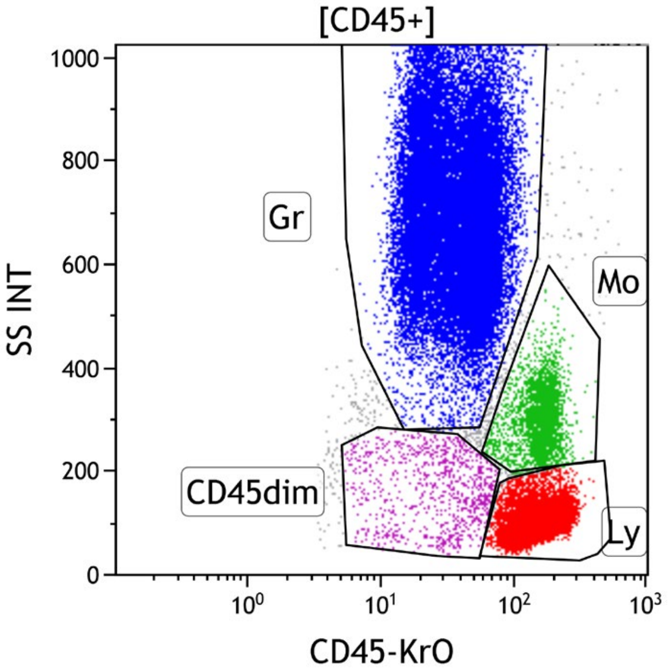

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

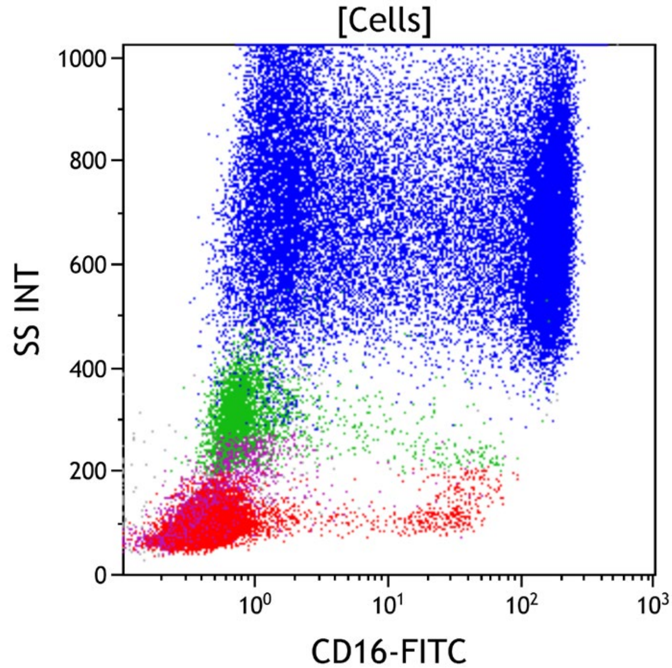

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

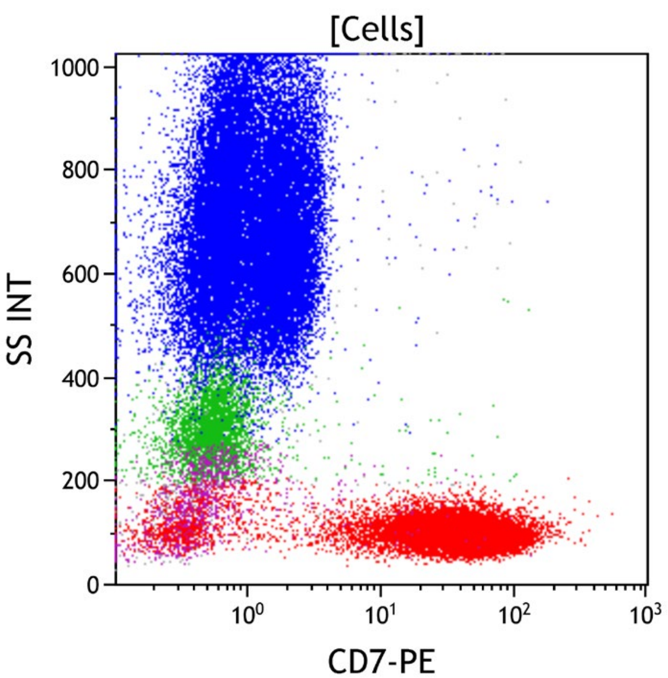

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage-committed progenitors (purple).

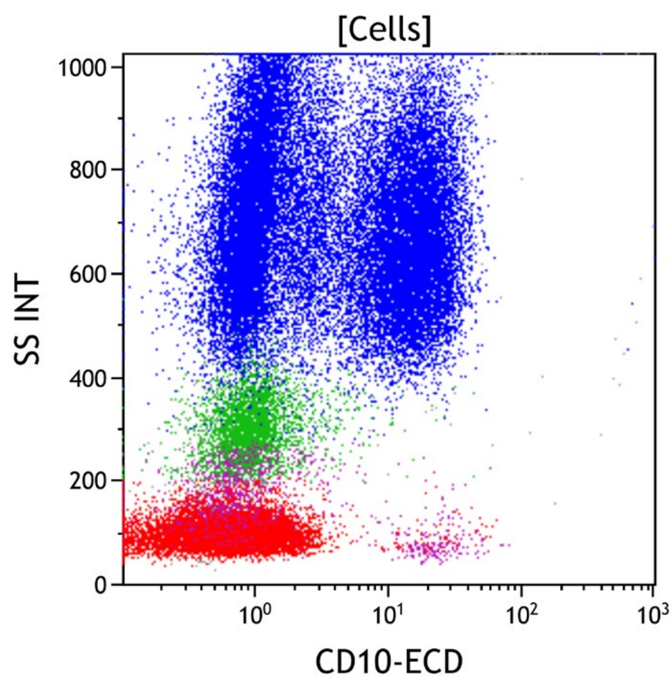

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

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M1 Cell Tube

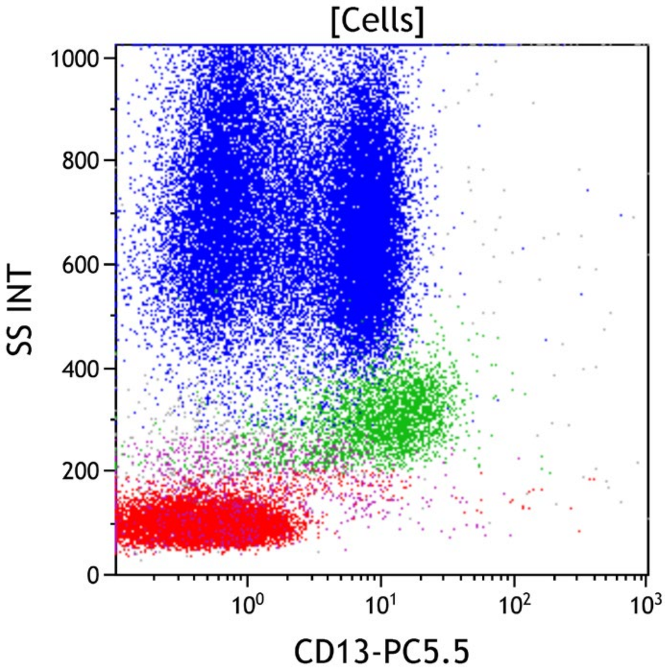


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

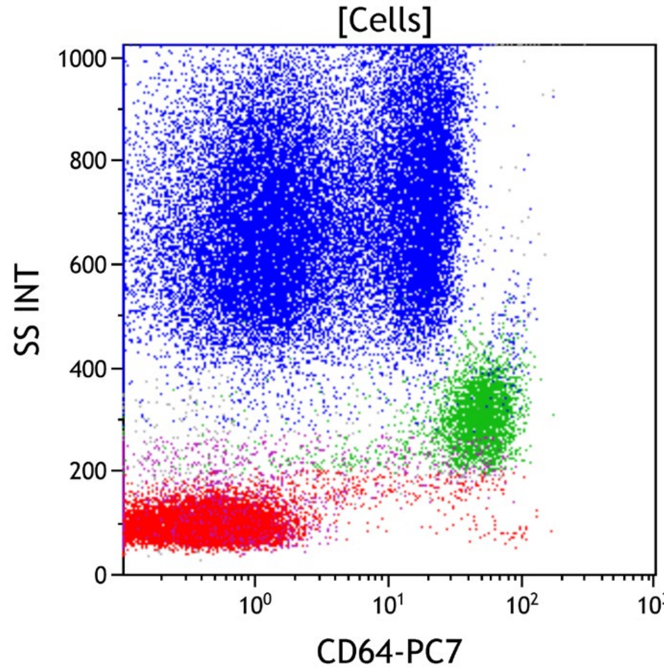


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34+ progenitors.

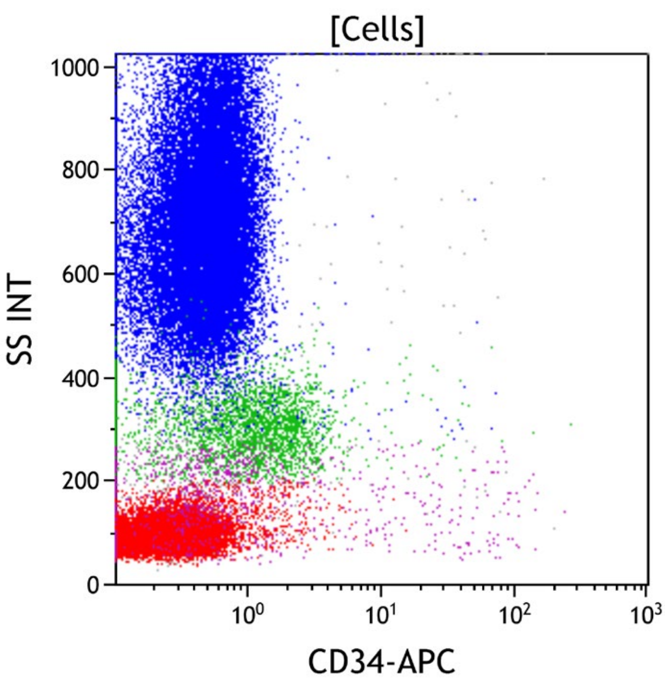


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

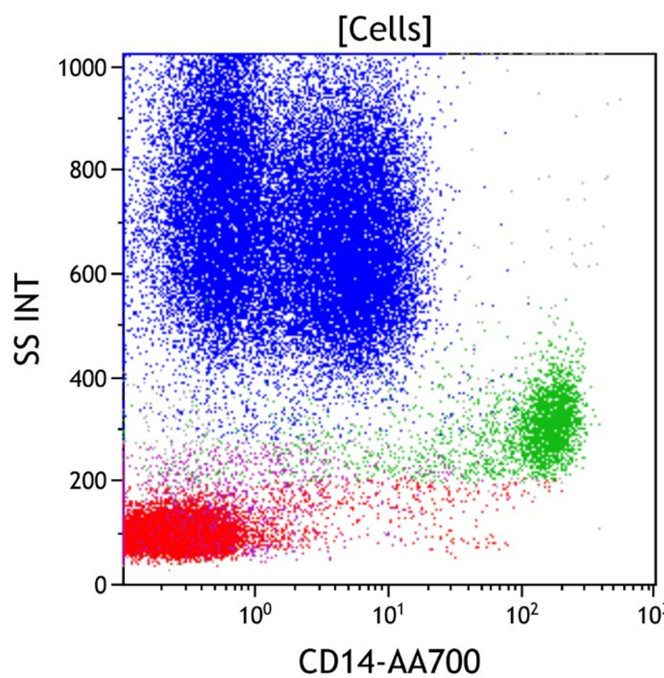


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

Table of Contents > No Immunophenotypic Abnormality > Case #2: Normal Bone Marrow

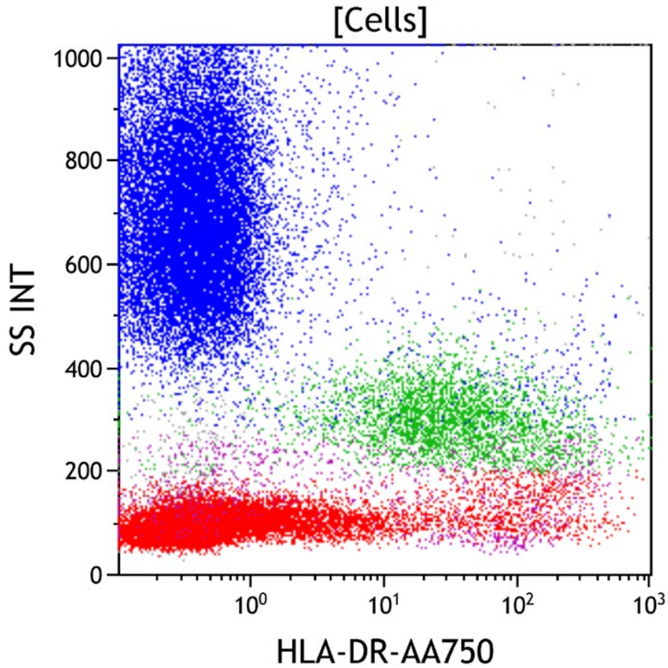


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red, lower right) and immature (purple) B cells, and activated T cells (red).

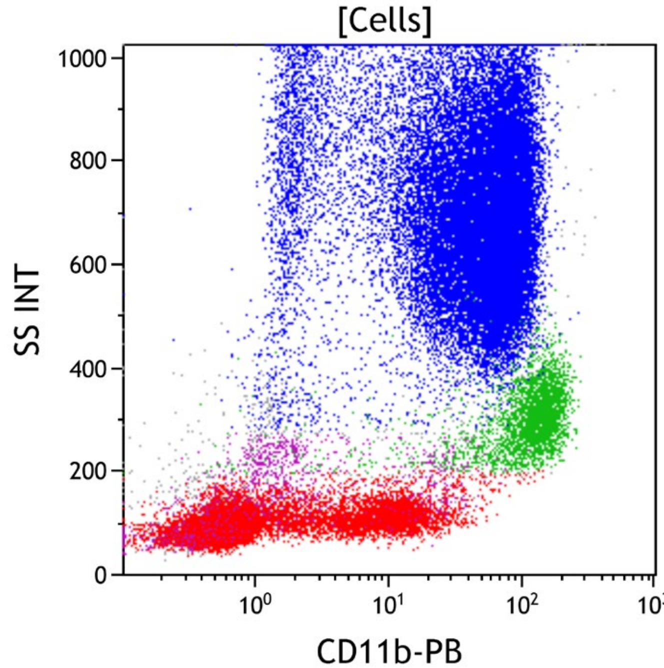


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

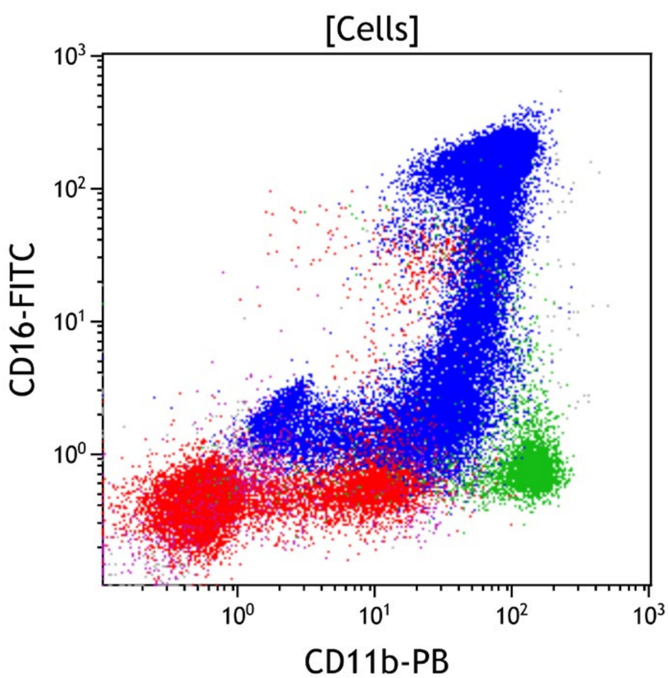


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue bottom left) and acquire CD11b as they mature toward myelocytes (blue bottom right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

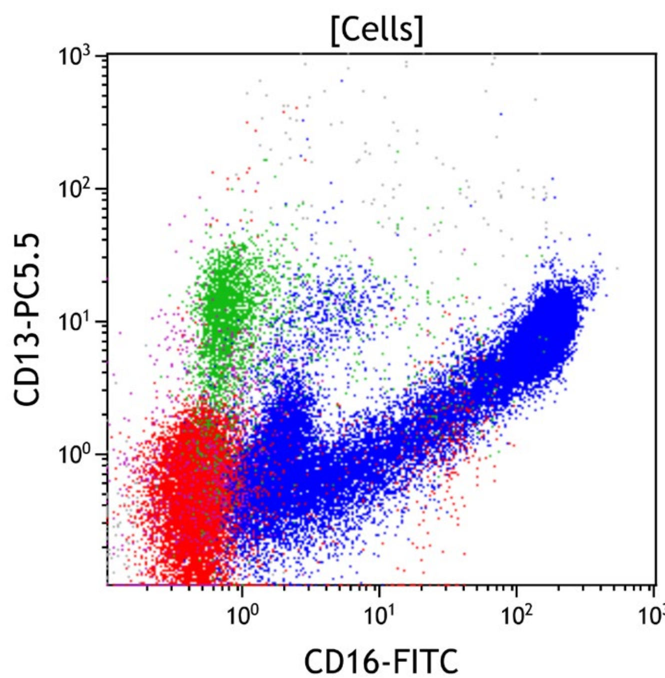


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue left) and lose CD13 as they mature to myelocytes (blue bottom left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue top right).

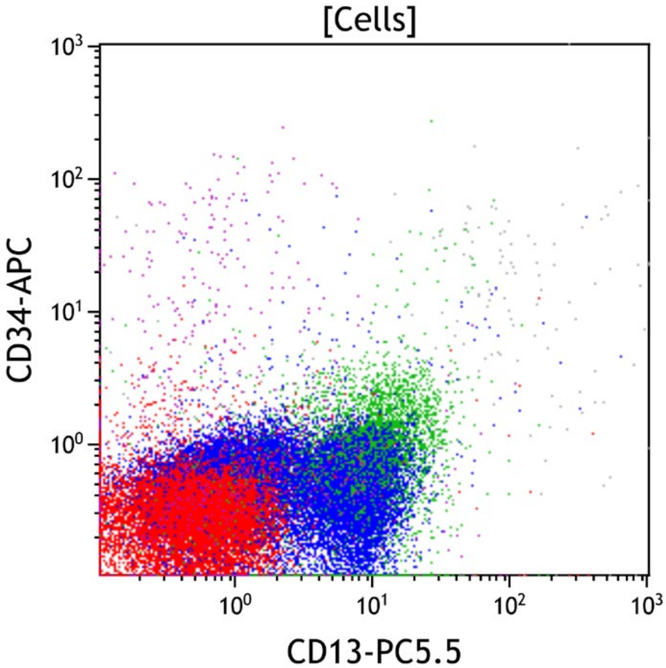


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphoid cells (red).

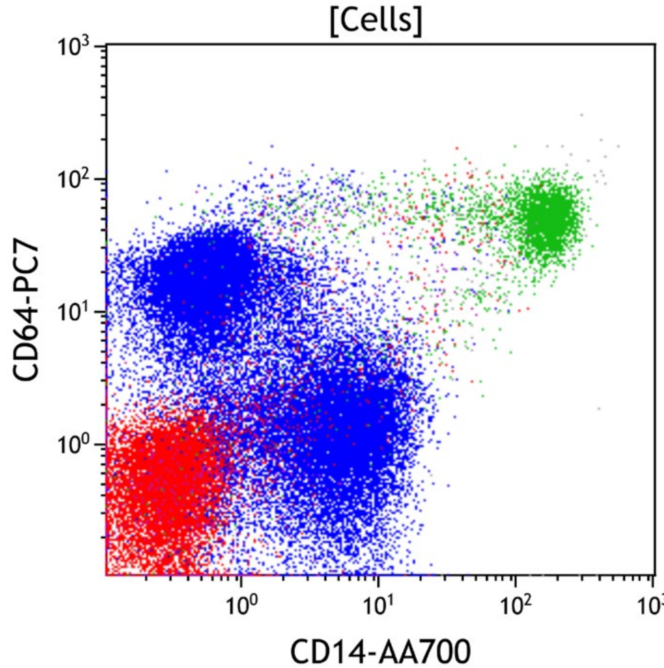


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (blue top left) and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64 (green). Immature granulocytes express moderate CD64 without CD14 (blue left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue bottom).

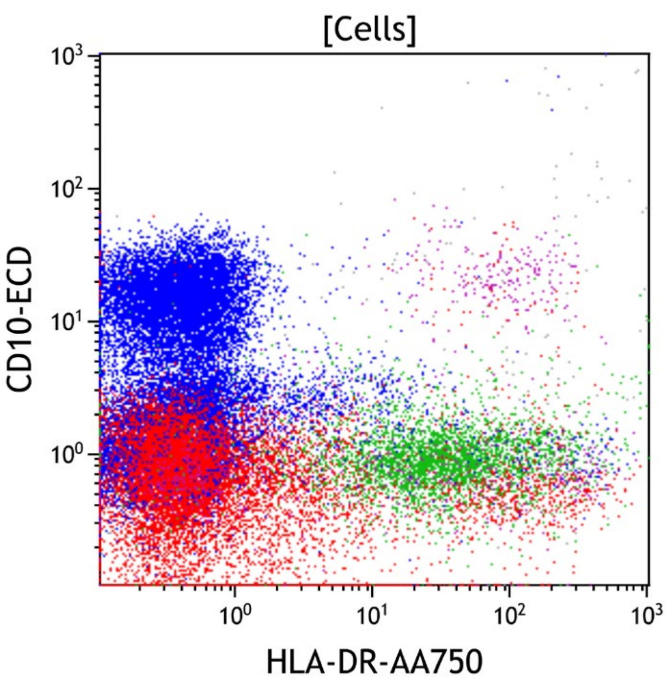


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

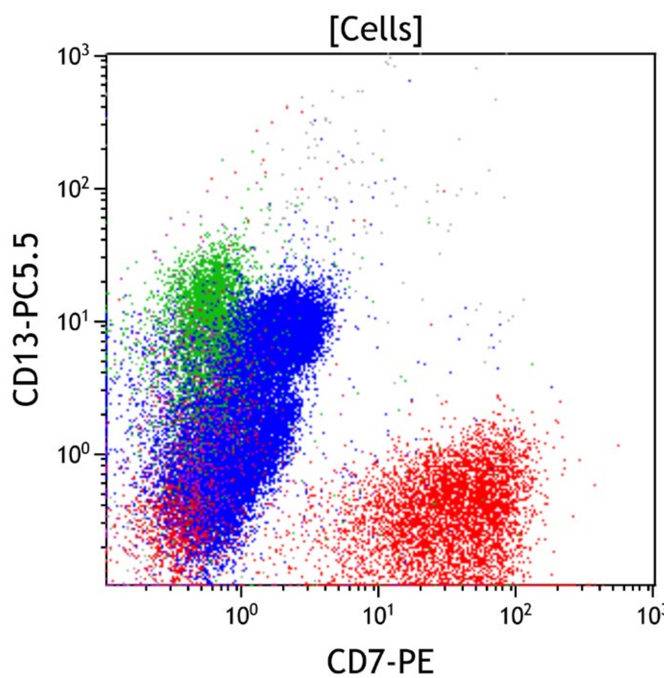
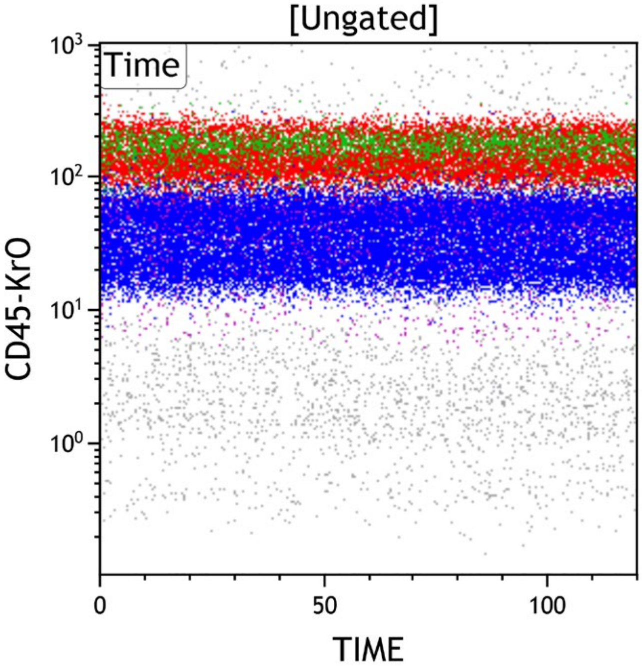


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

M2 Cell Tube



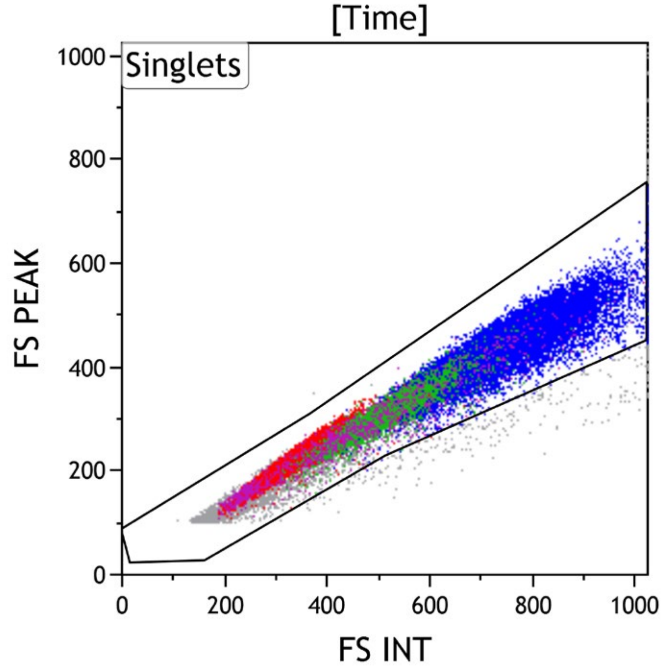


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

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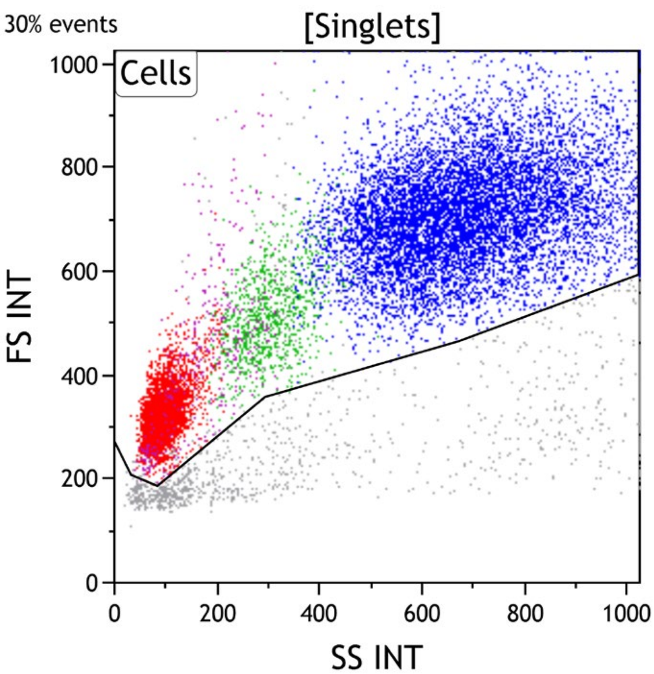

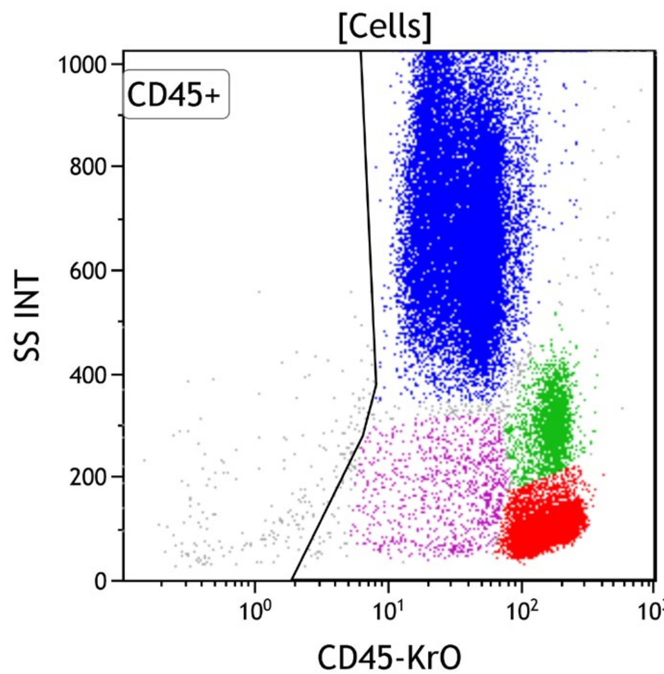

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

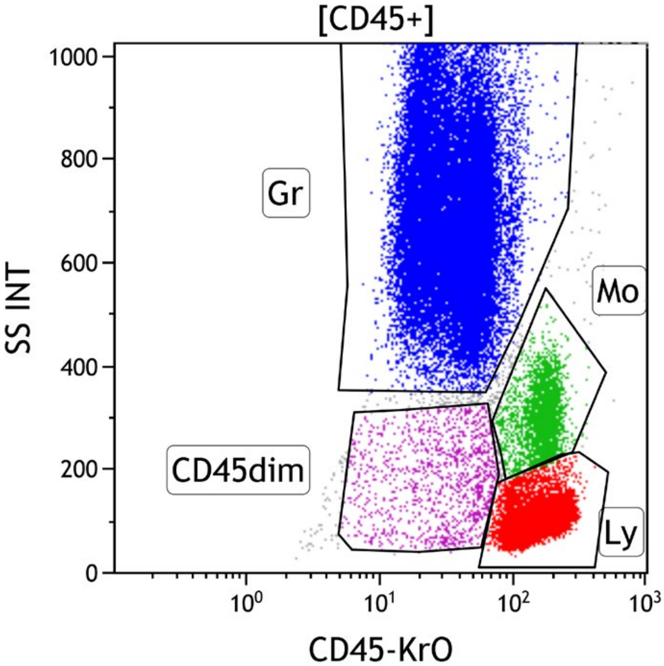

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

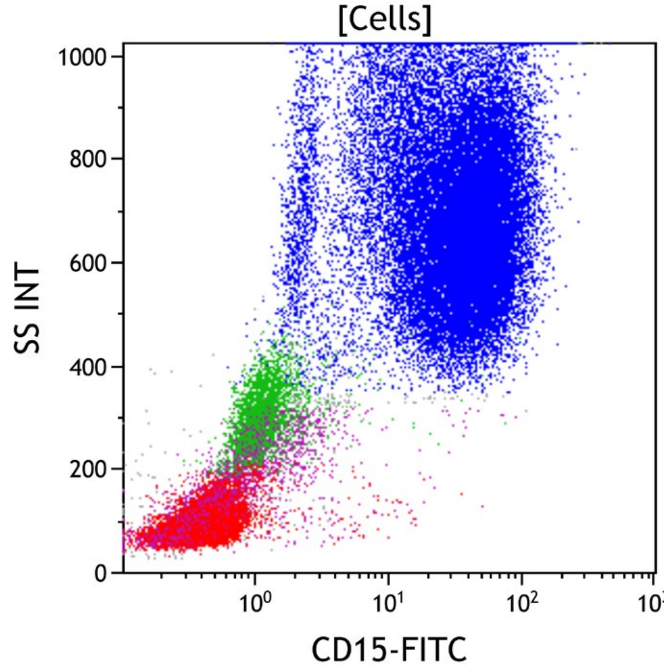

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

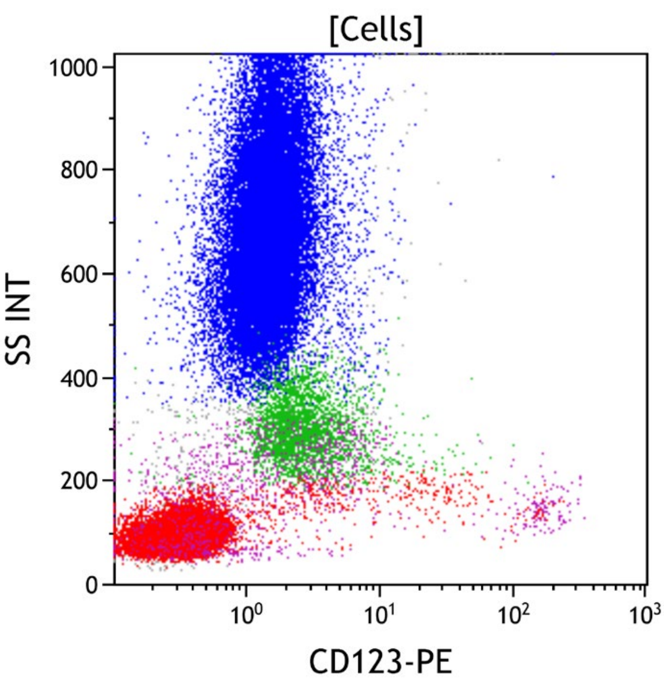

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green).

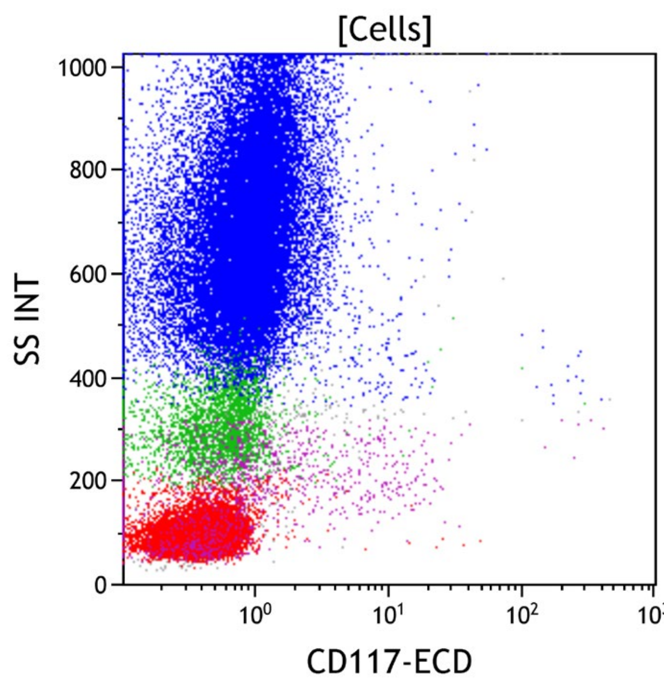

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

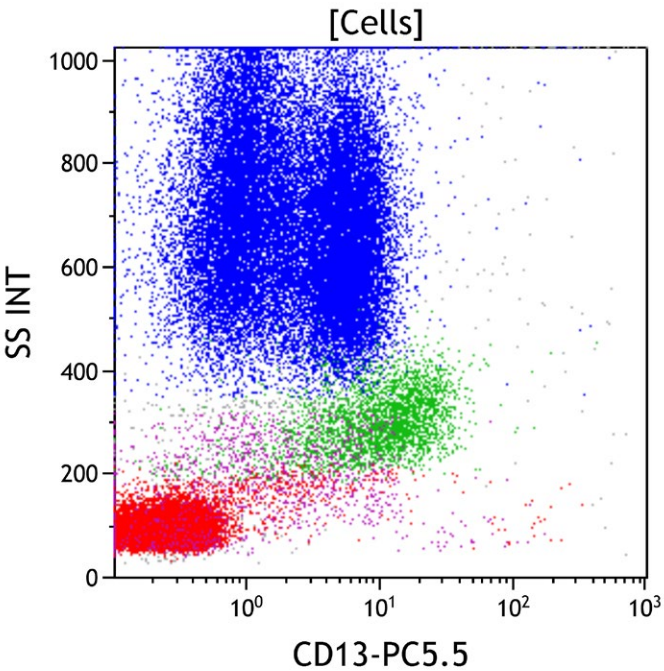

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

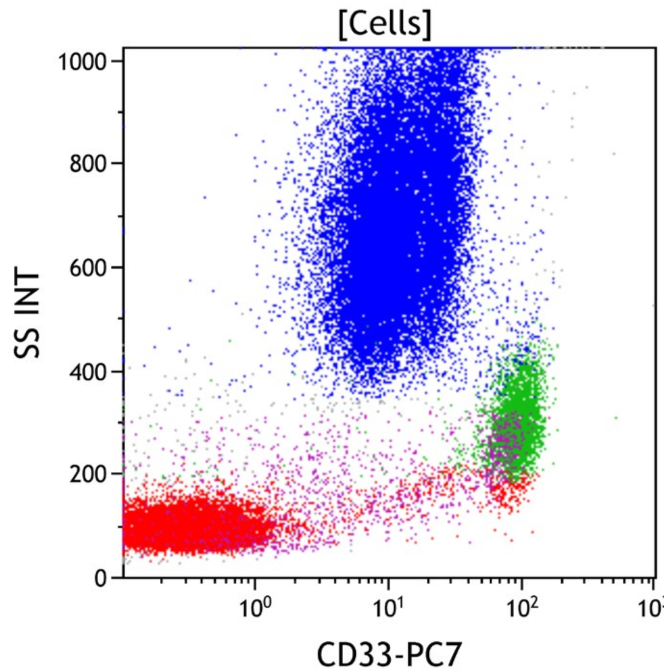

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34+ myeloid progenitors (purple).

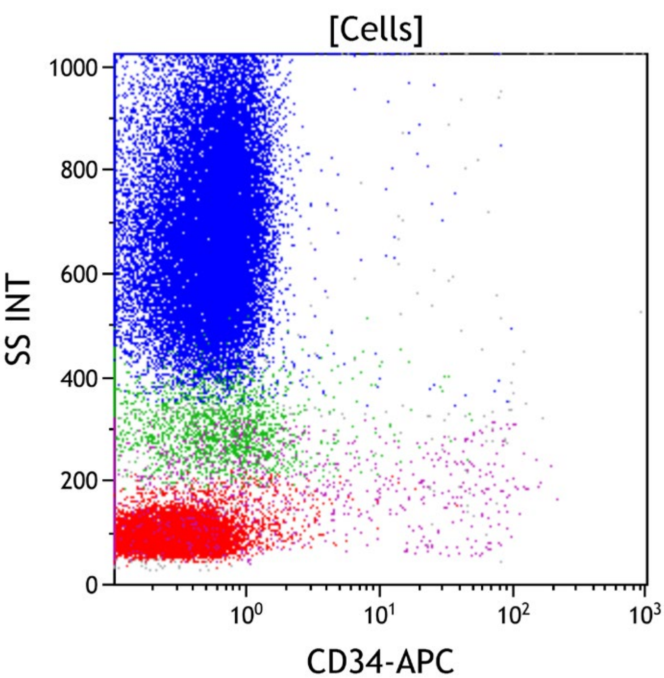

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

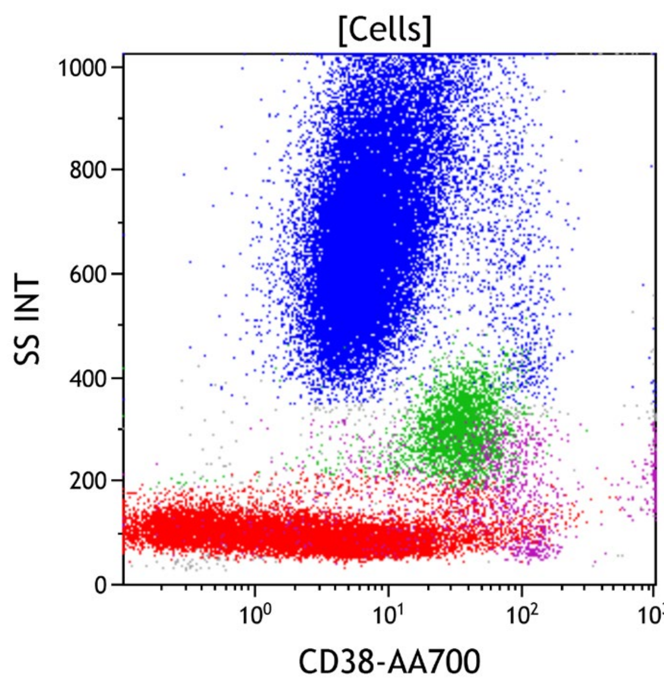

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level.

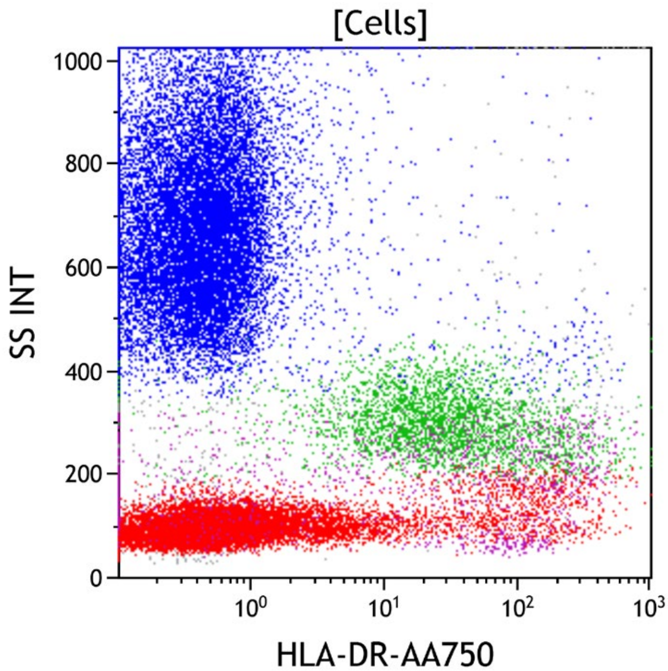

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

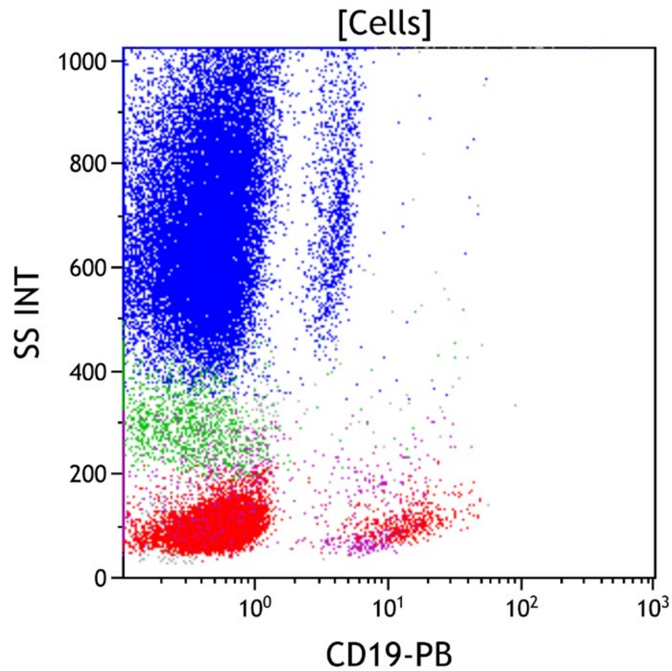

Figure 14: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

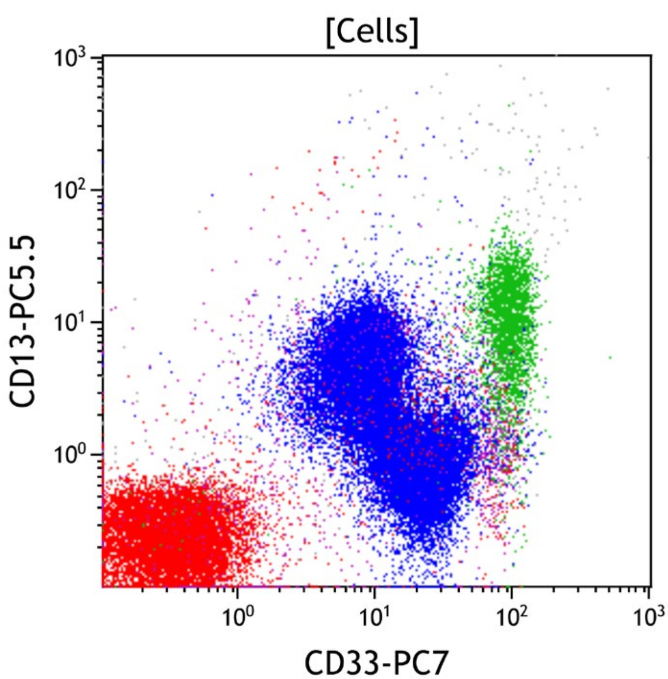

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue bottom) than more mature granulocytes (blue left). Lymphocytes largely do not express either CD13 or CD33 (red).

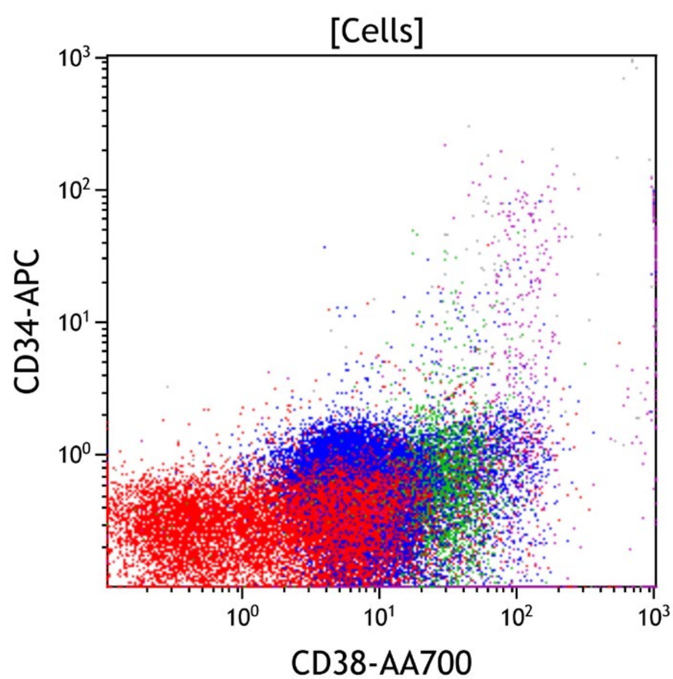

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable CD34 expression by plasma cells (purple extreme right) is a compensation artifact due to the extremely high level of CD38 that extends beyond the visible scale.

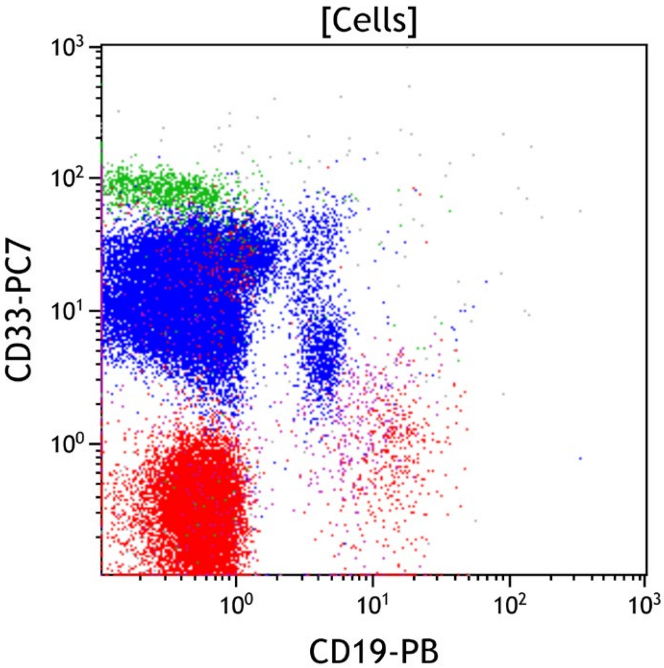

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

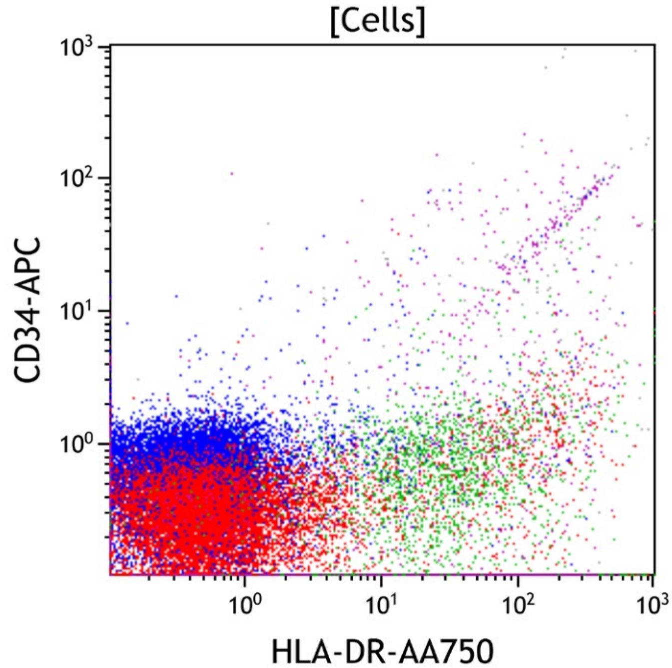

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes (purple right).

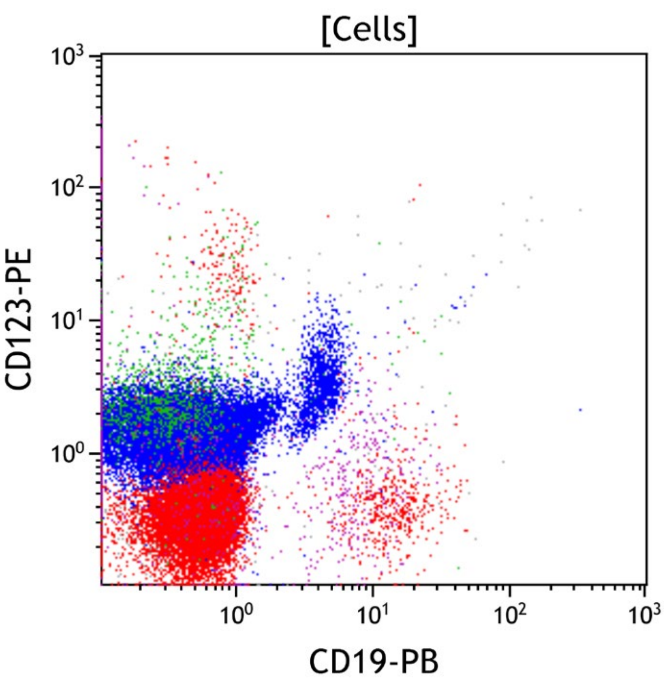

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not expression CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

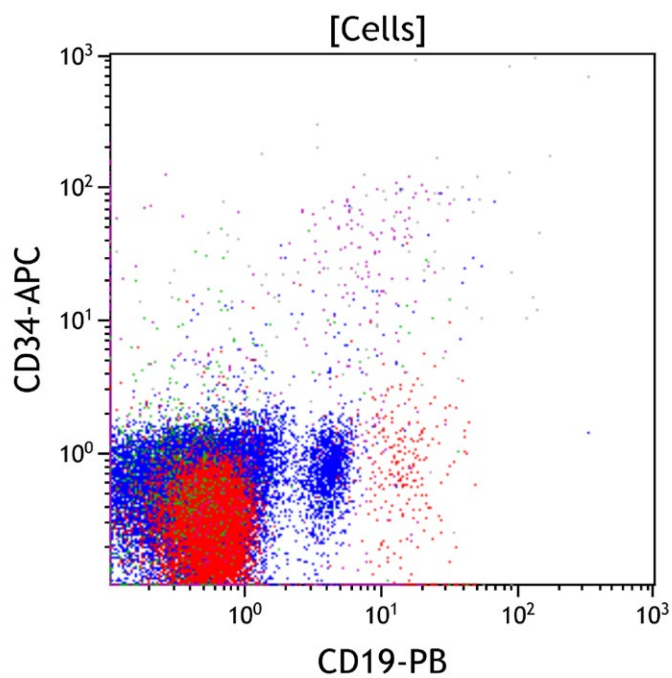

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19.

M2 Cell Tube

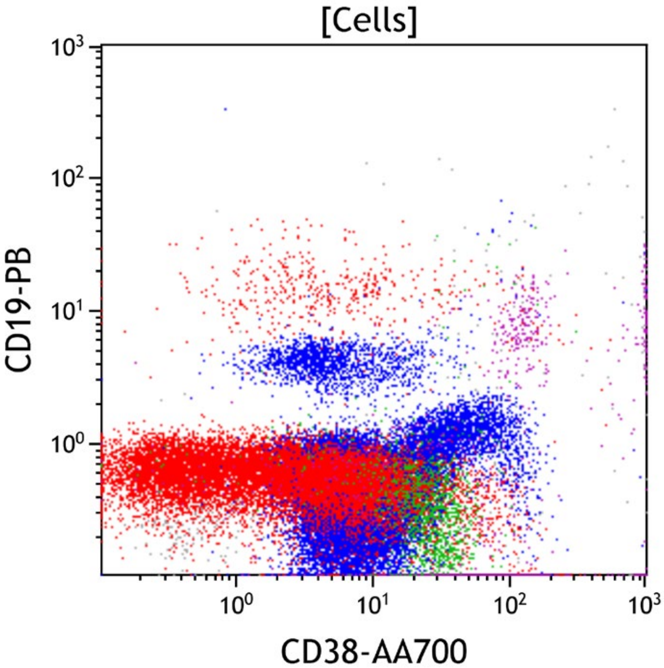

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression of CD38 (upper red). Plasma cells show extremely high CD38 expression that is largely off scale (purple extreme right), but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

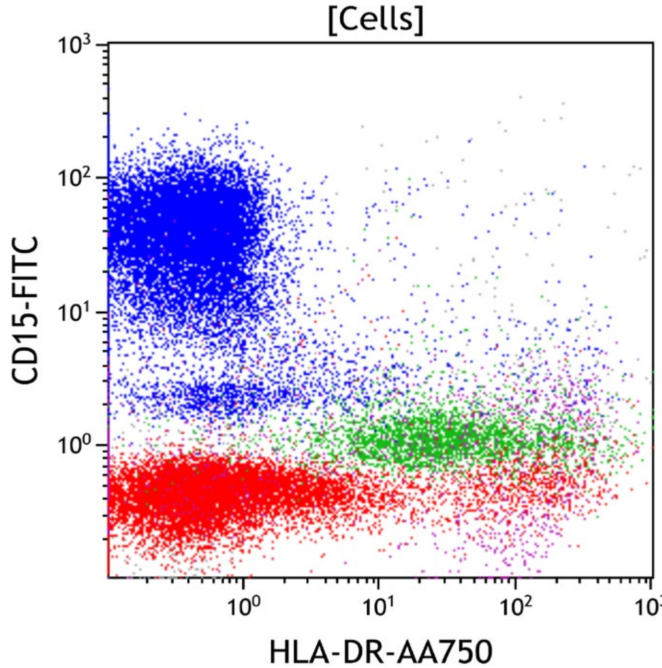

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34+ myeloid progenitors (purple) express HLA-DR but only transiently express CD15.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations in this case. Note that correlation with clinical and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay. The concurrent morphologic, immunohistochemical, and cytogenetic findings confirm the absence of a hematopoietic neoplasm.

Case #3: Normal Lymph Node

Clinical Vignette

This 53 year old female presents with a mass/lesion. A lymph node biopsy sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

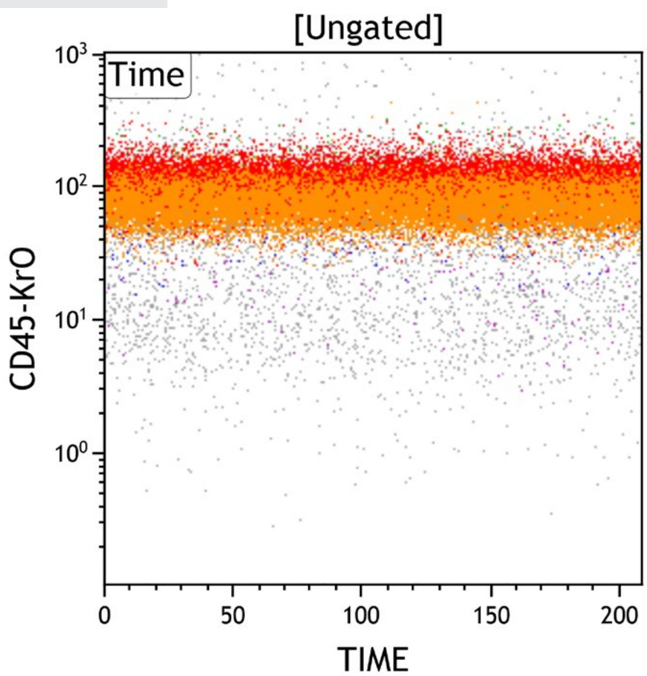

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

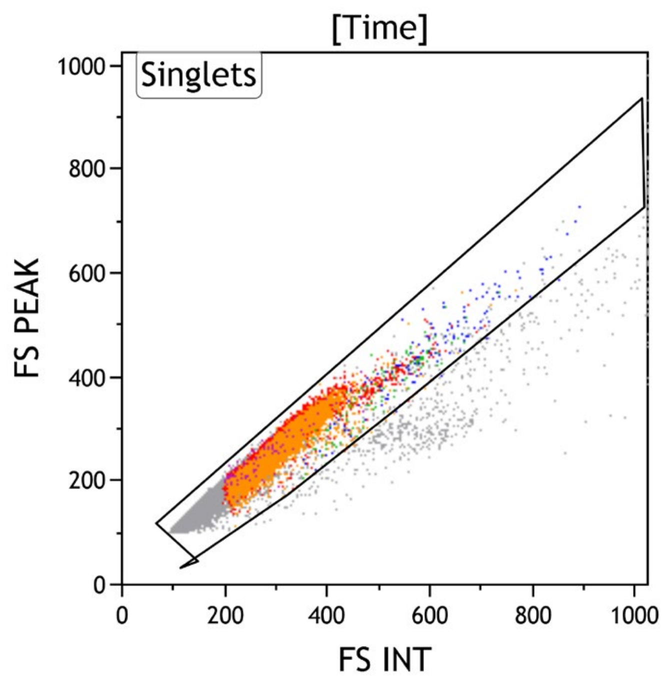

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

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B Cell Tube

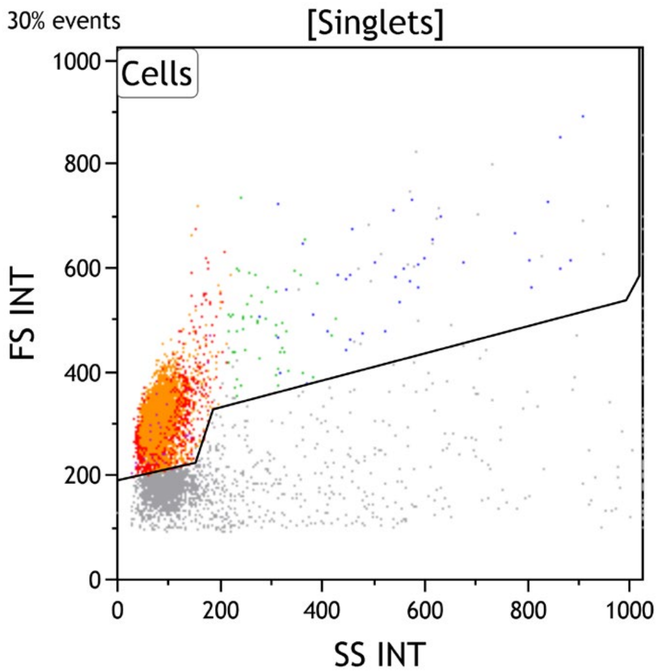

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. Exclusion of non-viable cells can be particularly important for tissue specimens to minimize artifact.

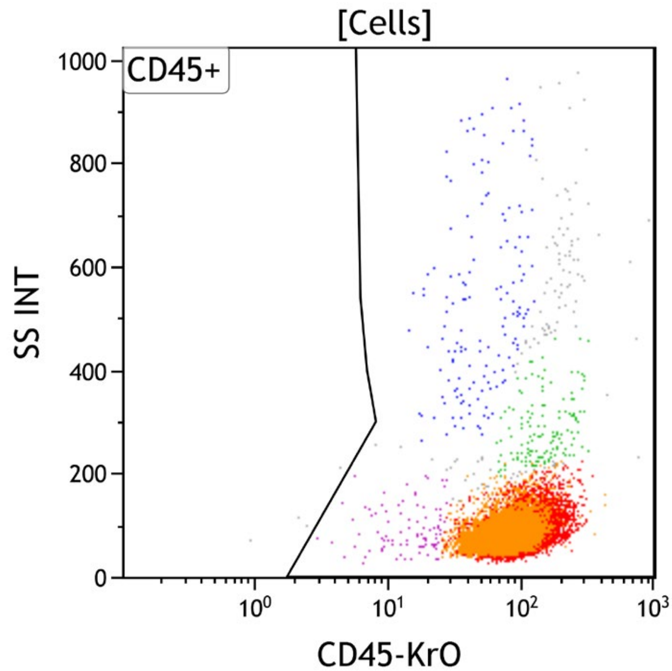

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

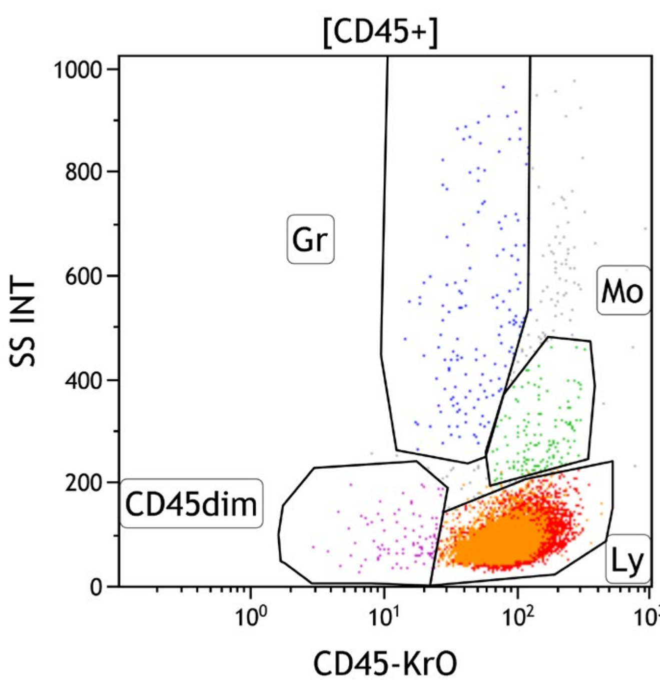

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) is occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. In this case, the white blood cells are composed predominantly of lymphocytes, as is typical for tissues.

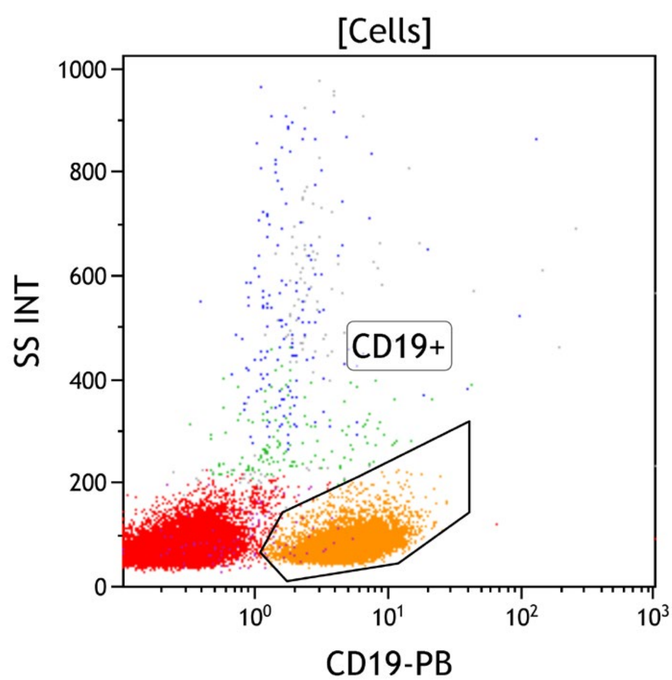

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B Cell Tube

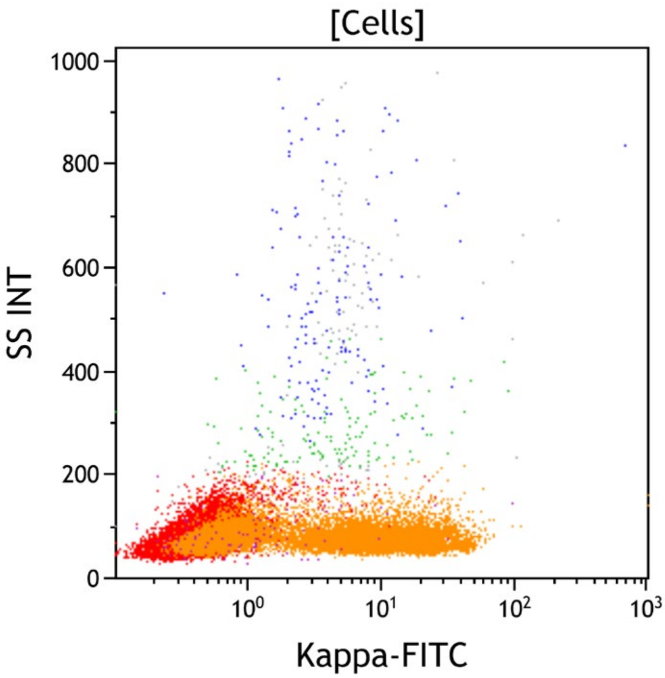

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The Kappa light chain positive cells are shown on the right side of the plot.

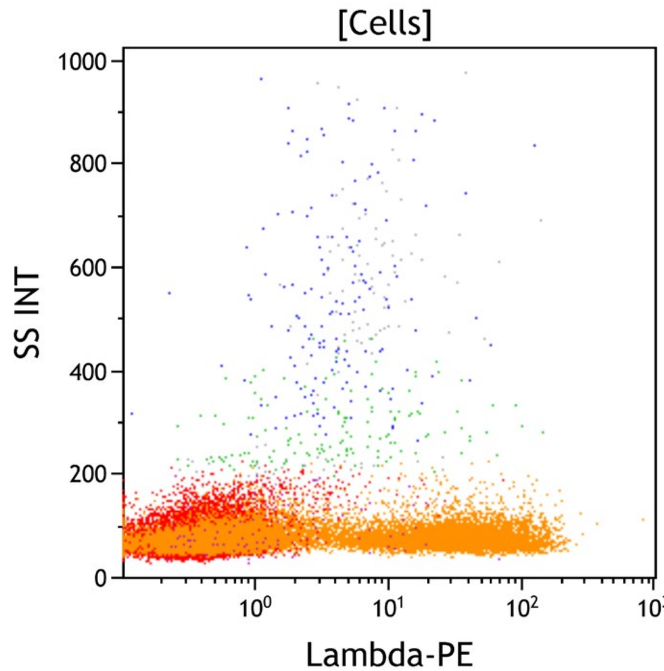

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The lambda light chain positive cells are shown on the right side of the plot.

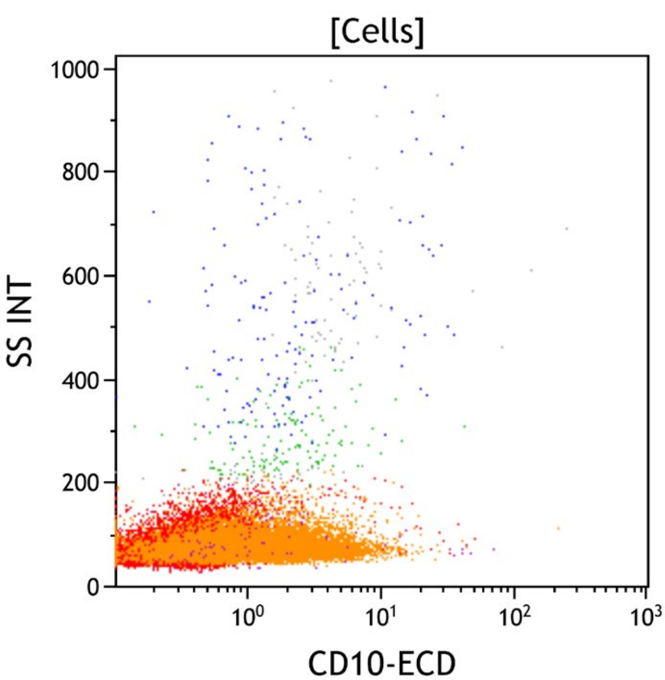

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes. A subset of CD19 positive B cells (orange) express CD10 and likely represent follicle center B cells.

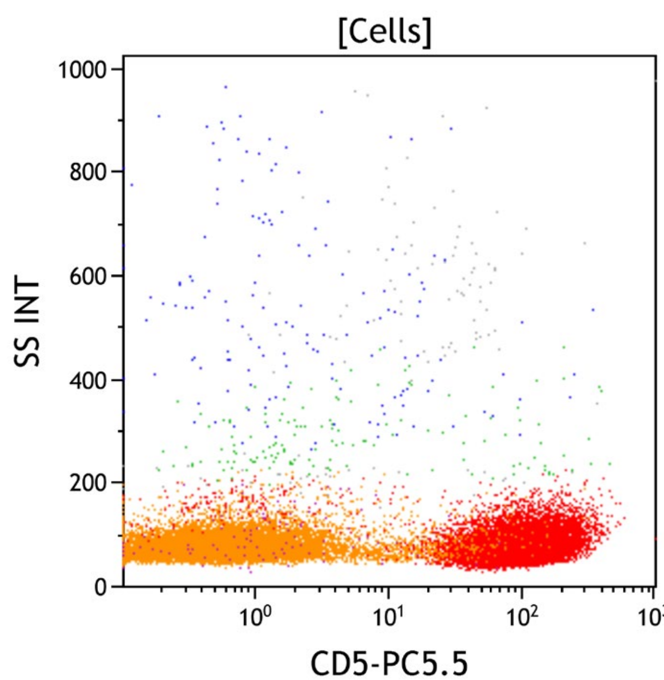

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter.

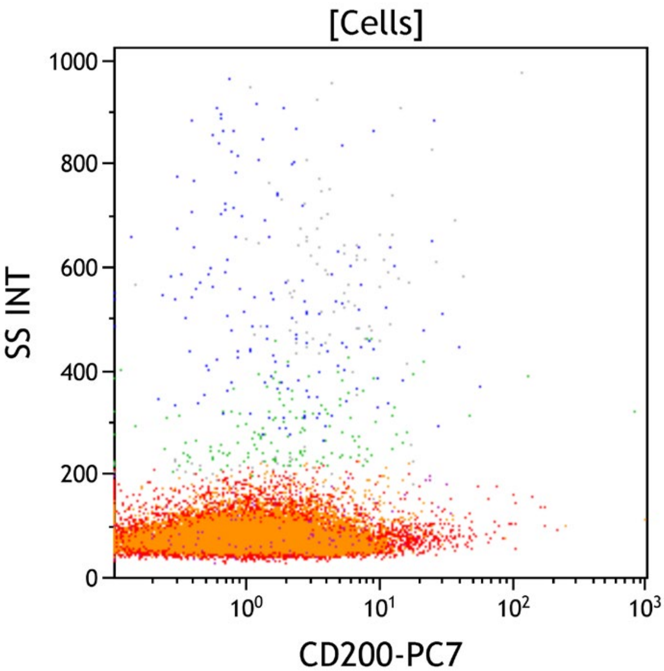

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

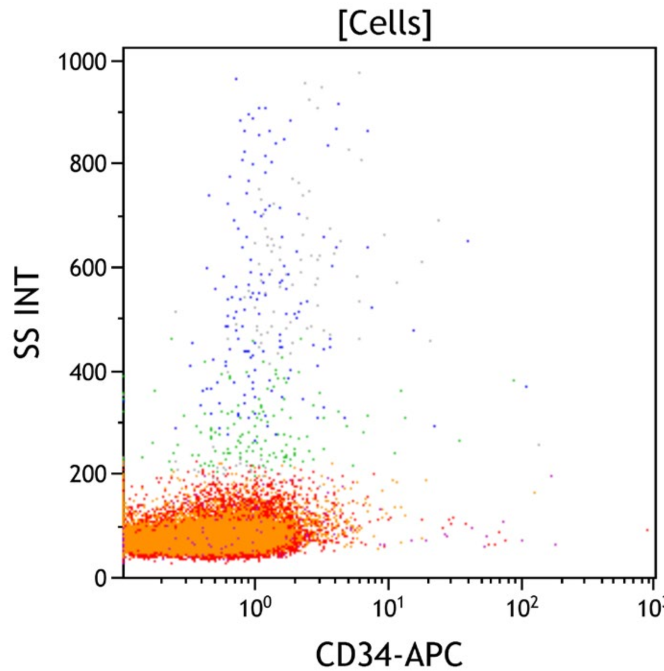

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature lymphocytes are negative for CD34.

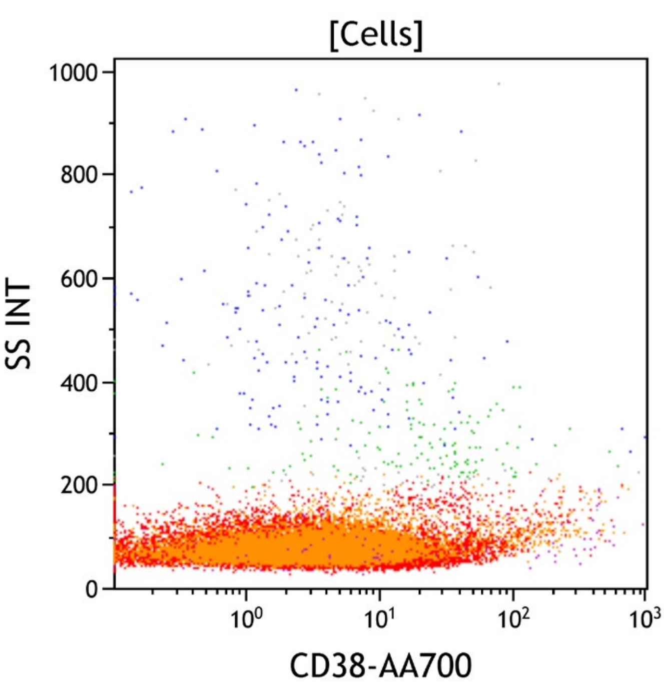

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variable level on activated mature lymphocytes (red and orange).

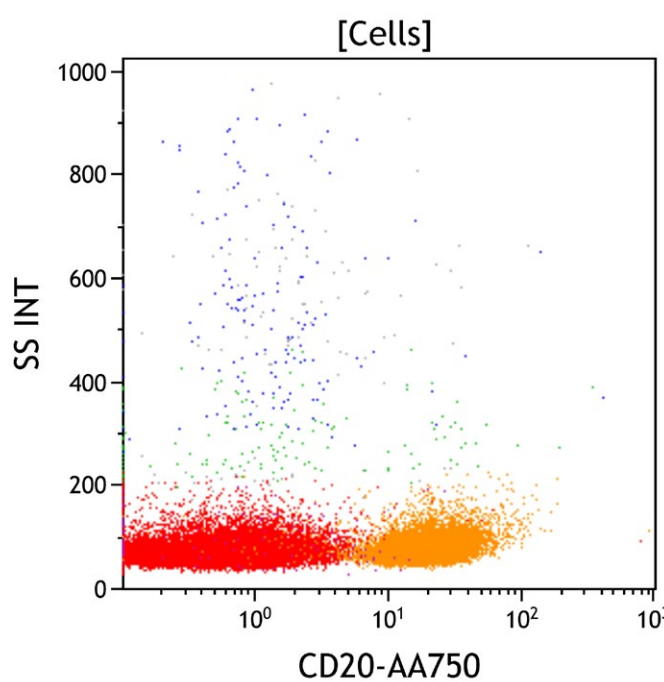

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.

B Cell Tube

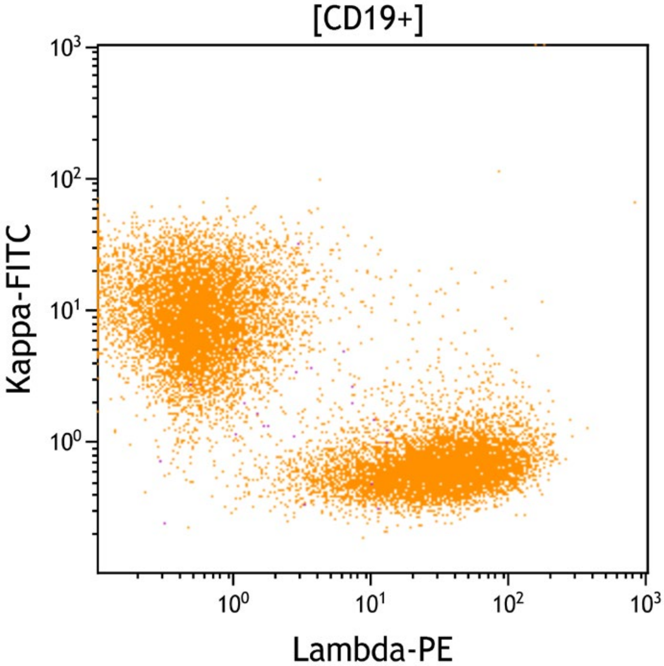

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

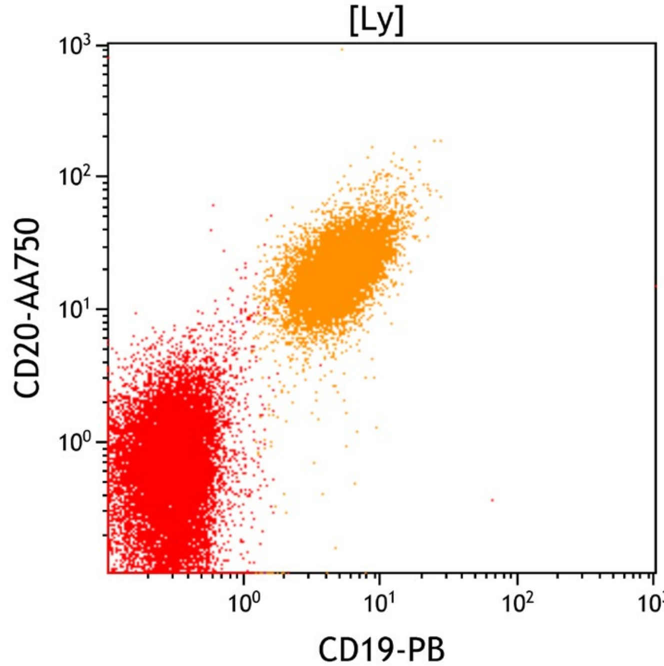

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression.

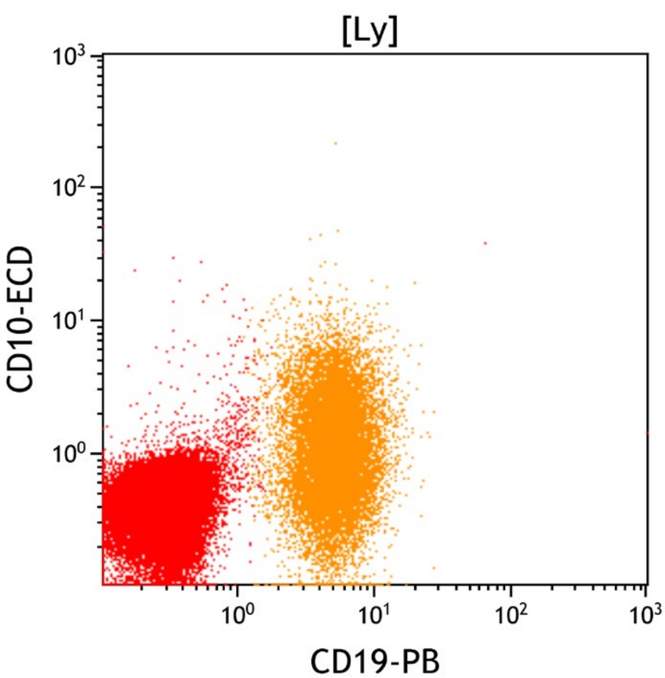

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate. B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. The dim CD10 positivity seen here is typical of germinal center B cells.

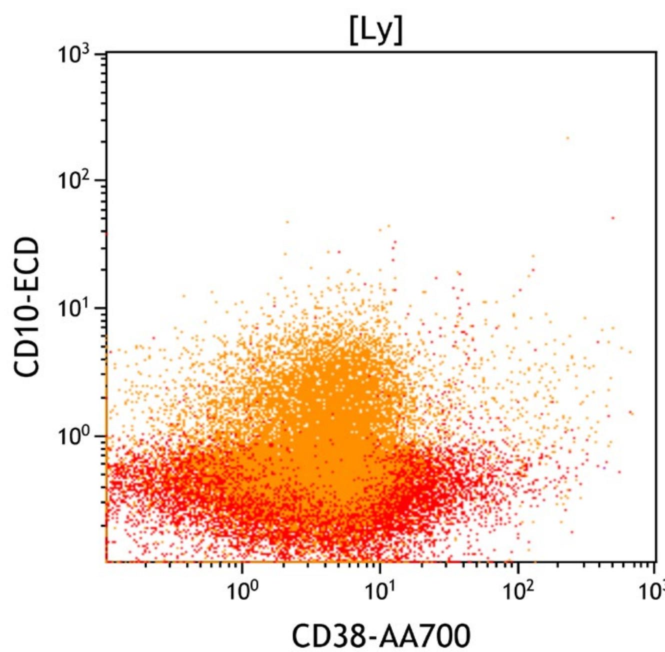

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. Germinal center B cells in the lymph node express CD10 and variable level of CD38. T cells (red) show variable CD38 expression dependent on activation state.

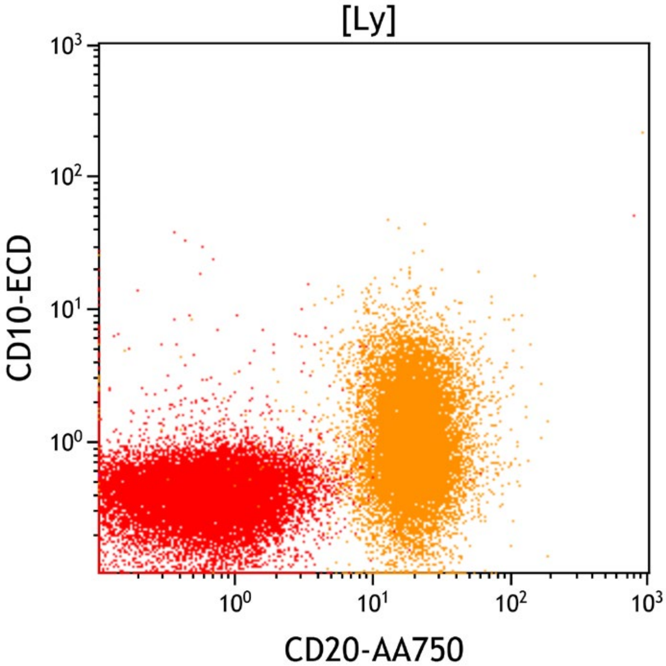

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20. Germinal center B cells in the lymph node express CD10 at a low level.

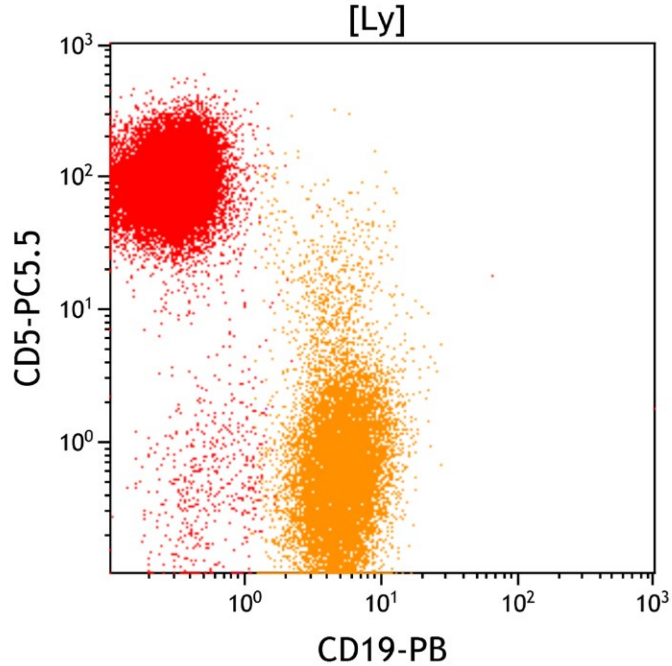

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper left), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

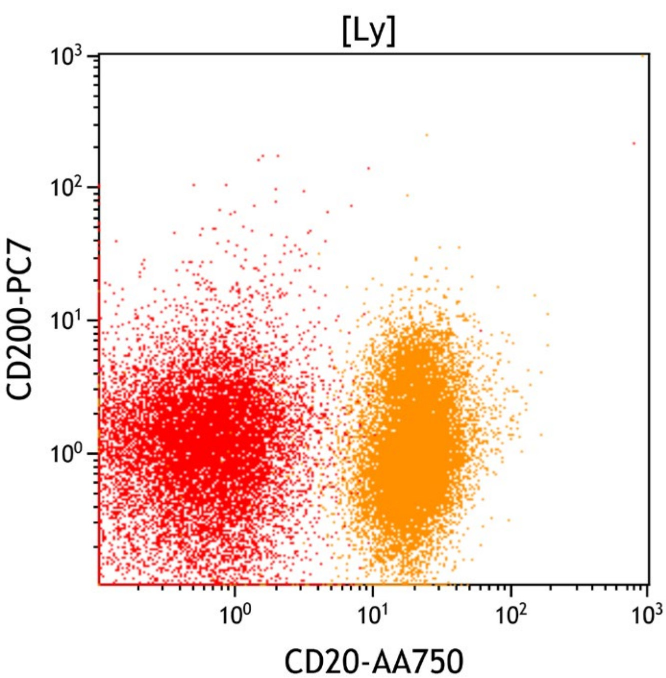

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

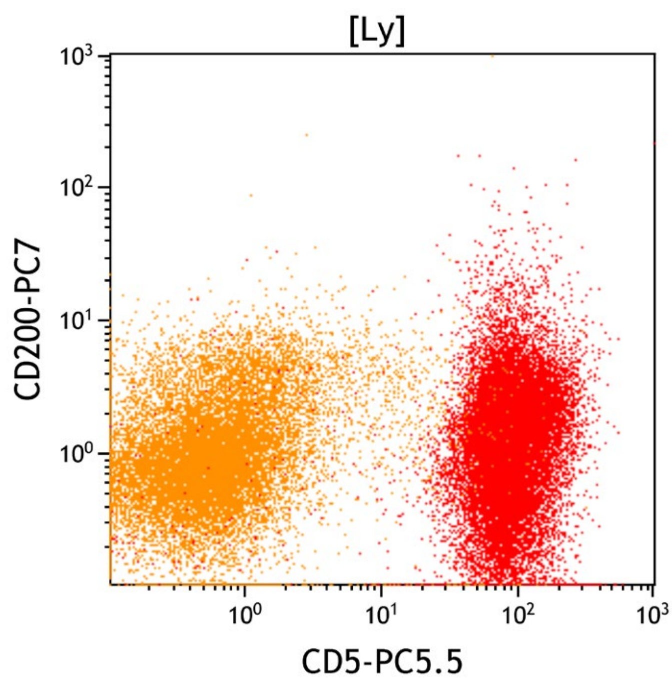

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 at a low to moderate level with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

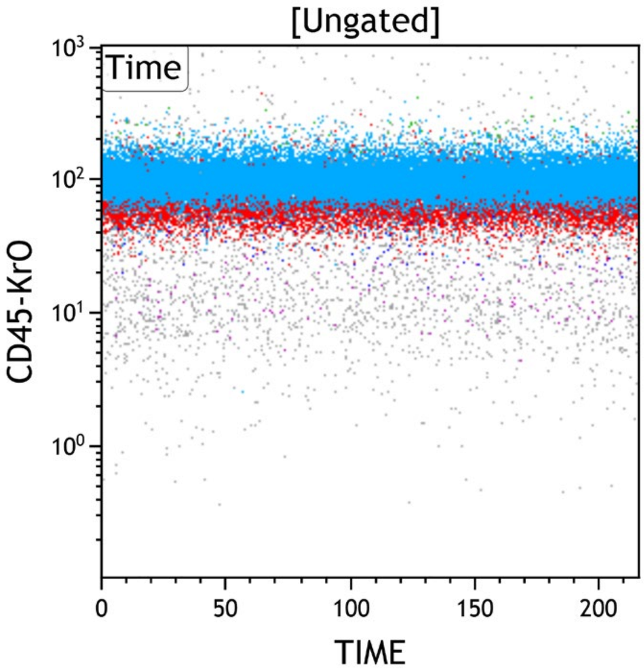

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

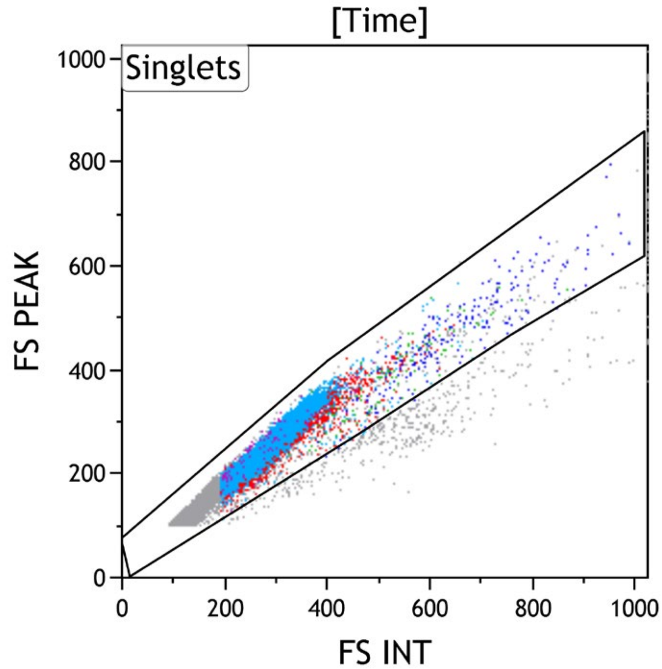

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

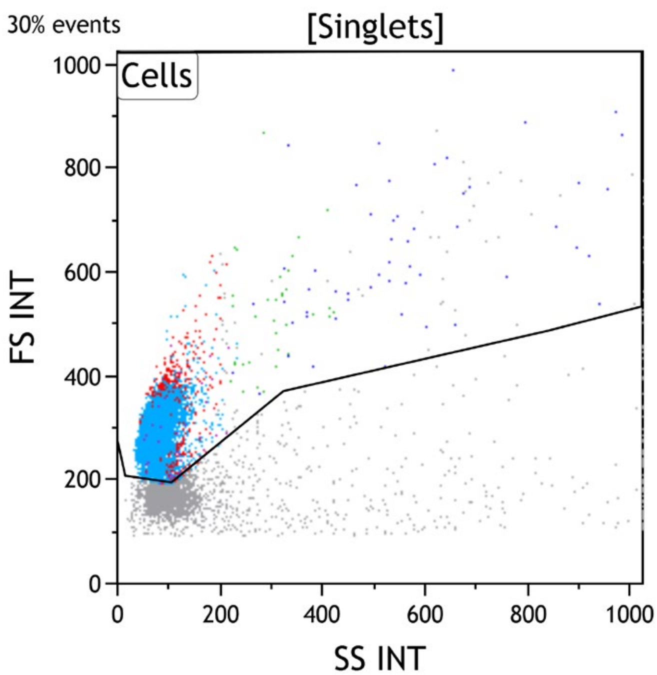

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

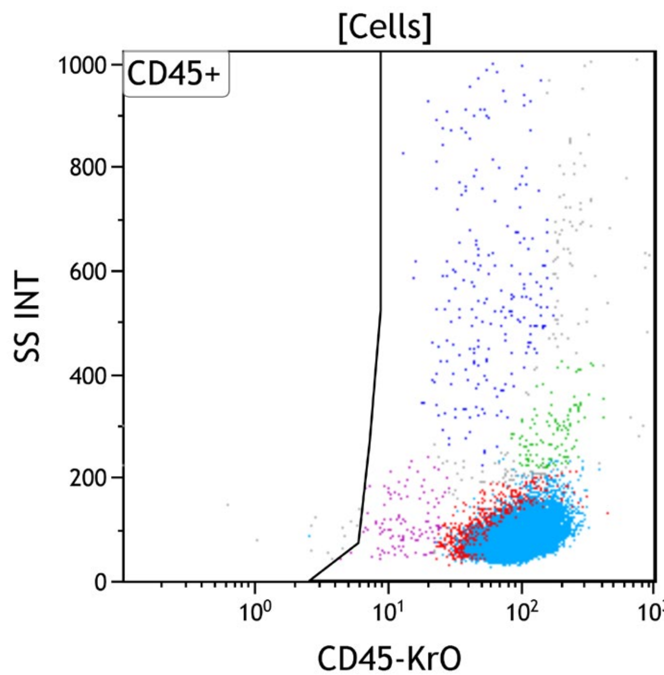

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

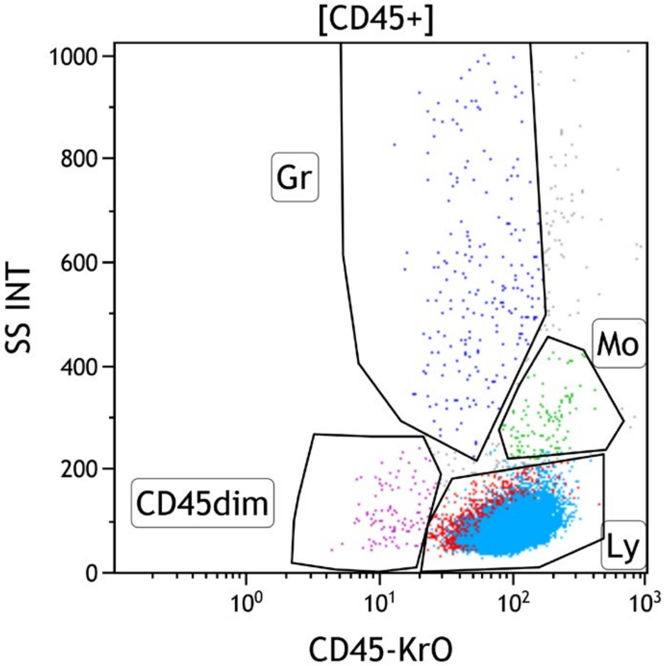

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. In this case, the white blood cells are composed predominantly of lymphocytes.

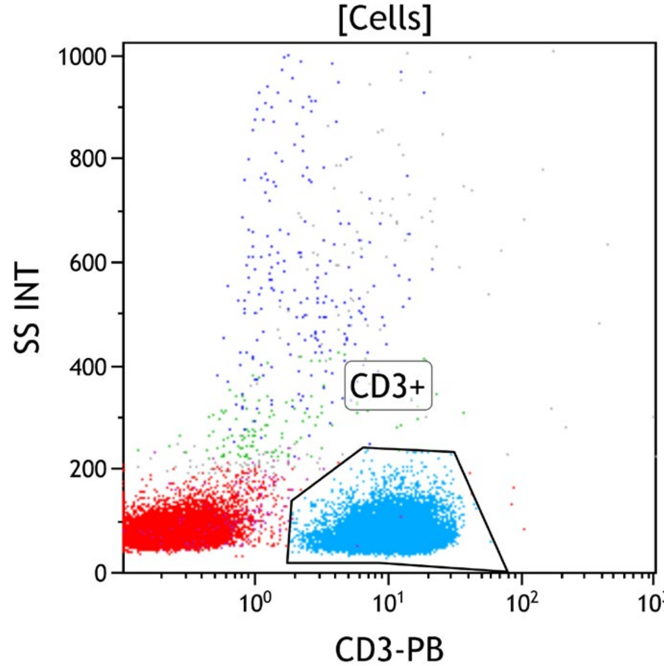

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

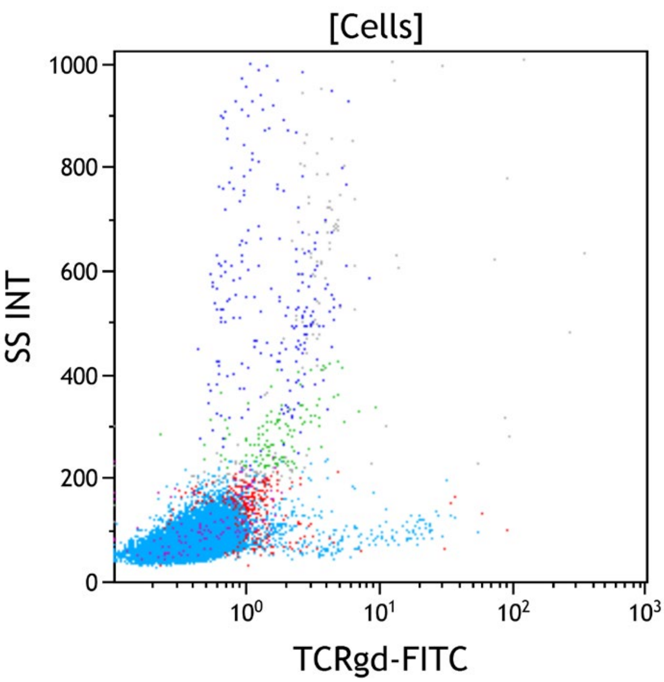

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua).

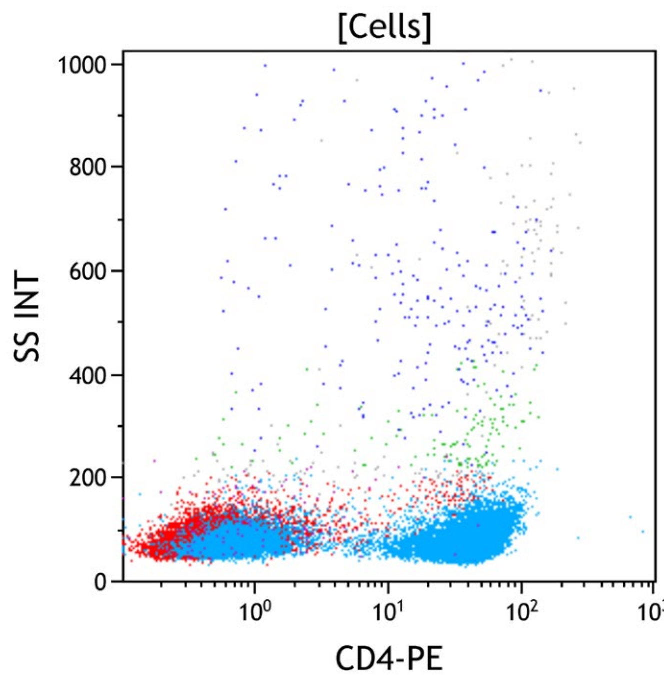

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells, but are essentially absent in this case.

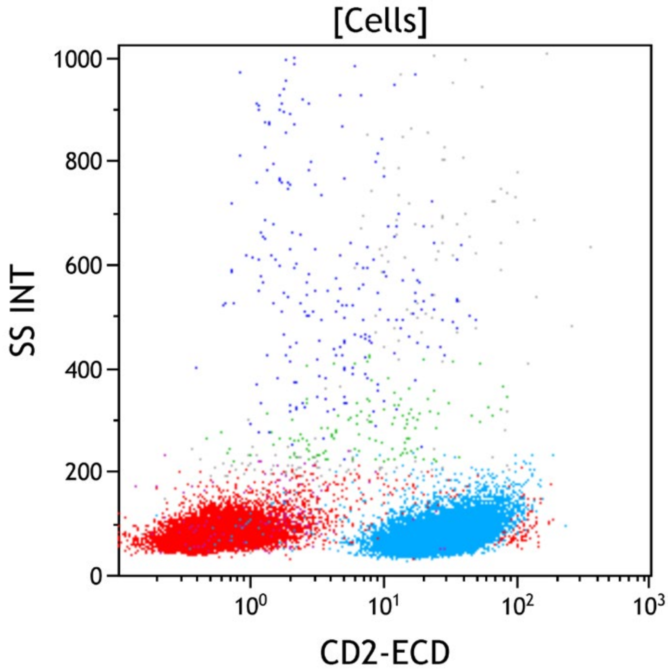

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells and at a low level on monocytes (green).

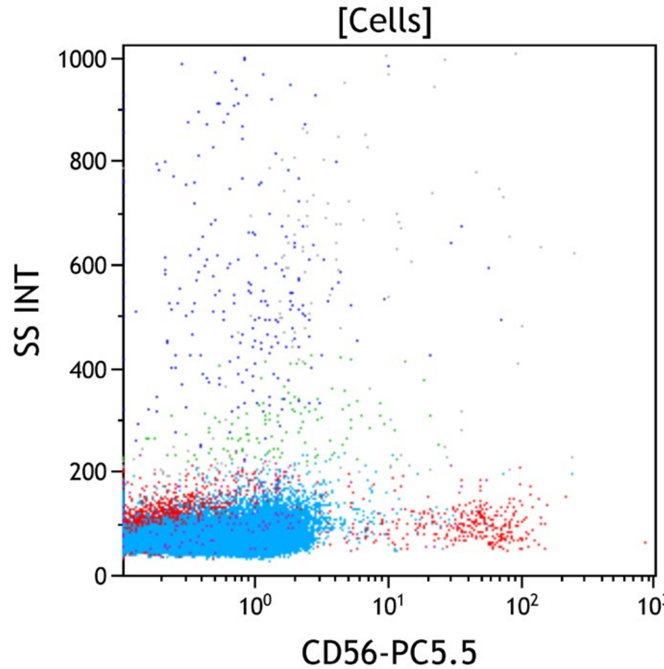

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red, lower right), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. NK cells are generally infrequent in normal tissues.

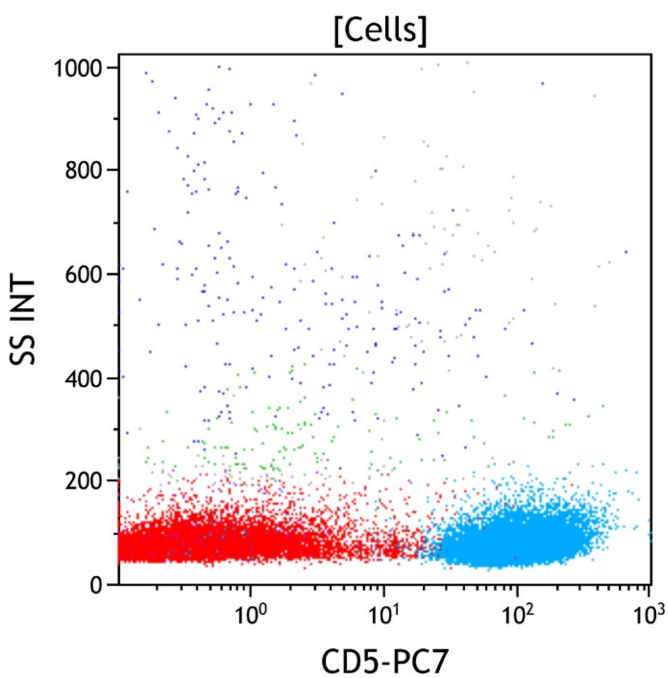

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

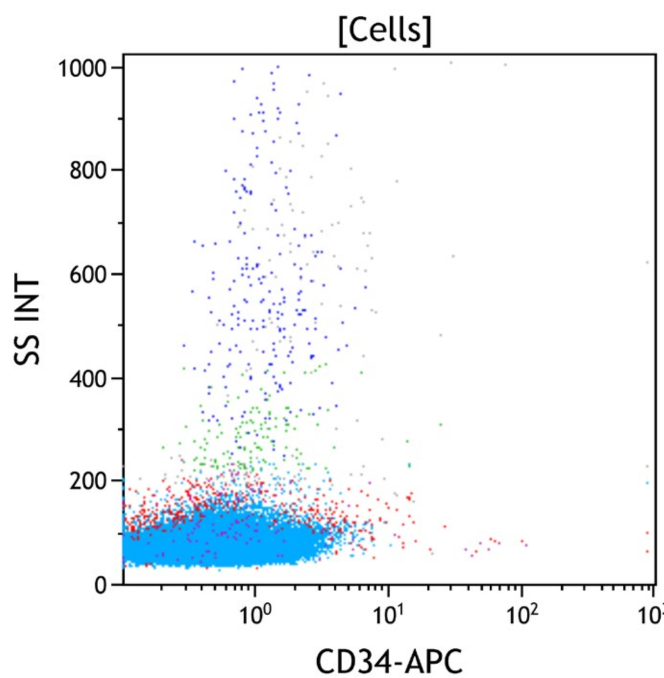

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature lymphocytes are negative for CD34. Tissues rarely contain CD34 positive progenitor populations.

T Cell Tube

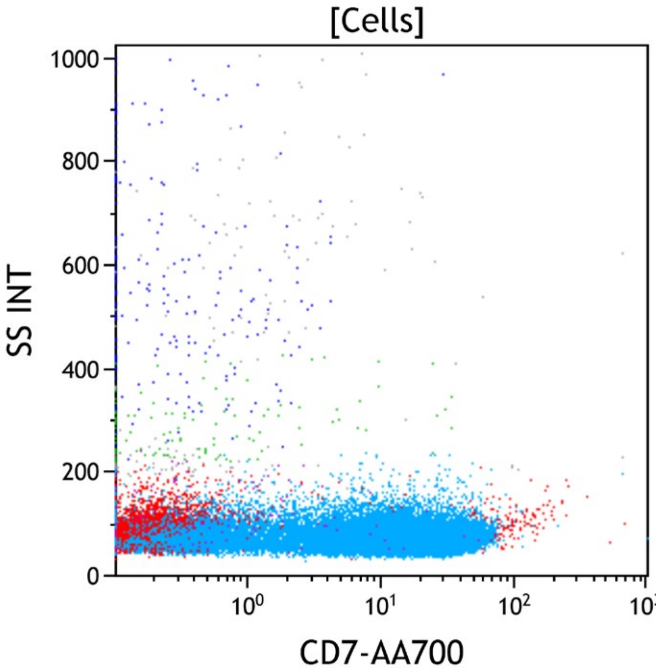

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

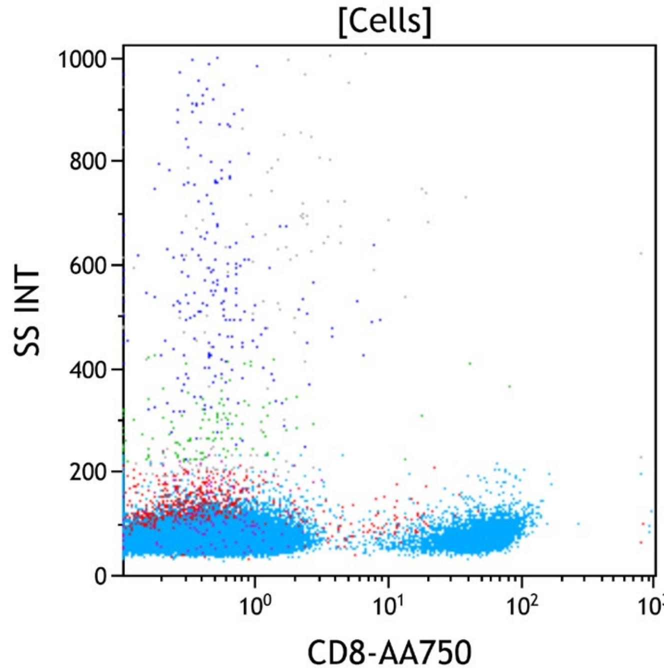

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

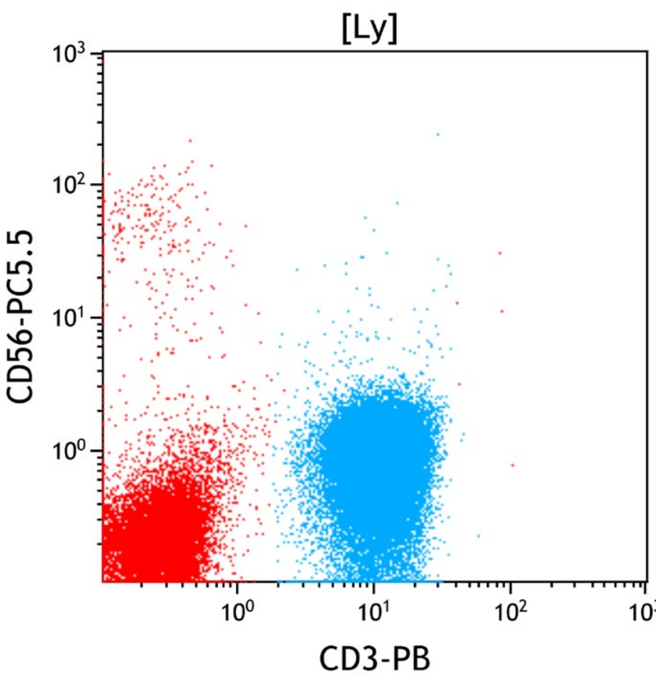

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

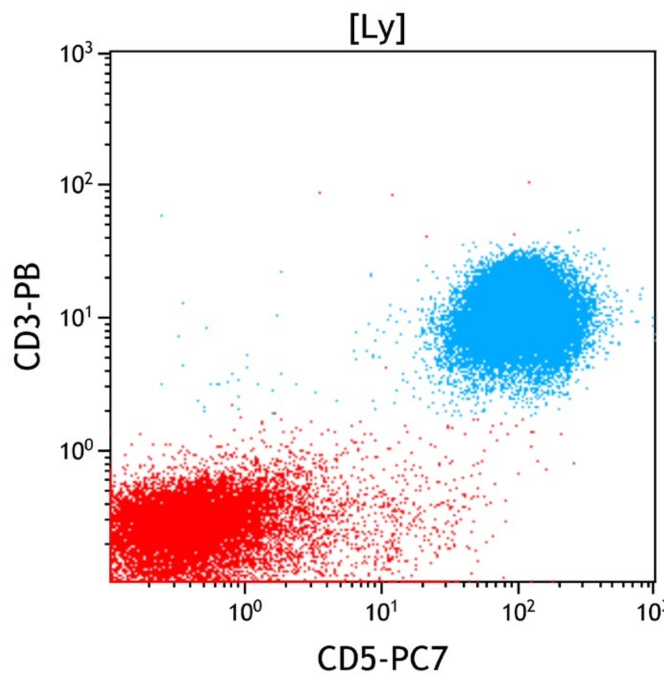

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5.

T Cell Tube

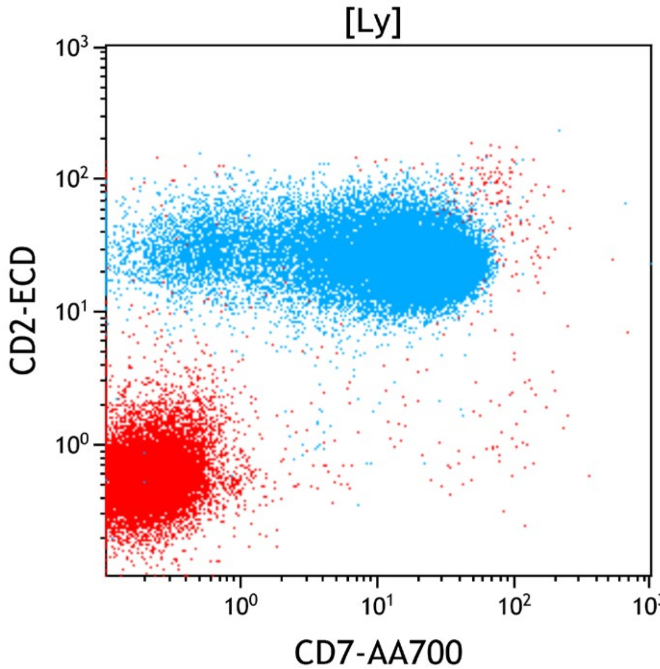

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

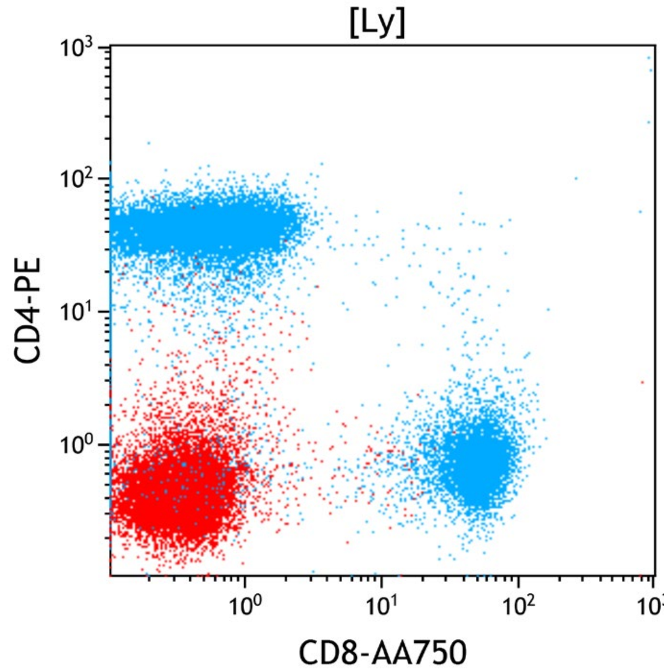

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells (aqua, lower left).

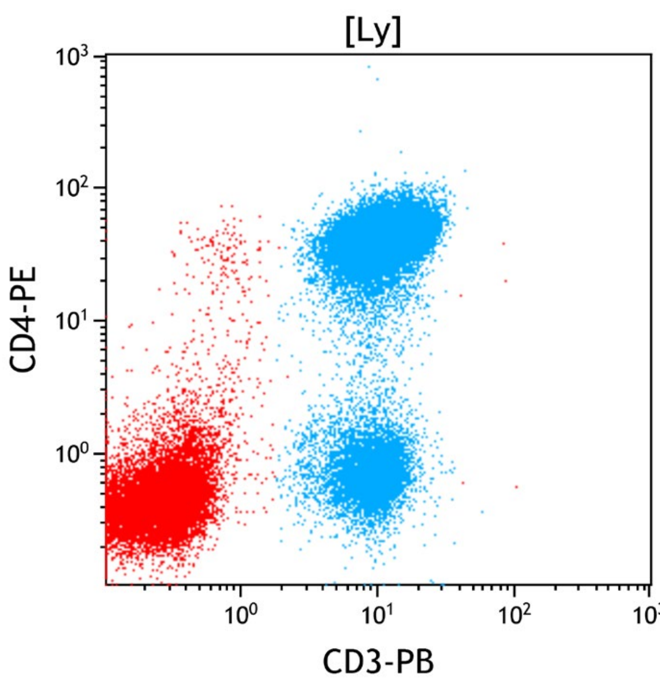

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression.

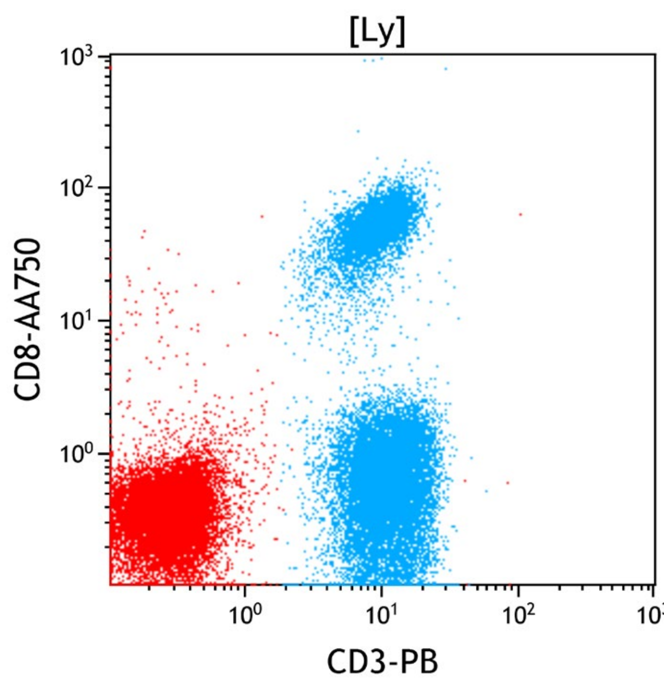

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

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T Cell Tube

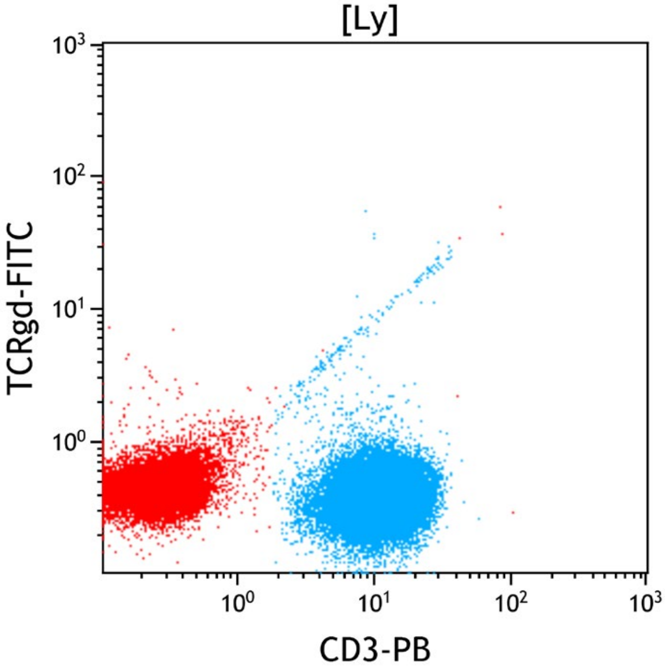

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

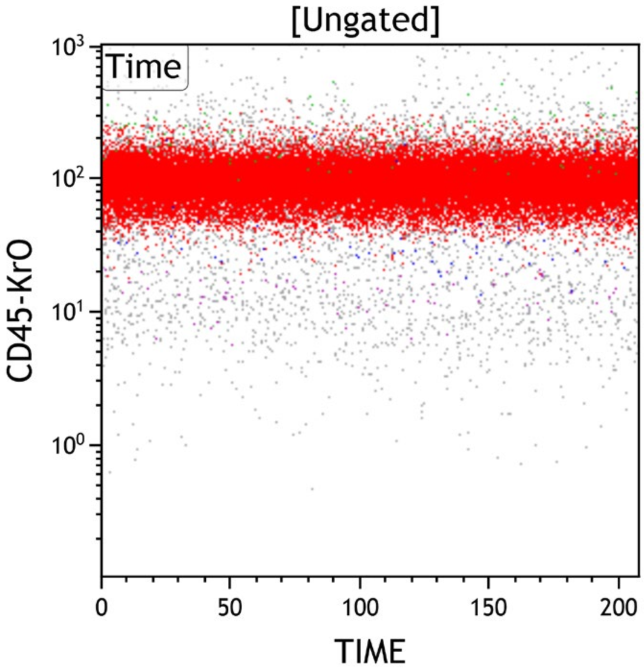

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

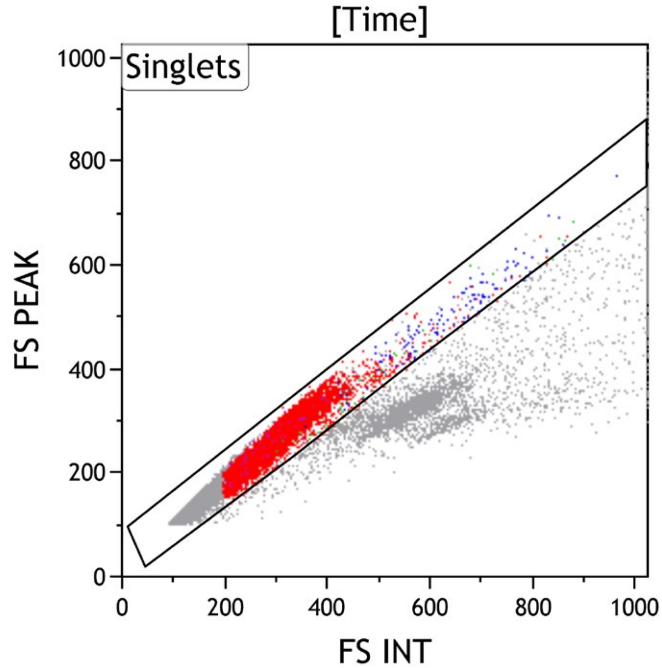

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

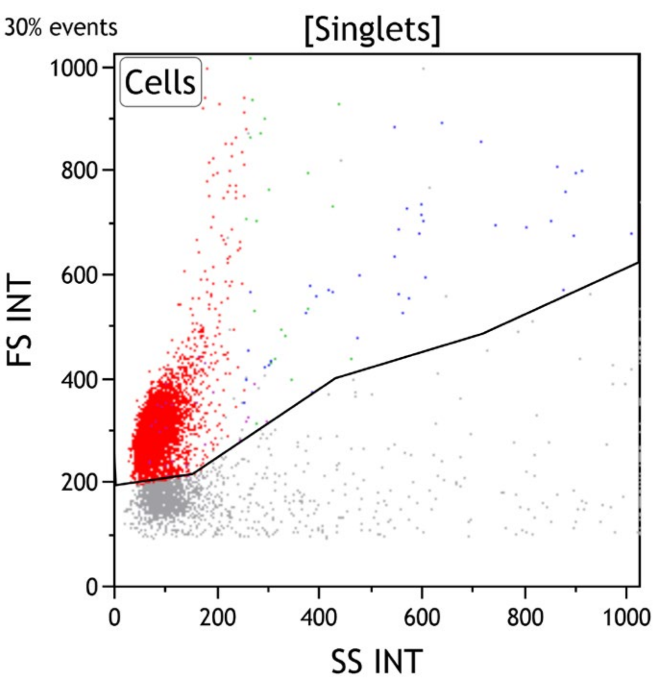

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

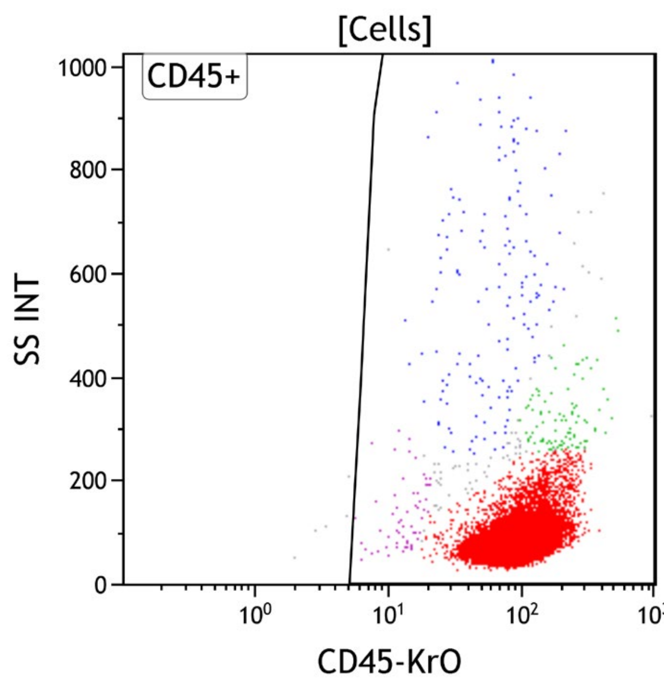

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

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M1 Cell Tube

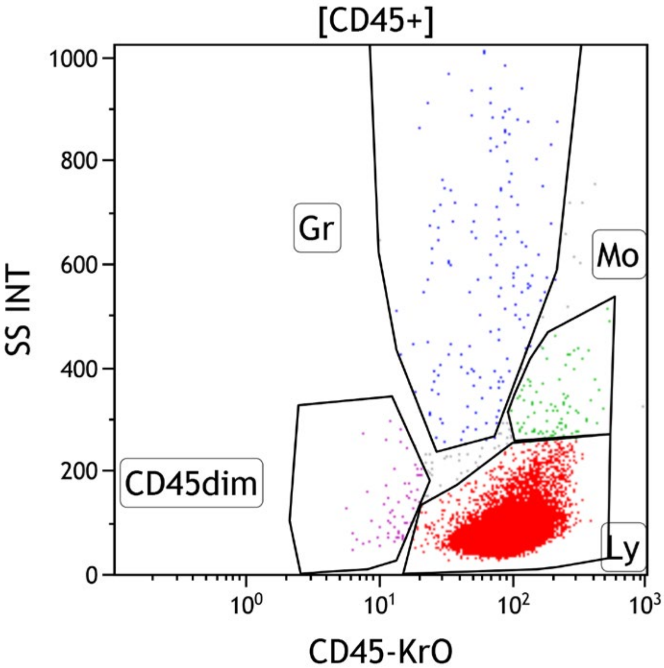

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. In this case, the white blood cells are composed predominantly of lymphocytes.

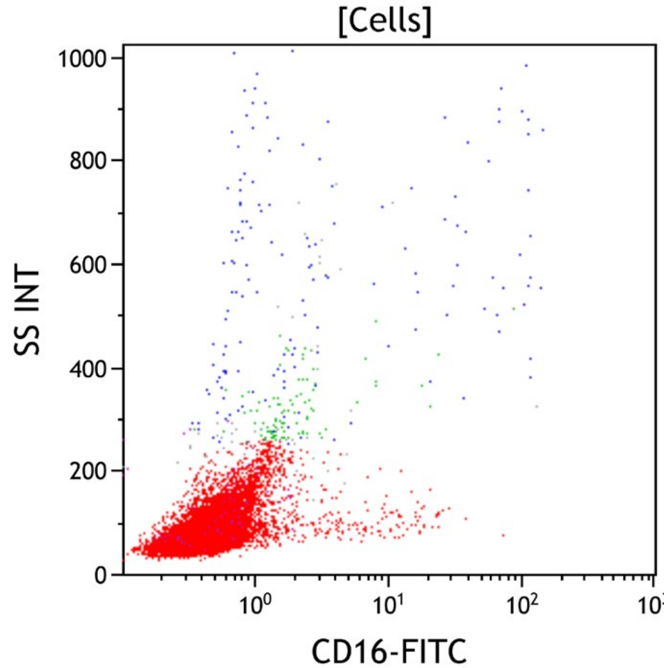

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands. Most NK cells express CD16 (red, right lower), as do a subset of activated monocytes. Mature lymphocytes (red, lower left) are negative for CD16.

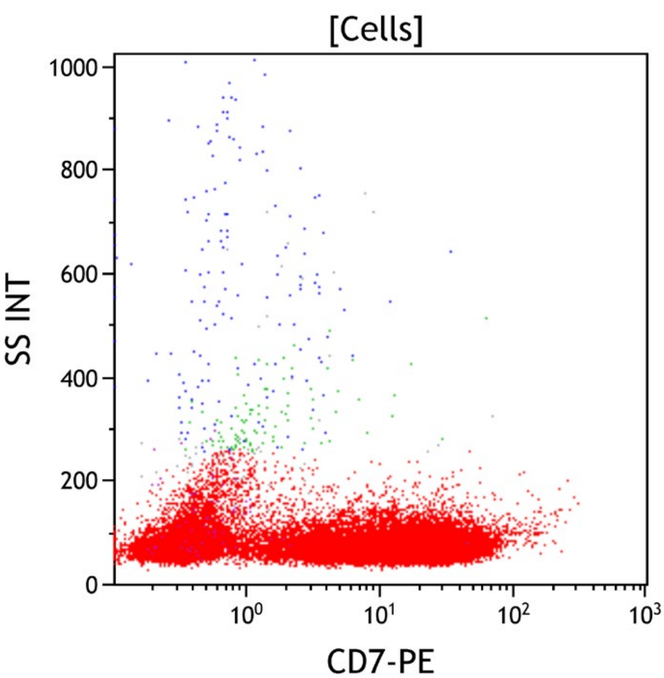

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red, lower right). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

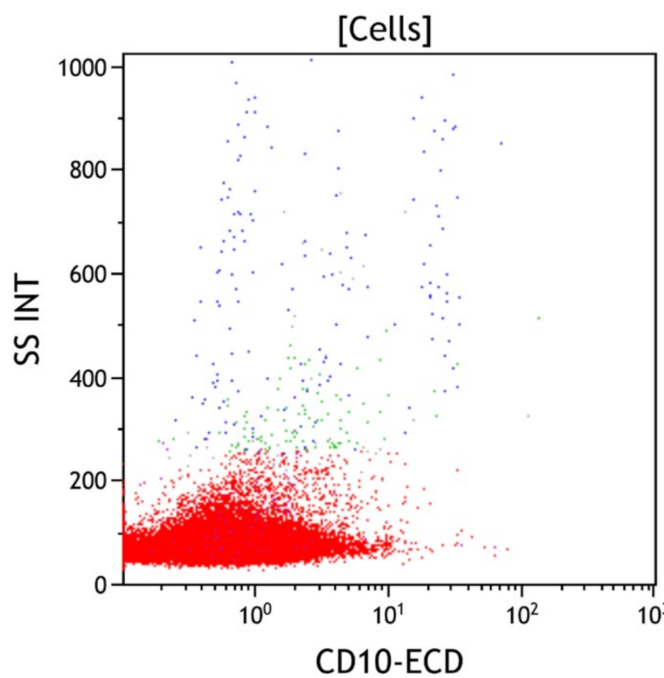

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes. The dim CD10 expression in this case is from germinal center B cells.

M1 Cell Tube

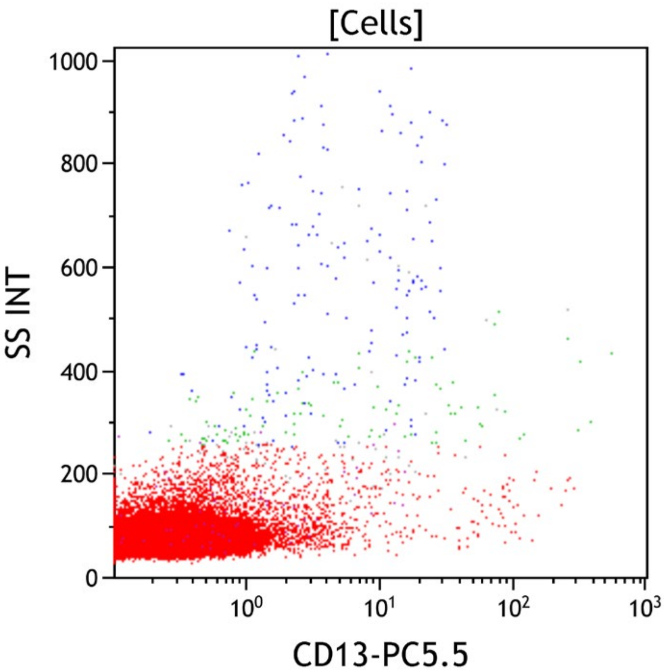

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes, monocytes, and myeloid progenitors. Lymphocytes (red) are largely negative for CD13.

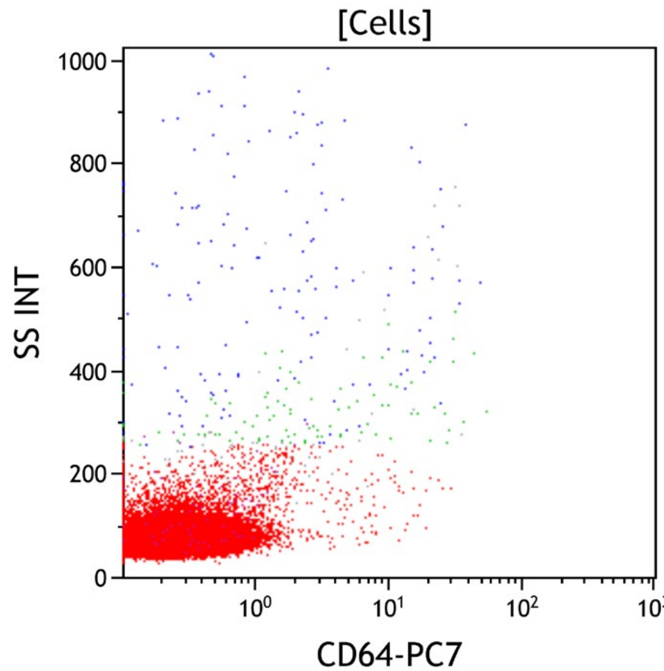

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes and at a slightly lower level on promyelocytes and myelocytes. CD64 is not well expressed on resting mature granulocytes, but increases in expression with granulocyte activation. Lymphocytes (red) are negative for CD64.

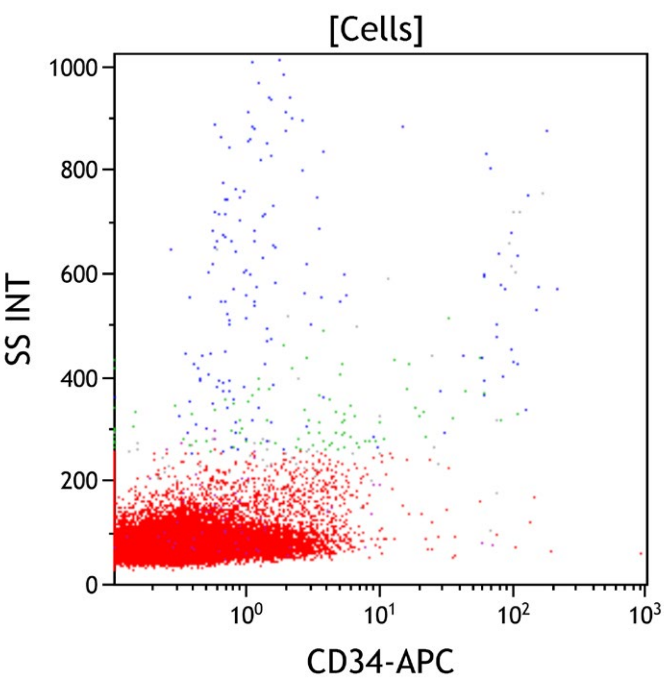

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature lymphocytes (red) are negative for CD34. Tissues rarely contain CD34 positive progenitors.

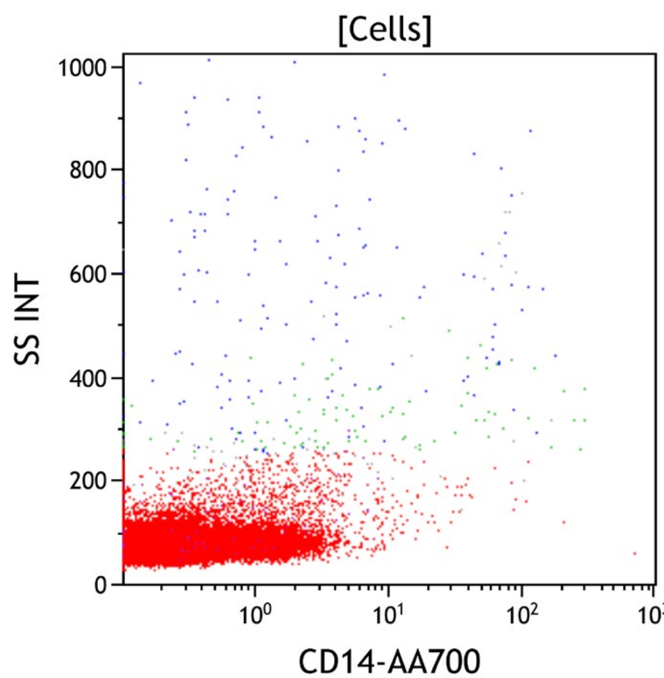

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes, at a variably lower level on immature monocytes, and at a low level on mature granulocytes. Mature lymphocytes (red) are negative for CD14.

M1 Cell Tube

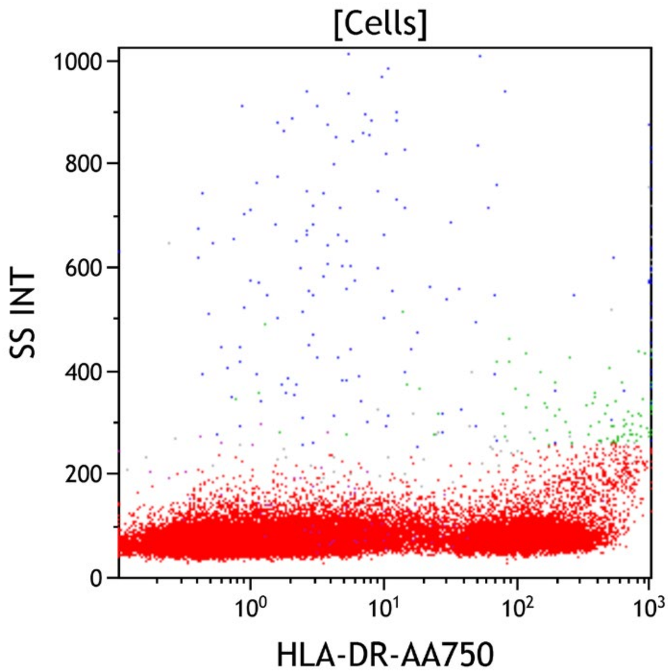

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells.

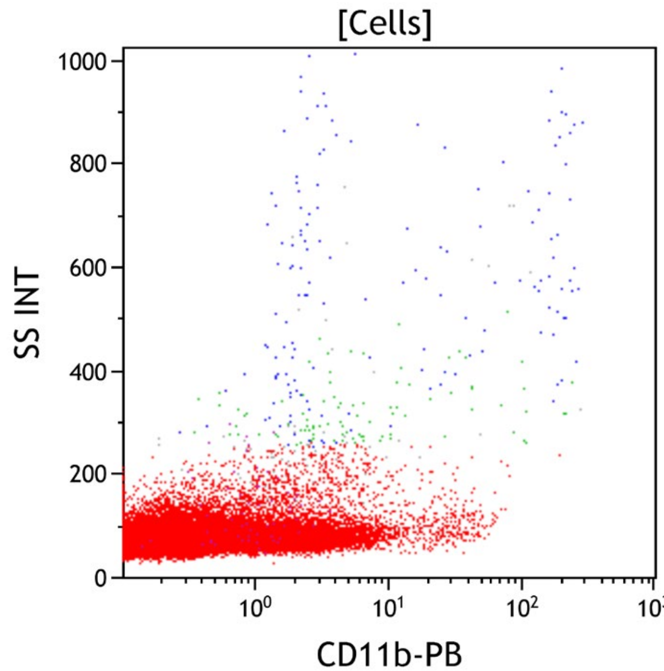

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes, NK cells (red, lower right) and basophils.

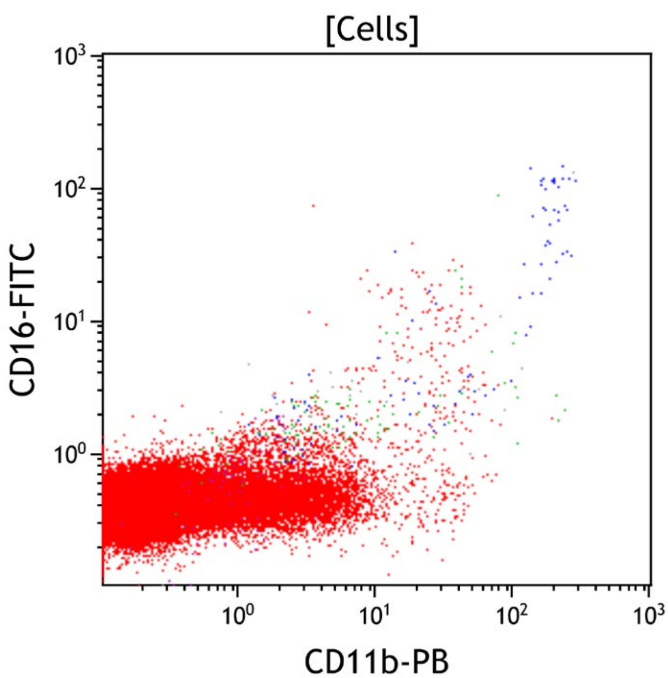

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes, and NK cells (red, lower right). CD16 is expressed on immature and mature granulocytes and a subset of NK cells.

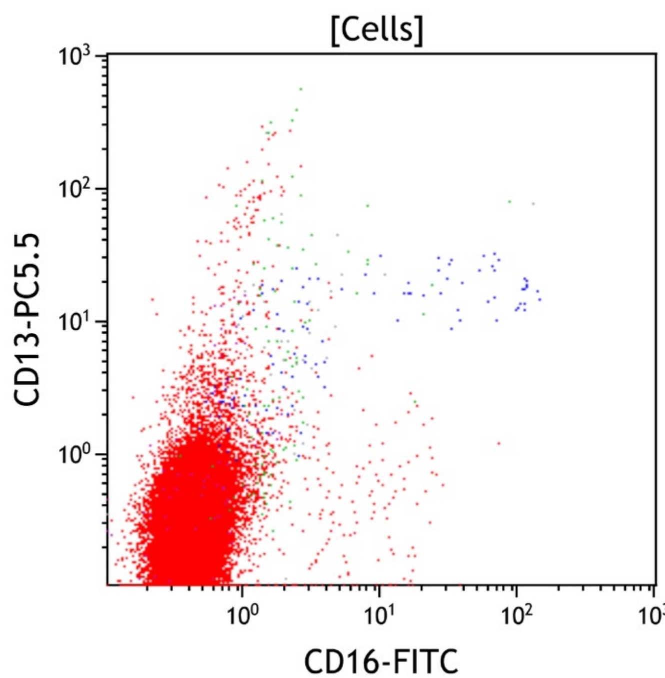

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells (red, lower right). Mature lymphocytes (red) are negative for CD13 and CD16.

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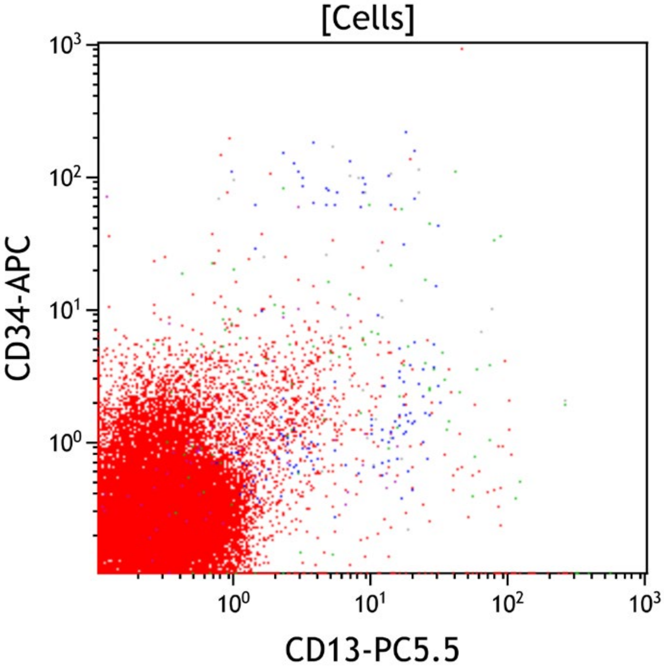

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. Mature lymphocytes (red) are negative for CD13 and CD34.

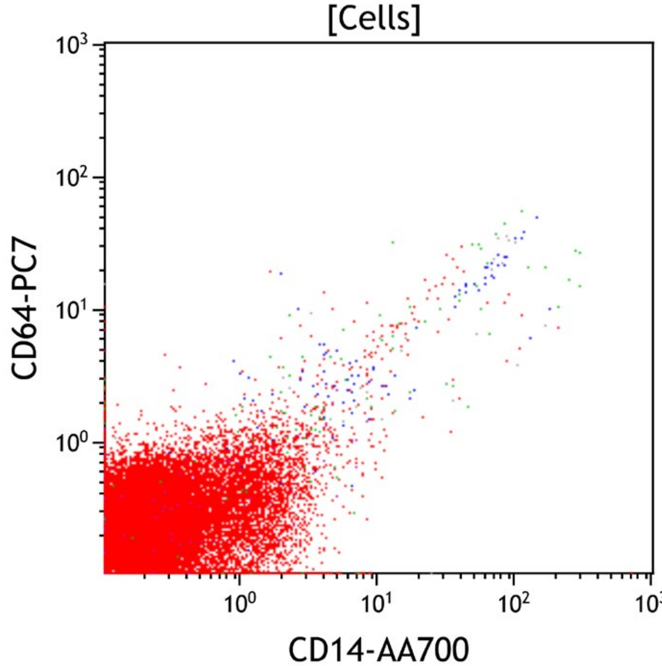

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Mature lymphocytes (red) are negative for CD14 and CD64.

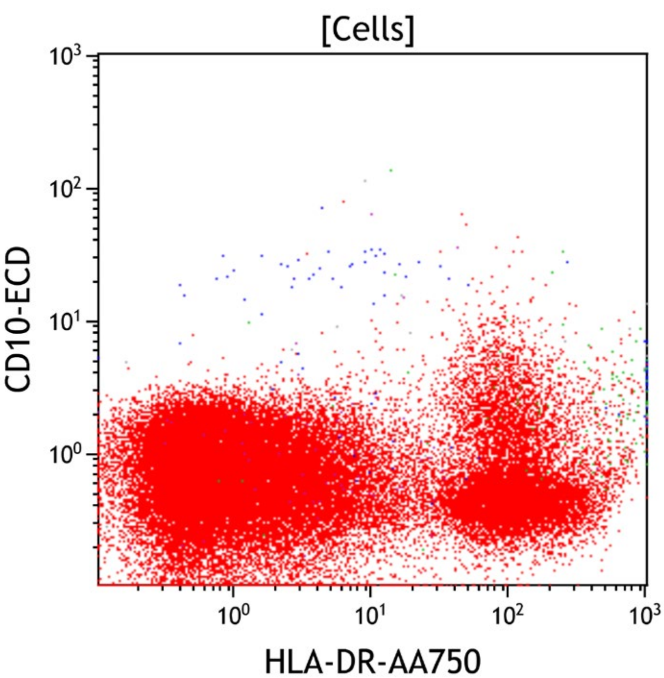

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells (red, lower right), plasmacytoid dendritic cells, and CD34-positive progenitors. CD10 is expressed on mature granulocytes, immature B cells, and germinal center B cells. The CD10 positive germinal center B cells also express HLA-DR at a level similar to other B cells.

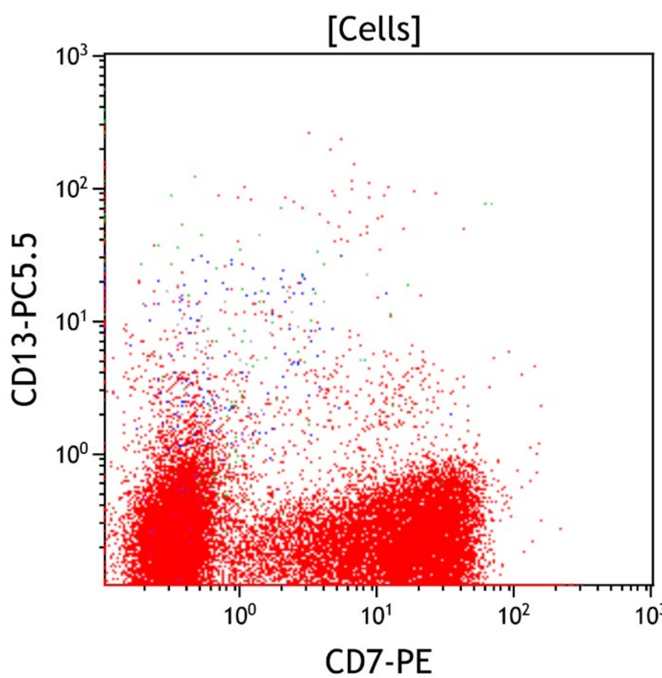

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on NK cells and T cells (red, lower right). CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

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M2 Cell Tube

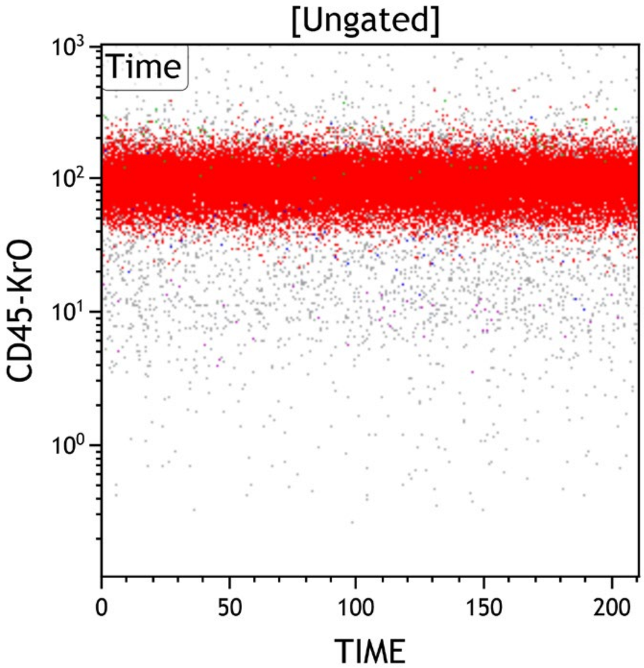

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

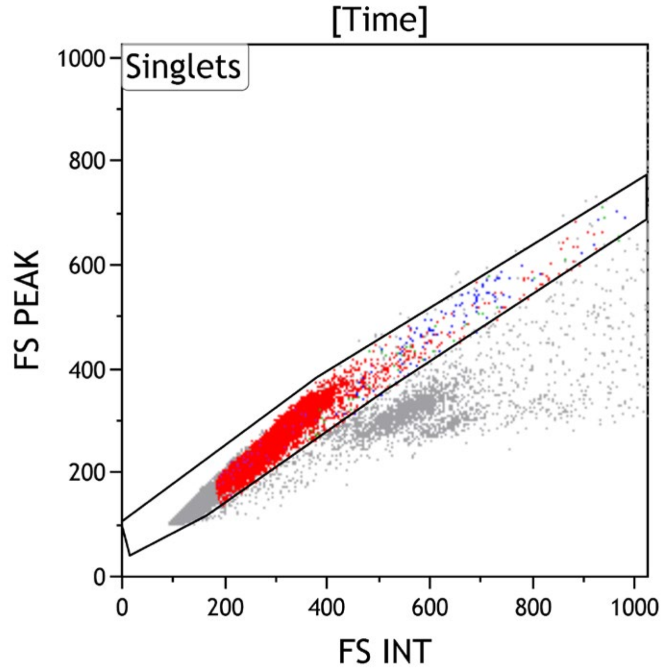

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

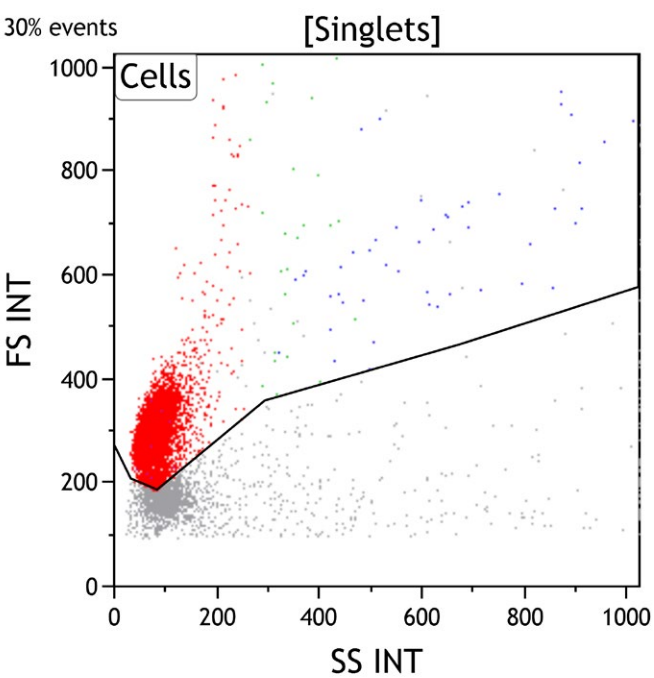

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

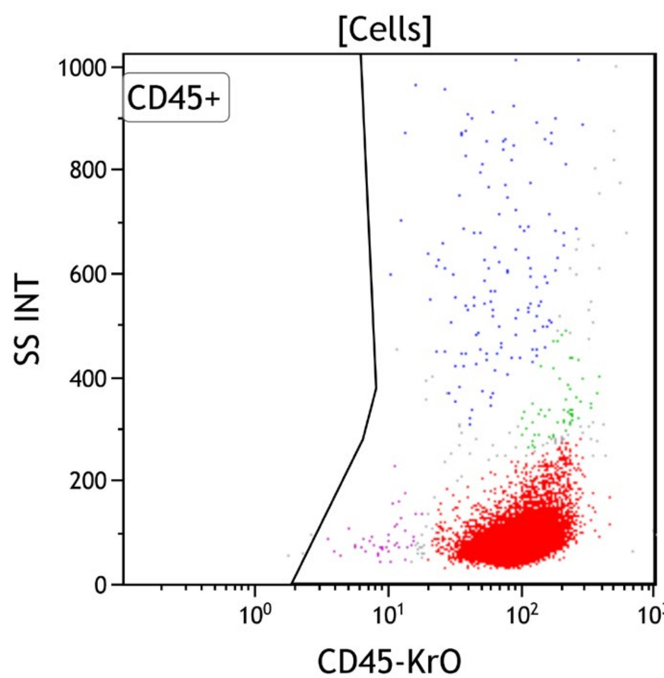

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

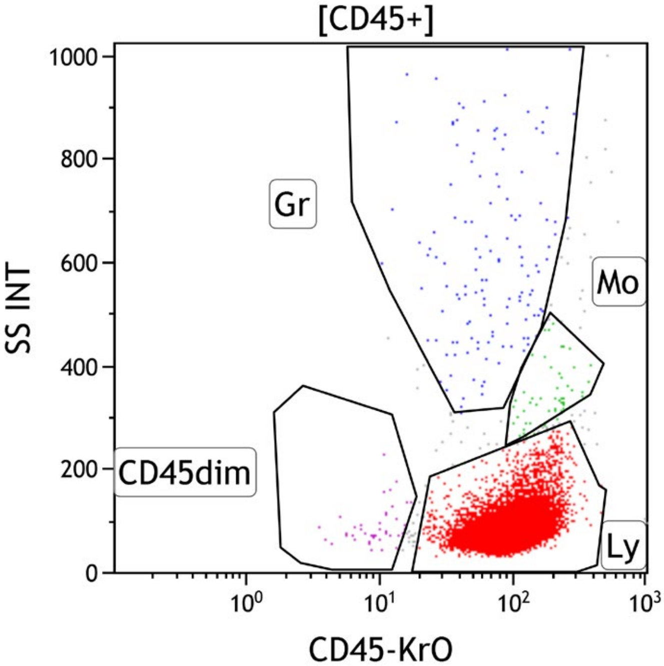

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. In this case, the white blood cells are composed predominantly of lymphocytes.

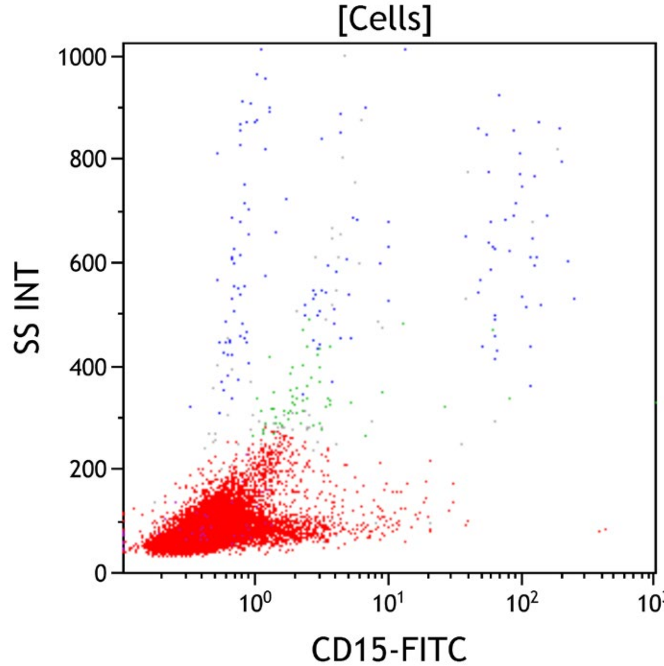

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes and at a lower level on monocytes. Mature lymphocytes (red) are negative for CD15.

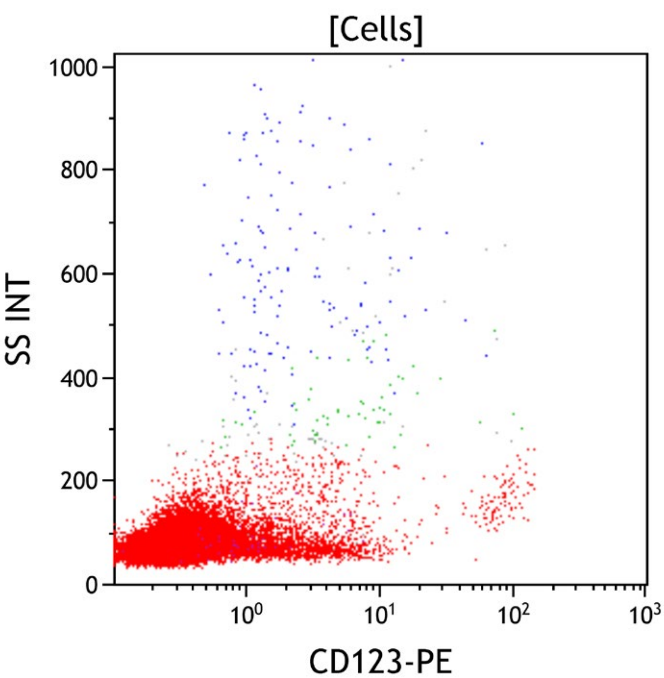

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes.

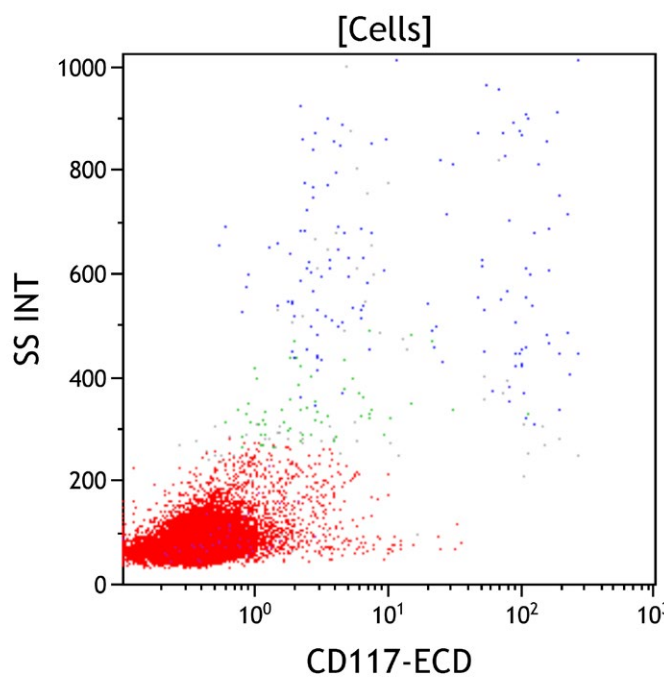

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Mature lymphocytes (red) are negative for CD117.

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M2 Cell Tube

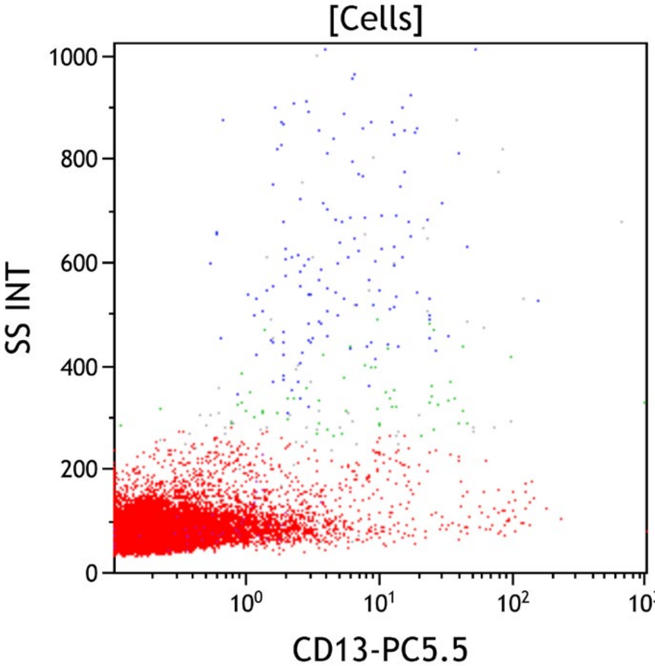


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, mature monocytes, and a lower level on immature monocytes with variable expression on myeloid progenitors. Mature lymphocytes (red) are negative for CD13.

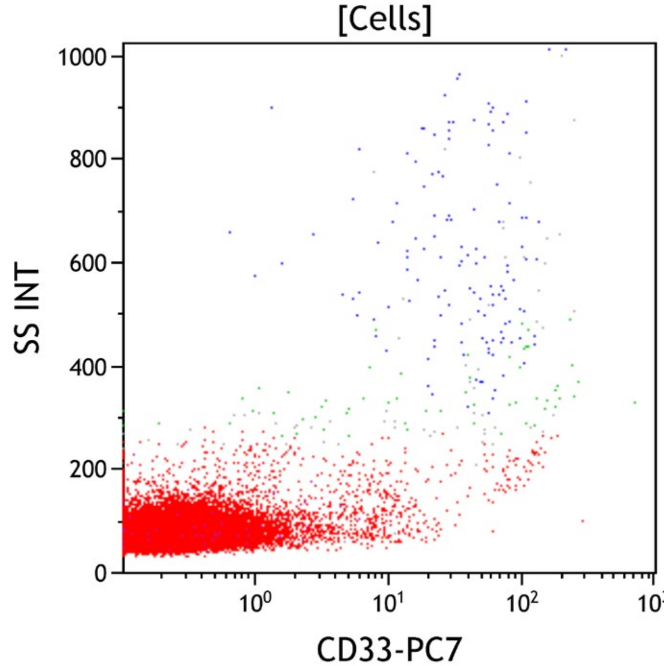


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes, and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. Mature lymphocytes (red) are negative for CD33.

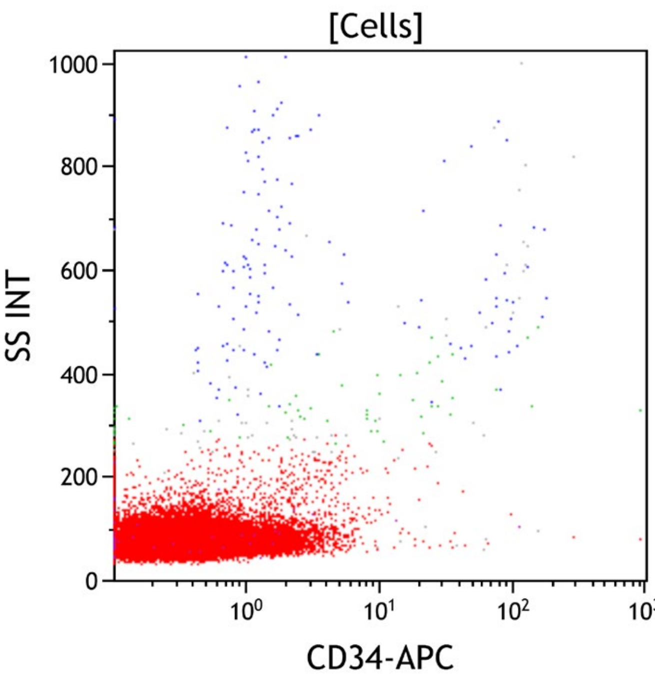


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). Mature lymphocytes (red) are negative for CD34. Tissues rarely contain CD34 positive progenitors.

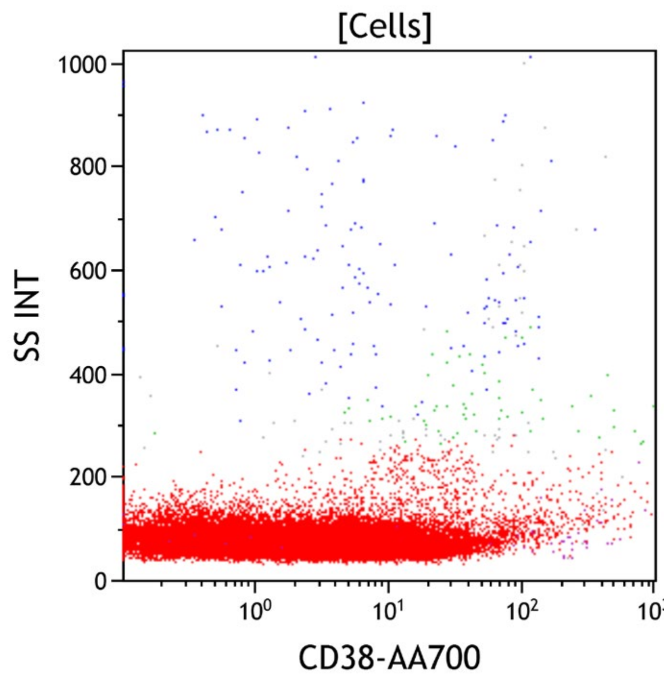


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes (red).

M2 Cell Tube

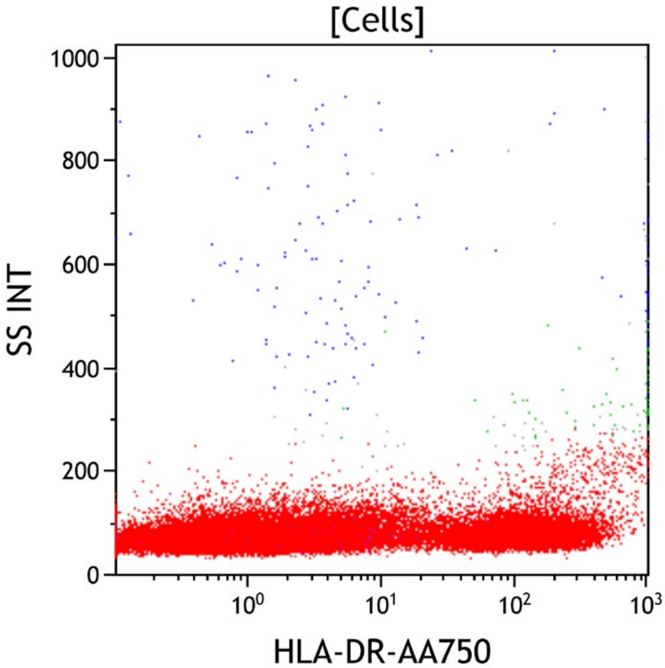


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells, and activated T cells.

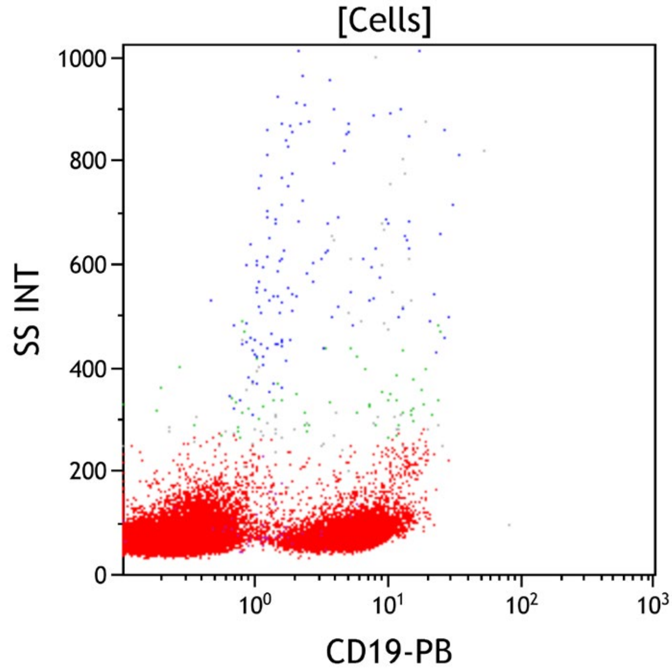


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on immature and mature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter.

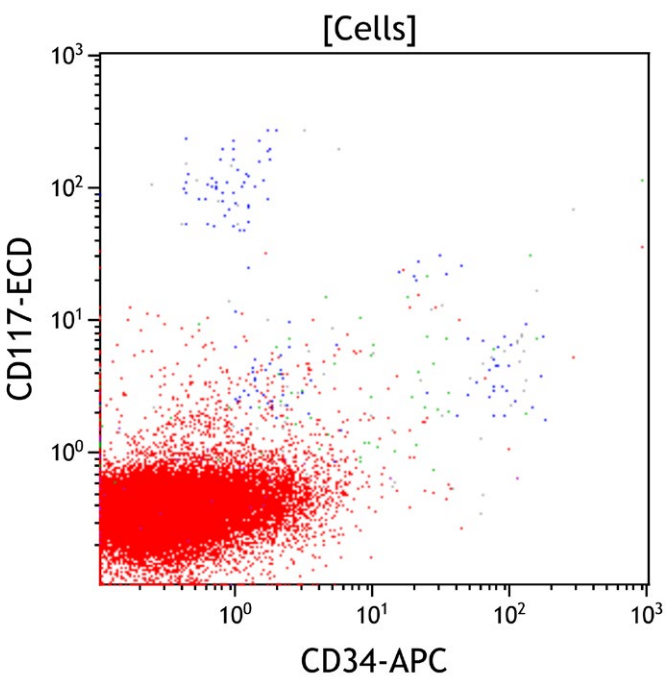


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. Mature lymphocytes (red) are negative for CD34 and CD117.

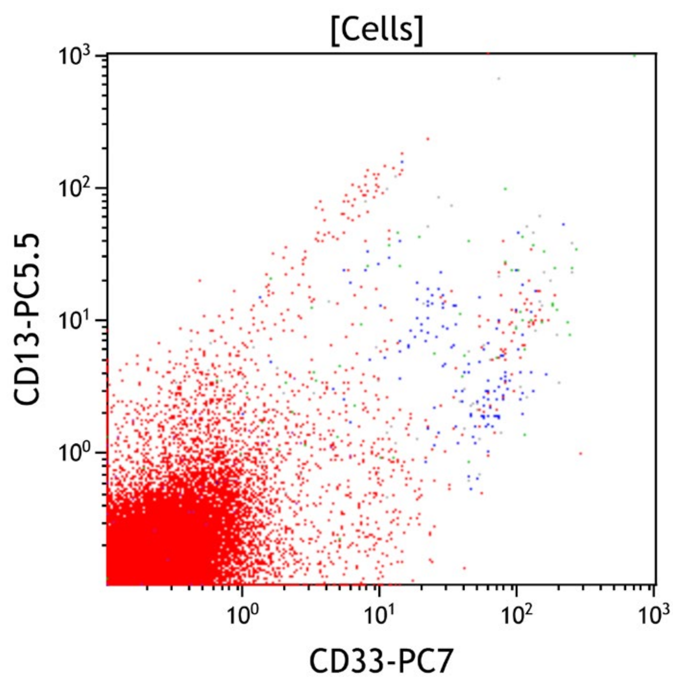


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Mature lymphocytes (red) largely do not express either CD13 or CD33.

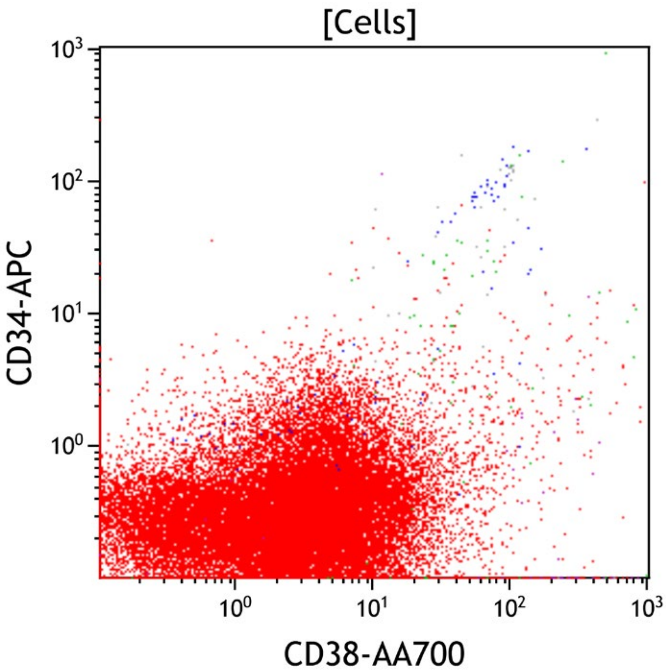


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature lymphocytes (red) show variable expression of CD38 without CD34.

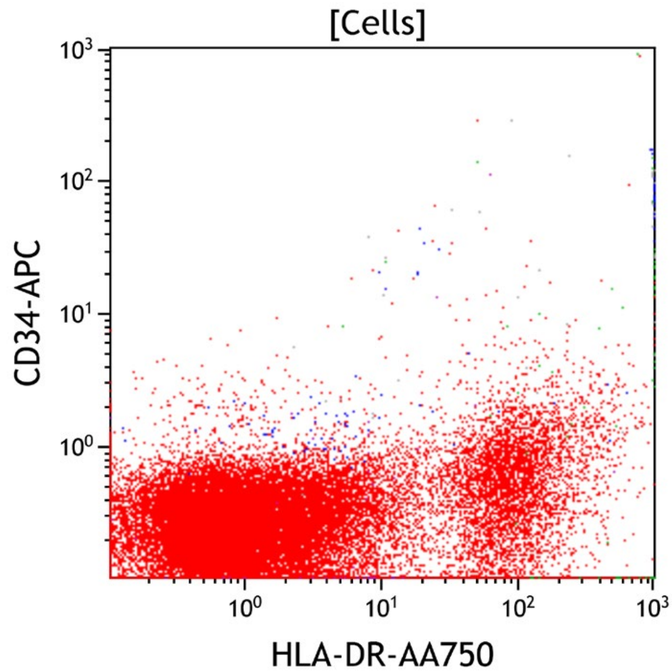


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells (red, lower right), monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors.

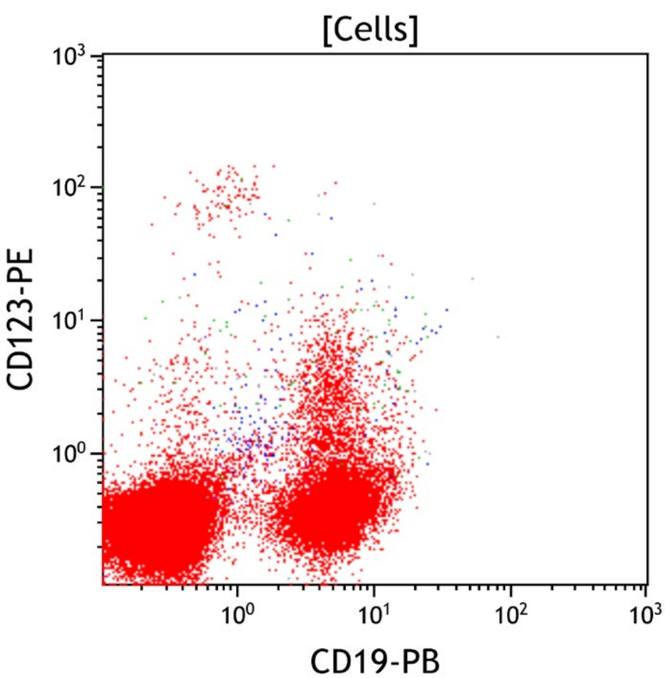


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes, and CD34 positive progenitors. CD19 positive B cells (red, lower right) normally do not express significant CD123.

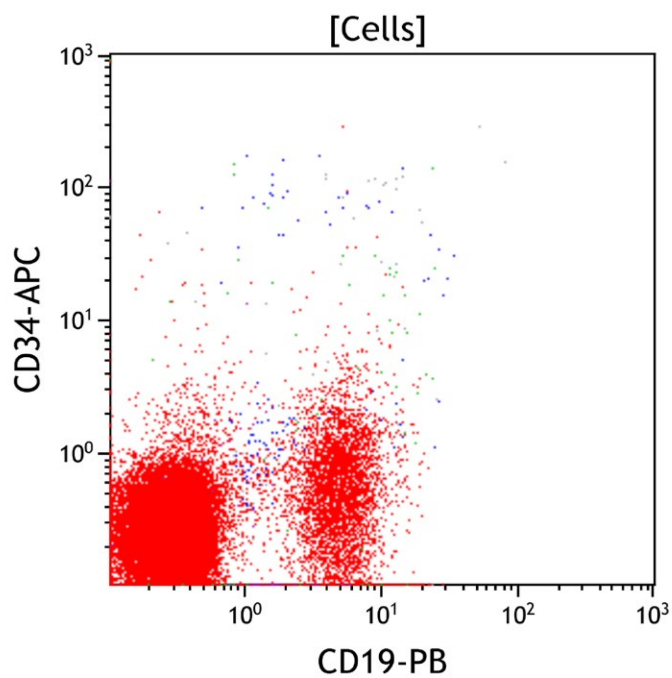


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while mature B cells (red, lower right) do not express CD34.

Table of Contents > No Immunophenotypic Abnormality > Case #3: Normal Lymph Node

M2 Cell Tube

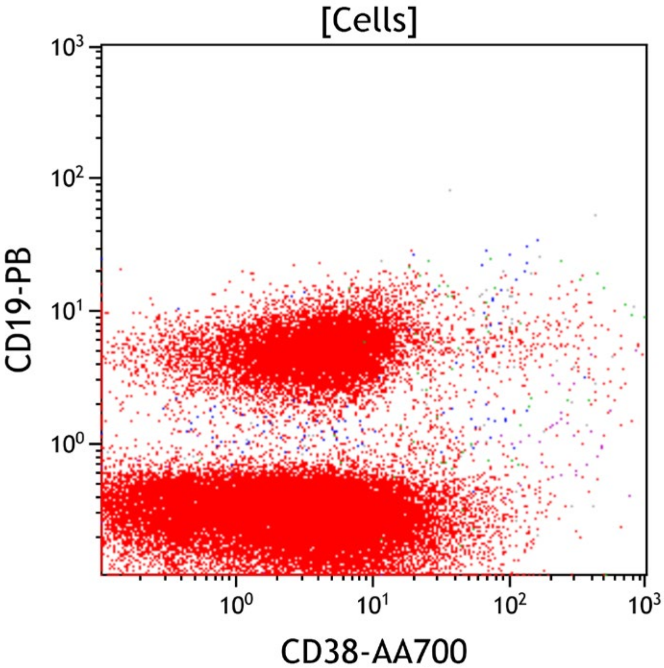


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show variable CD38 expression.

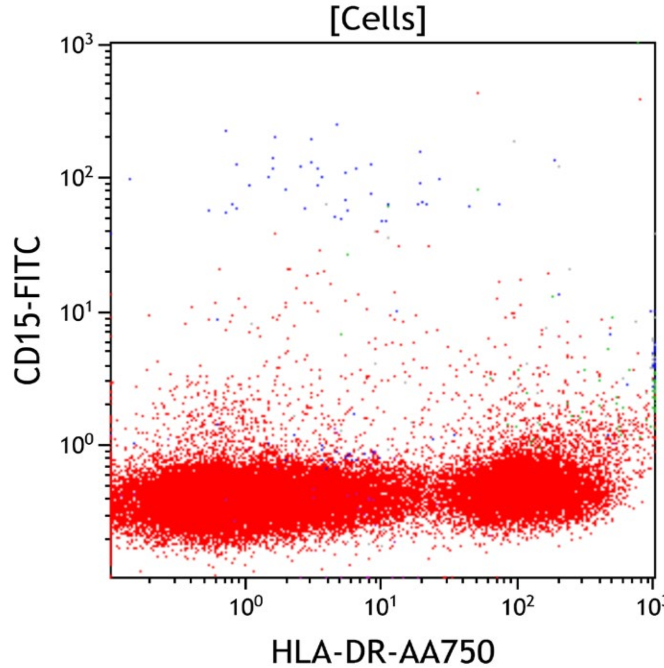


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells (red, lower right), monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors.

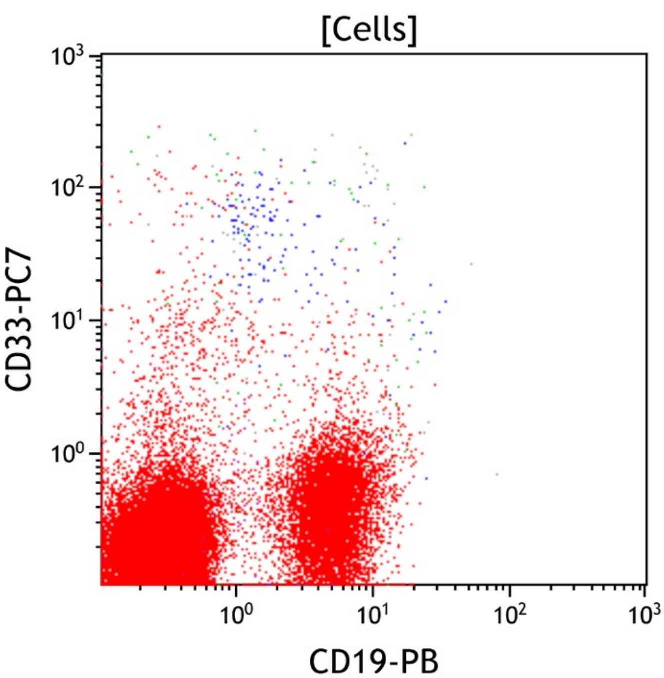


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red, right lower). CD33 is expressed by monocytes (green) and maturing granulocytes (blue), which are rare in this case. CD19 positive B cells normally do not express CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations in this case. Note that correlation with clinical, morphologic, and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay.

NEOPLASTIC PROCESS OF B-CELL ORIGIN

B cell neoplasms, which comprise the majority of all lymphoid neoplasms, are a diverse group of tumors that include acute lymphoblastic leukemias/lymphomas and mature B cell leukemias/lymphomas. To varying degrees, these neoplasms recapitulate normal stages of B cell differentiation and typically have distinctive immunophenotypes that permit classification according to their postulated cell of origin. In addition, cytogenetic profiles, genotype, and immunophenotype of the malignant cell have had considerable impact on prognostic and therapeutic stratifications of patients with B cell neoplasms.

B LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

Case #4: B Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

Clinical Vignette

This 70-year-old male presents with circulating blasts. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

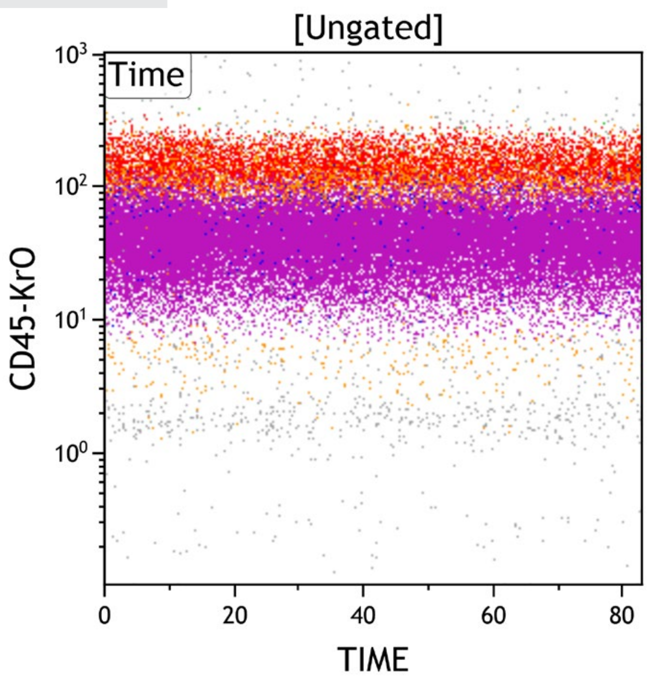


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

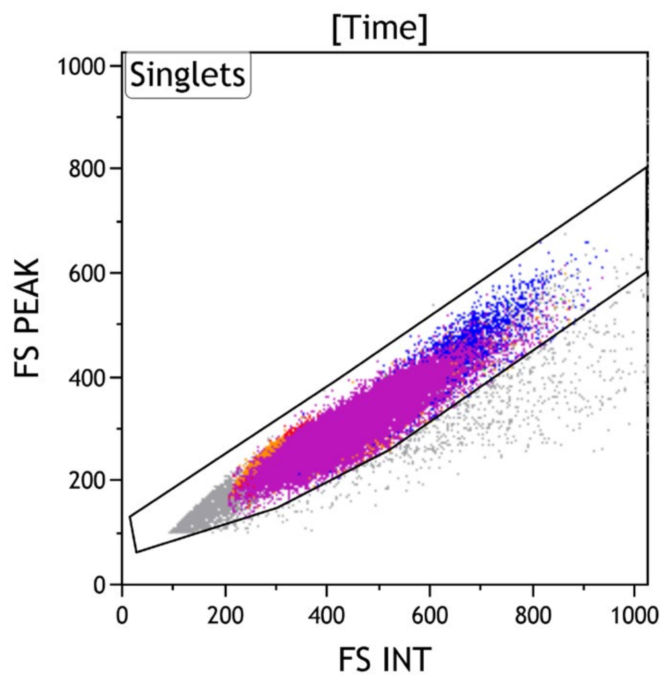


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

Table of Contents > Neoplastic Process of B-cell Origin > Case #4: B Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

B Cell Tube

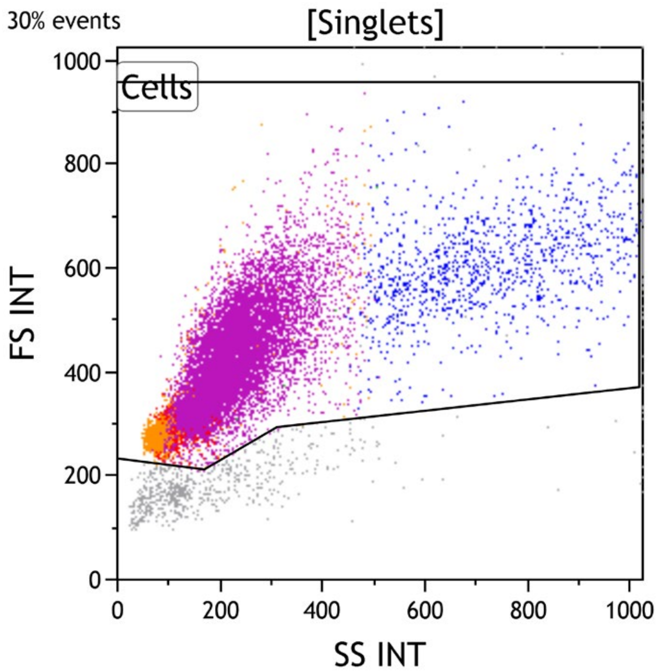


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

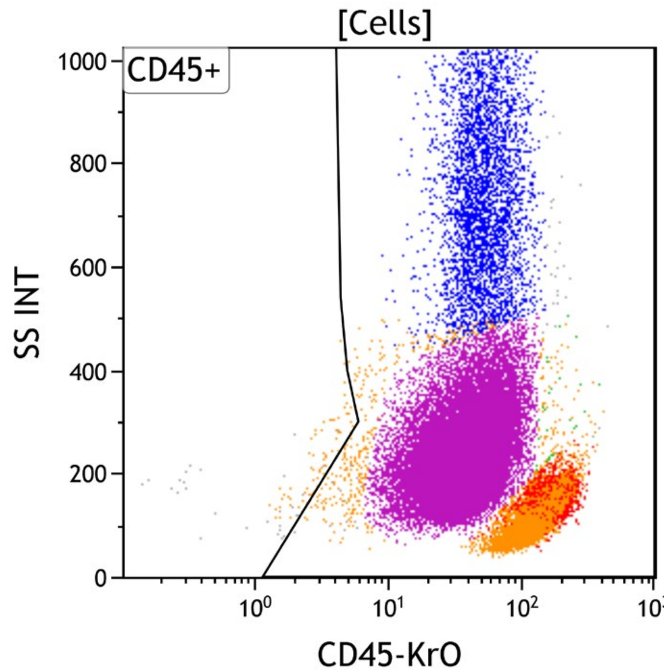


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

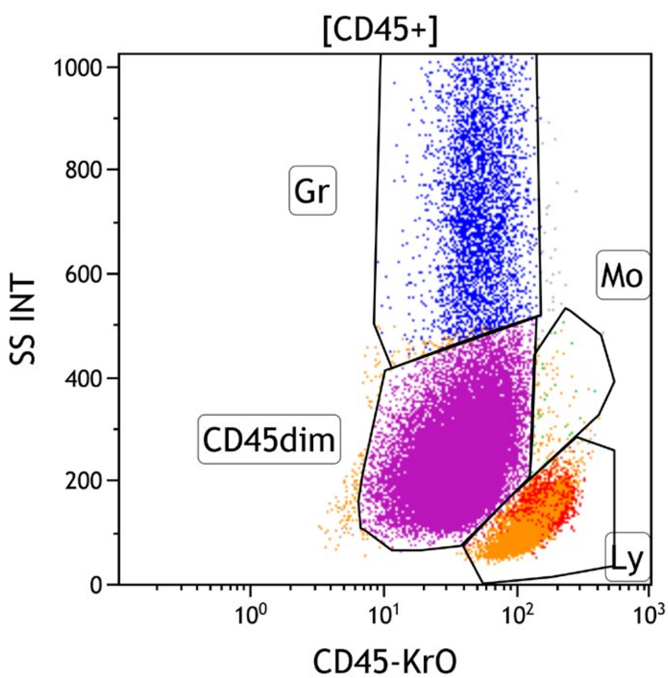


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note the increased number of progenitors (purple).

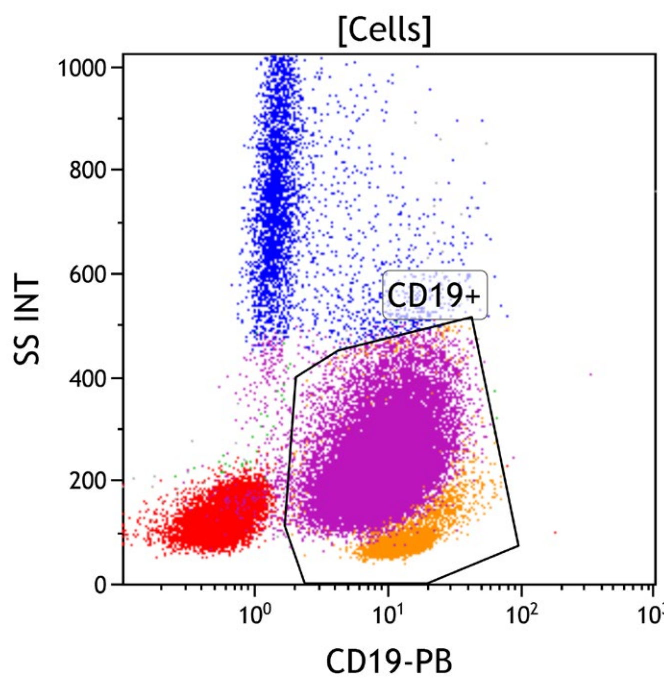


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is positive for CD19 and shows variably increased side scatter.

B Cell Tube

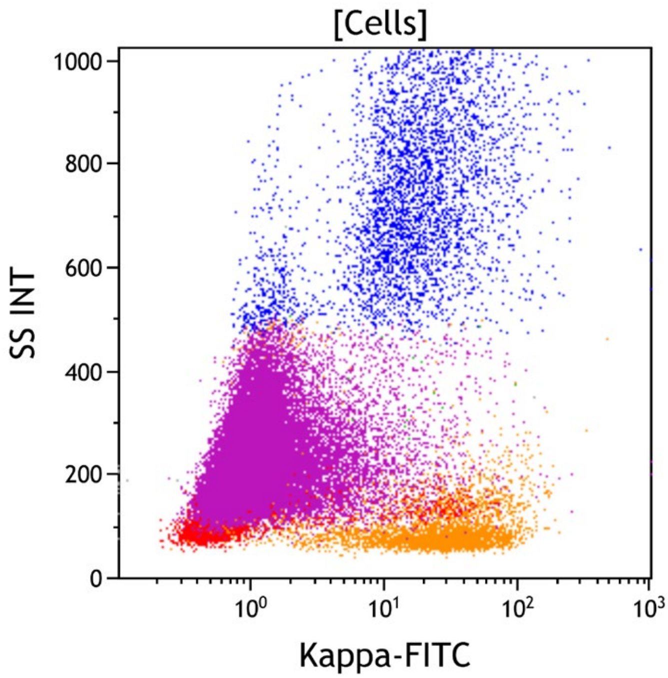


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The Kappa light chain positive cells are shown on the right side of the plot. The aberrant population (purple) is negative for surface kappa light chain.

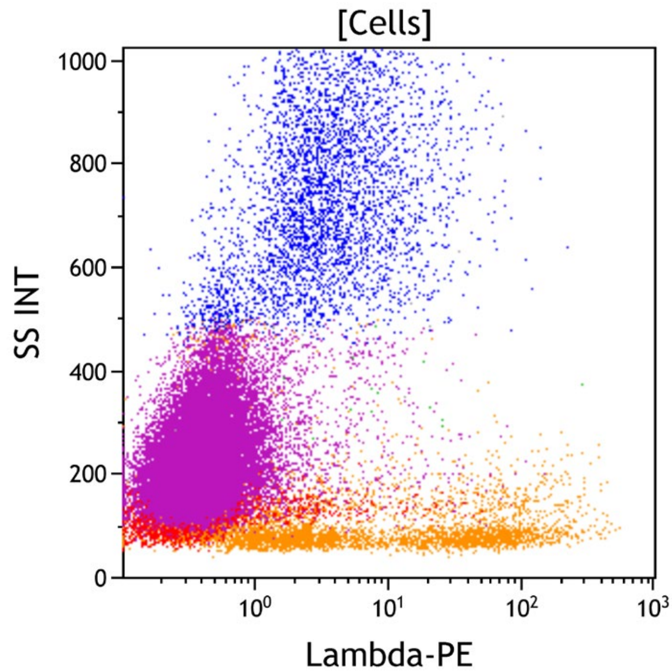


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The lambda light chain positive cells are shown on the right side of the plot. The aberrant population (purple) is negative for surface lambda light chain.

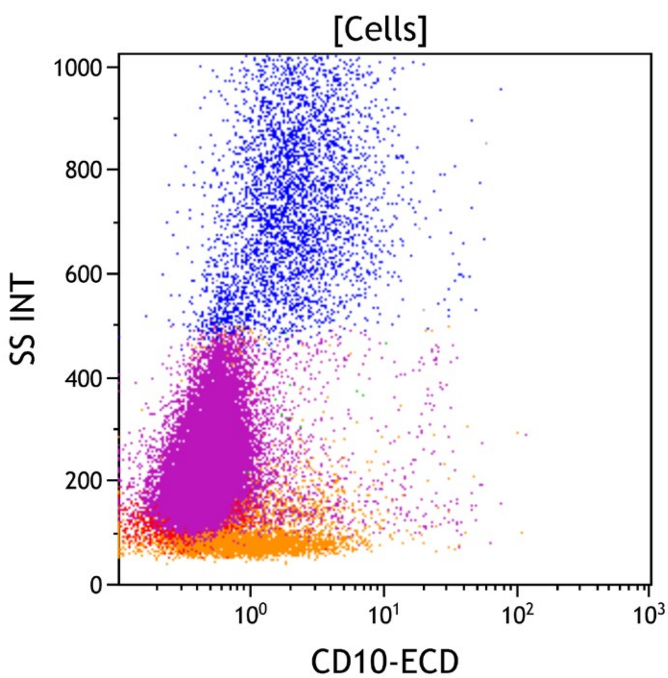


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple) is negative for CD10.

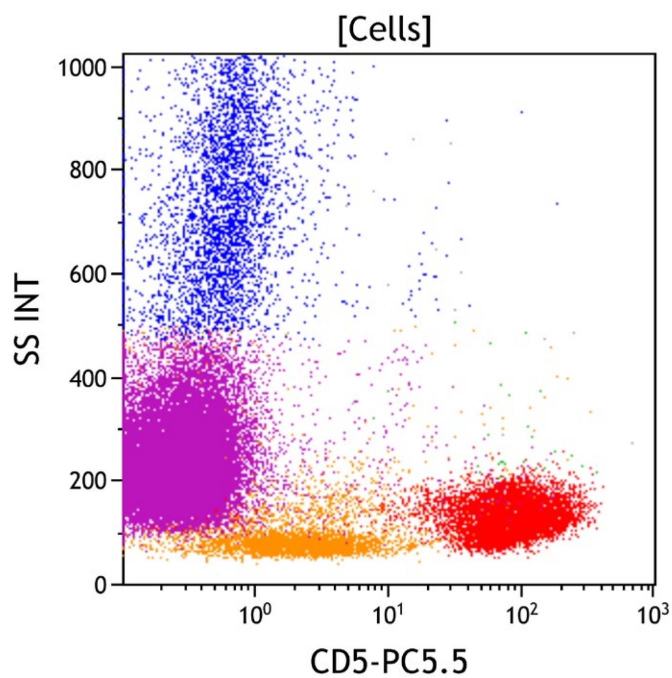


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly expressed in a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The aberrant population (purple) is negative for CD5.

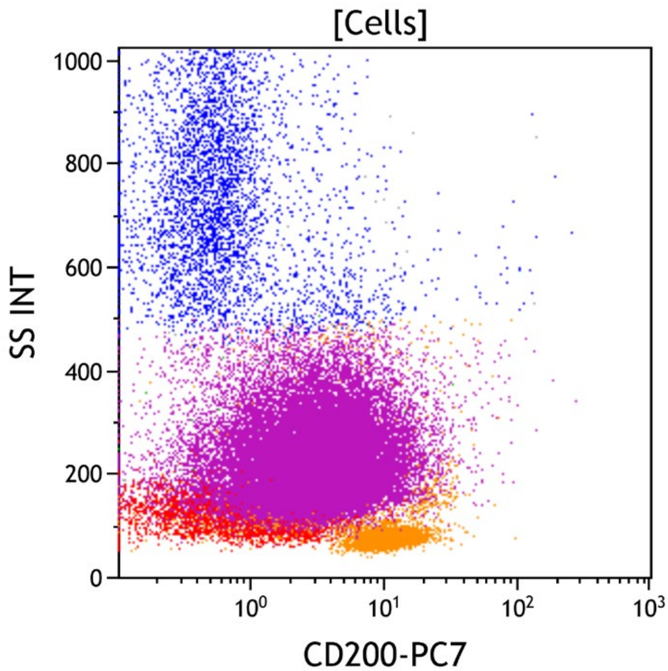


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The aberrant population (purple) has low level expression of CD200.

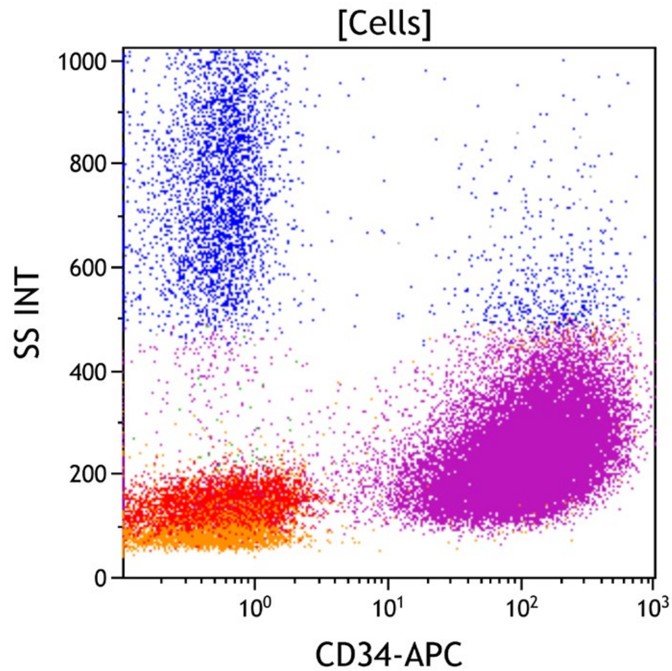


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive for CD34.

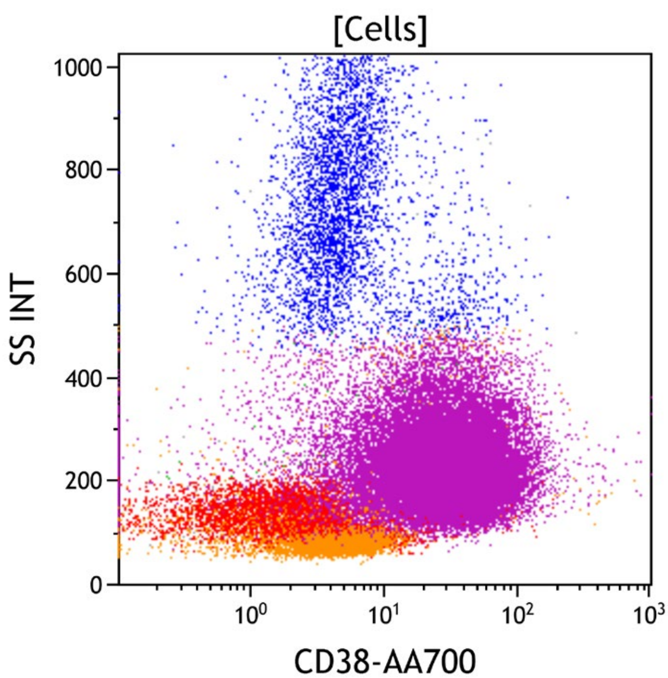


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes (red). The aberrant population (purple) is positive for CD38.

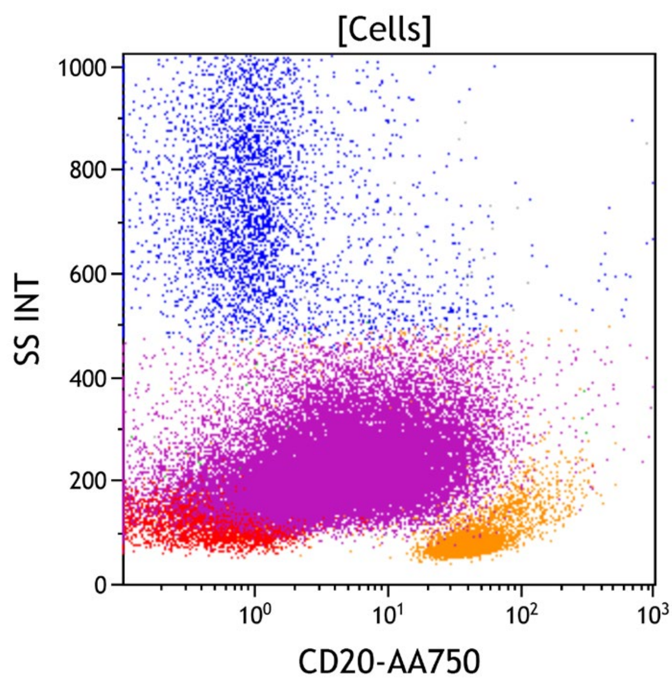


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20-positive cells are usually in the lymphocyte gate with low side scatter. The aberrant population (purple) displays variable CD20 expression.

B Cell Tube

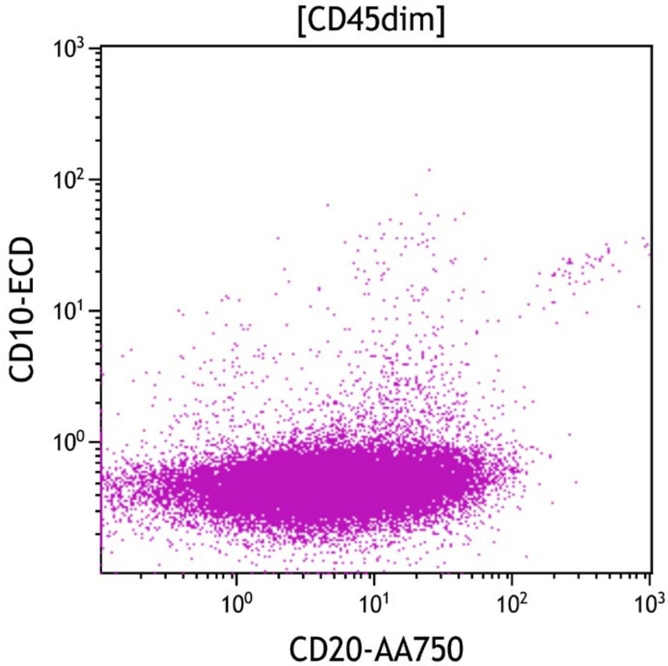


Figure 15: This CD20 vs CD10 dot plot shows all cells in the CD45dim gate. The aberrant population (purple) displays variable CD20 expression and lacks CD10 expression.

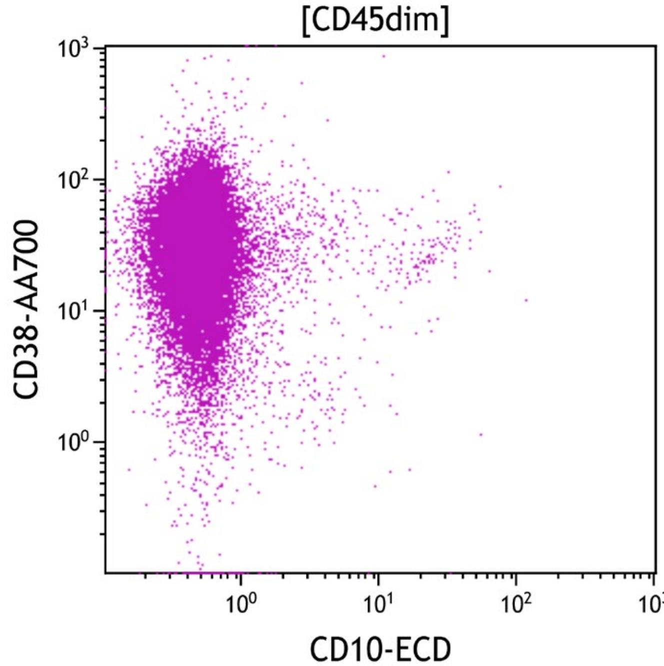


Figure 16: This CD10 vs CD38 dot plot shows all cells in the CD45dim gate. The aberrant population (purple) is positive for CD38 and negative for CD10.

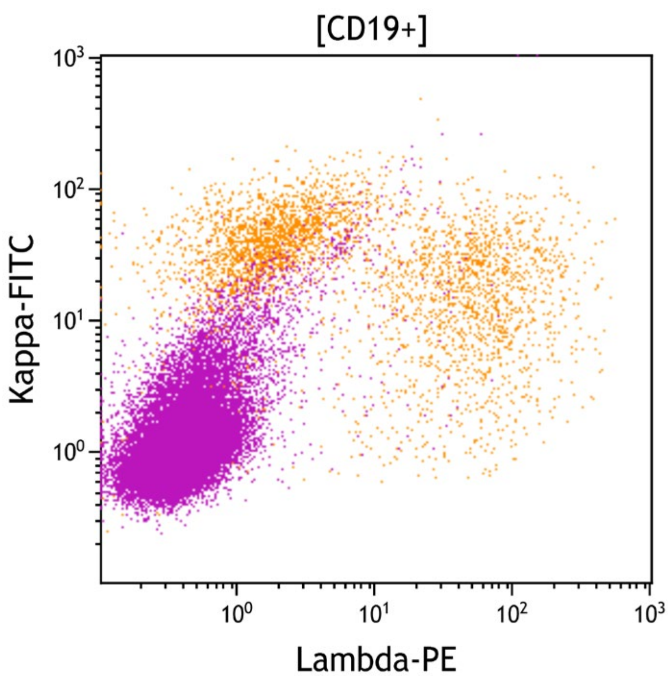


Figure 17. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. The aberrant population (purple) lacks kappa or lambda light chain expression.

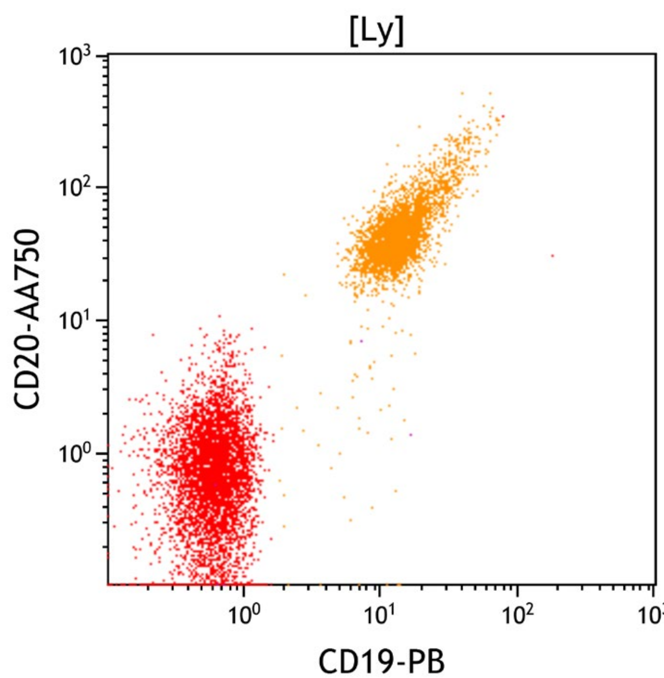


Figure 18. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression.

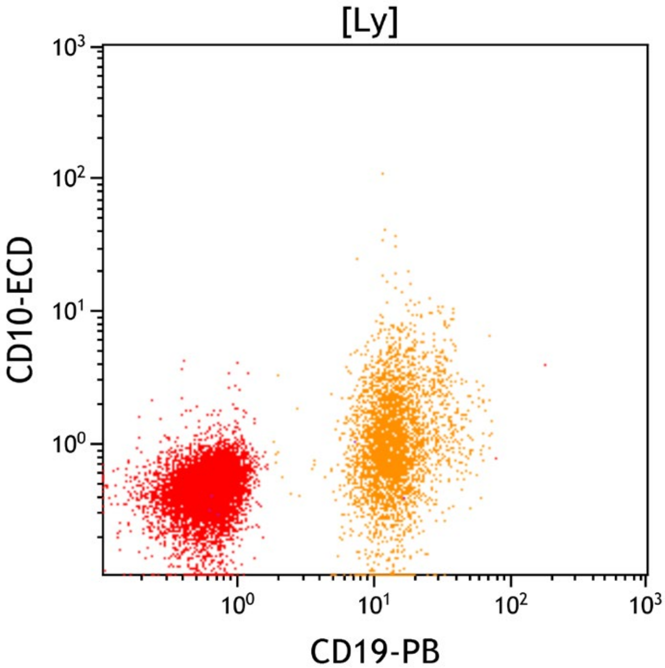


Figure 19. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells present in peripheral blood or bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45dim gate.

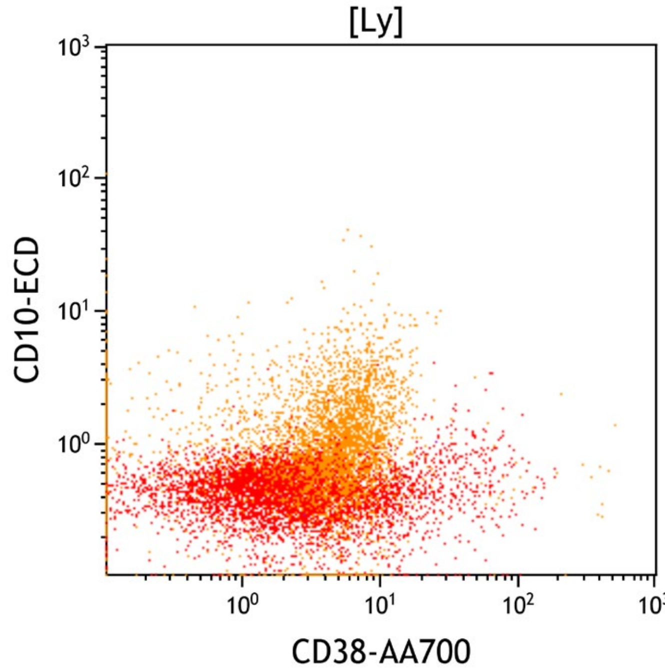


Figure 20. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45dim gate.

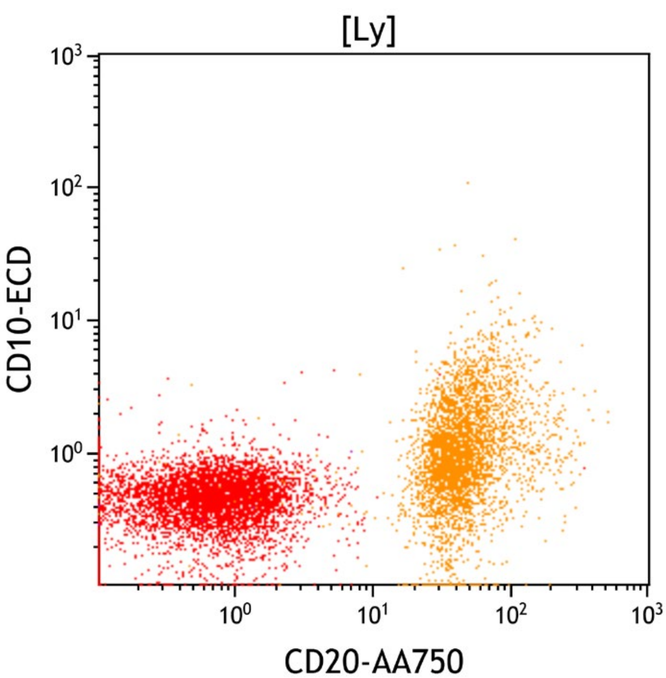


Figure 21. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20.

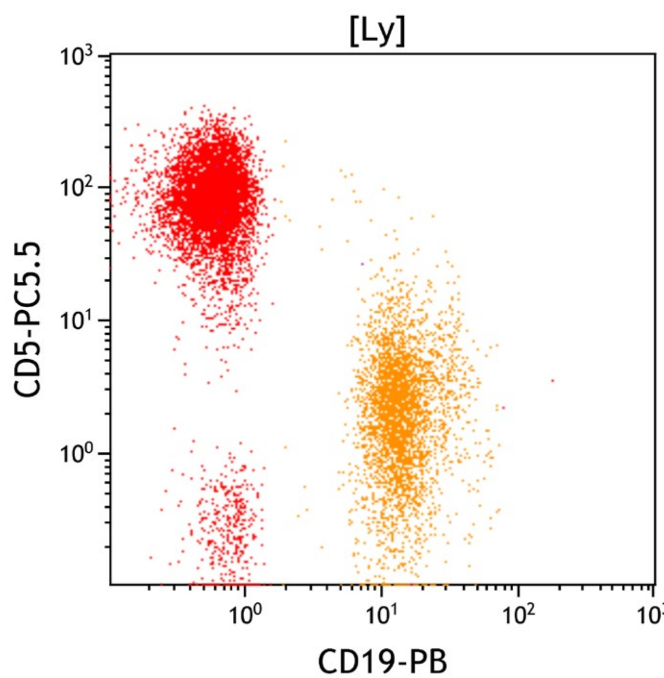


Figure 22. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

B Cell Tube

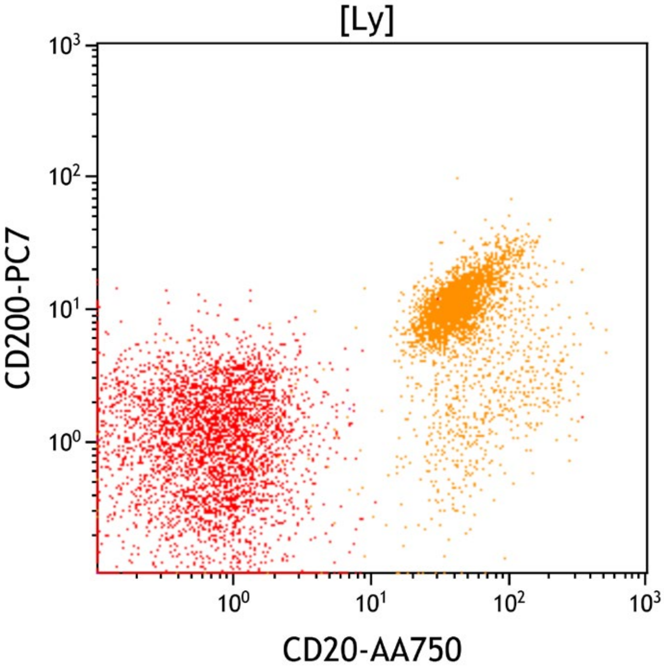


Figure 23. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

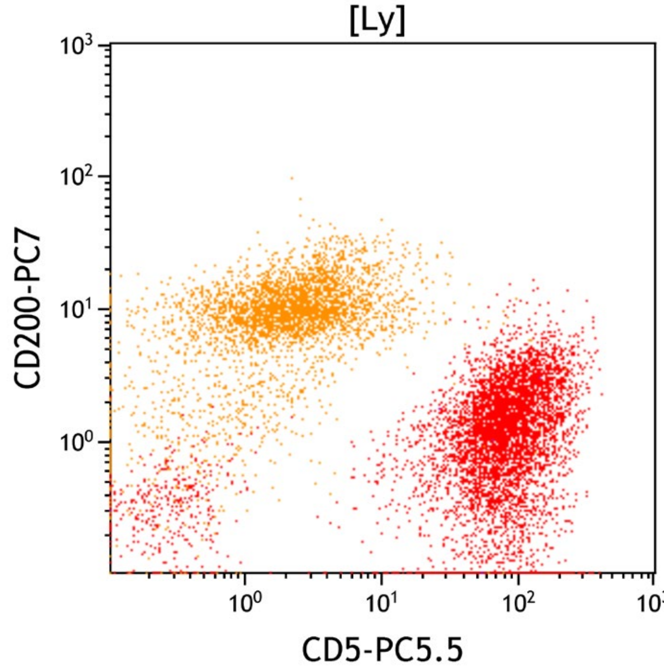


Figure 24. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

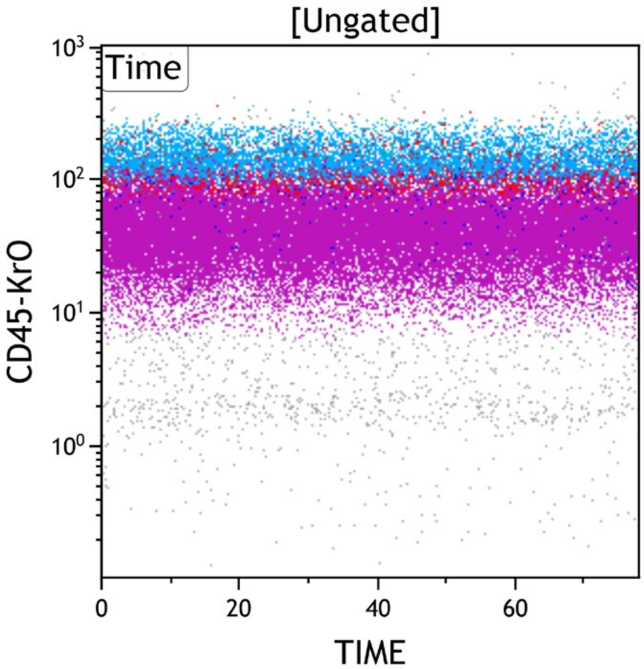


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

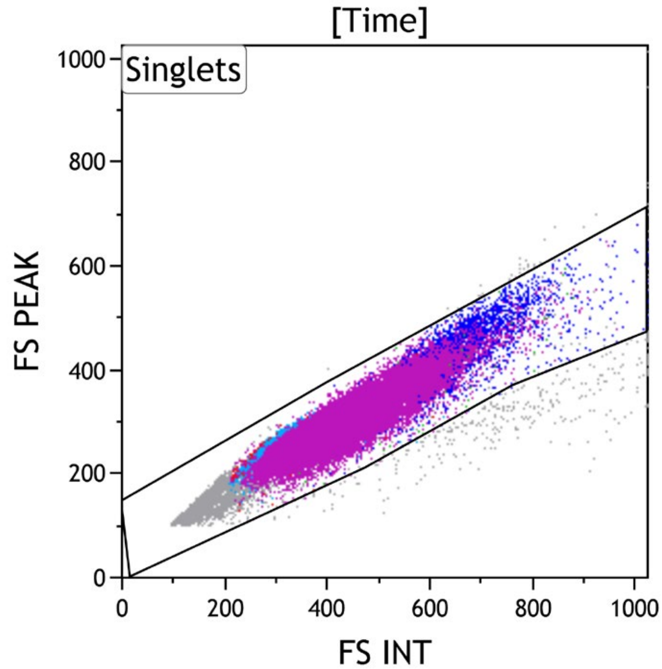


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

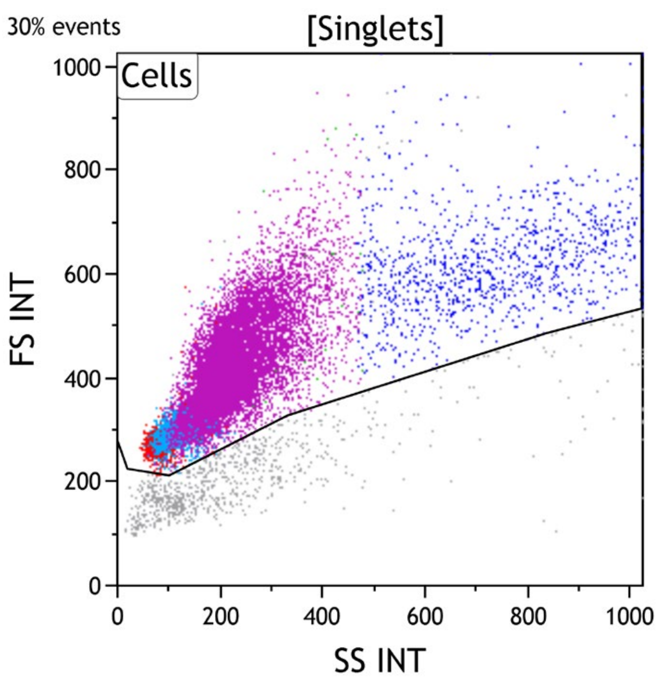


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

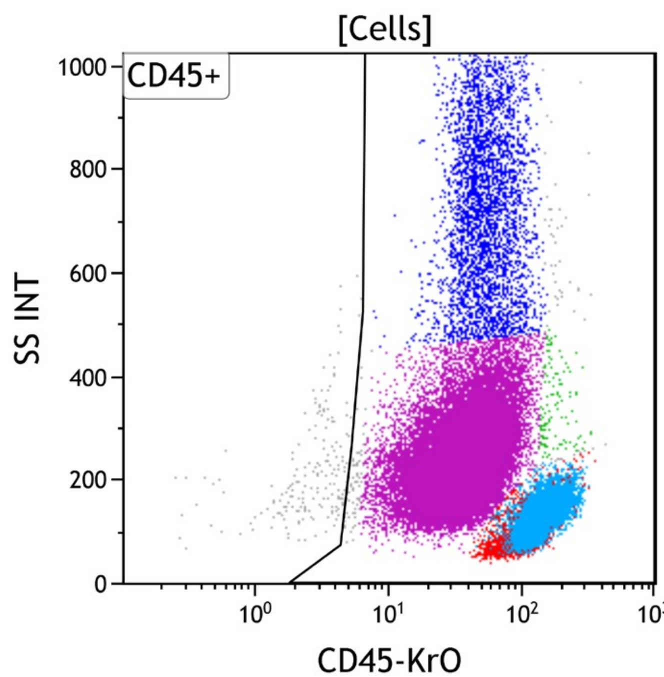


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

T Cell Tube

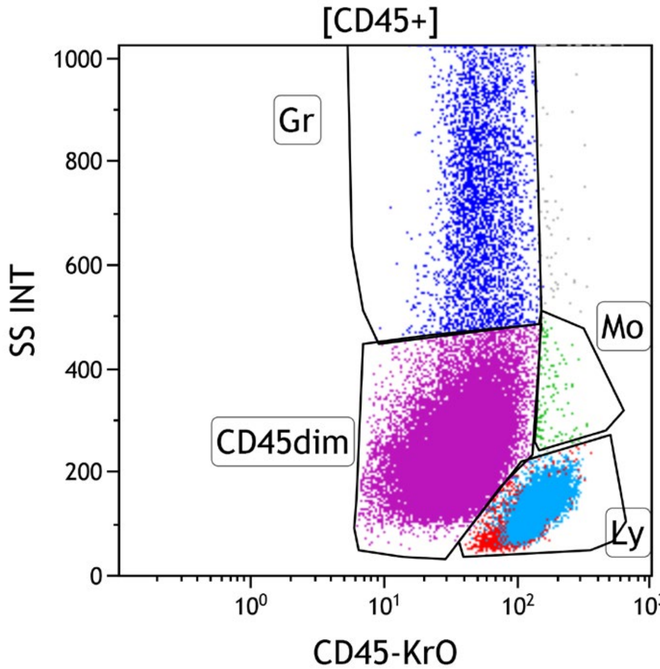


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the increased number of progenitors (purple).

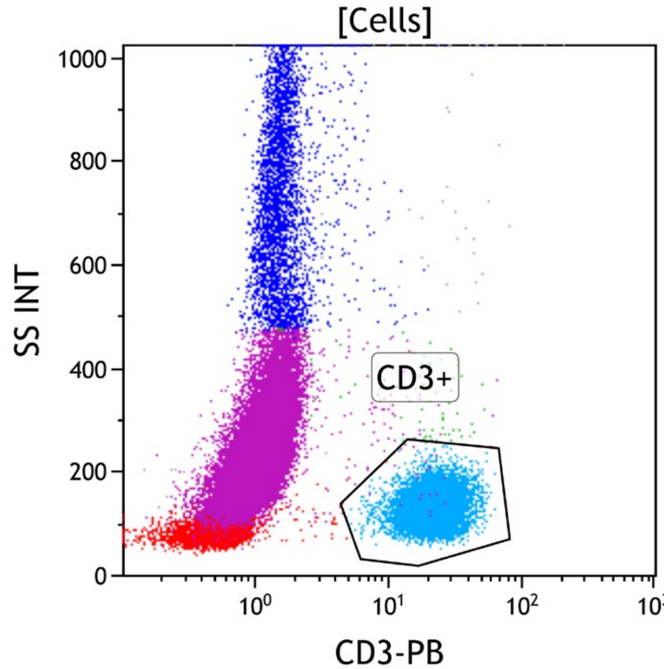


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies surface CD3 positive cells (in aqua). Surface CD3 typically expressed on mature T cell. The aberrant population (purple) is negative for CD3.

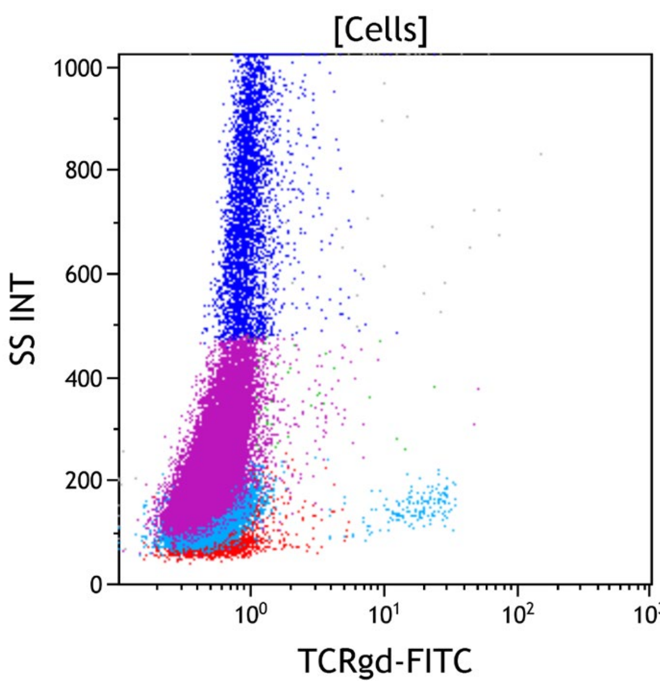


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The aberrant population (purple) is negative for TCRγδ.

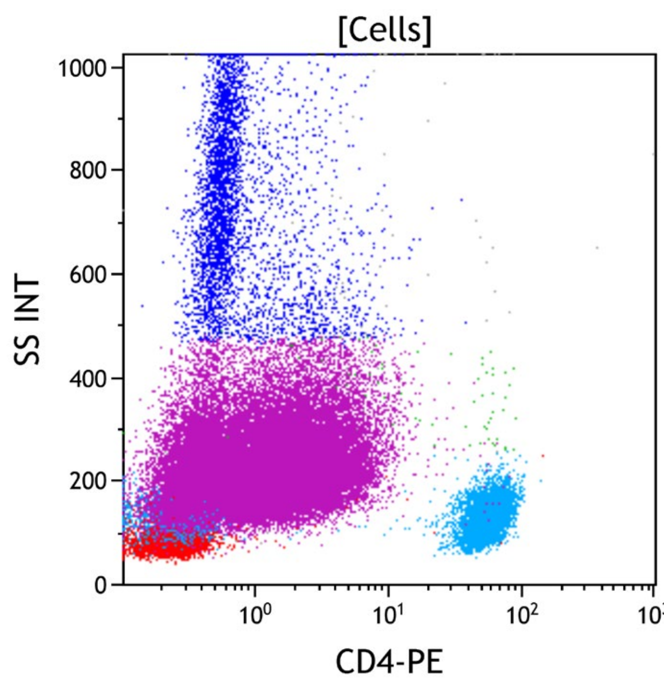


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). The aberrant population (purple) is dimly positive for CD4.

T Cell Tube

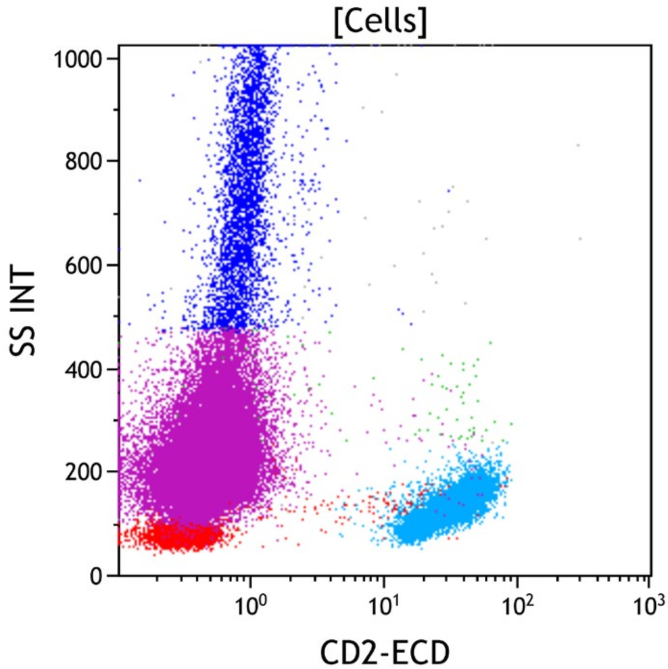


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells. The aberrant population (purple) is negative for CD2.

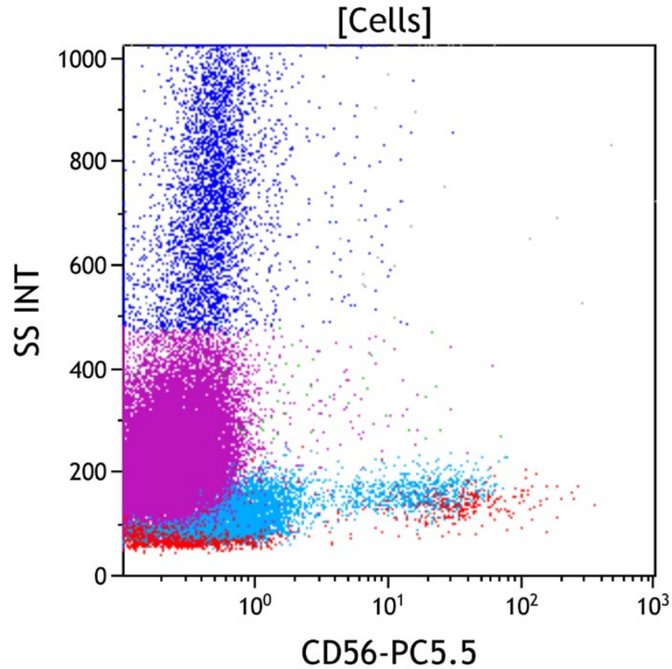


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). The aberrant population (purple) is negative for CD56.

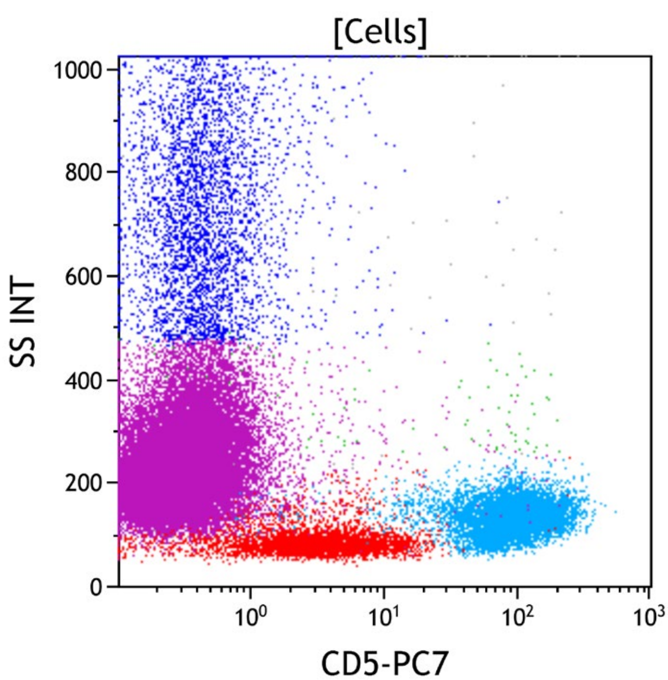


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. The aberrant population (purple) is negative for CD5.

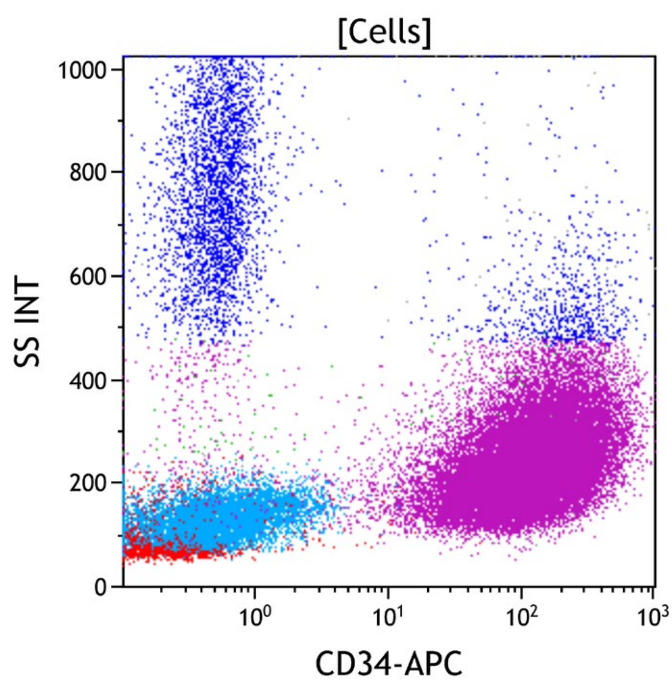


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The aberrant population (purple) is strongly positive for CD34.

T Cell Tube

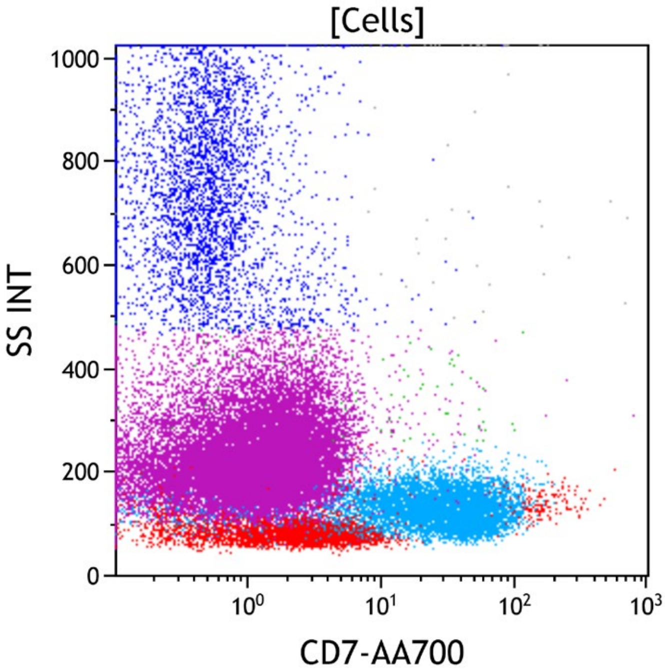


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage-committed CD34 positive progenitors. The aberrant population (purple) is negative for CD7.

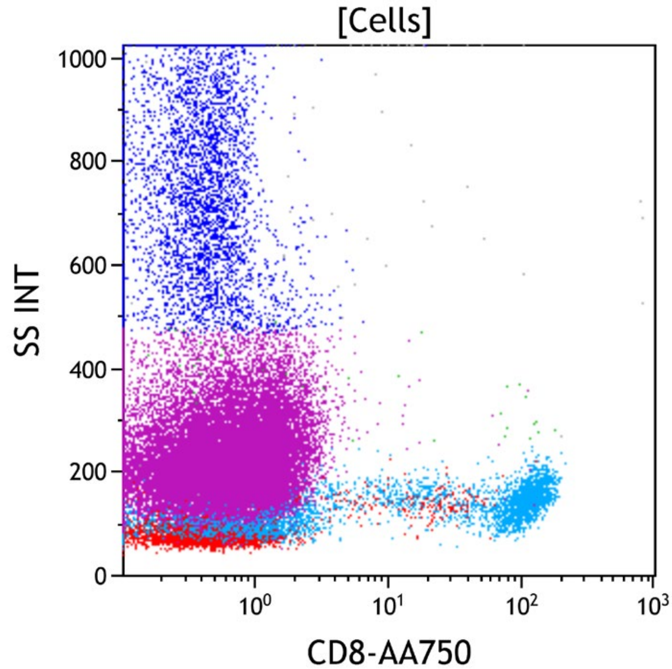


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The aberrant population (purple) is negative for CD8.

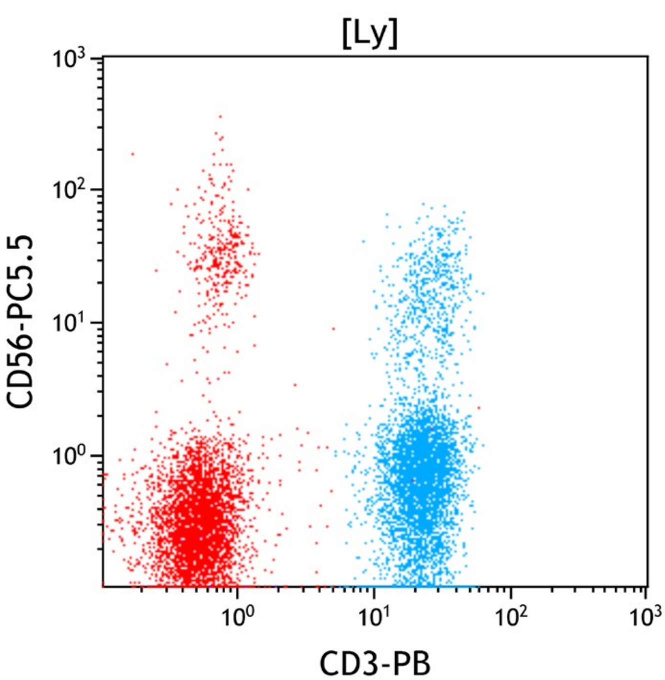


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

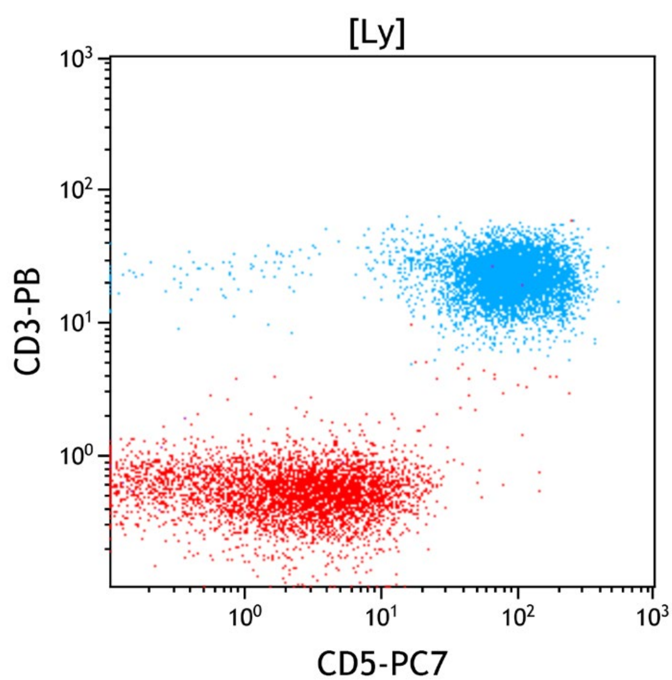


Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

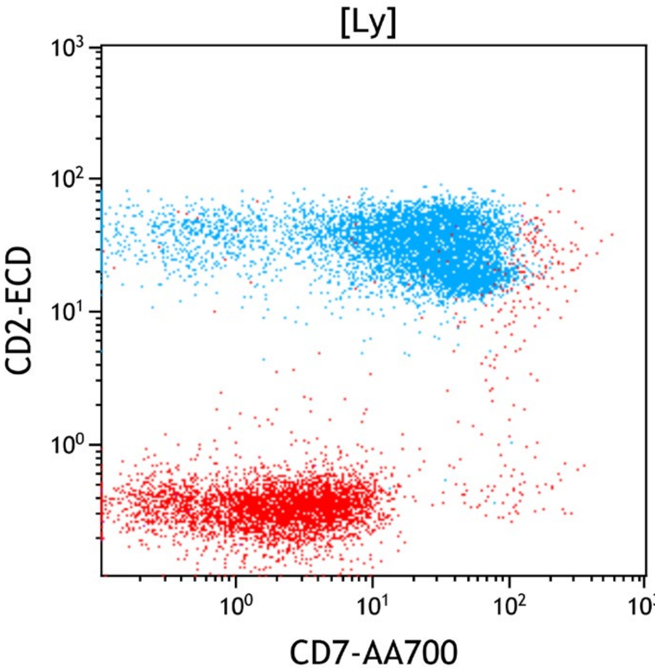


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

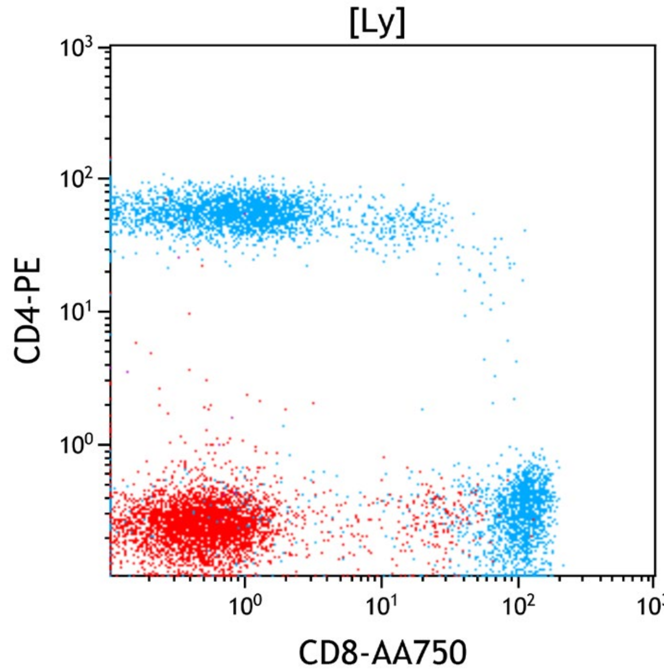


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells.

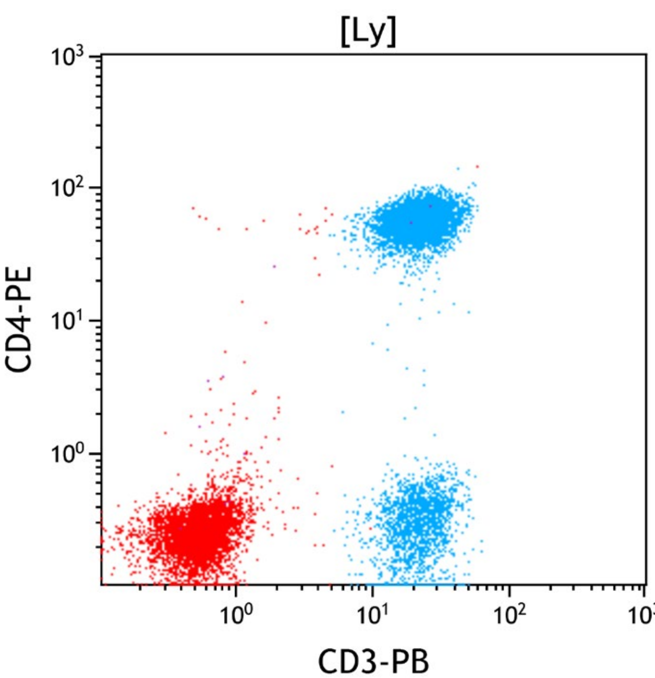


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

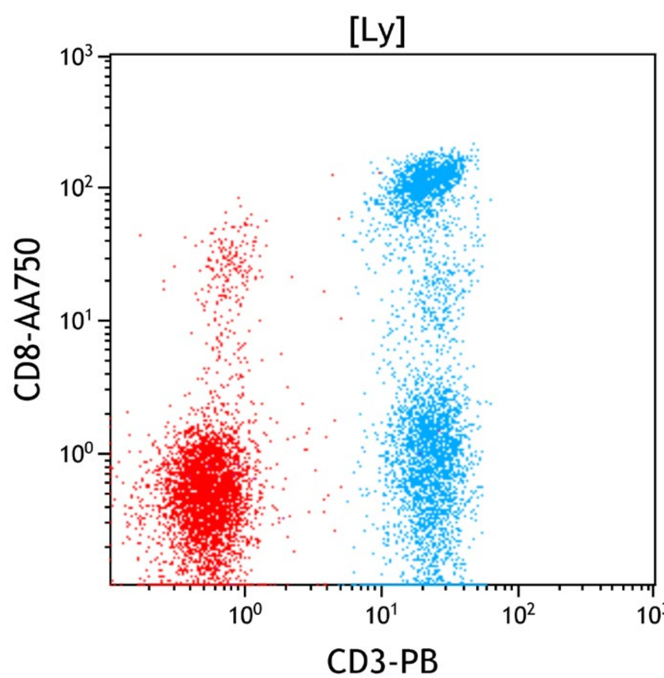


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) and lacks CD3 expression.

T Cell Tube

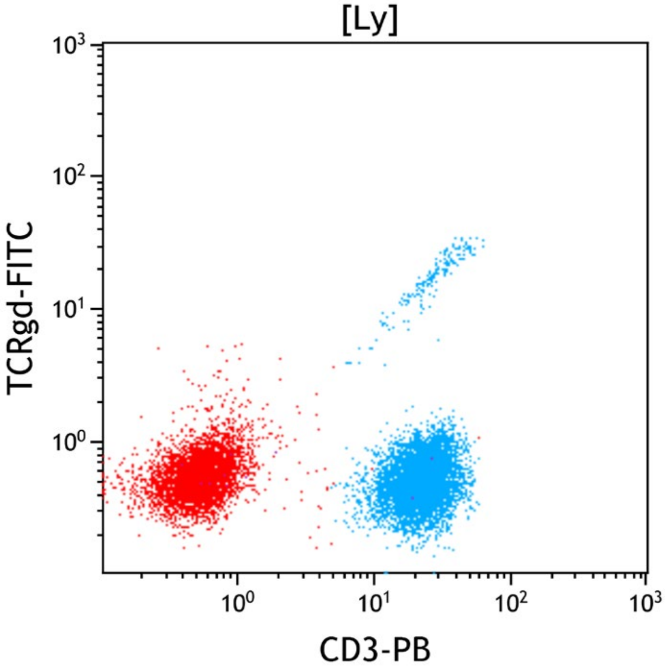


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCRγδ, which is co-expressed with CD3. The highly linear relationship between CD3 and TCRγδ is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

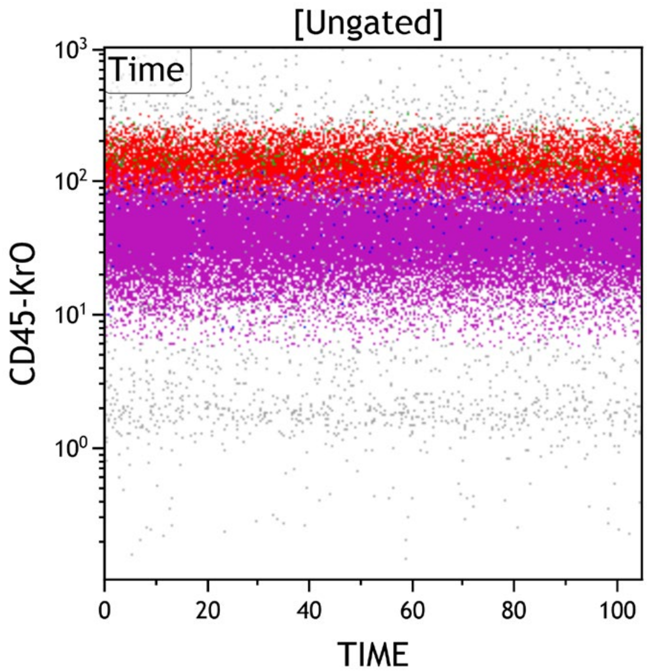


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

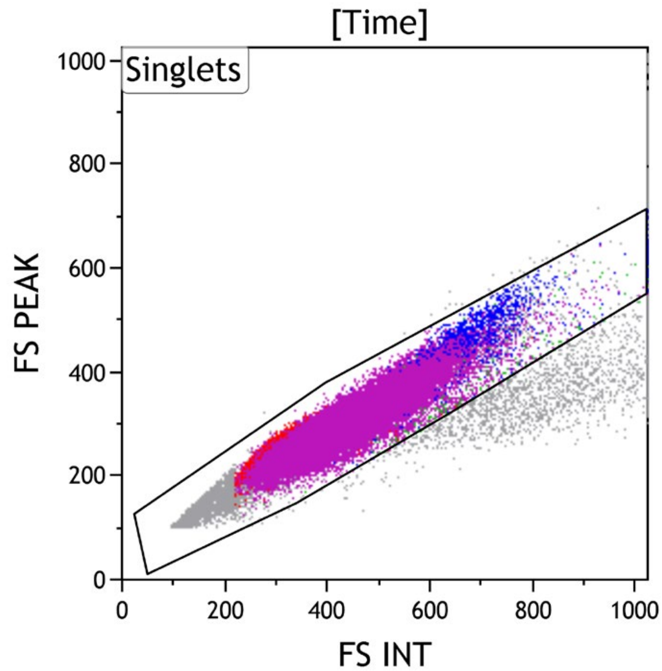


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

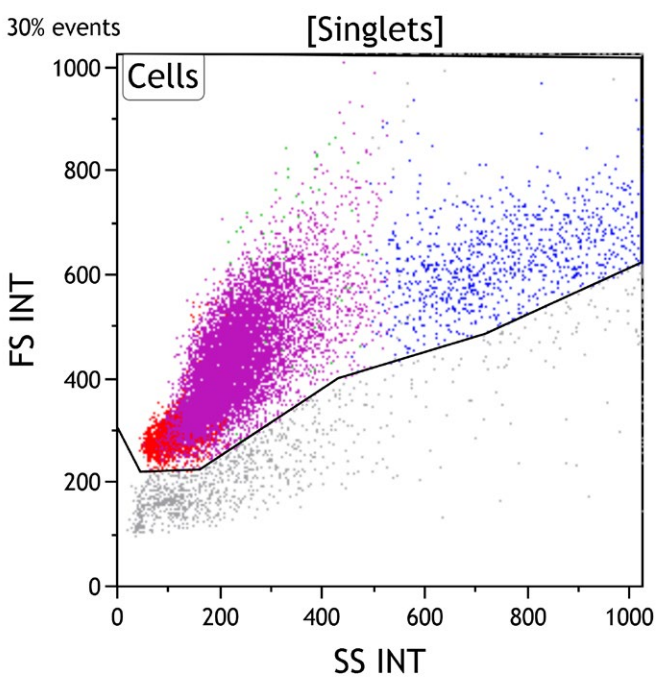


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

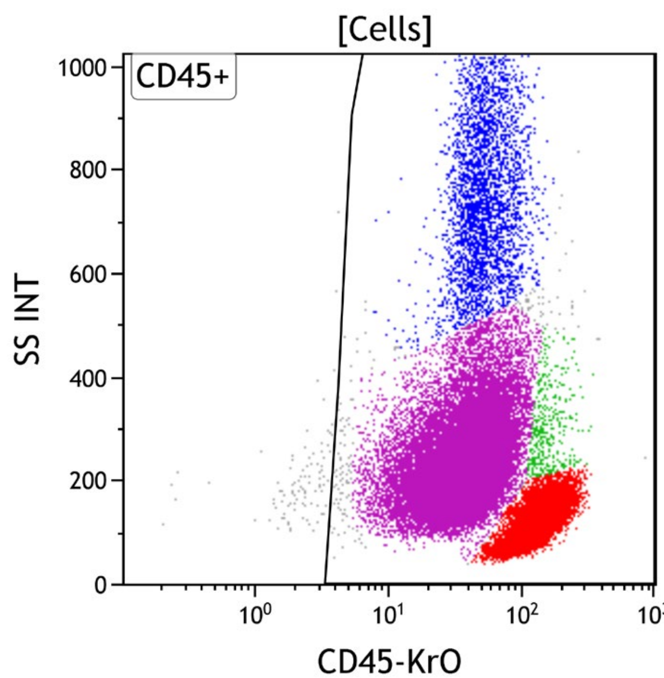
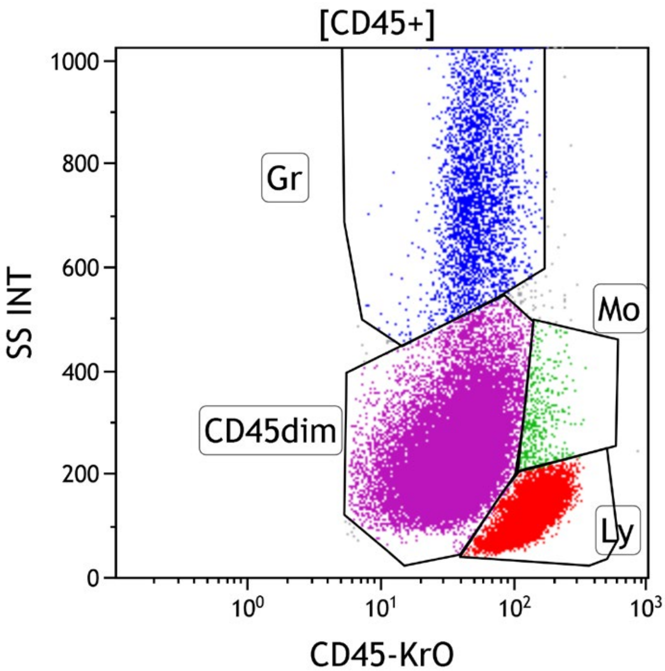


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.



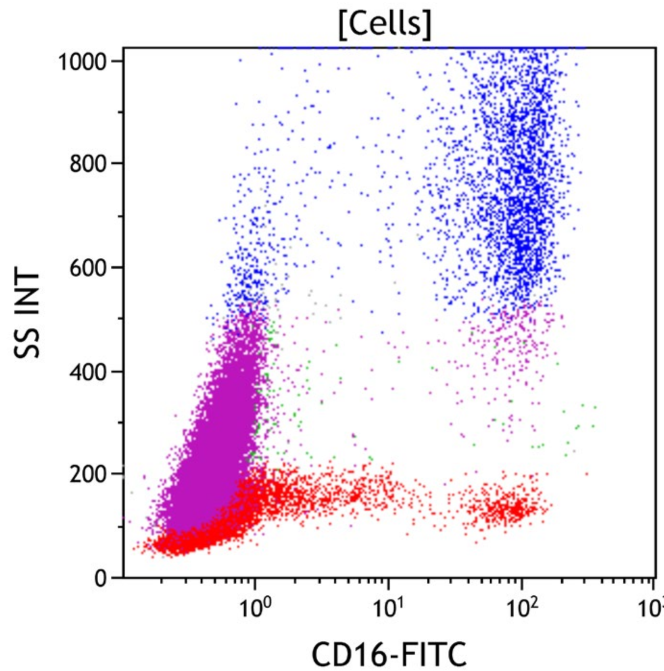
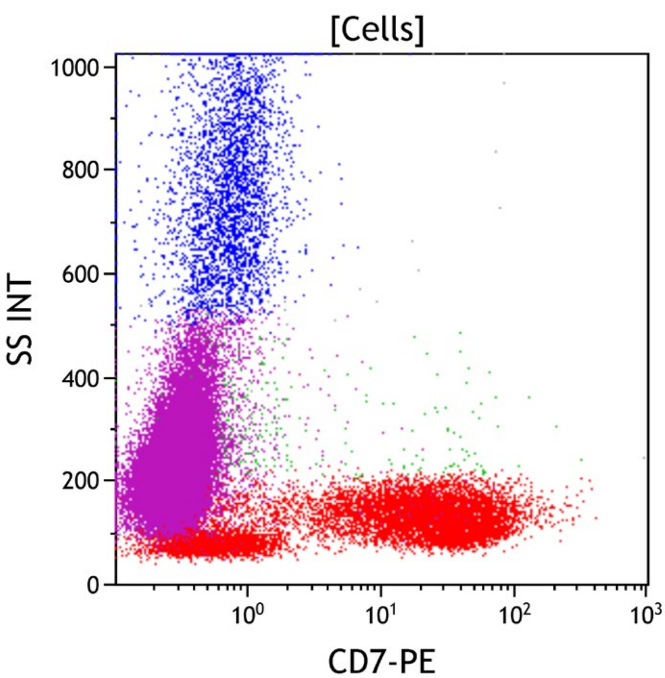


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the increased number of progenitors (purple).

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on granulocytes (blue). Most NK cells express CD16 (red). The aberrant population (purple) is negative for CD16.



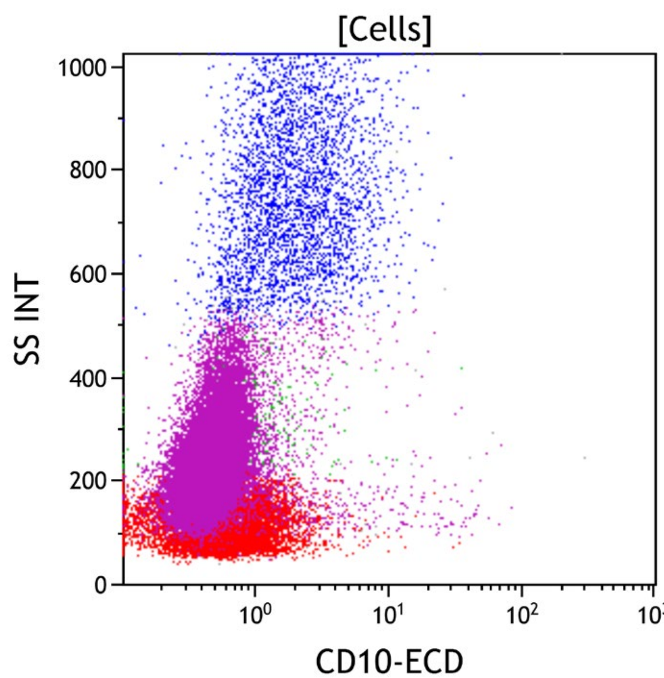


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The aberrant population (purple) is negative for CD7.

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show variable dim expression of CD10. The aberrant population (purple) is negative for CD10.

M1 Cell Tube

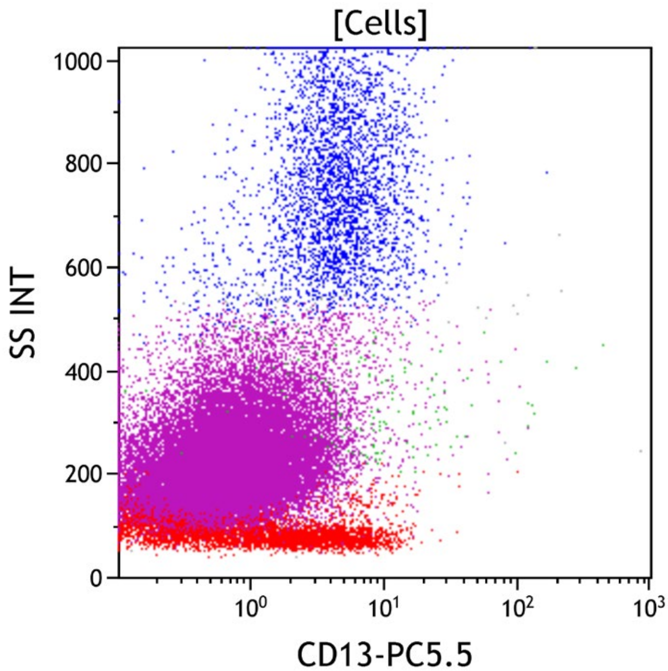


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature and a subset of immature granulocytes (in blue, with high side scatter). The aberrant population (purple) is dimly positive for CD13.

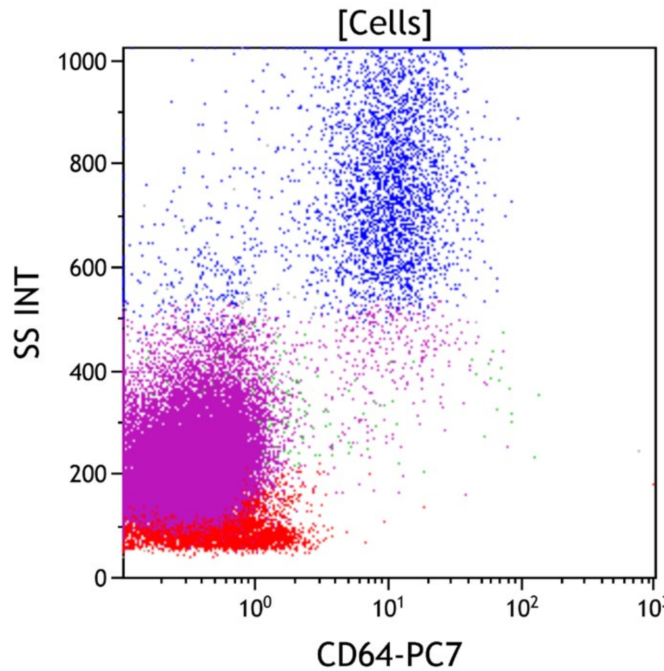


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The aberrant population (purple) is negative for CD64.

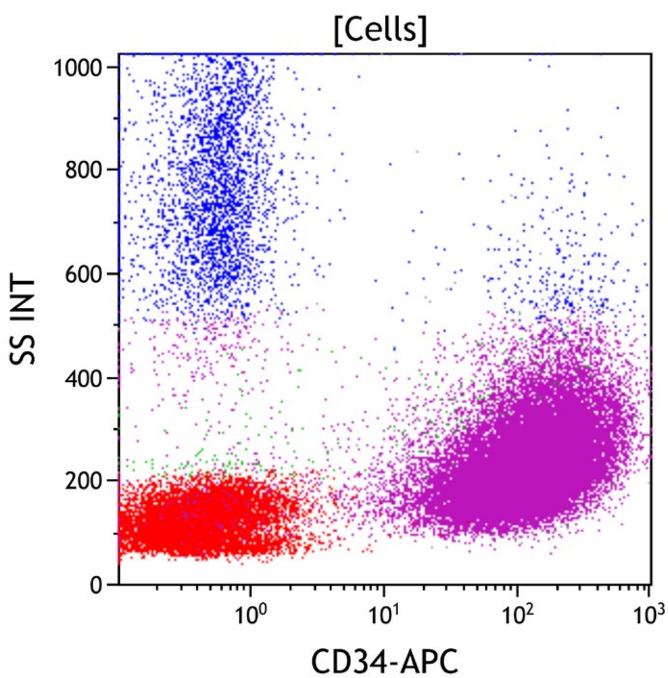


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive for CD34.

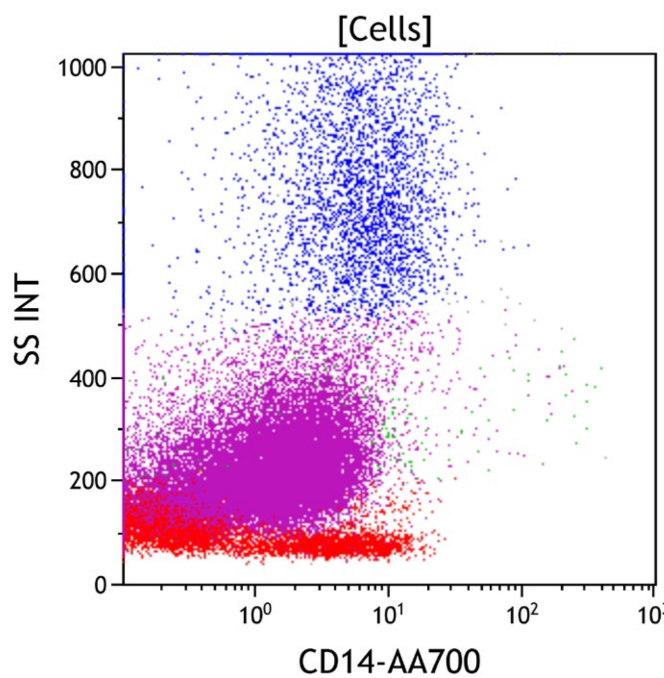


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed on granulocytes (blue) at a low level. The aberrant population (purple) is negative for CD14.

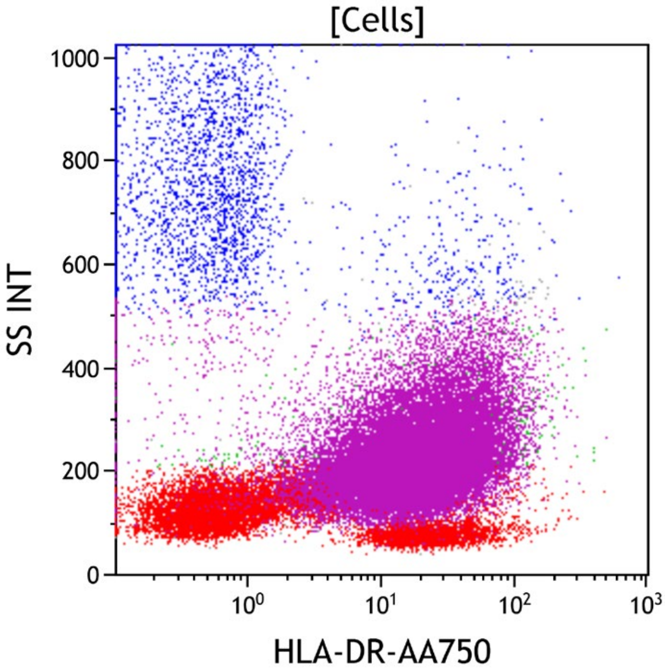


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The aberrant population (purple) is positive for HLA-DR.

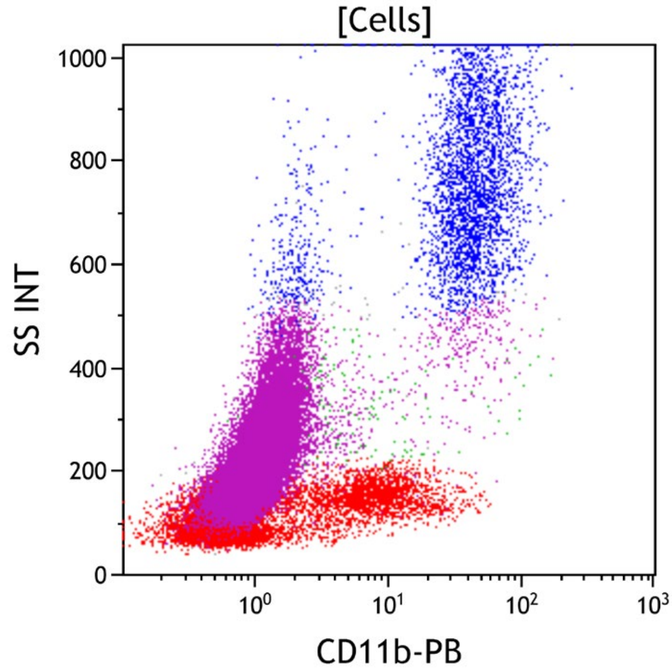


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (in blue), monocytes, basophils, and NK cells . The aberrant population (purple) is negative for CD11b.

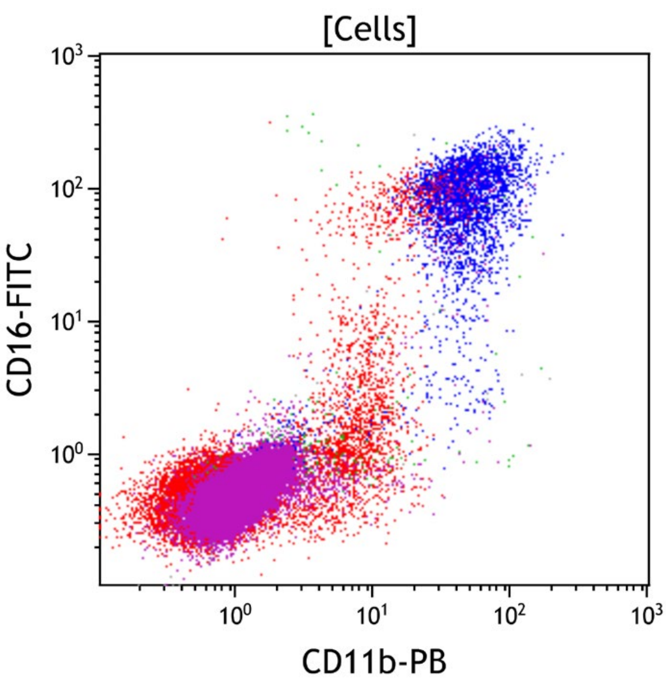


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, granulocytes (blue), and NK cells. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is negative for CD11b and CD16.

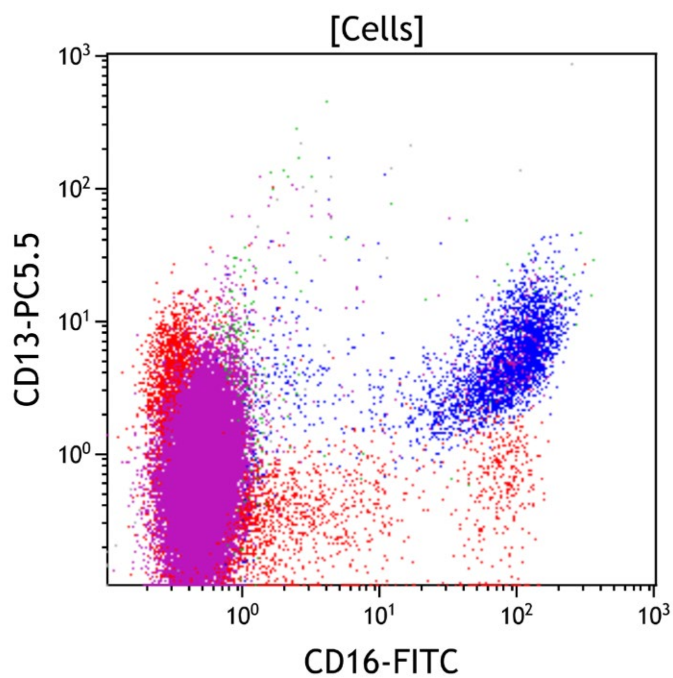


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is dimly positive for CD13 and negative for CD16.

M1 Cell Tube

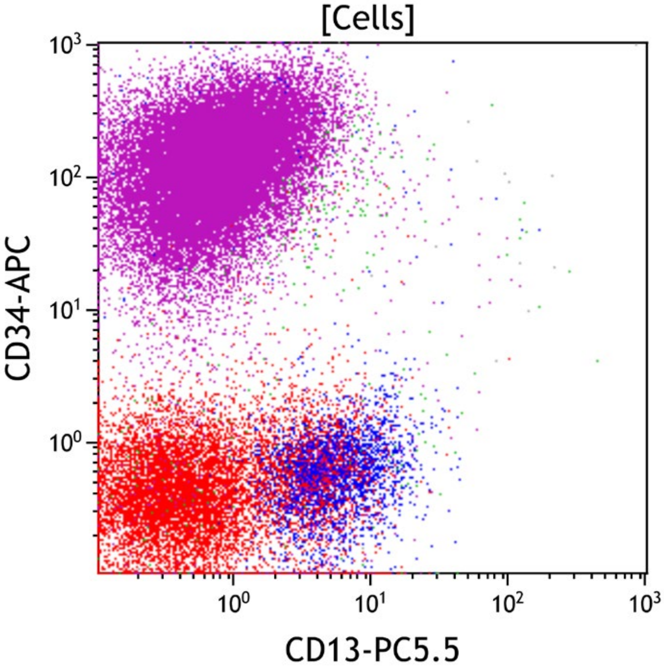


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. The aberrant population (purple) is strongly positive for CD34 and dimly positive for CD13.

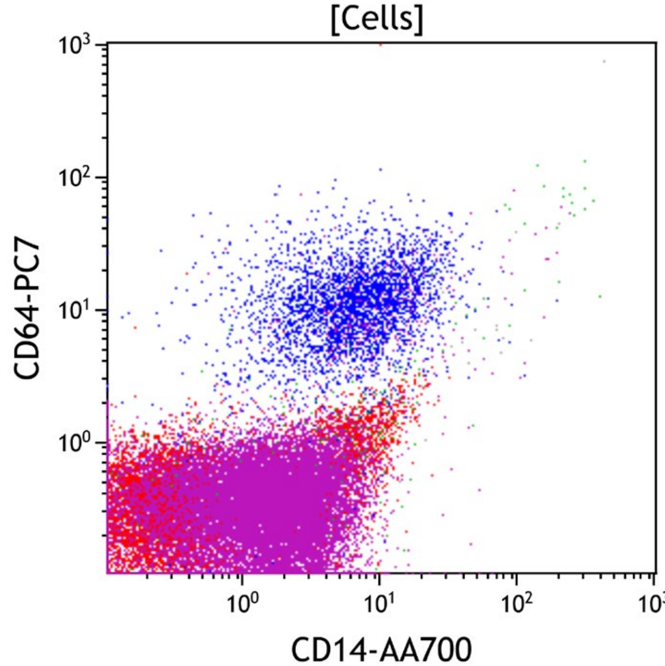


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes (blue). The aberrant population (purple) is negative for CD14 and CD64.

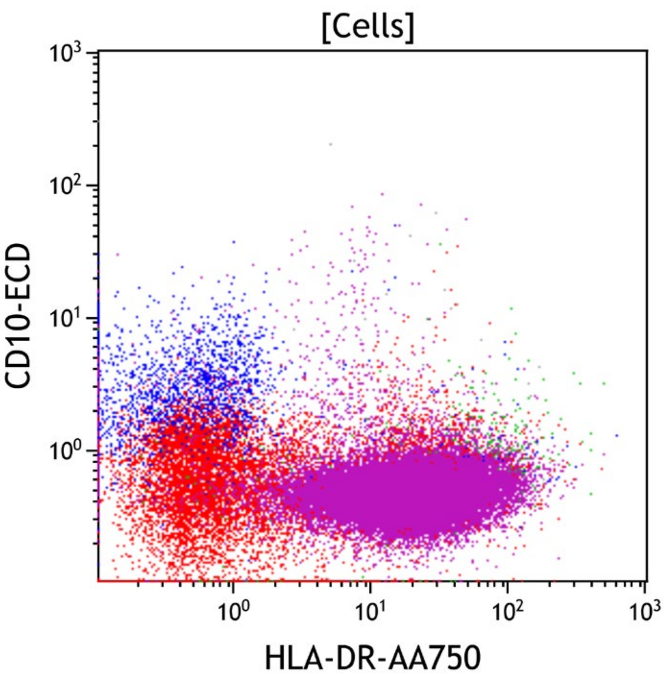


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34 positive progenitors. CD10 is expressed on granulocytes (blue). The aberrant population (purple) is positive for HLA-DR and negative for CD10.

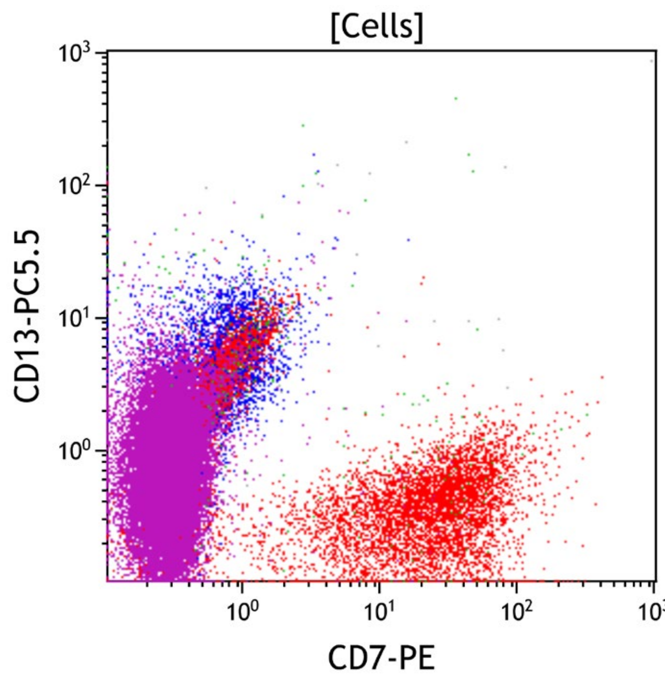


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on granulocytes (blue), monocytes, and basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The aberrant population (purple) is dimly positive for CD13 and negative for CD7.

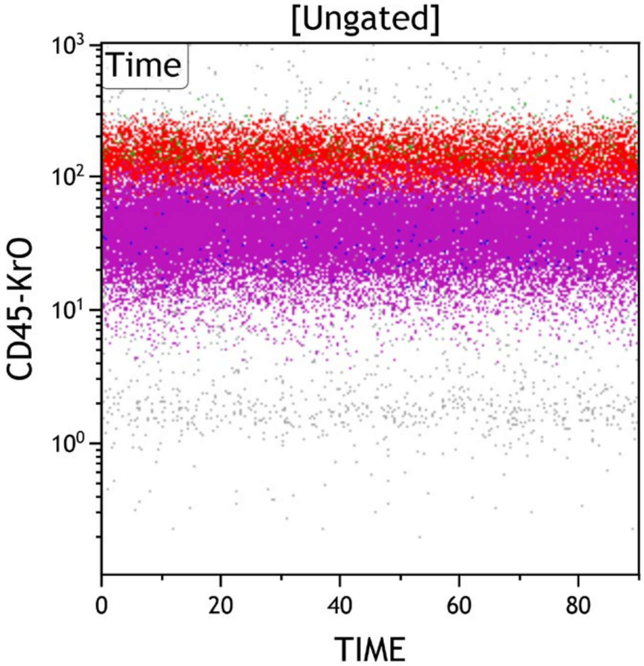


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

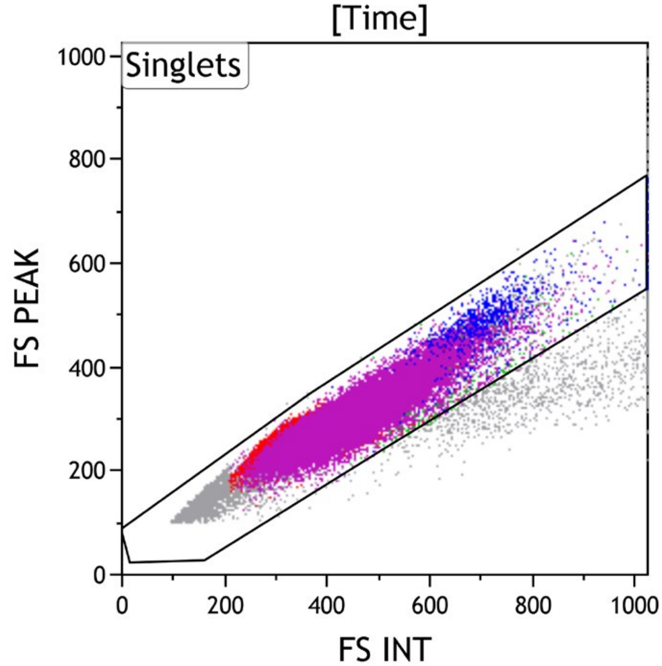


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

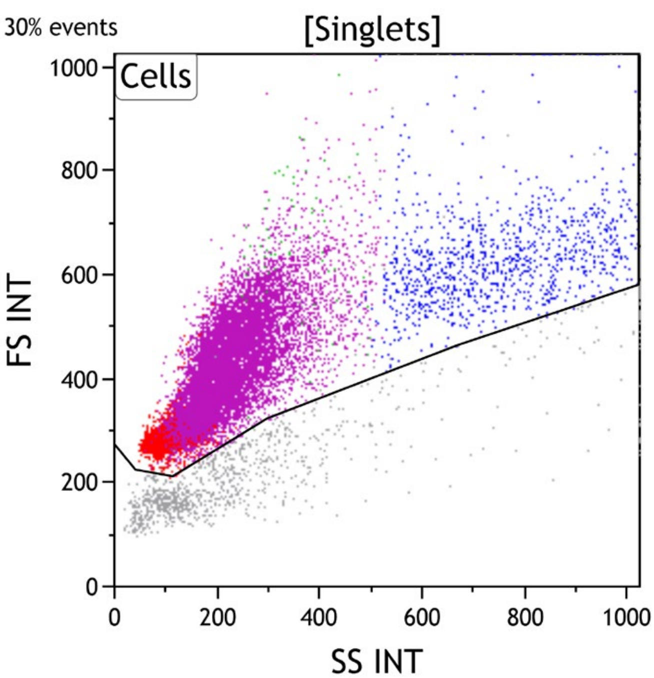


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

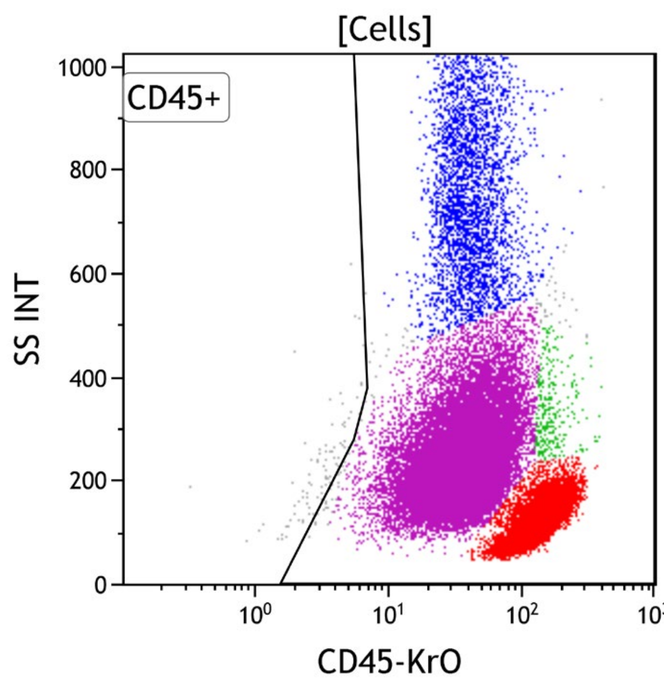


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

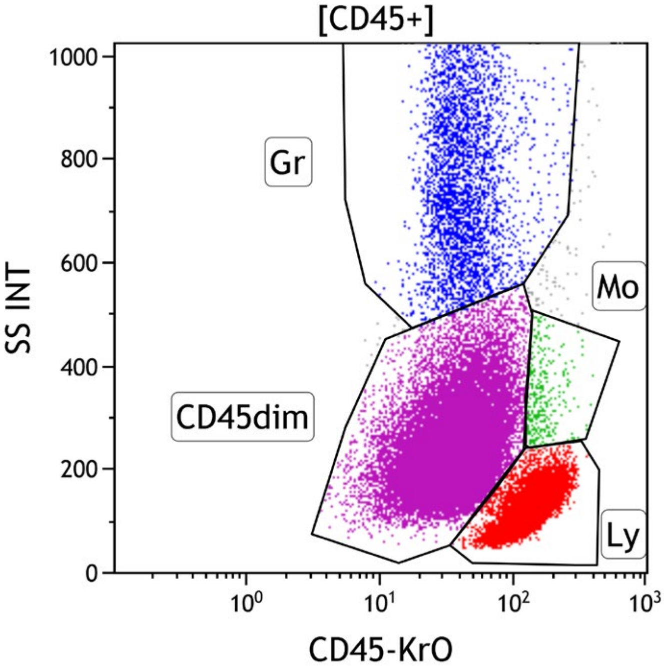


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the increased number of progenitors (purple).

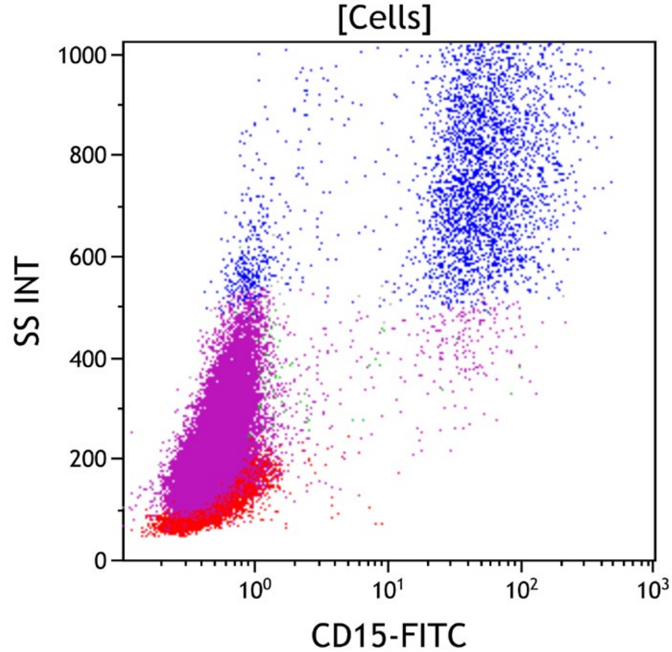


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on mature granulocytes (in blue). The aberrant population (purple) is negative for CD15.

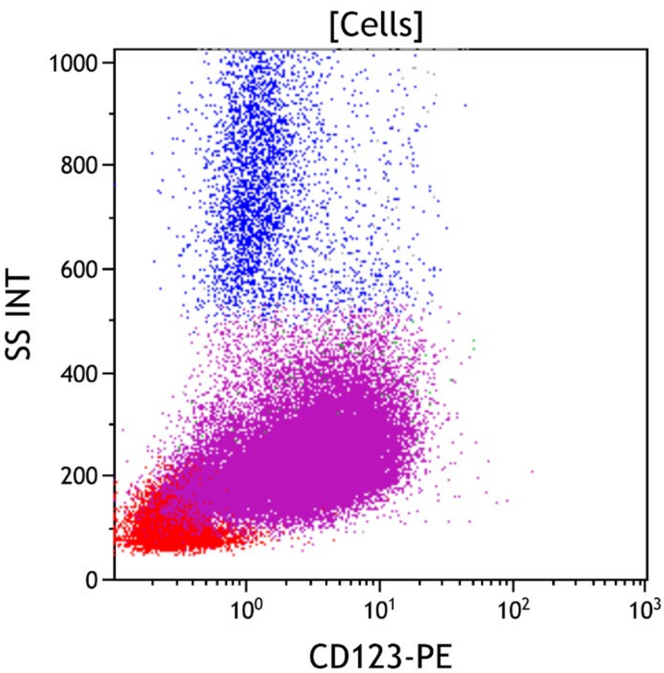


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. The aberrant population (purple) displays dim to intermediate CD123 expression.

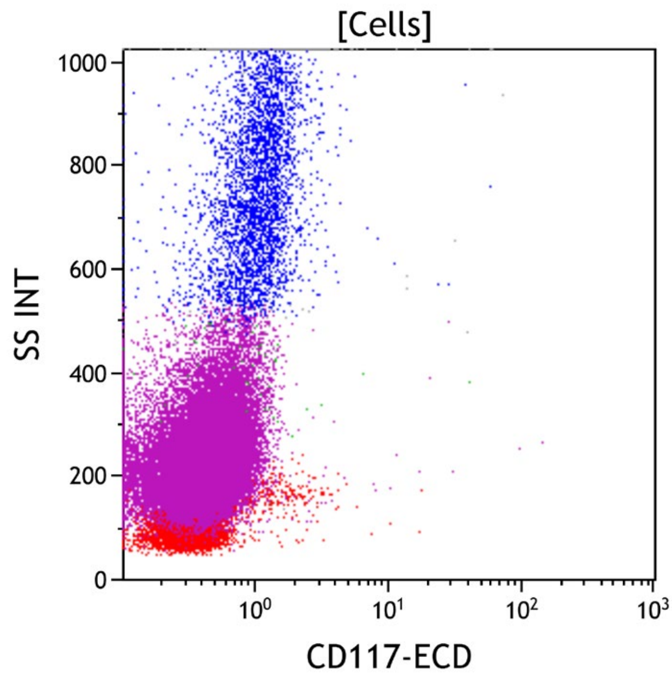


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The aberrant population (purple) is negative for CD117.

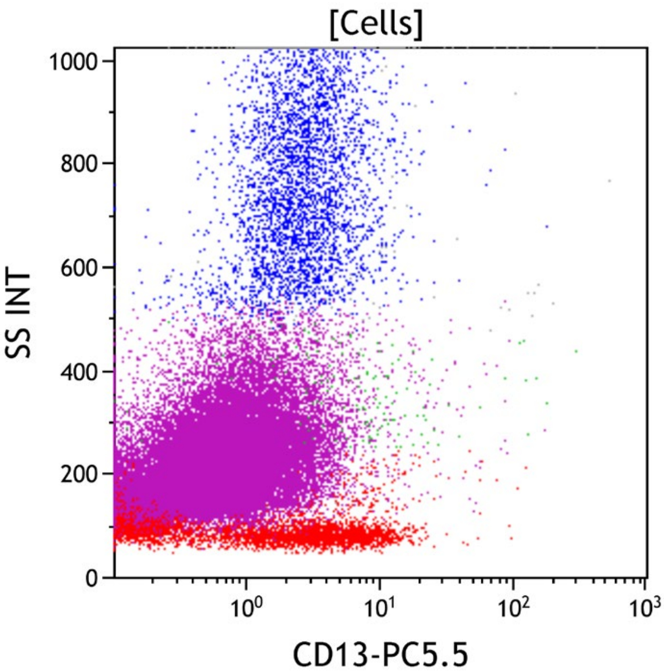


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (in blue, with high side scatter) and on mature monocytes. The aberrant population (purple) is dimly positive for CD13.

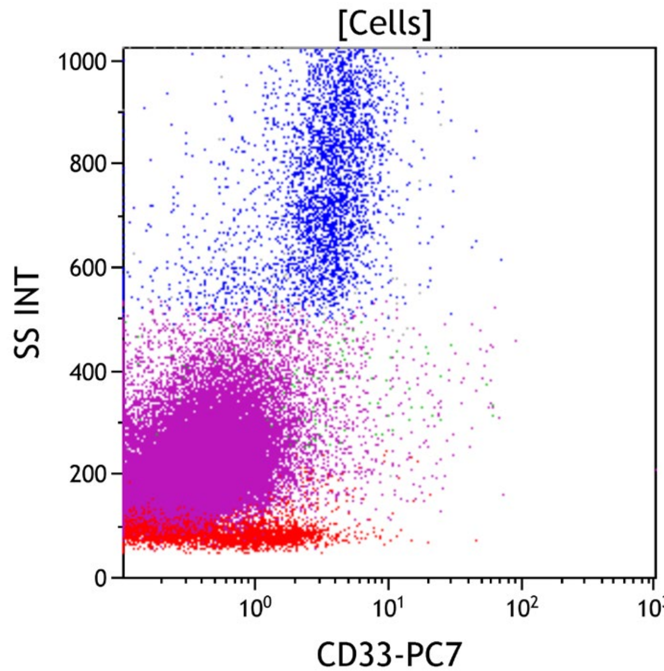


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The aberrant population (purple) is negative for CD33.

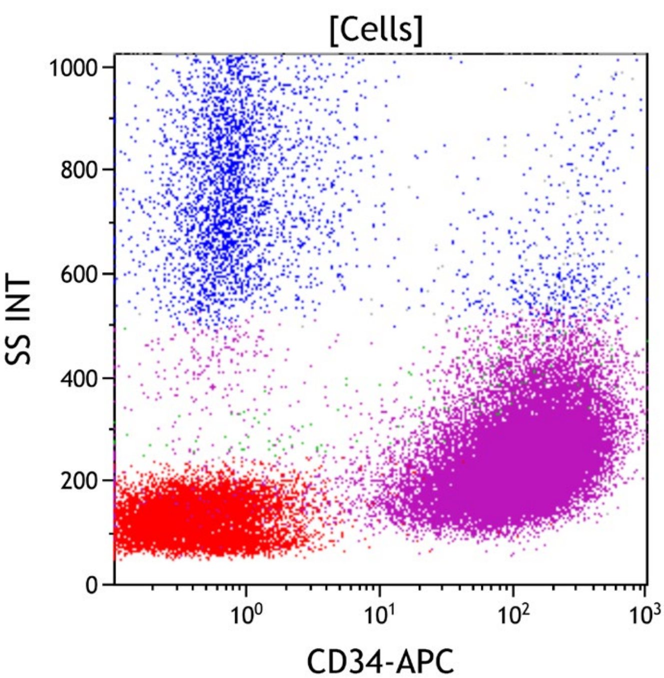


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive for CD34.

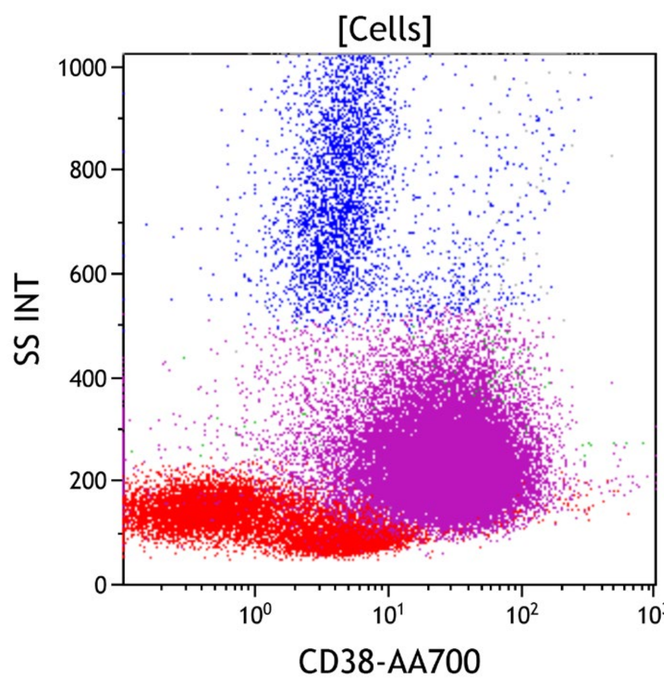


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variable level on activated lymphocytes (red). The aberrant population (purple) is positive for CD38.

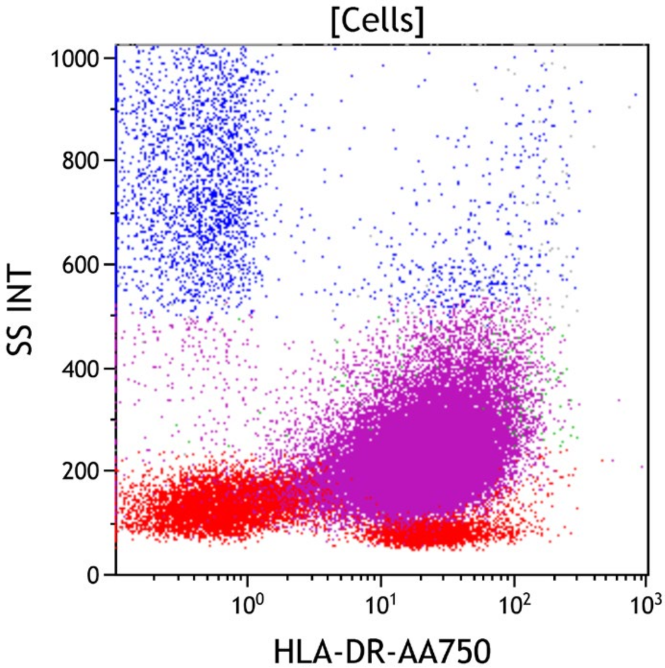


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red). The aberrant population (purple) is positive for HLA-DR.

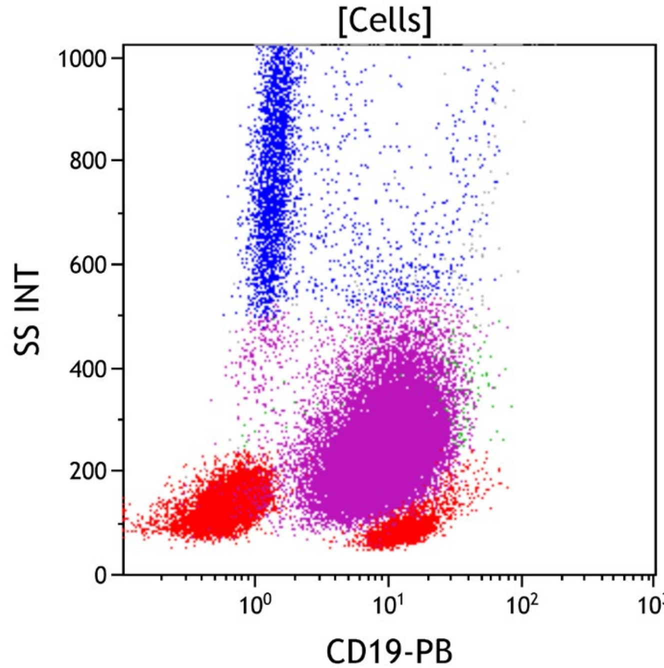


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is positive for CD19.

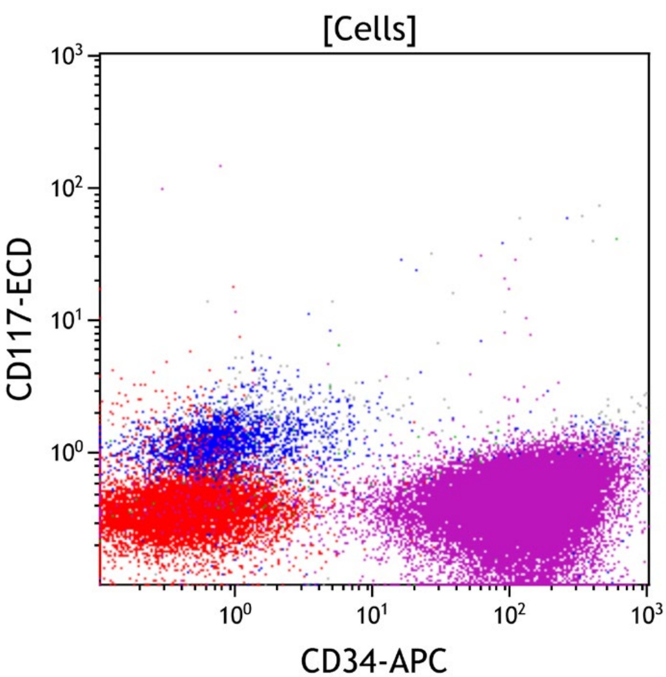


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes and early erythroid precursors, but negative on early B cell precursors. The aberrant population (purple) is strongly positive for CD34 and negative for CD117.

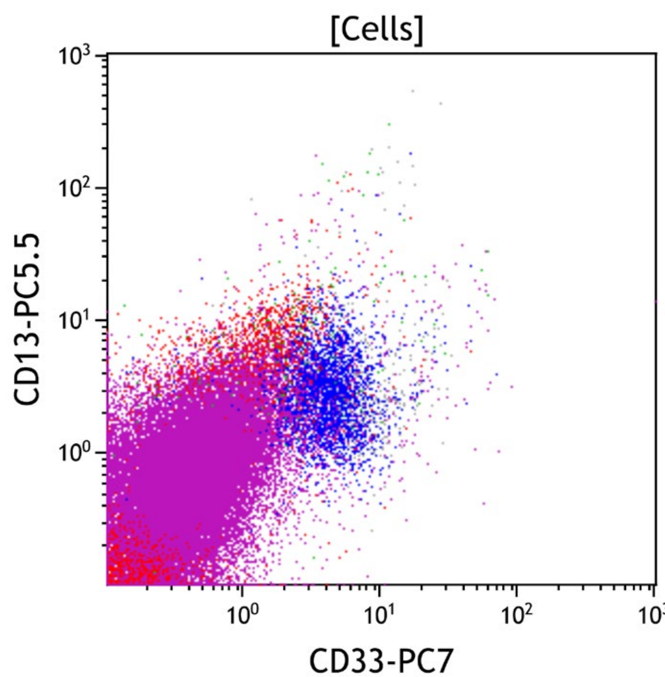


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes largely do not express either CD13 or CD33 (red). The aberrant population (purple) displays dim CD13 expression and is negative for CD33.

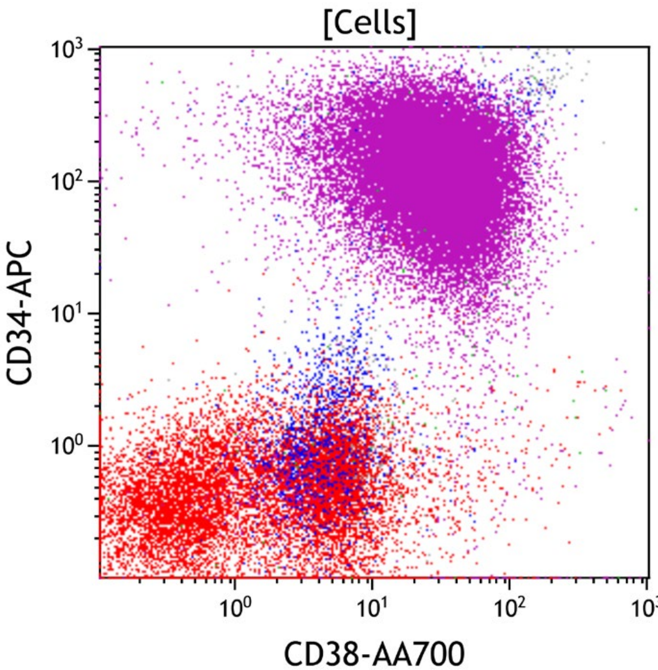


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. The aberrant population (purple) is positive for CD34 and CD38. The level of CD38 expression is slightly and variably lower than that seen on normal immature B cells.

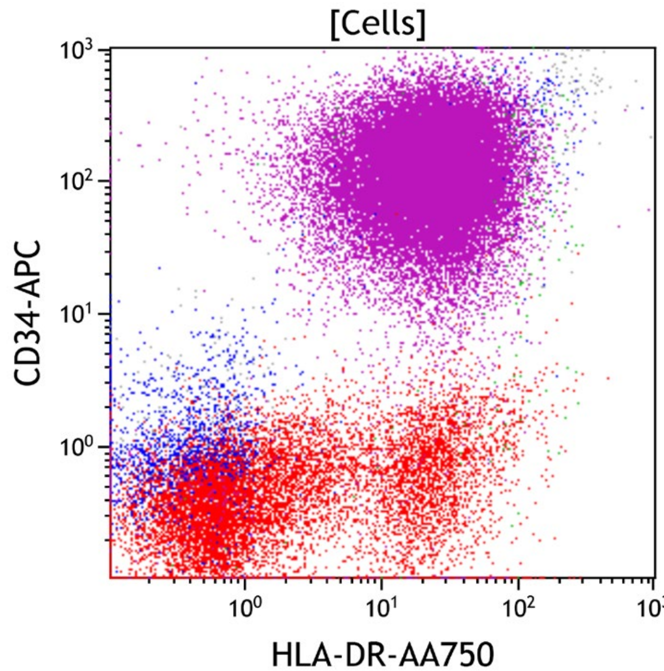


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors. CD34 is expressed on early progenitors. The aberrant population (purple) is strongly positive for CD34 and HLA-DR.

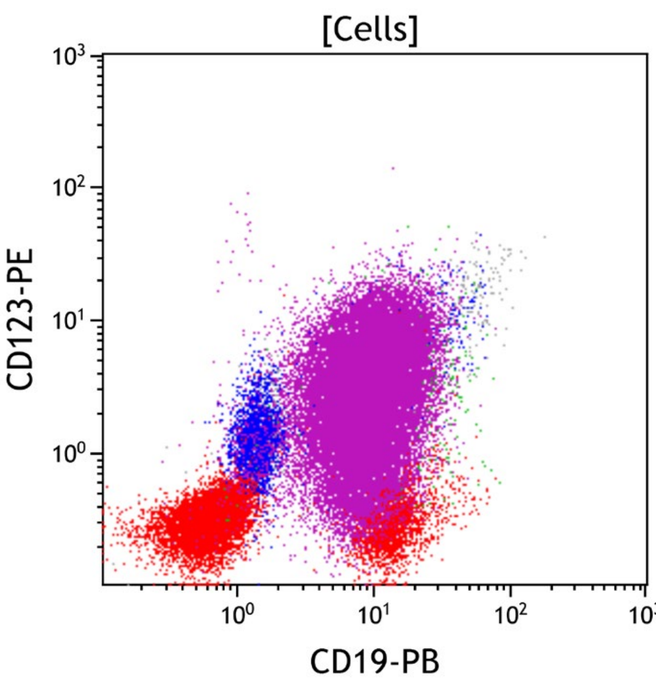


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 positive B cells normally do not express significant CD123. The aberrant population (purple) is positive for CD19 and CD123.

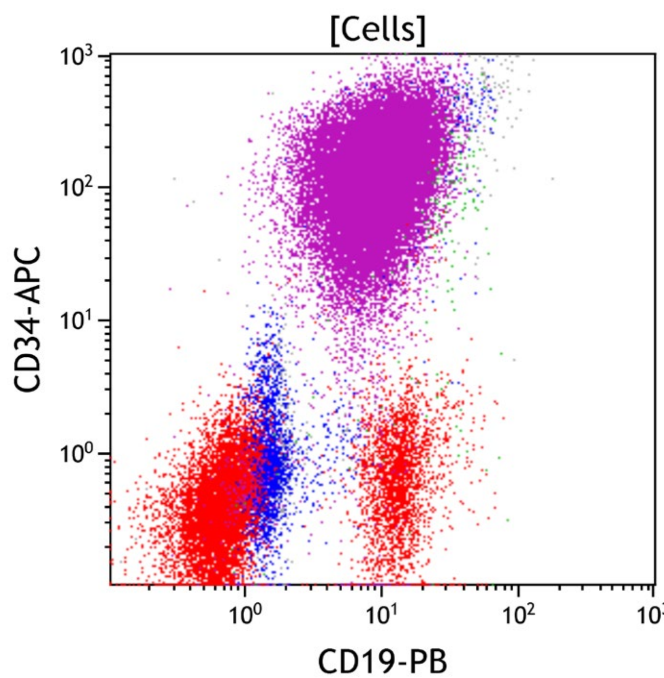


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red) do not express CD34. The aberrant population (purple) is positive for CD19 and CD34.

M2 Cell Tube

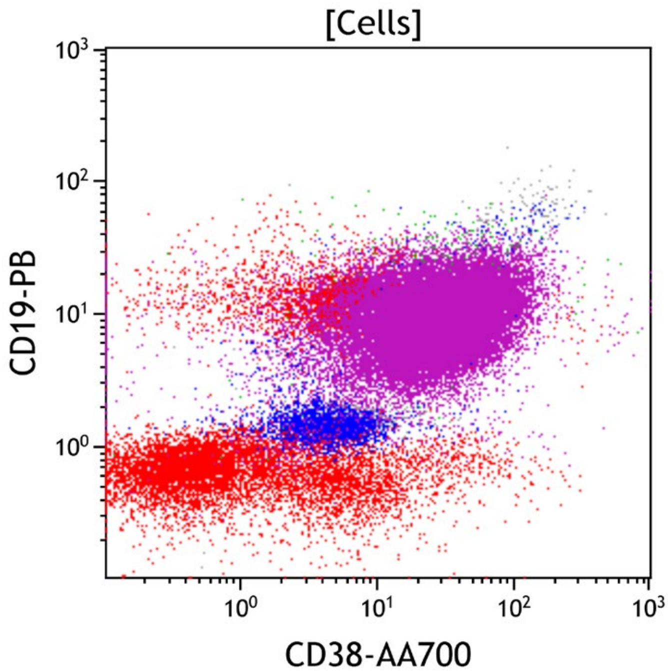


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show intermediate CD38. The aberrant population (purple) is positive for CD19 and CD38. The level of CD38 expression is slightly and variably lower than that seen on normal immature B cells.

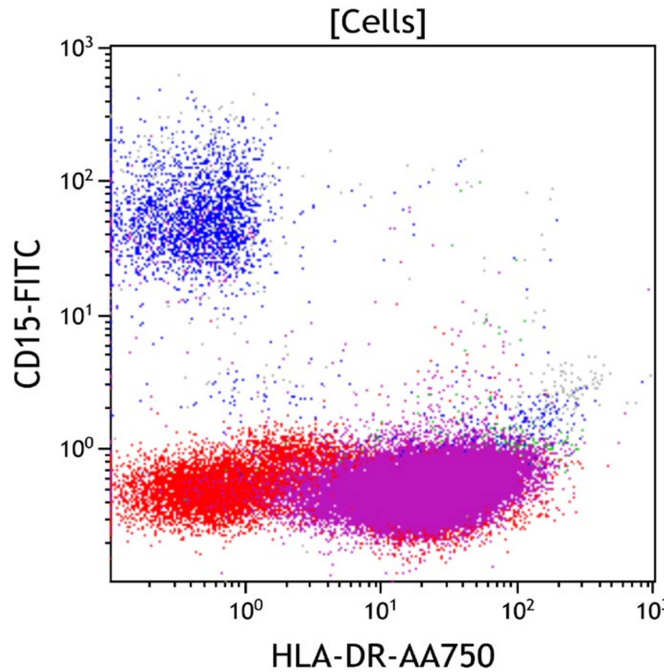


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by granulocytes (blue) and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes (blue) do not express HLA-DR. The aberrant population (purple) is positive for HLA-DR and negative for CD15.

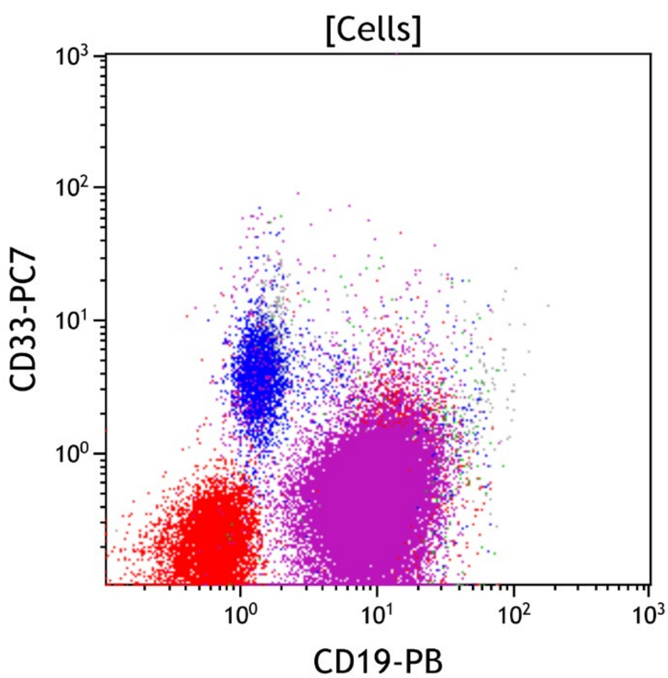


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes and granulocytes (blue). Mature CD19 positive B cells (red) do not normally express significant CD33. The aberrant population (purple) is positive for CD19 and negative for CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of dim CD13, intermediate CD19, variable CD20, bright CD34, intermediate CD38, dim CD45, low to intermediate CD123, and intermediate HLA-DR without CD10, CD33, CD117, or other B, T, or myeloid markers. Compared with normal B cell precursors, the increase in side scatter, absence of CD10, dim CD13, increased CD34, slight and variable decrease in CD38, and presence of CD123 are aberrant. Morphology shows 95% blasts, which in combination with the immunophenotypic findings is indicative of a B lymphoblastic leukemia/lymphoma.

Taken together, the findings in this case are most consistent with B lymphoblastic leukemia/lymphoma. Note that correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

Case #5: B Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

Clinical Vignette

This 7-year-old male presents with pancytopenia and circulating blasts. A peripheral blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

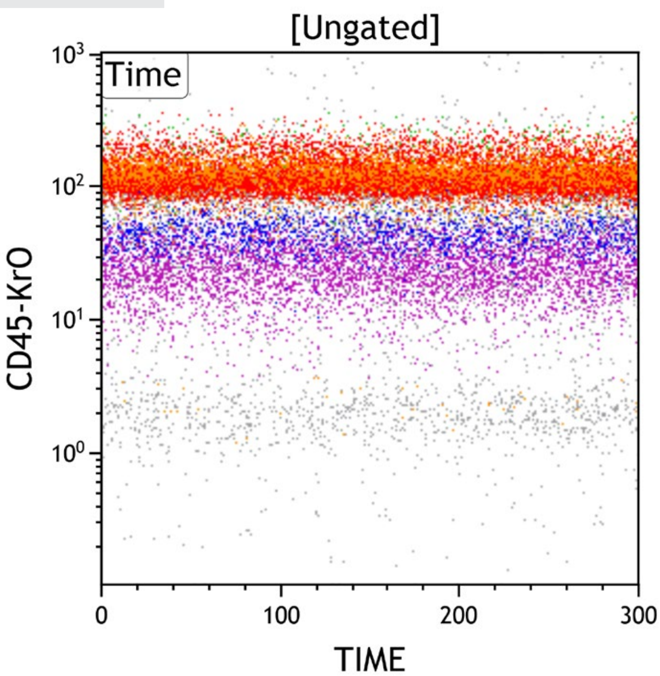


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

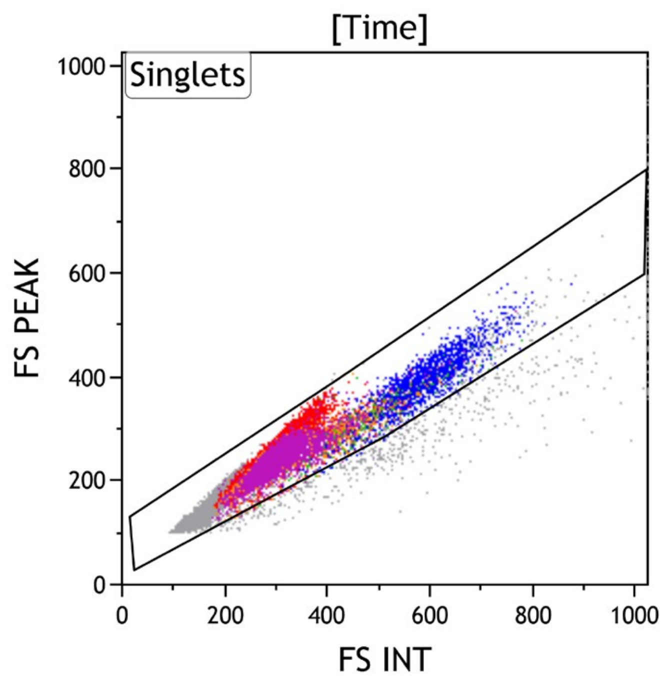


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

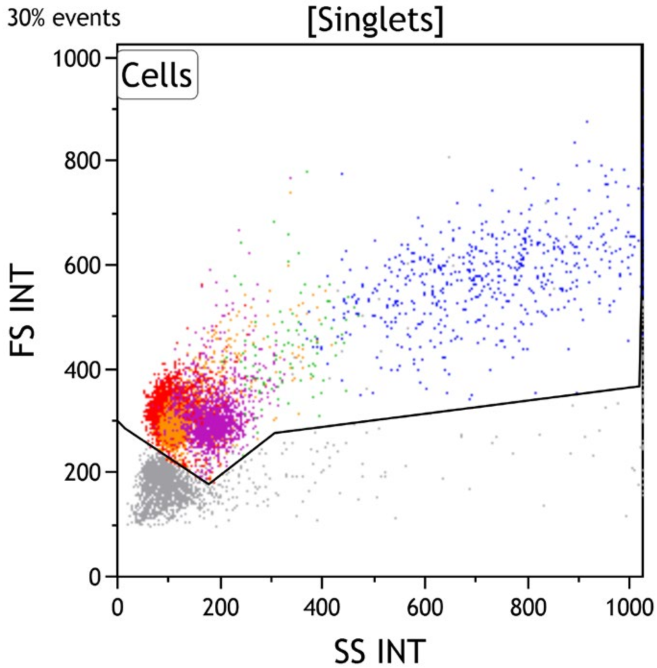


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

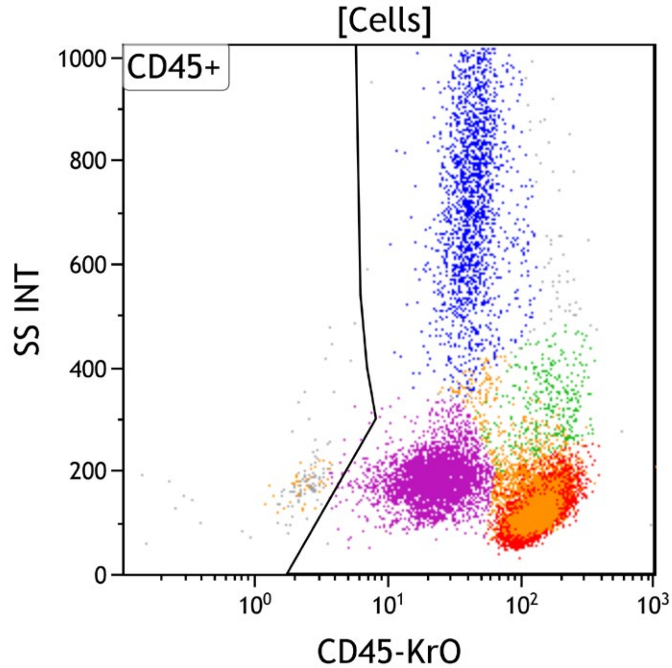


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

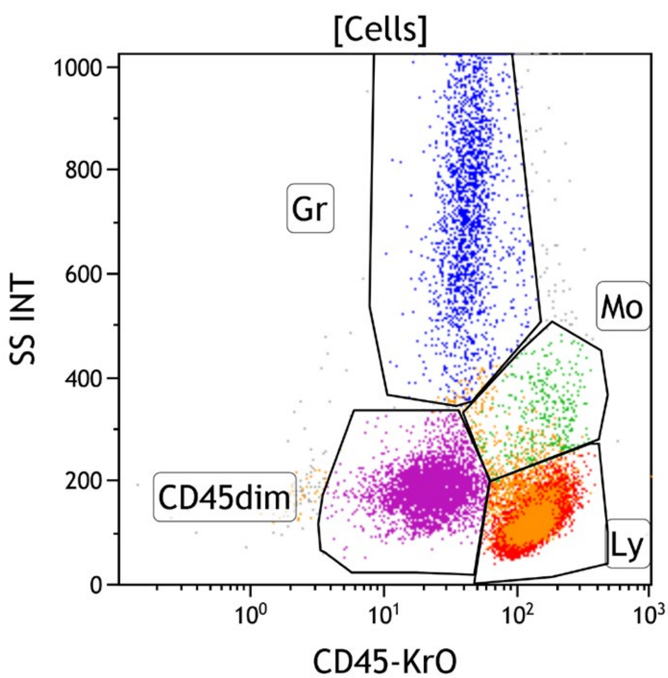


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note the increased number of progenitors (purple).

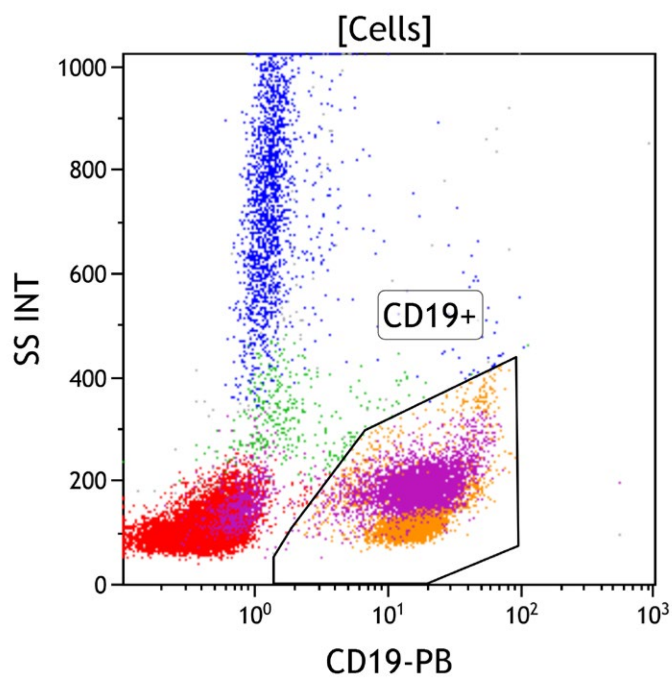


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is positive for CD19.

B Cell Tube

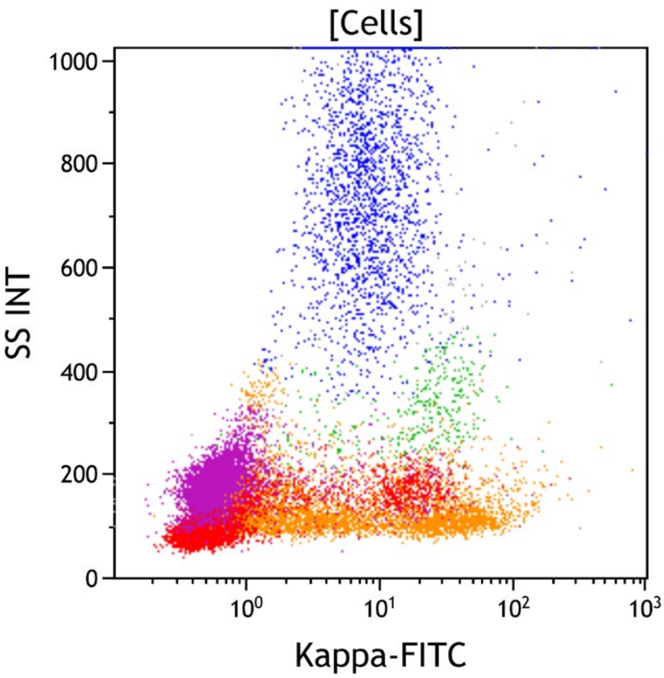


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The aberrant population (purple) is negative for surface kappa light chain.

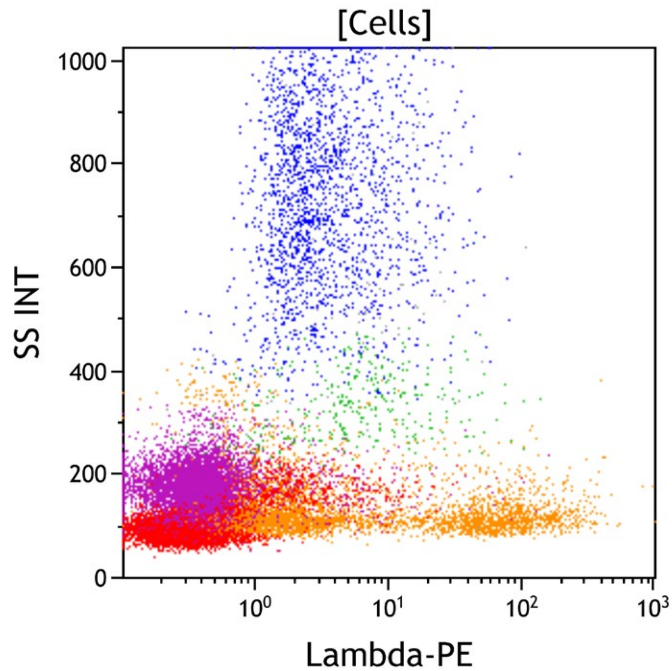


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The aberrant population (purple) is negative for surface lambda light chain.

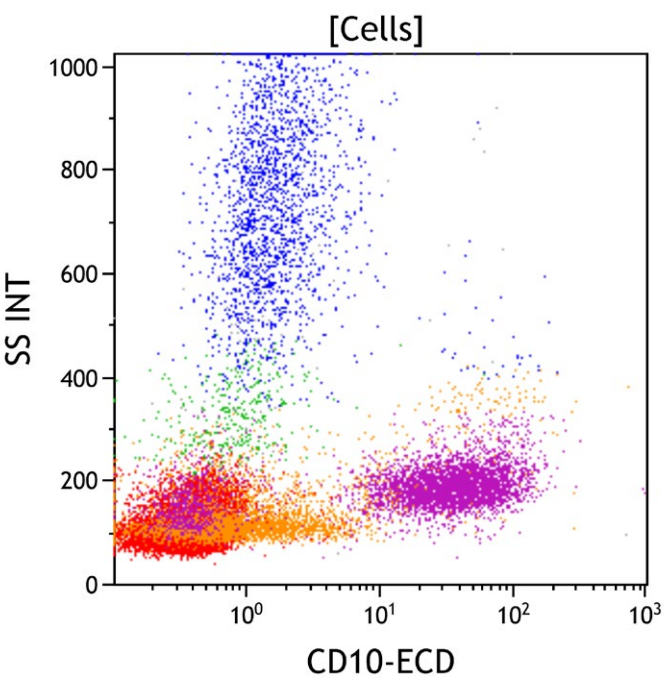


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple) is positive for CD10.

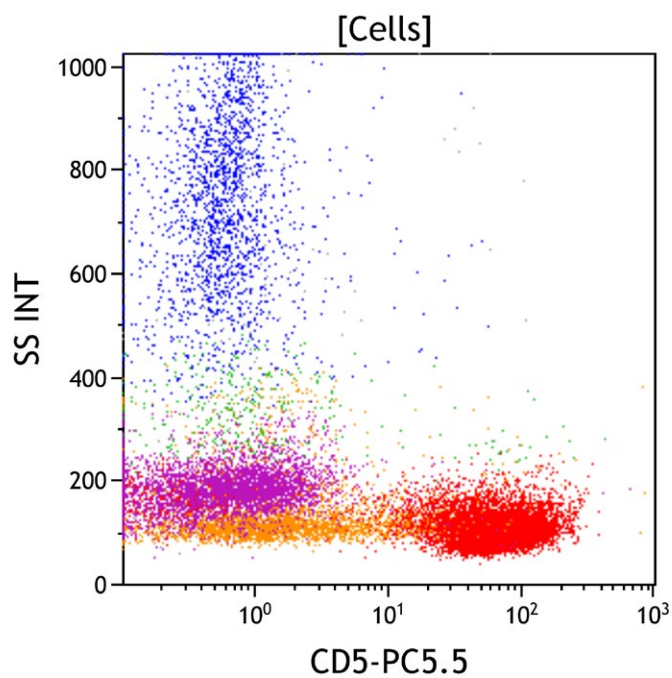


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The aberrant population (purple) is negative for CD5.

B Cell Tube

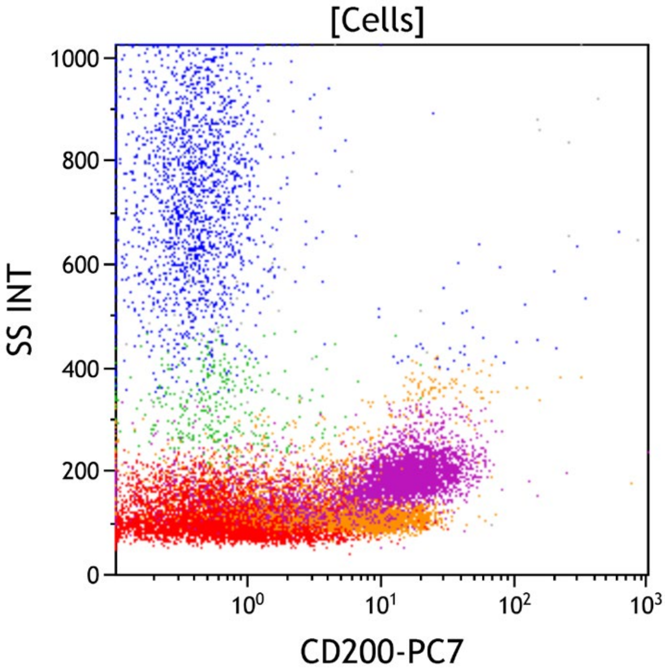


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The aberrant population (purple) is positive for CD200.

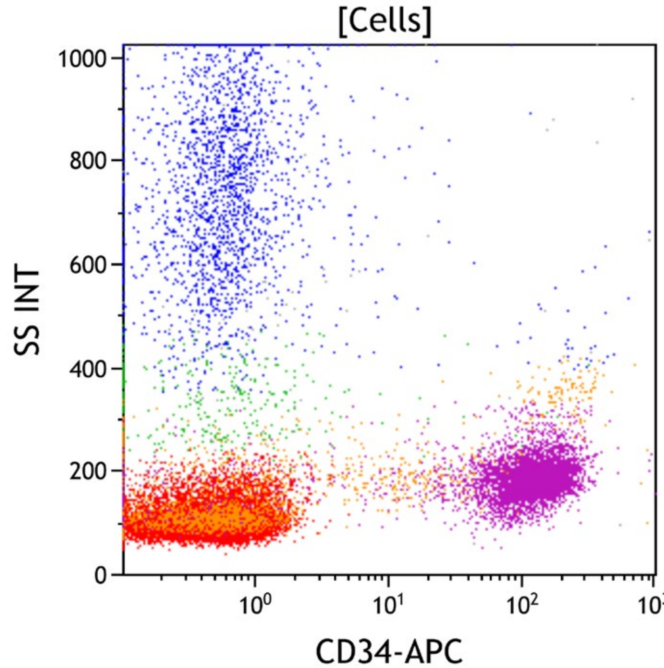


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive for CD34.

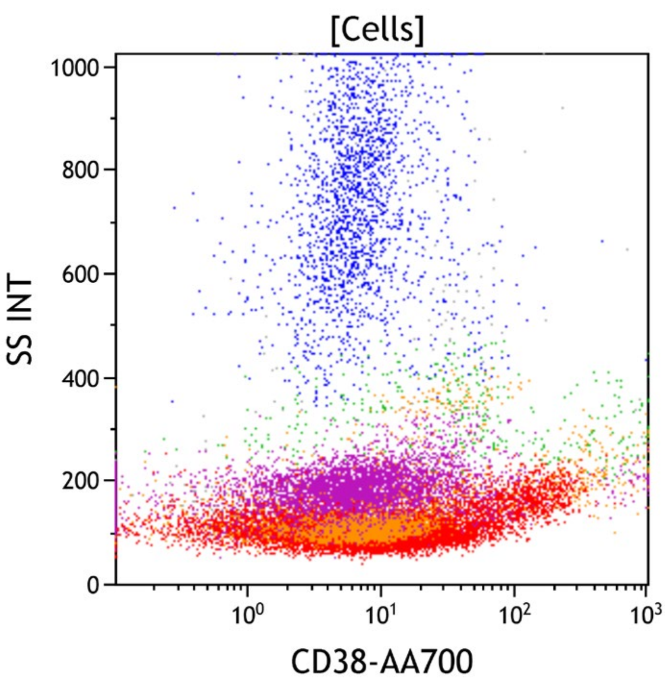


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). The aberrant population (purple) is positive for CD38 at level lower than that seen on normal immature B cells.

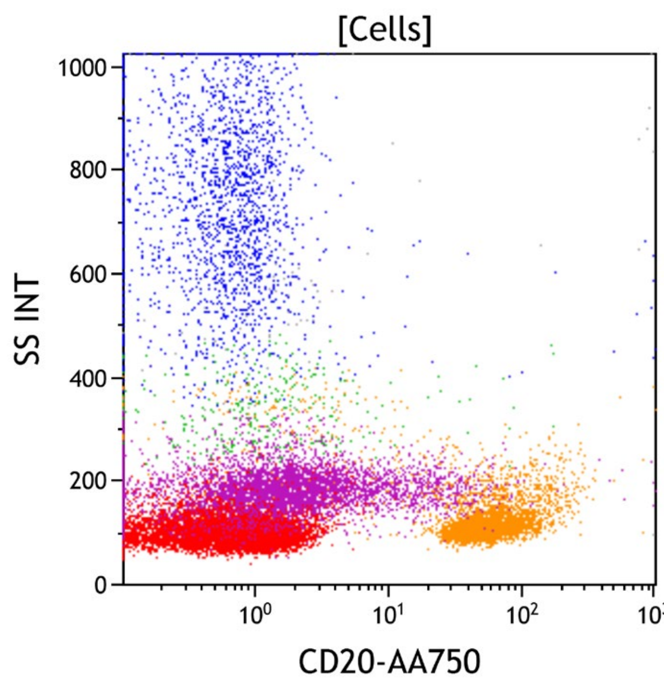


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20-positive cells are usually in the lymphocyte gate with low side scatter. The aberrant population (purple) displays partial CD20 expression.

B Cell Tube

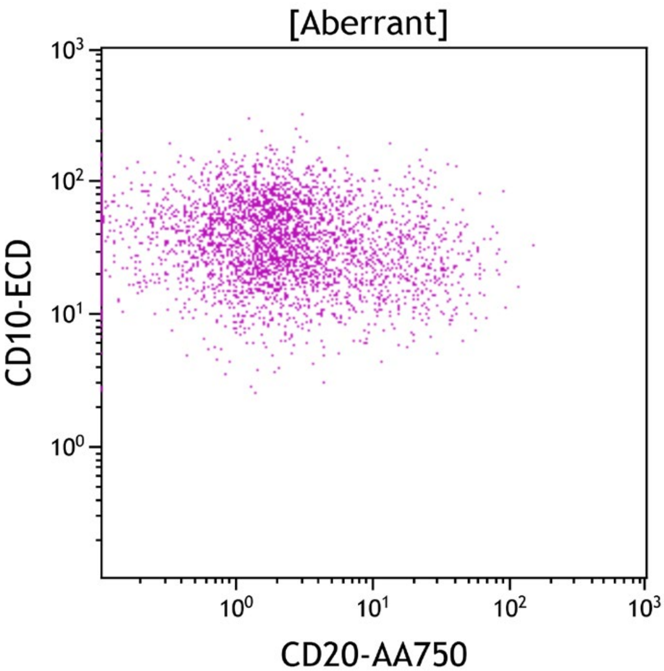


Figure 15: This CD20 vs CD10 dot plot shows the aberrant population in the CD45 dim gate. The aberrant population (purple) displays increased CD10 expression with low CD20 on a subset.

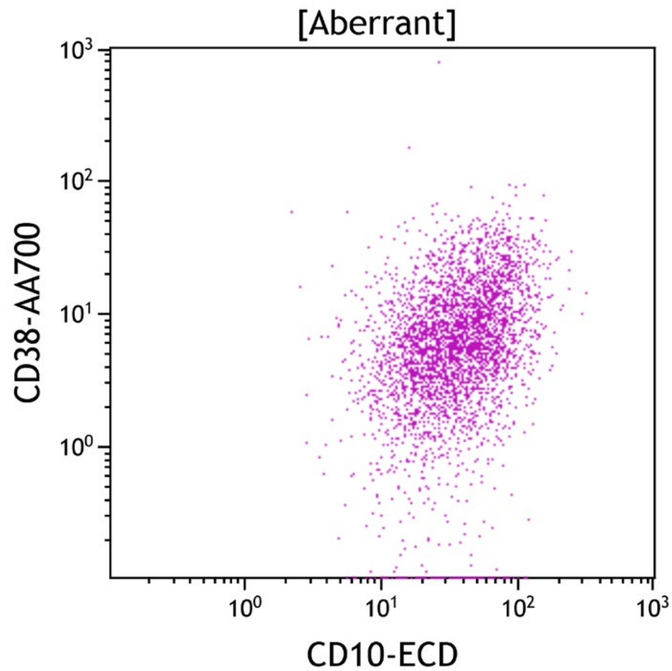


Figure 16: This CD10 vs CD38 dot plot shows the aberrant population in the CD45 dim gate. The aberrant population (purple) displays increased CD10 expression with lower level of CD38 than that seen on normal immature B cells.

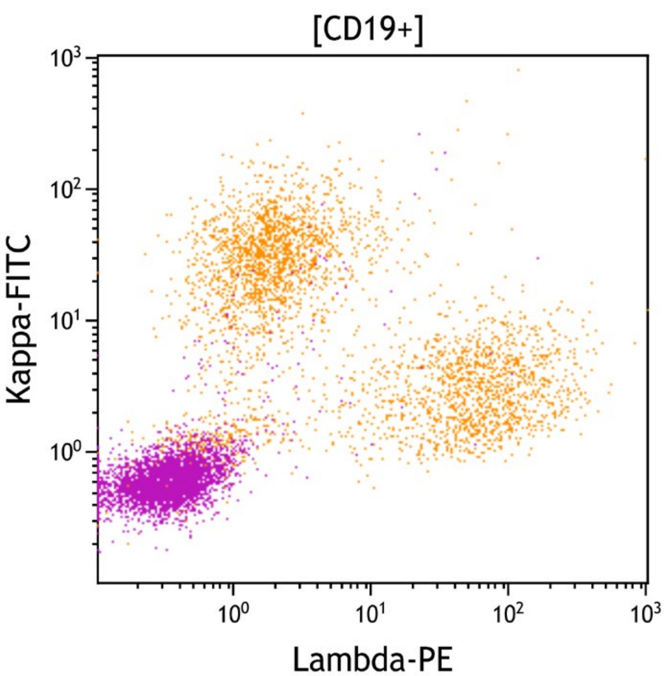


Figure 17. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. The aberrant population (purple) lacks kappa or lambda light chain expression.

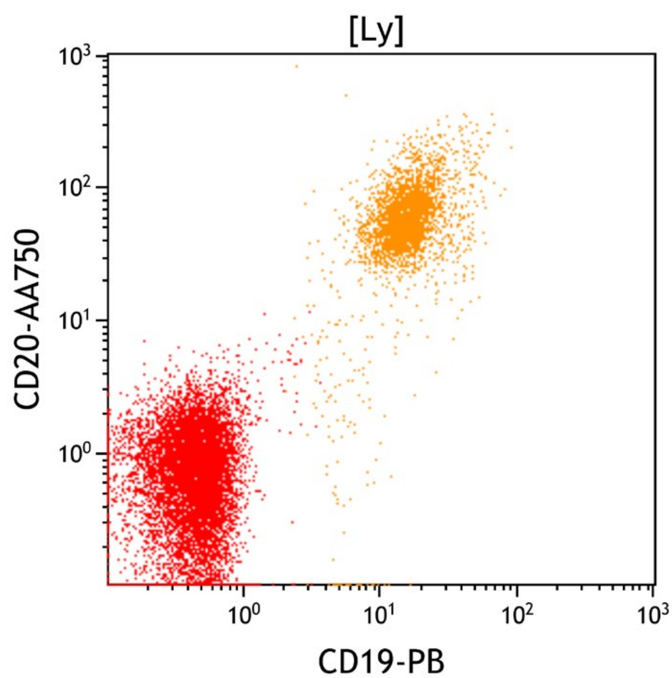


Figure 18. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression.

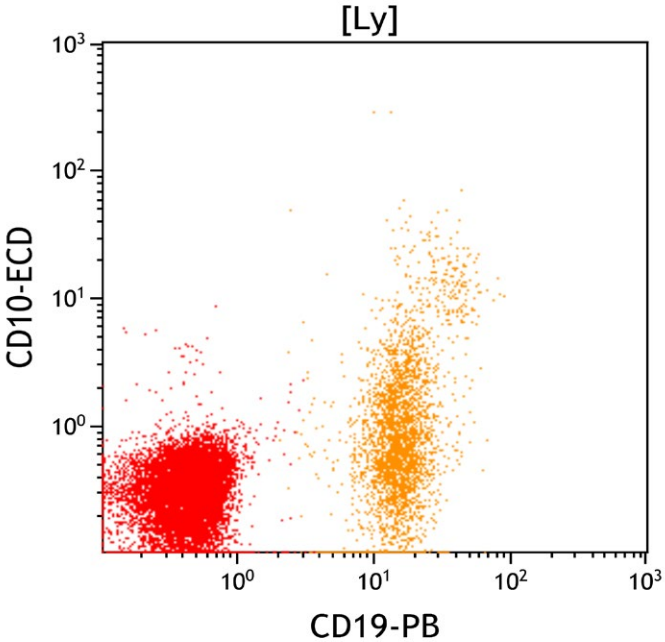


Figure 19. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45dim gate.

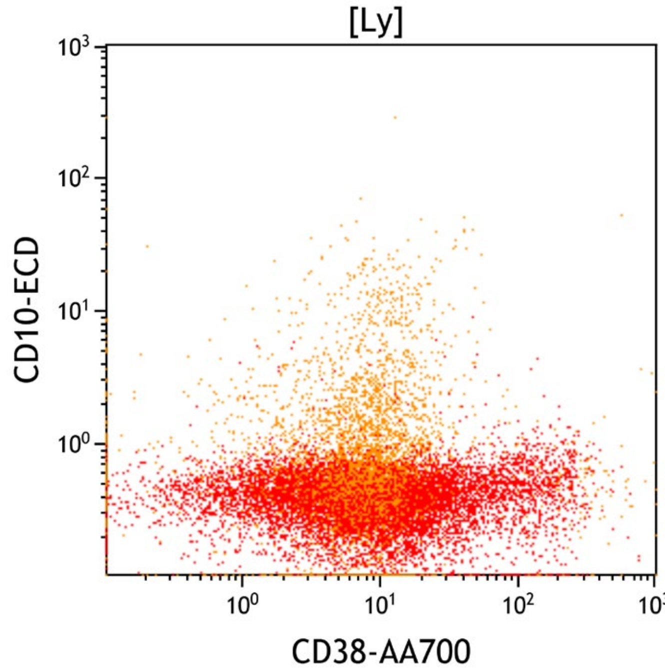


Figure 20. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45dim gate.

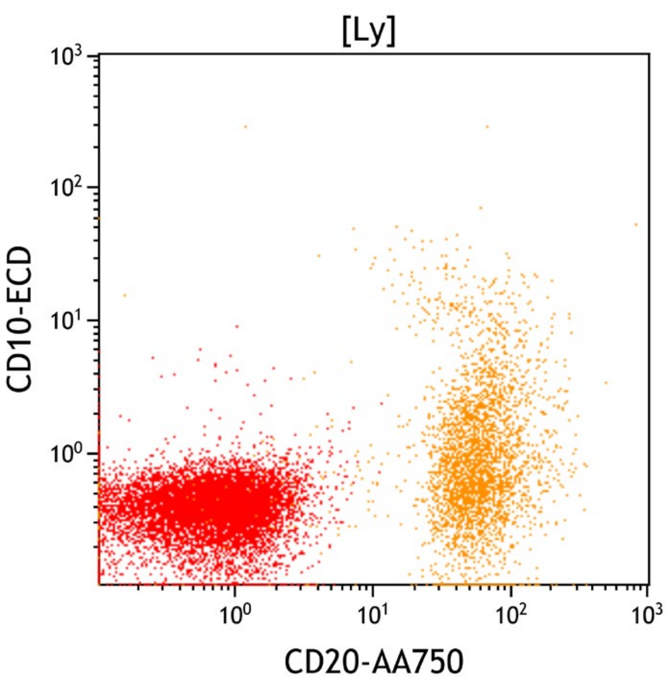


Figure 21. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20.

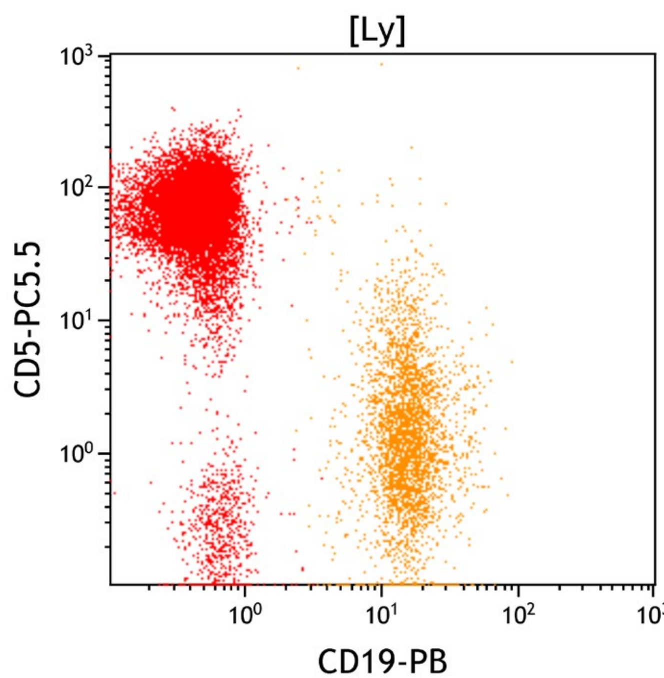


Figure 22. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

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B Cell Tube

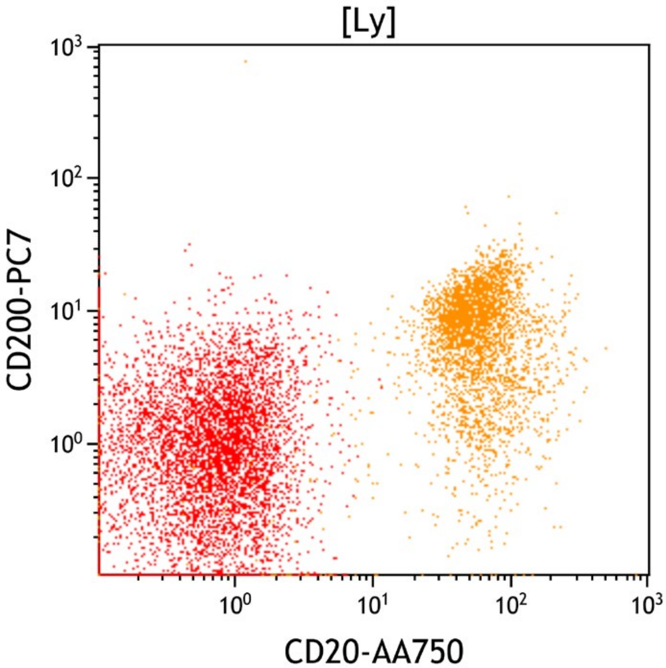

Figure 23. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

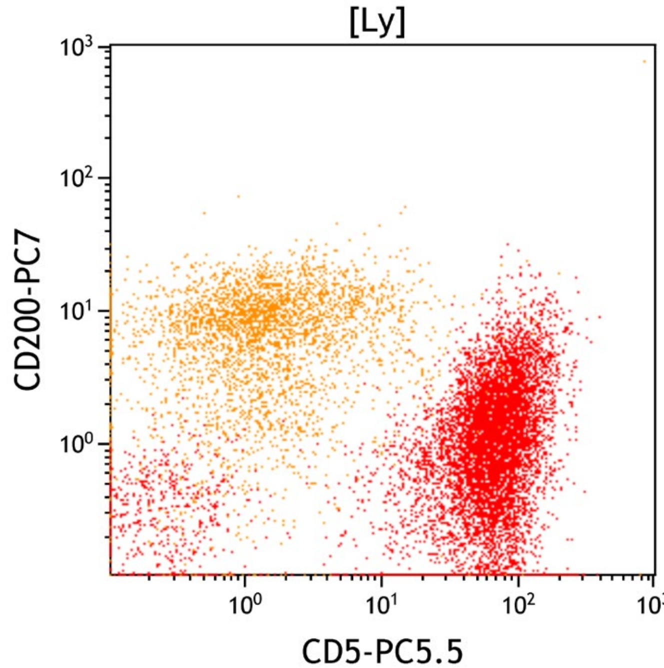

Figure 24. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

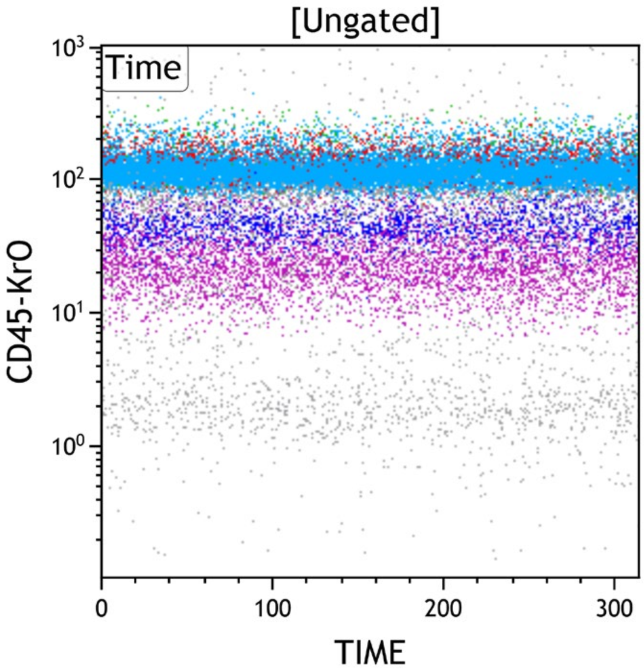

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

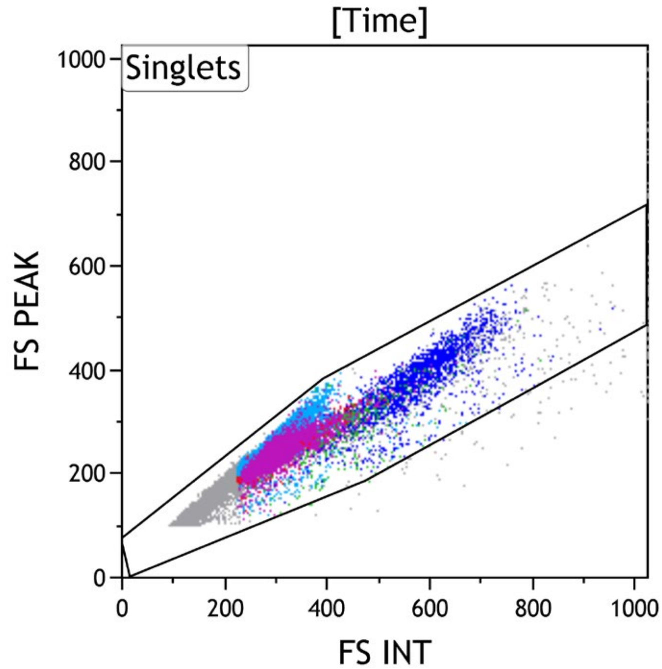

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

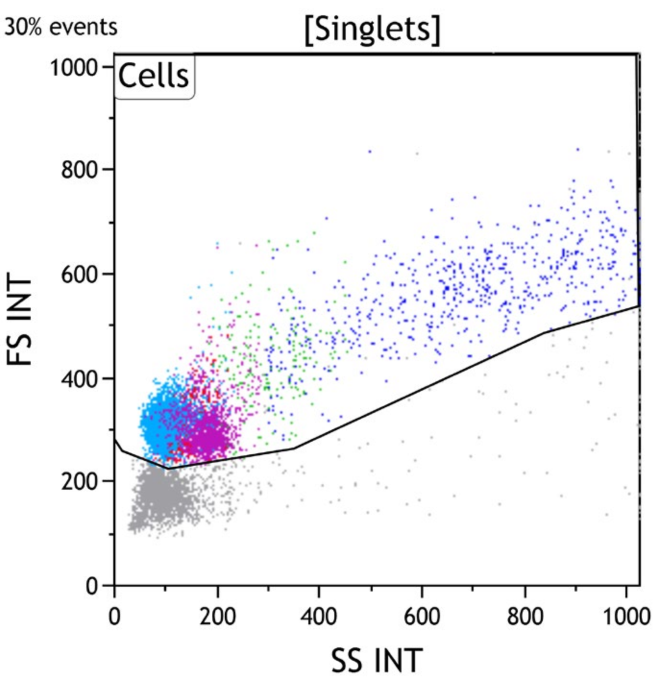

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

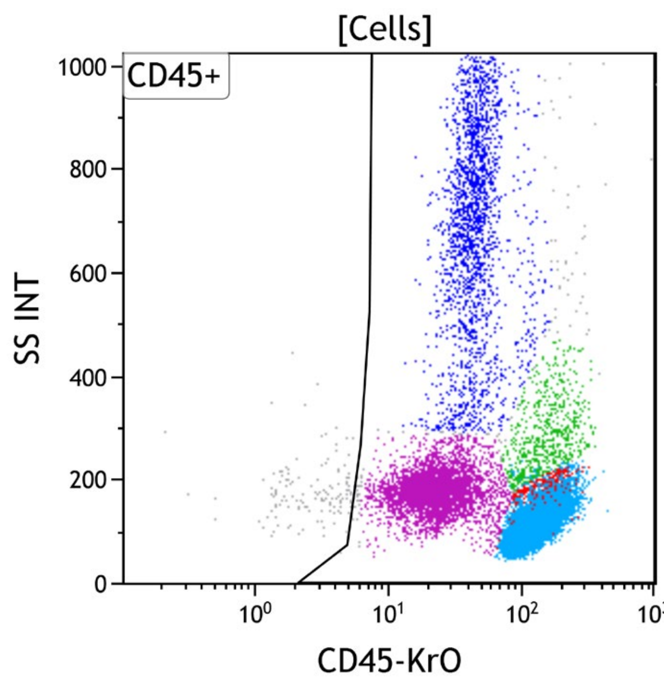

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

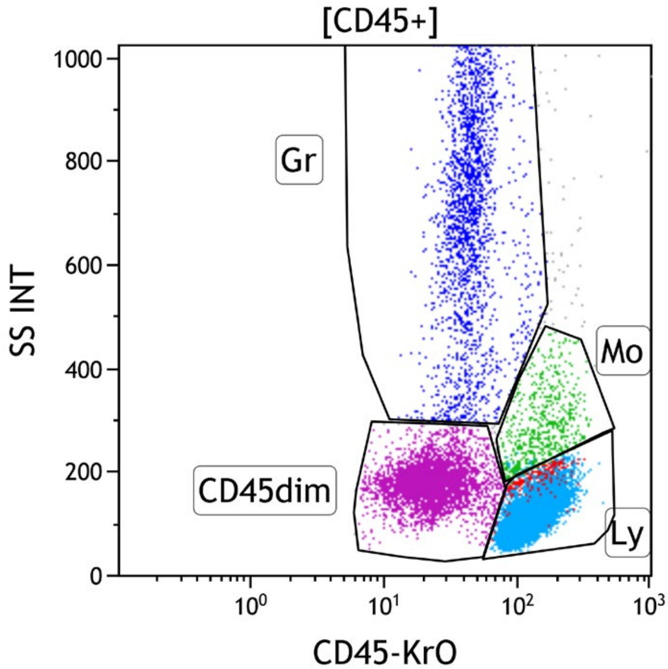

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the increased number of progenitors (purple).

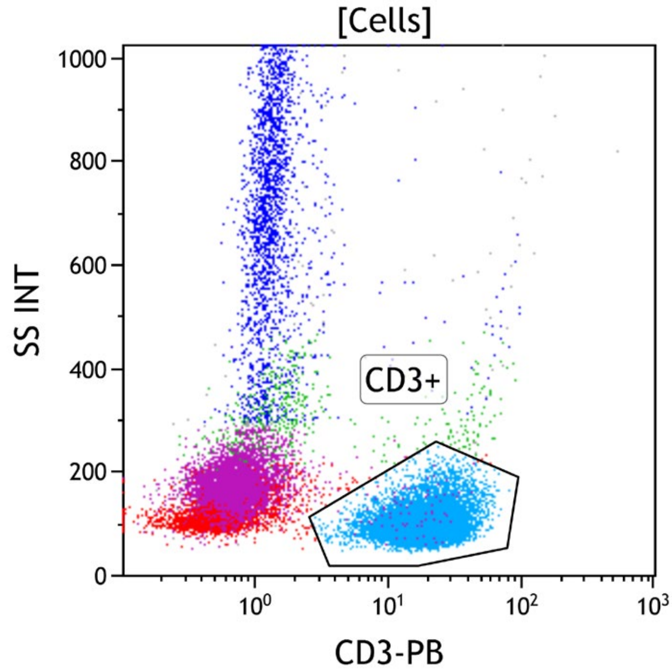

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies surface CD3 positive cells (aqua). Surface CD3 typically expressed on mature T cell. The aberrant population (purple) is negative for CD3.

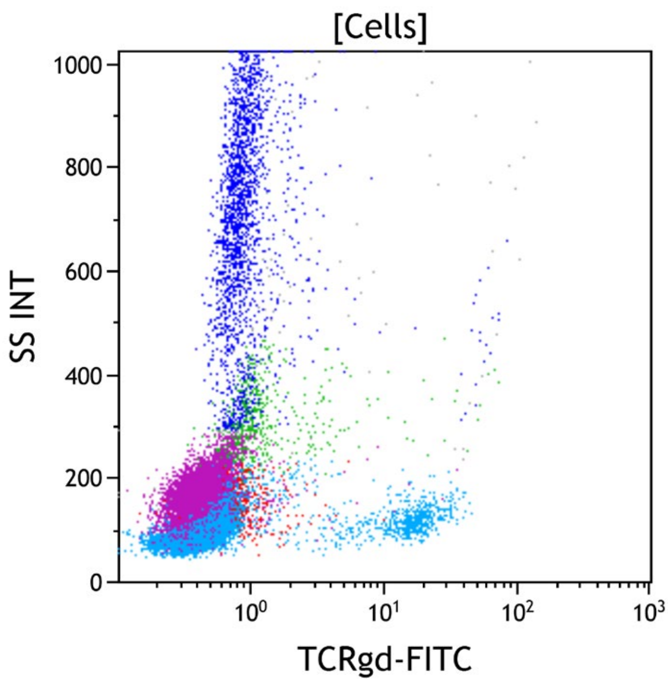

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). The aberrant population (purple) is negative for TCRγδ.

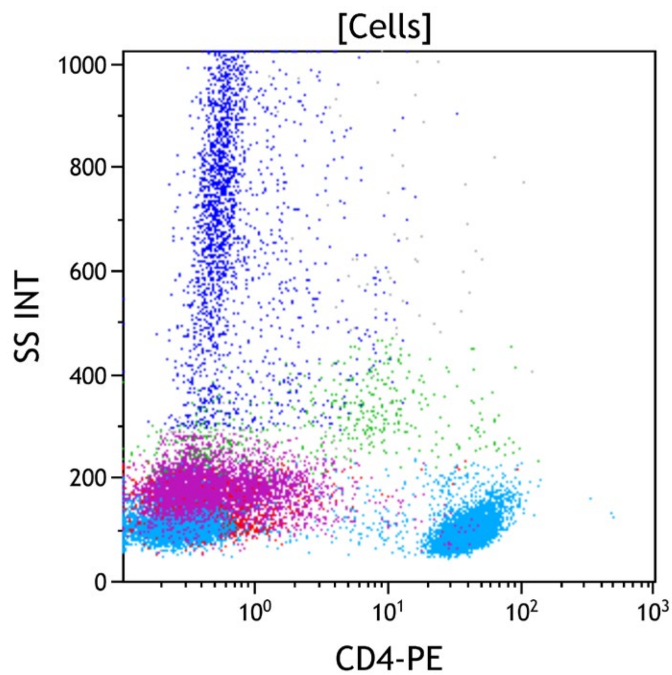

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. The aberrant population (purple) is negative for CD4.

T Cell Tube

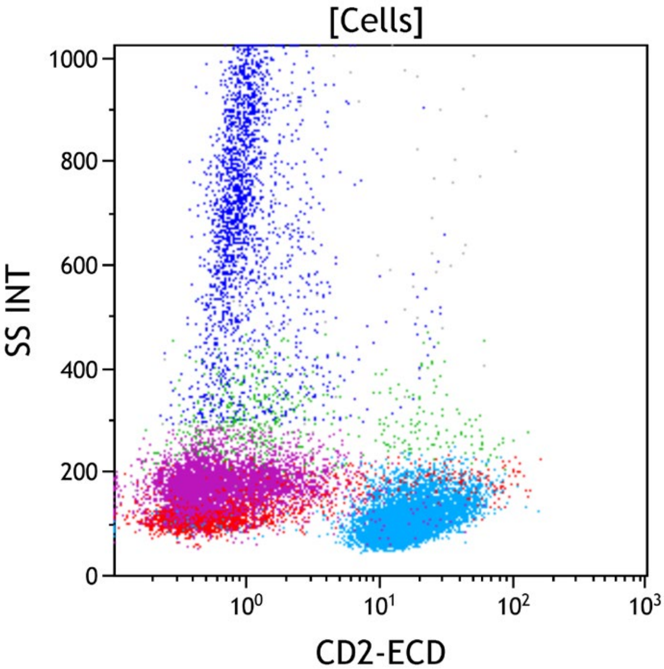

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The aberrant population (purple) is negative for CD2.

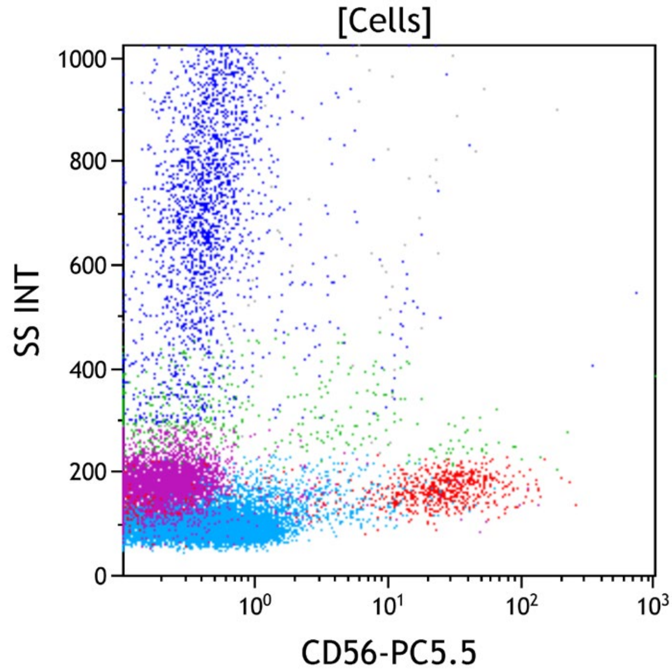

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The aberrant population (purple) is negative for CD56.

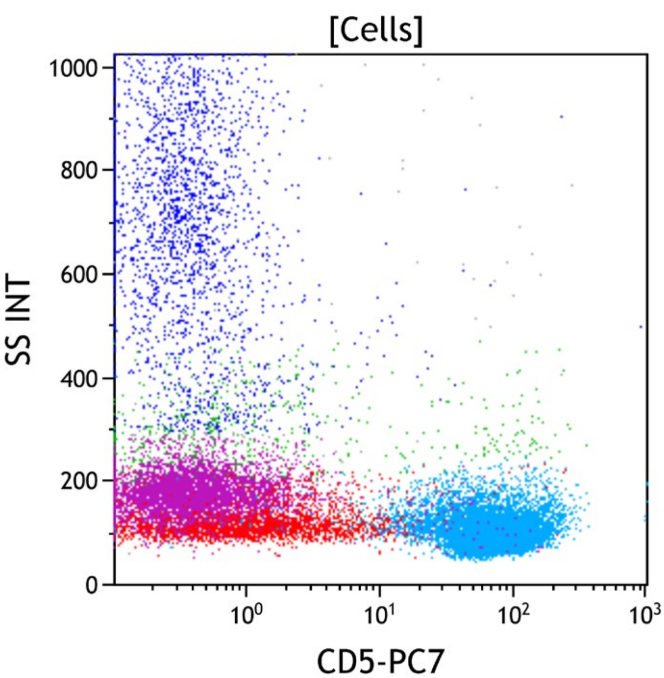

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The aberrant population (purple) is negative for CD5.

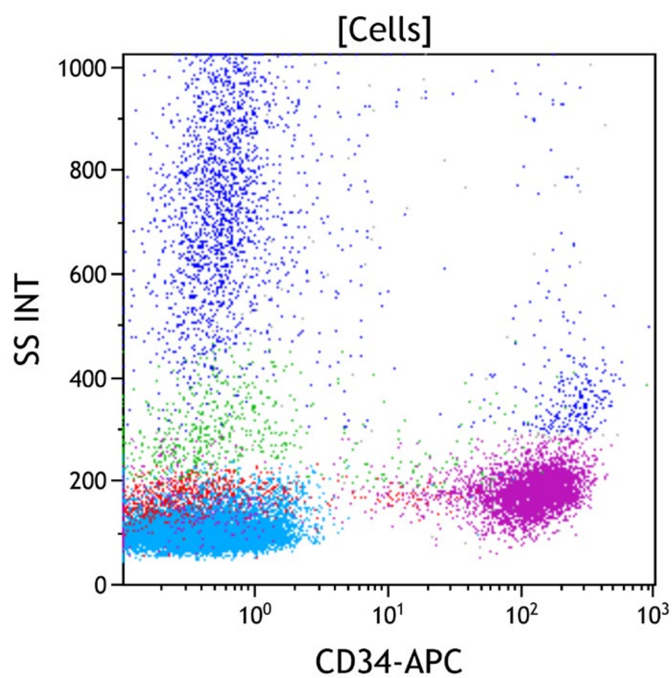

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The aberrant population (purple) is strongly positive for CD34.

T Cell Tube

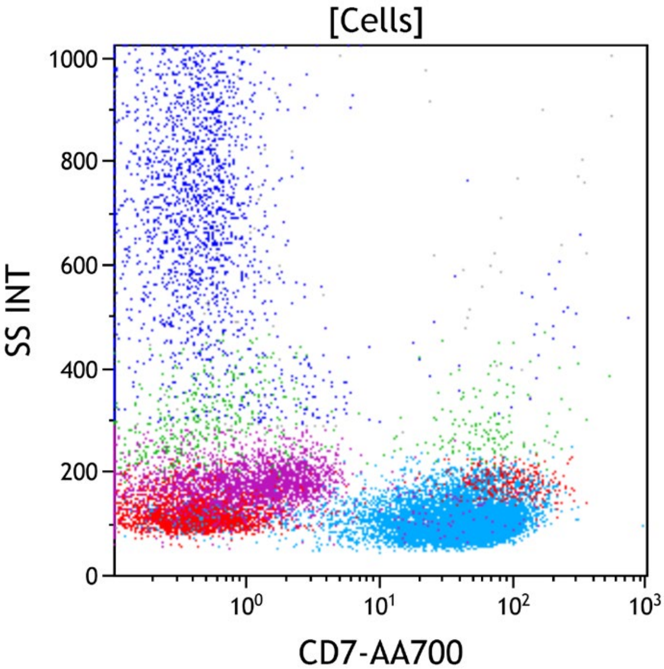

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The aberrant population (purple) is negative for CD7.

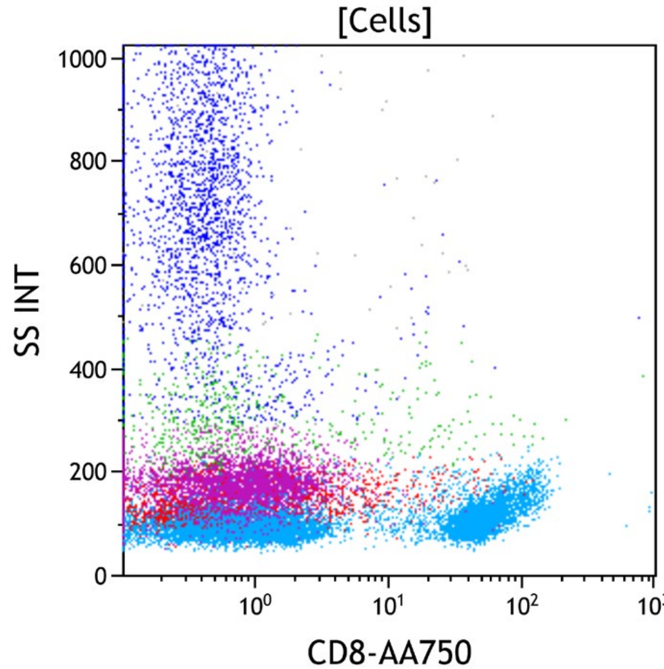

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The aberrant population (purple) is negative for CD8.

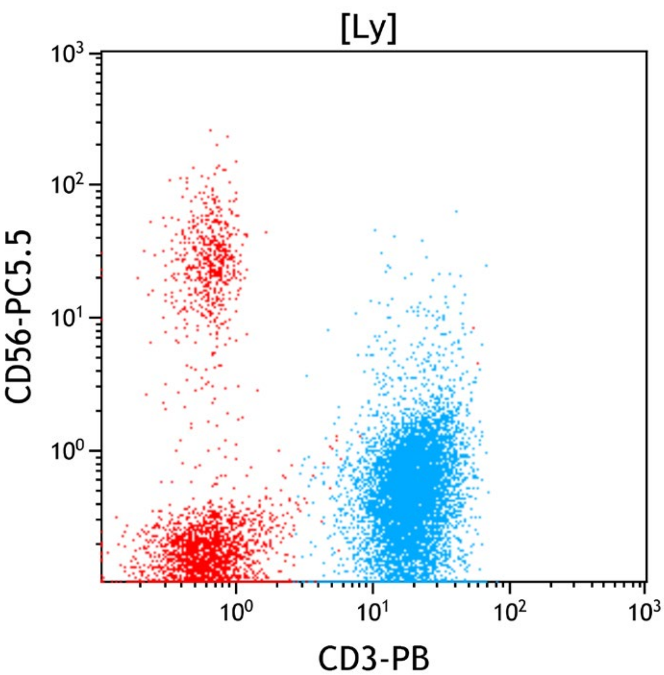

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

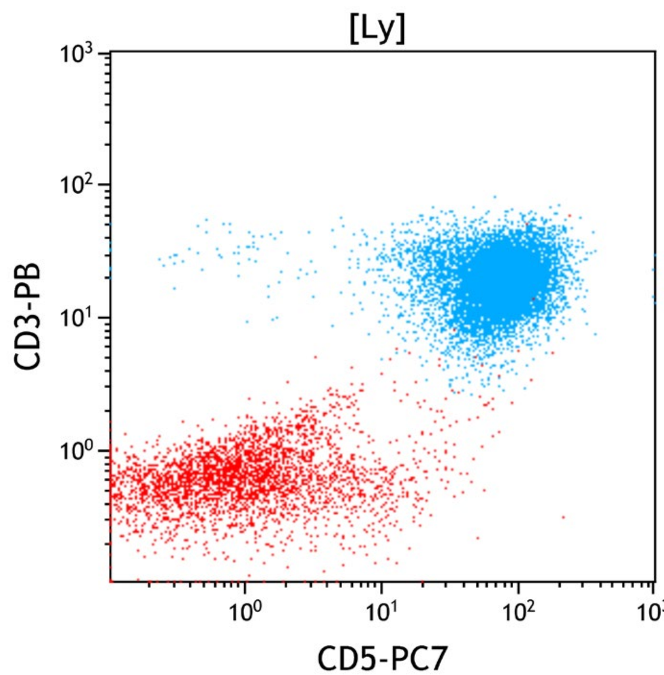

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red).

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T Cell Tube

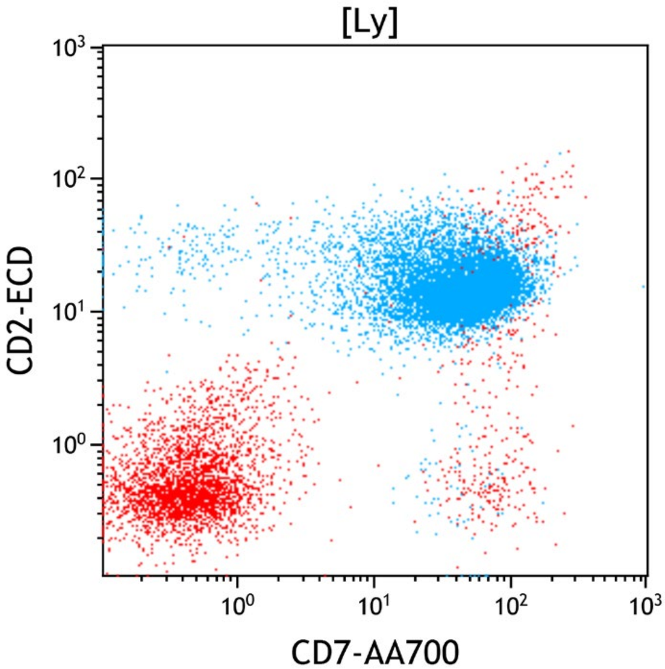


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

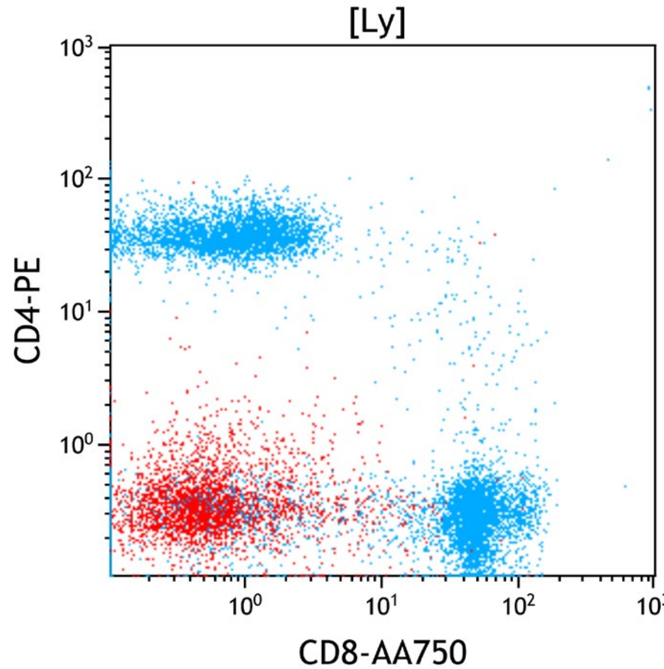


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

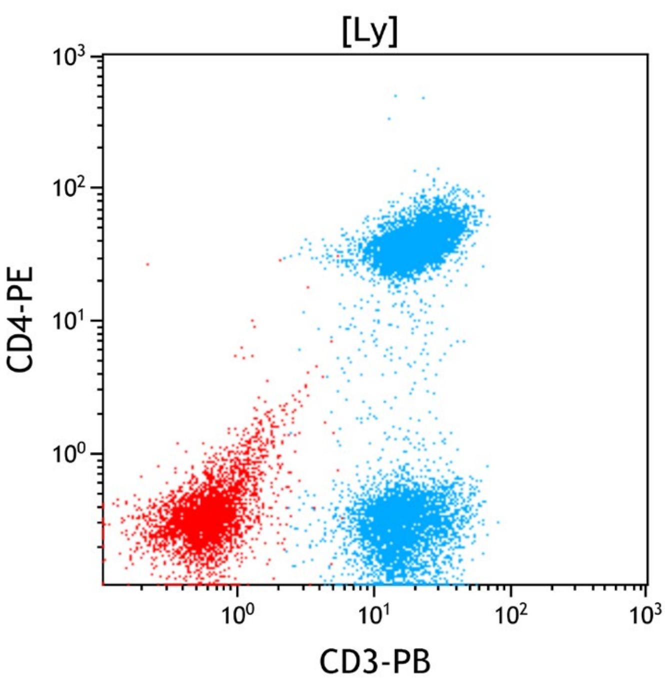


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

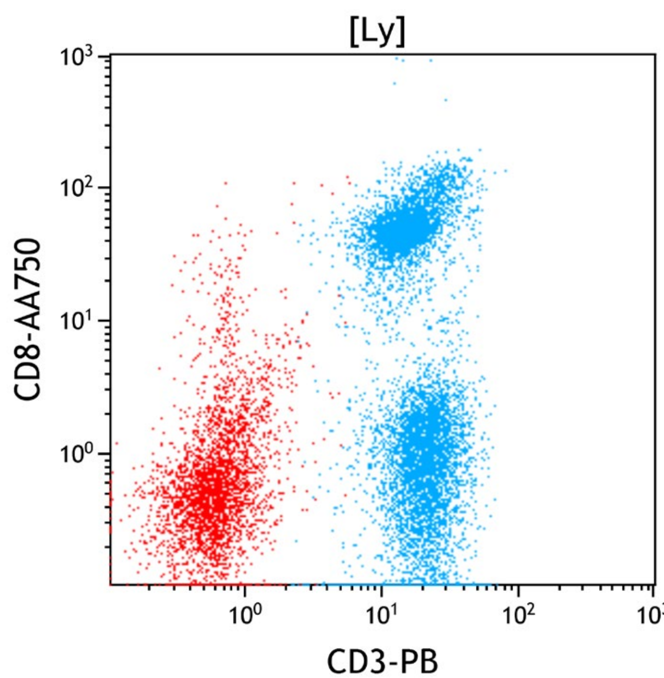


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

T Cell Tube

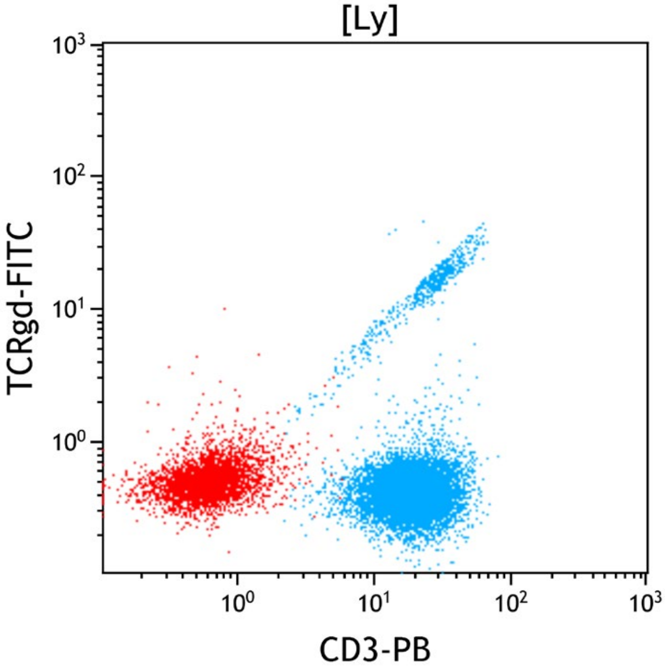
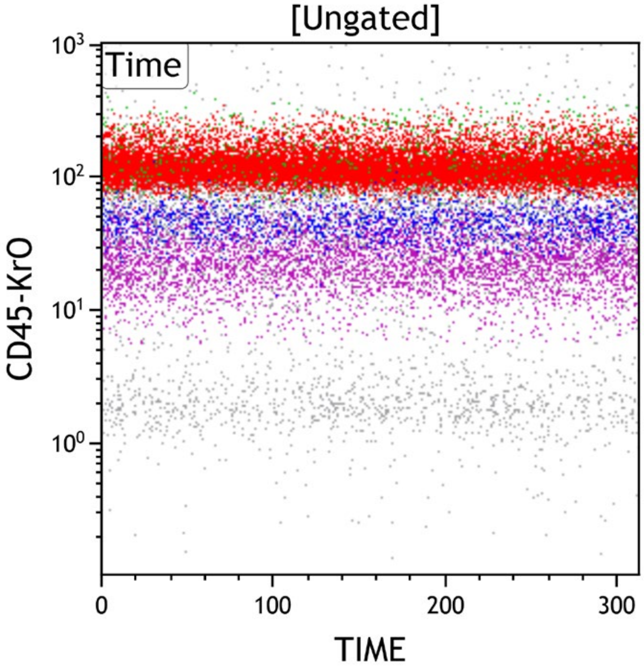


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.



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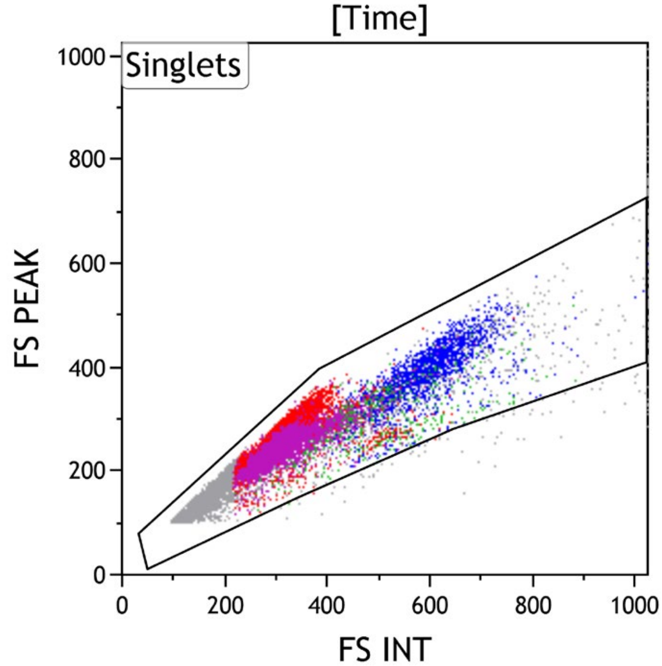

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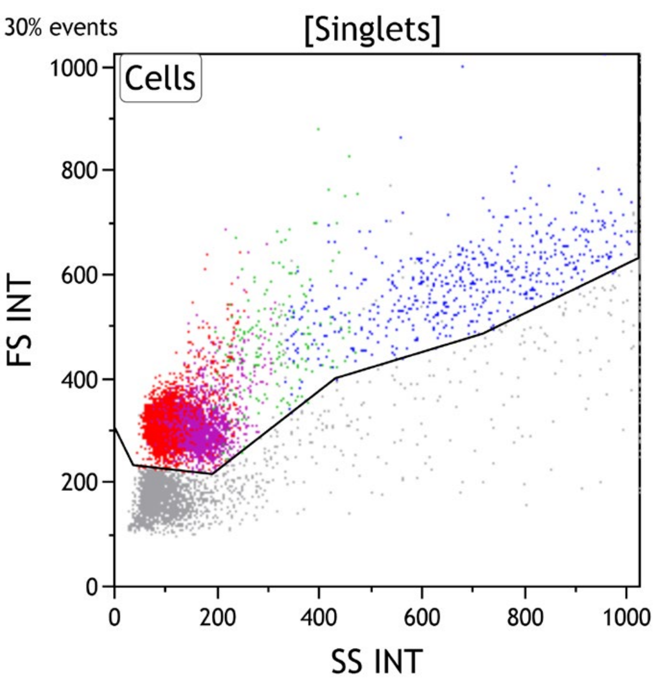

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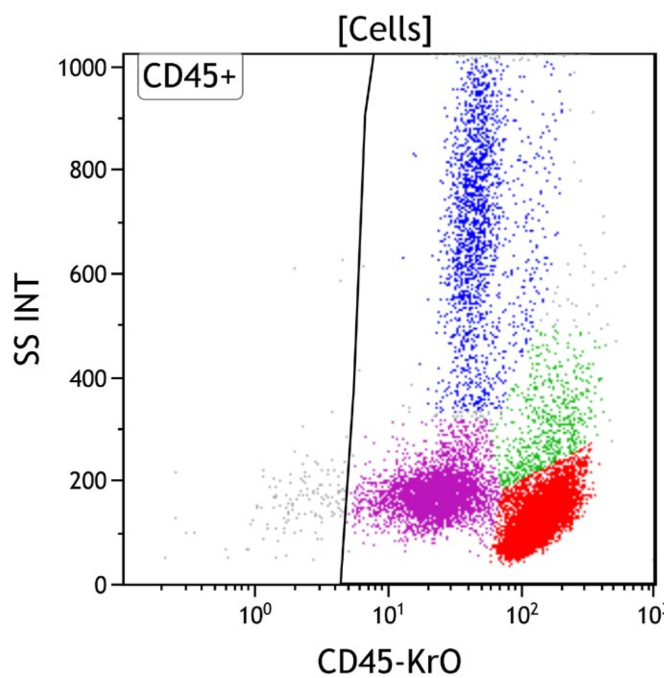

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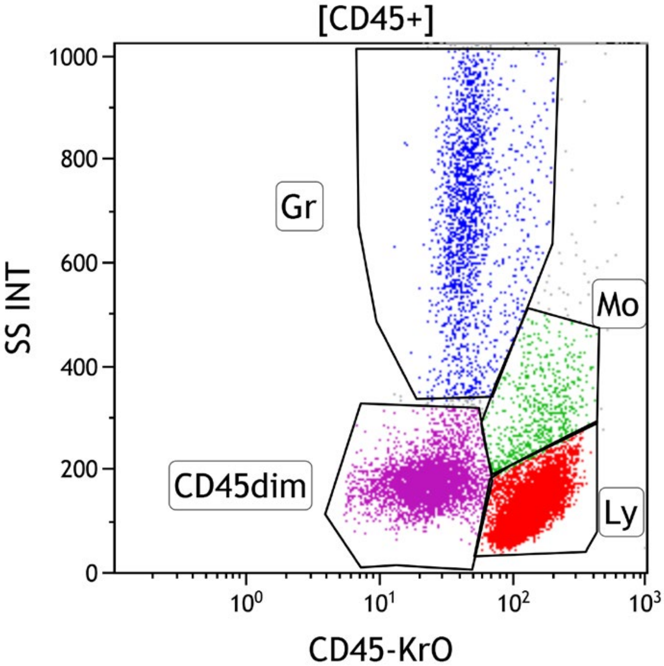

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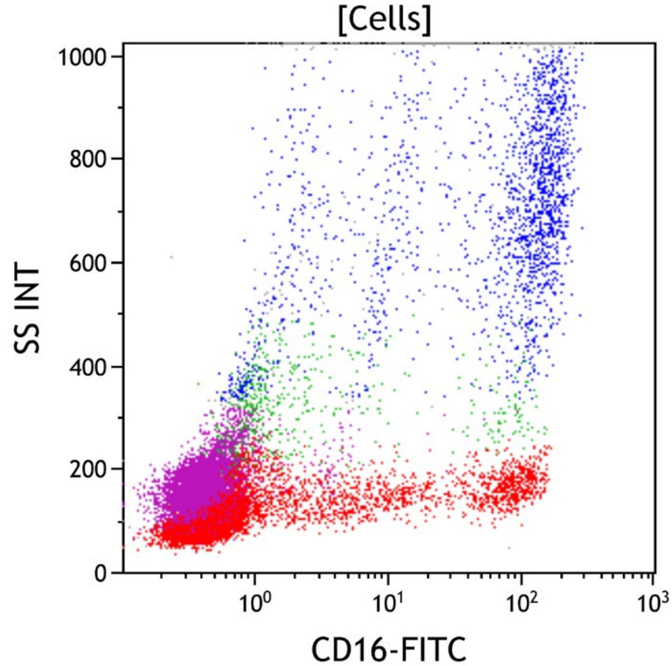


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on granulocytes (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The aberrant population (purple) is negative for CD16.

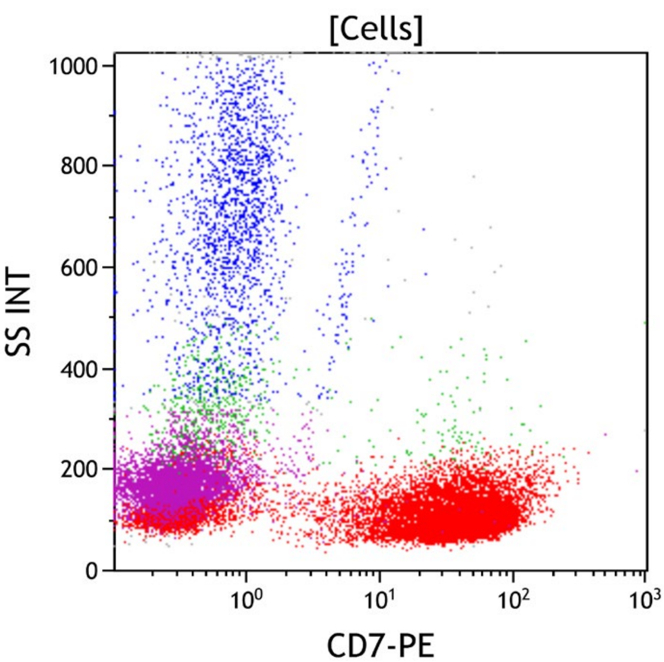


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The aberrant population (purple) is negative for CD7.

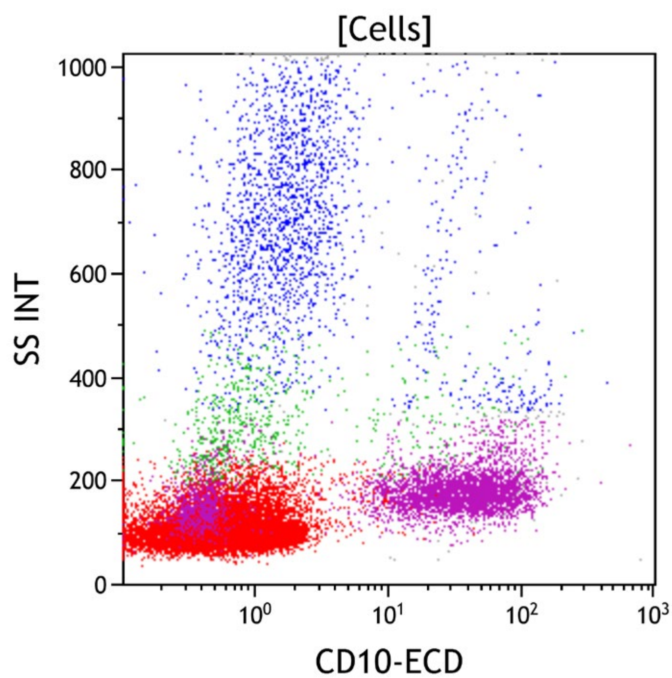


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show variable dim expression of CD10. The aberrant population (purple) is positive for CD10.

M1 Cell Tube

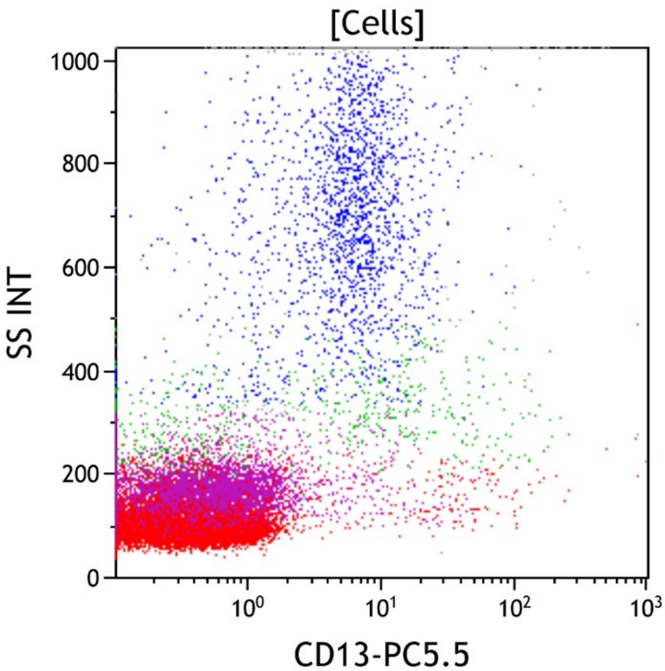


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature granulocytes (in blue, with high side scatter) and on mature monocytes (green). The aberrant population (purple) is negative for CD13.

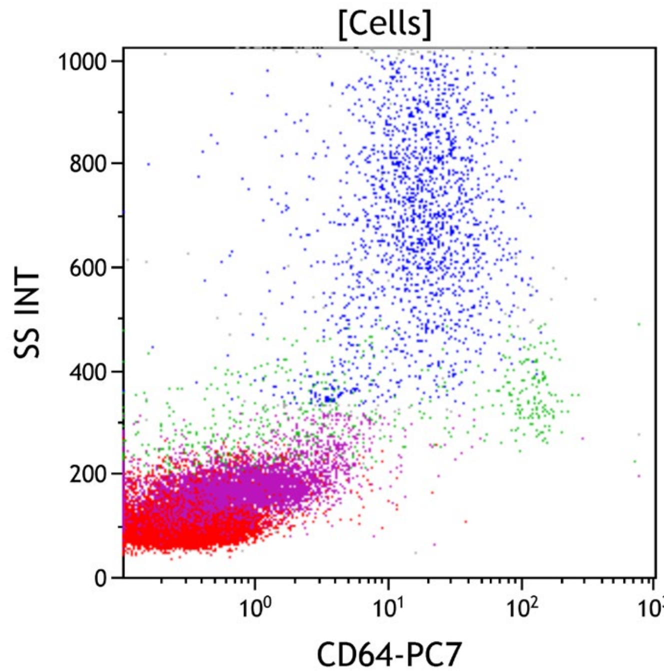


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green. CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The aberrant population (purple) is negative for CD64.

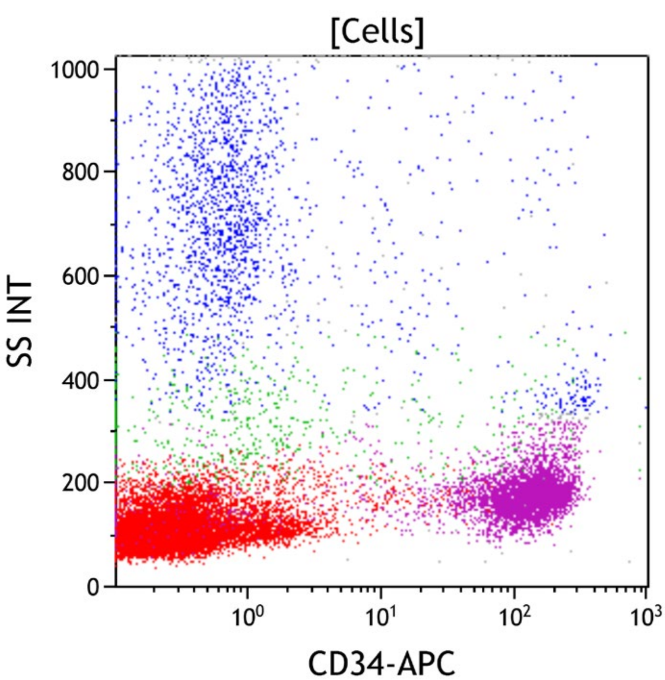


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The aberrant population (purple) is positive for CD34.

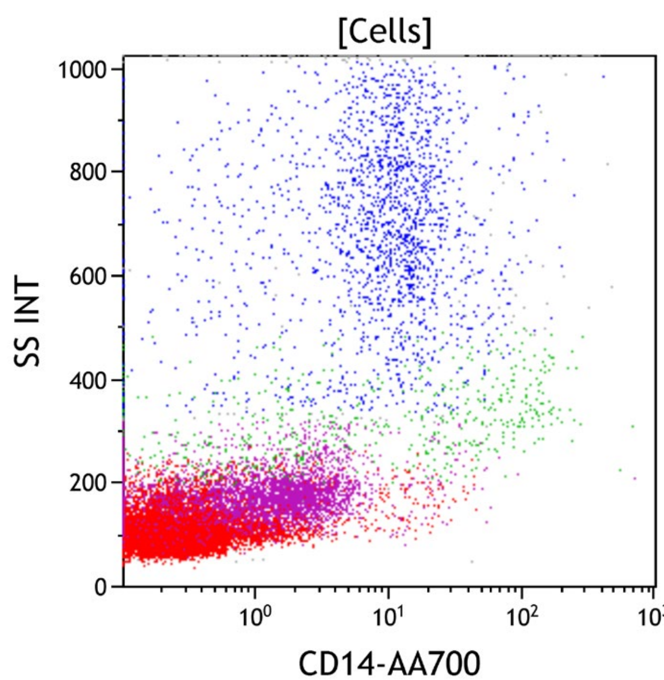


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on granulocytes (blue) at a low level. The aberrant population (purple) is negative for CD14.

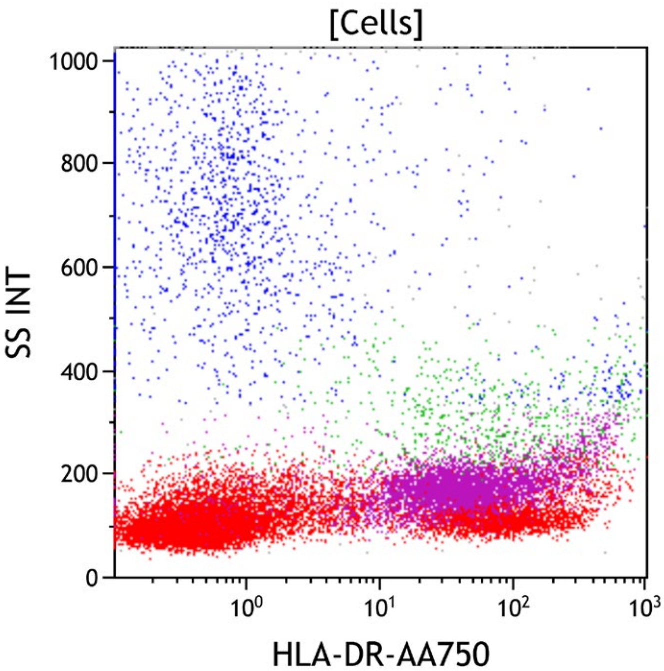


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). The aberrant population (purple) is positive for HLA-DR.

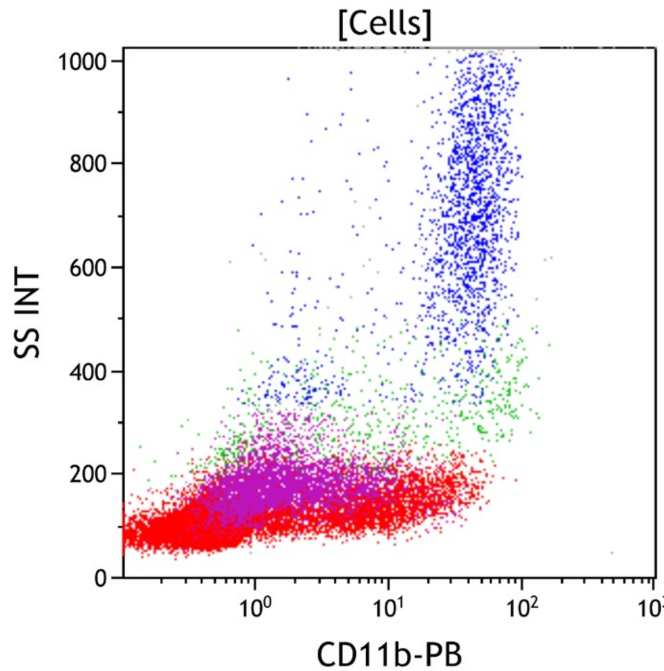


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (blue) and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The aberrant population (purple) is mostly negative for CD11b.

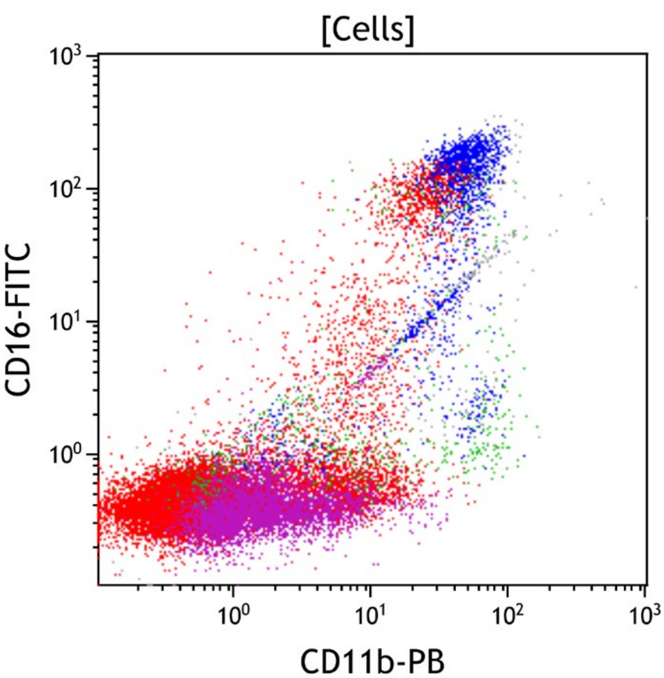


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue), and NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is negative for CD11b and CD16.

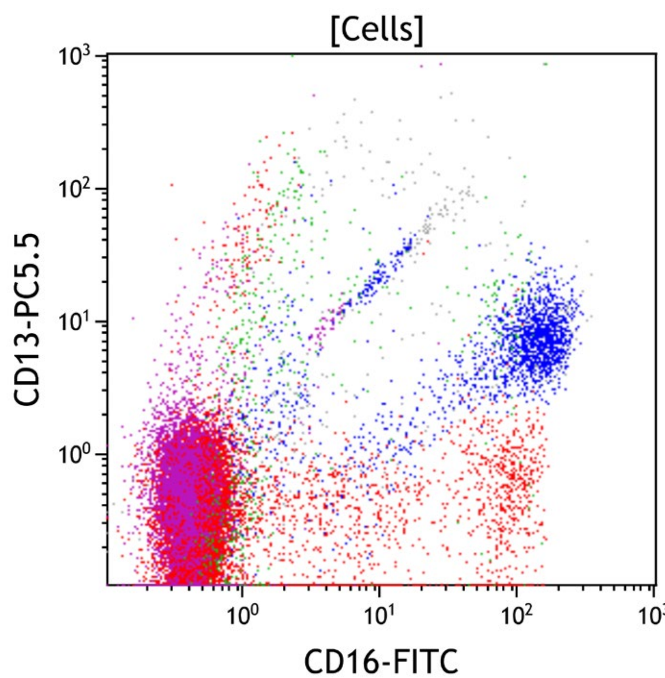


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is negative for CD13 and CD16.

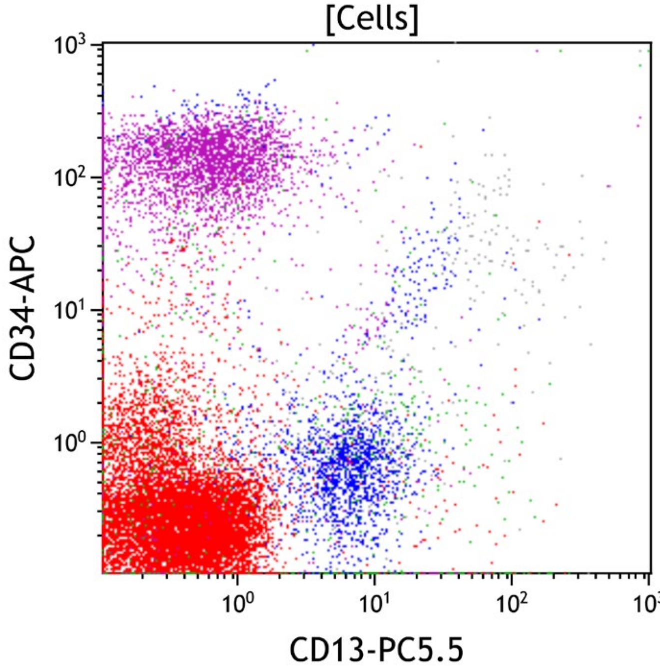


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. The aberrant population (purple) is positive for CD34 and negative for CD13.

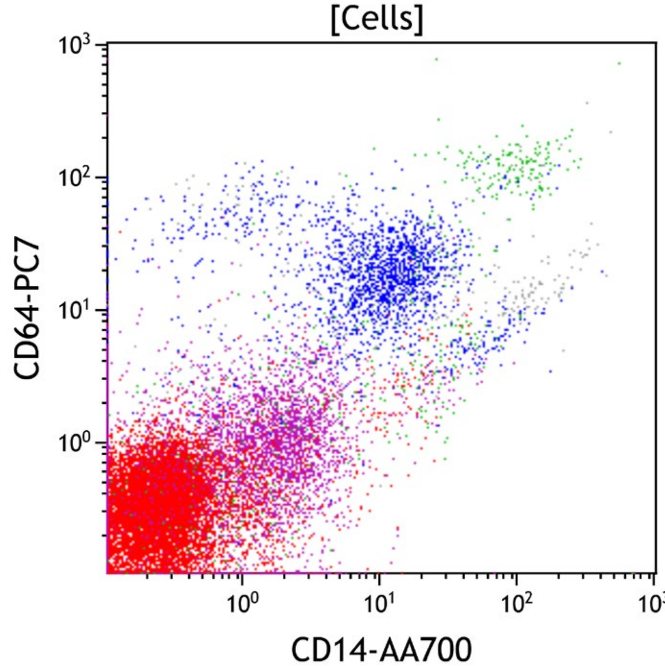


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes (blue). The aberrant population (purple) is negative for CD14 and CD64. The apparent CD14 expression is due to increased background from the adjacent bright CD34 APC.

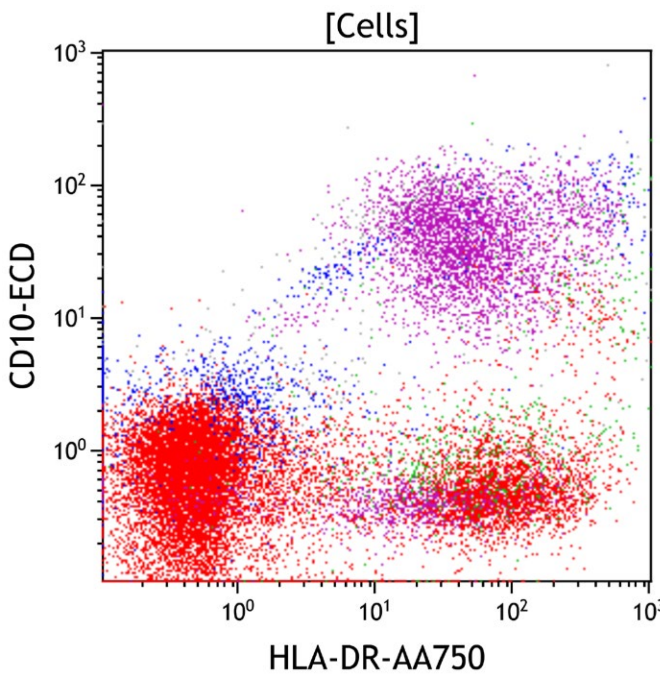


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells, plasmacytoid dendritic cells and CD34 positive progenitors. CD10 is expressed by granulocytes (blue).The aberrant population (purple) is positive for CD10 and HLA-DR.

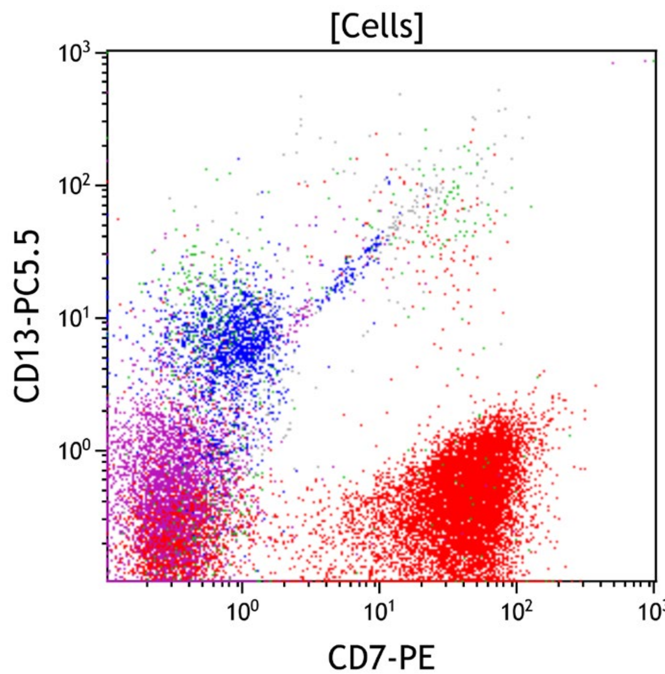
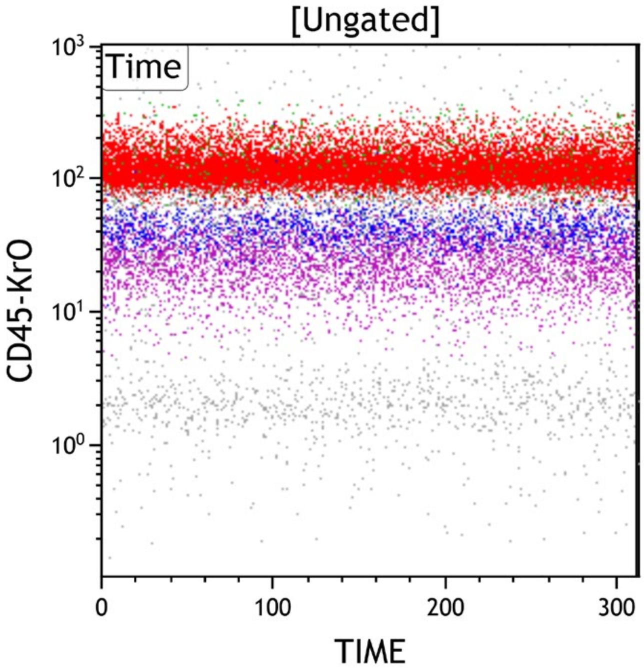


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes (green), and basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The aberrant population (purple) is negative for CD7 and CD13.



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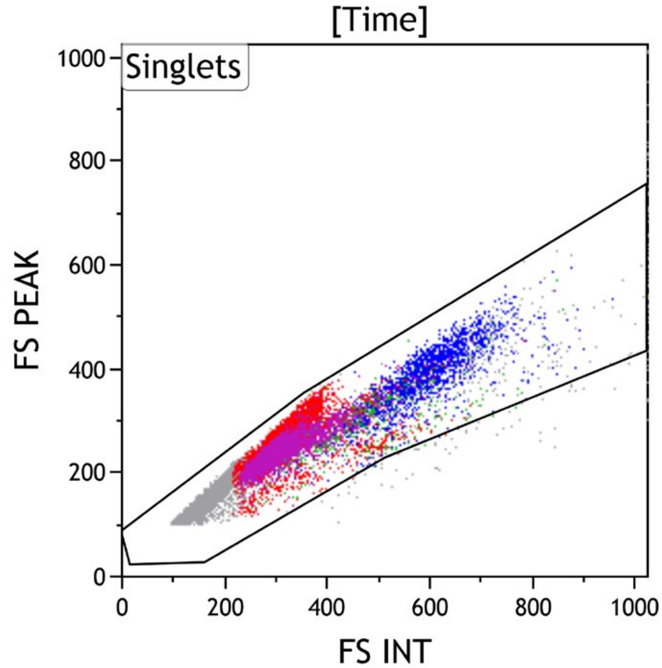

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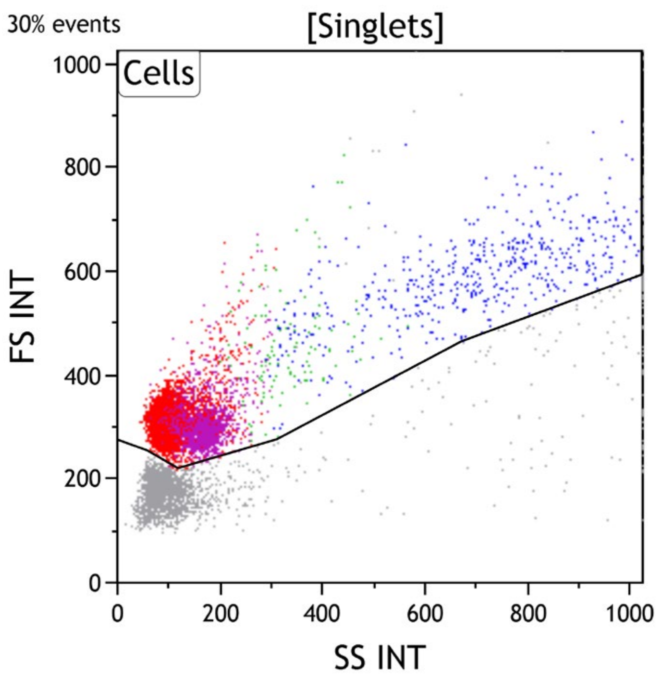

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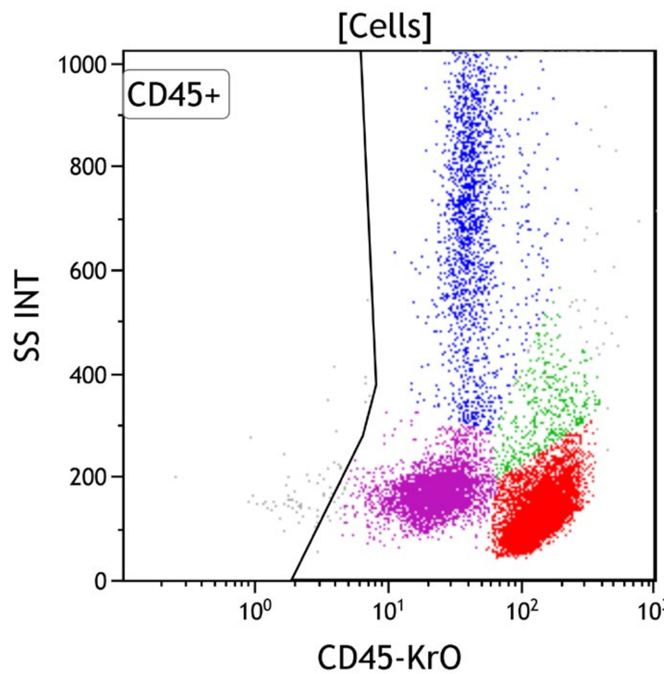

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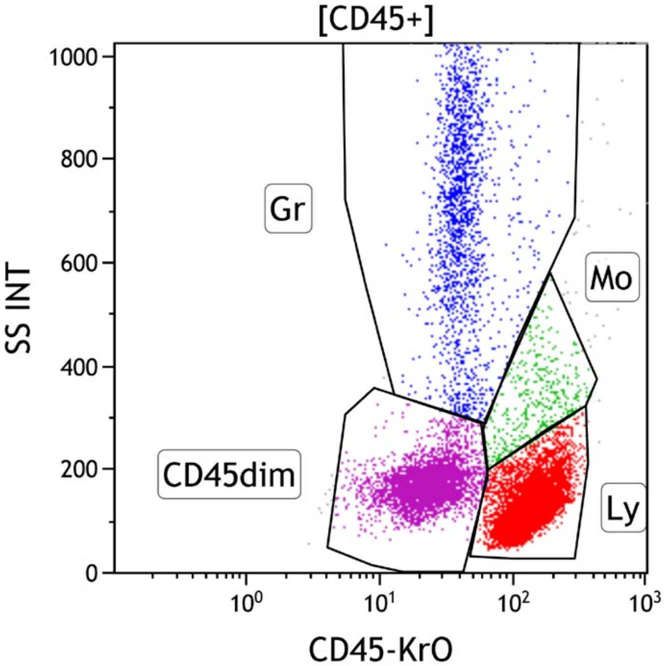


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the increased number of progenitors (purple).

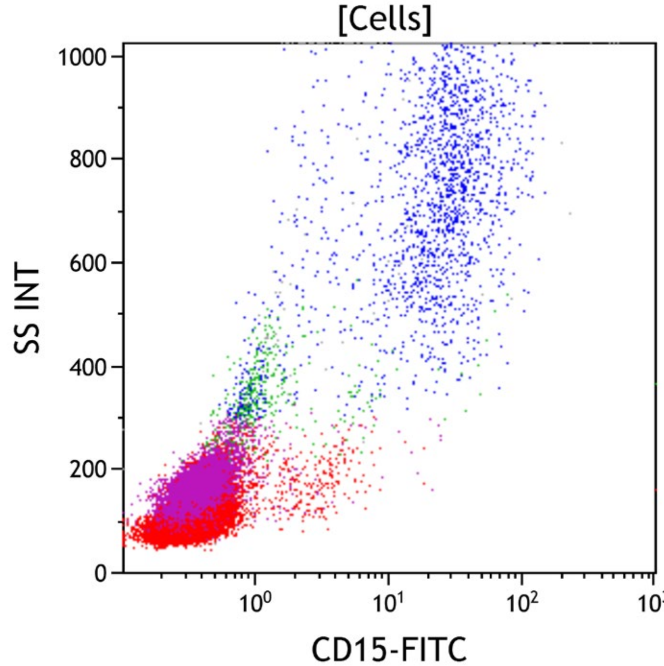


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on mature granulocytes (in blue) and at a lower level on monocytes (green). The aberrant population (purple) is negative for CD15.

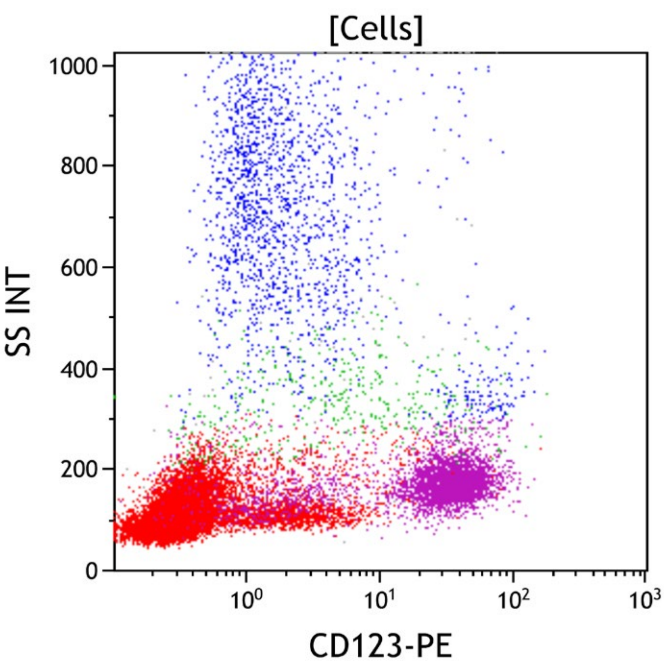


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The aberrant population (purple) is strongly positive for CD123.

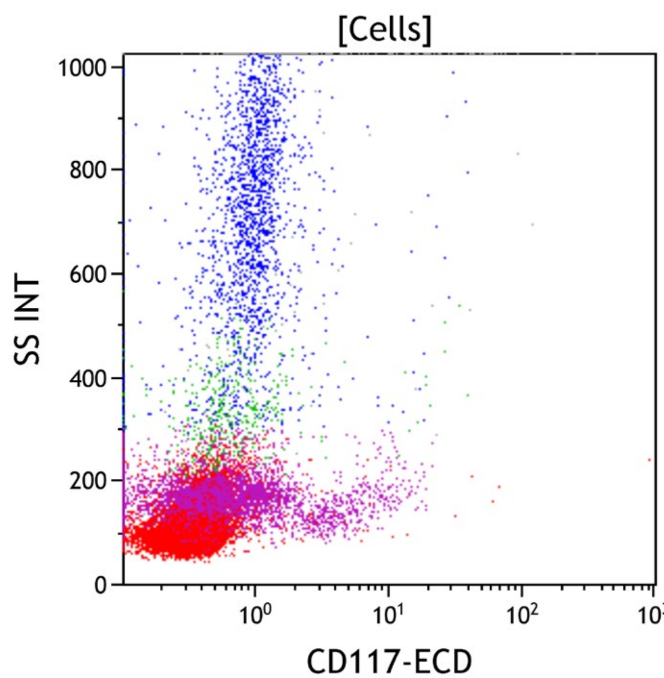


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The aberrant population (purple) is negative for CD117. The small number of CD117 positive cells are normal myeloid progenitors.

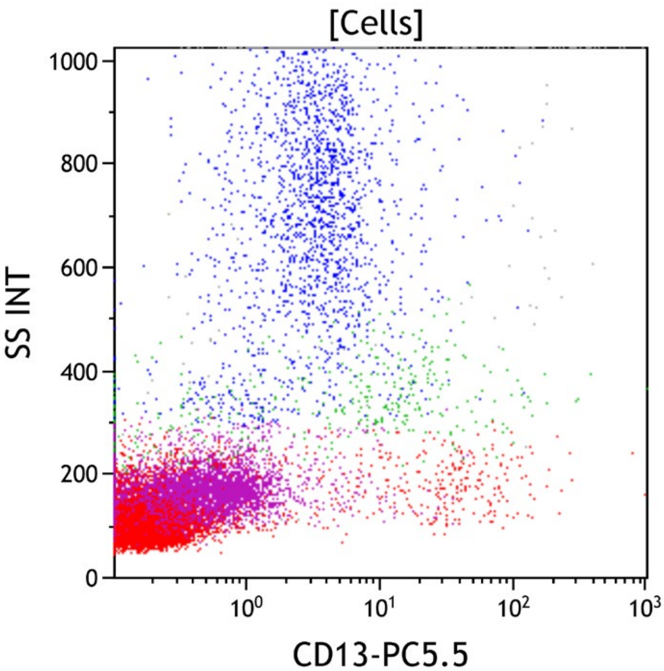


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (in blue, with high side scatter) and on mature monocytes (green). The aberrant population (purple) is negative for CD13.

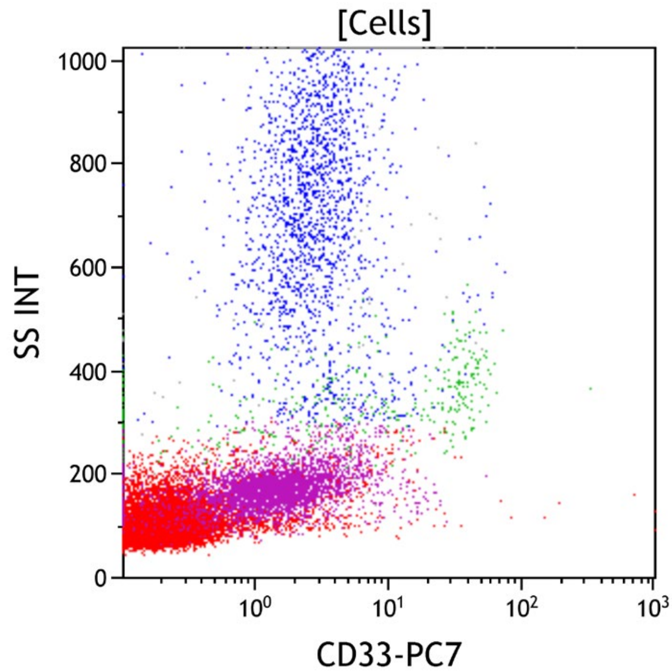


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The aberrant population (purple) displays low CD33 expression.

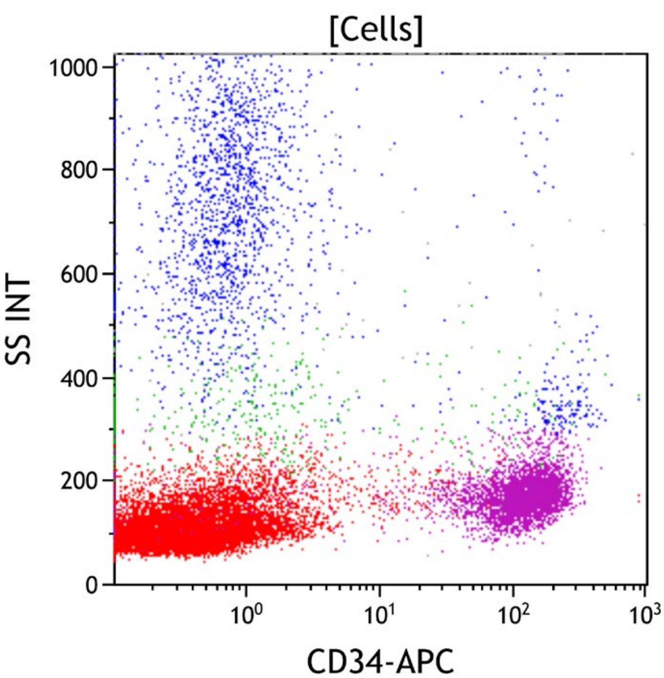


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive for CD34.

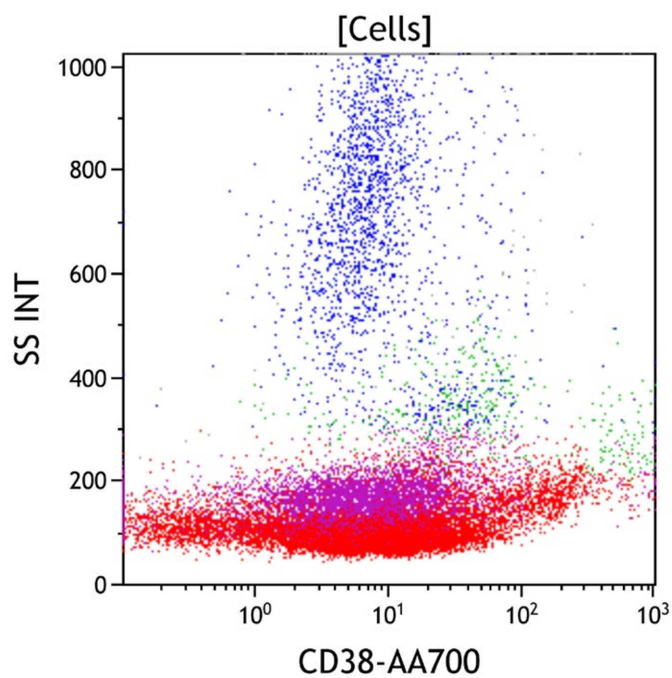


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated lymphocytes (red). The aberrant population (purple) is positive for CD38 at a level lower than that of normal immature B cells.

M2 Cell Tube

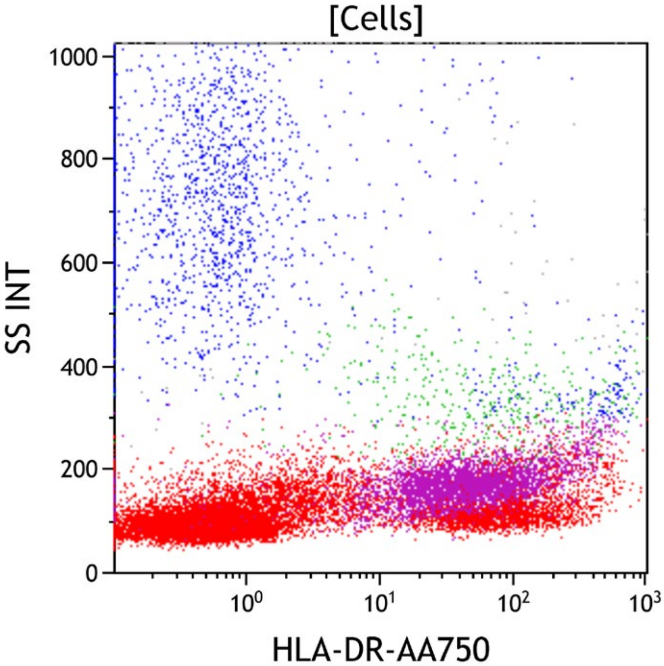


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red). The aberrant population (purple) is positive for HLA-DR.

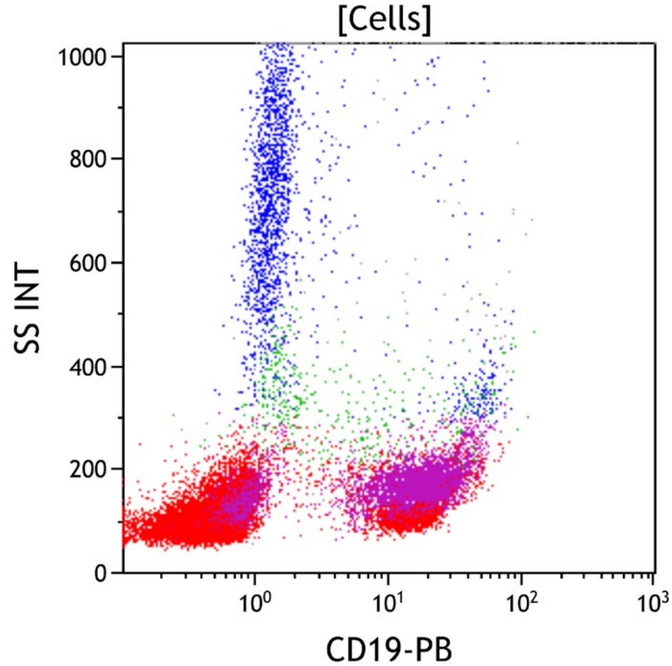


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The mature B cell population (red) is relatively expanded compared with normal. The aberrant population (purple) is positive for CD19.

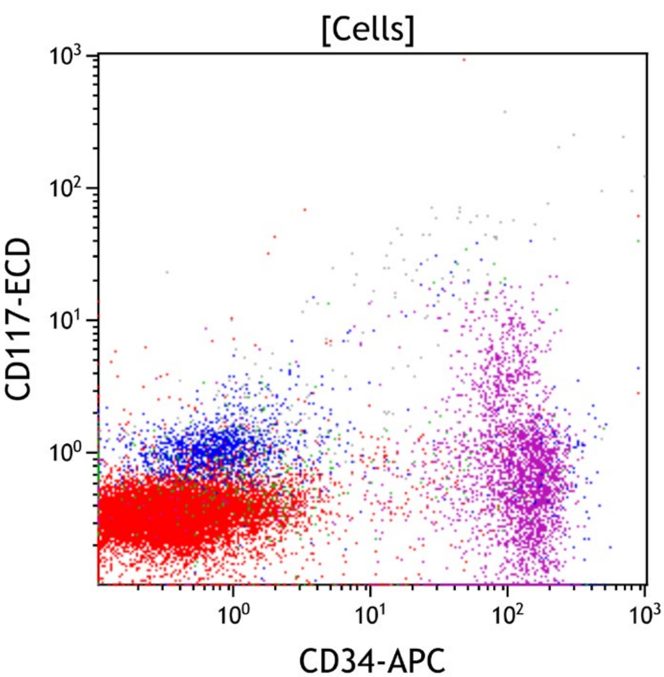


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The aberrant population (purple) is positive for CD34 and negative for CD117. The small number of CD34 and CD117positive cells are normal myeloid progenitors.

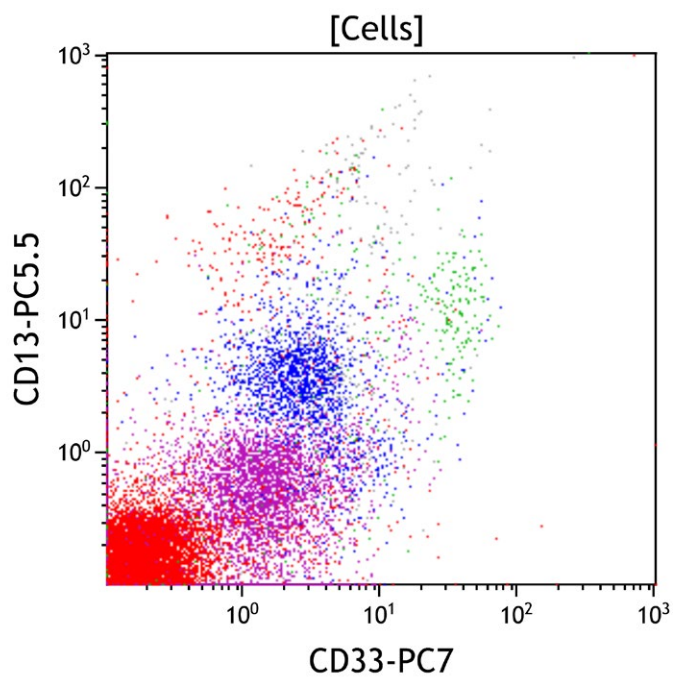


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Lymphocytes largely do not express either CD13 or CD33 (red). The aberrant population (purple) displays low CD33 expression and is negative for CD13.

M2 Cell Tube

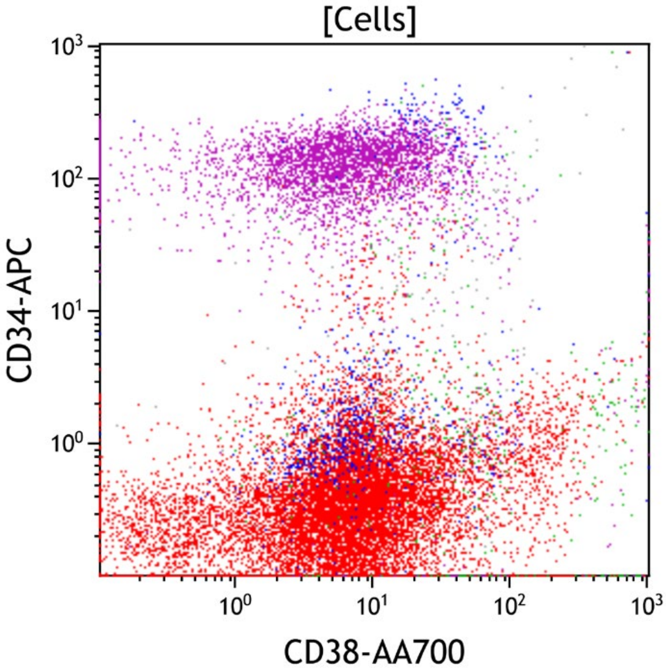


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. The aberrant population (purple) is positive for CD34 and express CD38 at lower and more variable level than that seen on normal immature B cells.

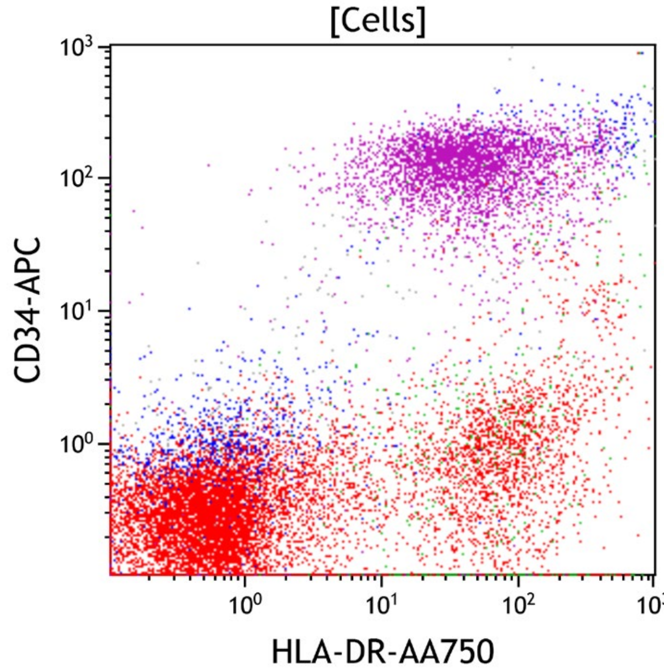


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors. CD34 is expressed on early progenitors. The aberrant population (purple) is positive for CD34 and HLA-DR.

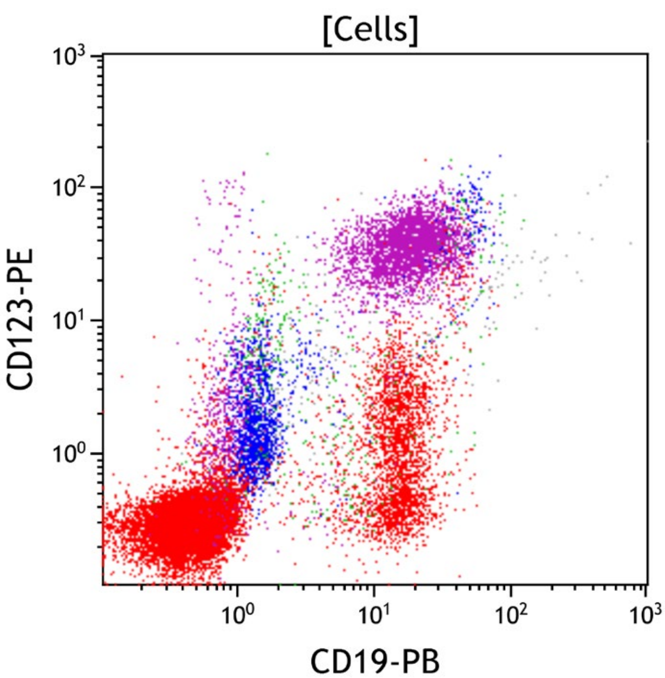


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34 positive progenitors. CD19 positive B cells (red) normally do not express significant CD123. The aberrant population (purple) is positive for CD19 and CD123.

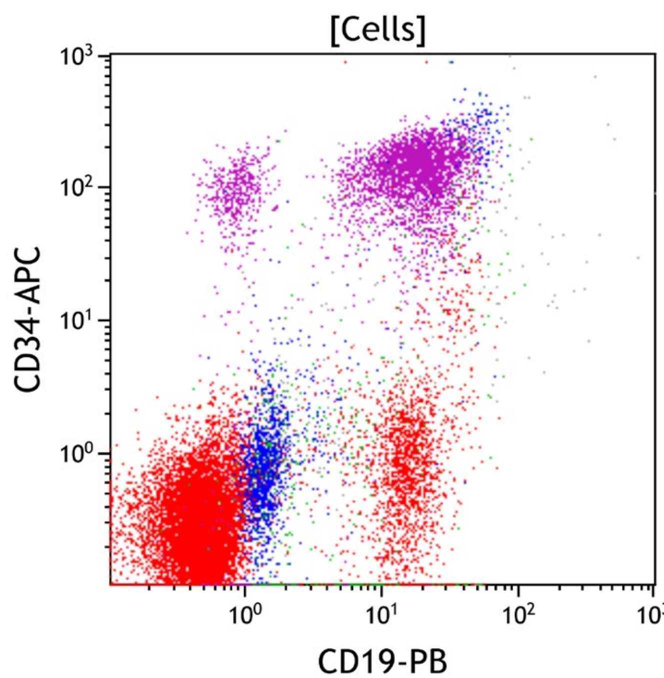


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red) do not express CD34. The aberrant population (purple) is positive for CD19 and CD34. The small population of CD34 positive and CD19 negative cells (top left) are normal myeloid progenitors.

M2 Cell Tube

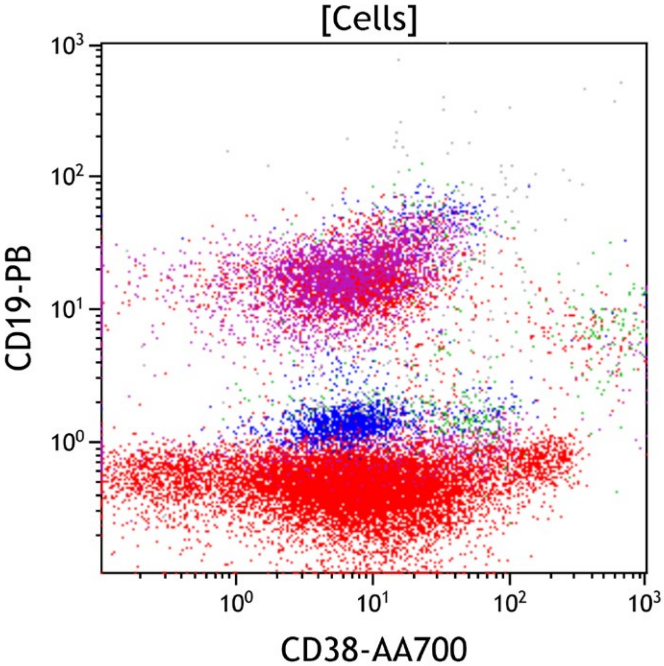


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show intermediate CD38. The aberrant population (purple) is positive for CD19 and CD38. The level of CD38 expression is lower than that seen on normal immature B cells.

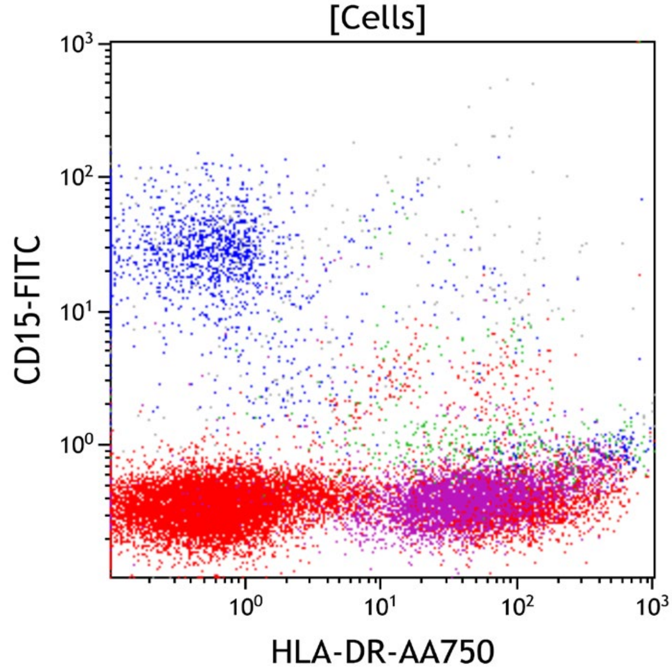


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes (blue) do not express HLA-DR. The aberrant population (purple) is positive for HLA-DR and negative for CD15.

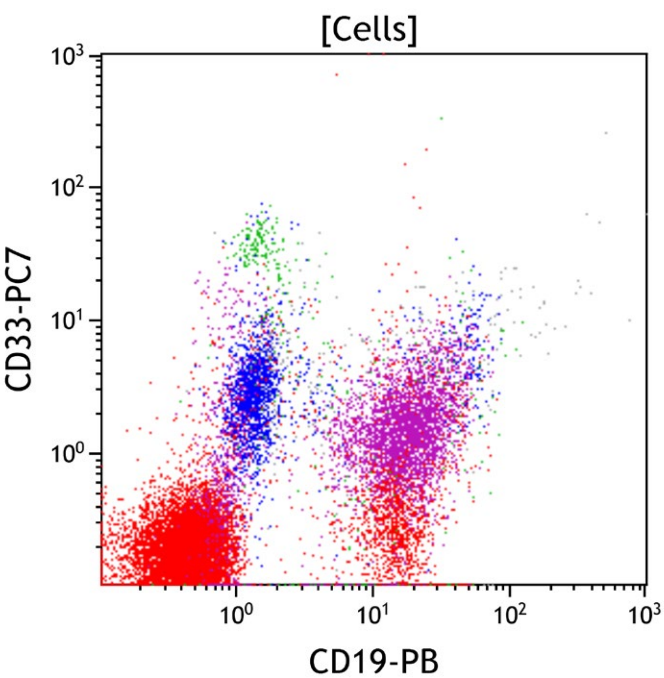


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed on monocytes (green) and granulocytes (blue). Mature CD19 positive B cells (red) do not normally express significant CD33. The aberrant population (purple) is positive for CD19 and low CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with low light scatter properties that express bright CD10, intermediate CD19, partial CD20, dim CD33, bright CD34, intermediate CD38, dim CD45, intermediate CD123, and bright HLA-DR without significant expression of other B, T, or myeloid markers. Compared with normal B cell precursors, the increased CD10, dim CD33, increased CD34, and presence of CD123 are aberrant. Morphology shows greater than 90% blasts, which in combination with the immunophenotypic findings is indicative of a B lymphoblastic leukemia/lymphoma.

Taken together, the findings in this case are most consistent with B lymphoblastic leukemia/lymphoma. Note that correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

Case #6: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Clinical Vignette

This 55-year-old male presents with lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

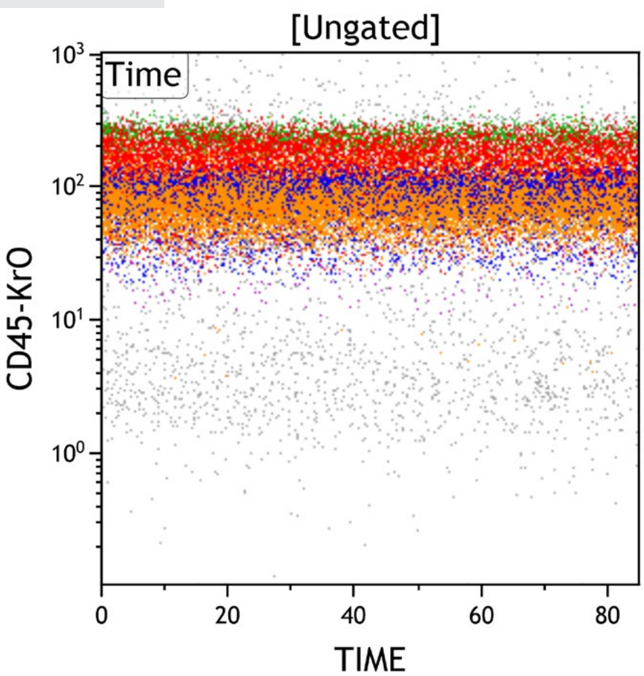


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

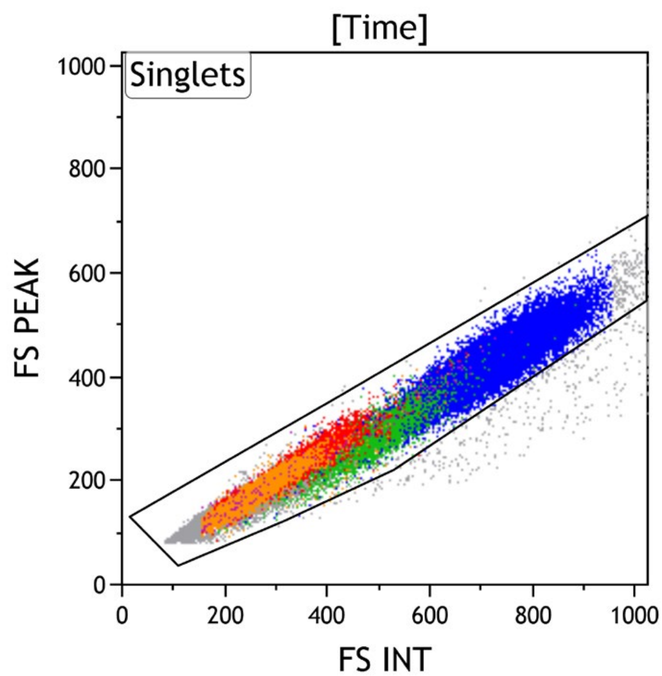
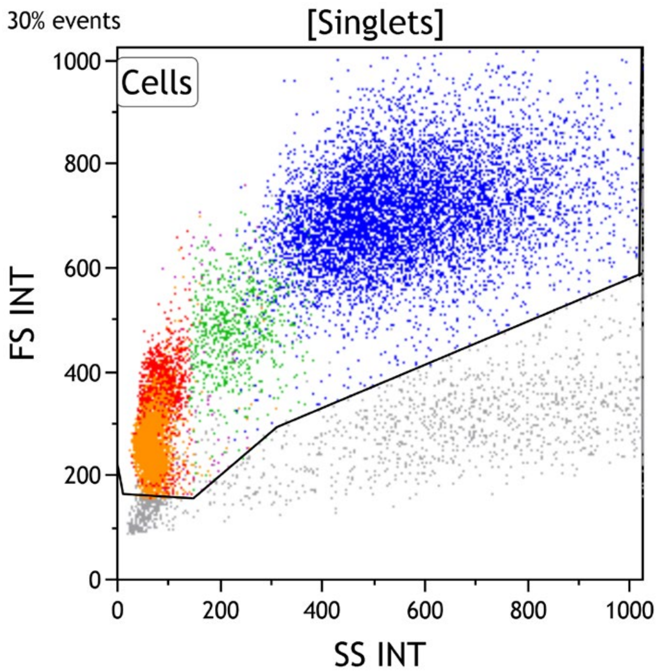


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube



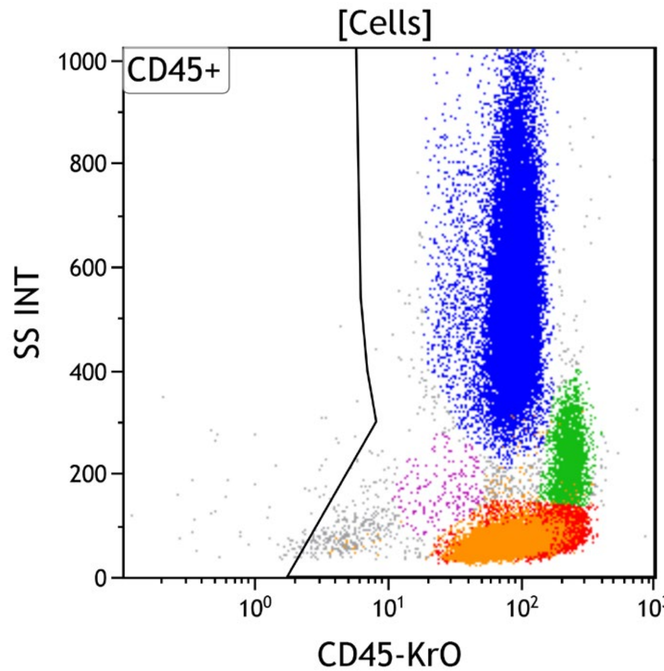
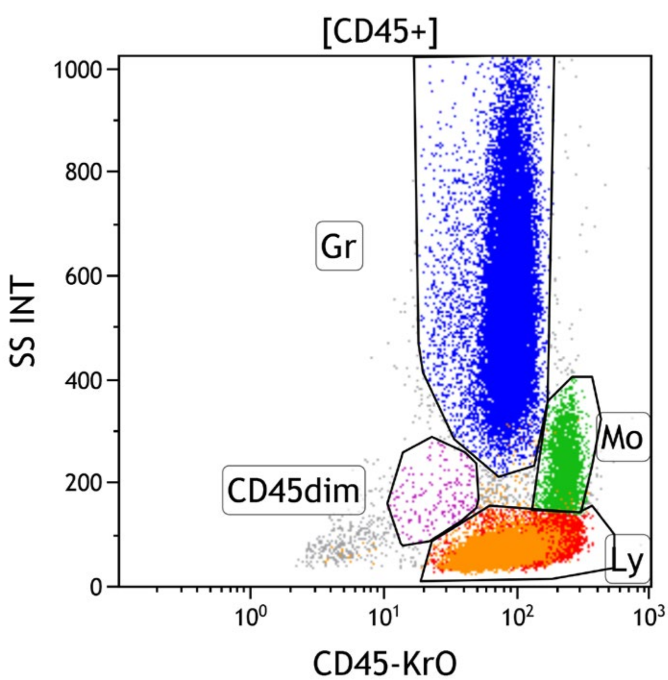


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.



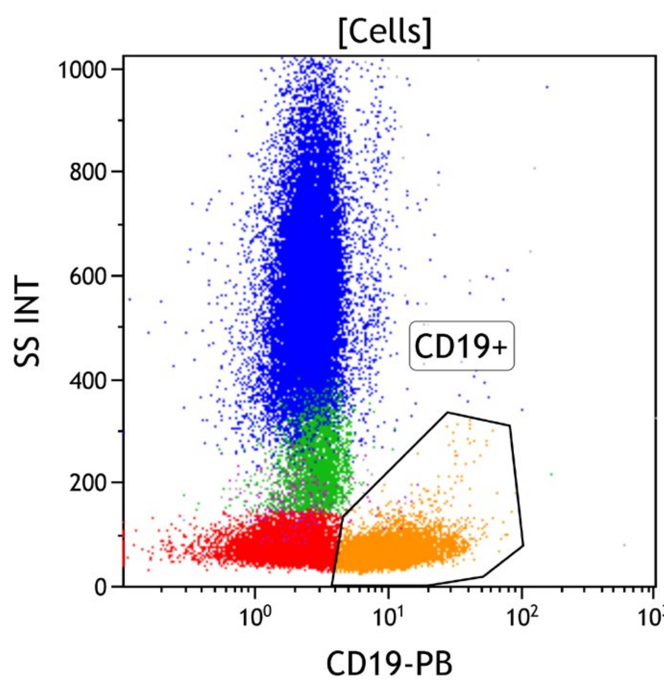


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note the relatively increased number of CD19 positive B cells in this sample.

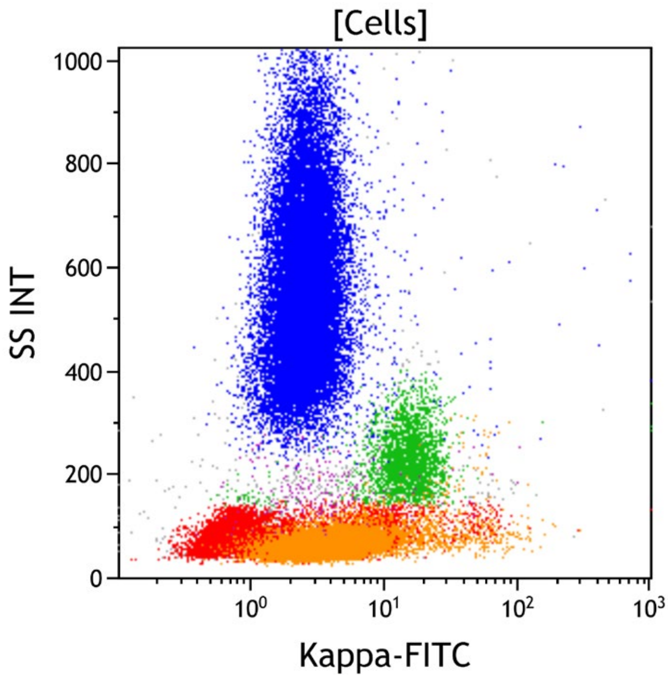


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) display low level kappa light chain expression.

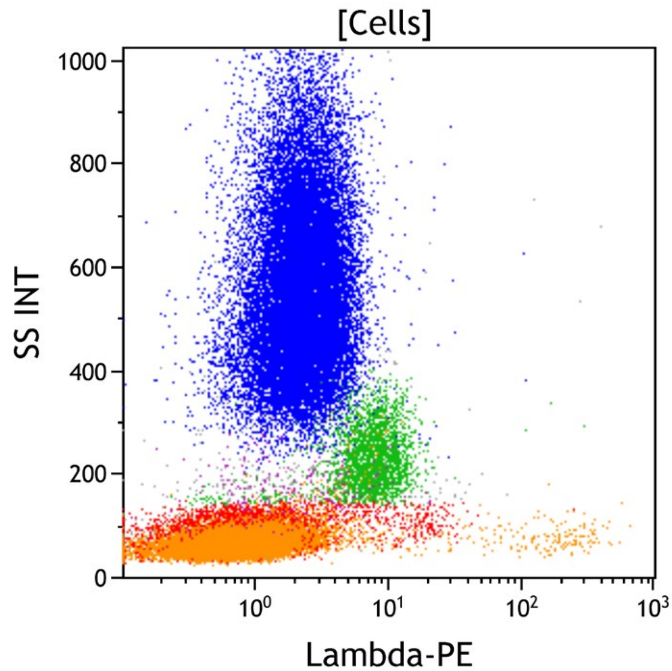


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) lacks surface lambda light chain expression. Note the small number of B cells with lambda light chain expression (also in orange, lower right), which represent normal B cells.

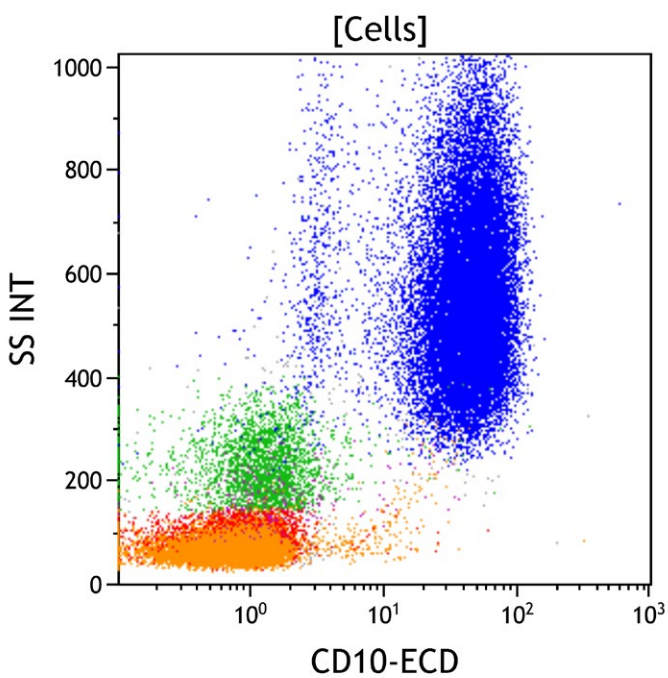


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange) is negative for CD10.

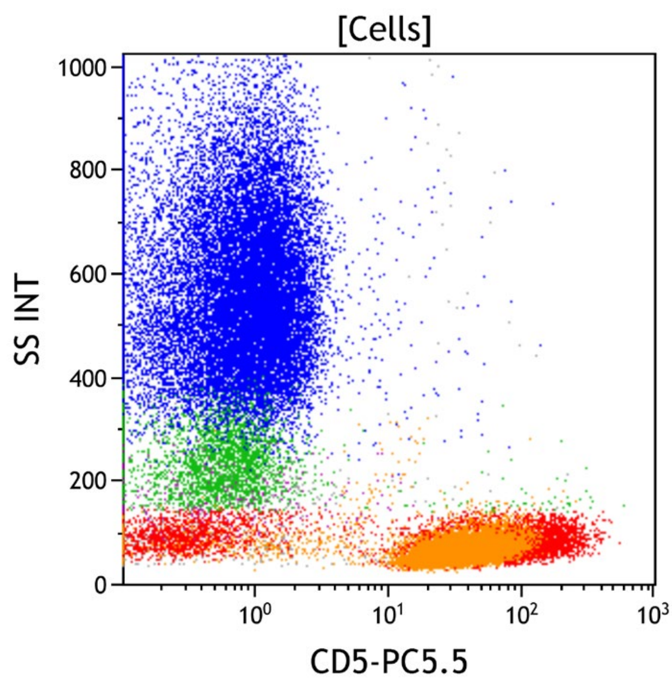


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature T cells (red), as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The CD19 positive population (orange) expresses CD5 at a level slightly below that of T cells (red).

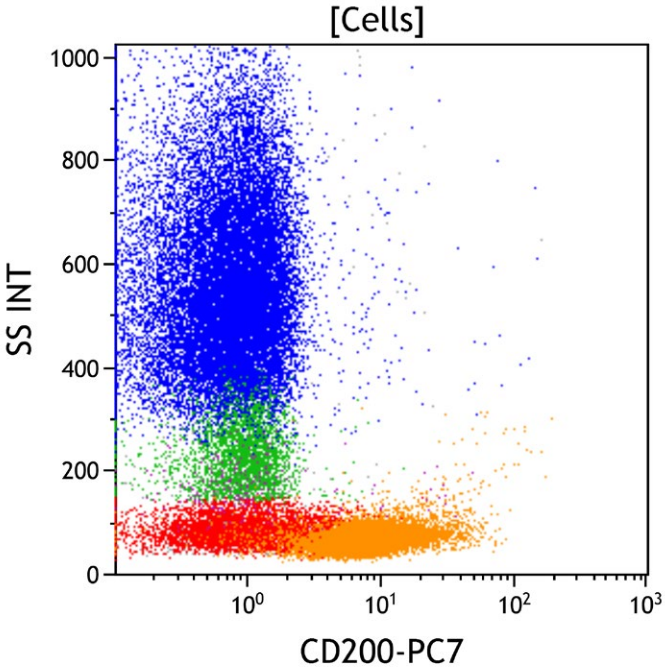


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative on some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The CD19 positive population (orange) is positive for CD200.

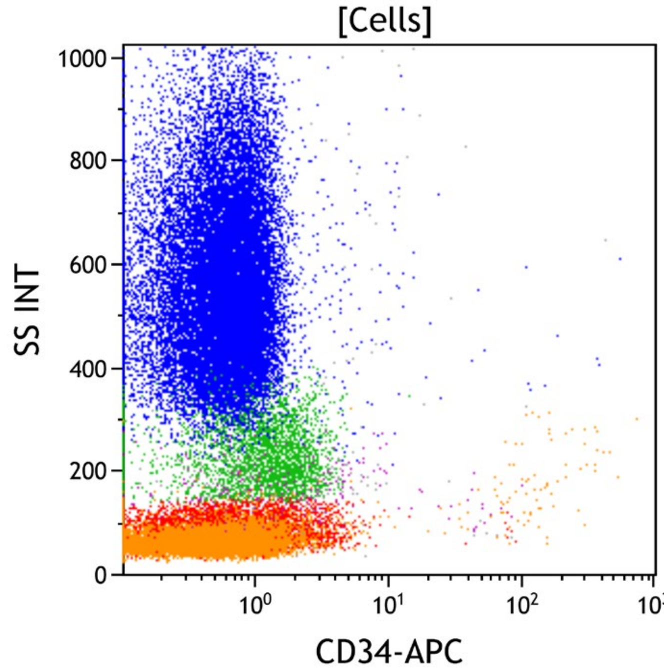


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The CD19 positive population (orange) is negative for CD34.

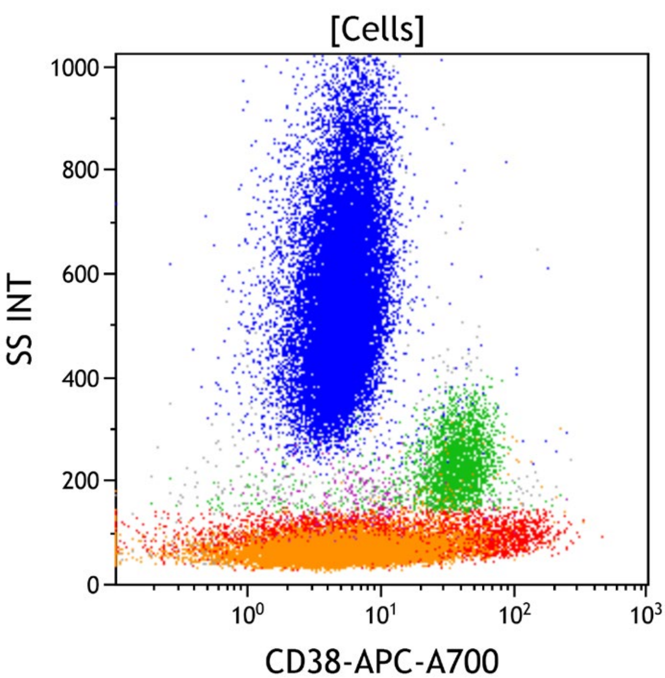


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). The CD19 positive population (orange) displays low to absent CD38 expression.

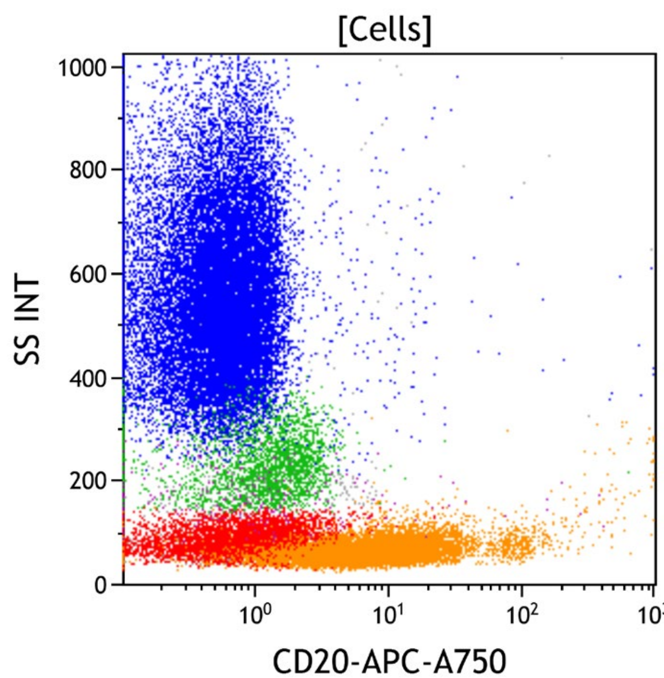


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The majority of the CD19 positive population (orange) displays variably decreased CD20 expression. The small population with a higher level (normal) of CD20 expression (also in orange, lower right) represents normal B cells.

B Cell Tube

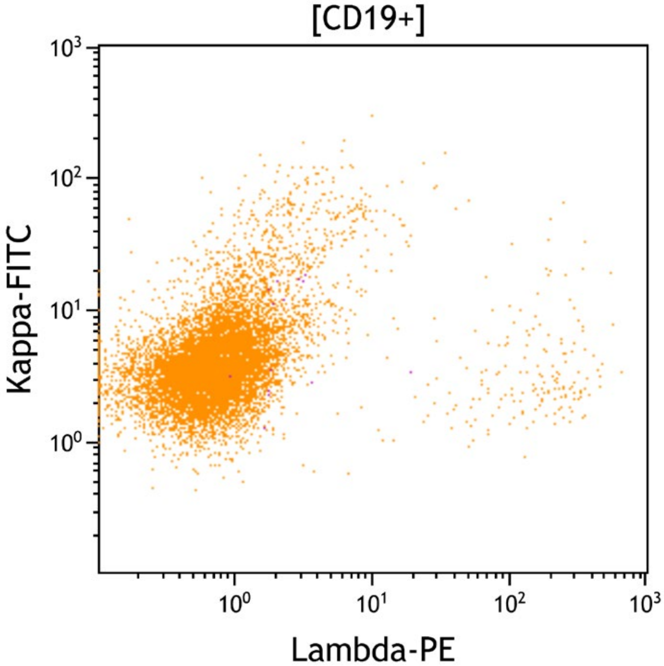


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. The CD19 positive cells (orange) predominantly have surface kappa light chain expression at a decreased level compared with normal mature B cells, indicating a clonal B cell population. A small population of polyclonal B cells (also in orange) with higher (normal) levels of kappa and lambda light chain expression is present.

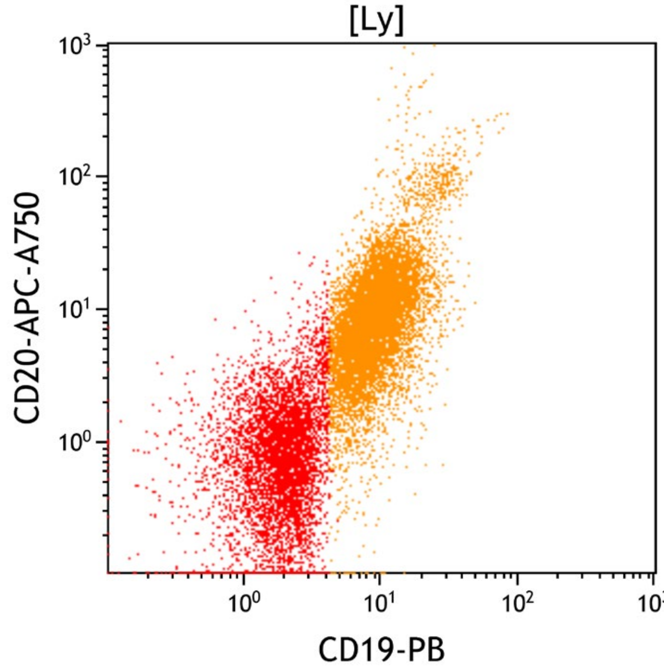


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cells (orange, center) displays decreased CD19 and CD20 expression compared with the higher level seen on normal mature B cells (orange, top right).

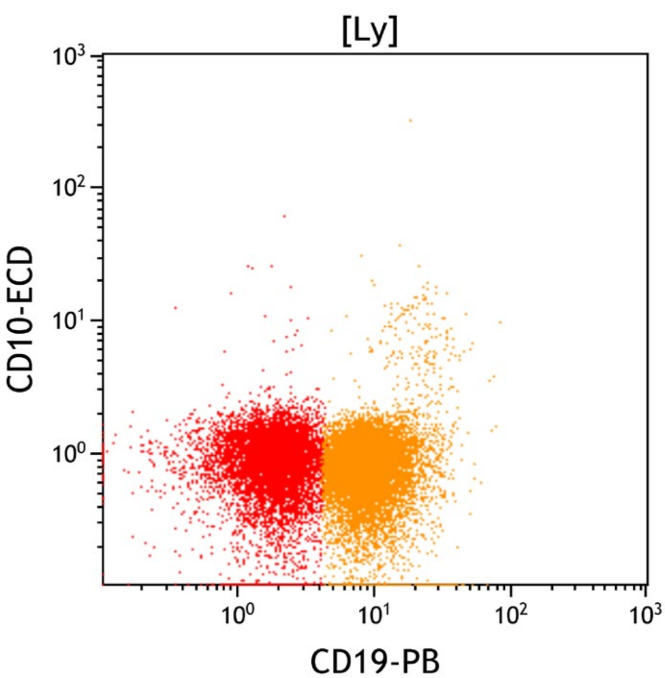


Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45dim gate. The CD19 positive clonal B cell population (orange) is negative for CD10.

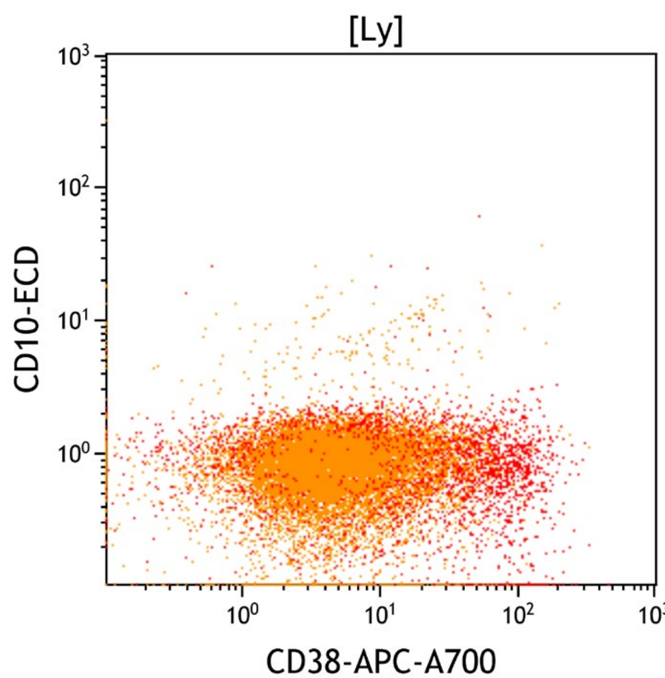


Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The clonal B cell population (orange) displays low to intermediate CD38 expression and is negative for CD10.

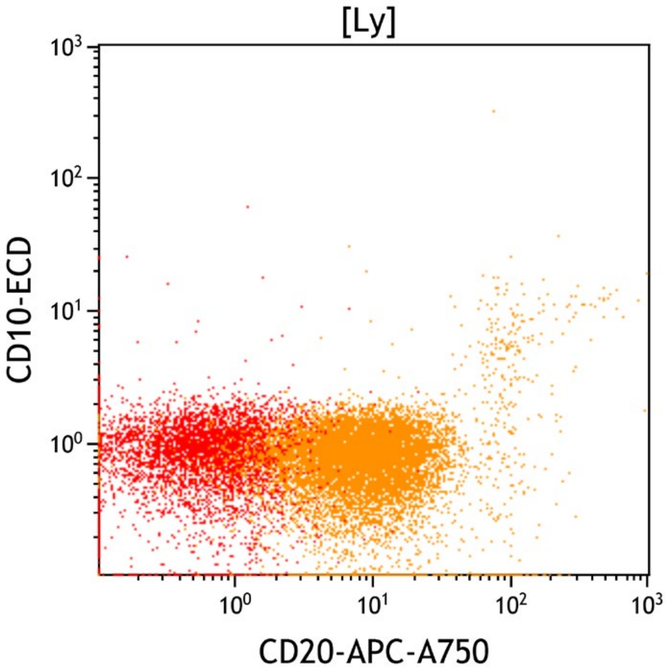


Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20. The clonal B cell population (orange) displays decreased CD20 expression and is negative for CD10. The small population with higher CD20 expression (also in orange, lower right) represents normal B cells.

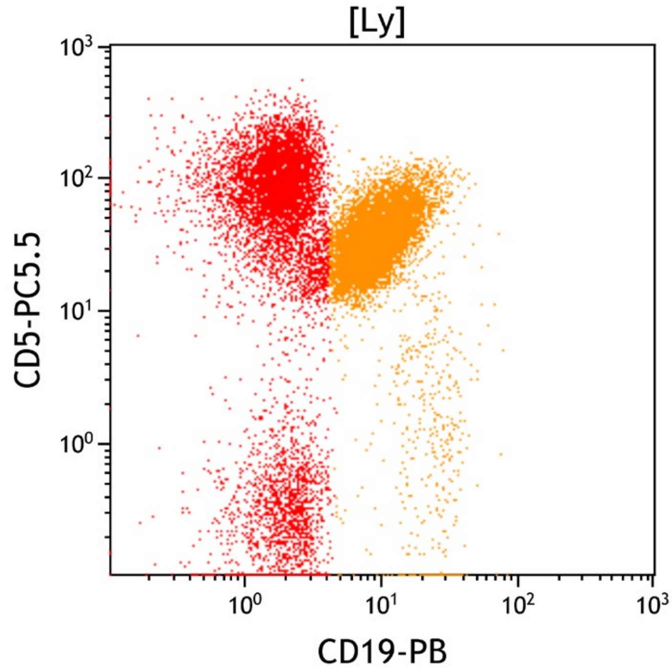


Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) expresses CD5 with slightly decreased CD19 expression. The small population with higher CD19 and mostly negative for CD5 (also in orange, lower right) represents normal B cells.

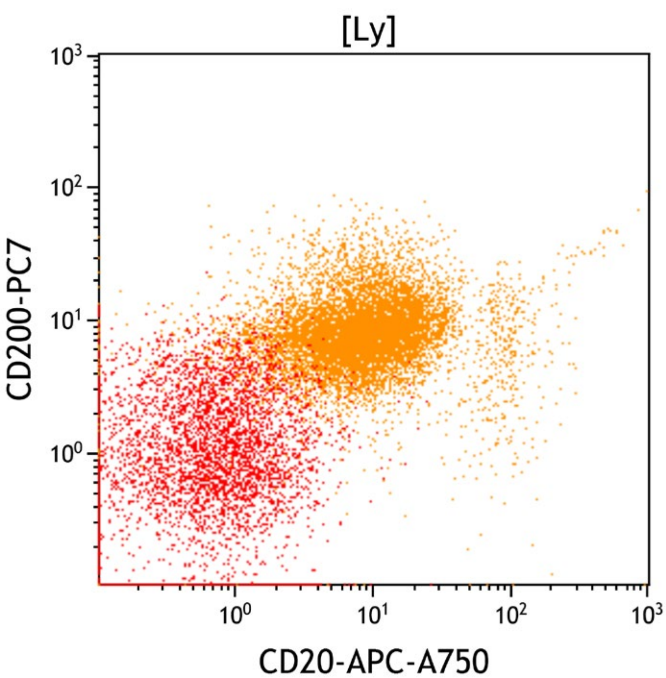


Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The clonal B cell population (orange) is positive for CD200 with decreased CD20. The small population with normal CD20 and CD200 expression (also in orange, right) represents normal B cells.

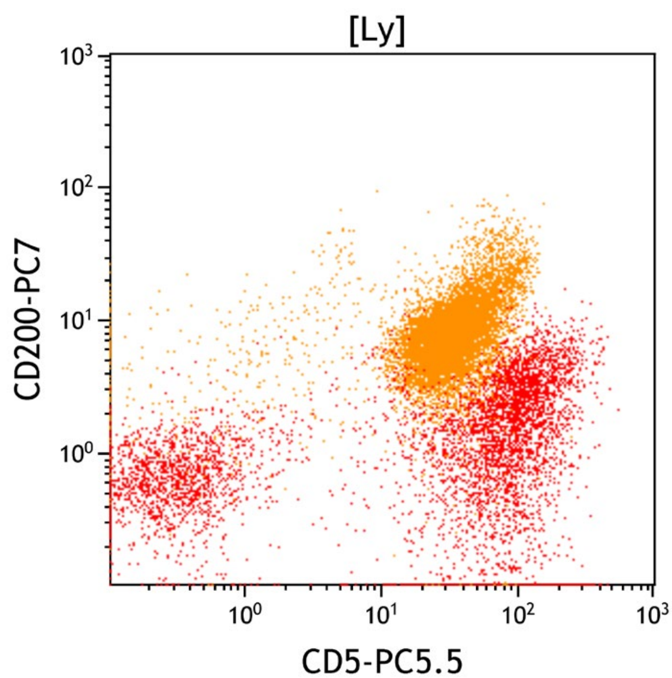


Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The clonal B cell population (orange) is positive for CD5 and CD200, favoring chronic lymphocytic leukemia/small lymphocytic lymphoma.

T Cell Tube

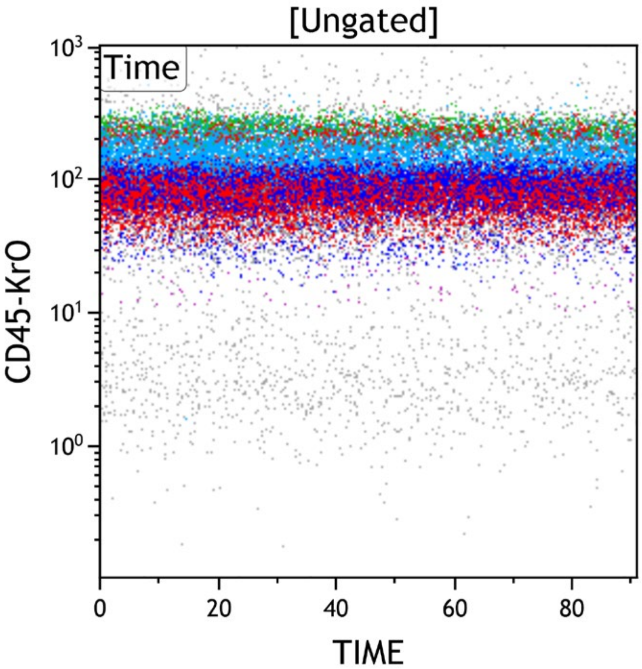


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

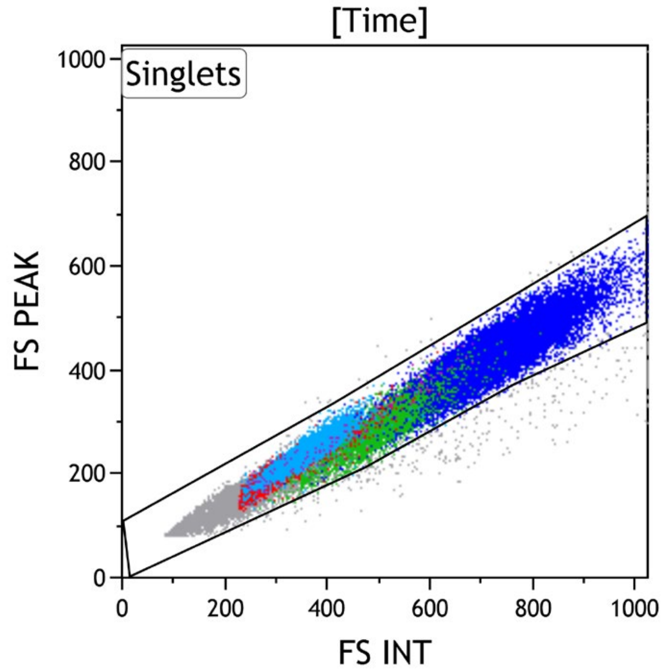


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

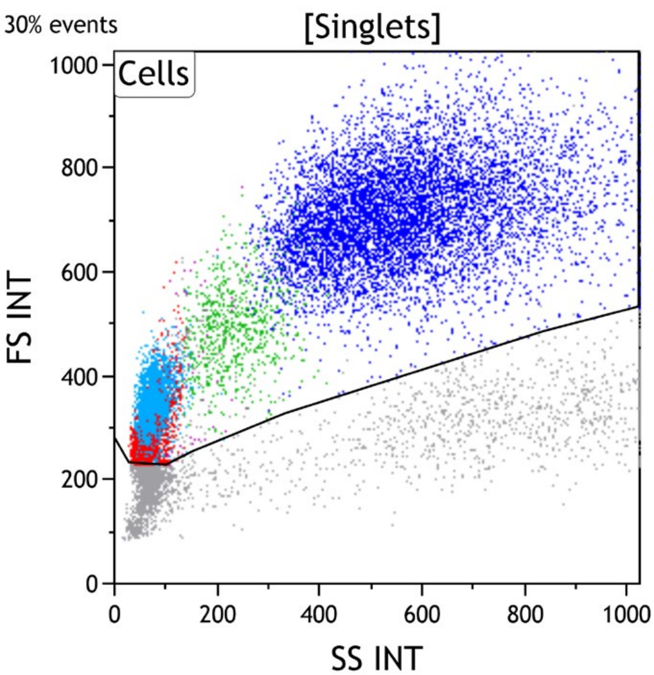


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

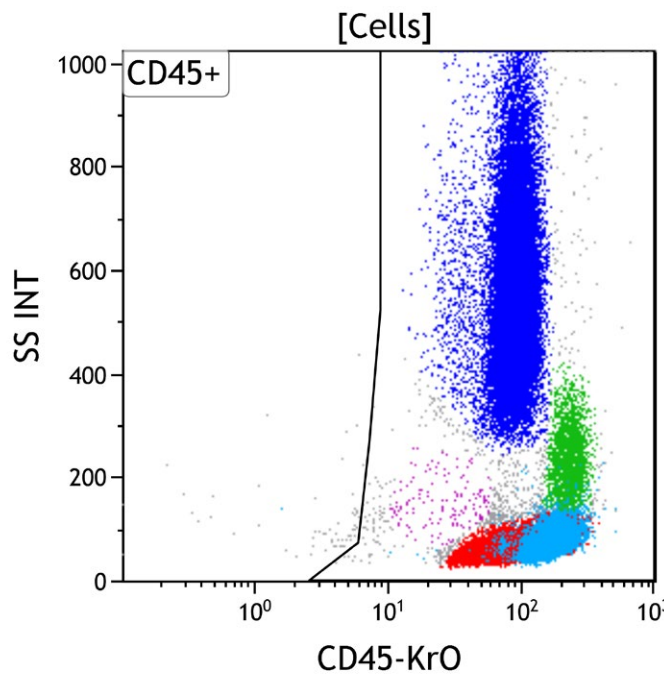


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

T Cell Tube

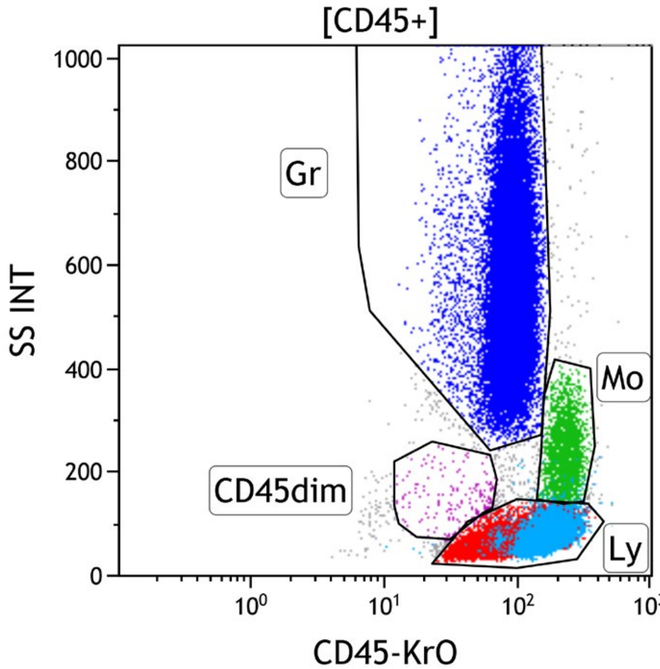


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, aqua/red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

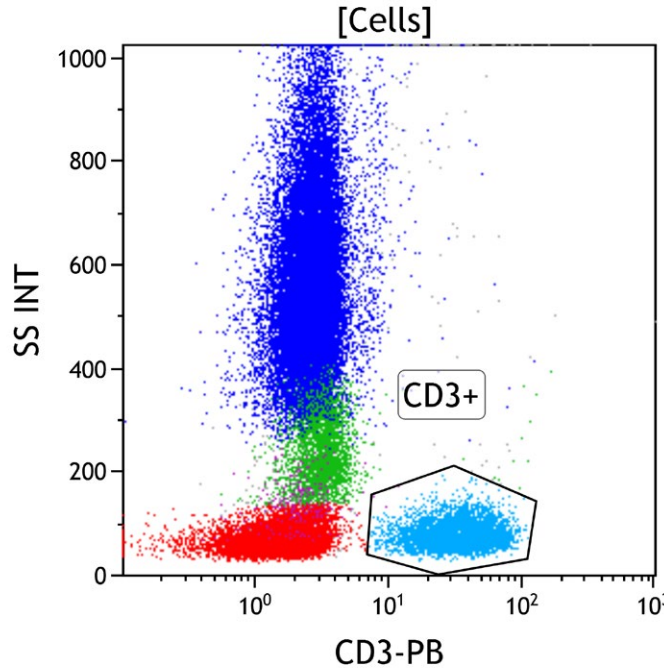


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies surface CD3 positive cells (aqua). CD3 is highly specific for T cells, being expressed on the surface of mature T cells and later stage immature T cells.

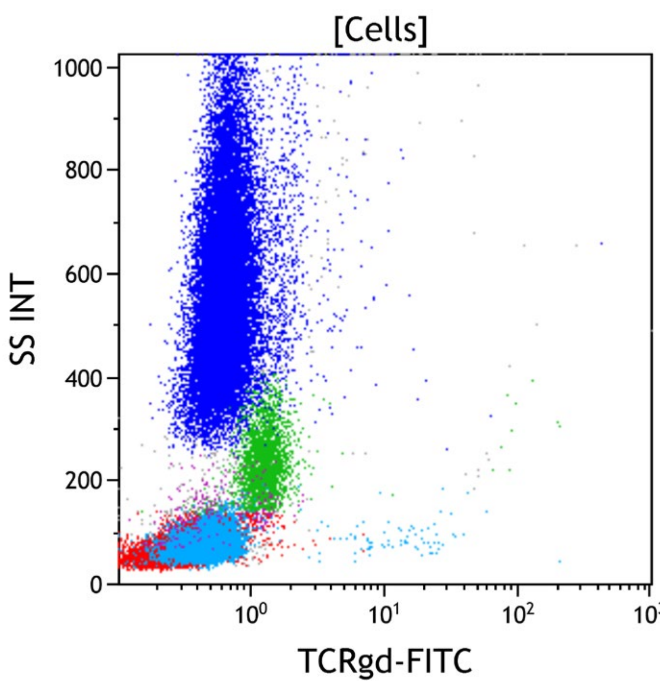


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

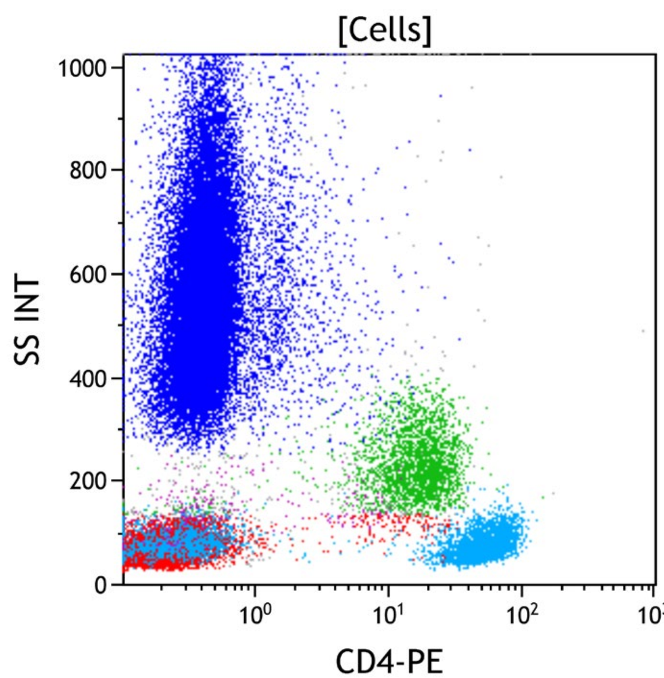


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells.

T Cell Tube

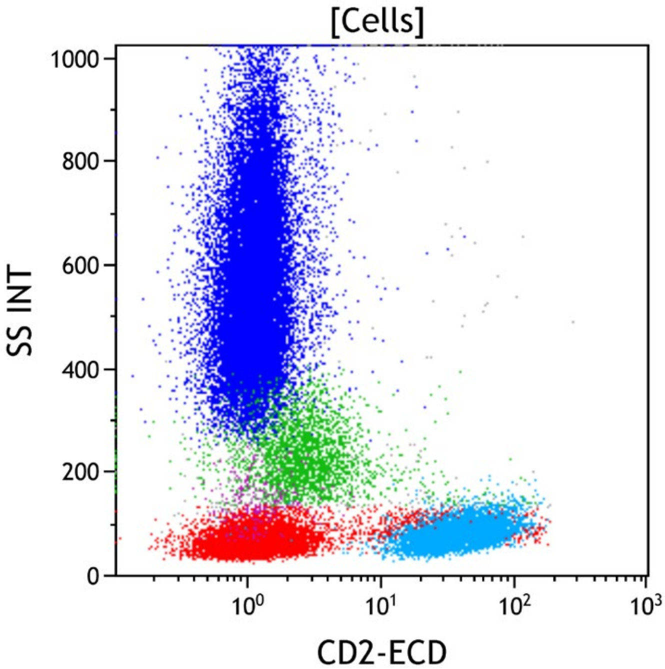


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

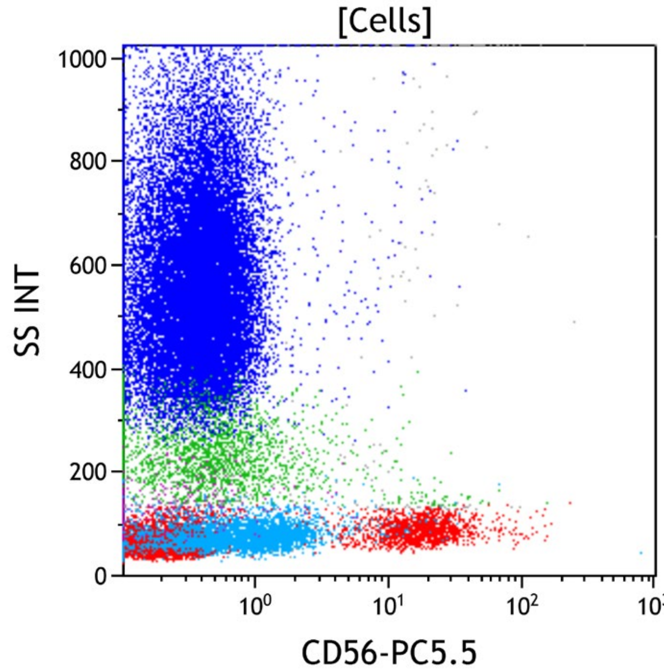


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

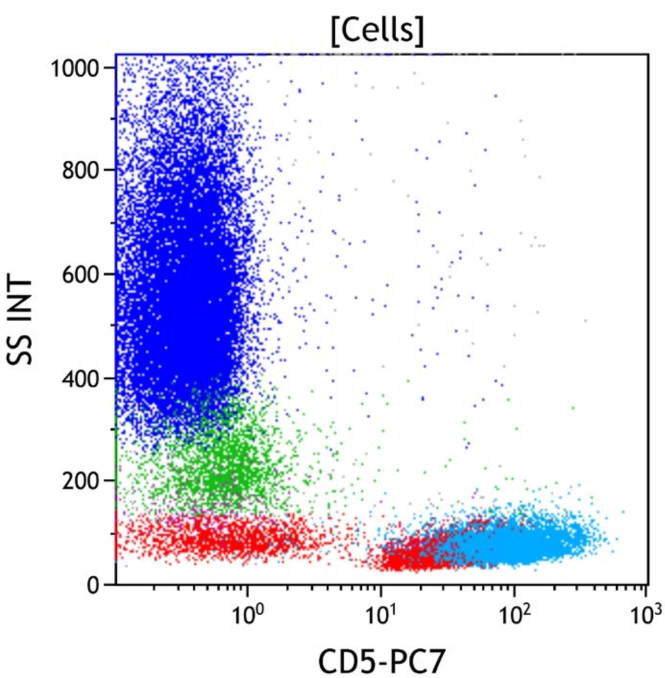


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. The CD3 negative cells in the lymphocyte gate (red, lower right) are aberrant B cells with CD5 expression.

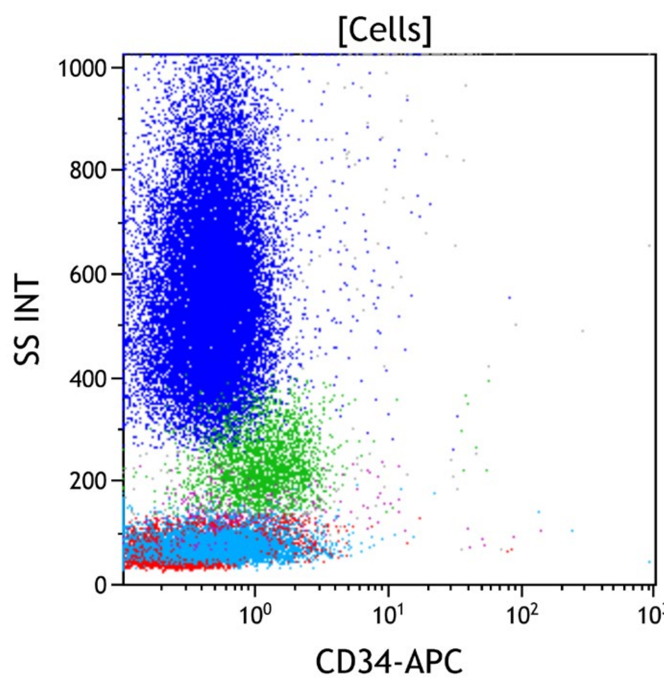


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The mature granulocytes, monocytes, and lymphocytes are negative for CD34.

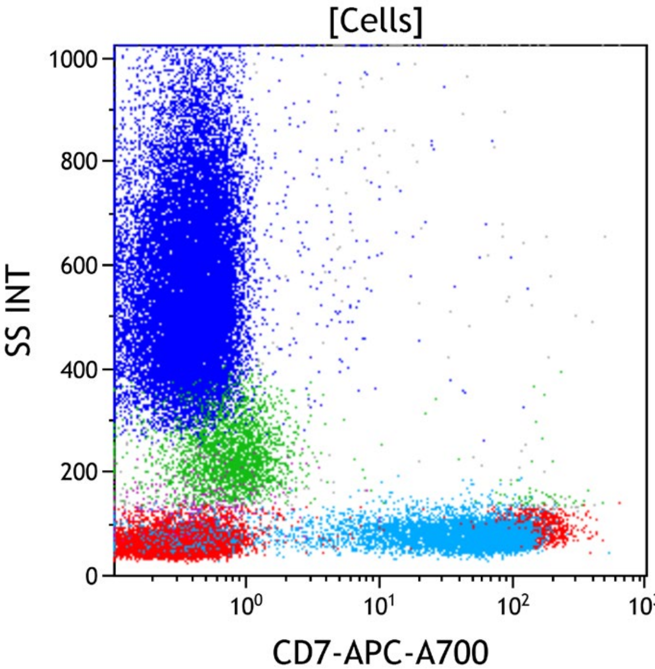


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

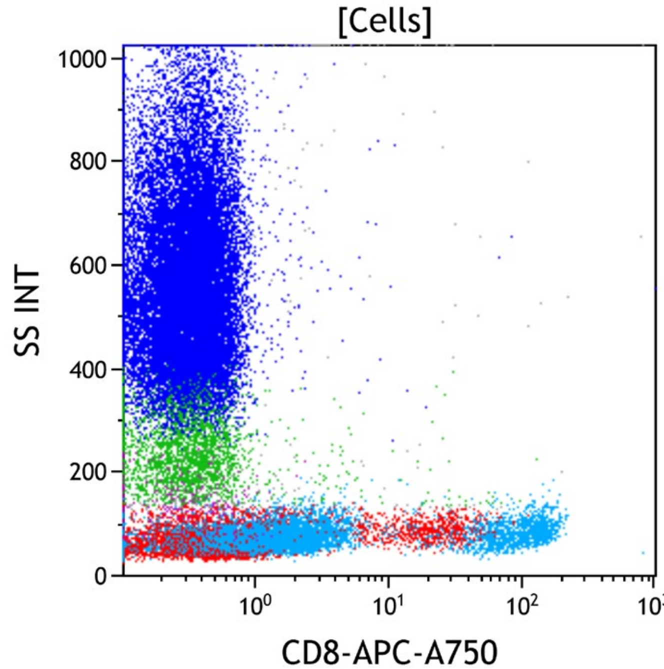


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

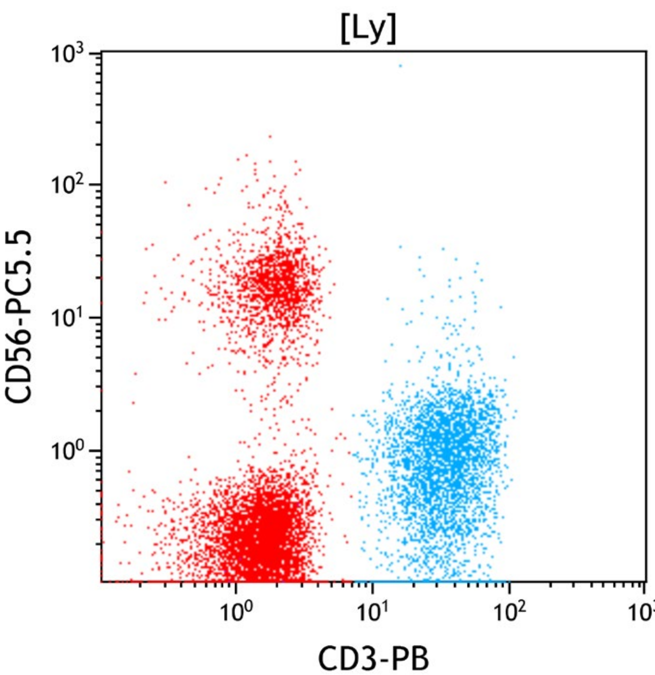


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells (aqua) and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

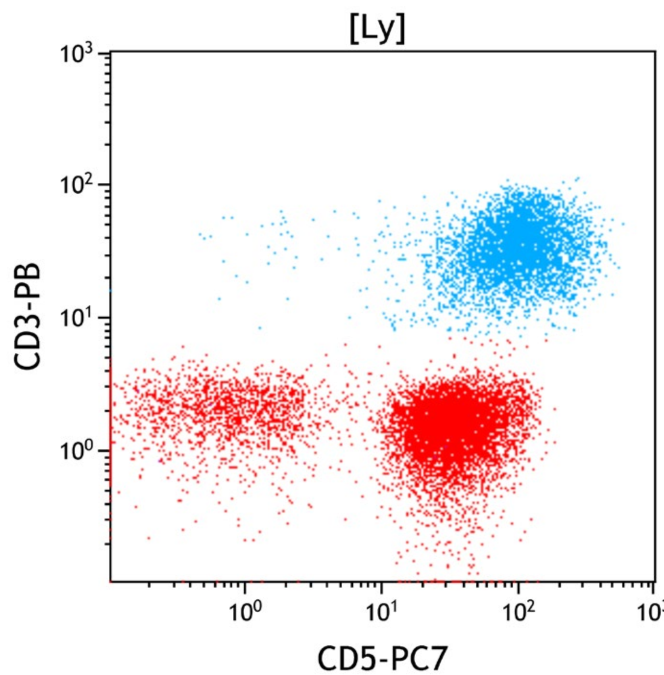


Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red). The CD5 positive cells without CD3 (red, lower right) are aberrant B cells.

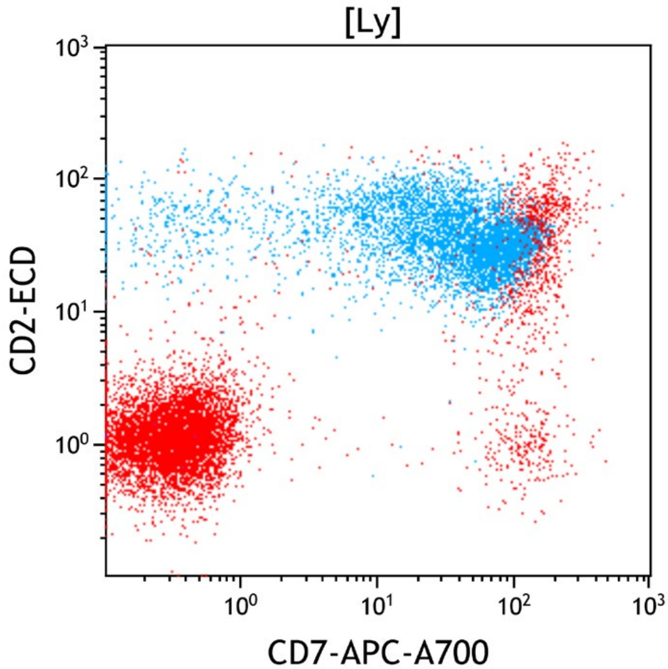


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

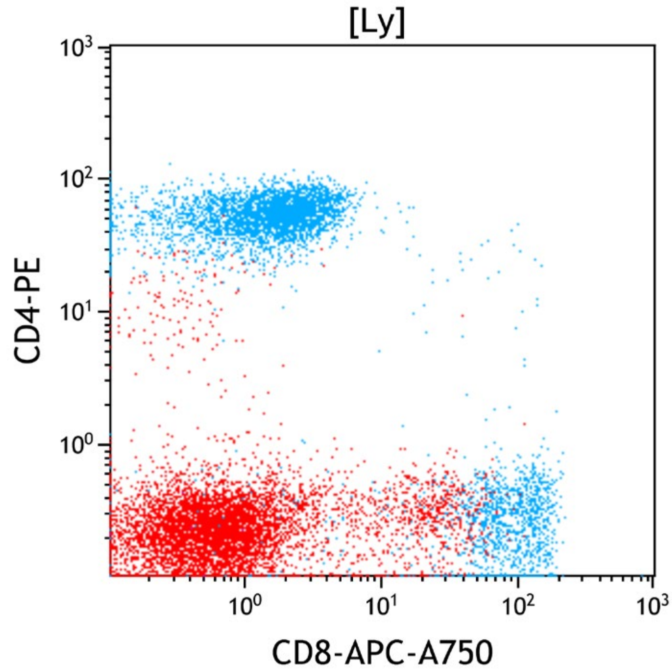


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells.

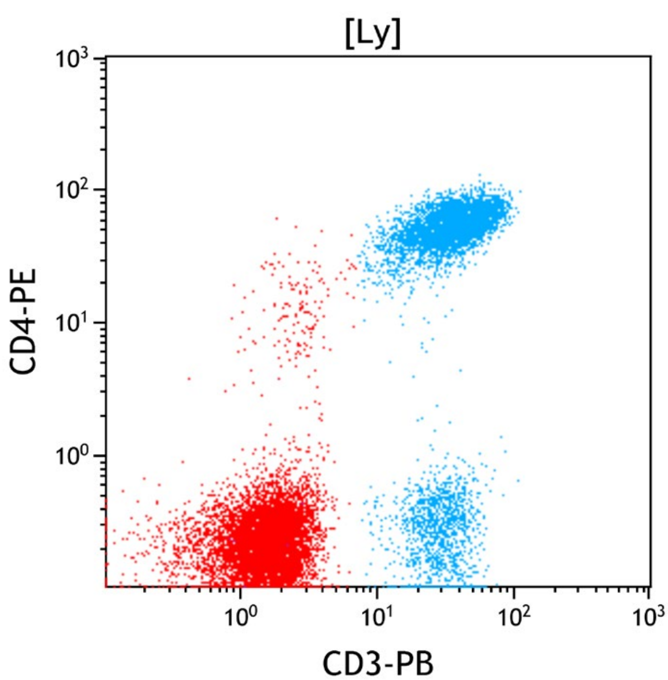


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. Of note, the CD4 positive but CD3 negative cells (red) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

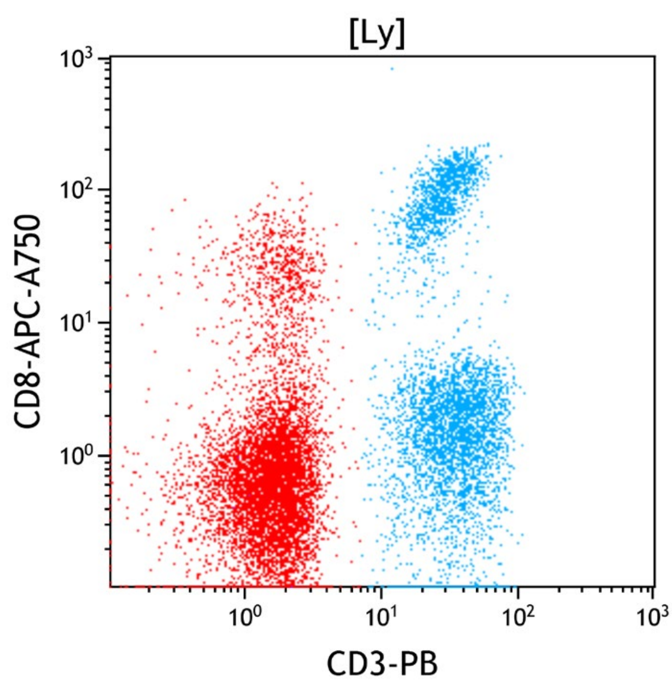


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

T Cell Tube

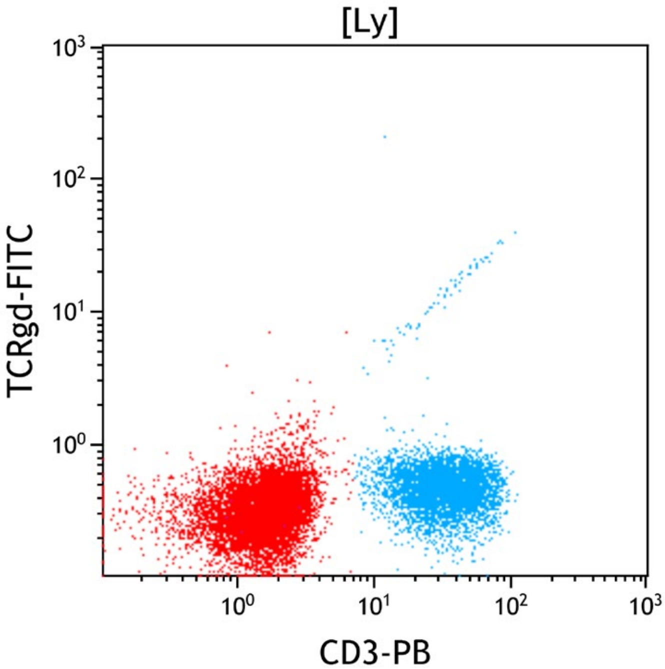
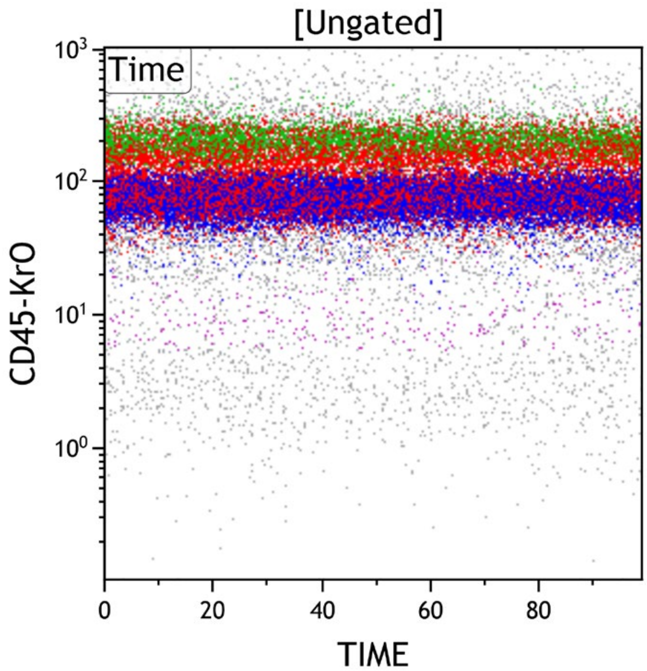
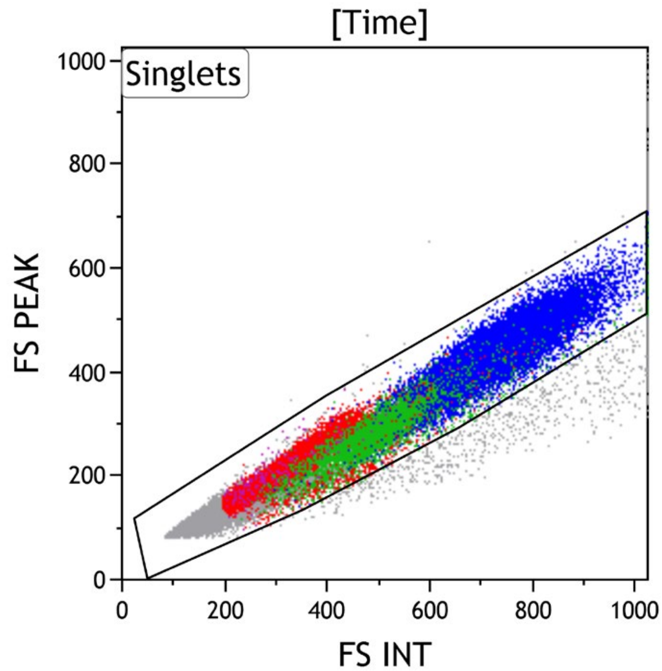


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the Lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube





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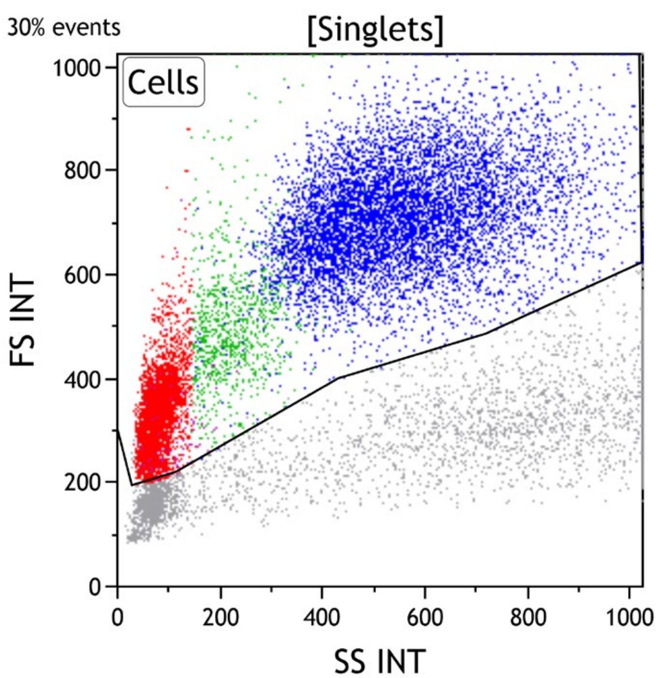

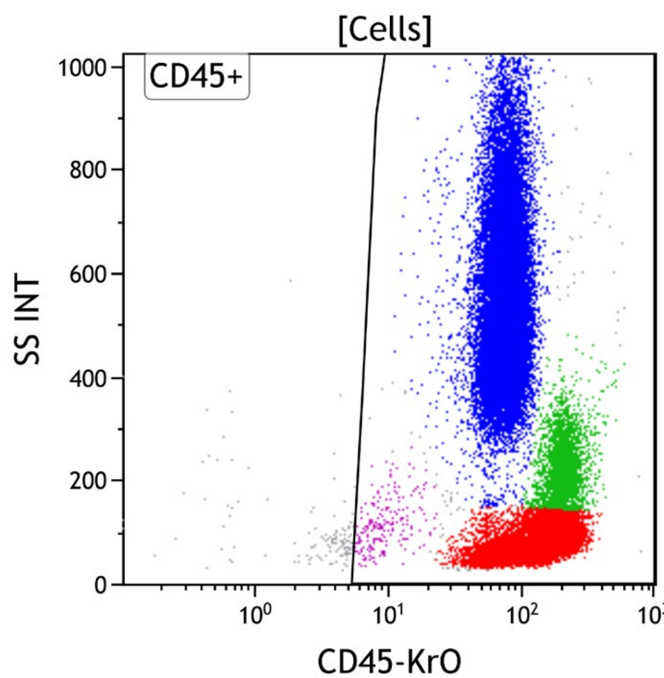

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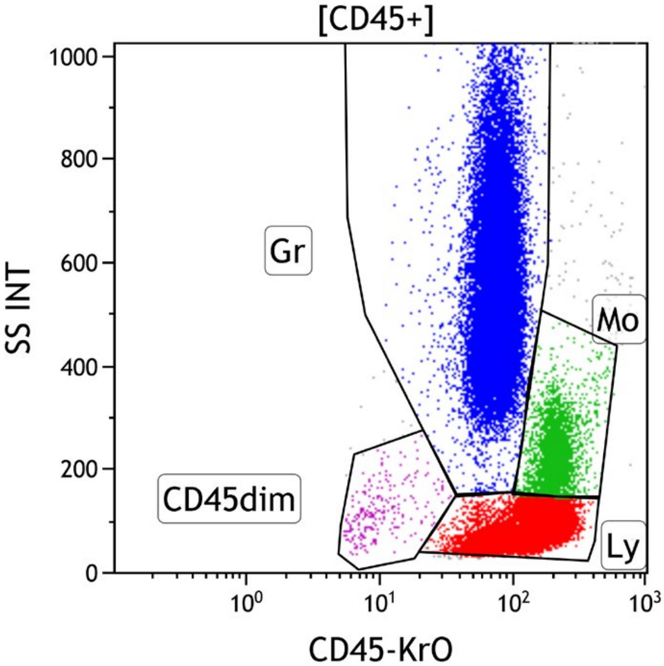
M1 Cell Tube



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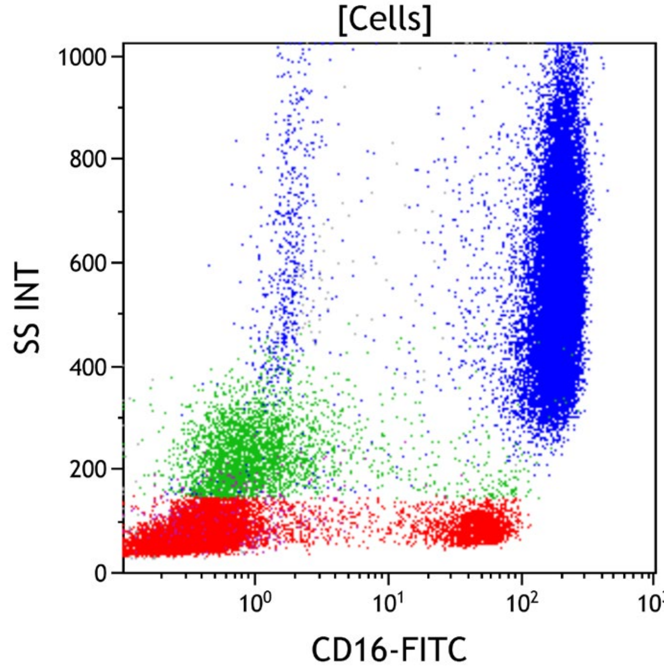


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on granulocytes (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

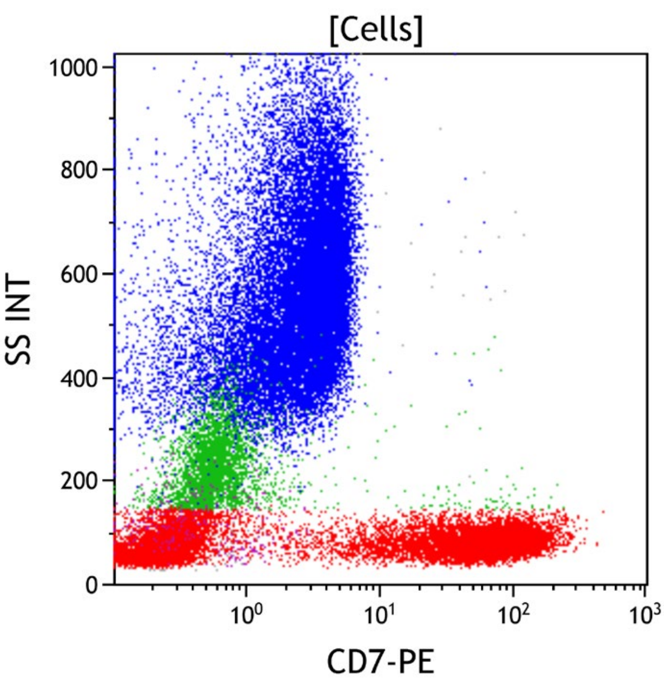


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

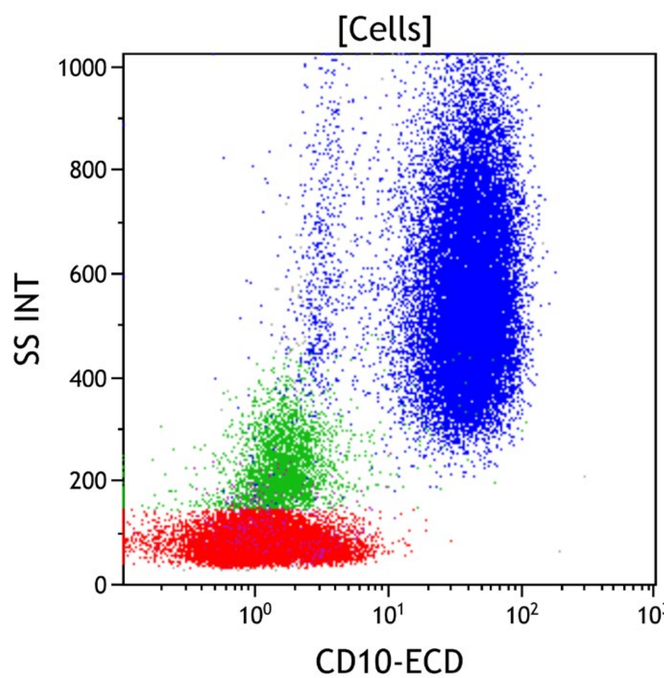


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show variable dim expression of CD10.

M1 Cell Tube

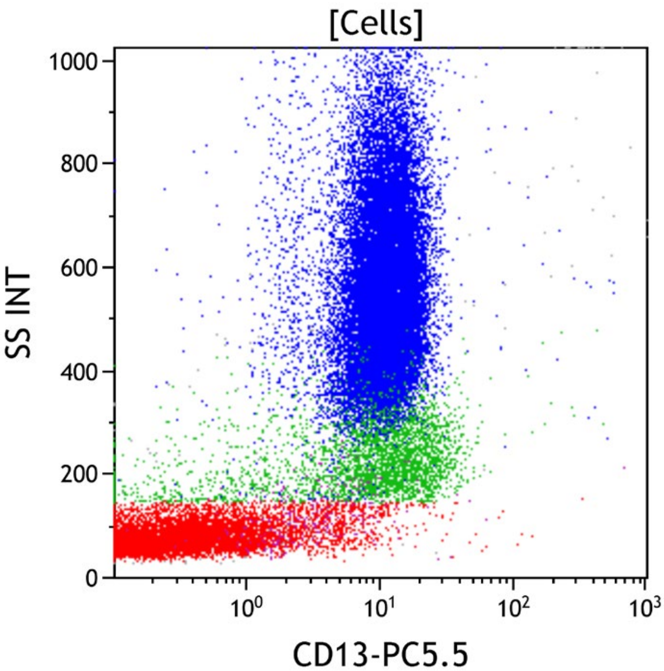


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (in blue, with high side scatter) and on mature monocytes (green).

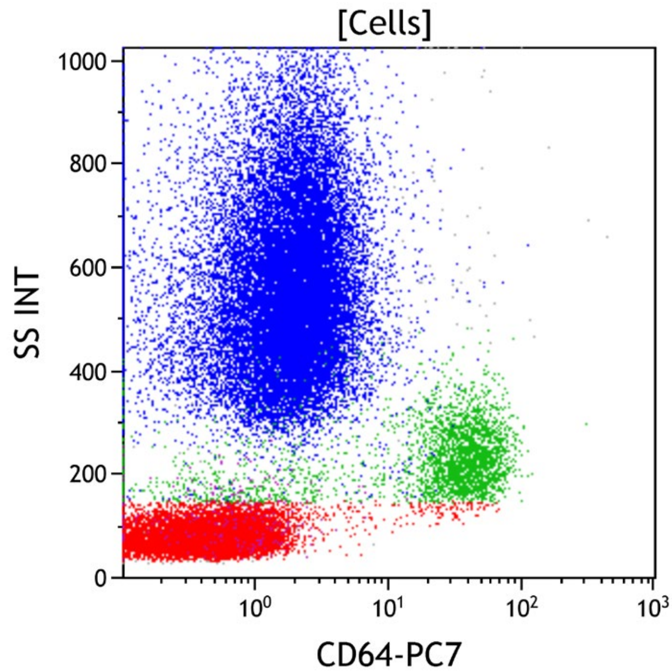


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors.

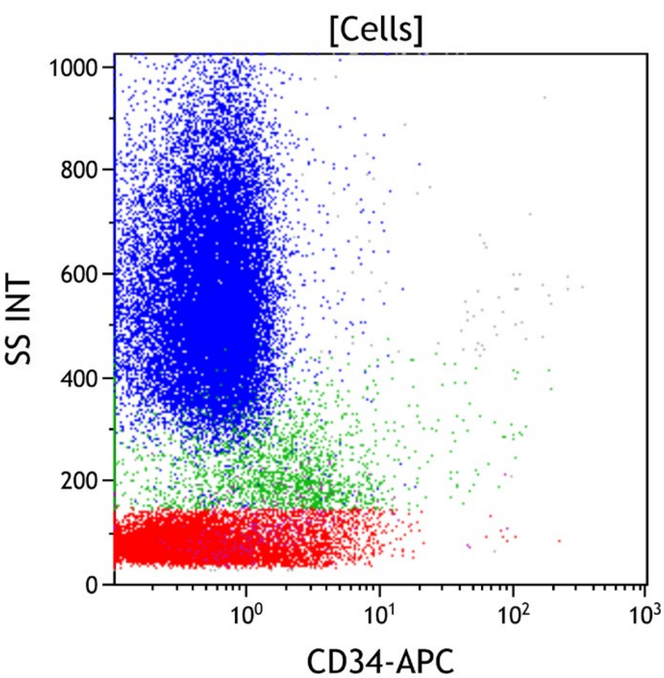


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34.

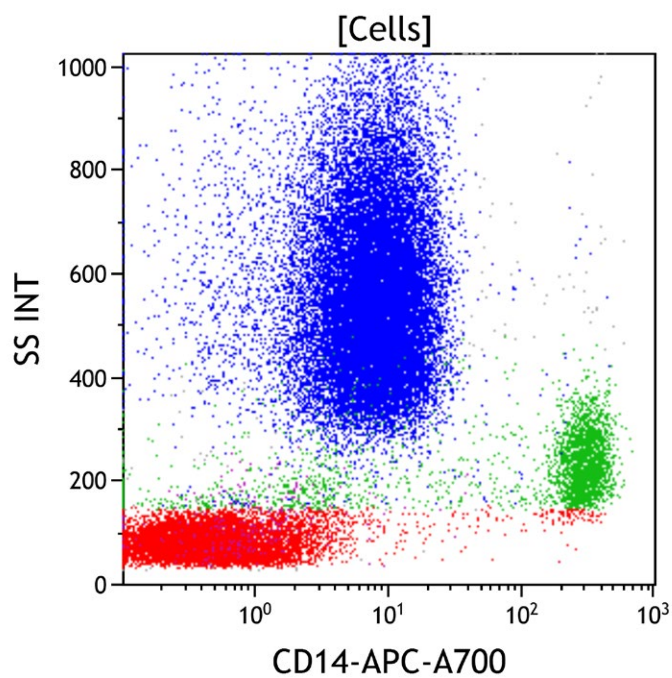


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on granulocytes (blue) at a low level.

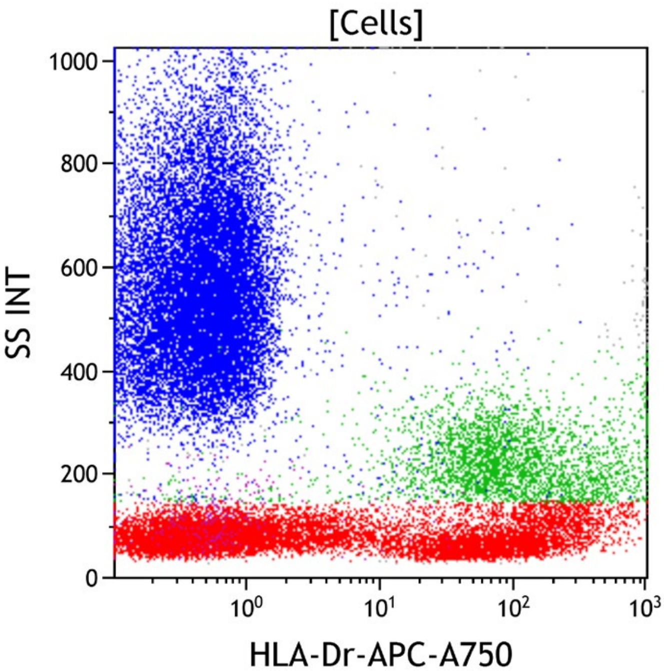


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red).

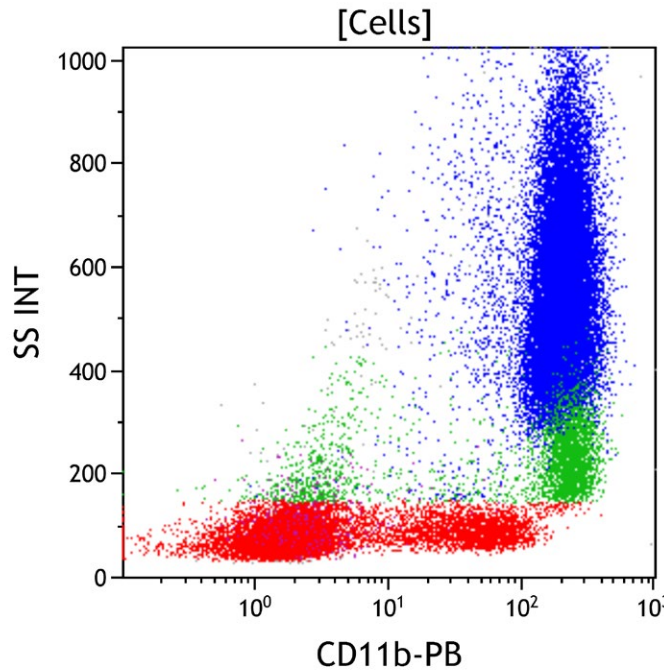


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (blue) and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils.

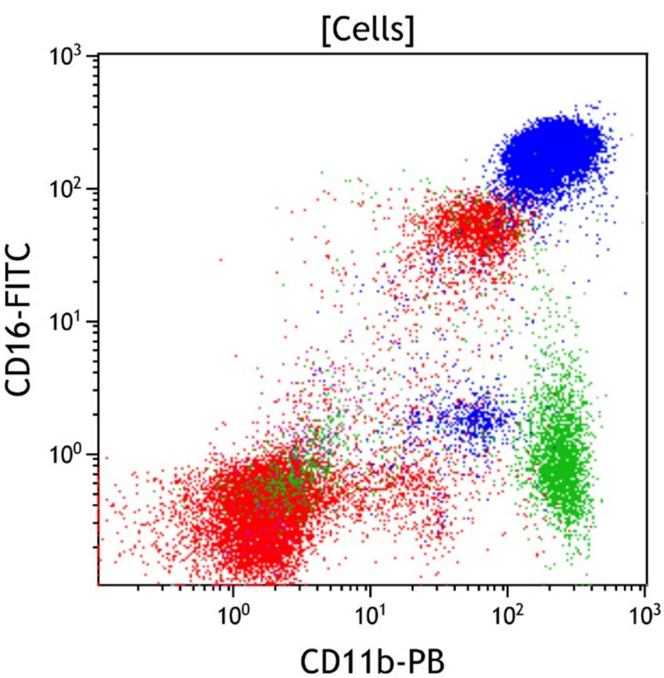


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue), and NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red).

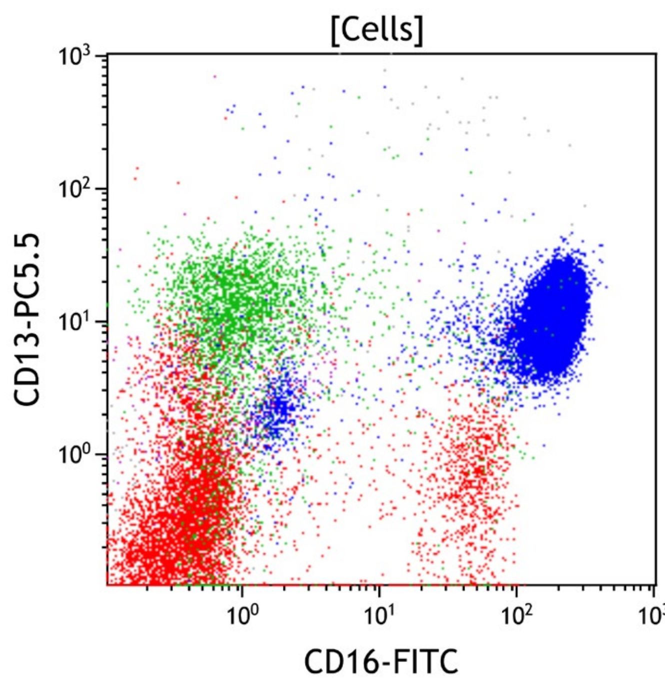


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red, lower right).

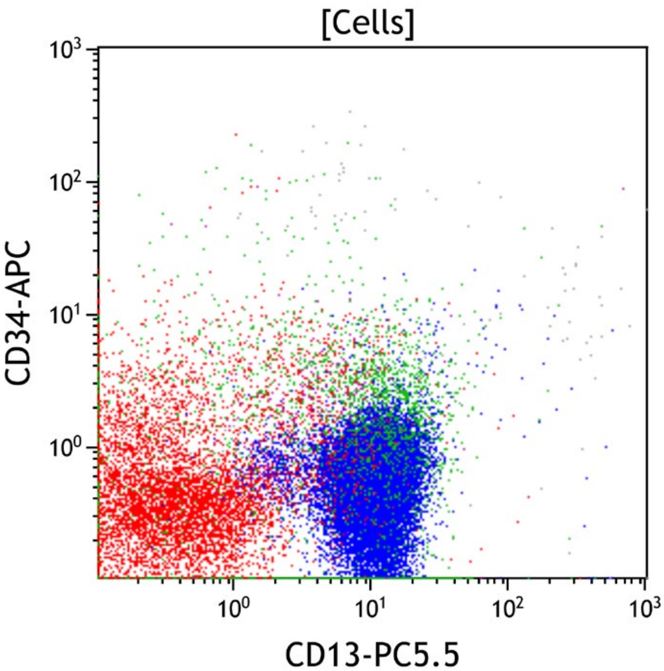


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors.

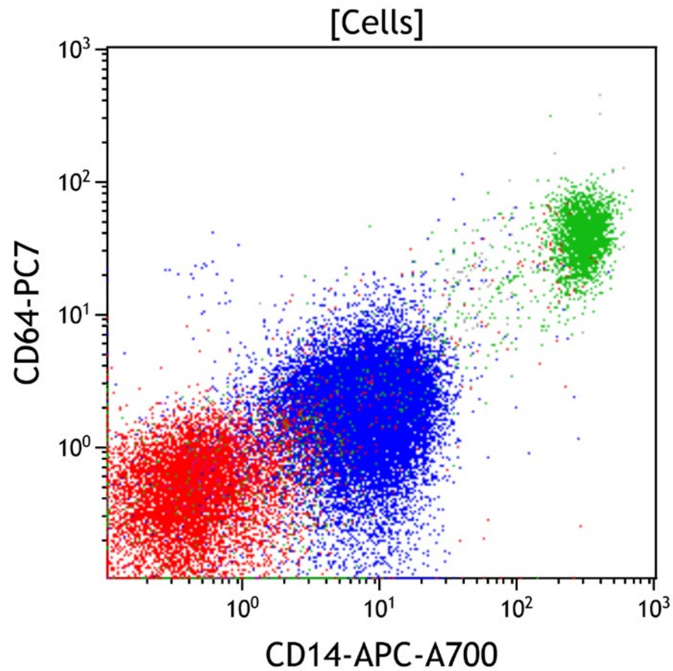


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes.

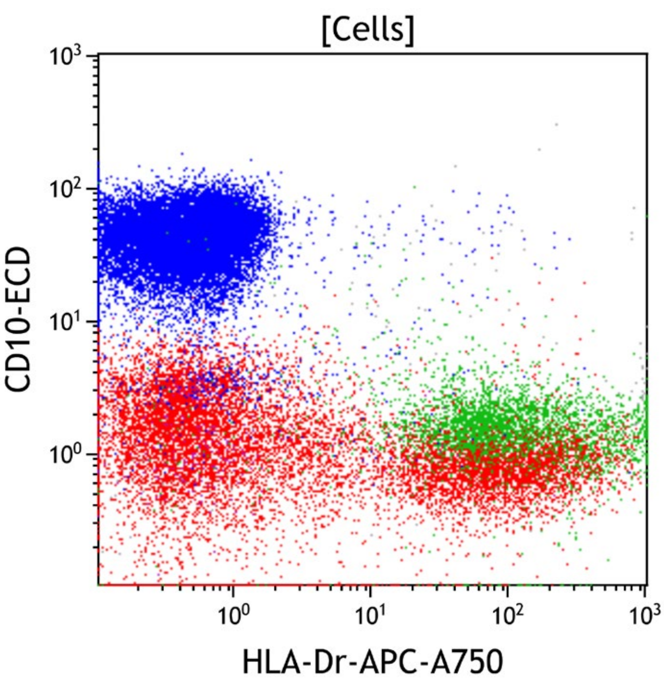


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells (red, lower right), plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by granulocytes (blue).

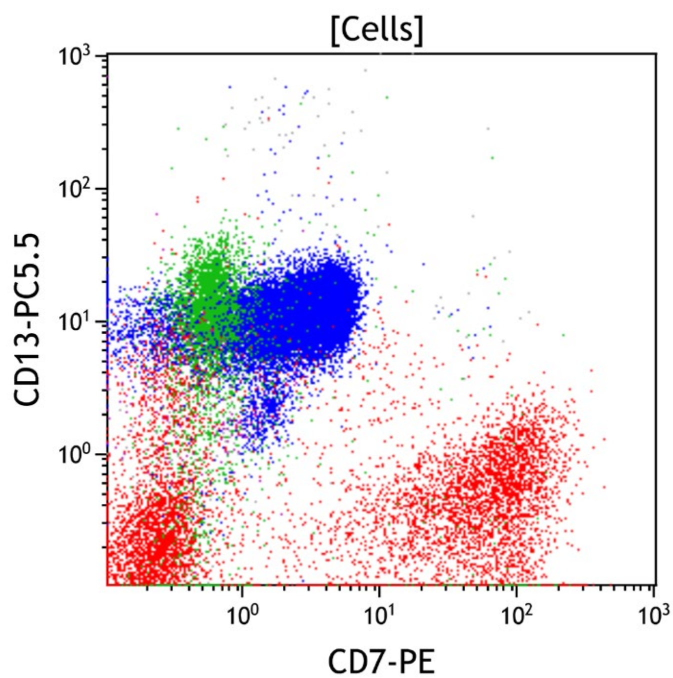
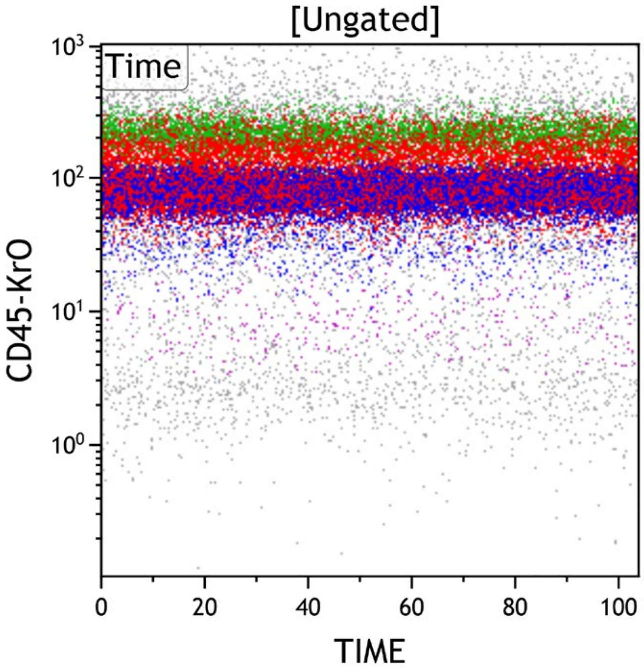


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

M2 Cell Tube



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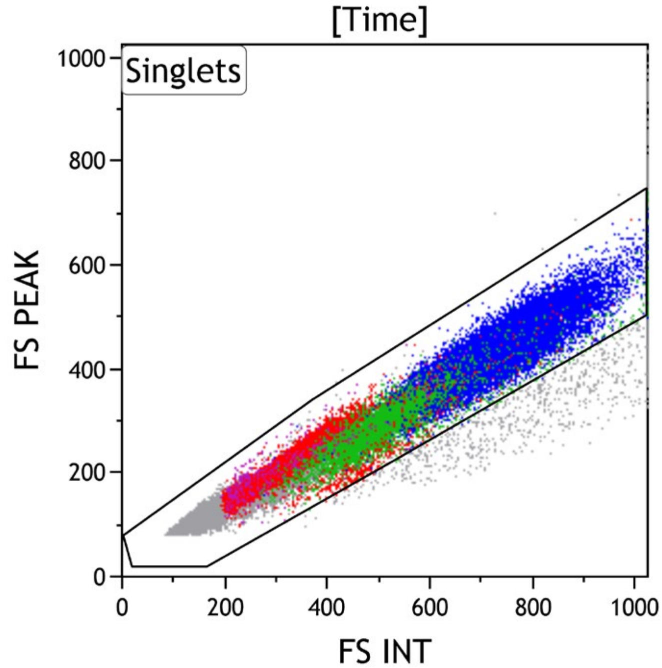

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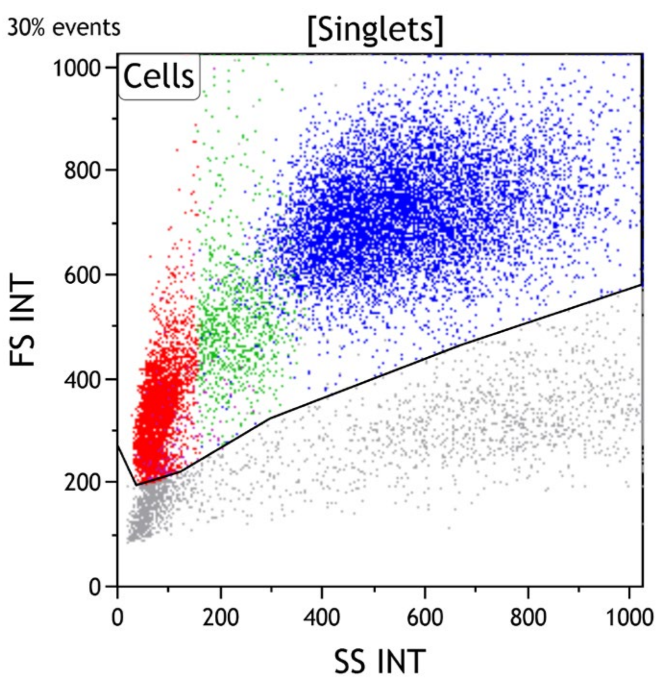

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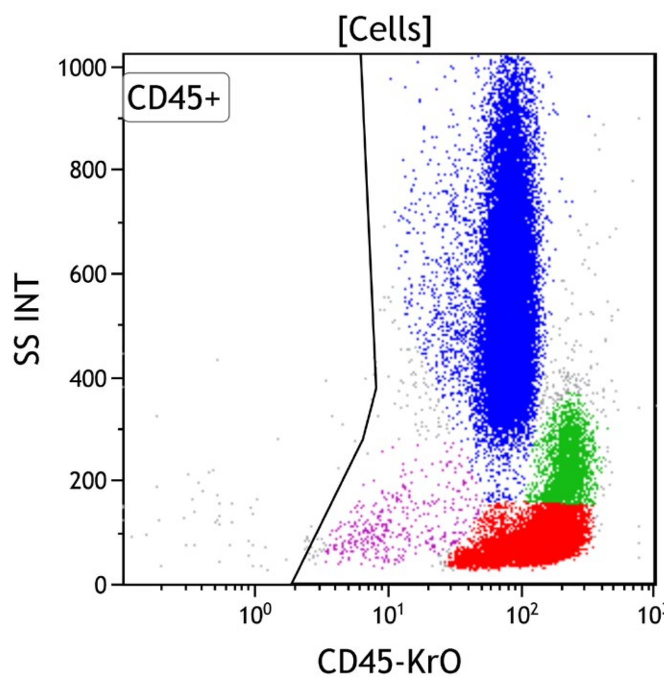


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

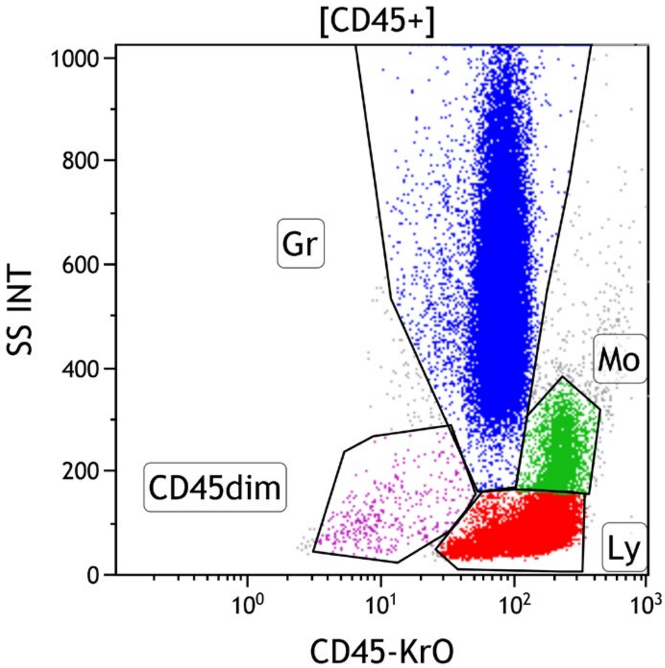


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

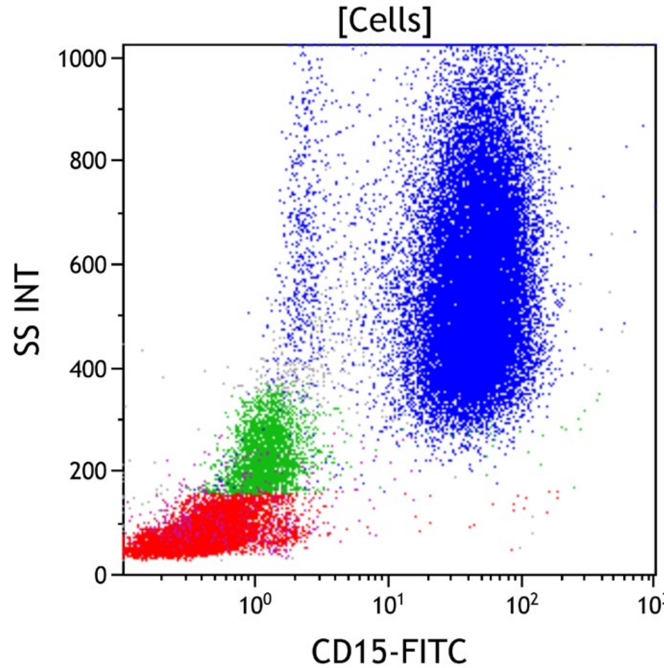


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (in blue) and at a lower level on monocytes (green).

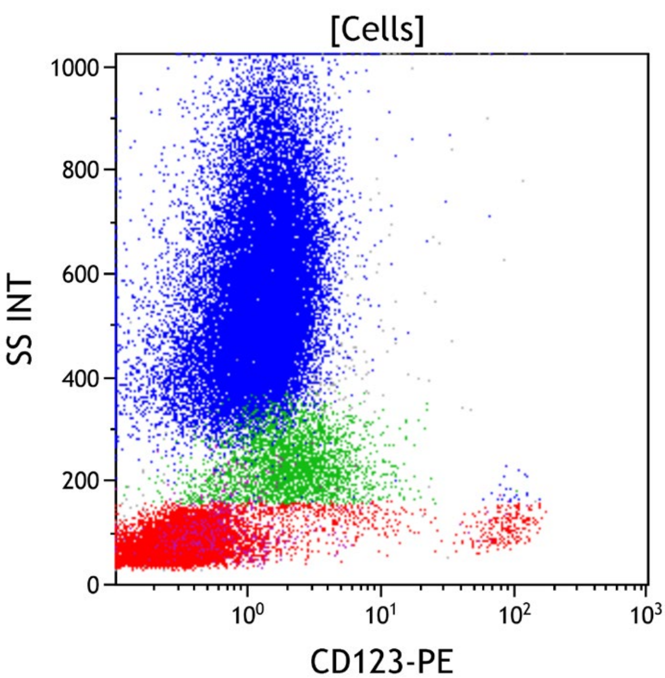


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

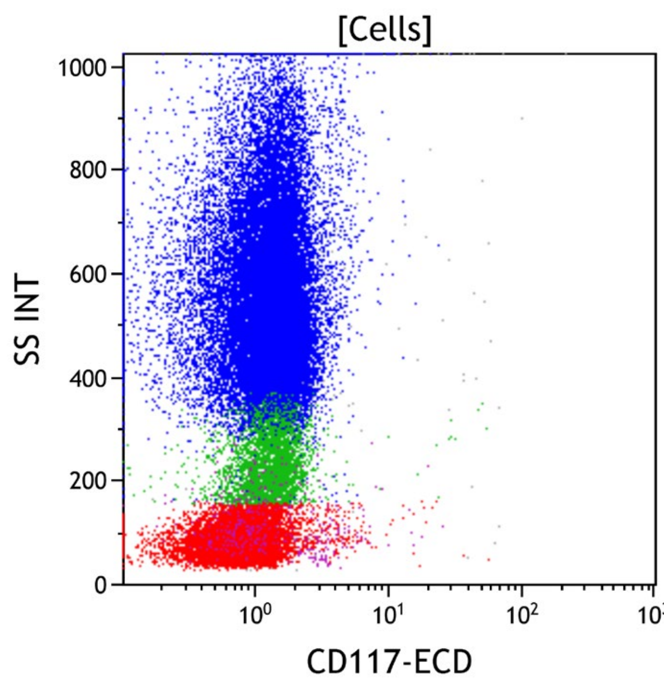


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

M2 Cell Tube

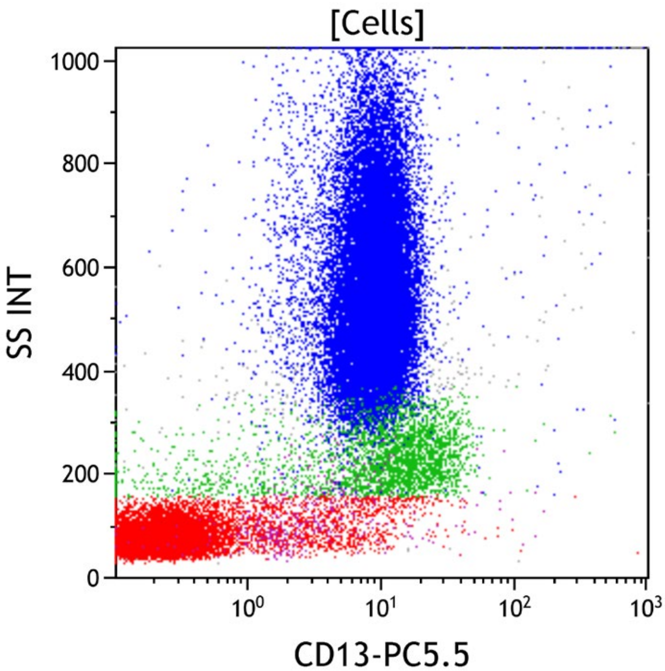


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and on mature monocytes (green).

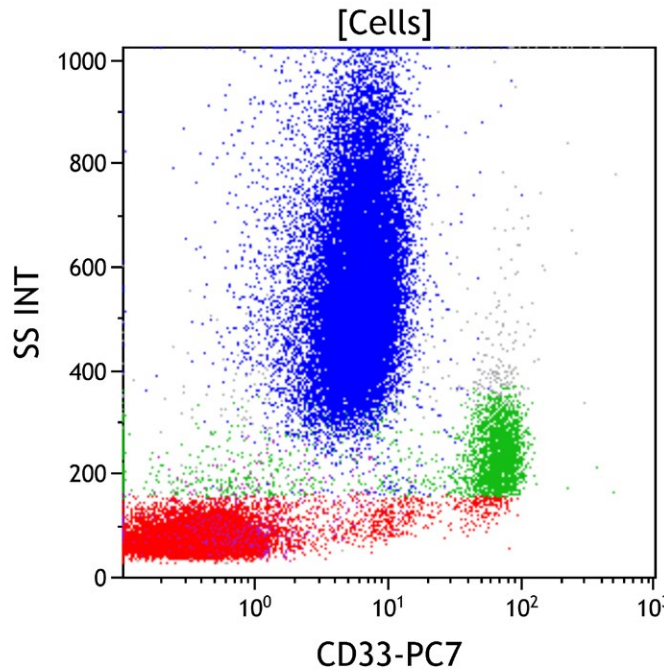


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors.

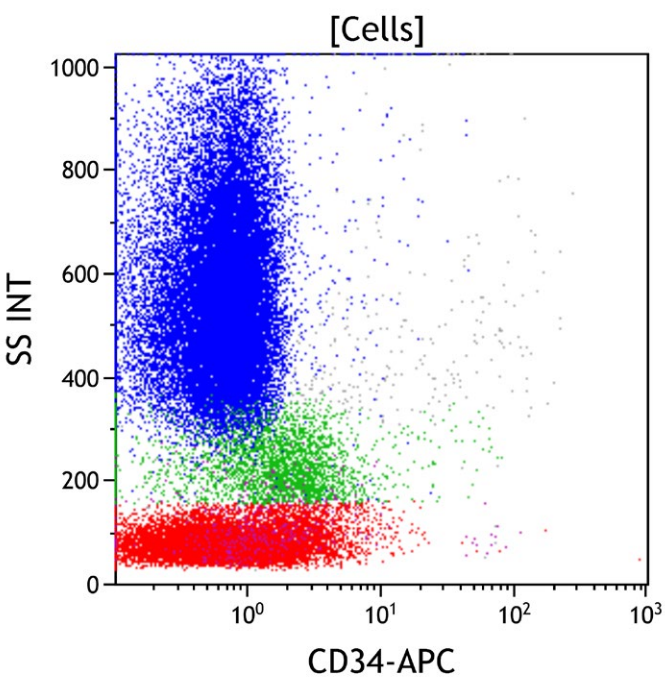


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts).

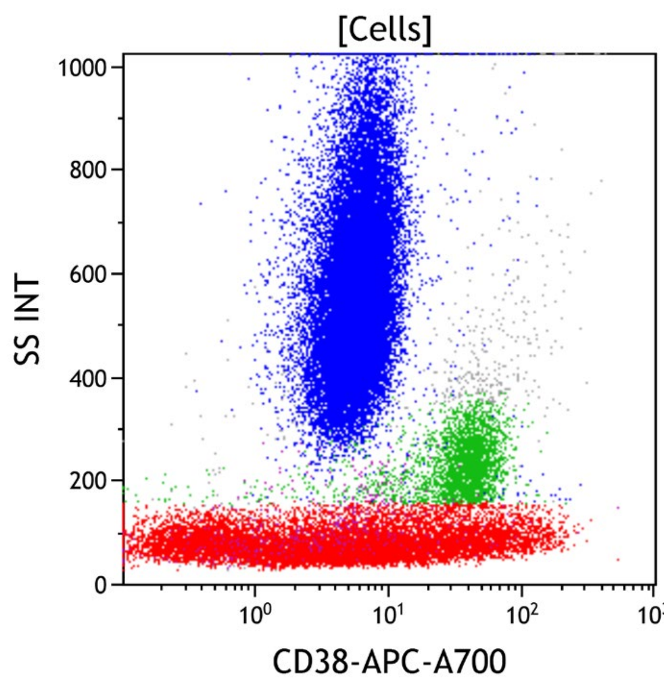


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and monocytes (green), and at a variable level on activated lymphocytes (red).

M2 Cell Tube

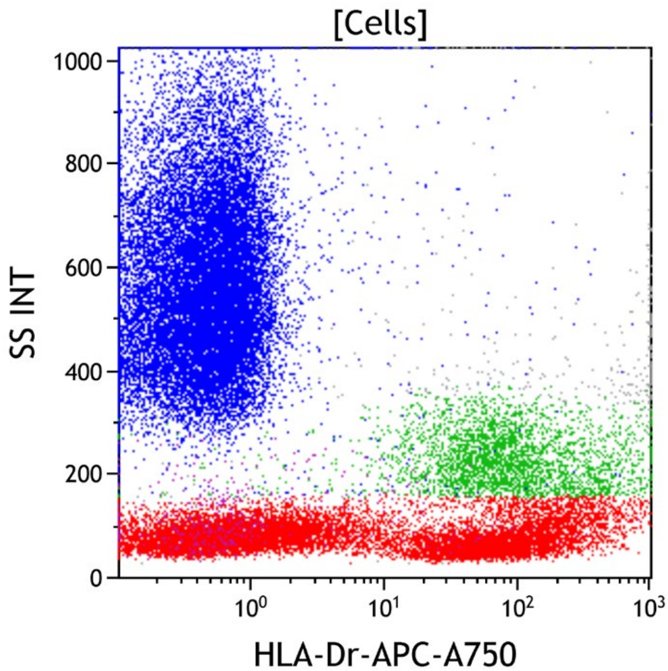


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red).

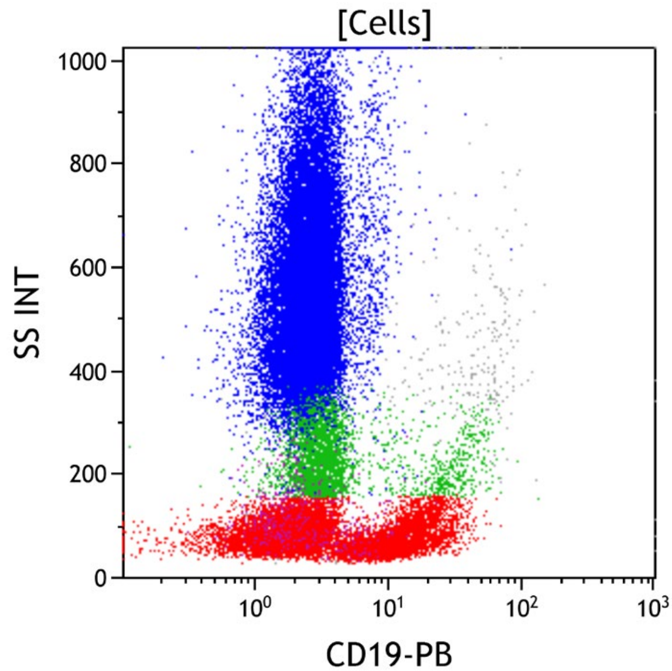


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The mature B cell population (red) is relatively expanded compared with normal.

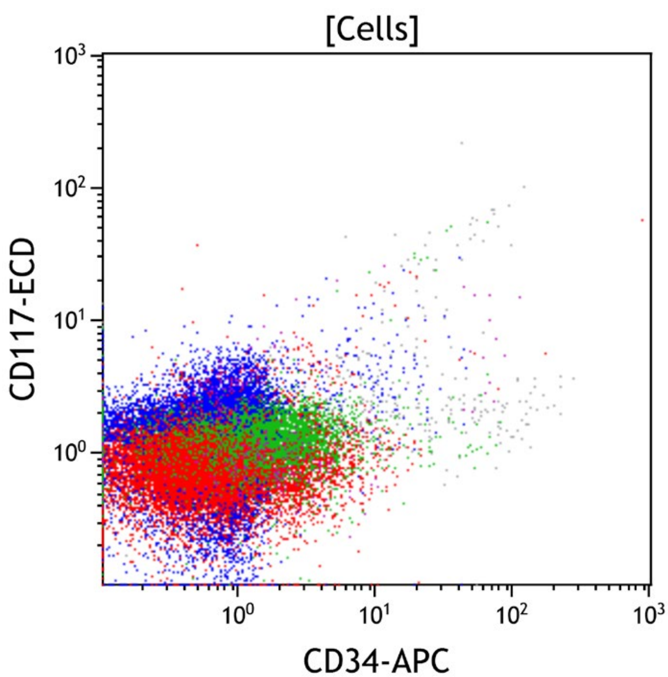


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors.

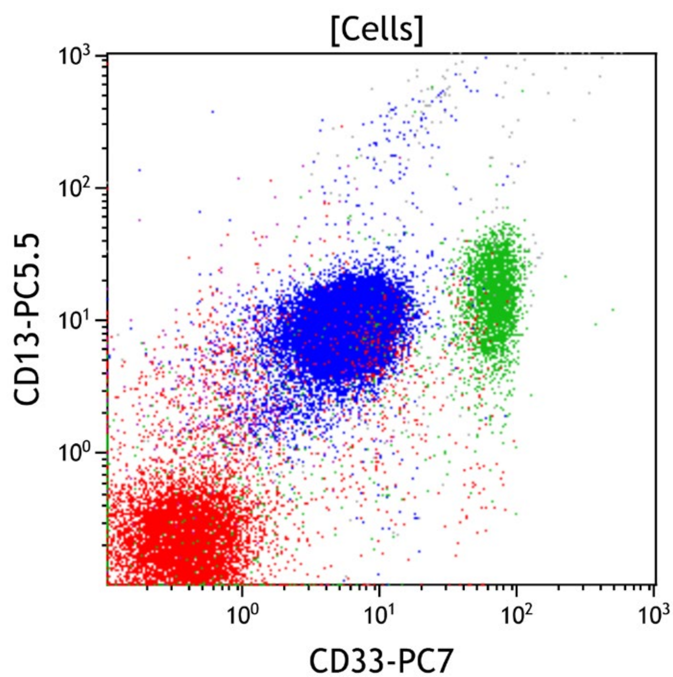


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Lymphocytes largely do not express either CD13 or CD33 (red).

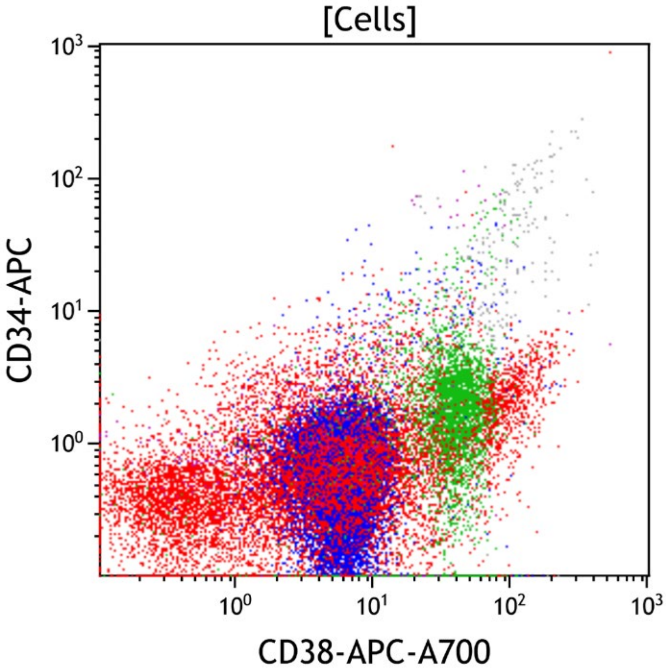


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34.

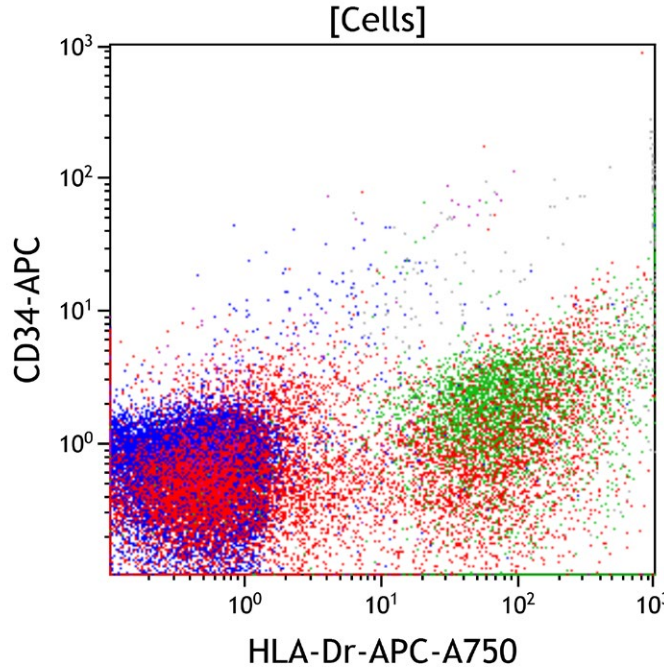


Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors.

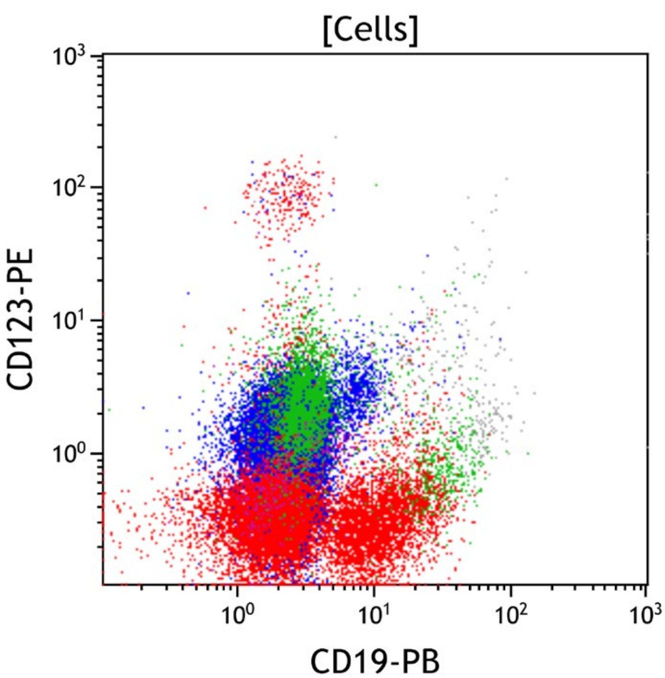


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red, lower middle) normally do not express significant CD123.

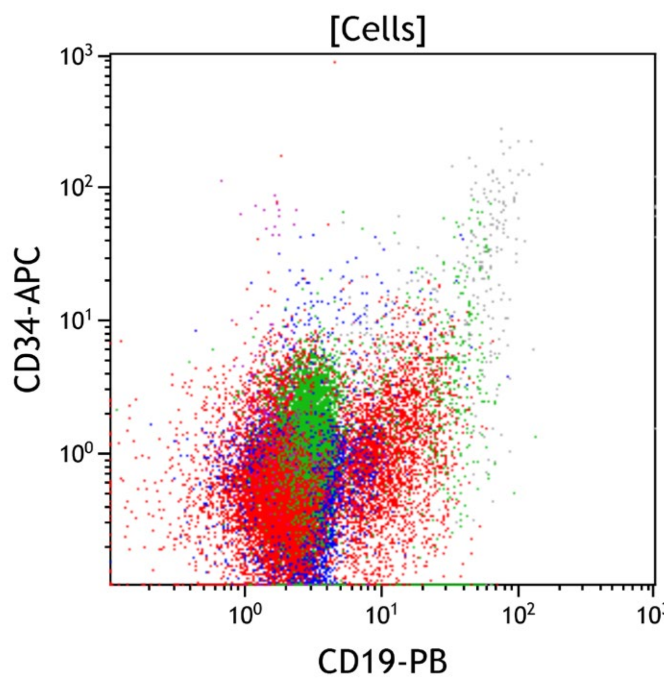


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red, lower middle) do not express CD34.

M2 Cell Tube

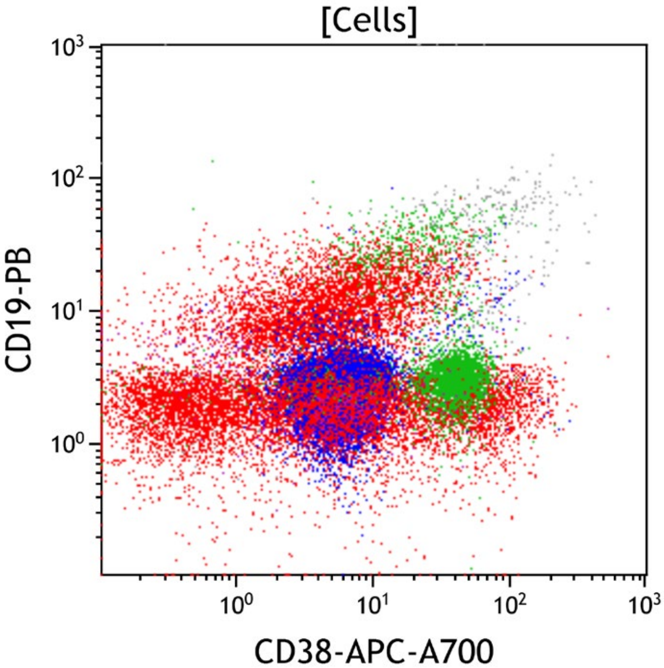


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show intermediate CD38 expression.

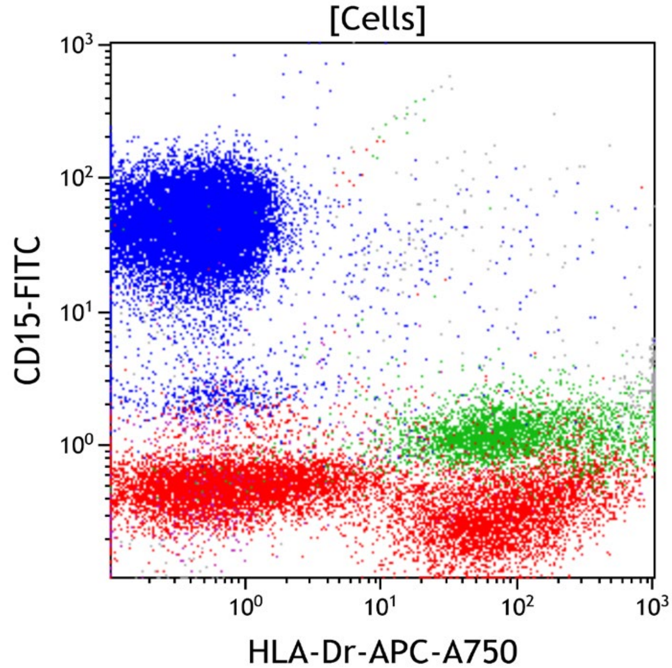


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes (blue) do not express HLA-DR.

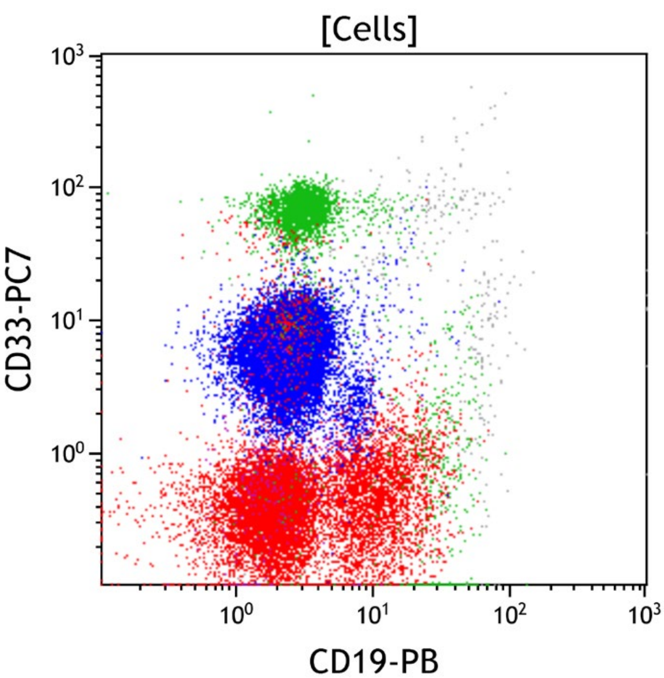


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes (green) and granulocytes (blue). Mature CD19 positive B cells (red, lower middle) do not normally express significant CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate to bright CD5, intermediate CD19, low to intermediate CD20, low to intermediate CD38, bright CD45, intermediate CD200, and dim surface kappa light chain expression without CD10 or other T or myeloid markers. Compared with normal B cells, the expression of CD5, decreased CD20, and kappa light chain restriction of low intensity are aberrant.

Taken together, the immunophenotype of the aberrant population is most consistent with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). However, a definitive diagnosis of CLL/SLL using current WHO criteria requires the demonstration of disease related clinical and/or laboratory findings and/or the presence of greater than 5,000 neoplastic cells per microliter in the peripheral blood. Therefore, correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

Case #7: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Clinical Vignette

This 65-year-old male presents with lymphocytosis. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

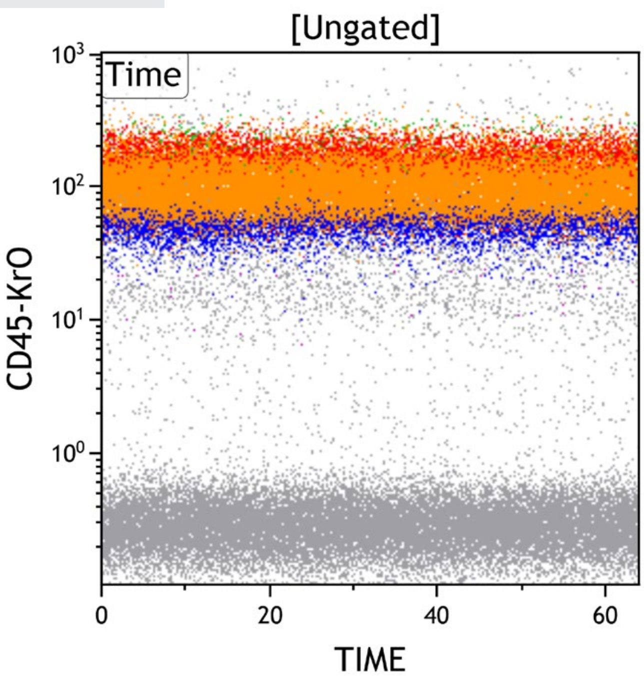


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

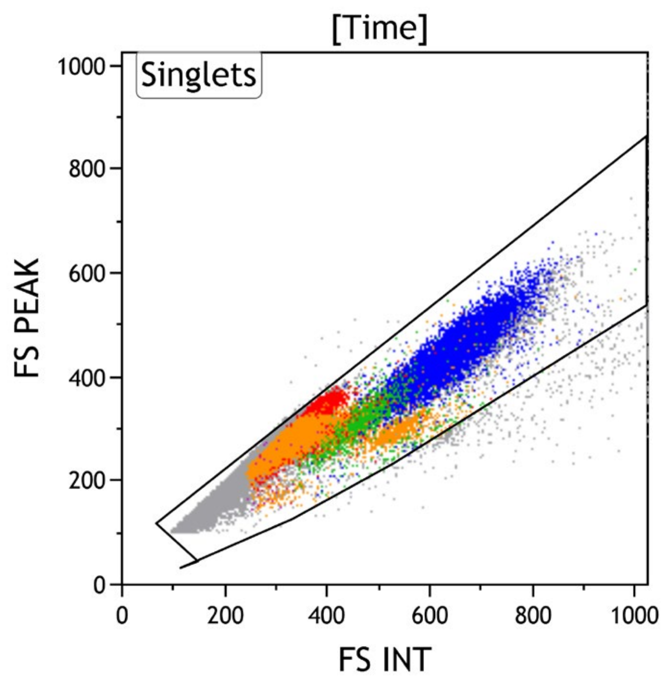


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

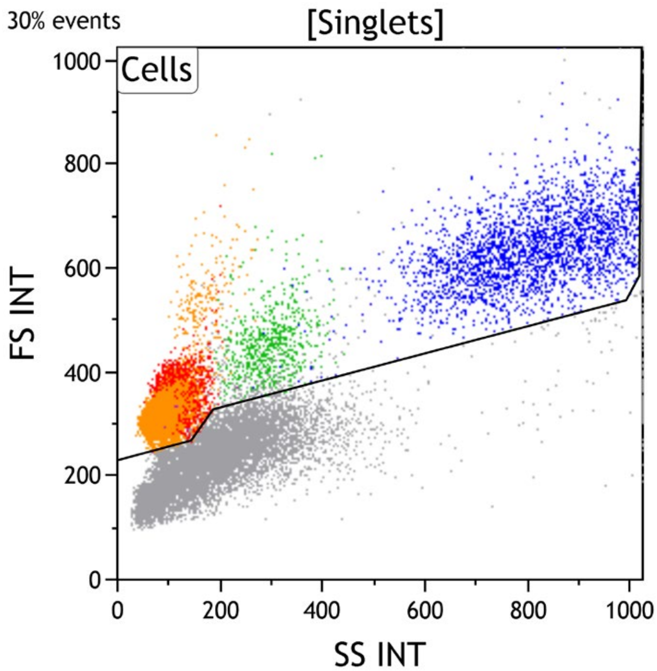


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

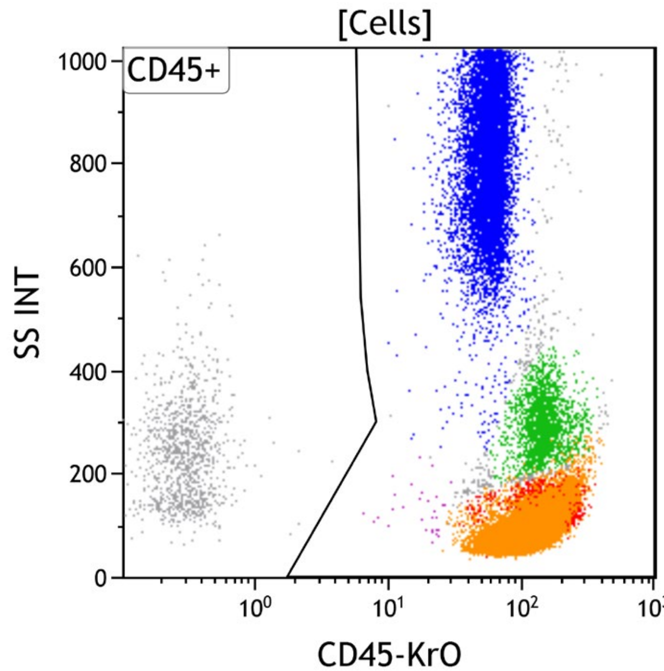


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

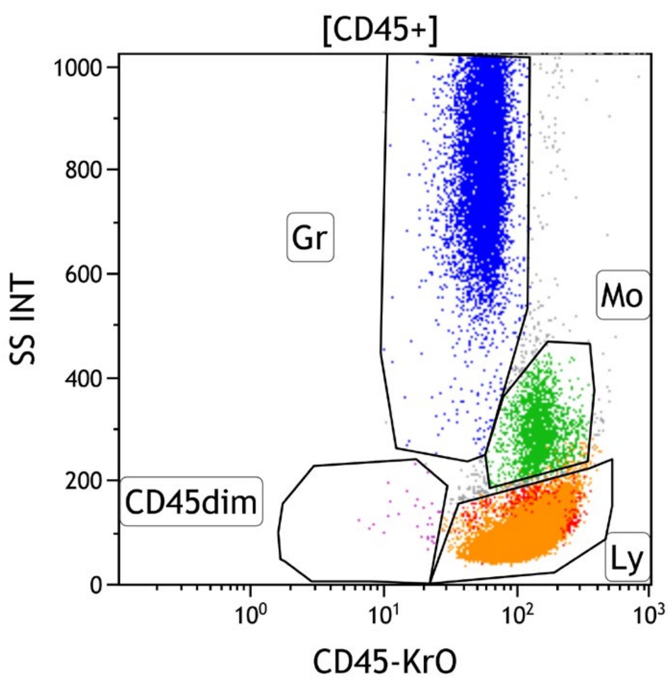


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

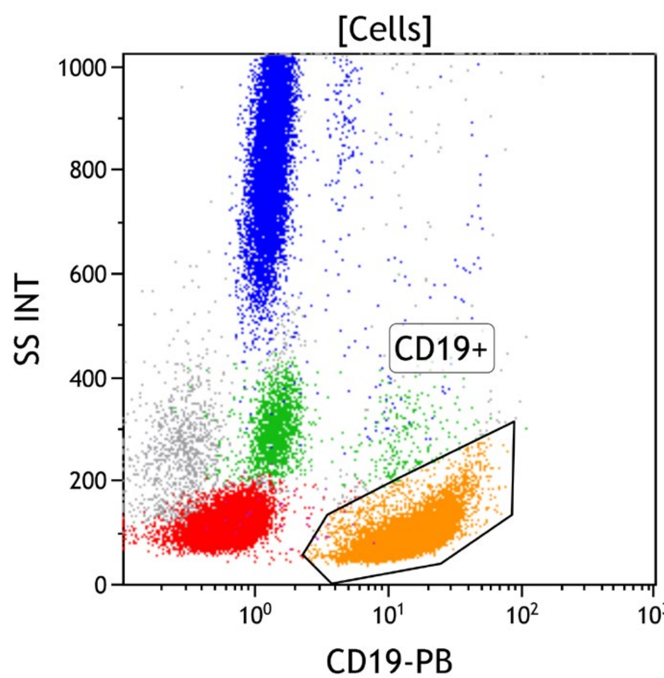


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note the relatively increased number of CD19 positive B cells in this sample.

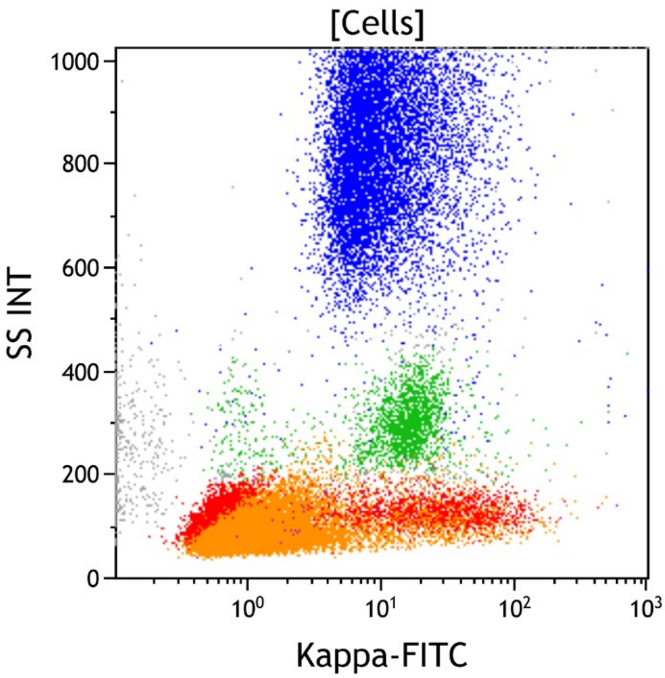


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) lack kappa light chain expression.

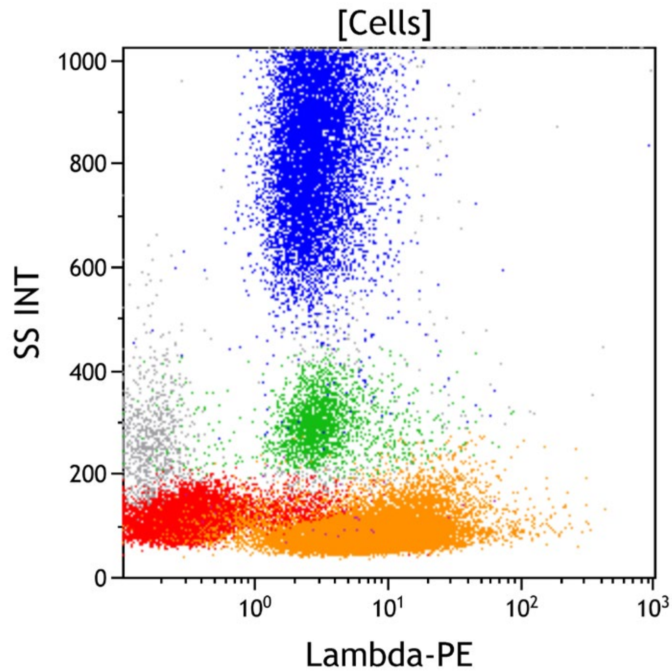


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) have surface lambda light chain expression.

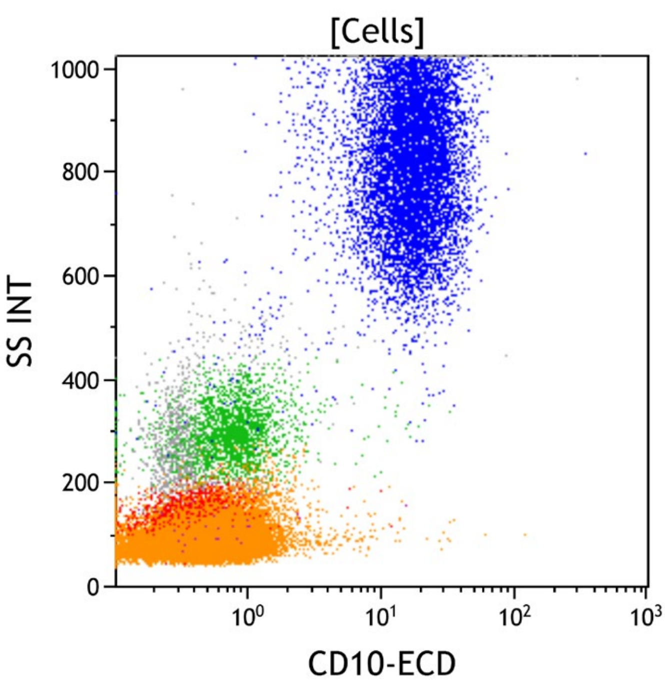


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange) is negative for CD10.

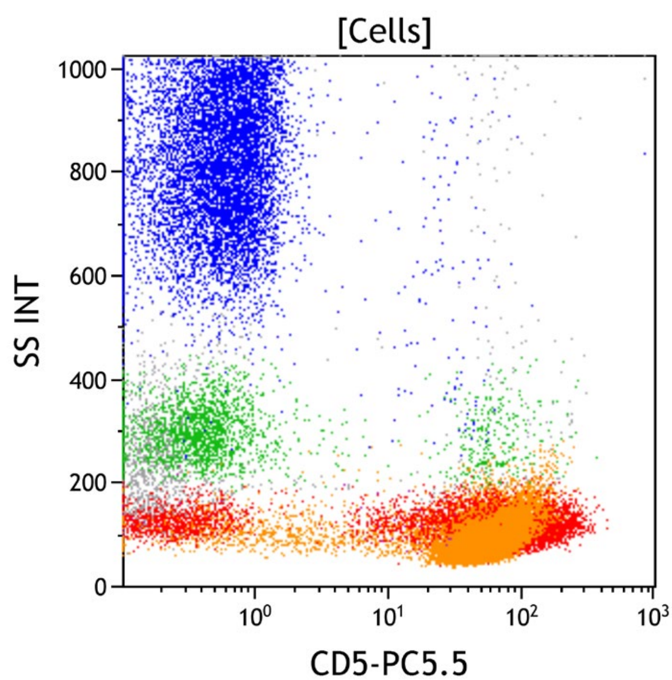


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature T cells (red), as well as dimly expressed in a subset of mature B cells. These lymphoid cells typically have low side scatter. The CD19 positive population (orange) expresses CD5 at a level similar to T cells (red).

B Cell Tube

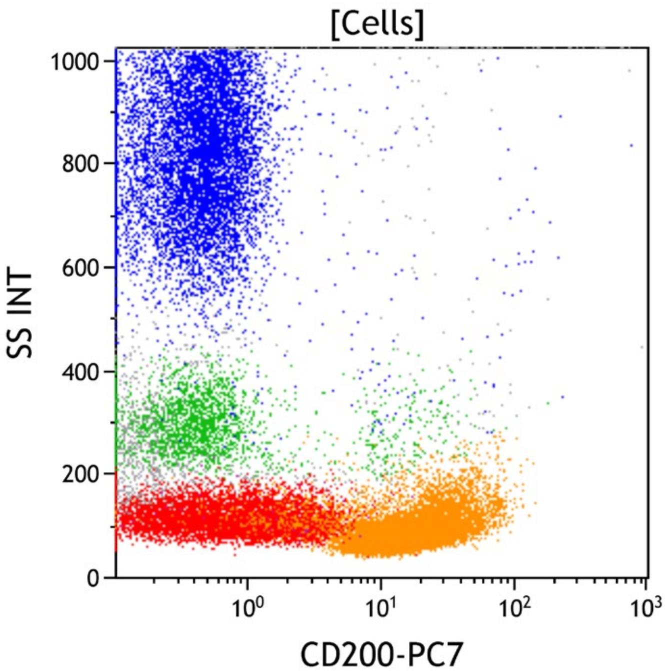


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The CD19 positive population (orange) is positive for CD200.

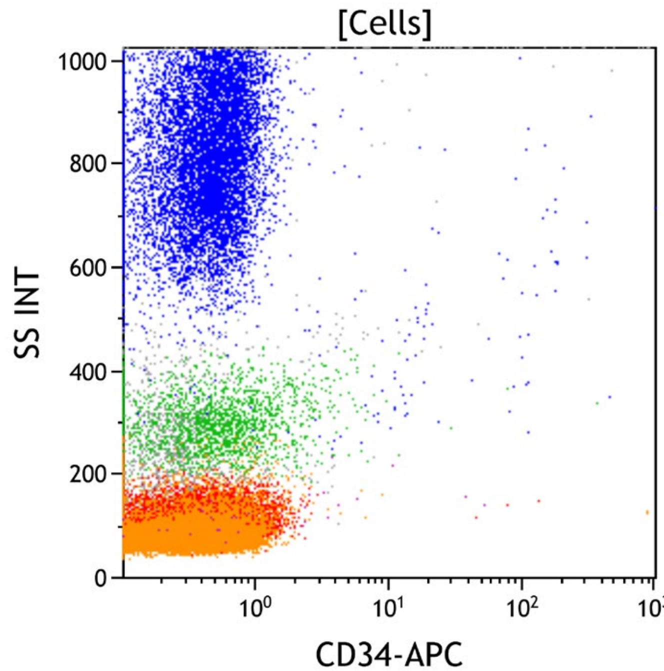


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The CD19 positive population (orange) is negative for CD34.

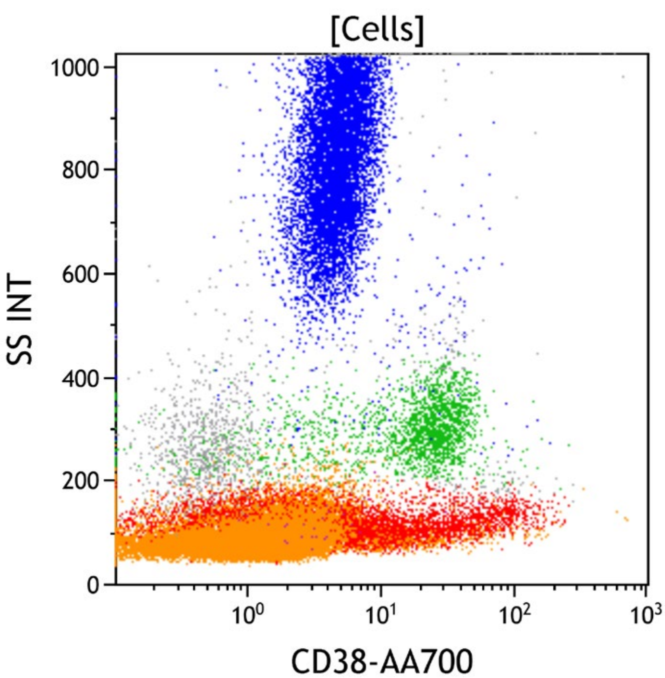


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). The CD19 positive population (orange) displays low to intermediate CD38 expression.

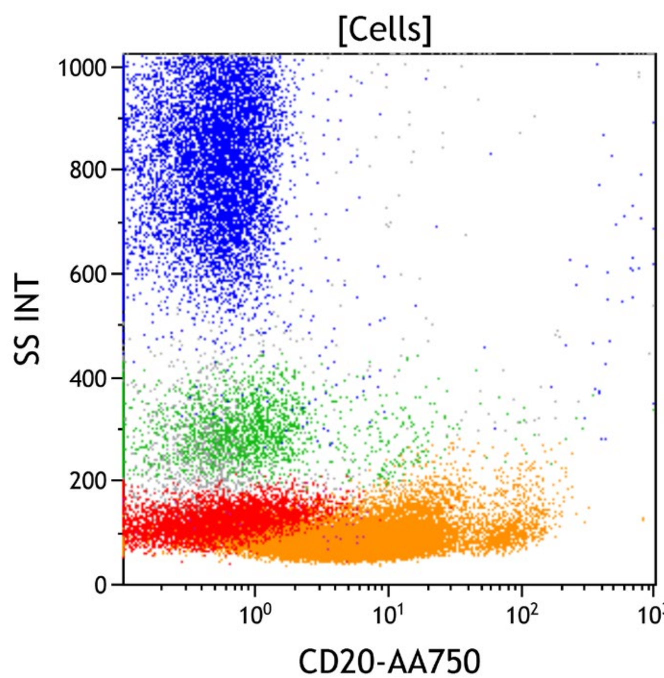


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The CD19 positive population (orange) displays variably decreased CD20 expression. In comparison to the high level seen on normal B cells (also in orange, lower right).

B Cell Tube

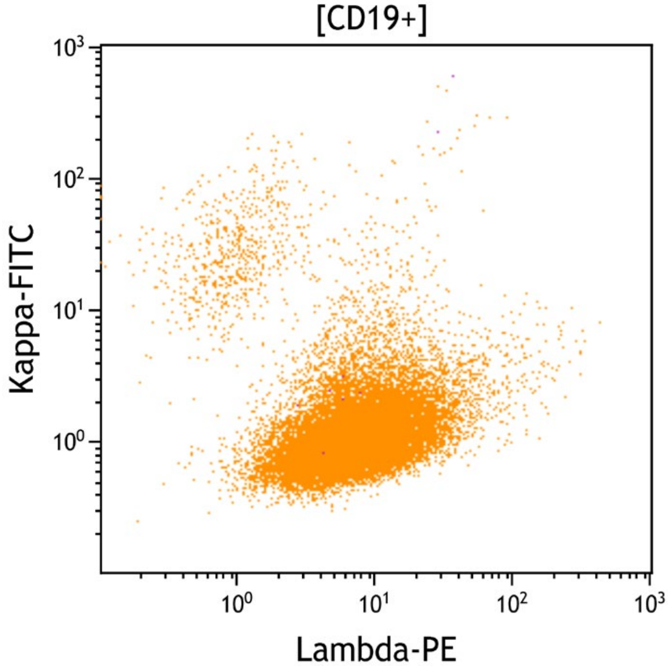


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. The CD19 positive cells (orange) predominantly have surface lambda light chain expression at a decreased level compared with normal mature B cells, indicating a clonal B cell population. A small population of polyclonal B cells (also in orange) with normal levels of kappa and lambda light chain expression is present.

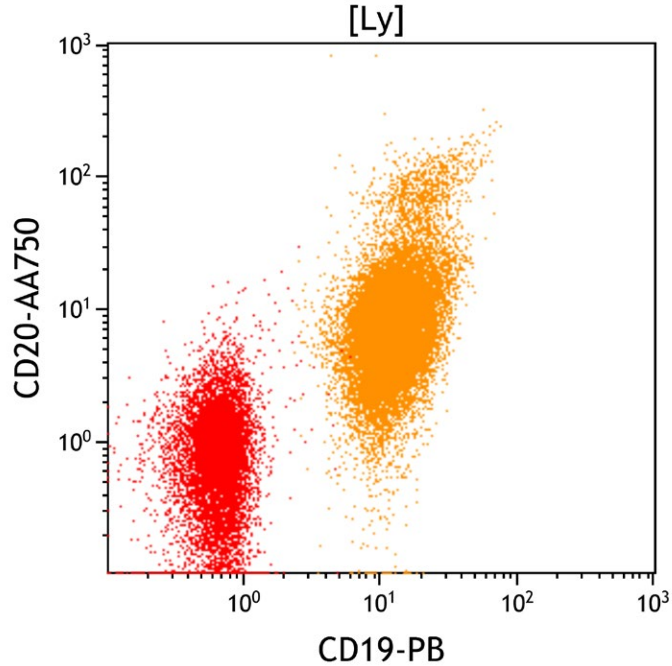


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cells (orange) display decreased CD19 and CD20 expression compared with the higher level seen on normal mature B cells (orange, upper right).

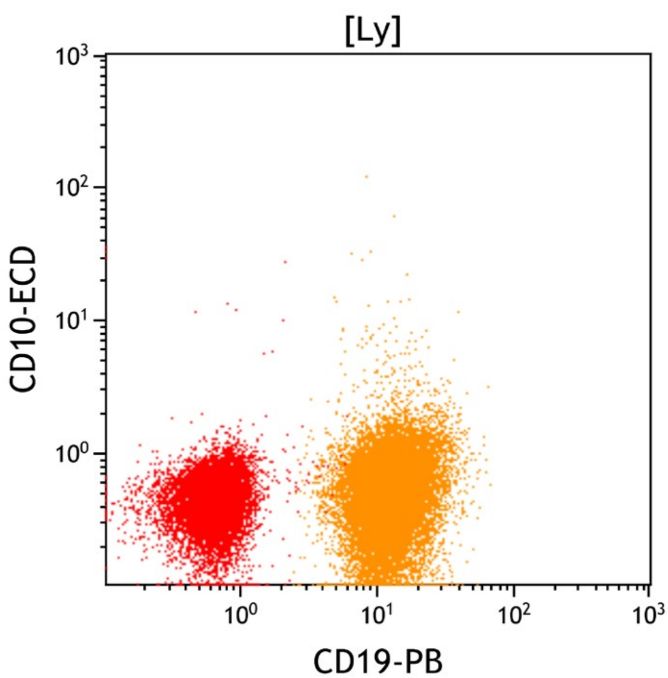


Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45dim gate. The CD19 positive clonal B cell population (orange) is negative for CD10.

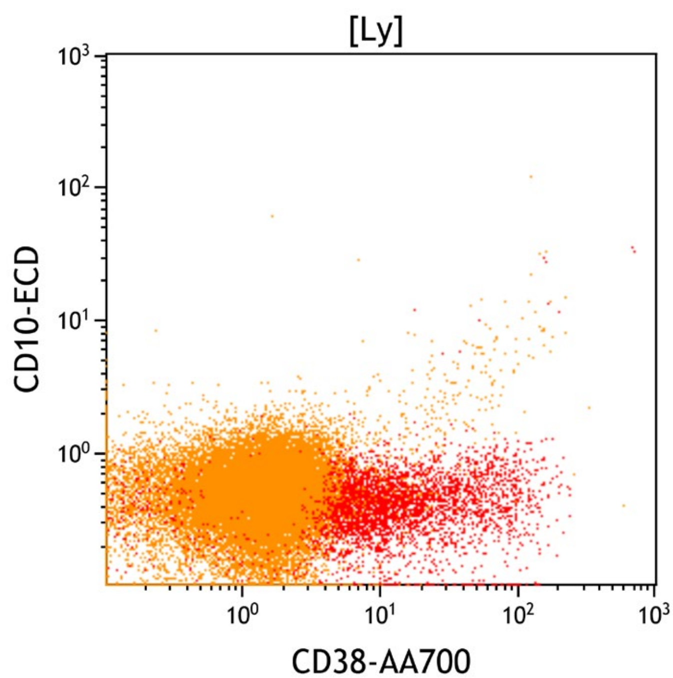


Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The clonal B cell population (orange) displays low to absent CD38 expression and is negative for CD10.

B Cell Tube

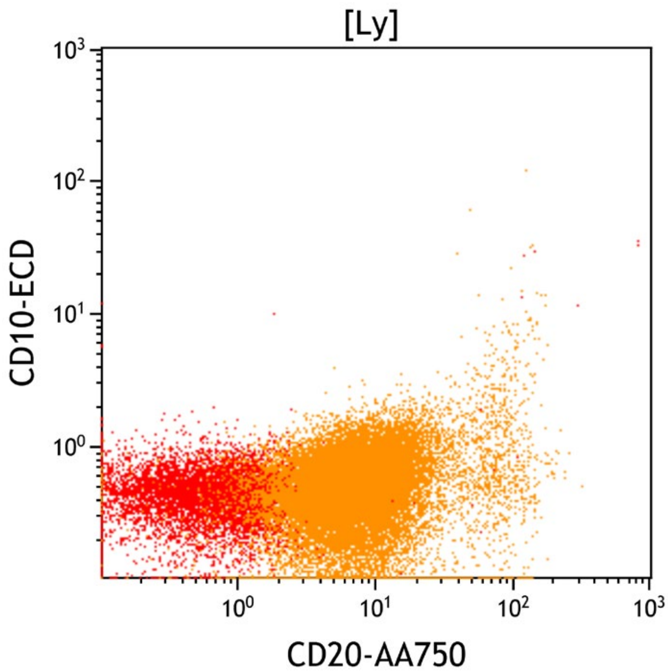


Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20. The clonal B cell population (orange) displays decreased CD20 expression and is negative for CD10. The small population with normal CD20 expression (also in orange, lower right) represents normal B cells.

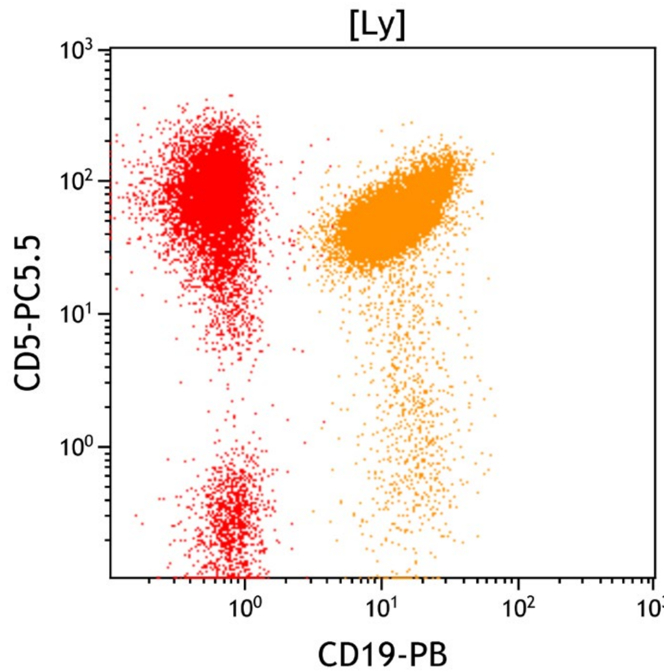


Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper right), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) expresses CD5 and CD19. The small population with normal CD19 and low to absent CD5 expression (also in orange, lower right) represents normal B cells.

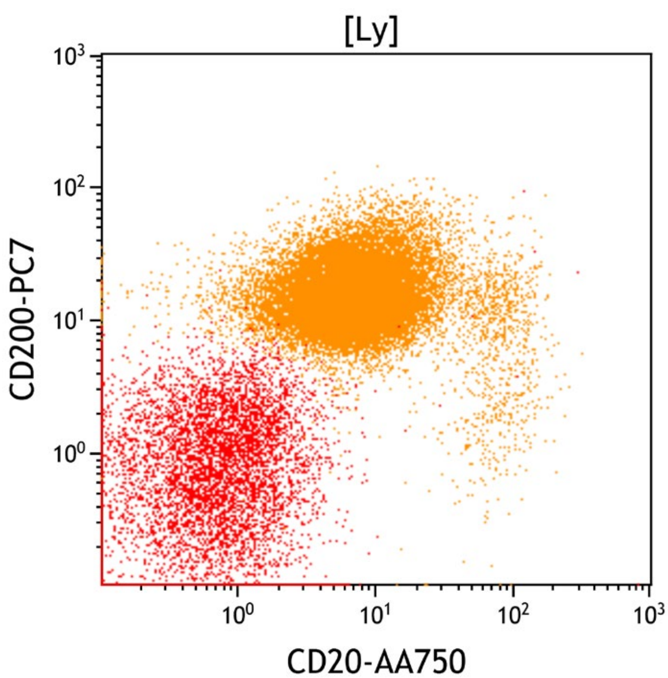


Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The clonal B cell population (orange) is positive for CD200 with decreased CD20. The small population with normal CD20 and CD200 expression (also in orange, right) represents normal B cells.

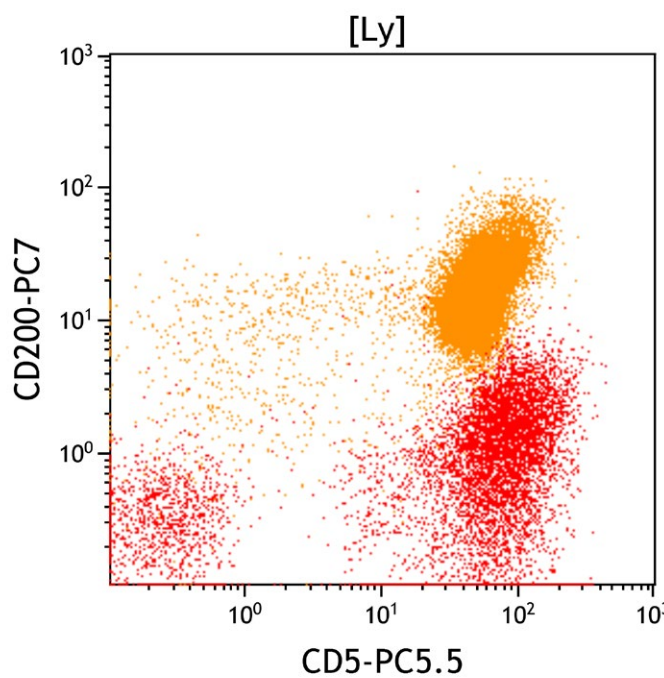


Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The clonal B cell population (orange) is positive for CD5 and CD200, favoring chronic lymphocytic leukemia/small lymphocytic lymphoma.

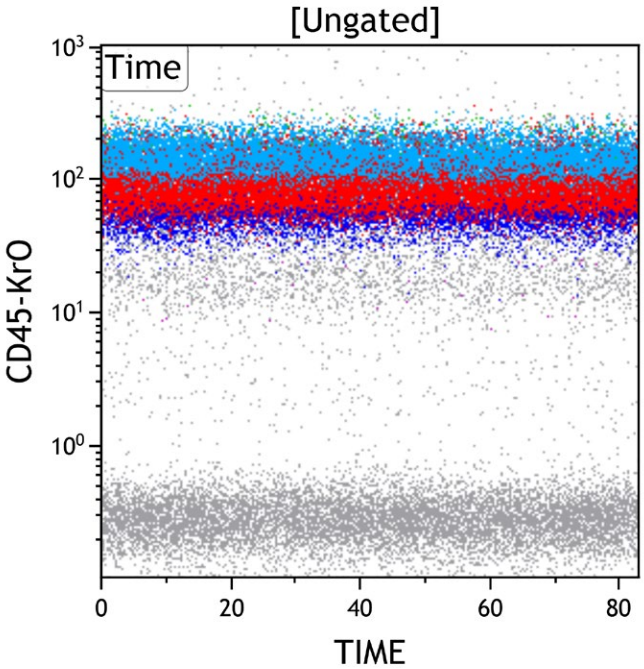


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

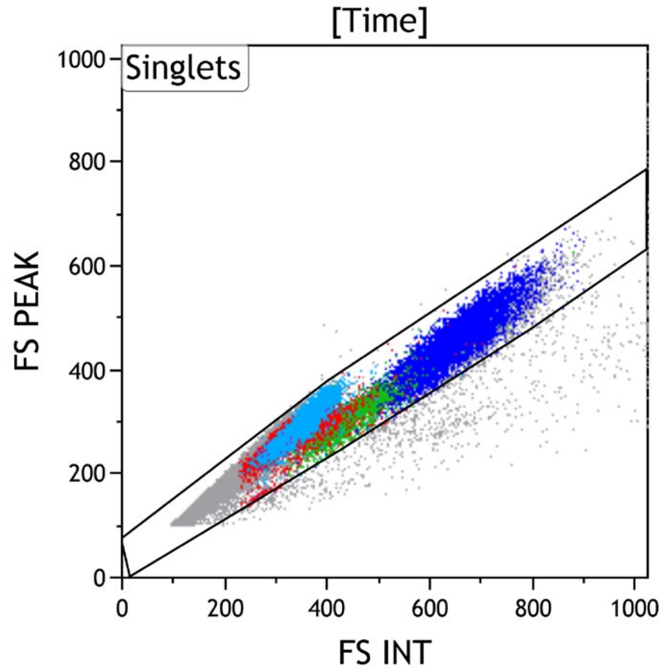


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

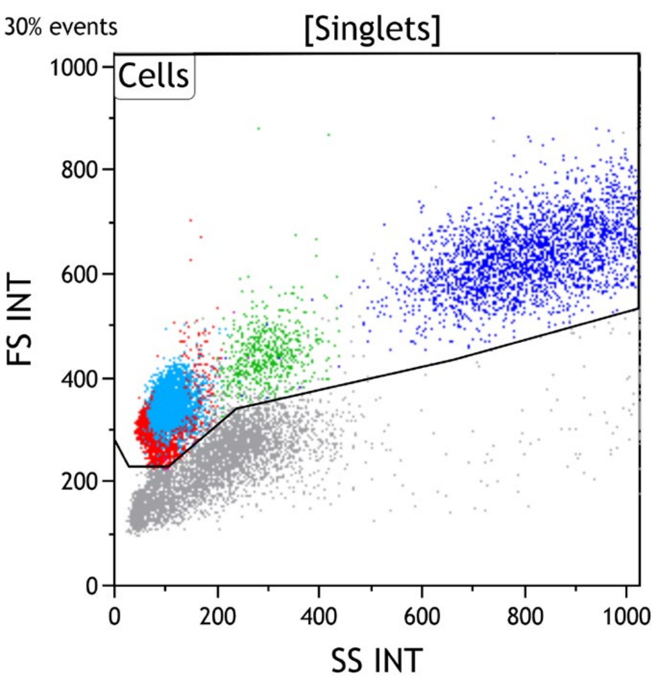


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

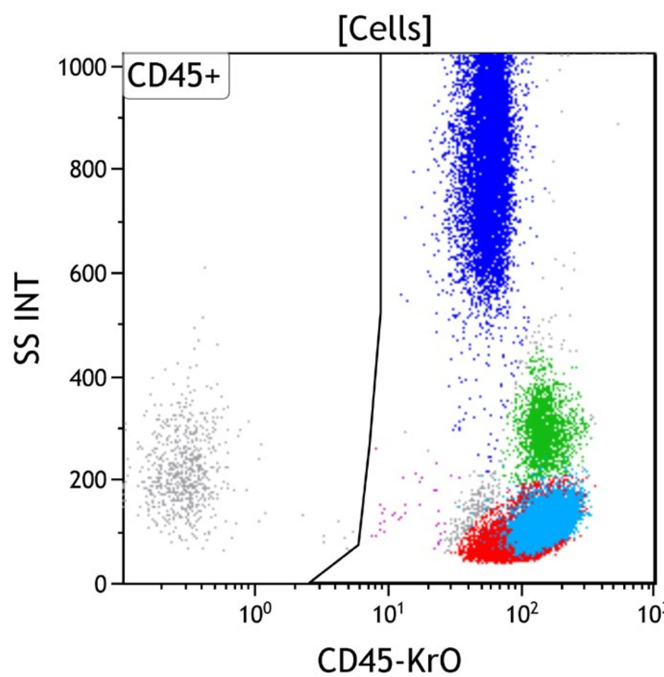


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

T Cell Tube

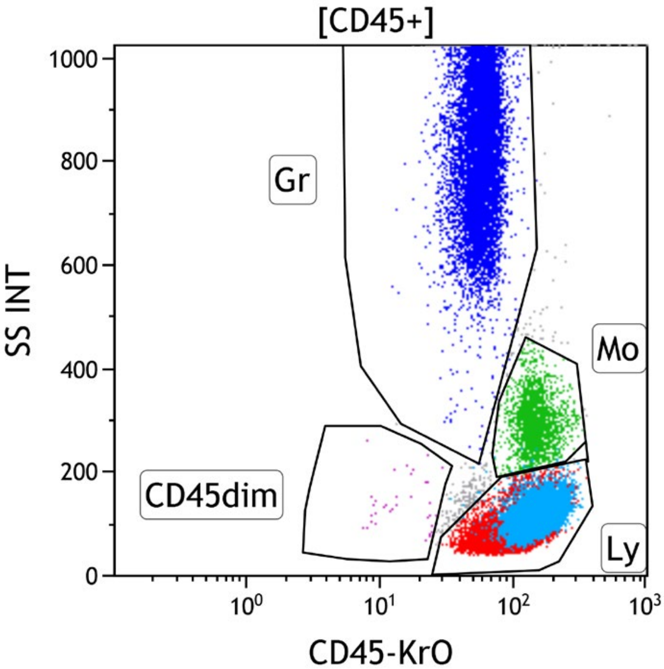


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, aqua/red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

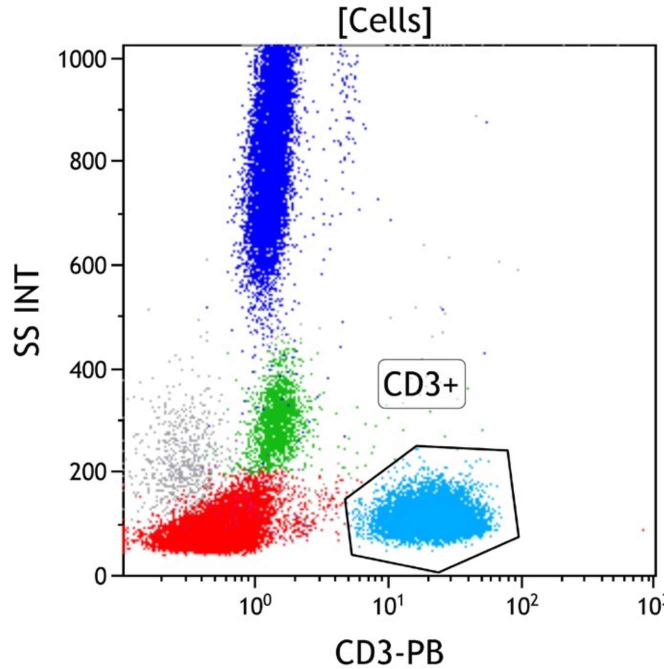


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies surface CD3 positive cells (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells.

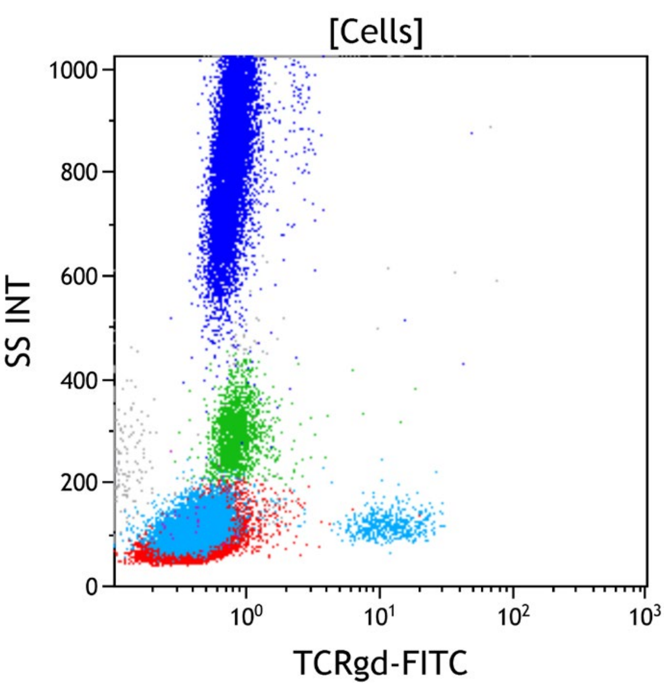


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

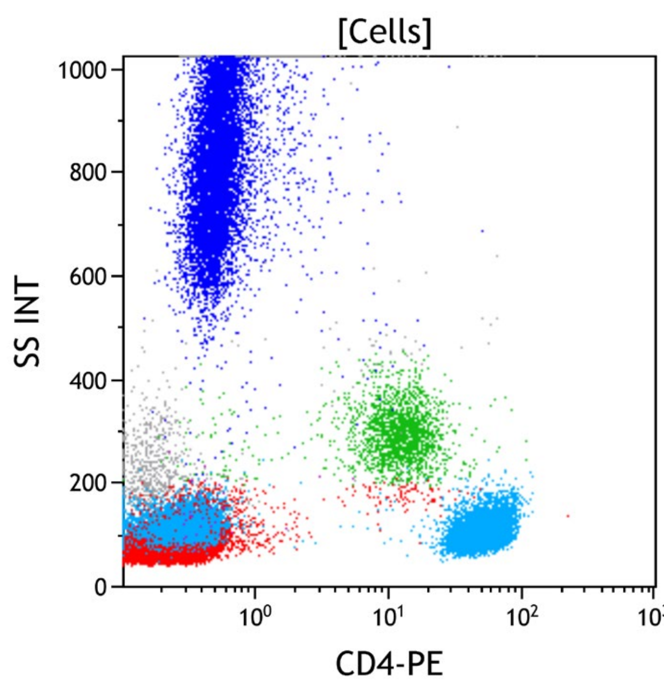


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells.

T Cell Tube

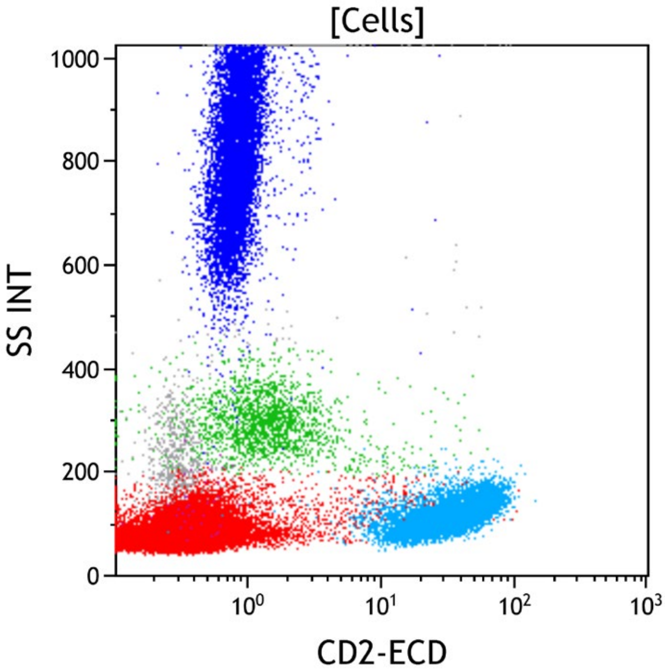


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

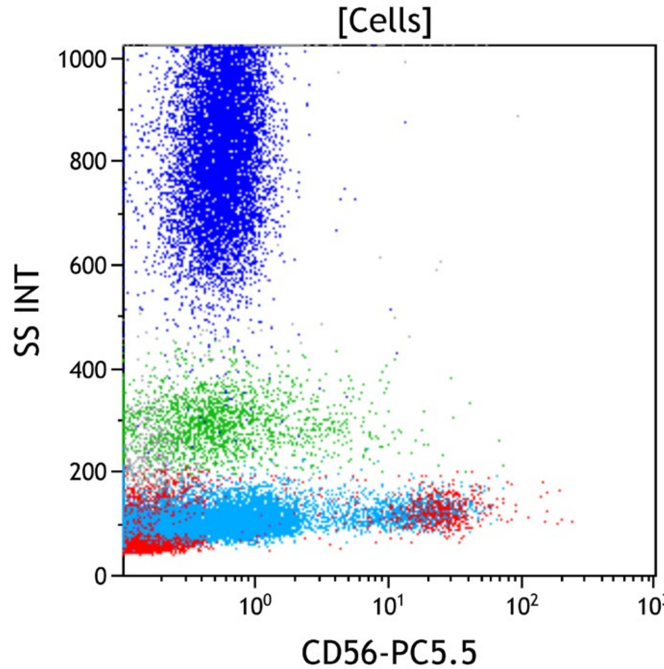


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

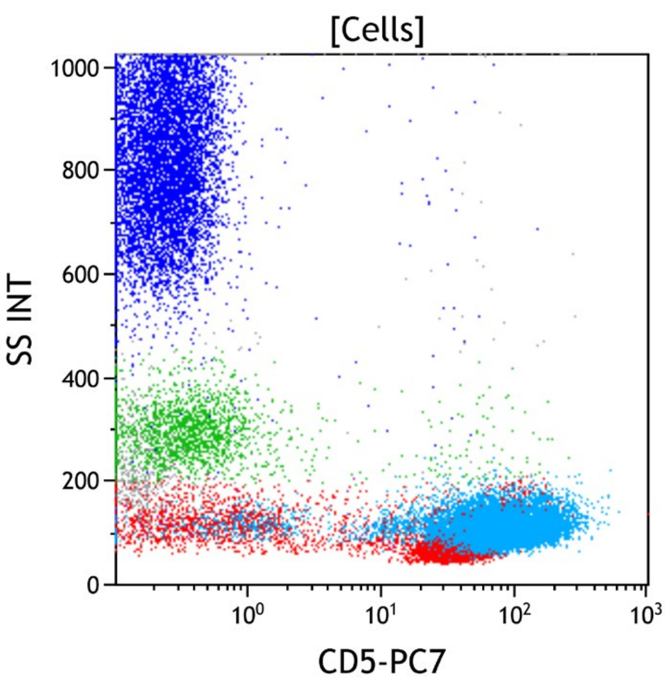


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The CD3 negative cells in the lymphocyte gate (red, lower right) are aberrant B cells with CD5 expression.

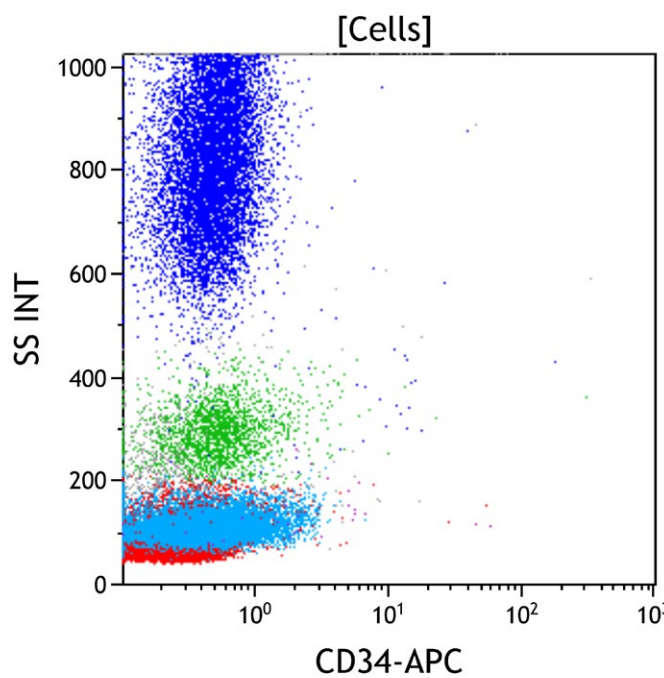


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The mature granulocytes, monocytes, and lymphocytes are negative for CD34.

T Cell Tube

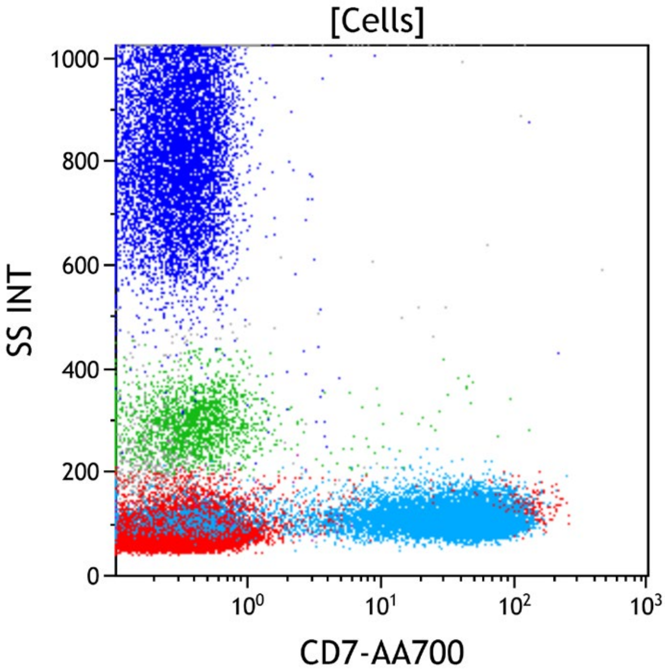


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

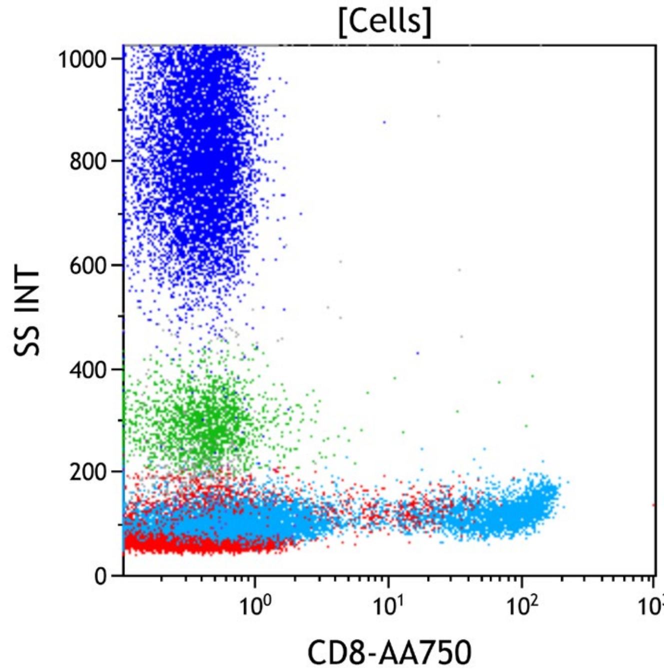


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

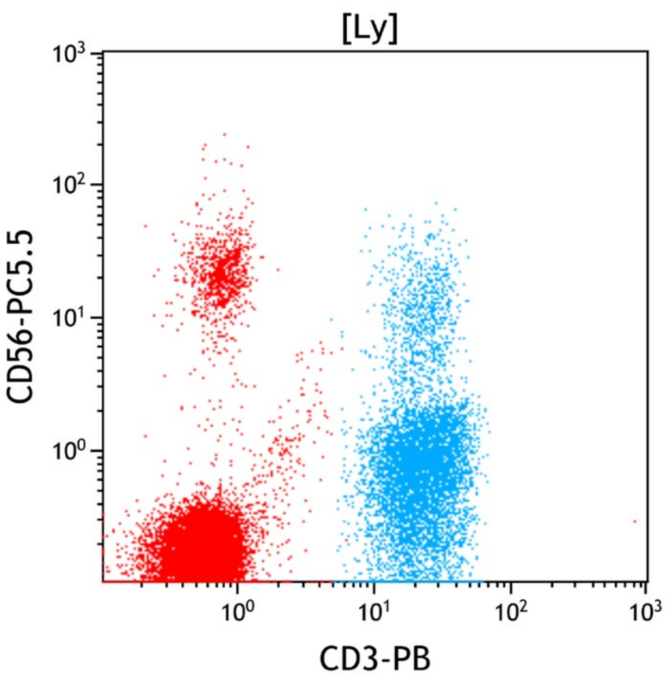


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells (aqua) and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

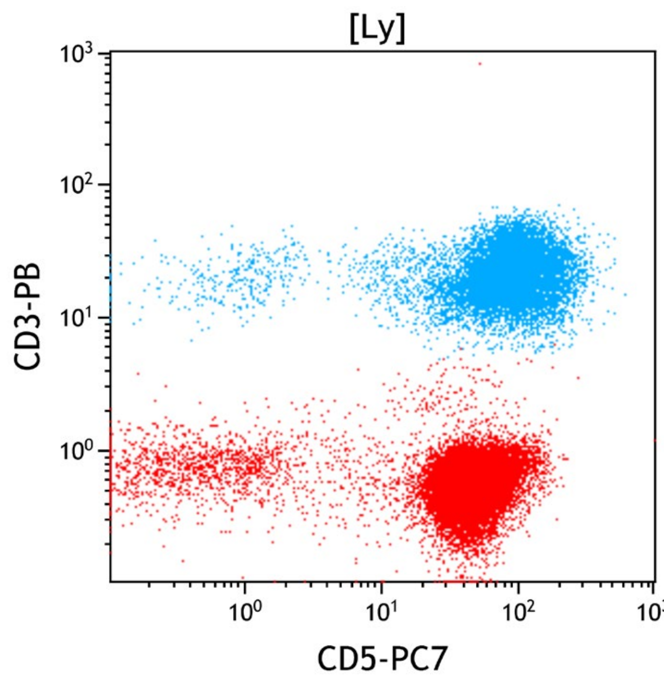


Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red). The CD5 positive cells without CD3 (red, lower right) are aberrant B cells.

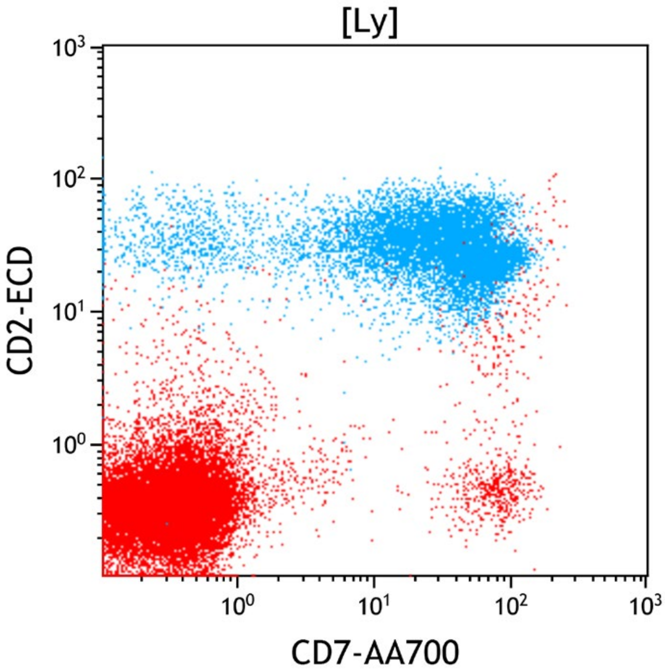


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

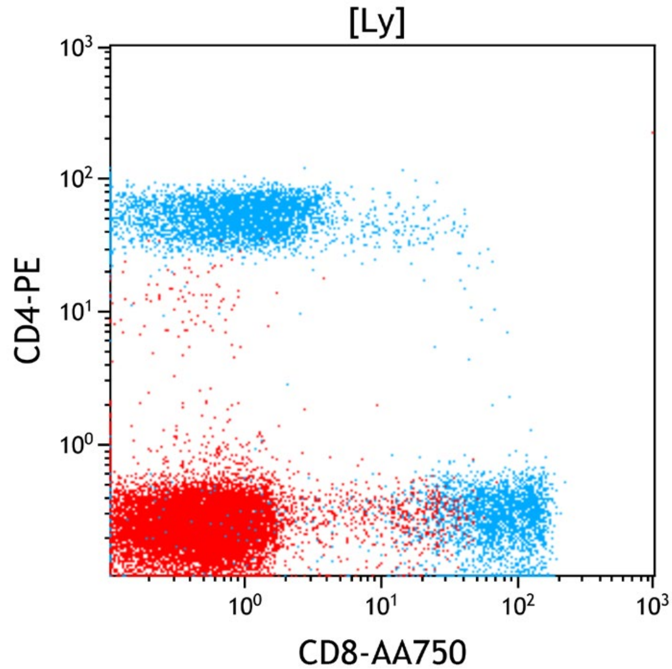


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells.

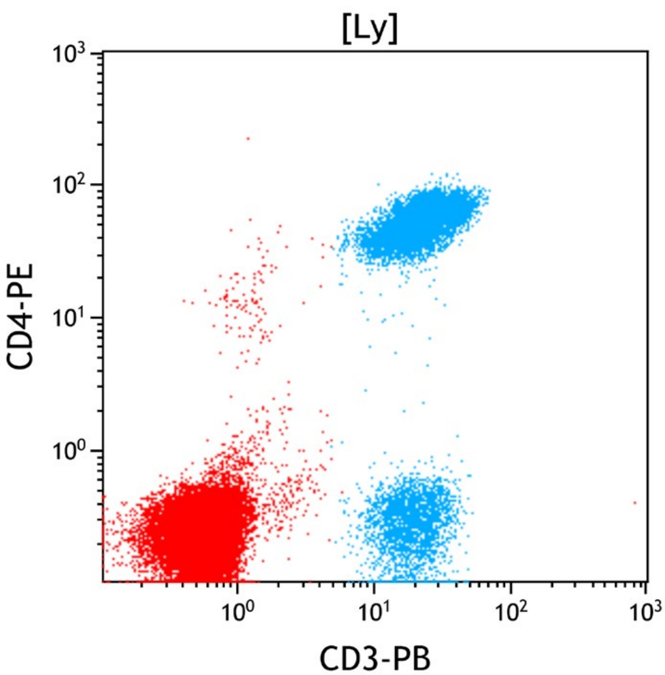


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. Of note, the CD4 positive but CD3 negative cells (red, upper left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

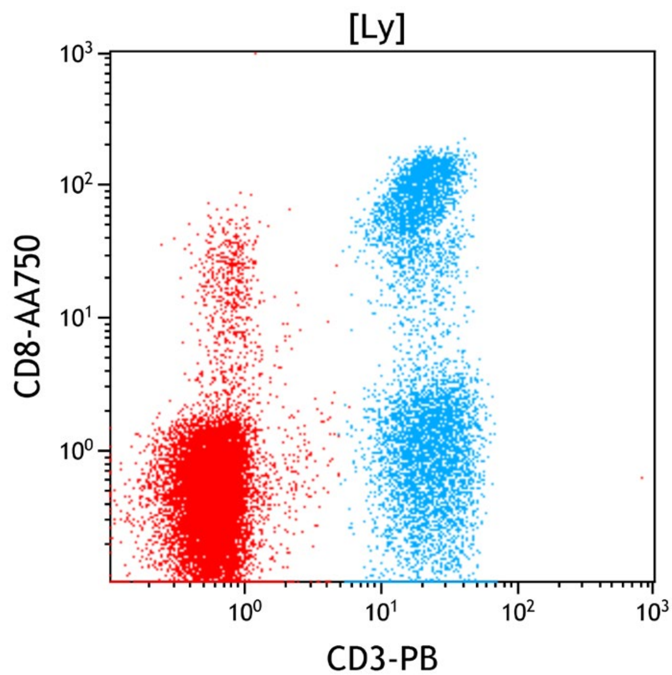


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

T Cell Tube

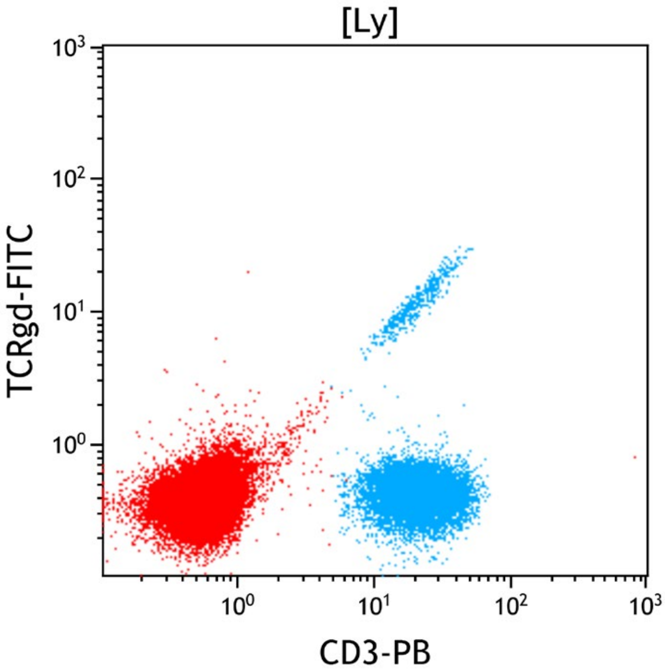


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

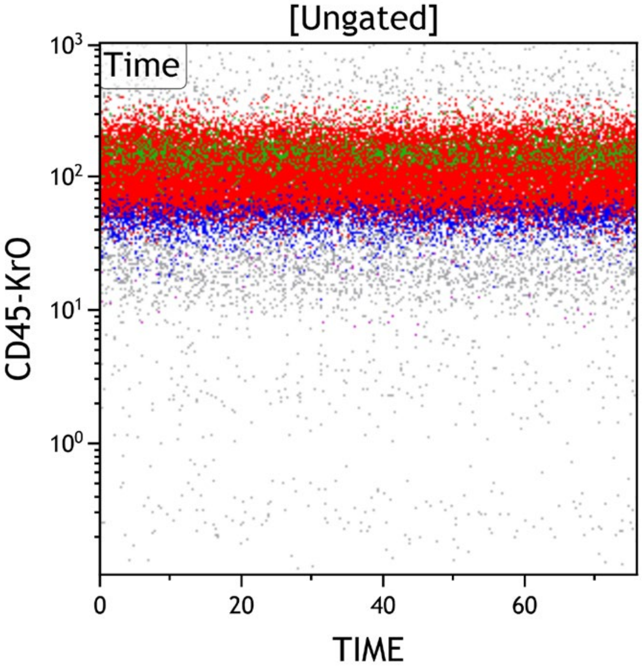
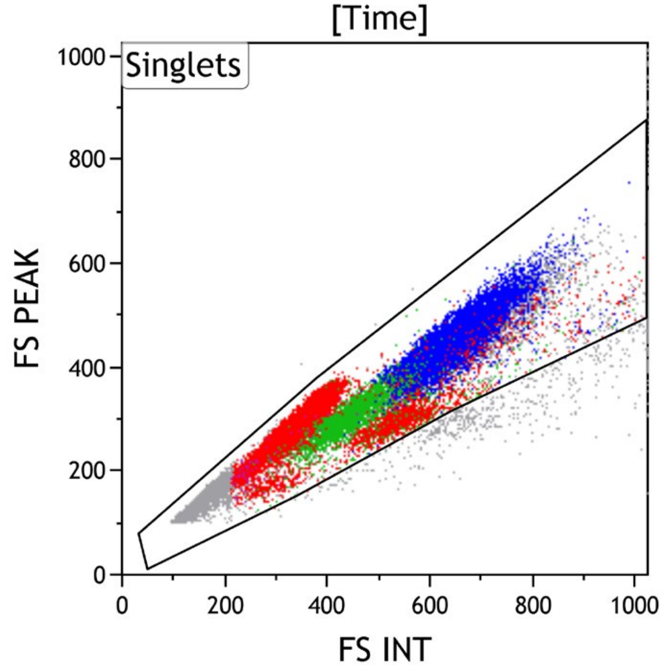


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.



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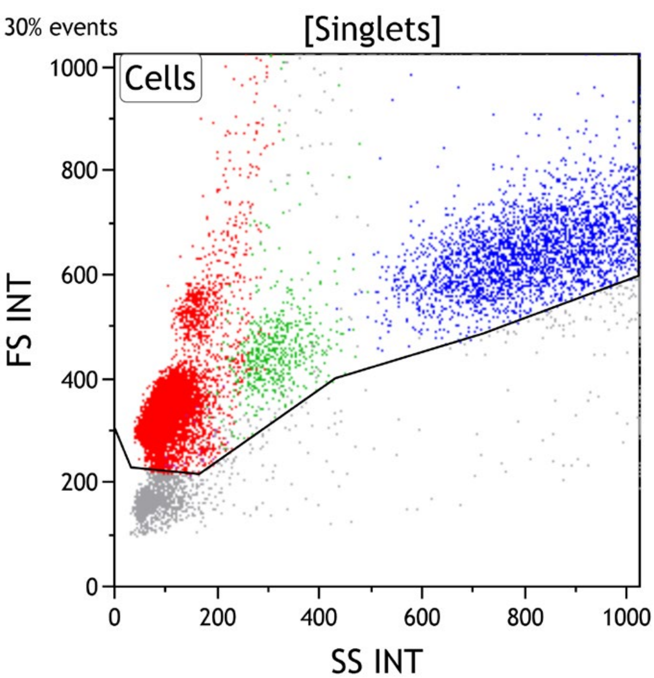


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

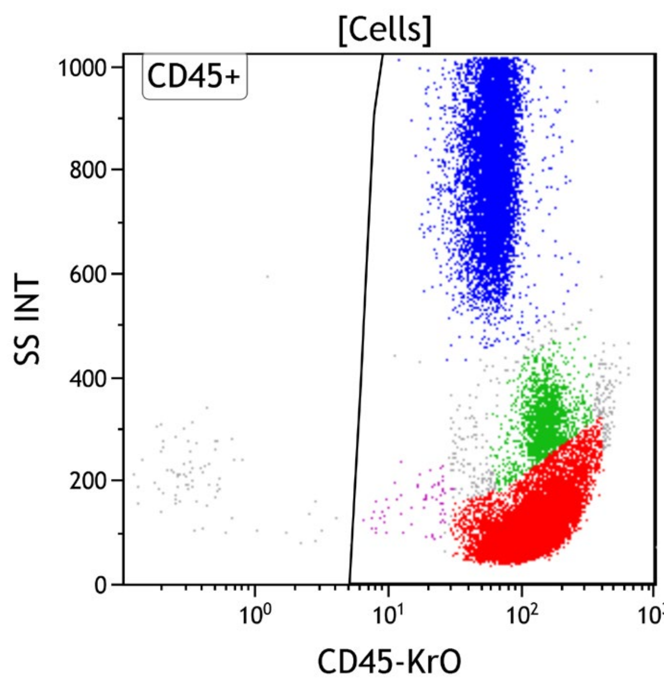


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

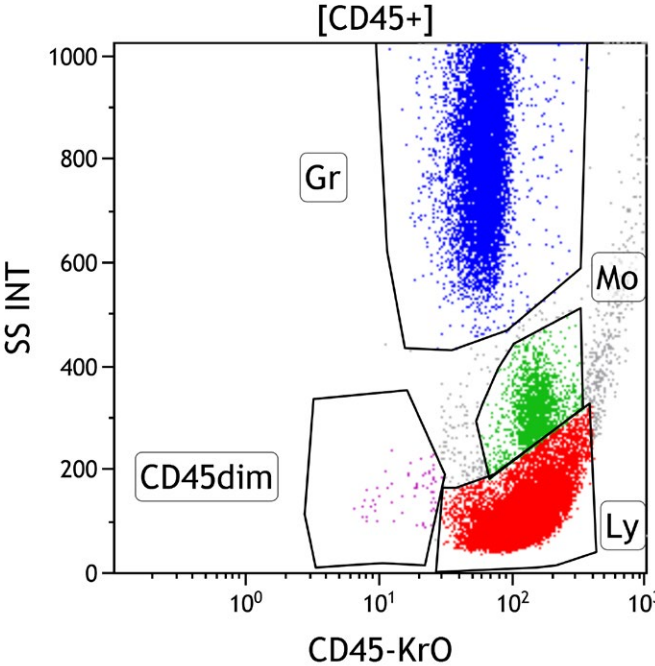


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

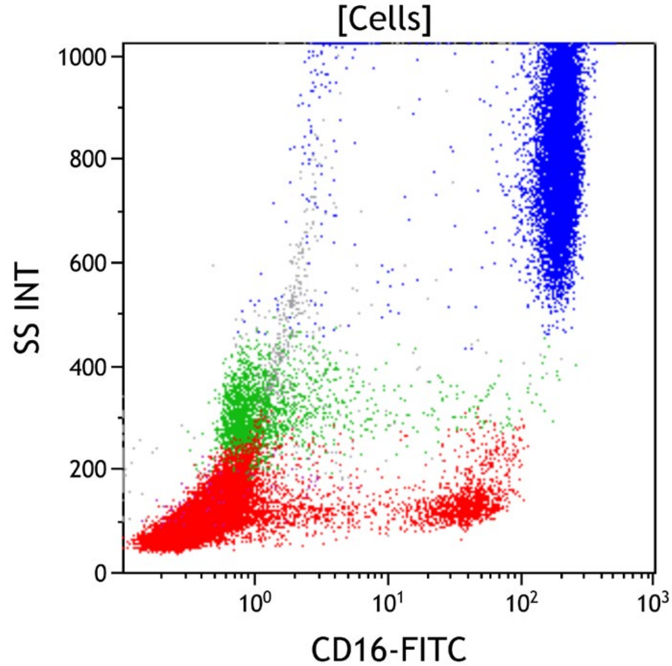


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on granulocytes (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

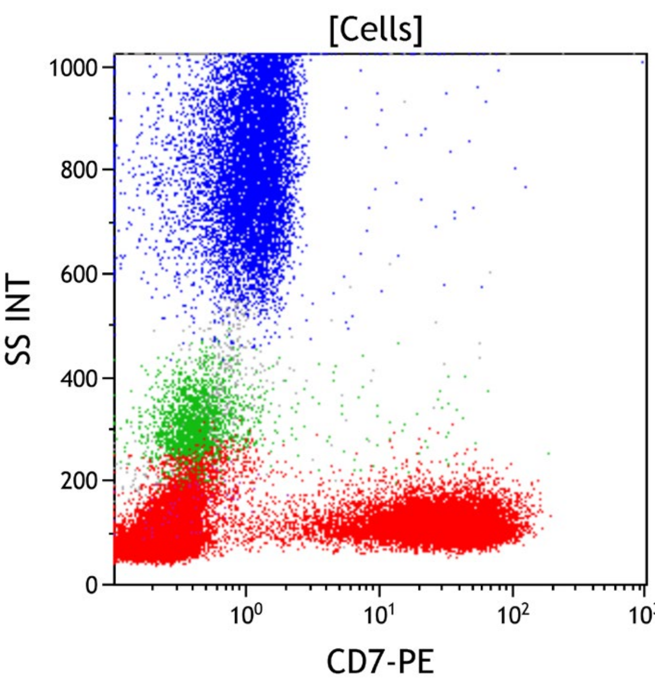


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red lower, right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

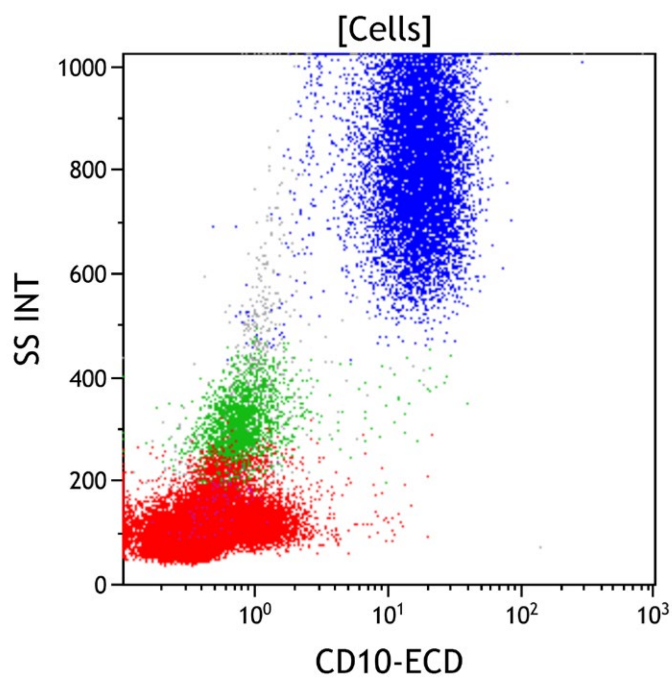


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show variable dim expression of CD10.

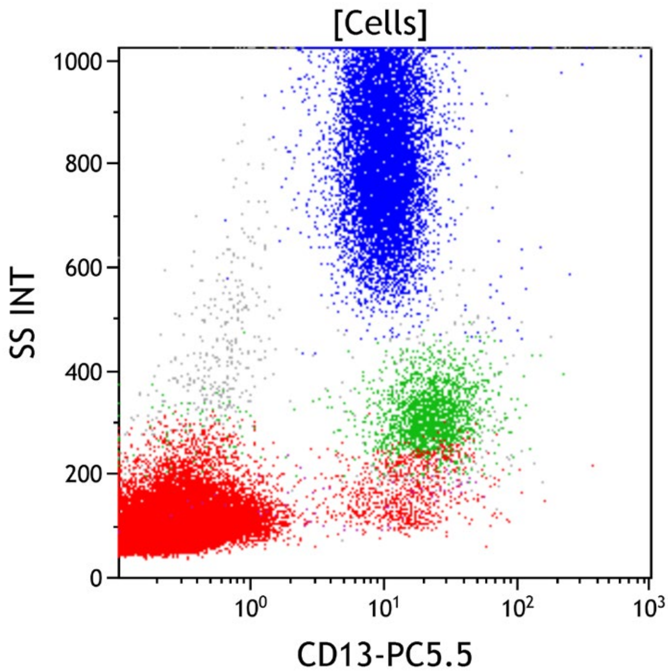


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue, with high side scatter) and on mature monocytes (green).

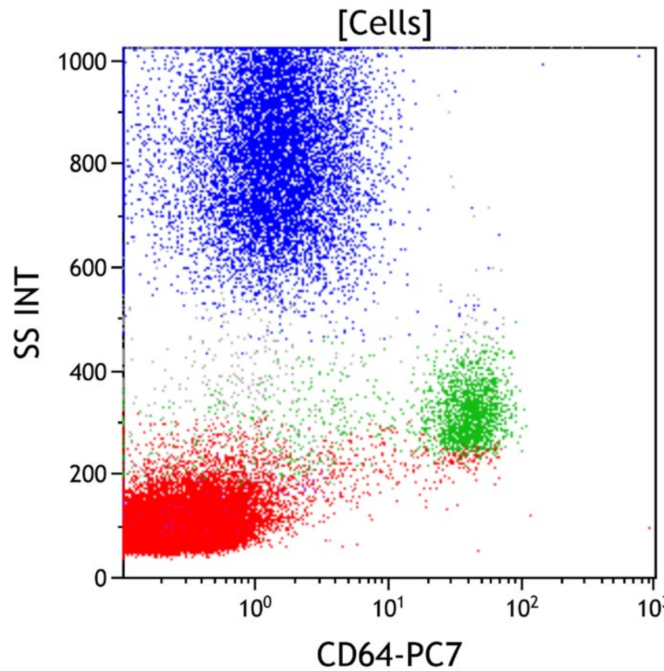


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors.

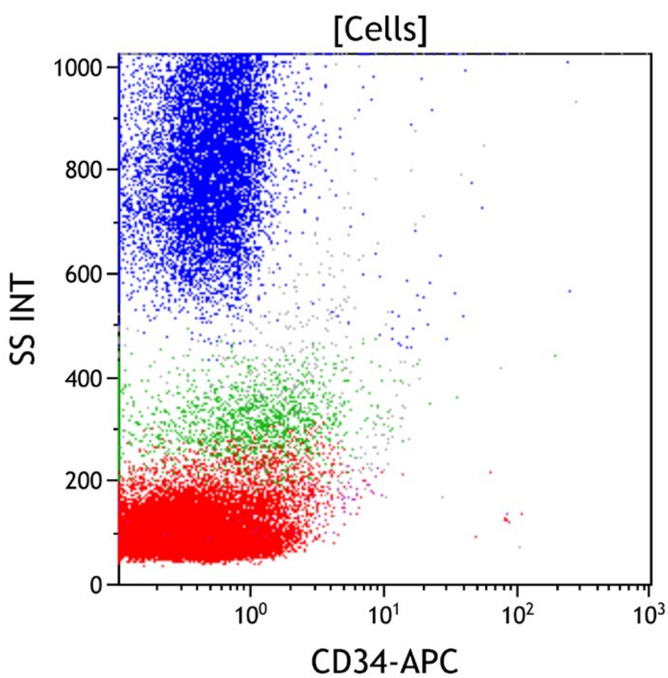


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34.

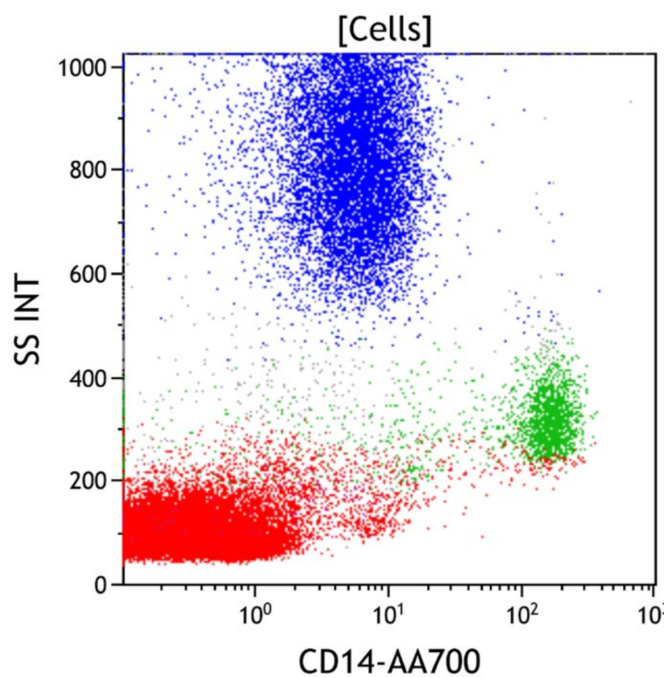


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on granulocytes (blue) at a low level.

M1 Cell Tube

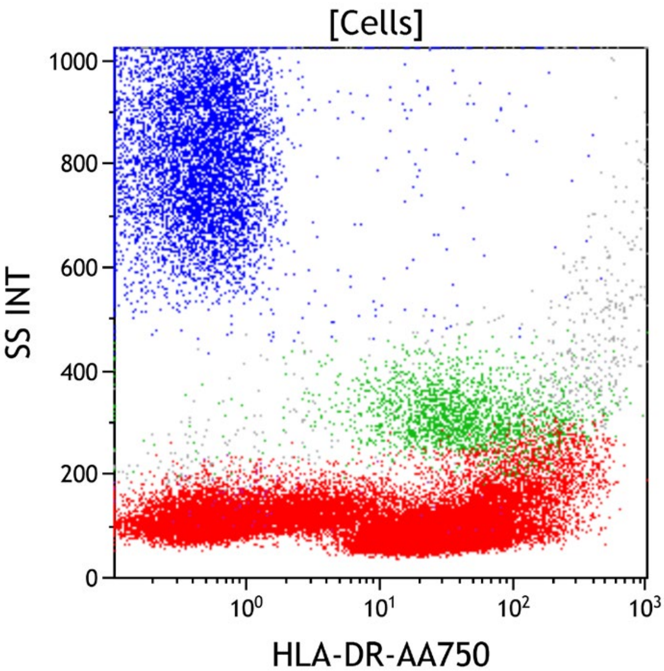


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red) and immature B cells, and activated T cells (red).

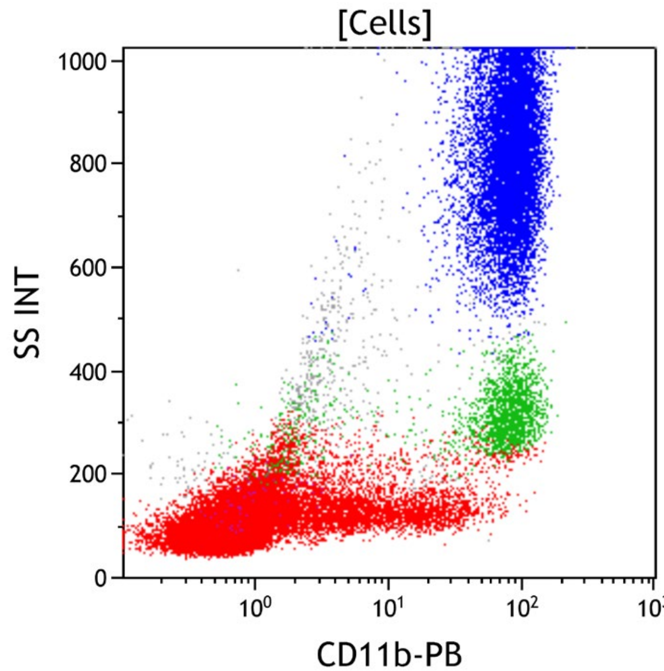


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (blue) and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils.

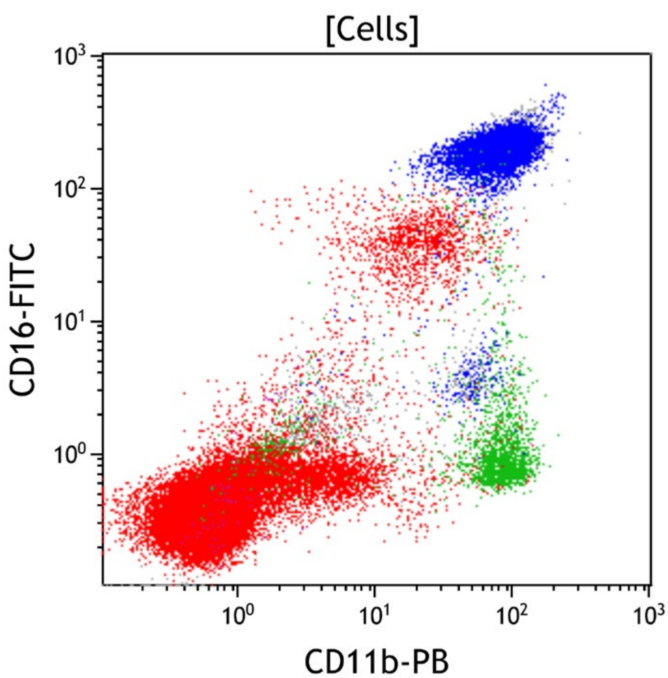


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue), and NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red).

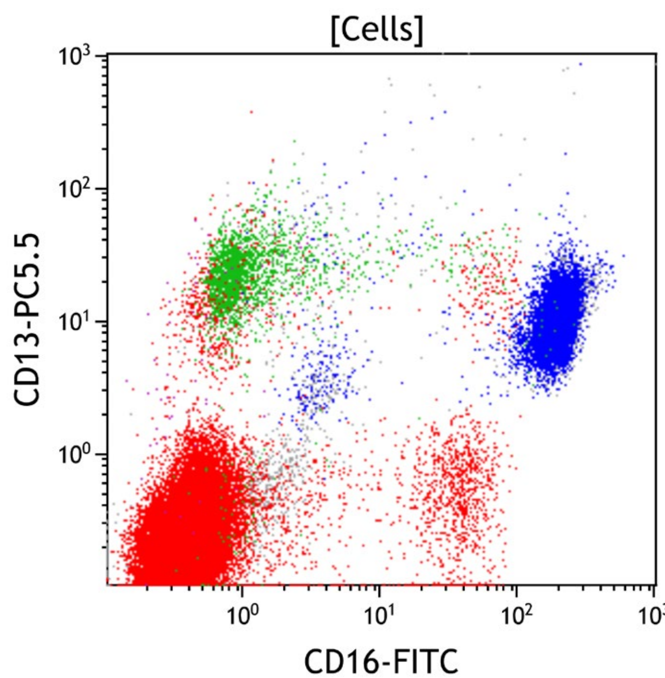


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red, lower right).

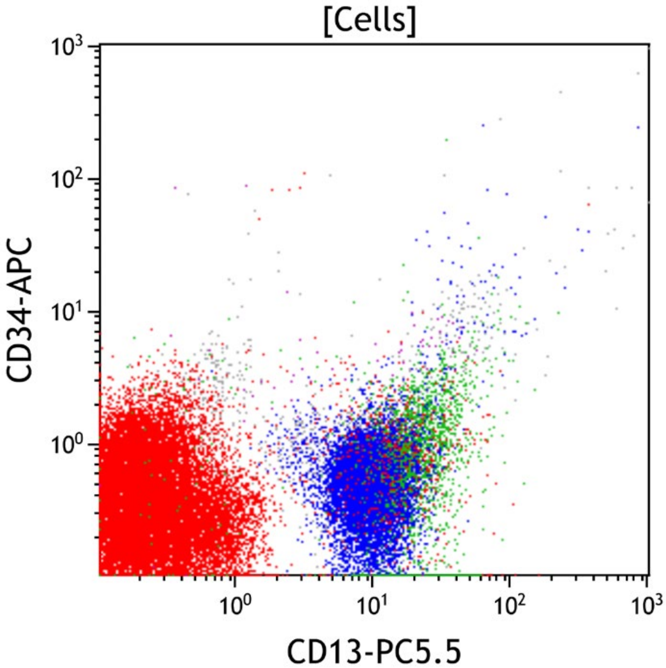


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors.

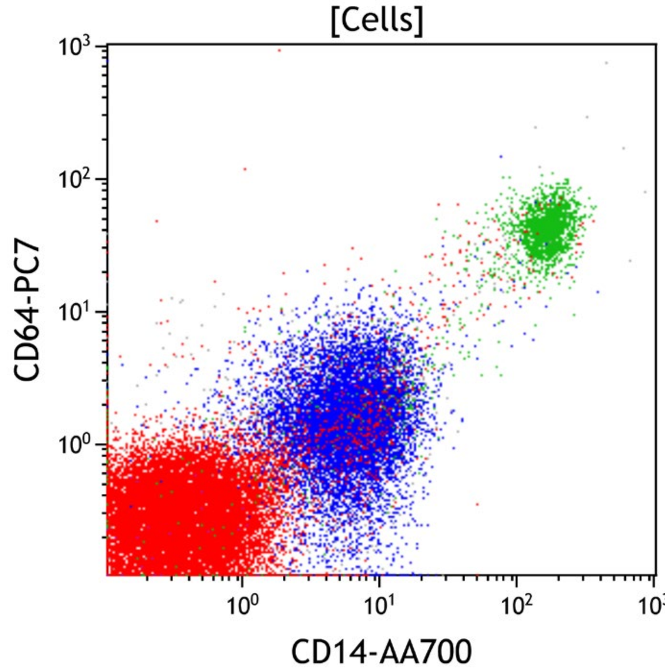


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes.

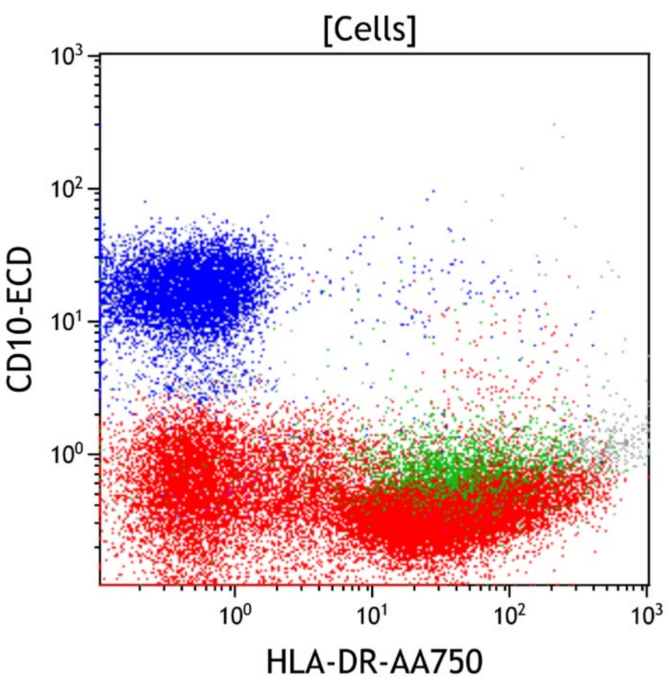


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells (red, lower right), plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on granulocytes (blue).

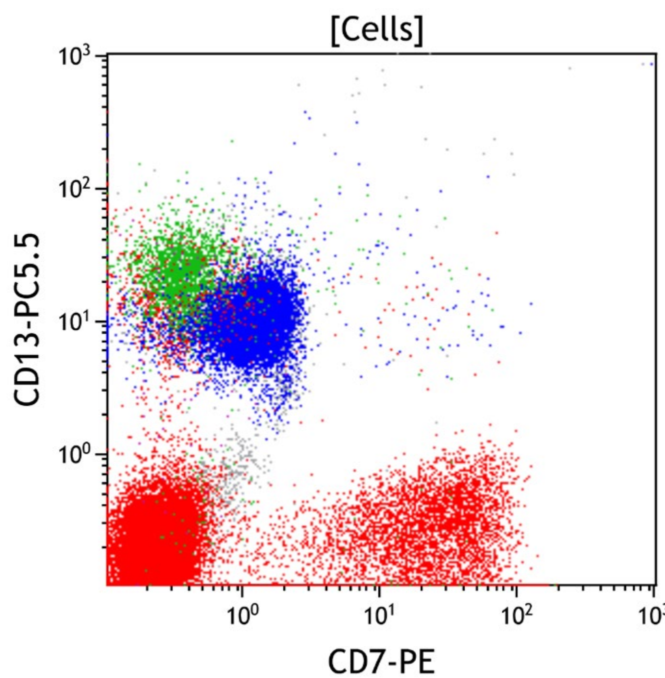
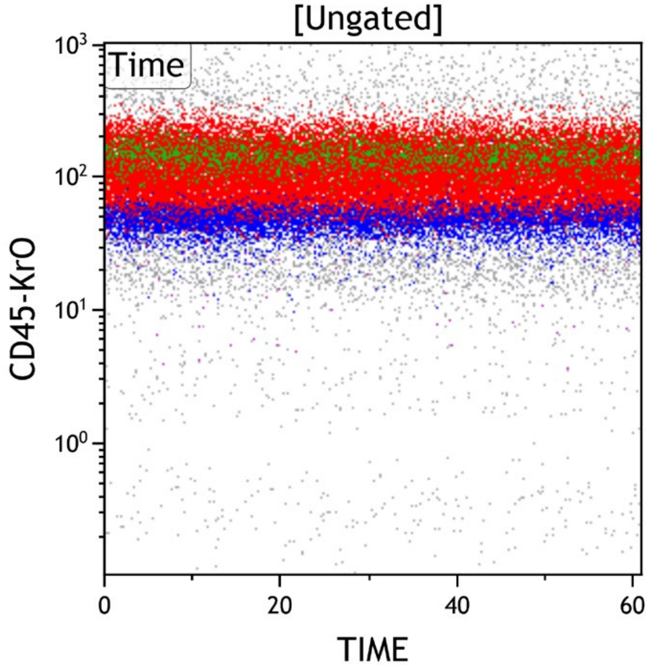


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.



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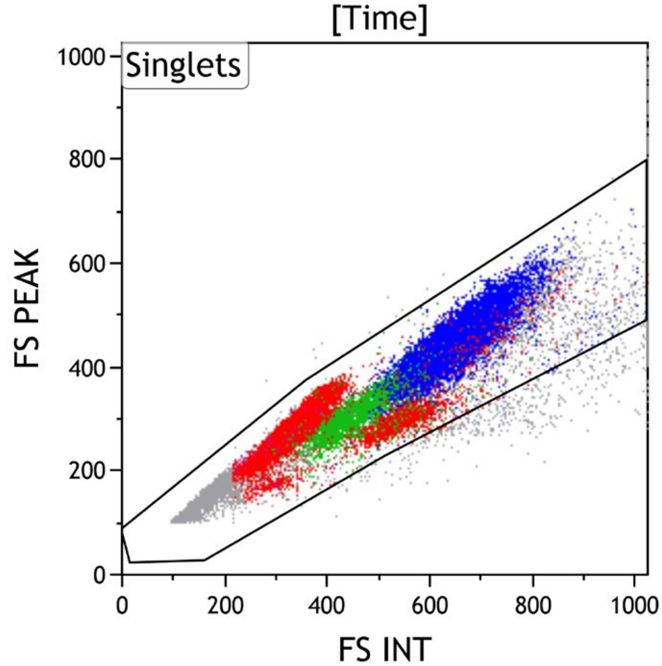

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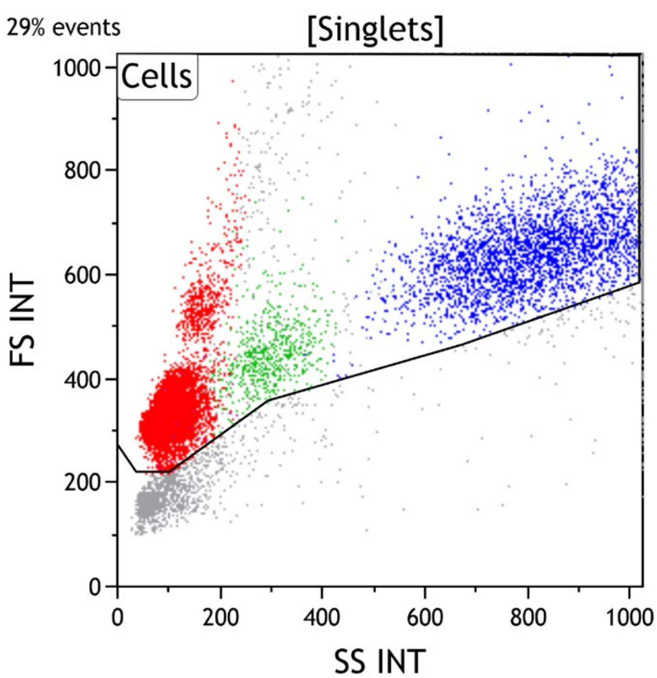
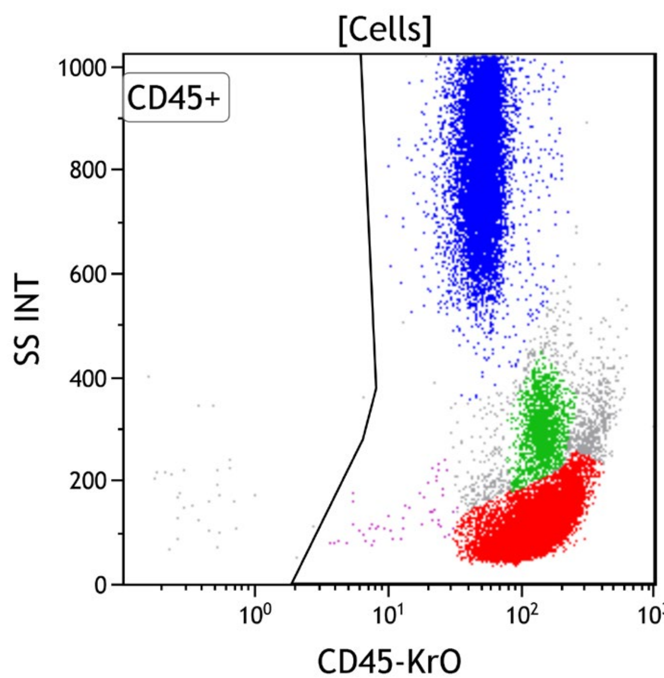


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.



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M2 Cell Tube

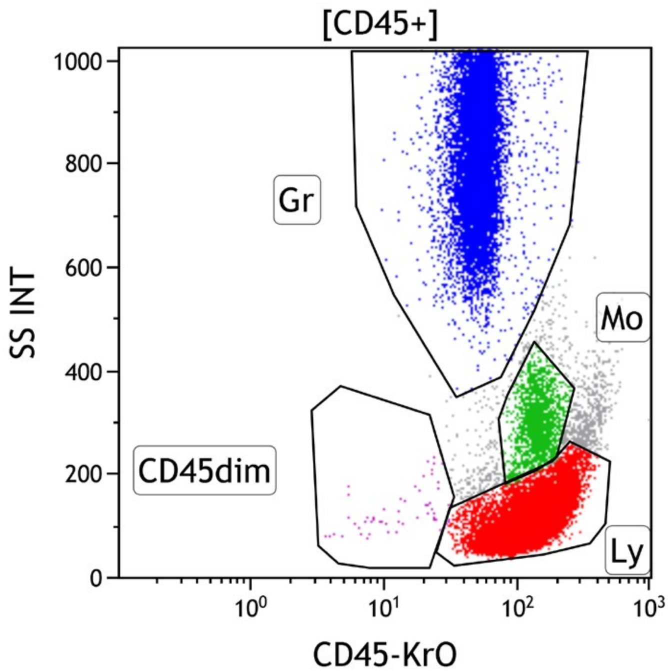

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

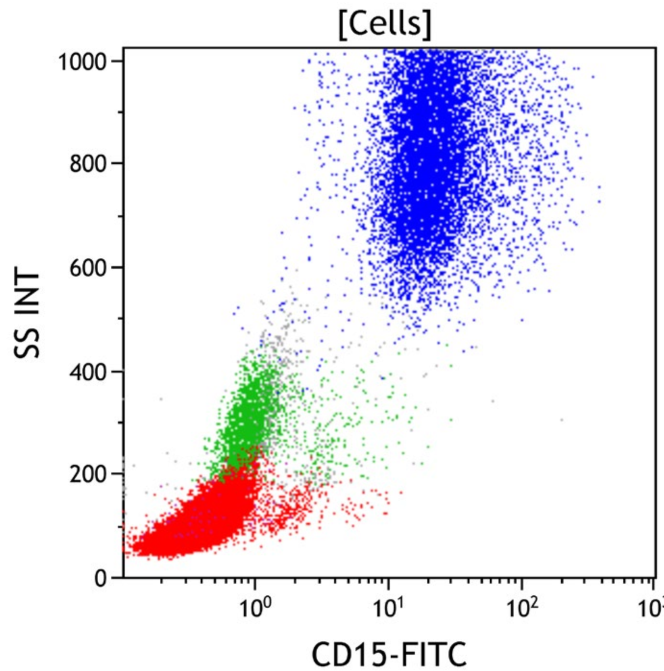

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (in blue) and at a lower level on monocytes (green).

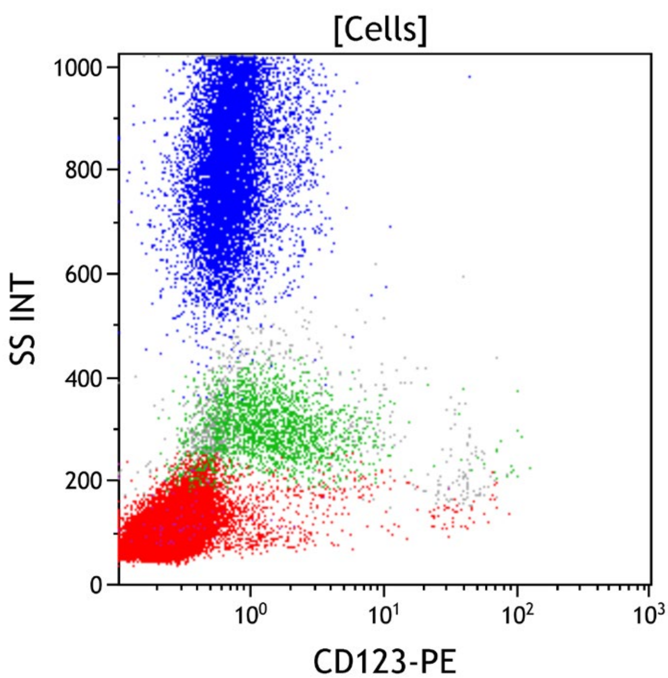

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

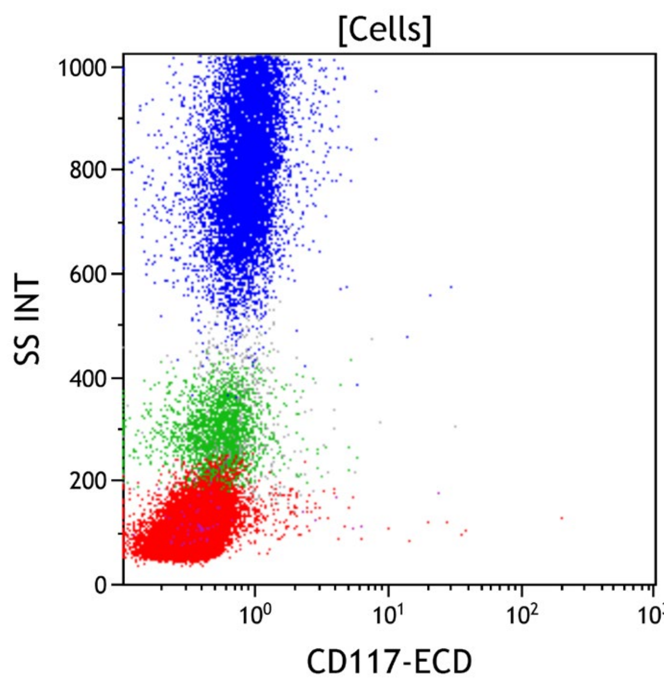

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

M2 Cell Tube

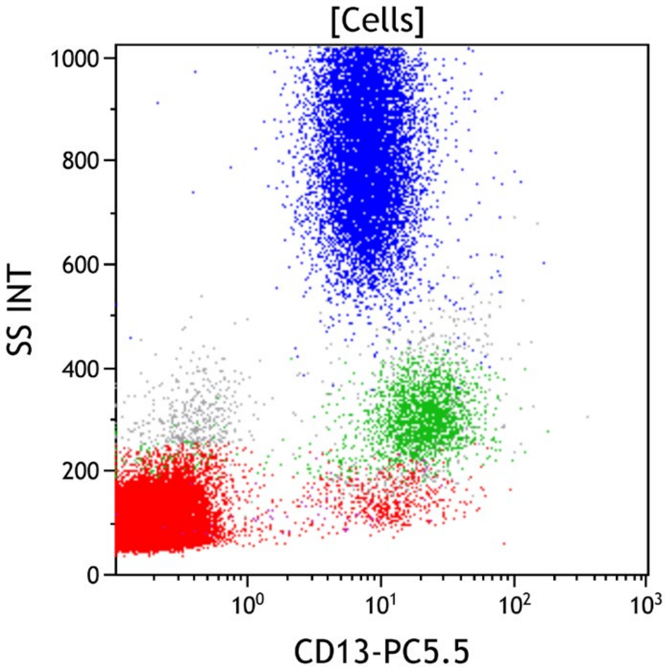

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and on mature monocytes (green).

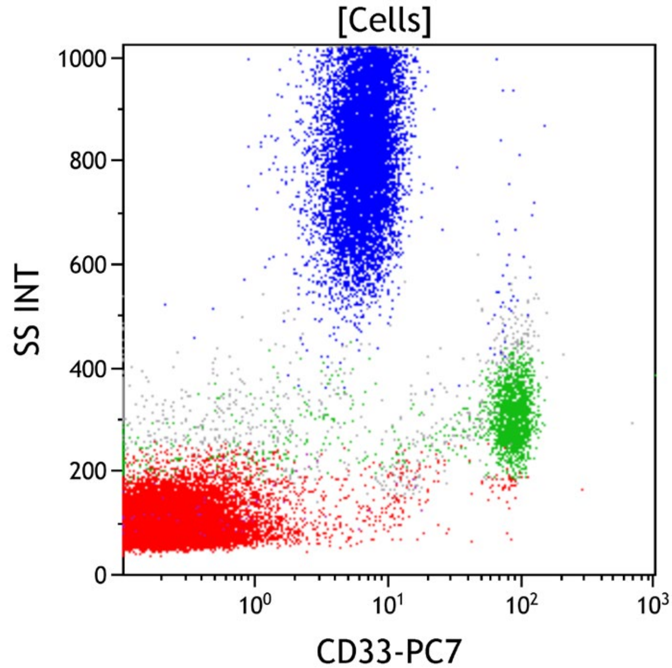

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors.

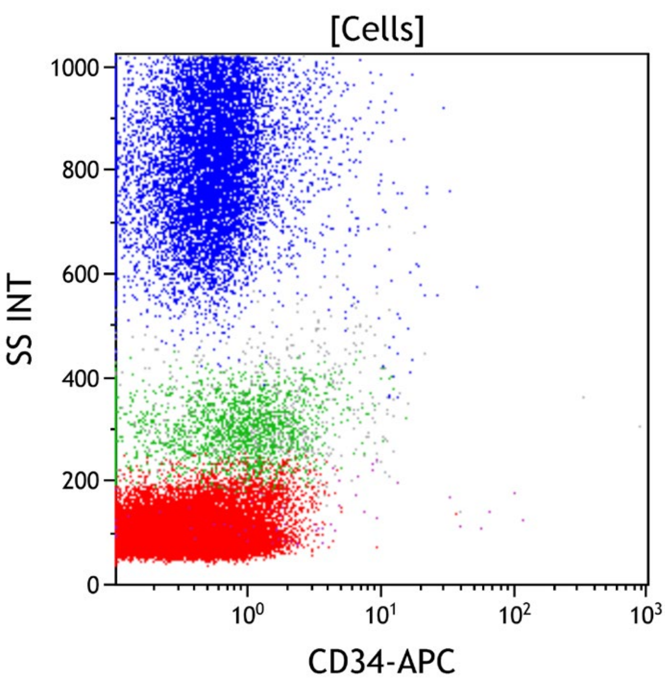

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are negative for CD34.

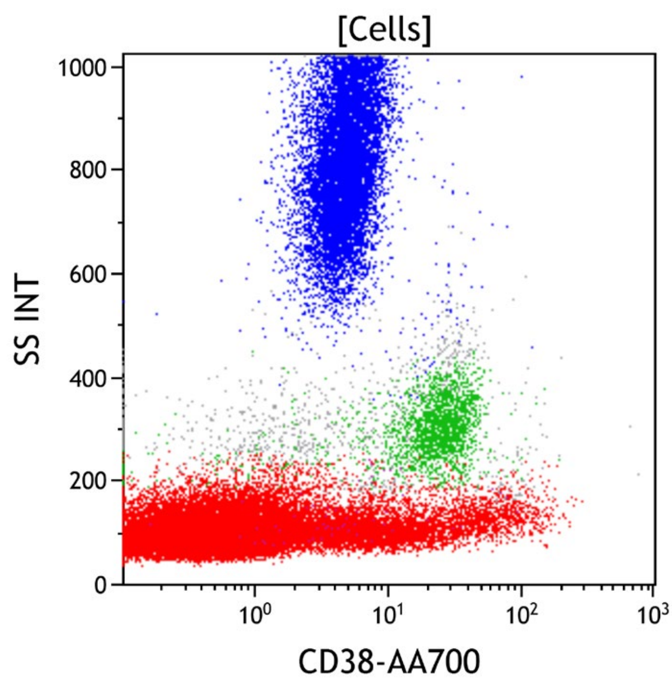

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and monocytes (green), and at a variable level on activated lymphocytes (red).

M2 Cell Tube

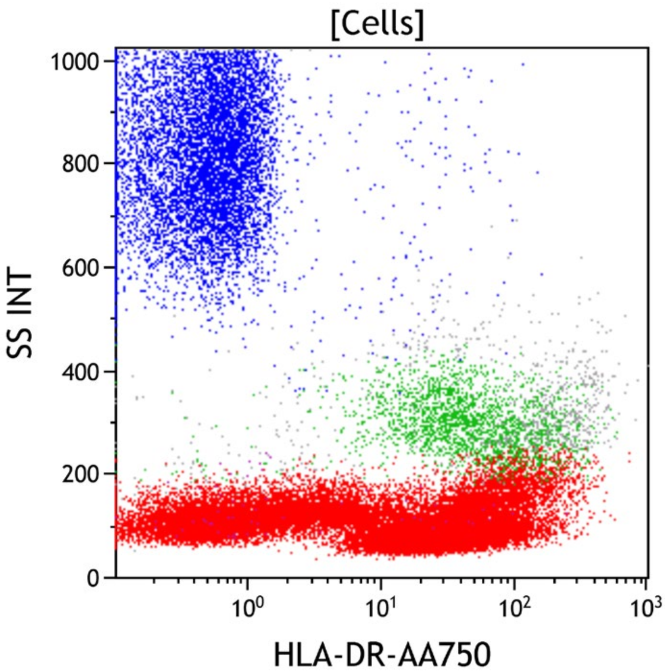

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red).

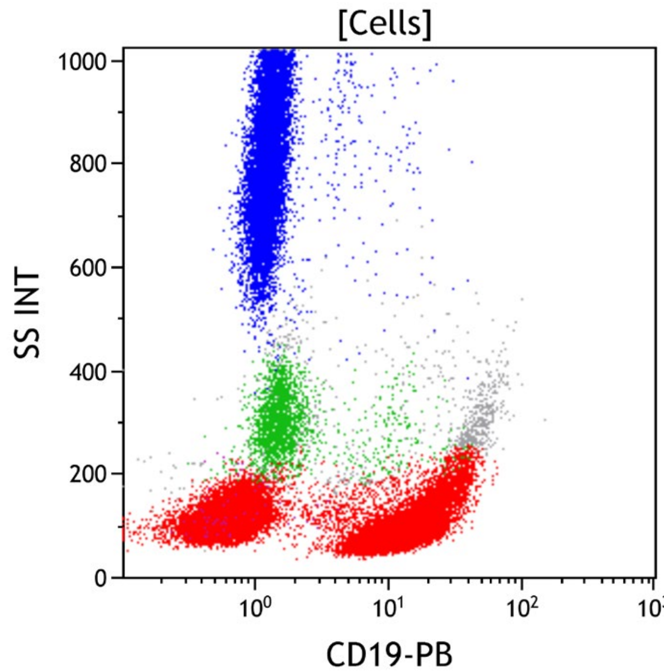

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on immature and mature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The mature B cell population (red) is relatively expanded compared with normal.

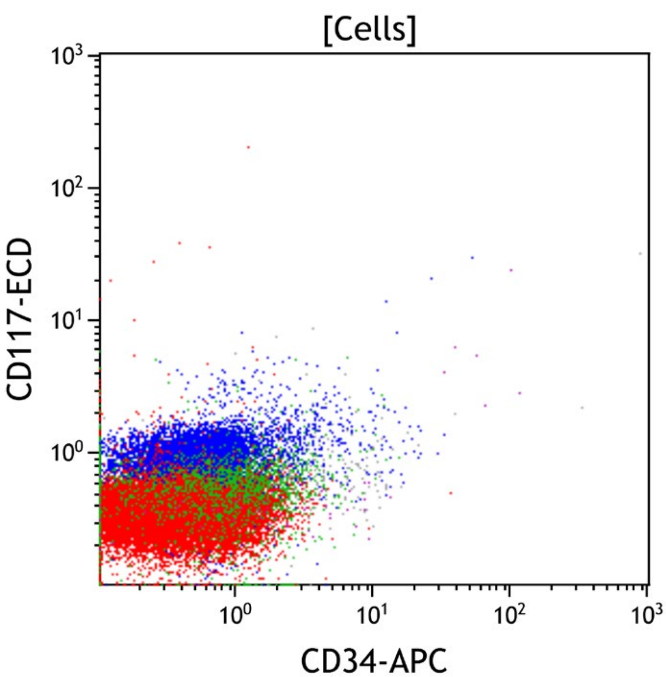

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors.

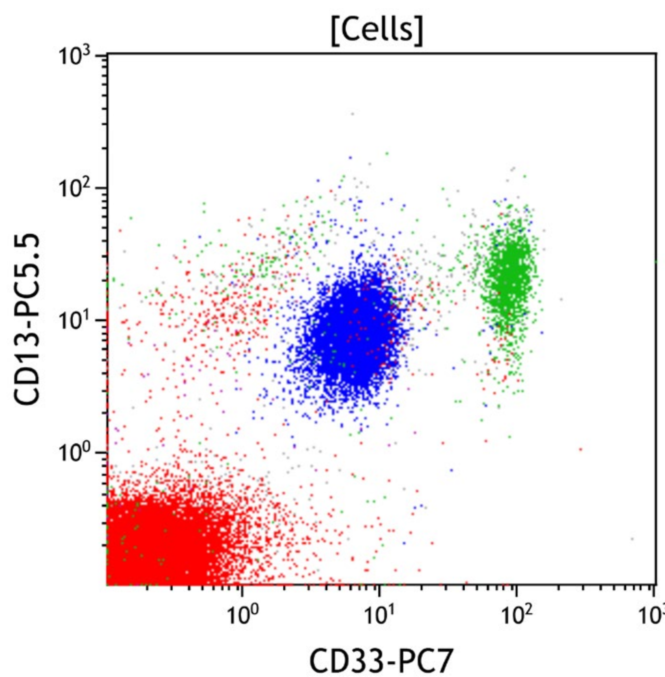

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Lymphocytes largely do not express either CD13 or CD33 (red).

M2 Cell Tube

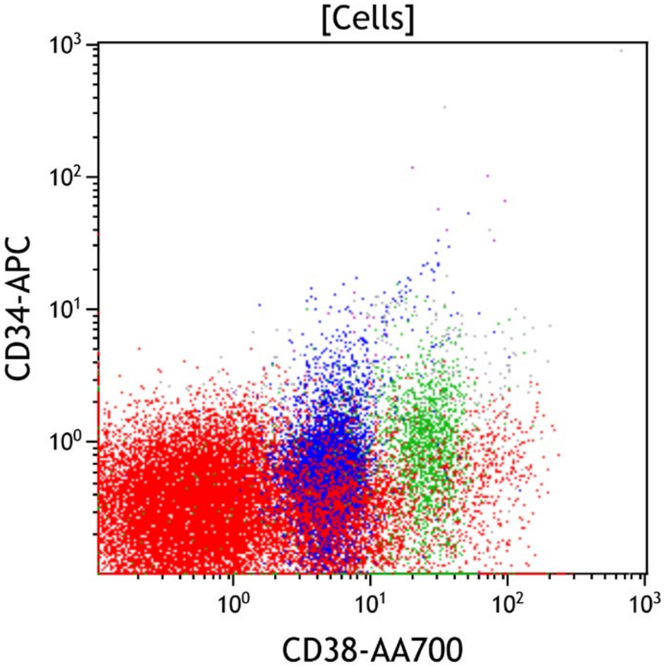

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34.

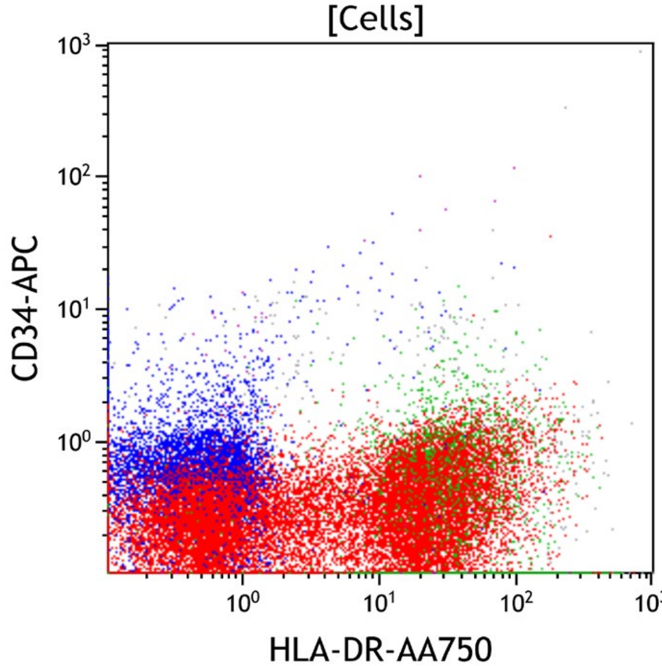

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors.

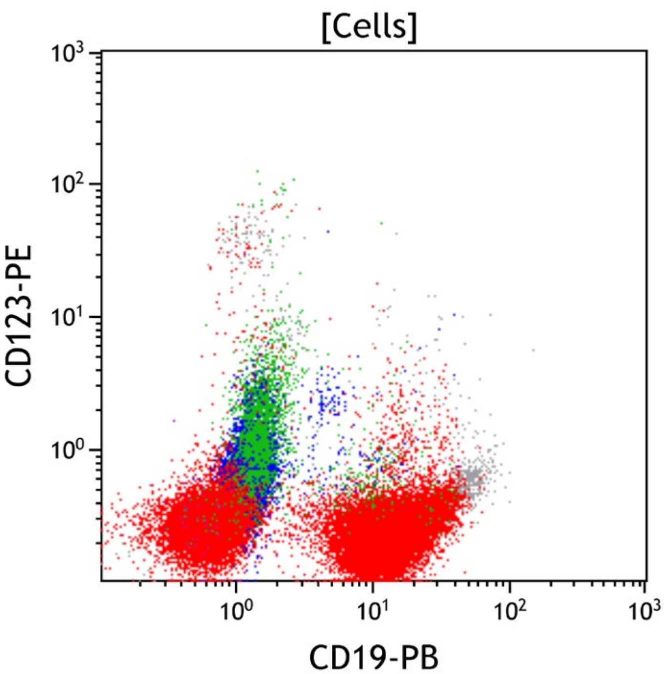

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red, lower middle) normally do not express significant CD123.

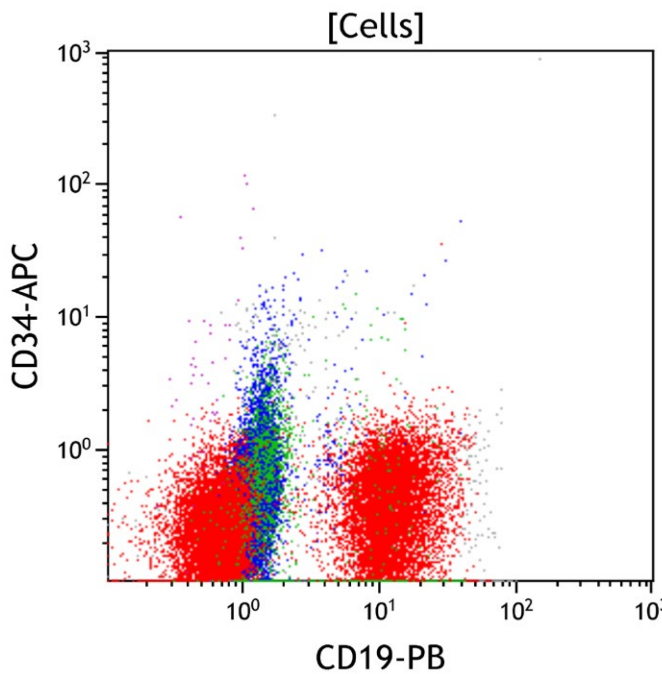

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red, lower middle) do not express CD34.

M2 Cell Tube

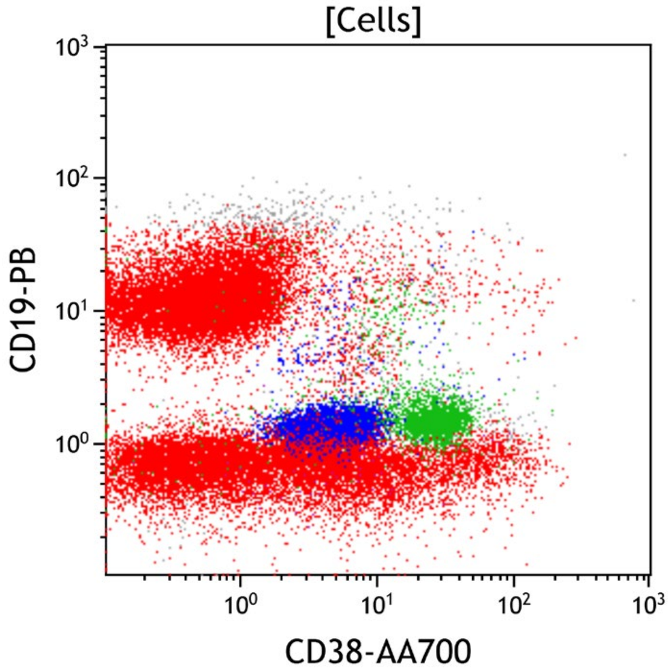

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. The CD19 positive B cells (red, right middle) have low to absent CD38 expression.

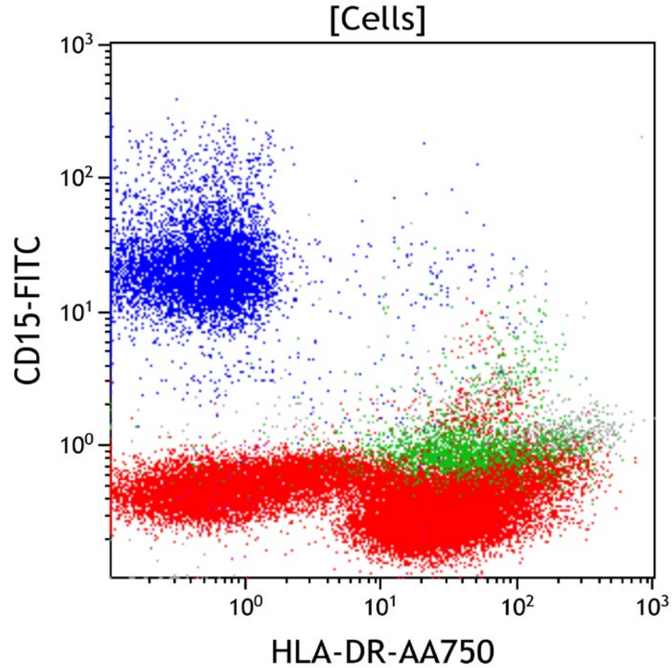

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes (blue) do not express HLA-DR.

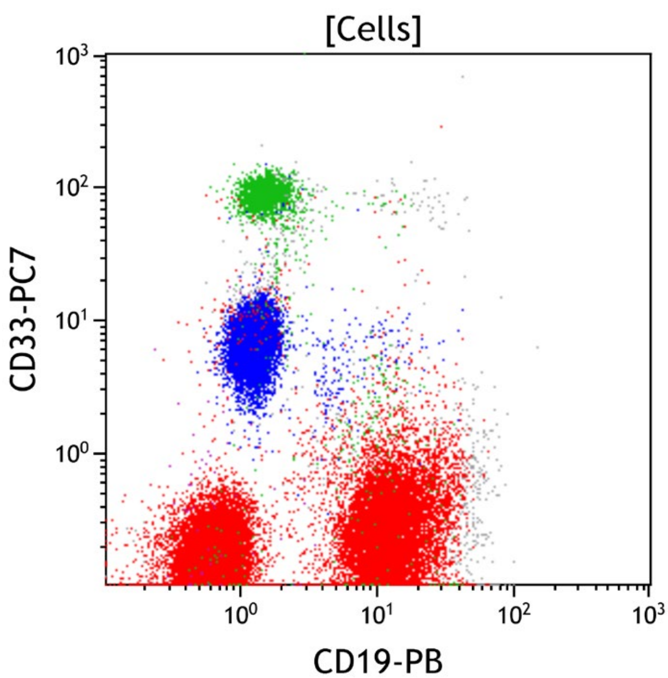

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes (green) and granulocytes (blue). Mature CD19 positive B cells (red, lower middle) do not normally express significant CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate to bright CD5, intermediate CD19, low to intermediate CD20, low to absent CD38, bright CD45, intermediate CD200, and dim surface lambda light chain expression without CD10 or other T or myeloid markers. Compared with normal B cells, the expression of CD5, decreased CD20, and lambda light chain restriction of low intensity are aberrant.

Taken together, the immunophenotype of the aberrant population is most consistent with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). FISH shows a deletion of chromosome 13q, an abnormality seen in a subset of patients with CLL/SLL. However, a definitive diagnosis of CLL/SLL using current WHO criteria requires the demonstration of disease related clinical and/or laboratory findings and/or the presence of greater than 5,000 neoplastic cells per microliter in the peripheral blood. Therefore, correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

Case #8: Mantle cell lymphoma

Clinical Vignette

This 55-year-old male presents with organomegaly. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

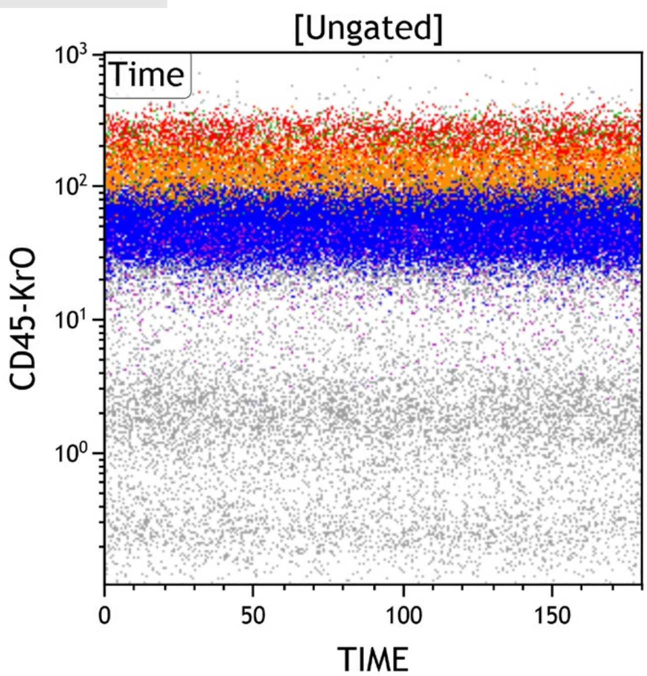

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

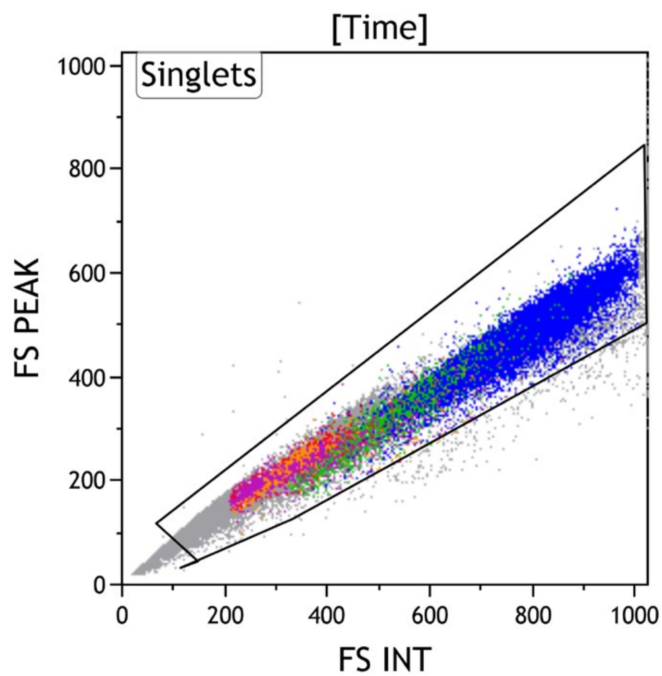

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

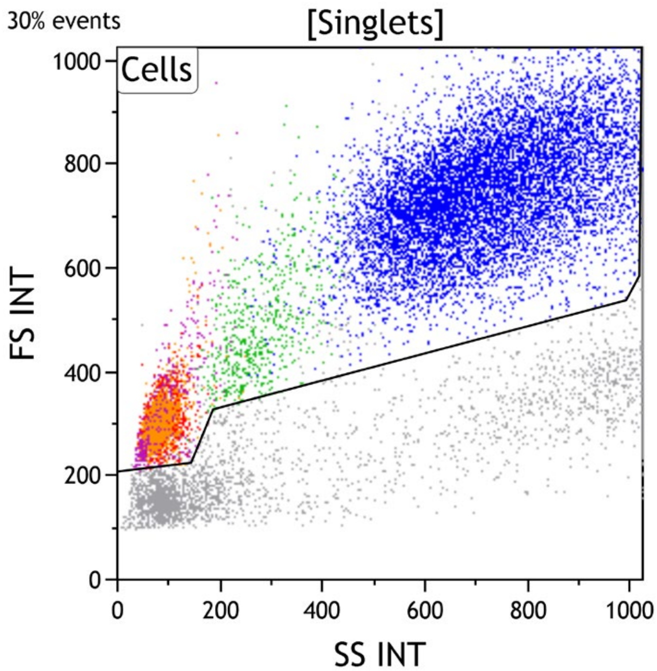

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

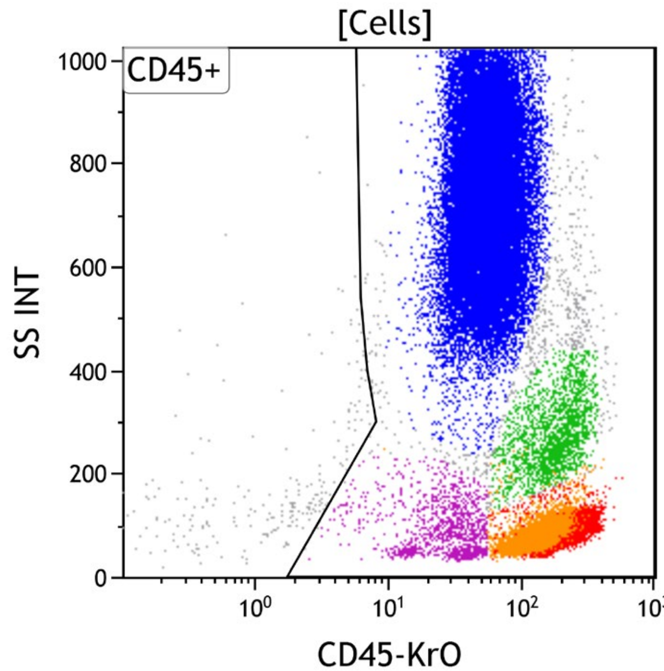

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

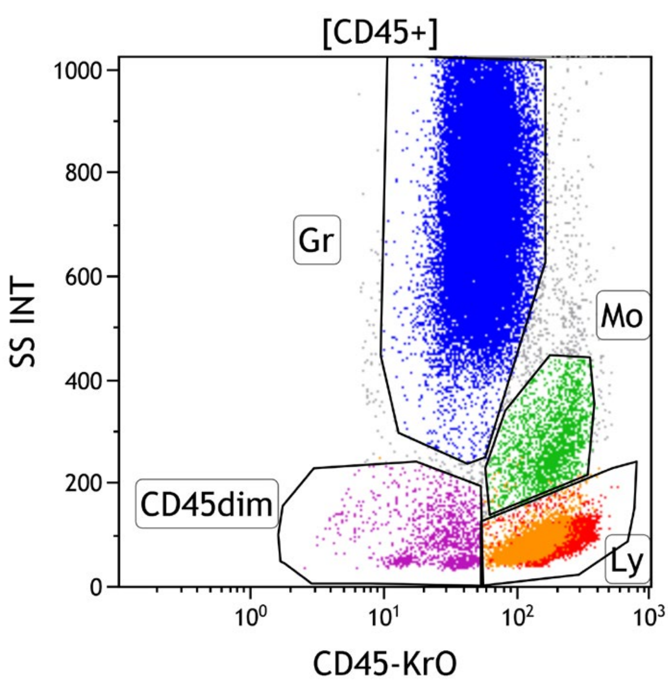

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

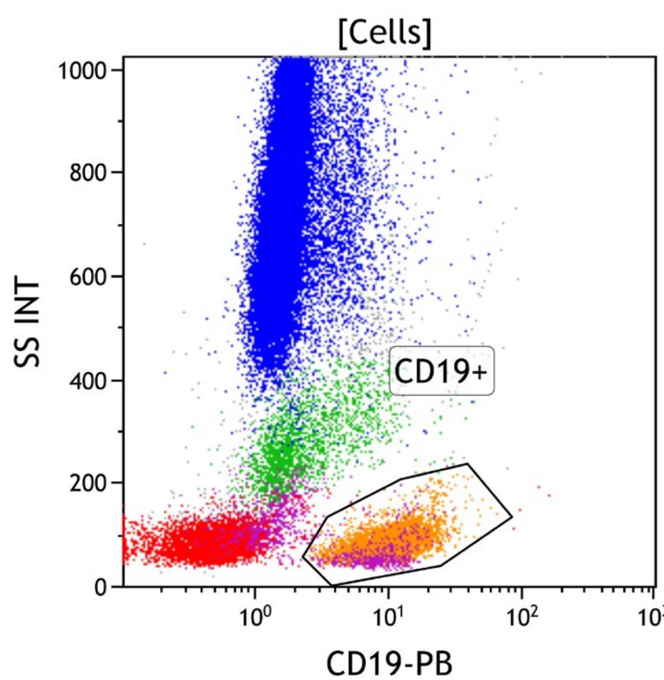

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

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B Cell Tube

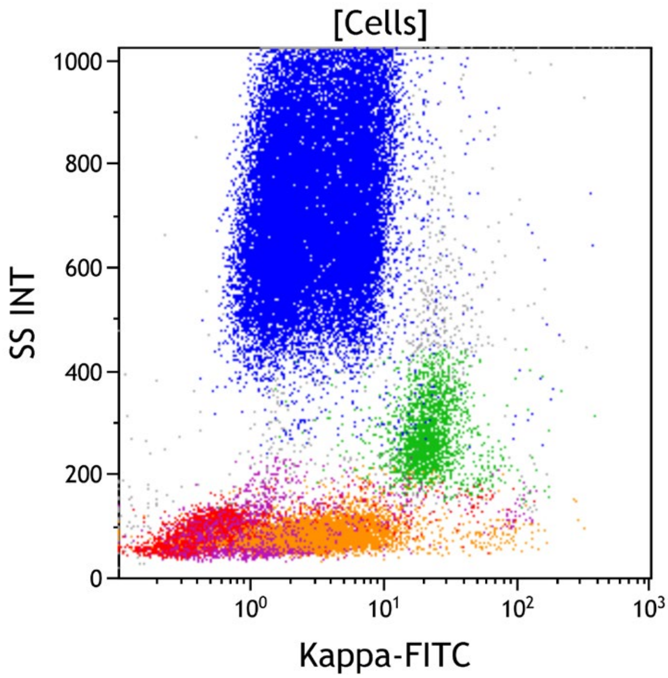

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) lack kappa light chain expression.

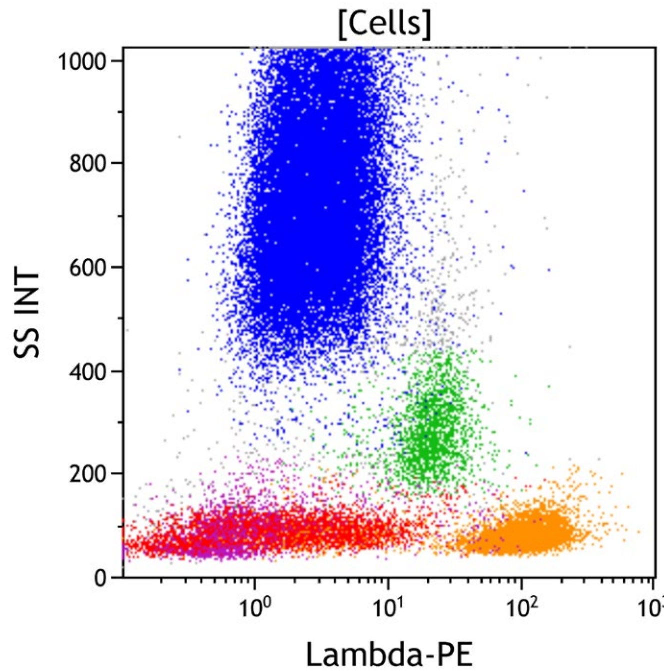

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) have surface lambda light chain expression.

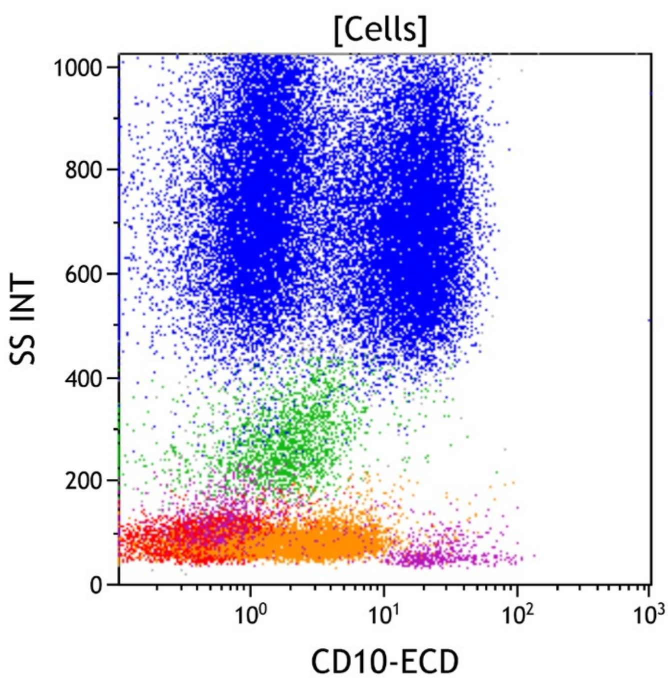

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange) is negative for CD10. The apparent CD10 positivity on the aberrant population (orange) is likely due to bright lambda light chain causing an increase in ECD background.

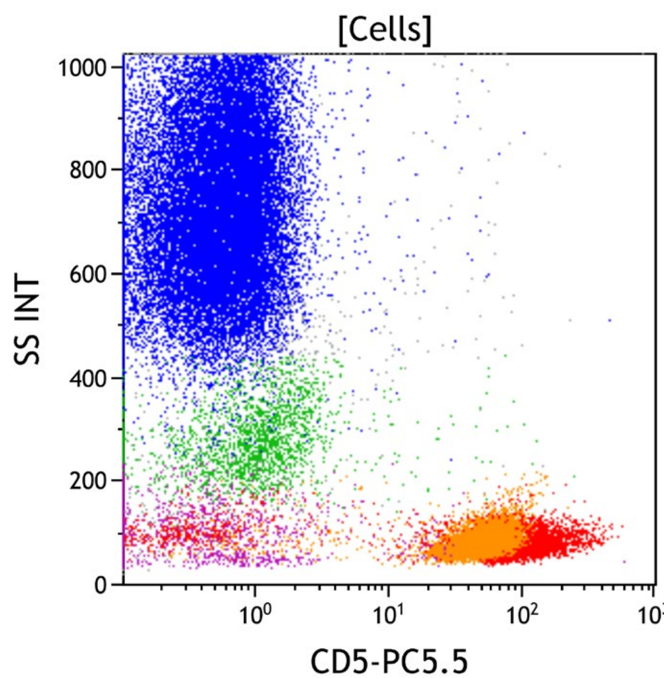

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The CD19 positive population (orange) expresses CD5 at a slightly lower level than that seen on T cells (red).

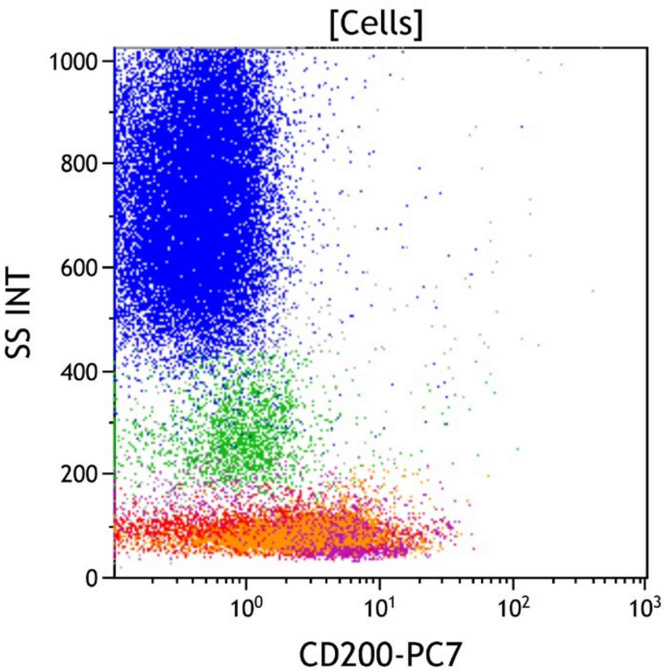

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The CD19 positive population (orange) is negative for CD200. The apparent expression of low CD200 is due to spectral overlap from CD5 PE-Cy5.5 that is causing an increase in background for PC7.

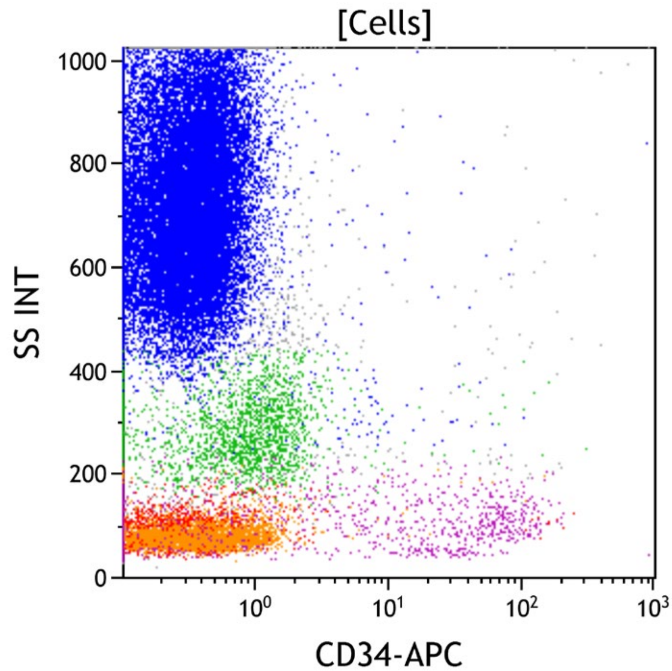

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The CD19 positive population (orange) is negative for CD34.

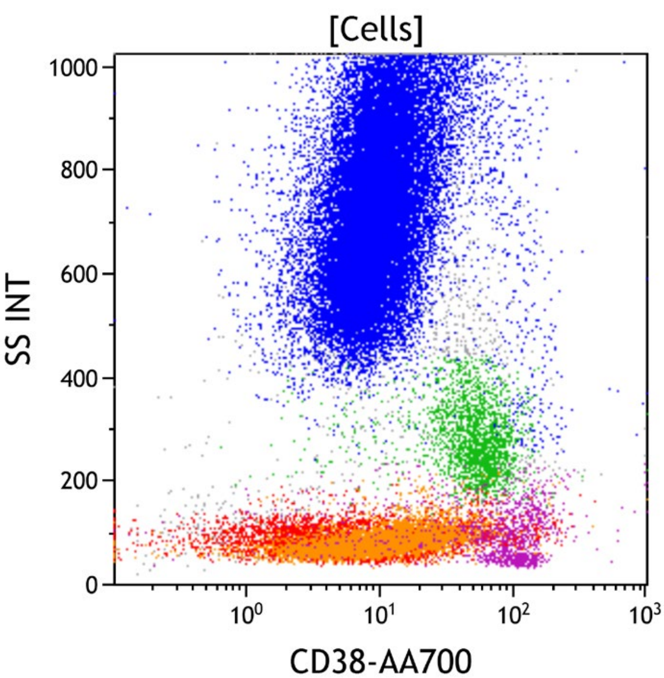

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The CD19 positive population (orange) displays intermediate CD38 expression.

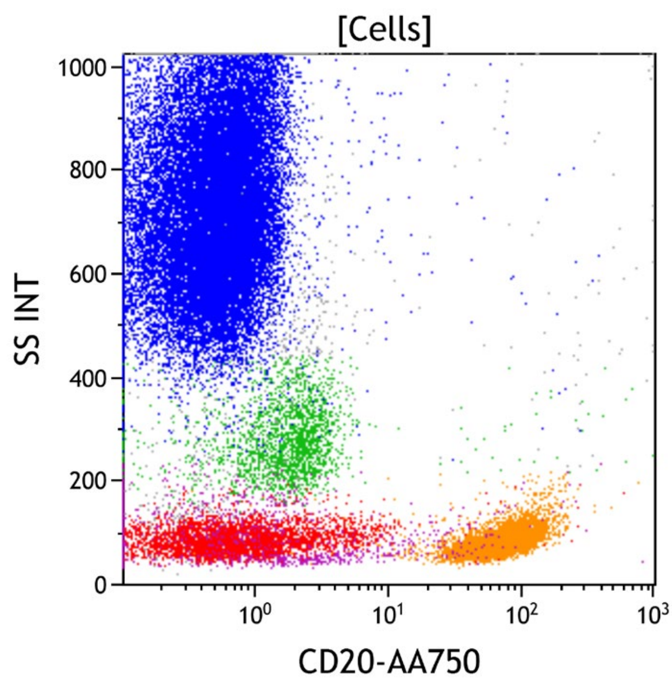

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The CD19 positive population (orange) is positive for CD20.

B Cell Tube

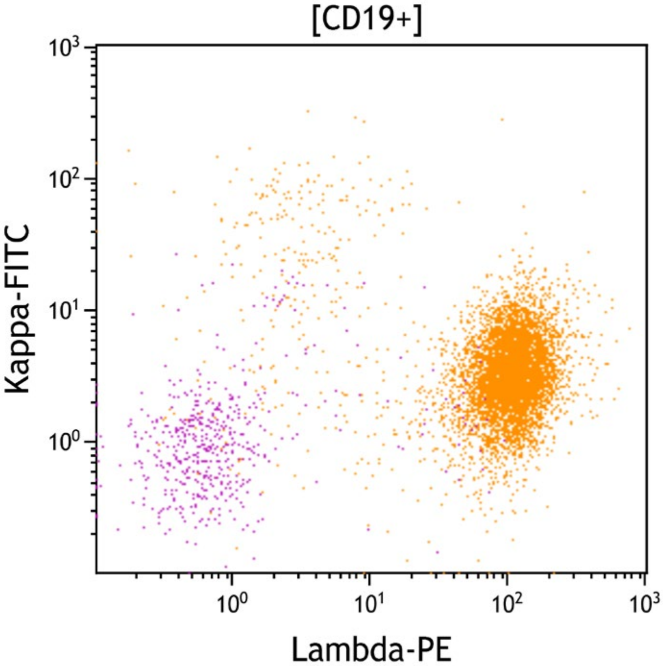

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. The CD19 positive cells (orange) predominantly have surface lambda light chain expression, indicating a clonal B cell population. A small population of polyclonal B cells (also in orange) with normal levels of kappa and lambda light chain expression is present.

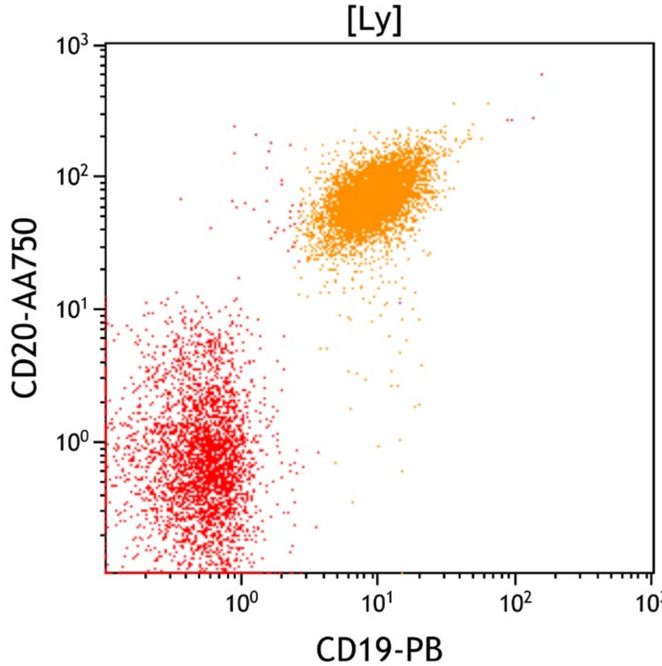

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cells (orange) is positive for CD19 and CD20.

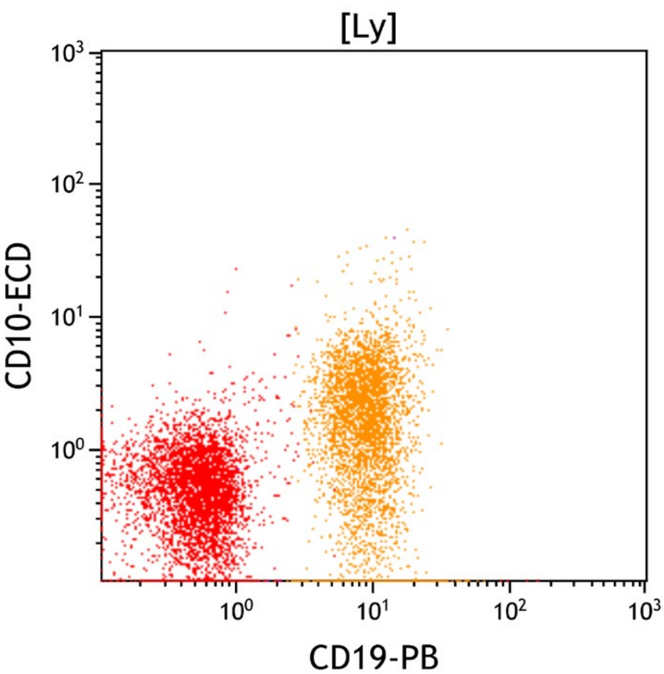

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. The CD19 positive clonal B cell population (orange) is negative for CD10. The apparent CD10 positivity on the aberrant population (orange) is likely due to bright lambda light chain causing an increase in ECD background.

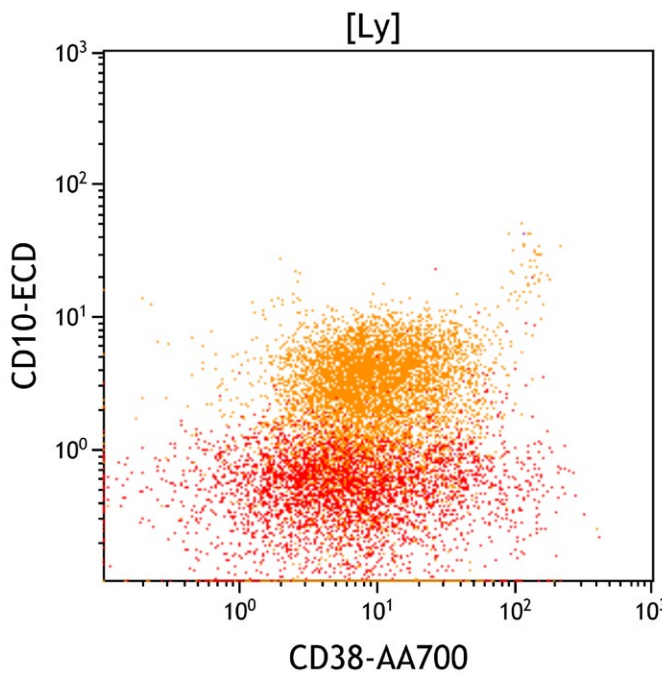

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 and CD38 positive cells (orange, upper right) represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. The clonal B cell population (orange) displays intermediate CD38 expression and is negative for CD10. The apparent CD10 positivity on the aberrant population (orange) is likely due to bright lambda light chain causing an increase in ECD background.

B Cell Tube

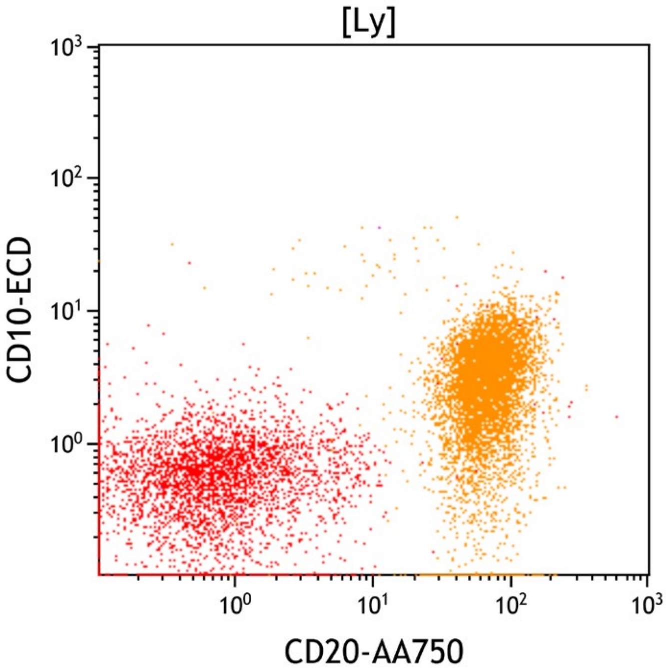

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 (orange, upper middle) represent late stage immature B cells. The clonal B cell population (orange) is positive for CD20 and largely negative for CD10. The apparent CD10 positivity on the aberrant population (orange) is likely due to bright lambda light chain causing an increase in ECD background.

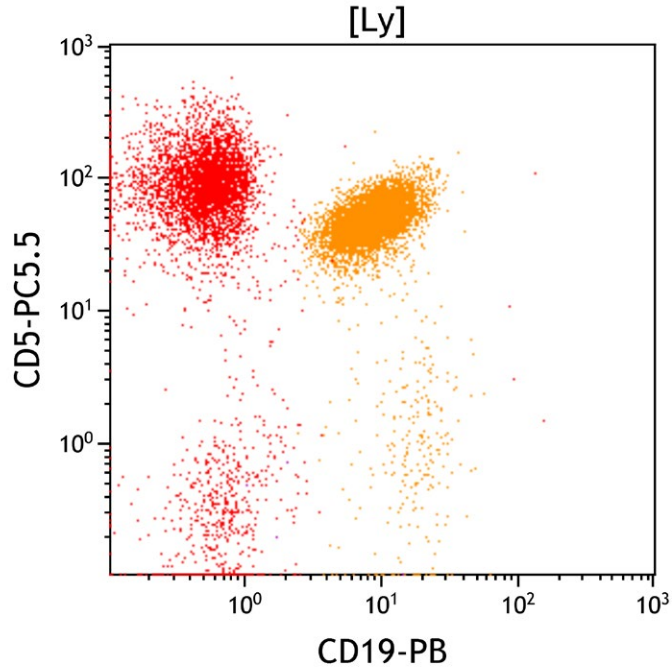

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) is positive for CD5 and CD19.

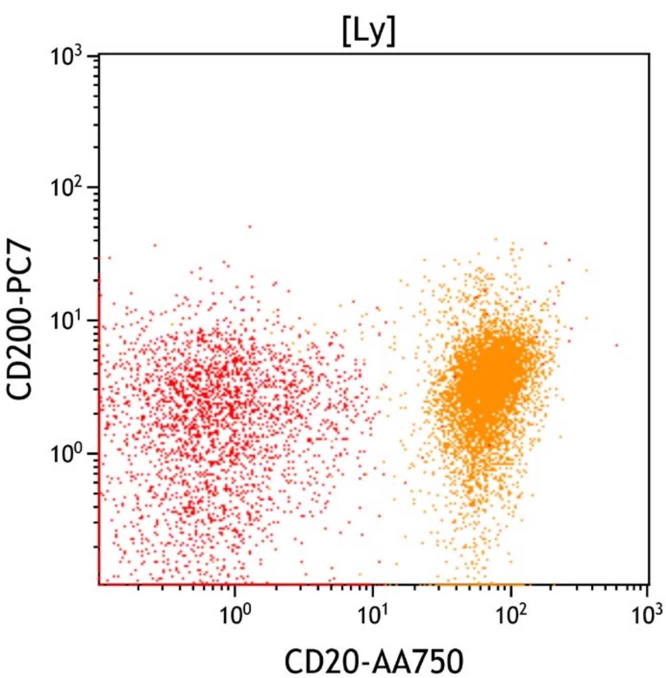

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The clonal B cell population (orange) is positive for CD20 and negative for CD200. The apparent expression of low CD200 is due to spectral overlap from CD5 PC5.5 that is causing an increase in background for PC7.

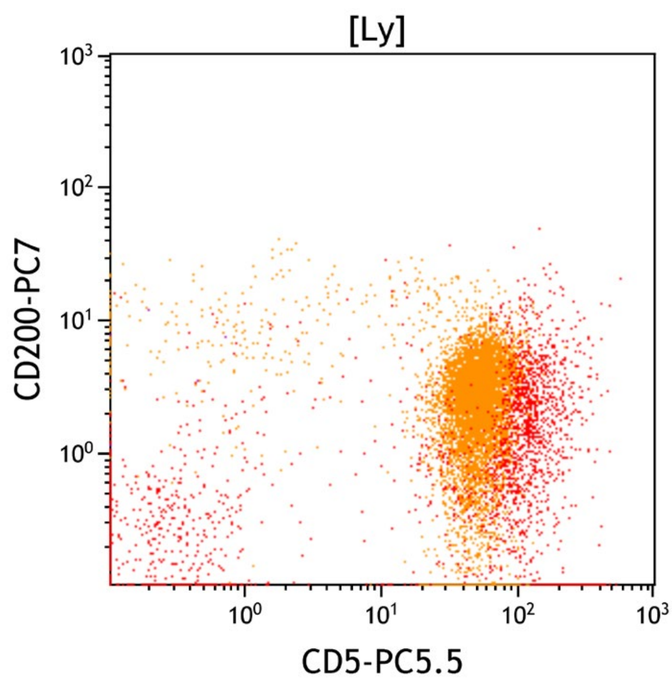

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The clonal B cell population (orange) is positive for CD5 and negative for CD200, favoring mantle cell lymphoma.

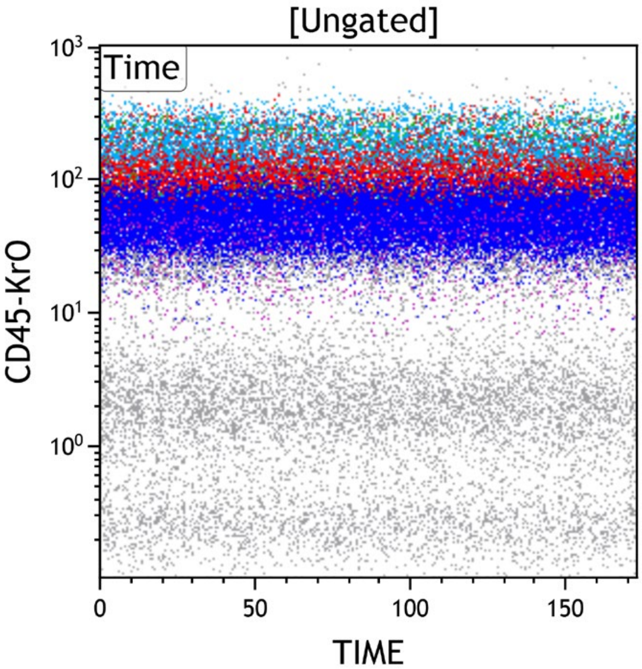

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

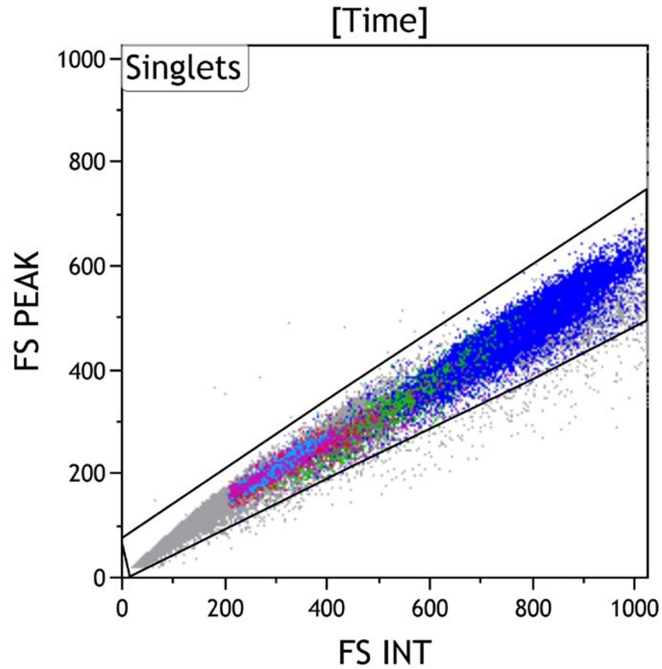

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

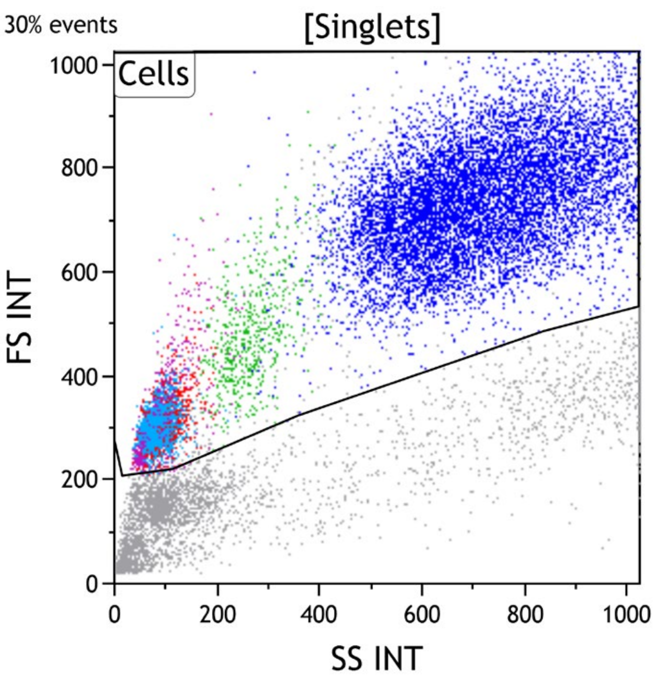

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

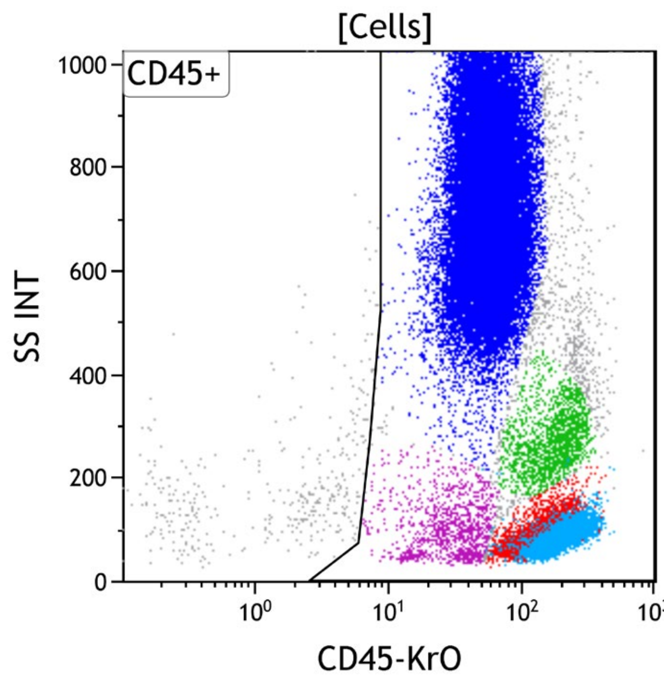

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

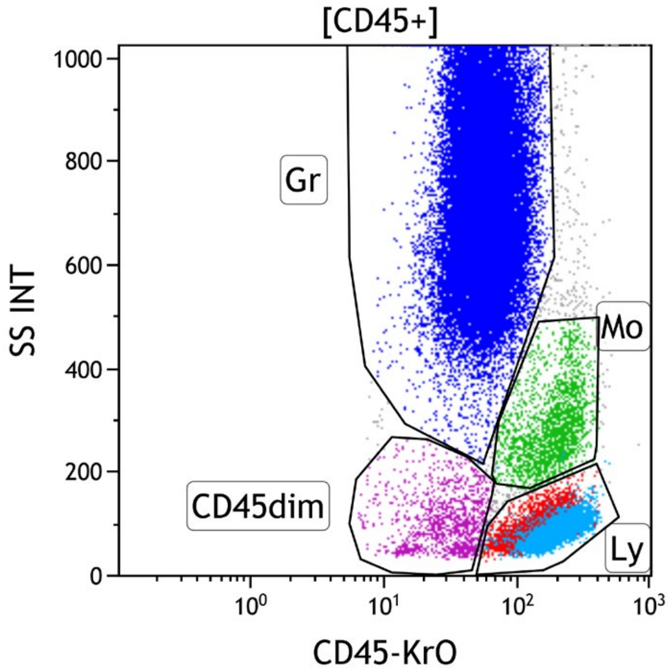

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, aqua/red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

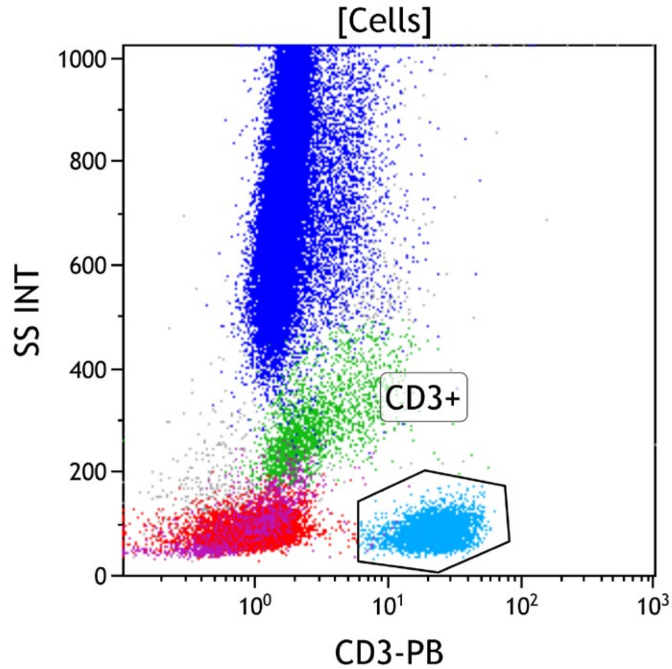

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

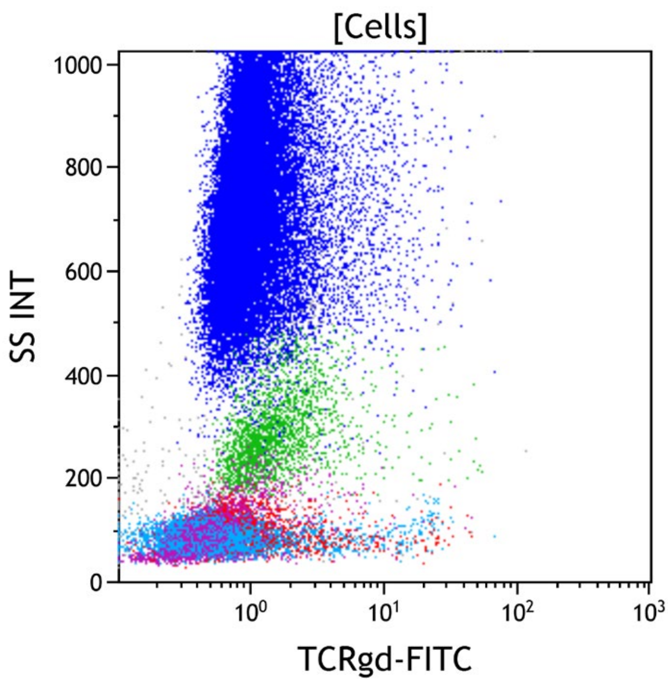

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua, lower right).

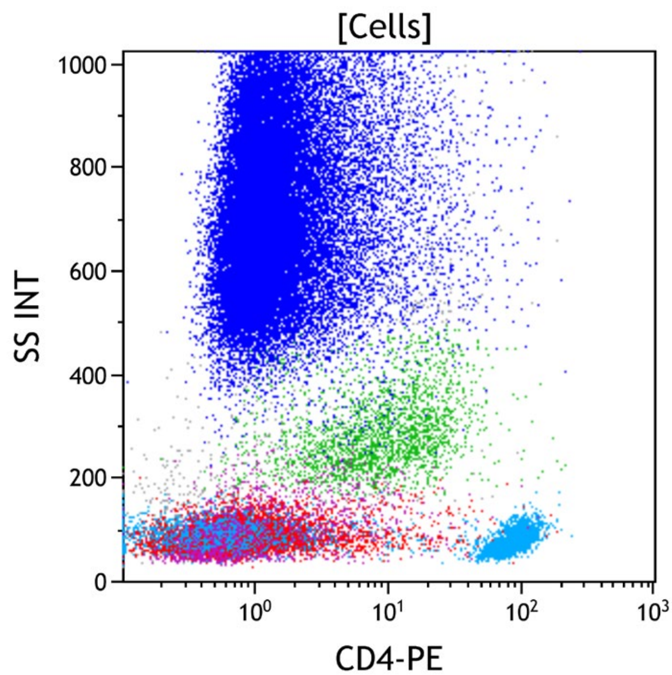

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

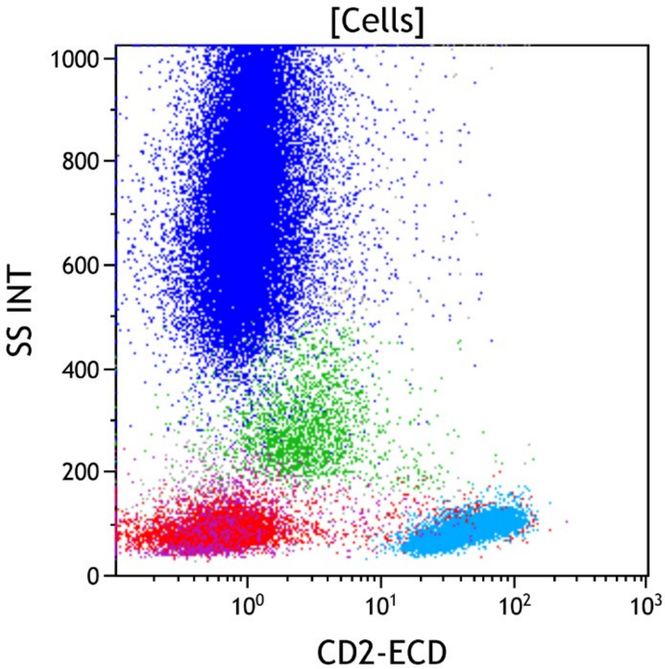

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

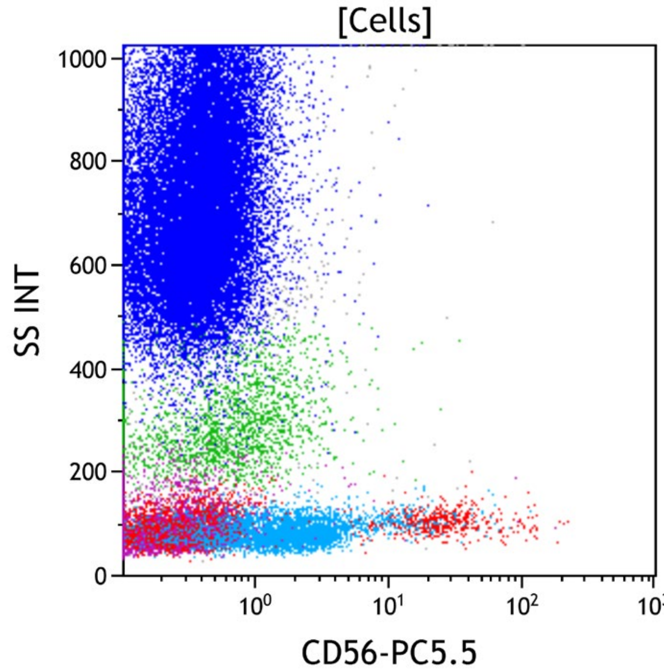

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

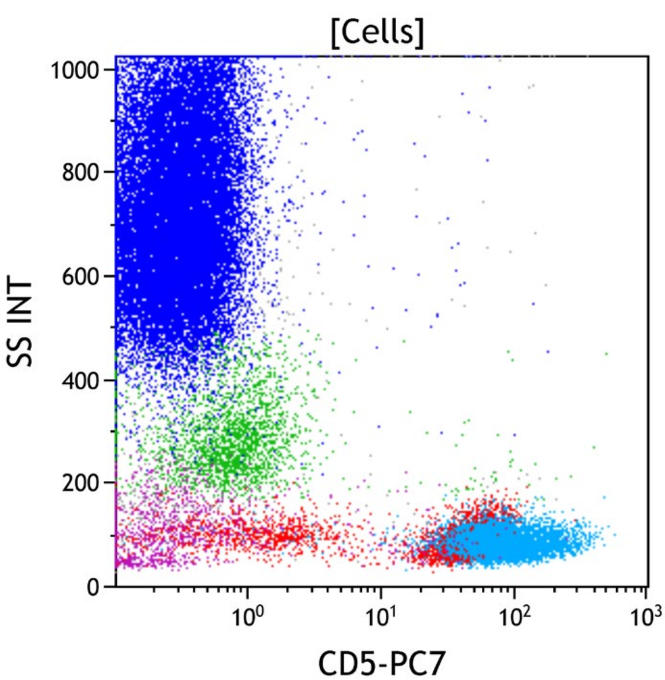

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The CD3 negative cells in the lymphocyte gate (red, lower right) are aberrant B cells with CD5 expression.

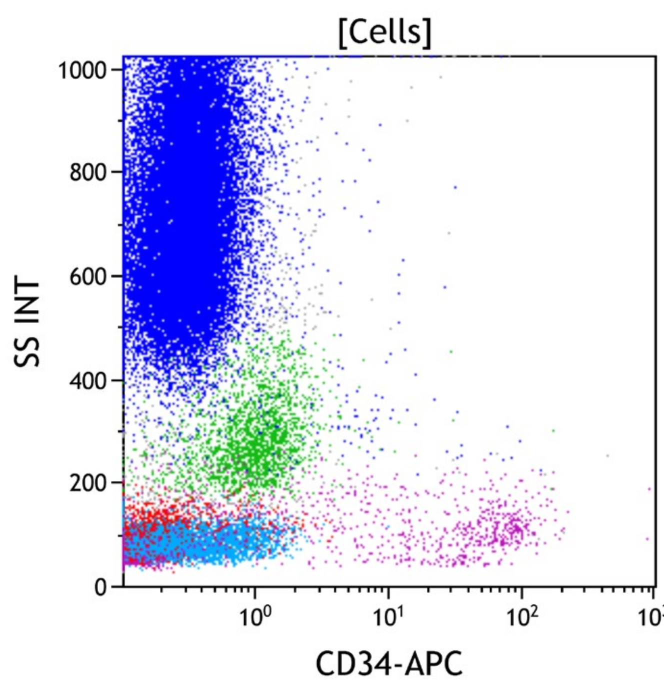

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The mature granulocytes (blue), monocytes (green), and lymphocytes (aqua and red) are negative for CD34.

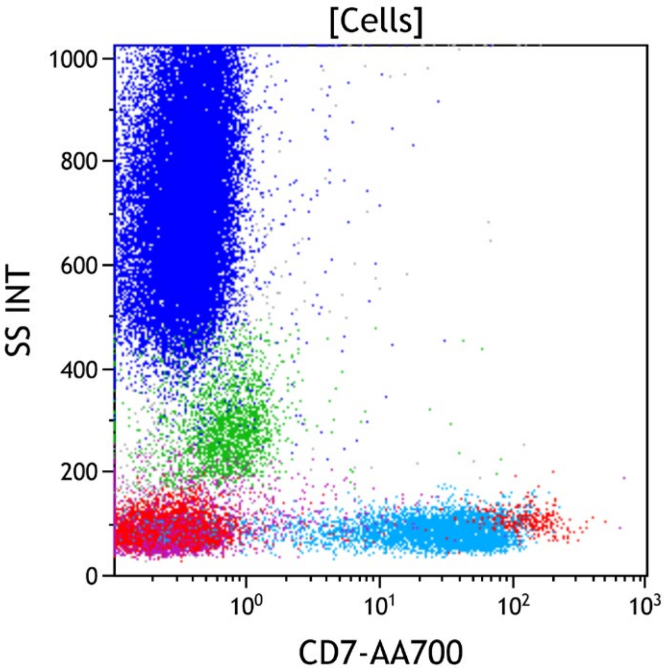

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

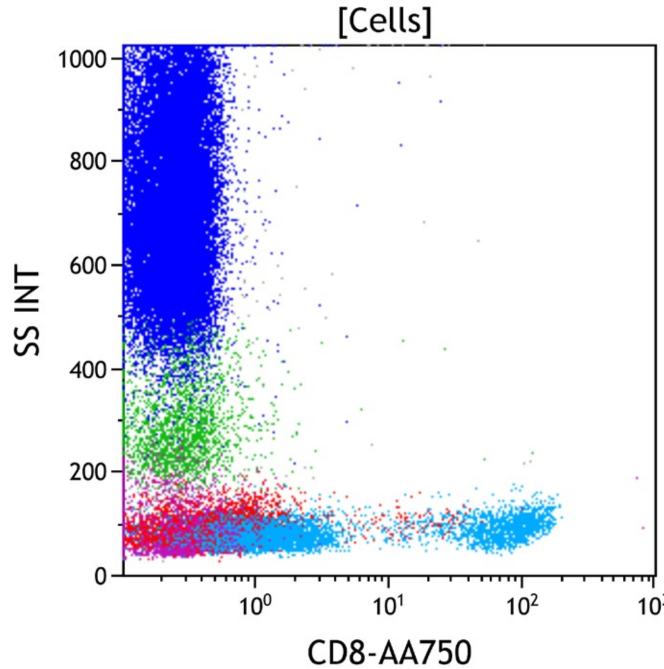

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

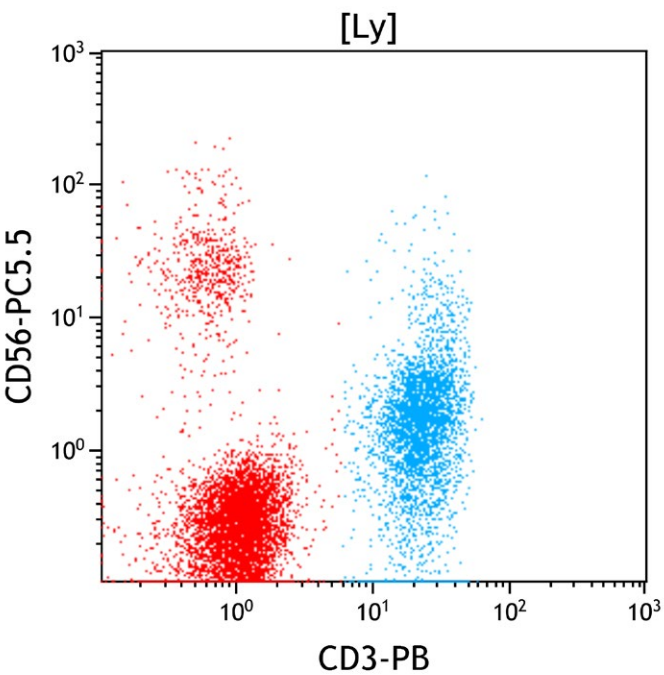

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells. The aberrant population lacks expression of CD3 and CD56.

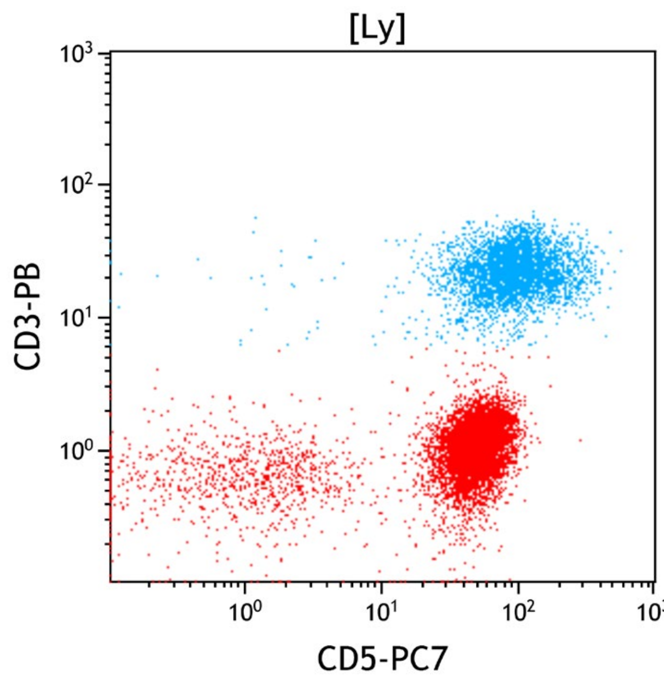

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red). The CD5 positive cells without CD3 (red, lower right) are aberrant B cells.

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T Cell Tube

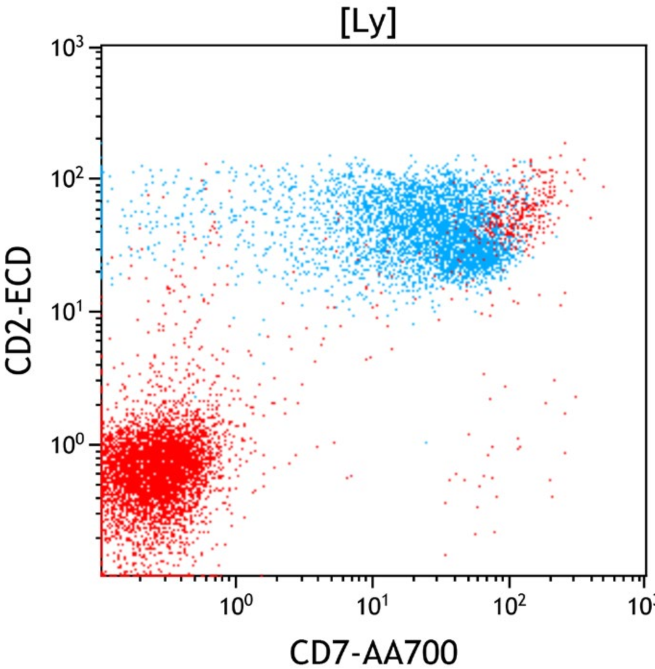


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right). The aberrant B cell population lacks expression of CD2 and CD7.

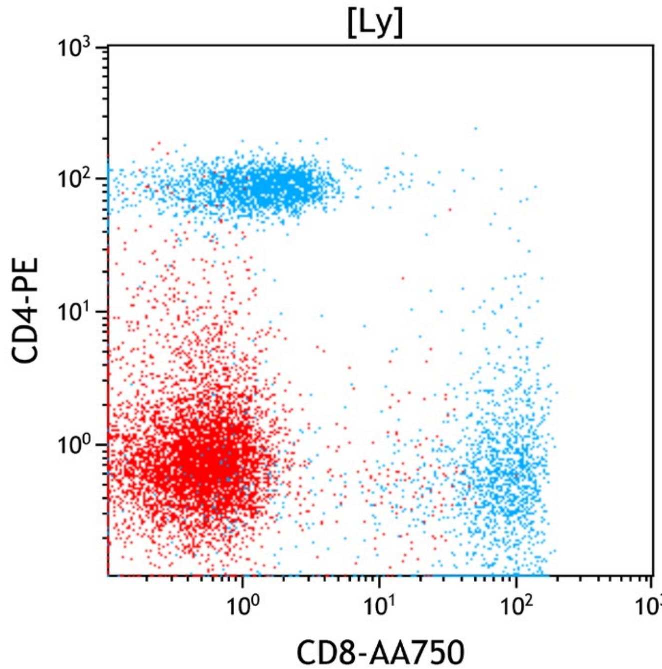


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. The aberrant B cell population lacks expression of CD4 and CD8.

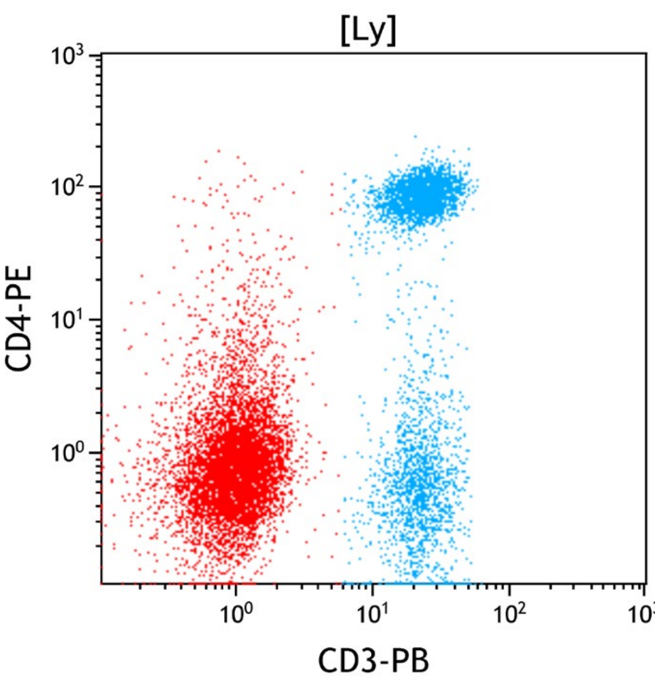


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. The aberrant B cell population lacks expression of CD3 and CD4.

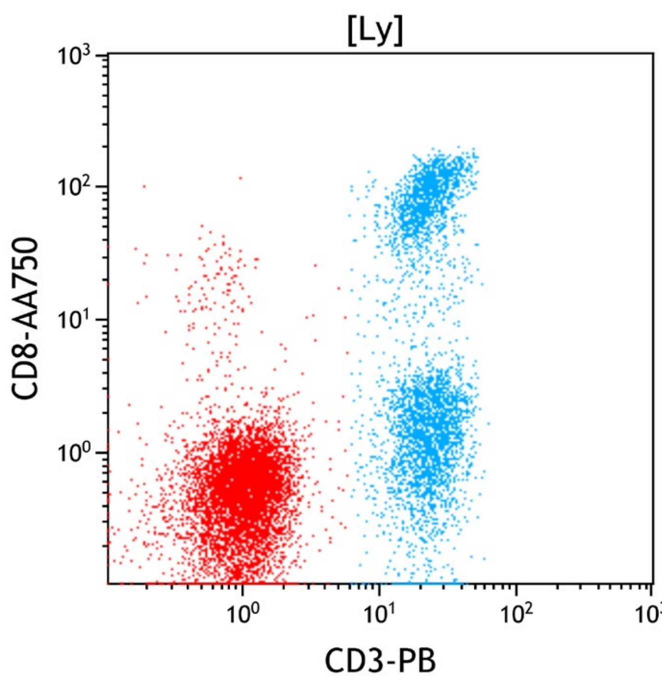


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red) also expresses CD8 without CD3. The aberrant B cell population lacks expression of CD3 and CD8.

T Cell Tube

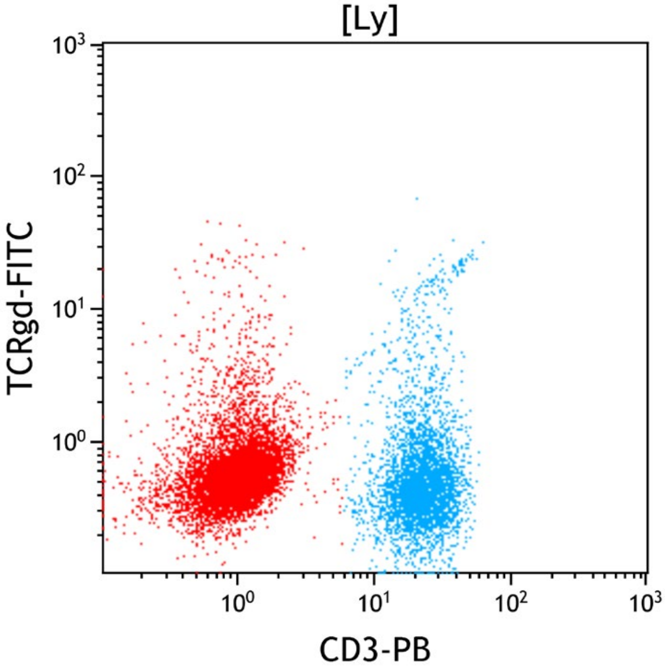
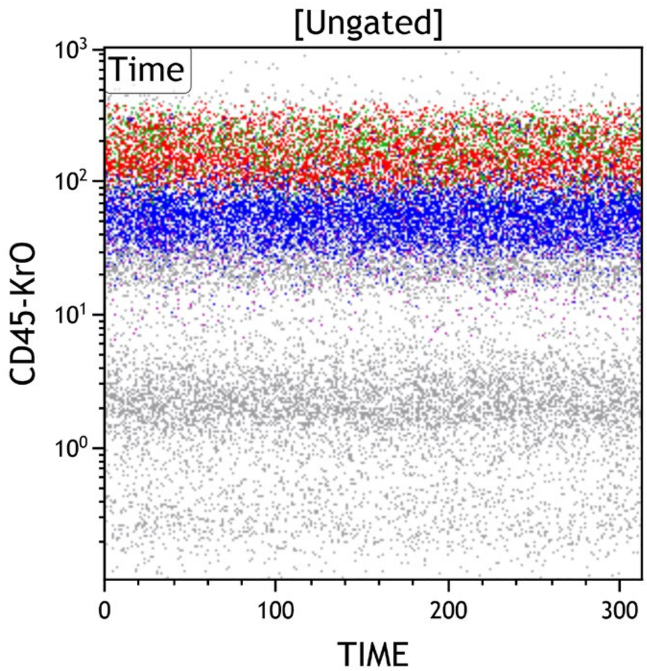


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other. The aberrant B cell population lacks expression of CD3 and TCRγδ.



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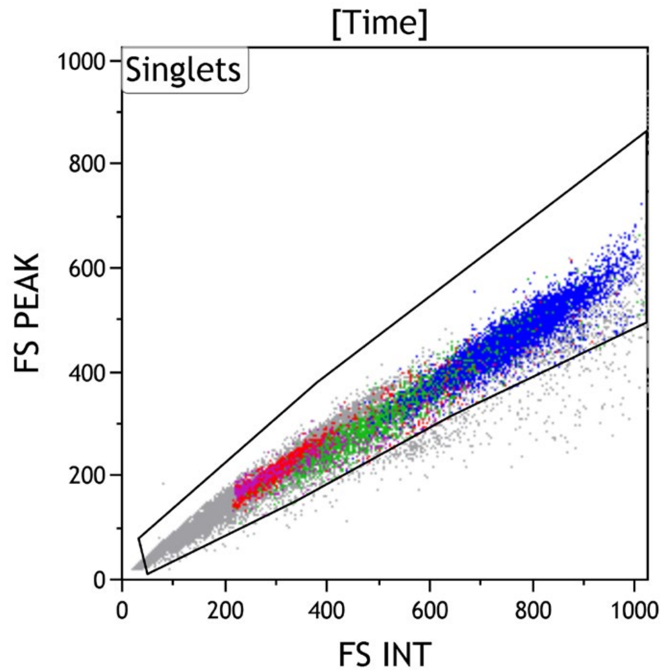

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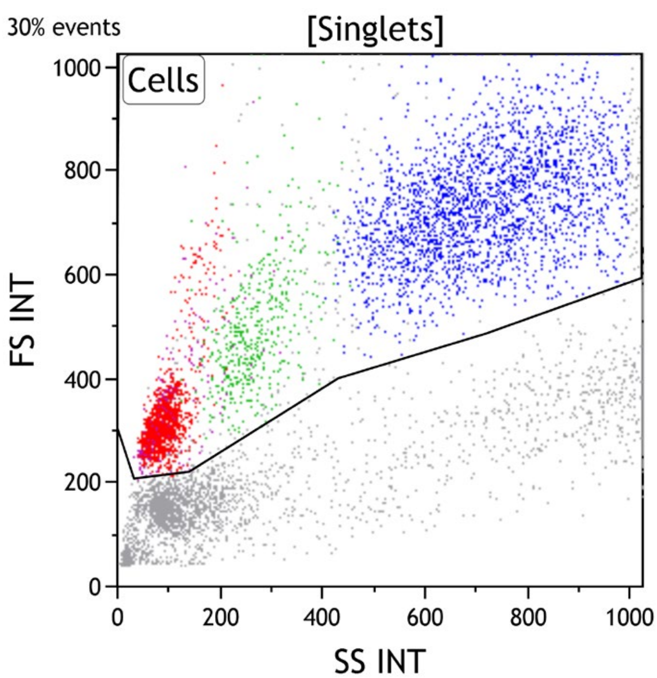

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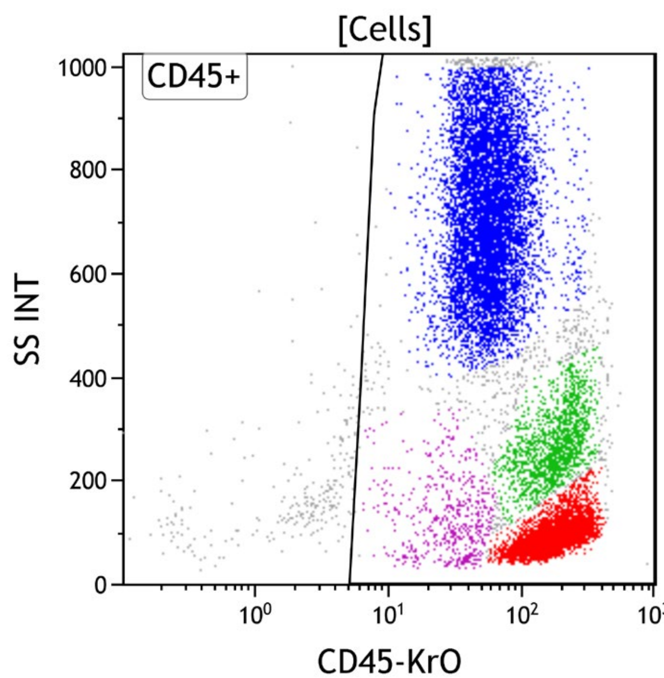
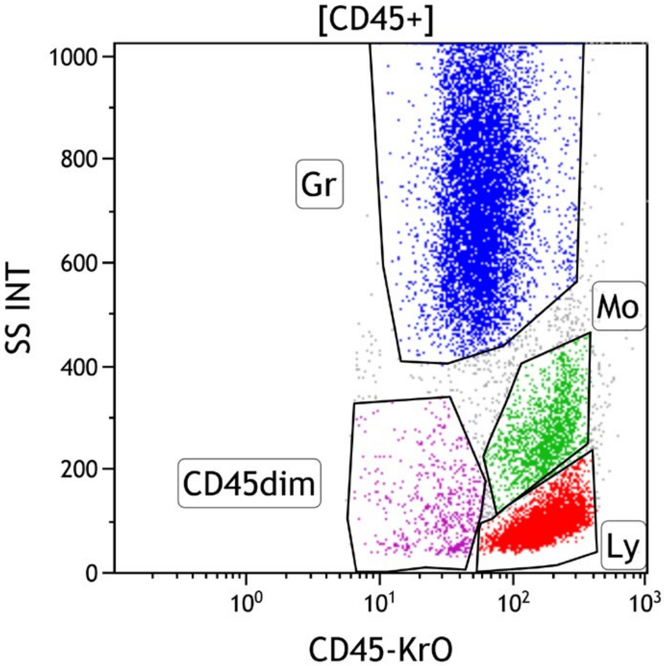


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.



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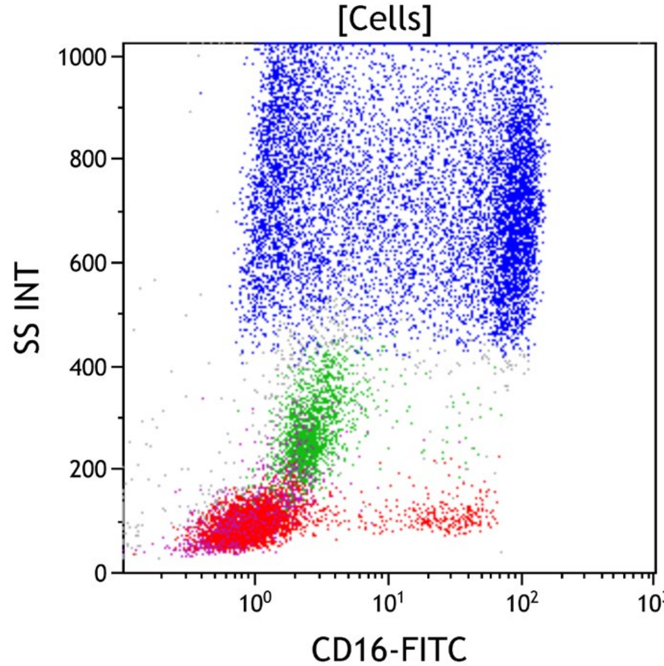

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

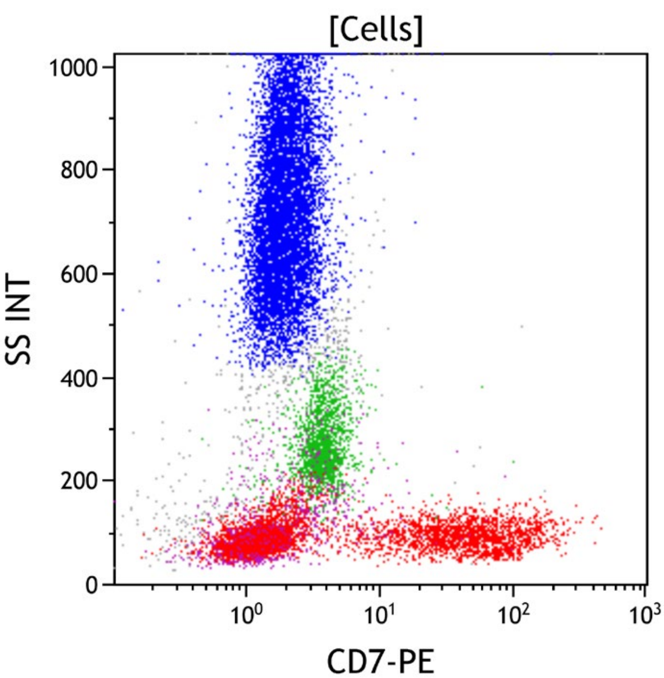

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

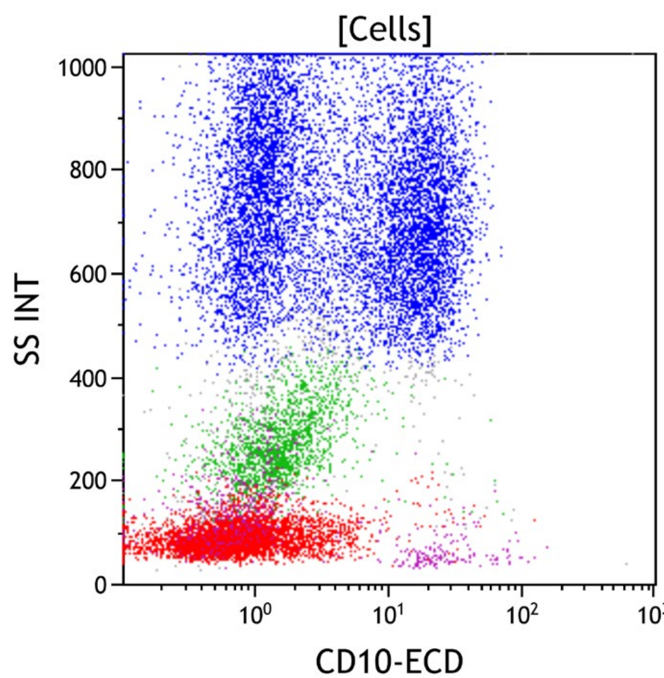

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

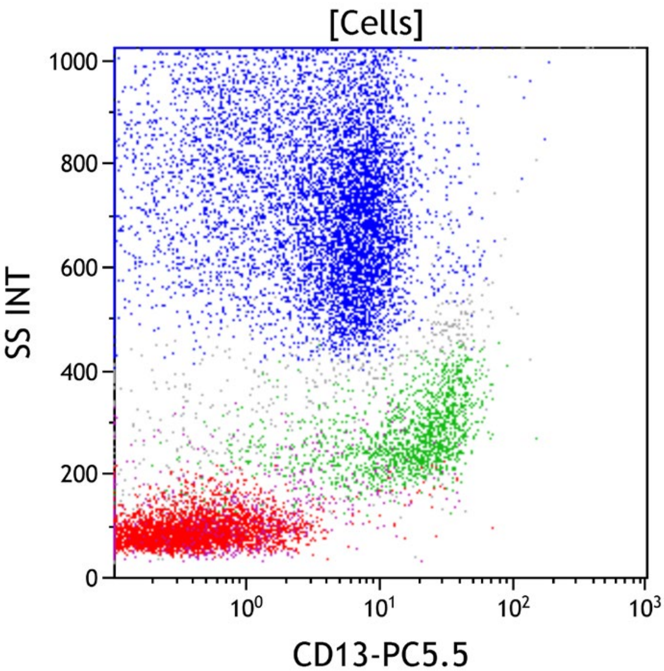

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

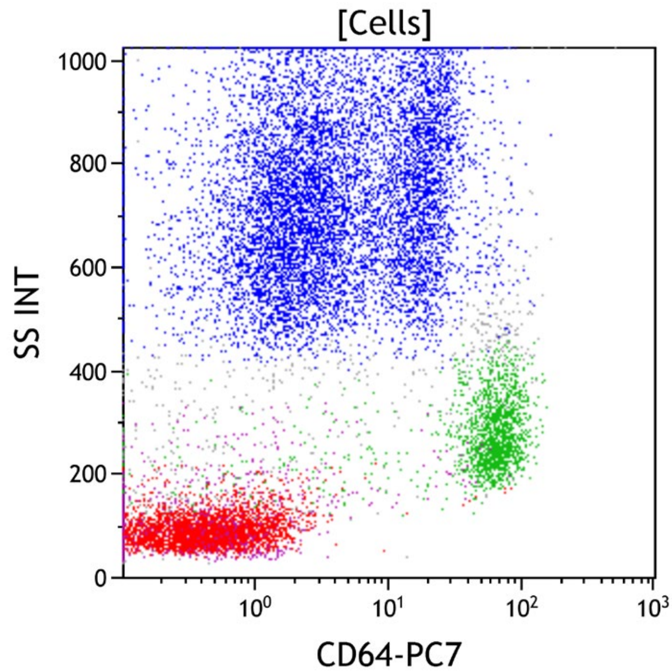

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors.

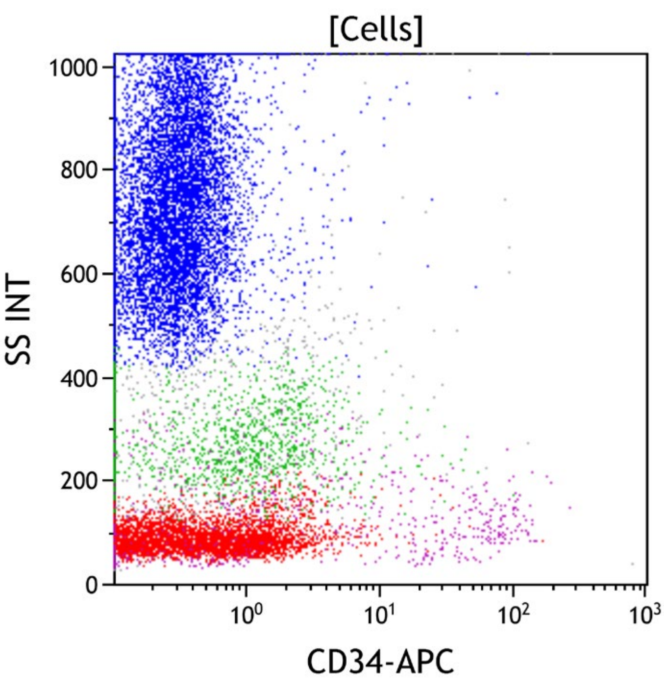

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes (blue), monocytes (green), and lymphocytes (Red) are negative for CD34.

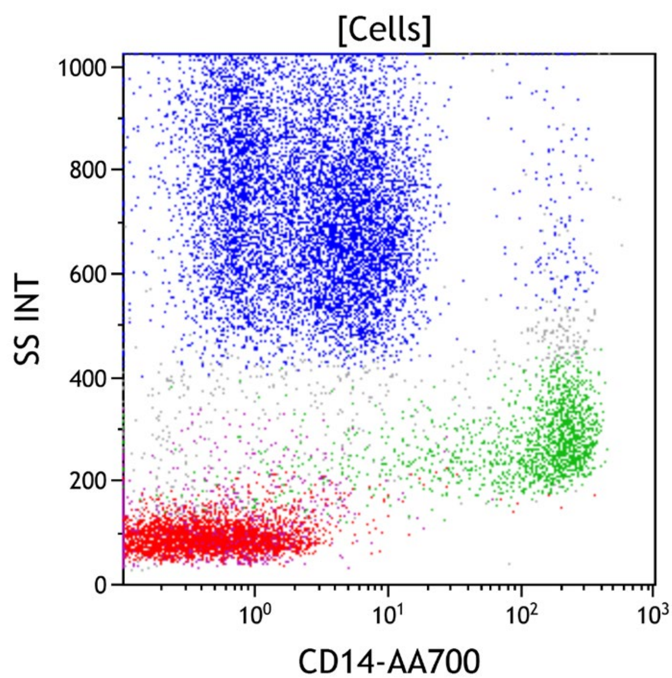

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

M1 Cell Tube

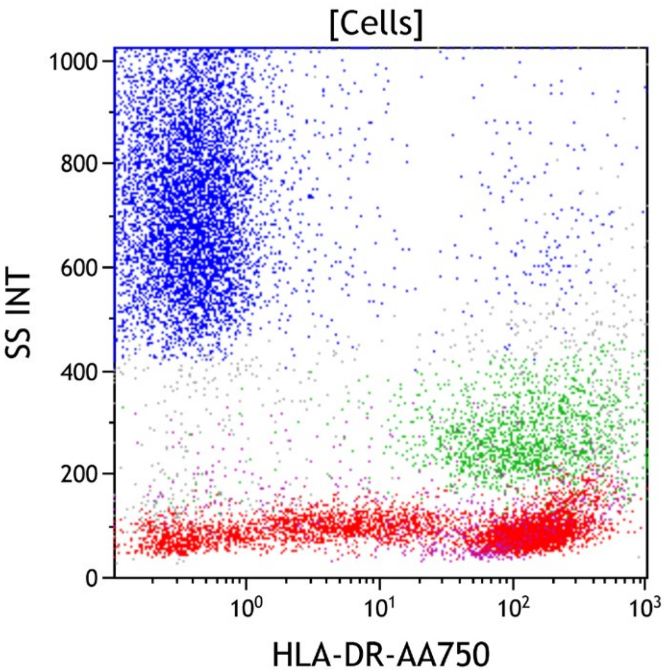

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

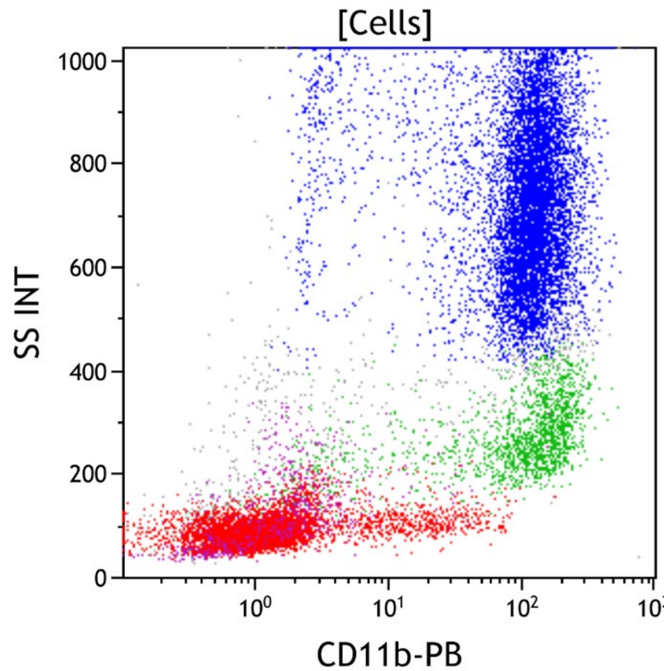

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils (purple).

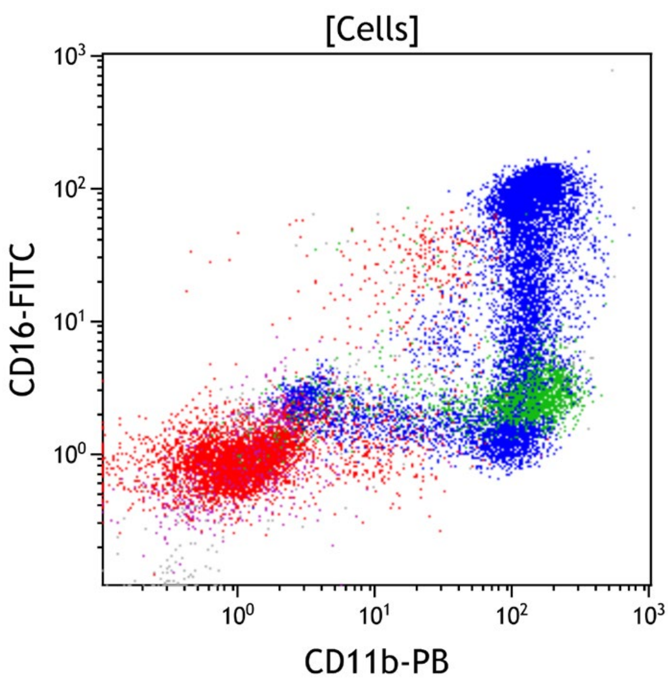

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

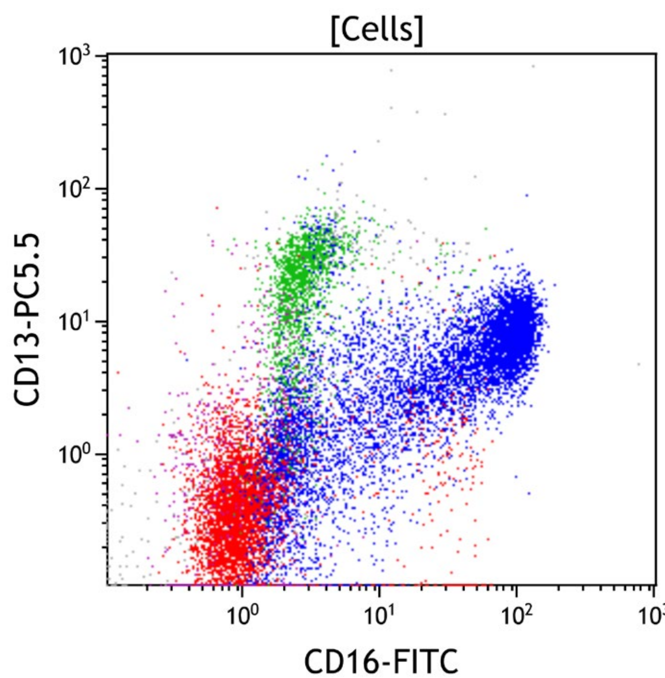

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, upper left) and lose CD13 as they mature to myelocytes (blue, lower left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue, upper right).

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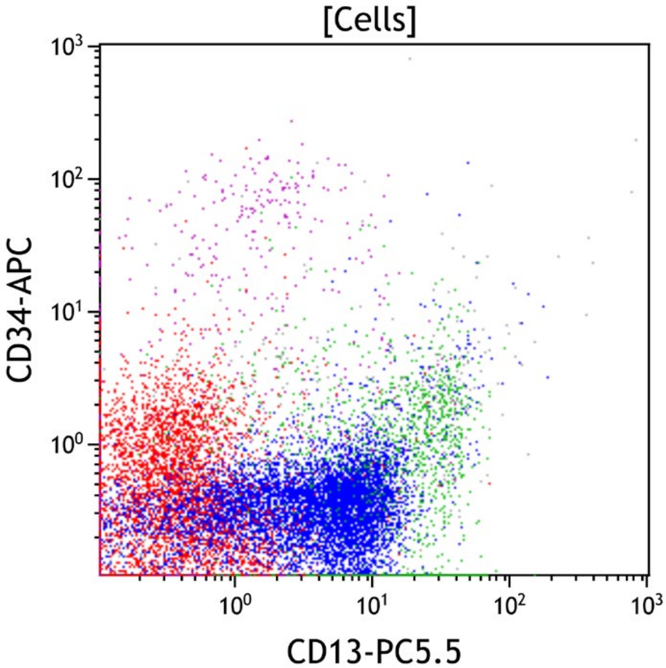

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphocytes (red).

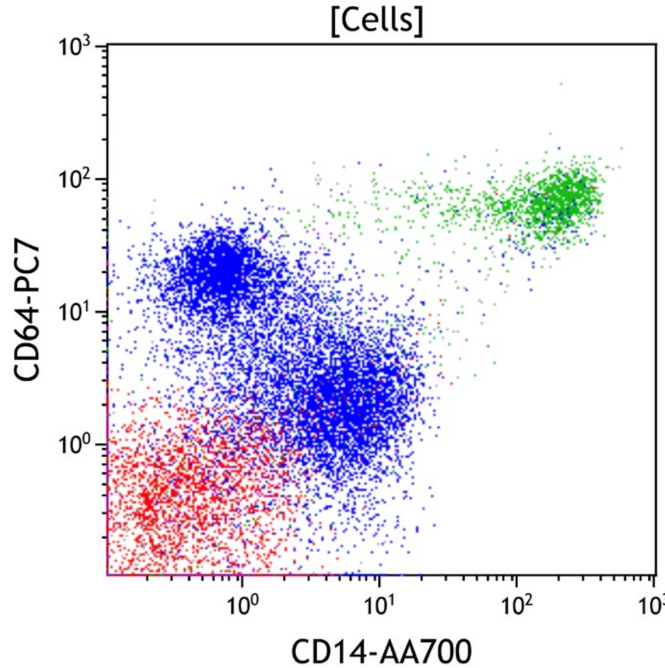

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 green, upper left) and progressively acquire CD14 during maturation to mature monocytes while retaining high level CD64 (green, upper right). Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left).

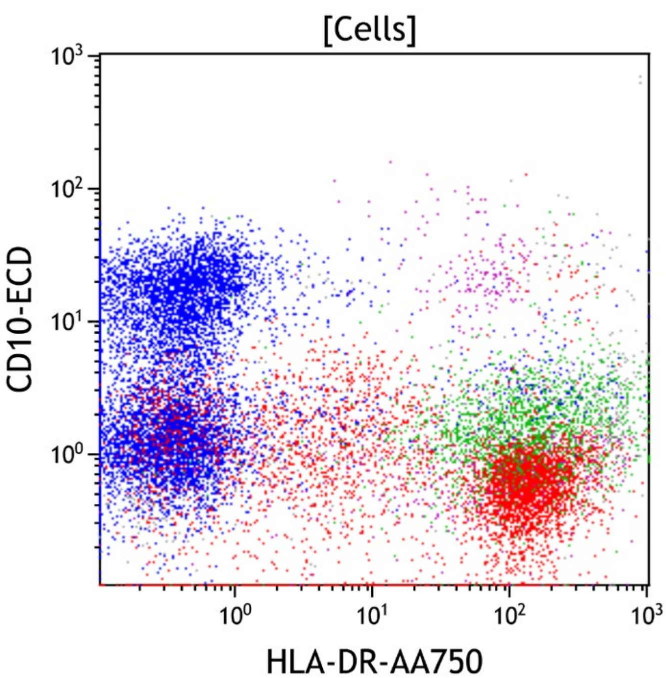

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

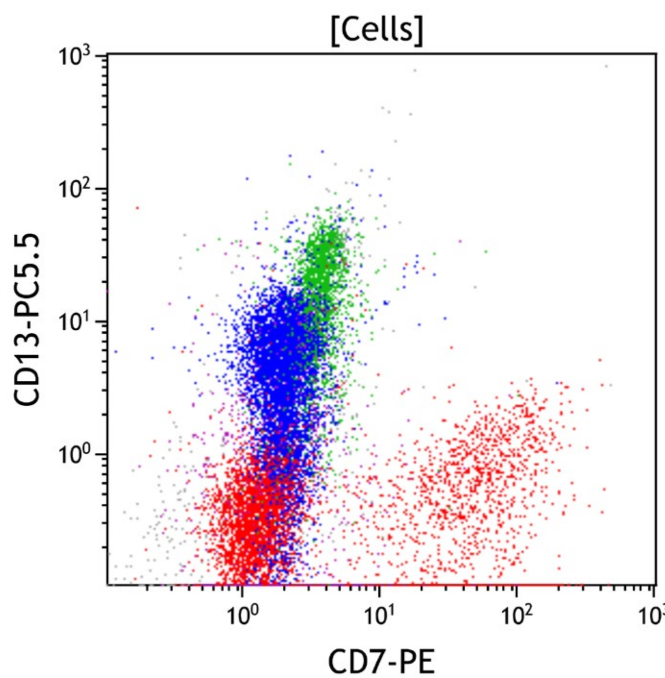

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

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M2 Cell Tube

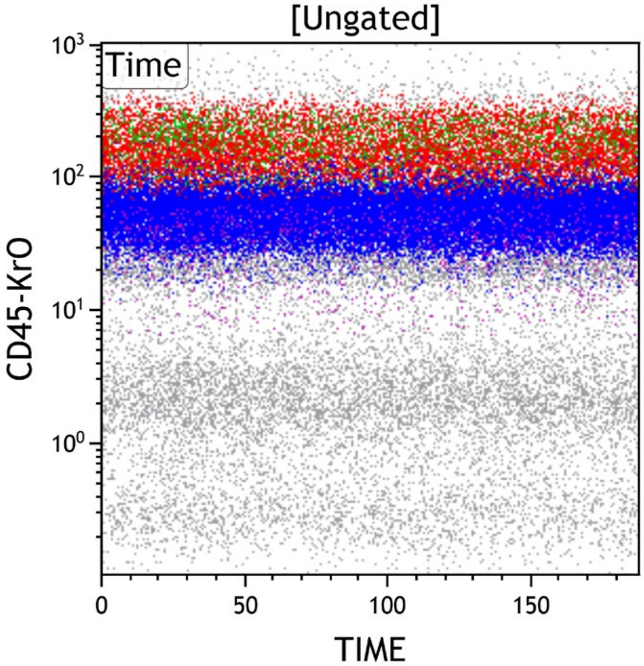

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

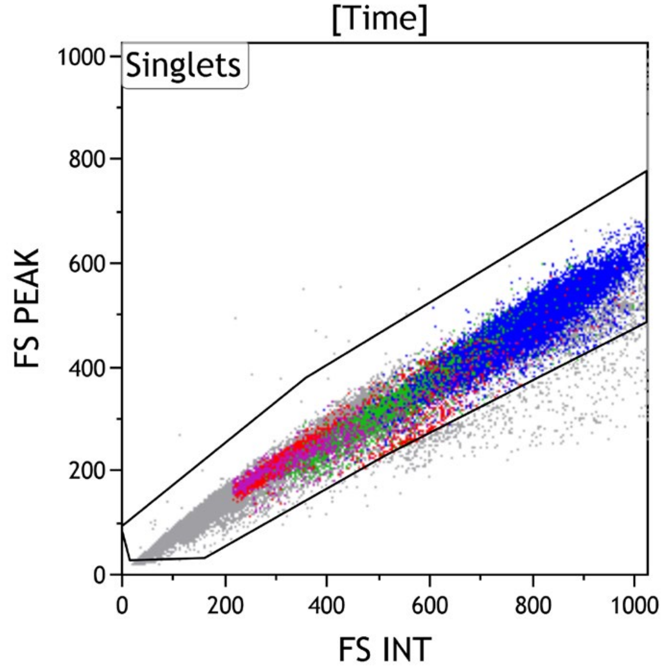

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

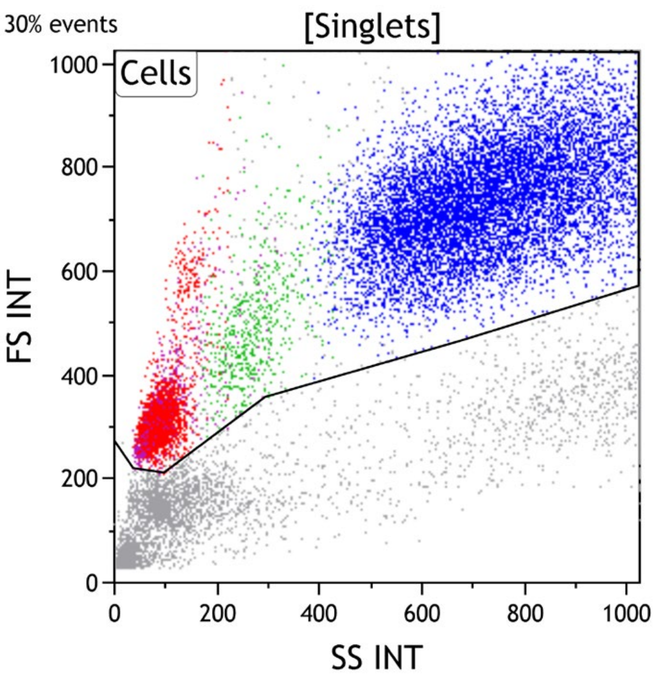

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

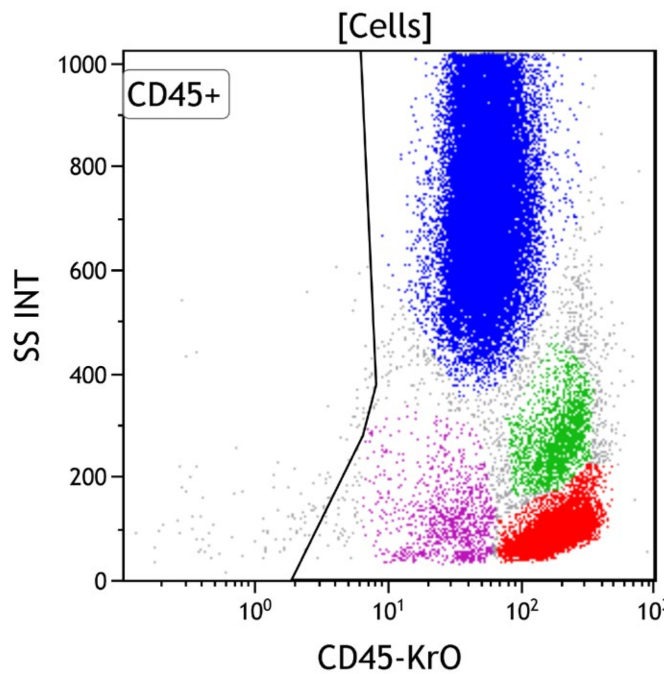

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

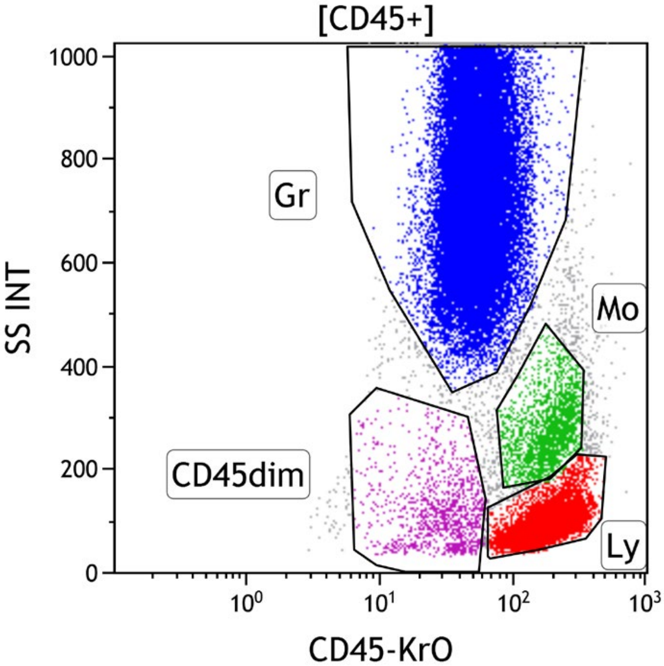

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

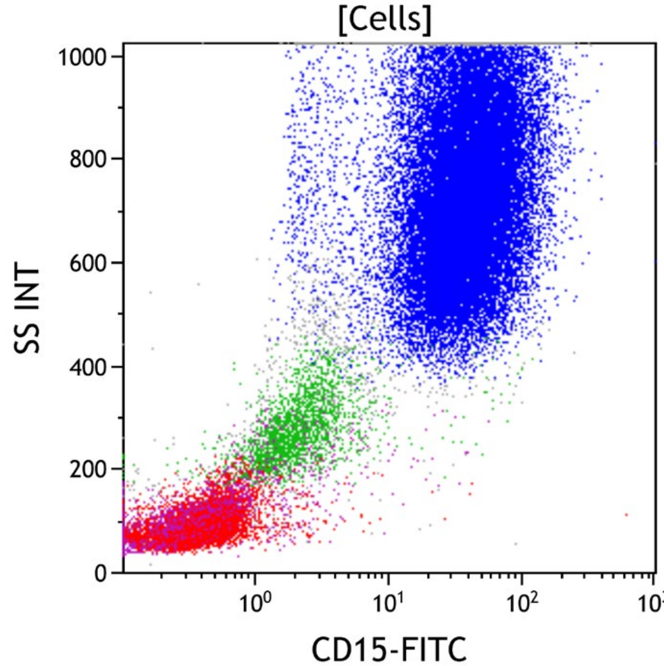

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

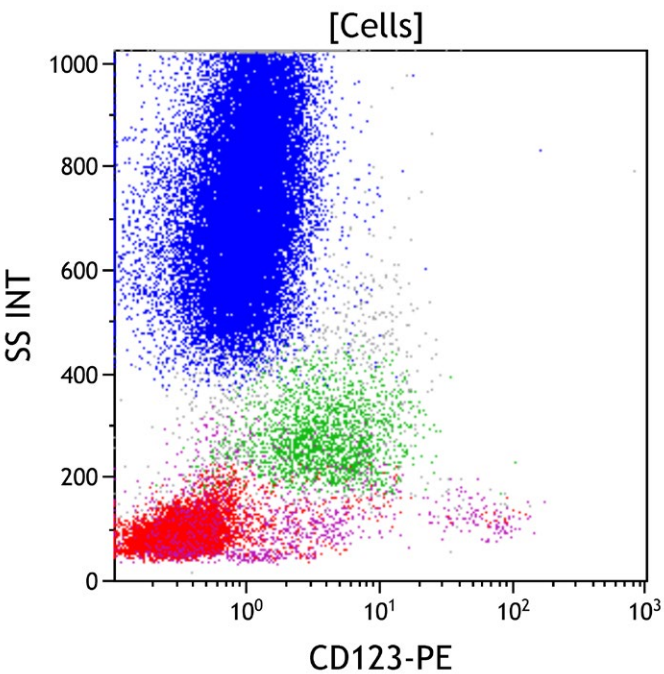

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple, lower right) and at a lower level on CD34 positive myeloid progenitors (purple, lower left) and monocytes (green).

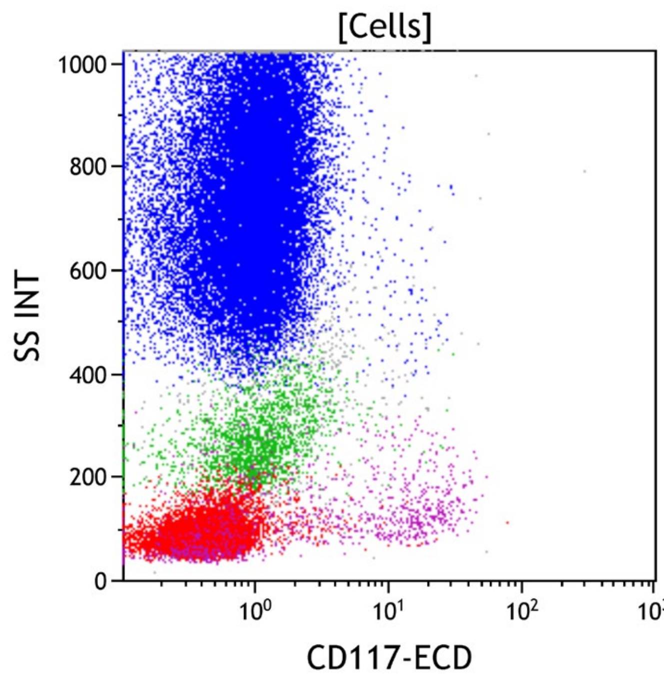

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors (purple), early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

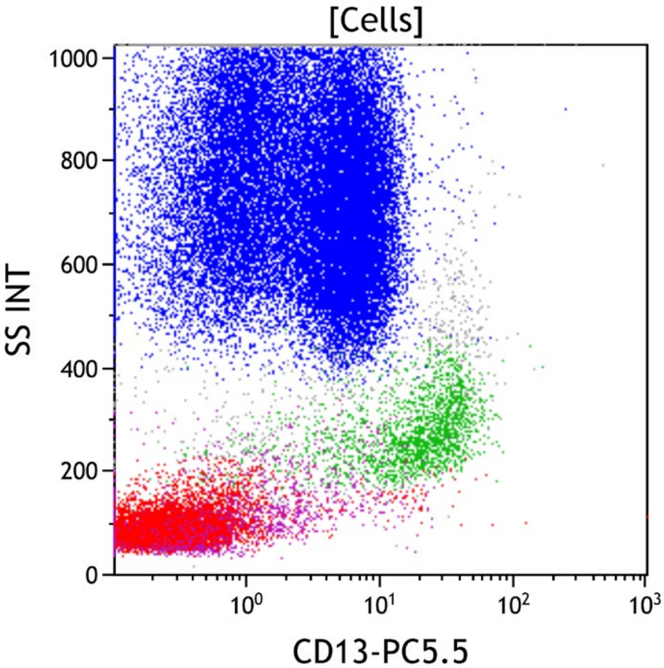

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

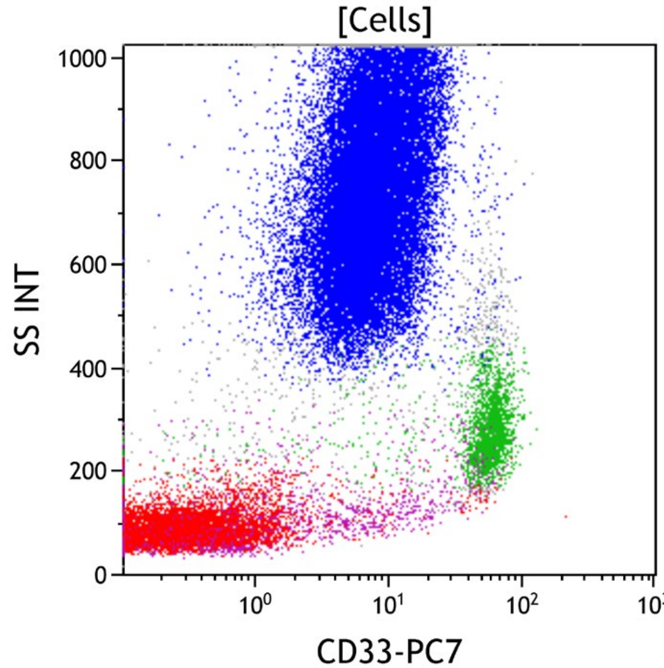

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red, lower right), and a subset of CD34 positive myeloid progenitors (purple).

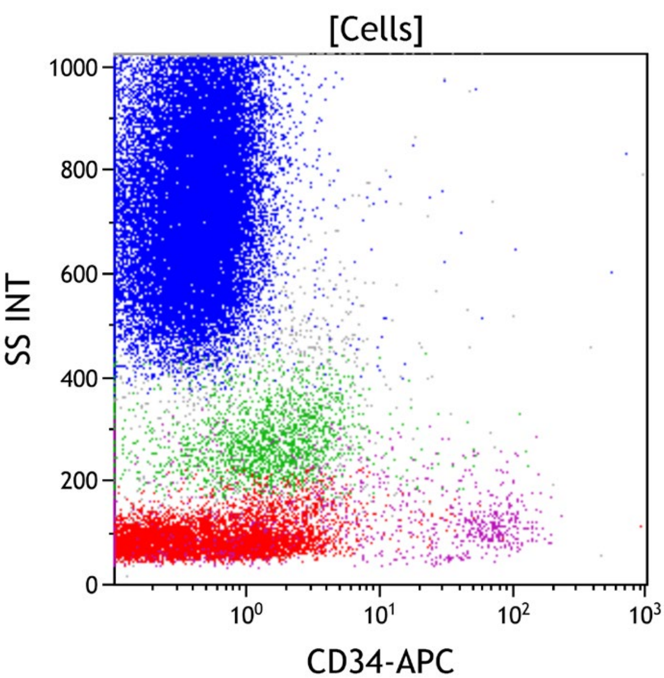

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45 dim gate (purple).

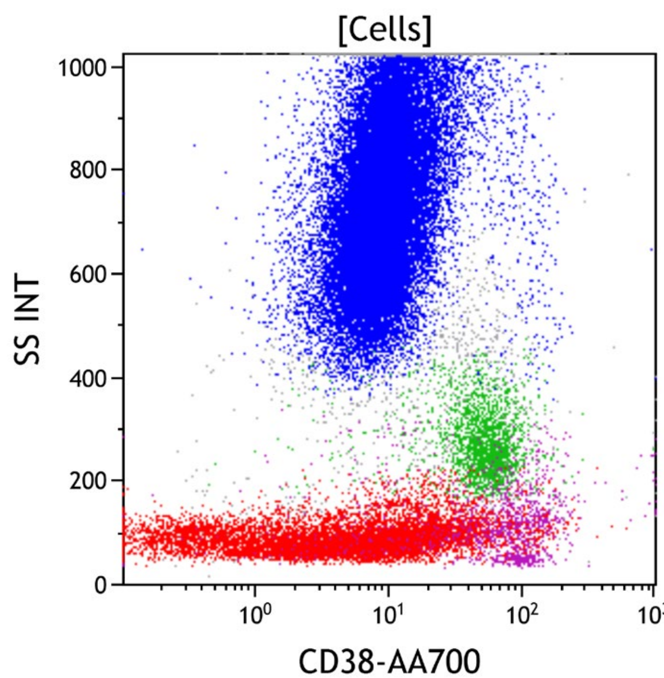

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red).

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M2 Cell Tube

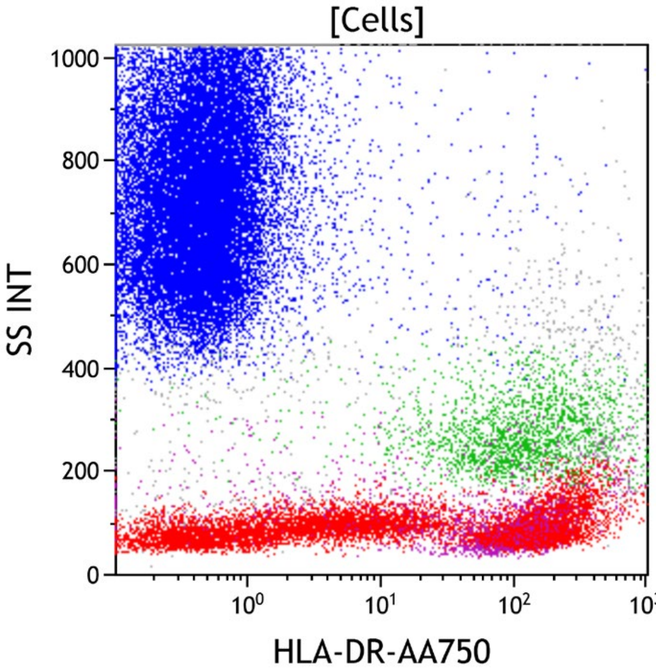

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

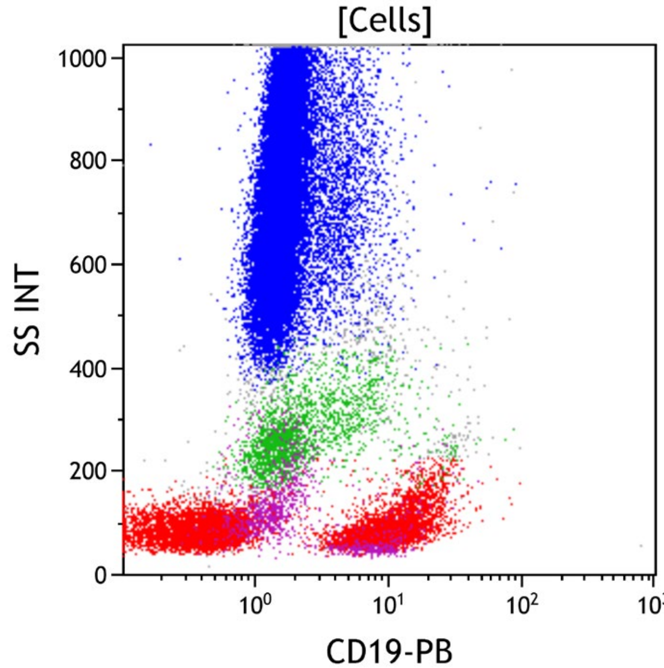

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

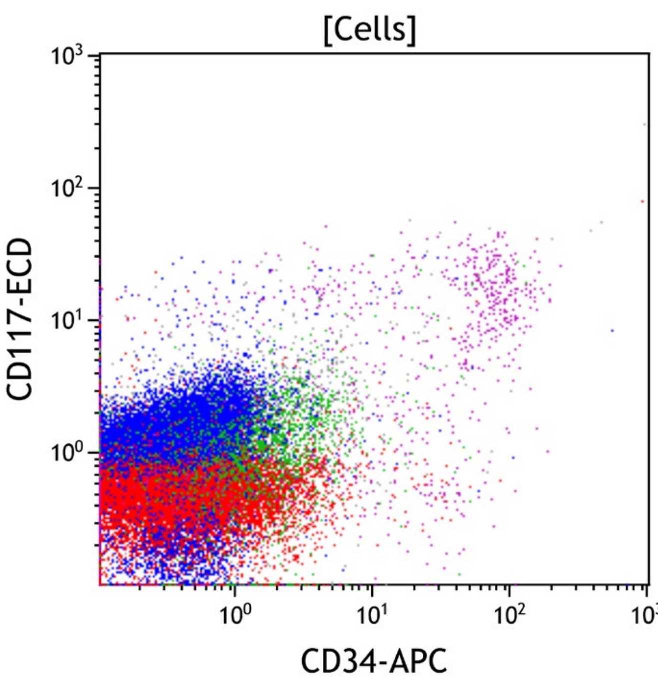

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (purple, upper right), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (purple, lower right).

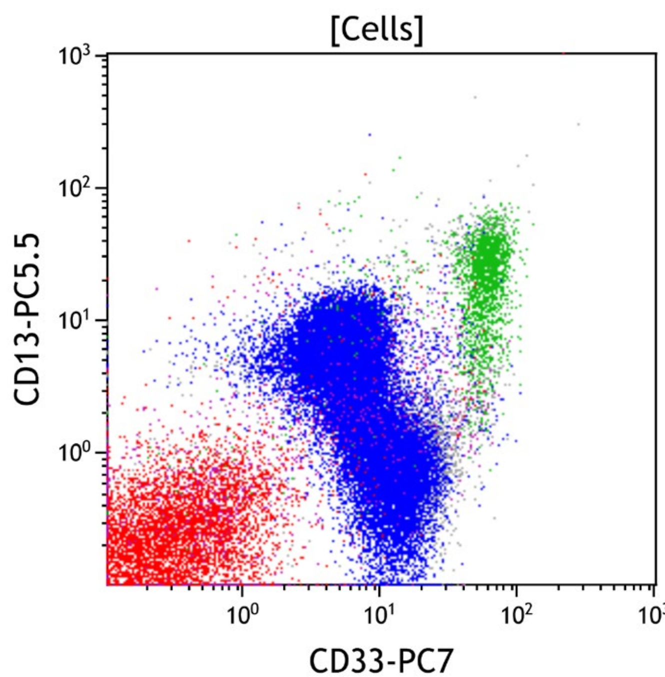

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue, bottom) than more mature granulocytes (blue, upper left). Lymphocytes largely do not express either CD13 or CD33 (red).

M2 Cell Tube

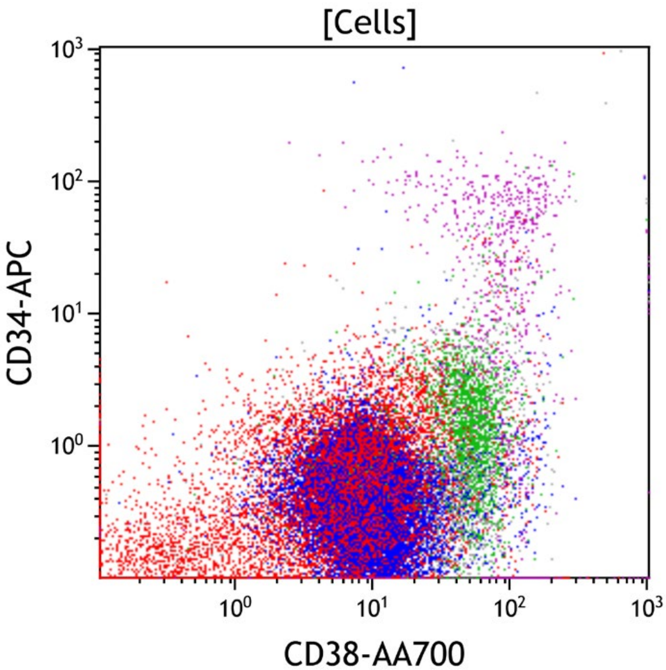

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple).

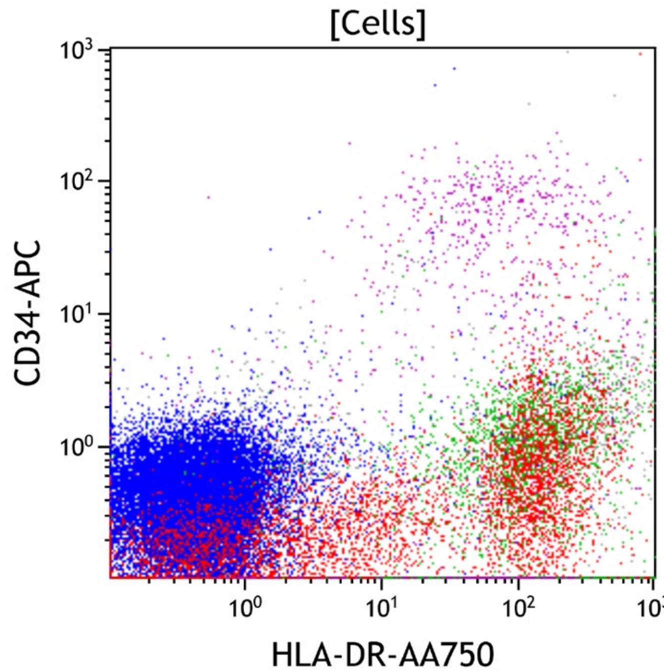

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes.

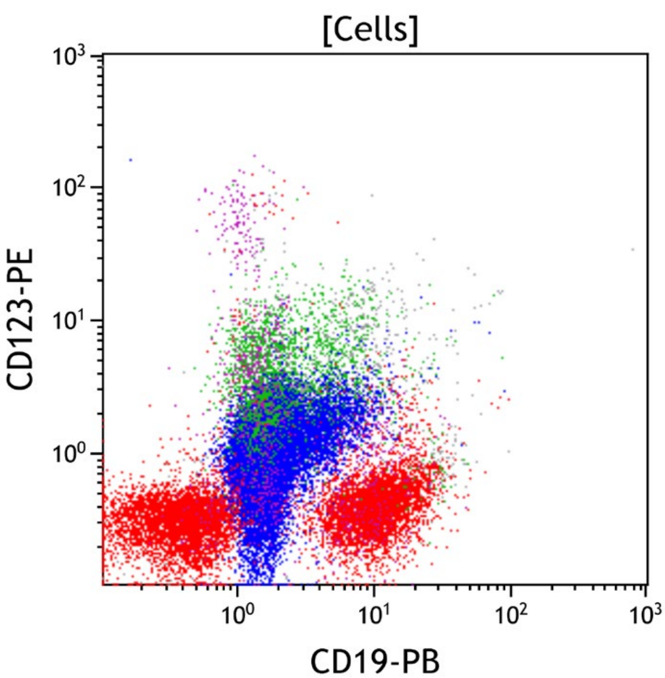

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors (purple). CD19 positive B cells (red, lower middle) normally do not express significant CD123. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

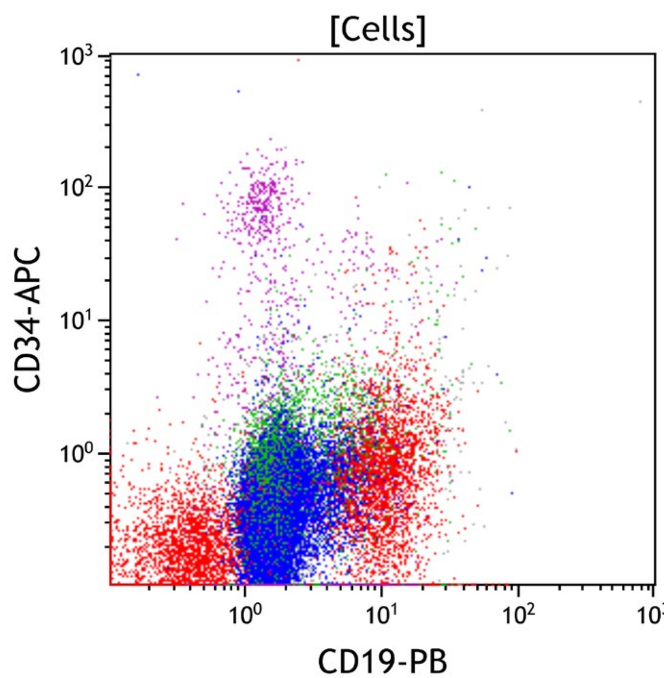

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors (purple). Mature CD19 positive B cells (red, lower middle) do not express CD34.

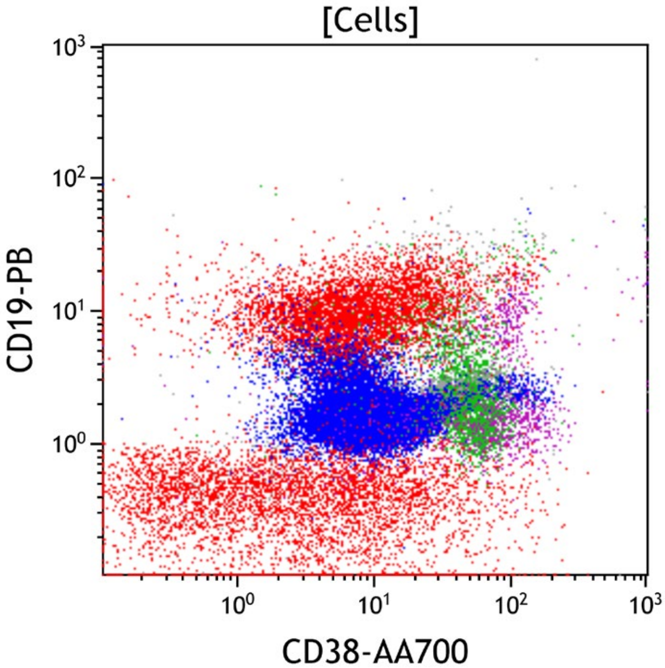

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Mature CD19 positive B cells show intermediate expression of CD38 (red, upper middle). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

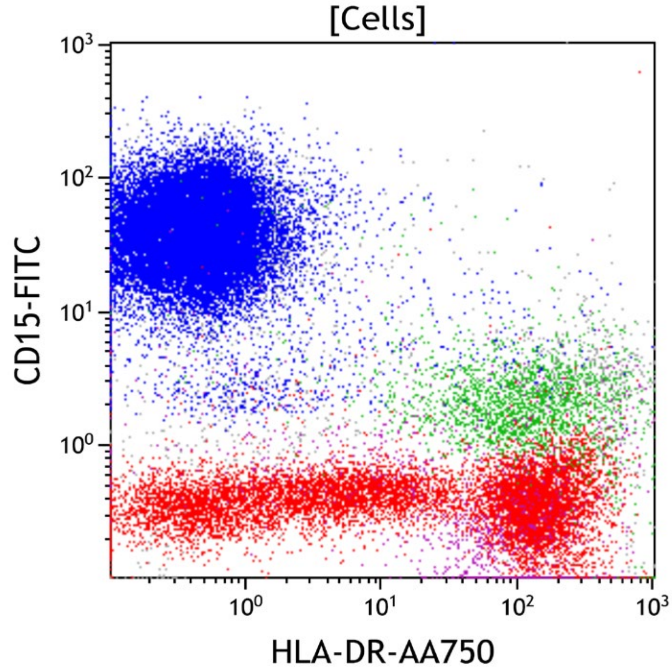

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired.

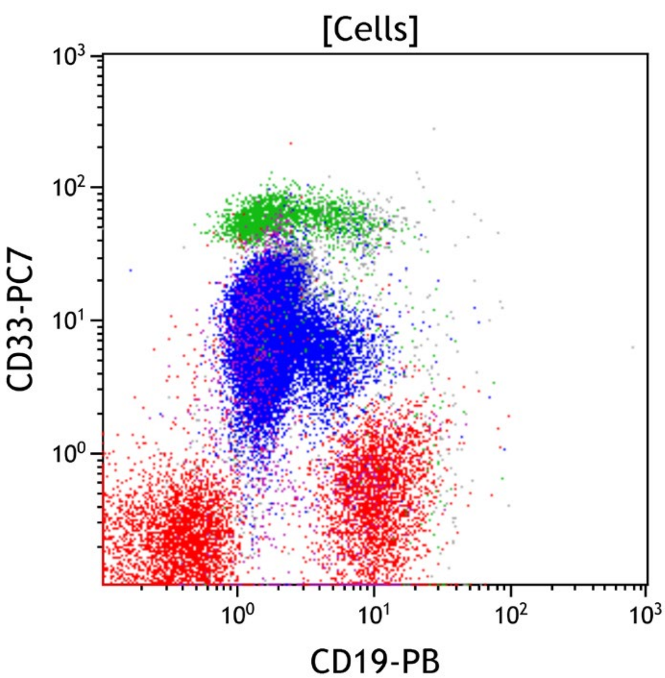

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red, lower right). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate to bright CD5, intermediate CD19, bright CD20, intermediate CD38, bright CD45, and surface lambda light chain restriction without CD10, CD200 or other T or myeloid markers. Compared with normal B cells, the expression of CD5, absence of CD200, and lambda light chain restriction are aberrant.

Taken together, the immunophenotype of the aberrant population is most consistent with mantle cell lymphoma. Correlation with clinical, morphologic and laboratory data and FISH studies for t(11;14) is recommended, and that additional immunophenotyping may be warranted.

Case #9: Plasma Cell Myeloma

Clinical Vignette

This 80-year-old male presents with plasmacytosis. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

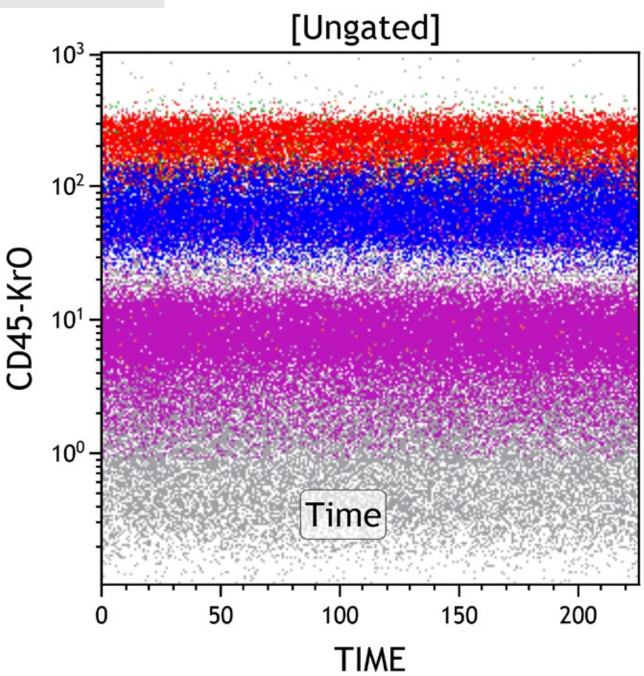

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

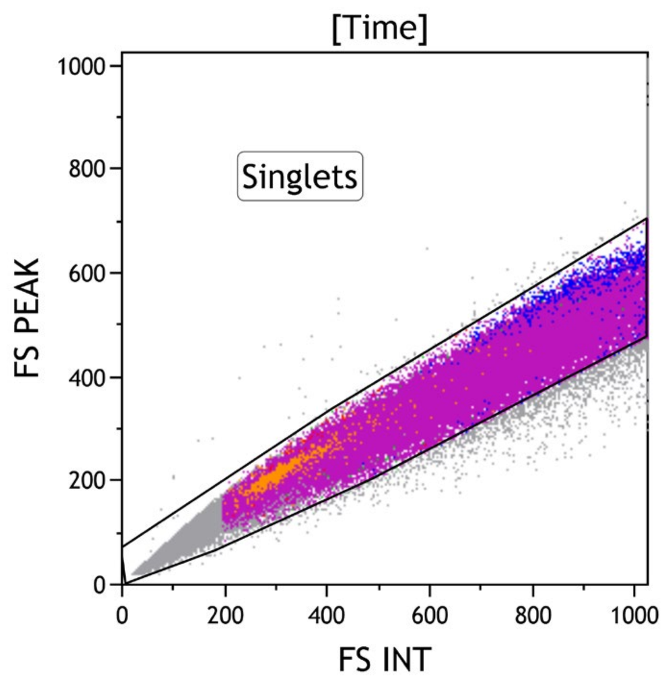

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

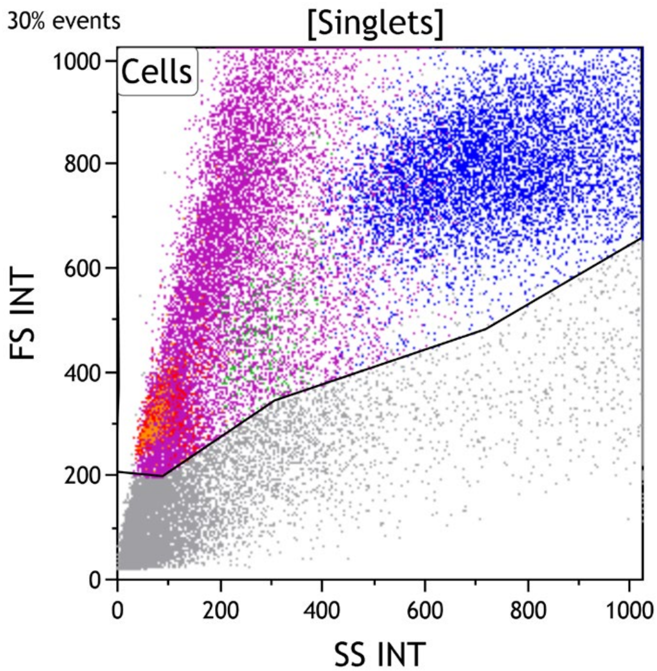

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. The aberrant population (purple) has increased forward and side scatter.

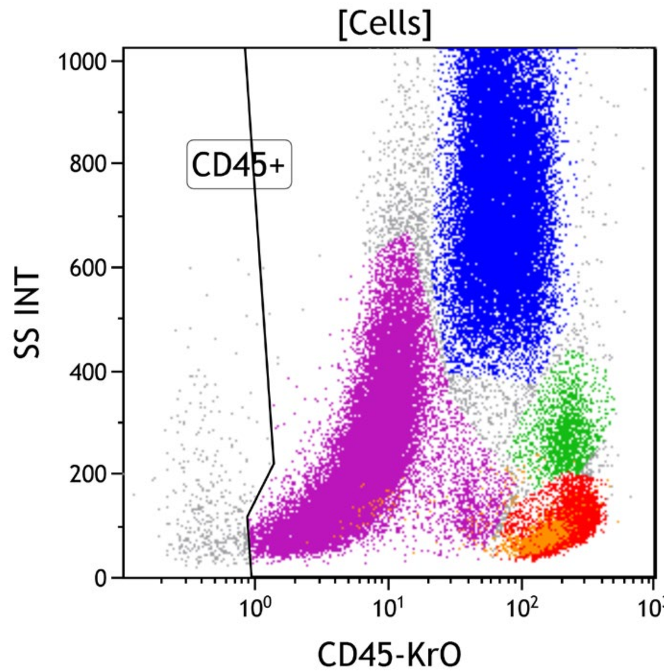

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells. The aberrant population (purple) has low level expression of CD45.

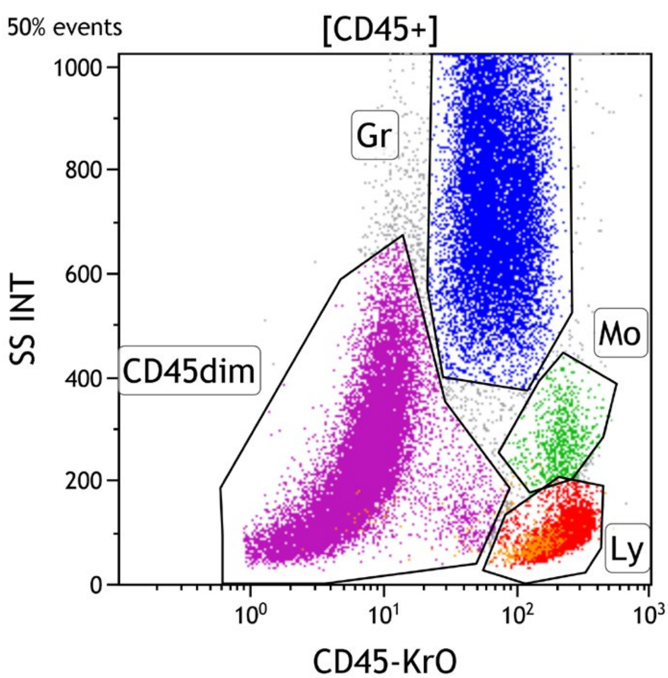

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. The aberrant population (purple) has low level expression of CD45.

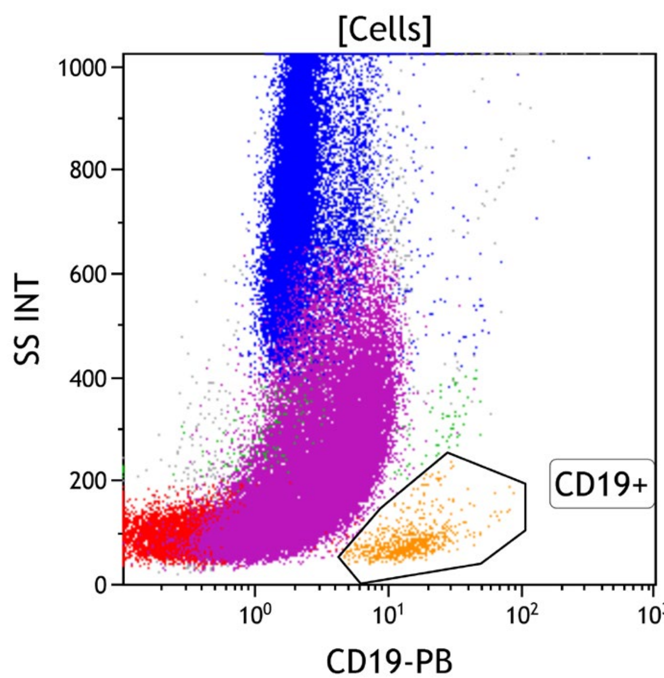

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) has increased side scatter and low-level expression of CD19.

B Cell Tube

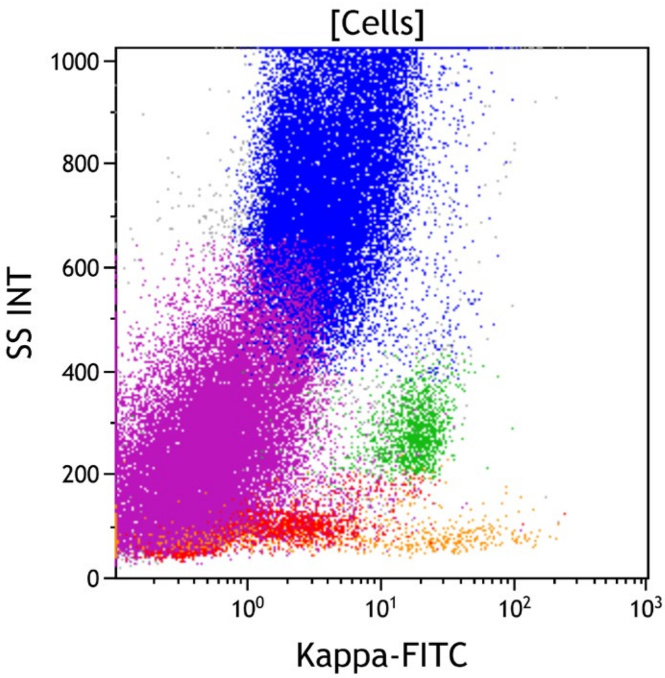

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The aberrant population (purple) lacks surface kappa light chain expression.

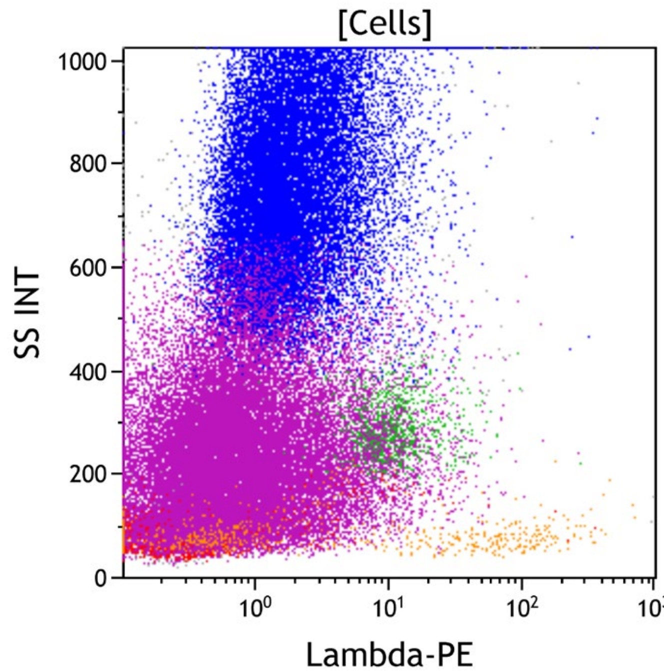

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The aberrant population (purple) lacks surface lambda light chain expression.

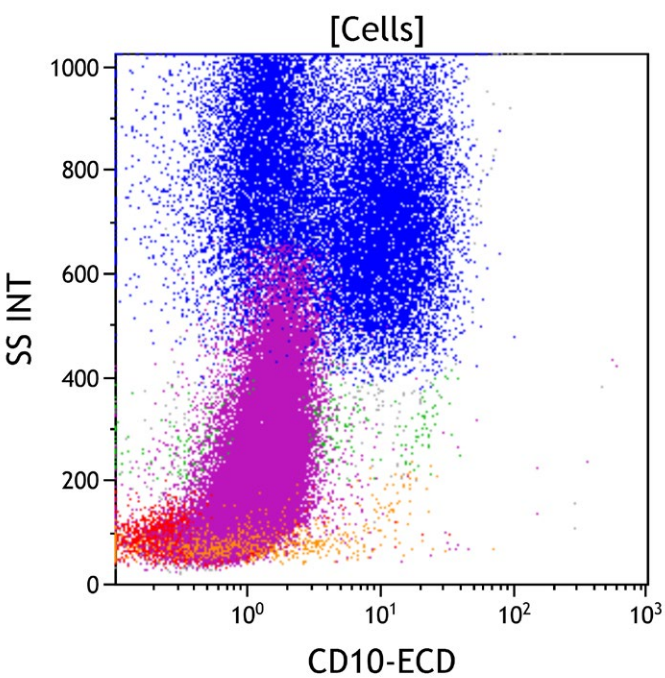

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple) is negative for CD10.

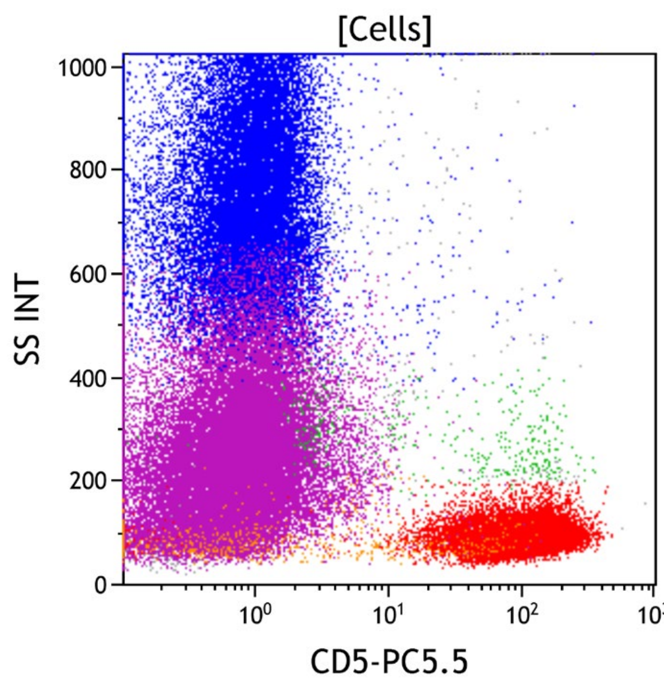

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The aberrant population (purple) is negative for CD5.

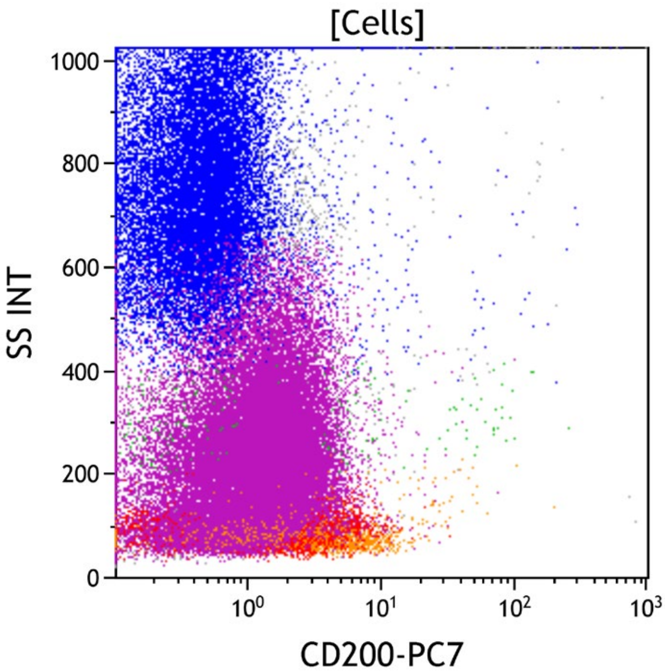

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The aberrant population (purple) is dimly positive for CD200.

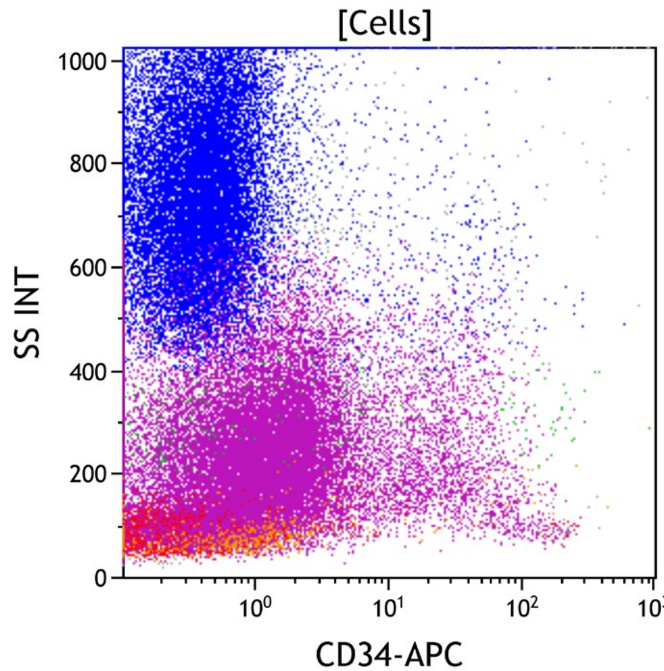

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The apparent variable CD34 positivity on the aberrant population (purple) is a compensation artifact due to the extremely high level of CD38 that extends beyond the visible scale.

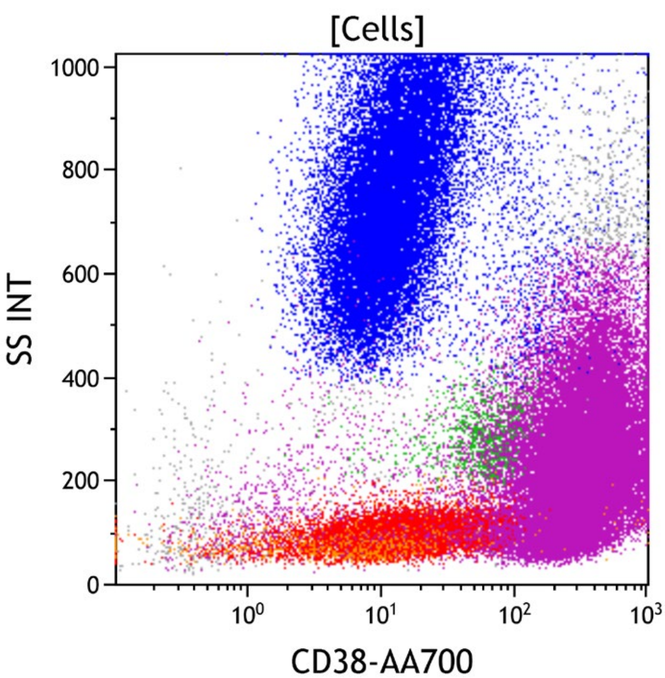

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The aberrant population (purple) has bright CD38 expression.

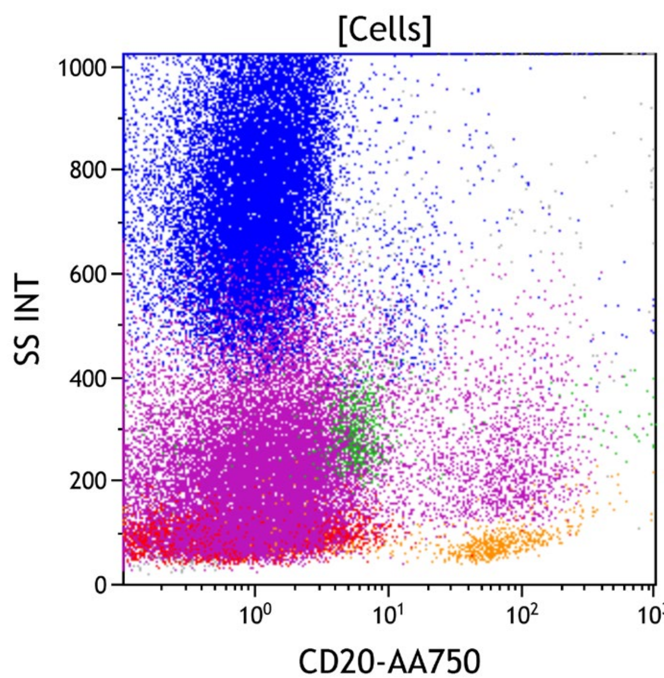

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The aberrant population (purple) expresses CD20 on a small subset.

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B Cell Tube

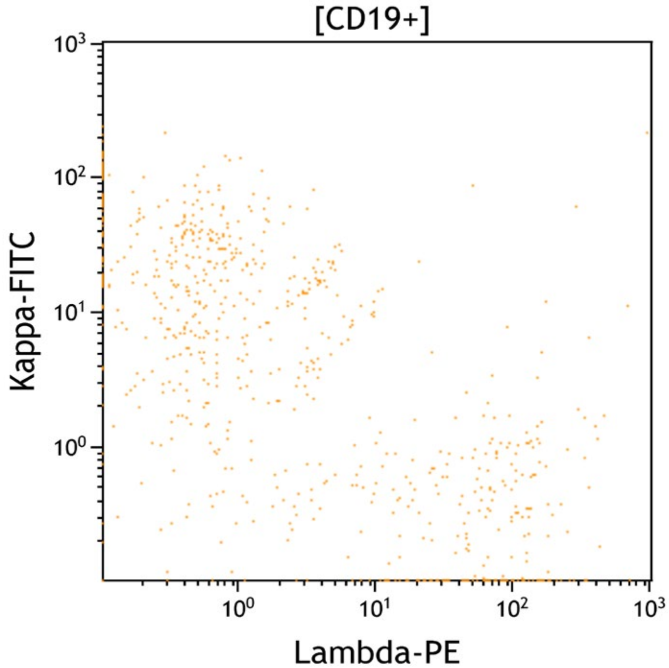

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells (orange) are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

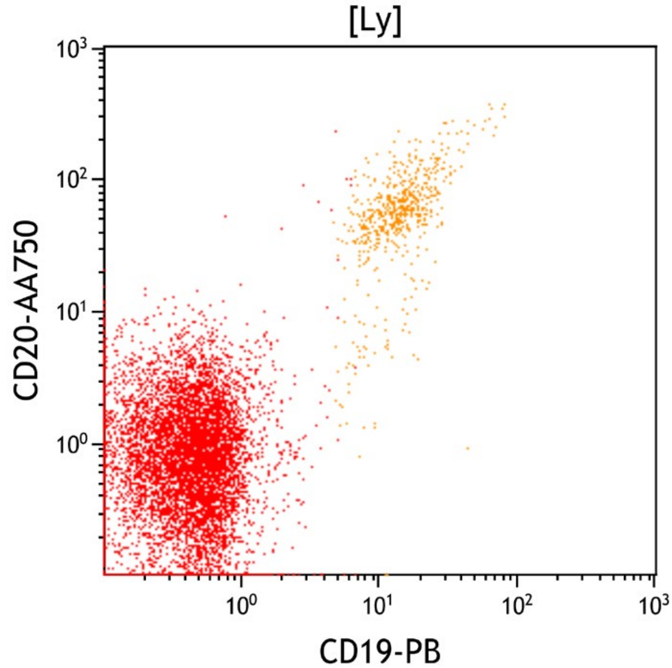

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

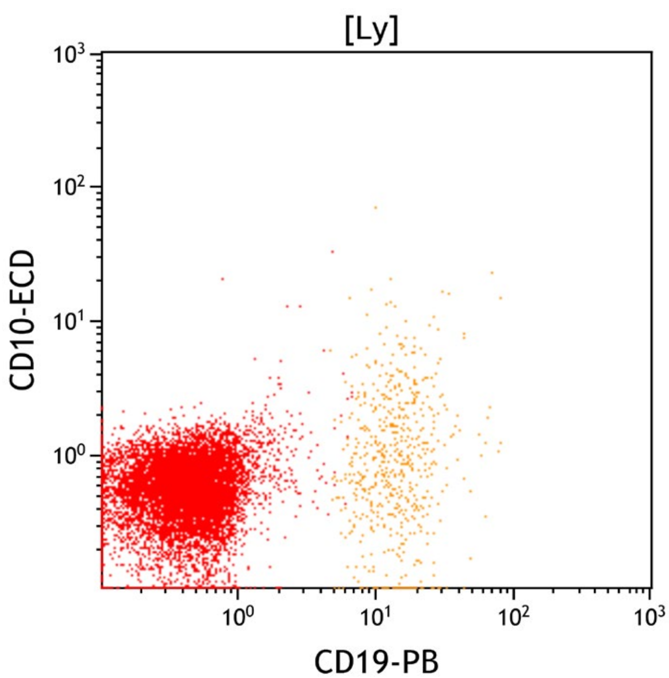

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates.

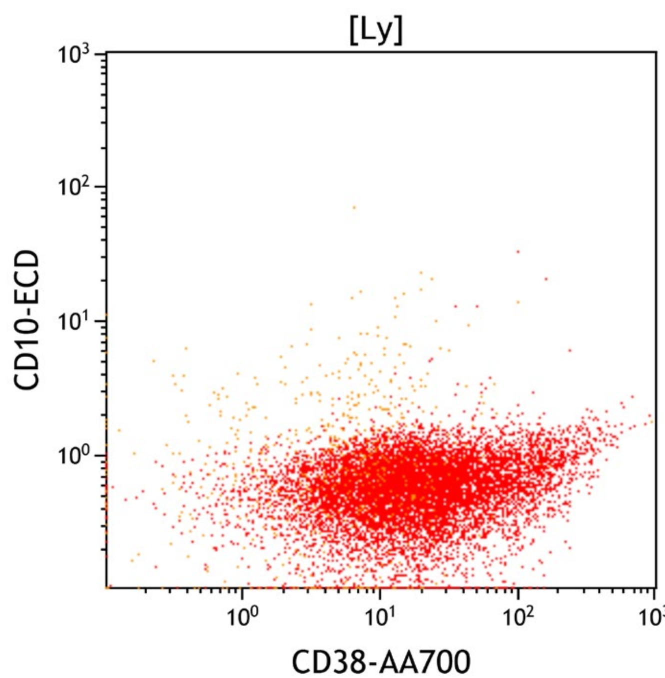

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells display low to absent CD38 expression. T cells (red) show variable CD38 expression dependent on activation state.

B Cell Tube

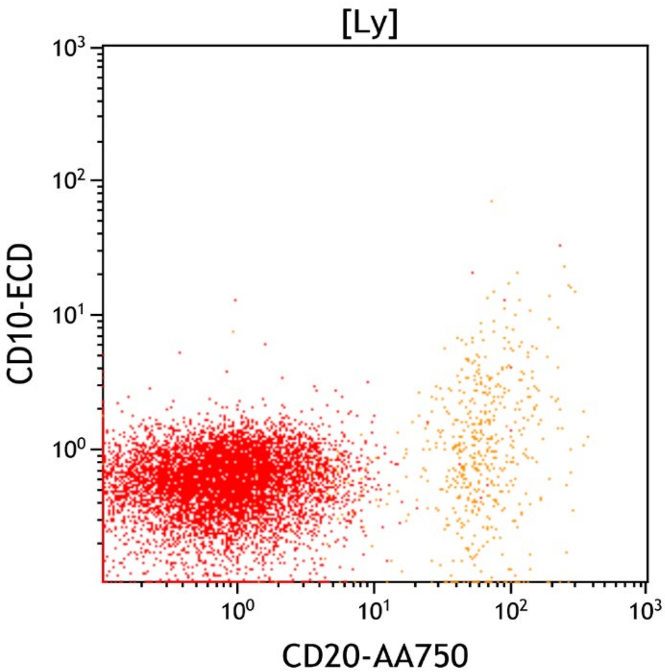

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20 without CD10.

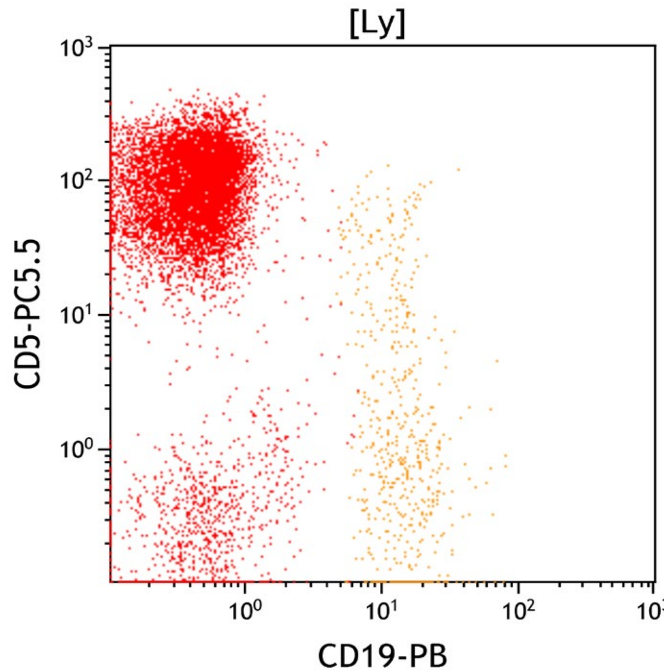

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

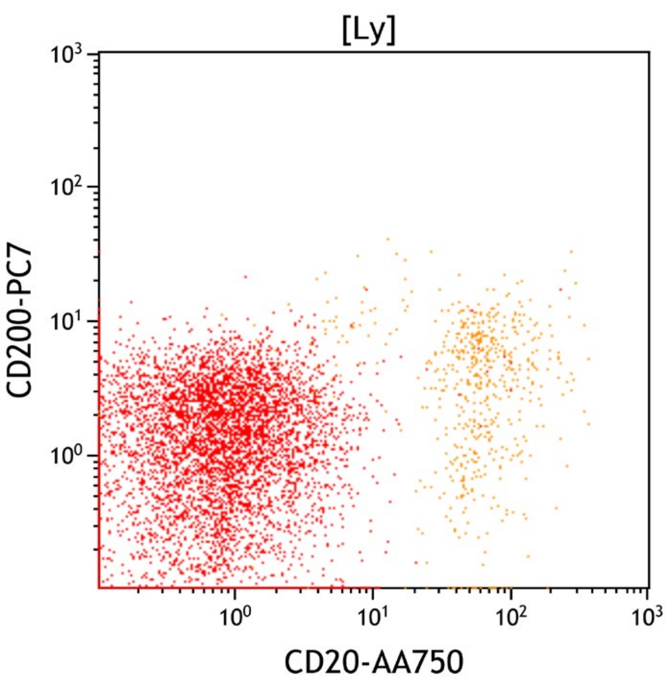

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to intermediate level.

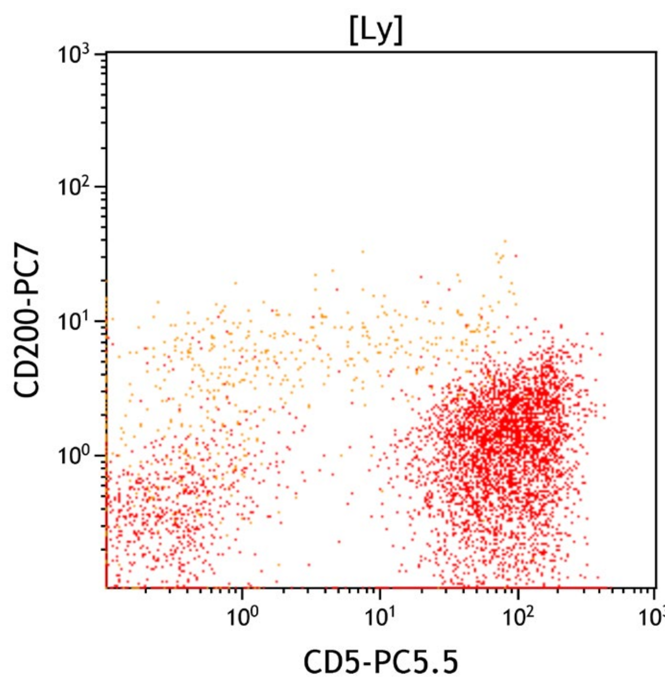

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

T Cell Tube

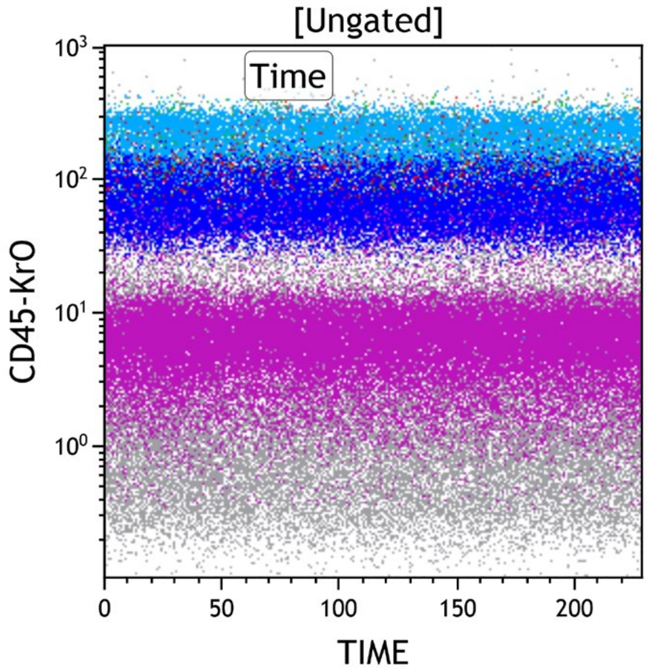

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

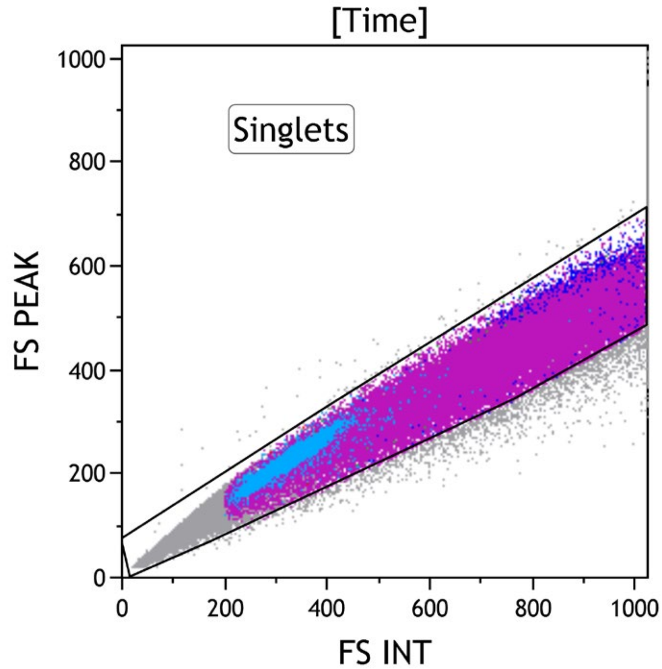

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

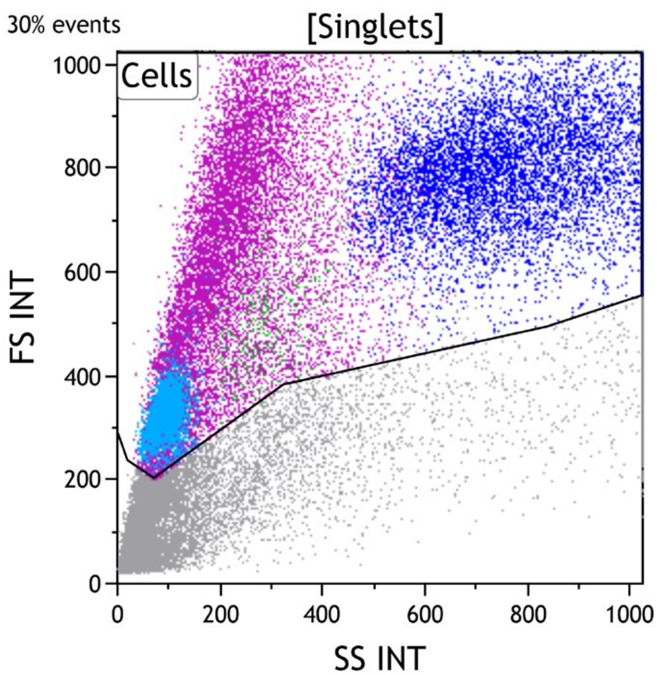

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. The aberrant population (purple) has increased forward and side scatter.

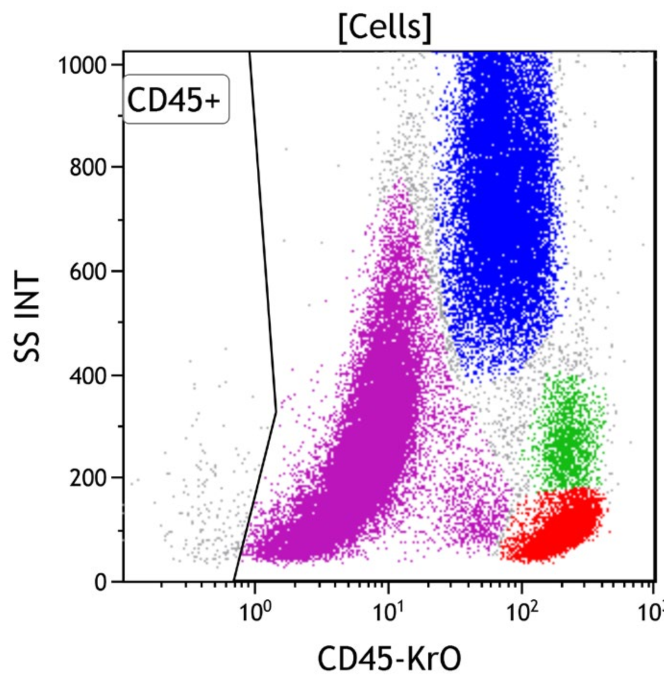

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells. The aberrant population (purple) has low level expression of CD45.

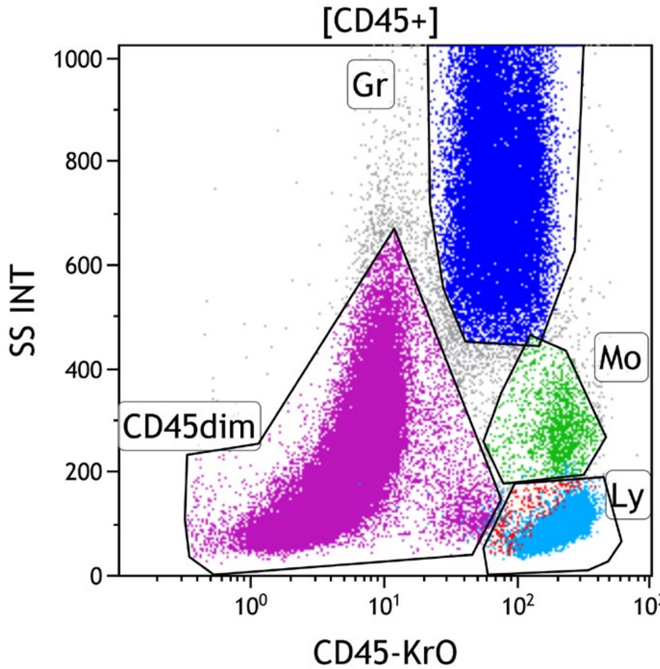

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, aqua/red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The aberrant population (purple) has low level expression of CD45.

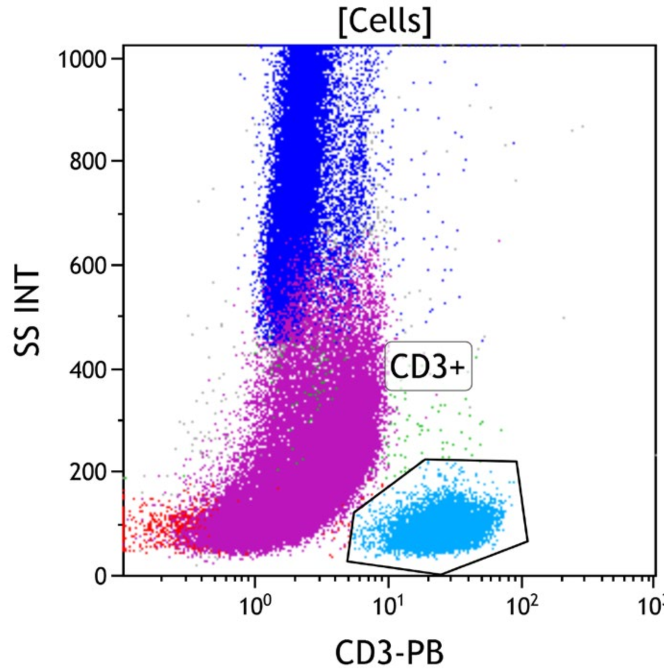

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is negative for CD3.

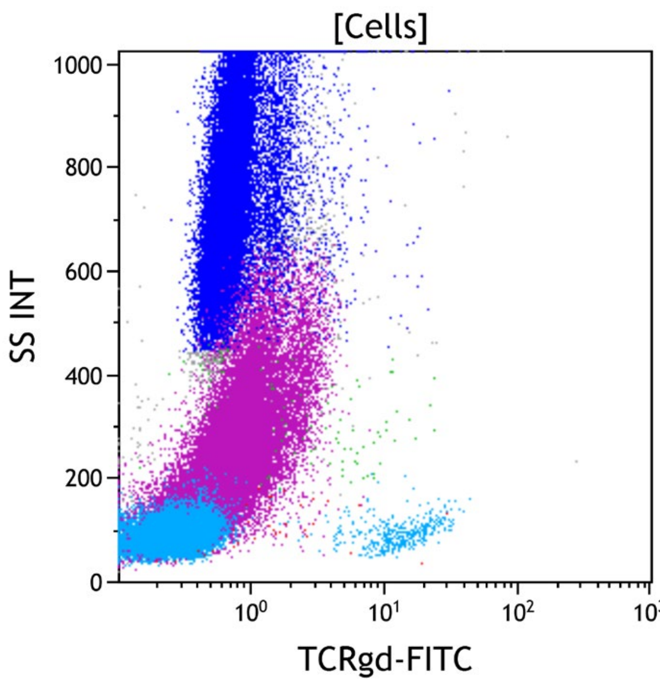

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua, lower right). The aberrant population (purple) is negative for TCRγδ.

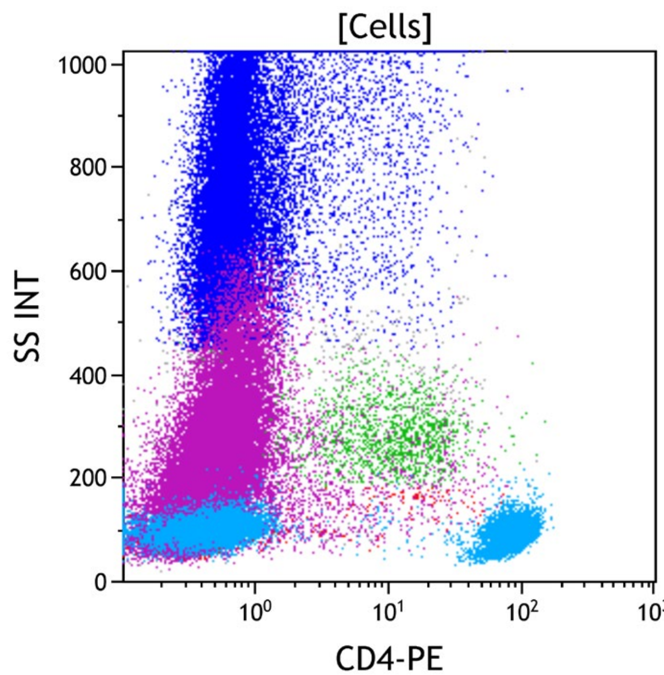

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The aberrant population (purple) is negative for CD4.

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T Cell Tube

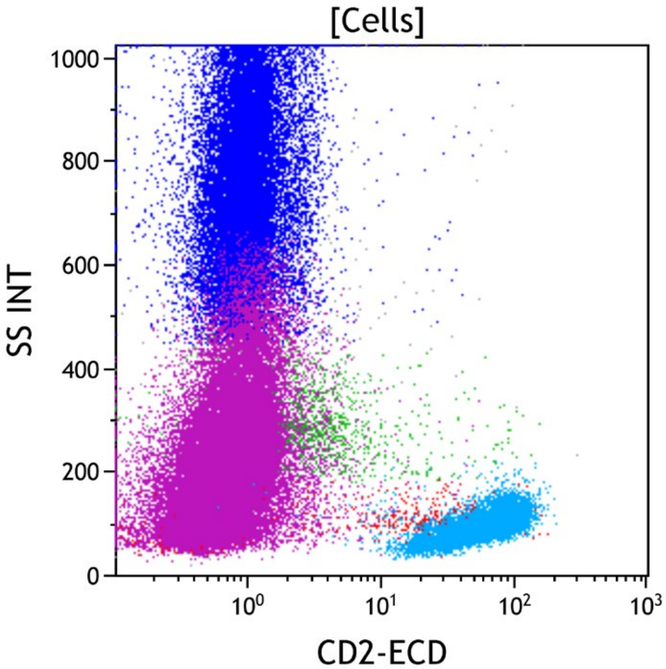


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The aberrant population (purple) is negative for CD2.

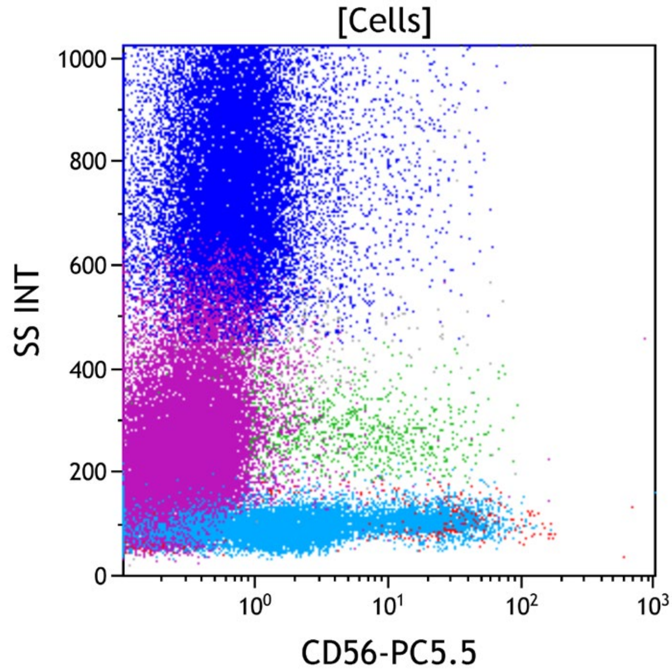


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The aberrant population (purple) is negative for CD56.

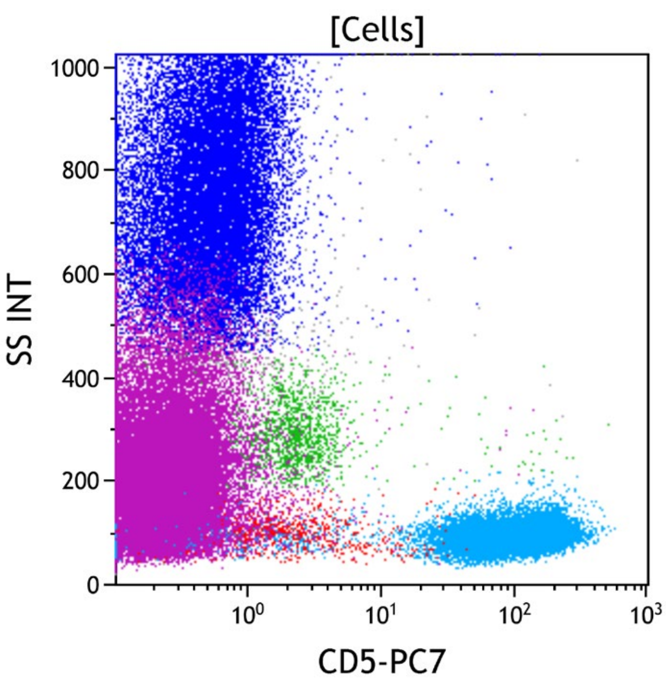


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The aberrant population (purple) is negative for CD5.

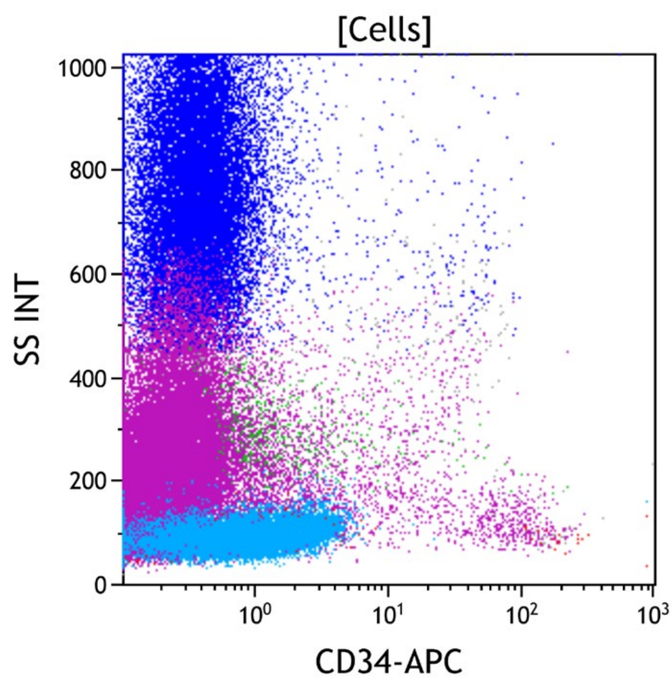


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The mature granulocytes (blue), monocytes (green), and lymphocytes (aqua and red) are negative for CD34. The aberrant population (purple) is negative for CD34.

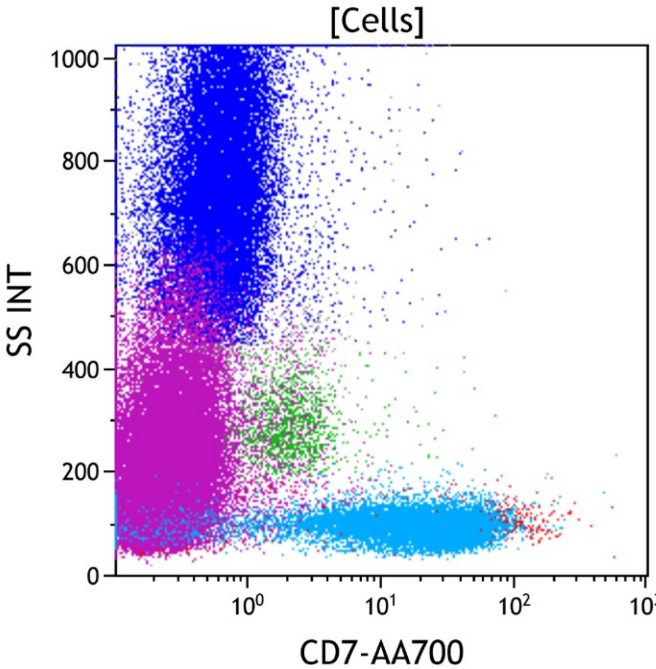


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The aberrant population (purple) is negative for CD7.

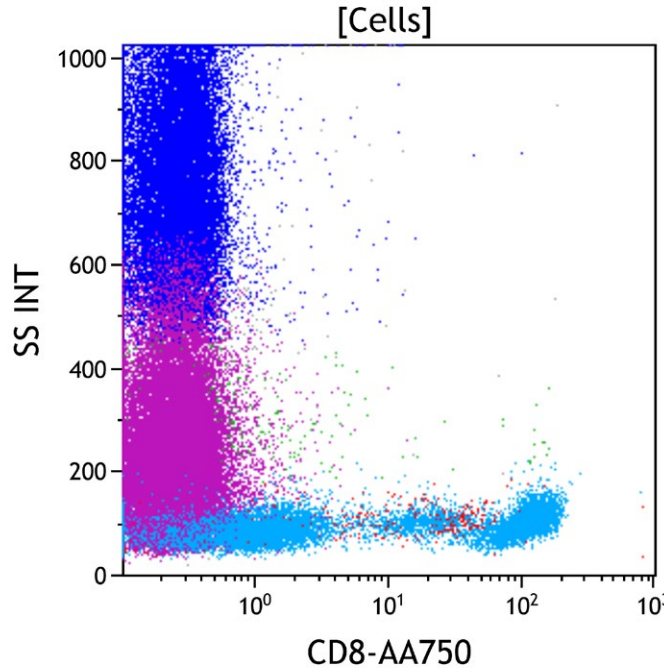


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The aberrant population (purple) is negative for CD8.

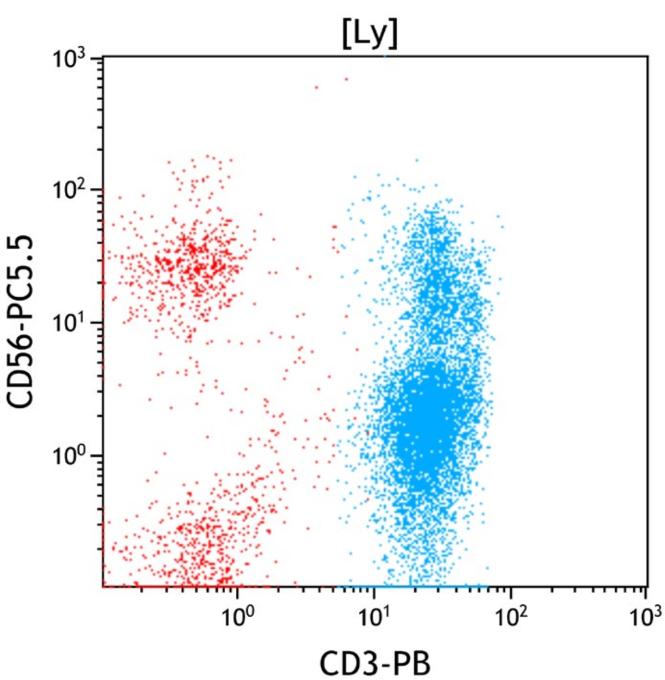


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

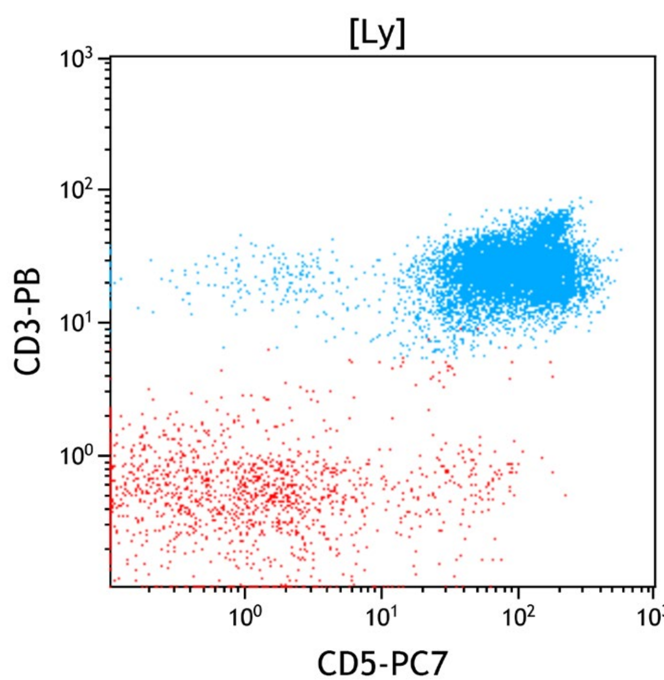


Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red).

T Cell Tube

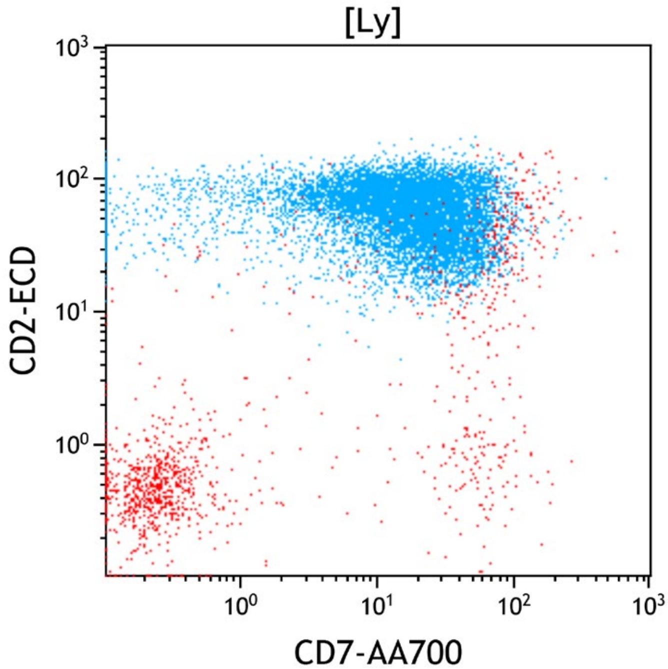


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

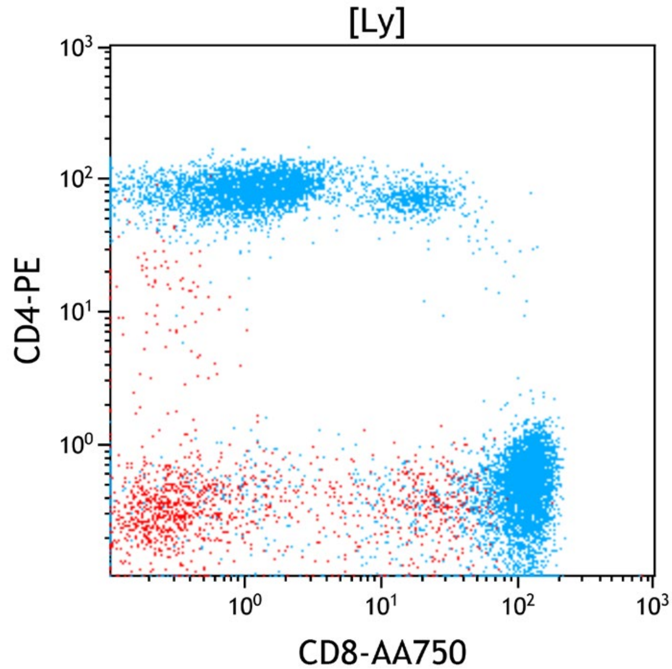


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells.

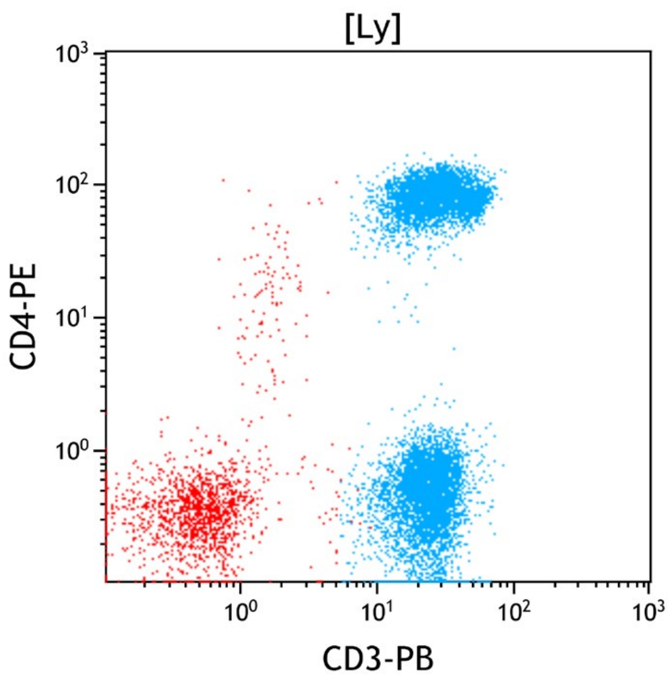


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells (red, lower left) lack expression of both CD3 and CD4.

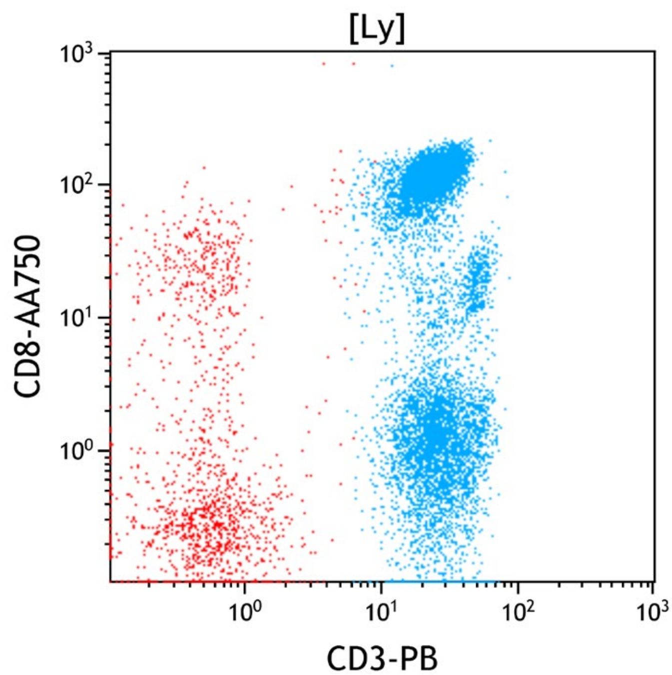


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

T Cell Tube

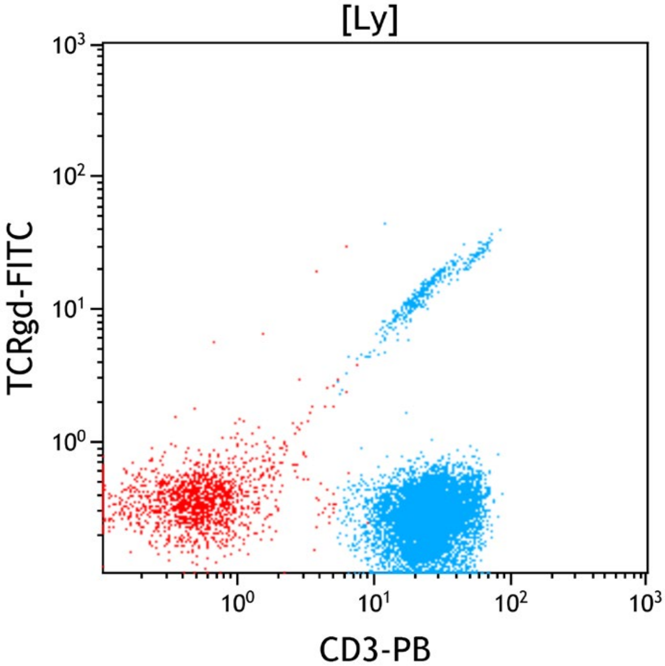


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

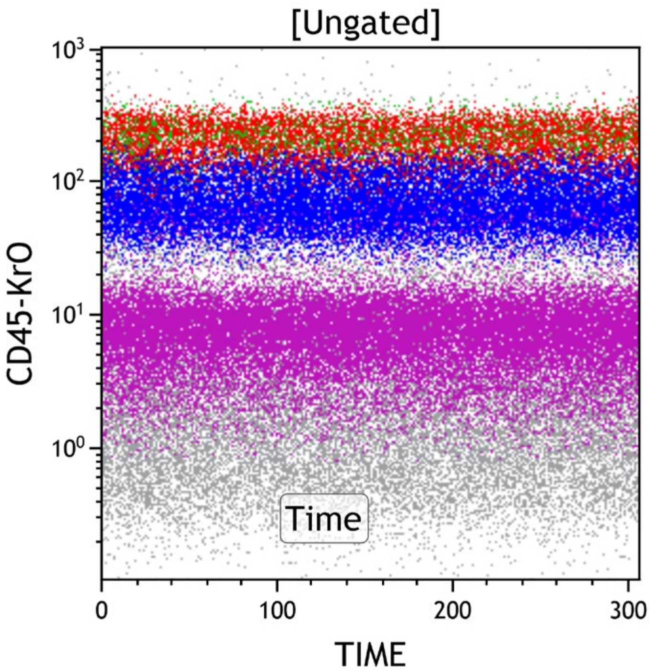


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

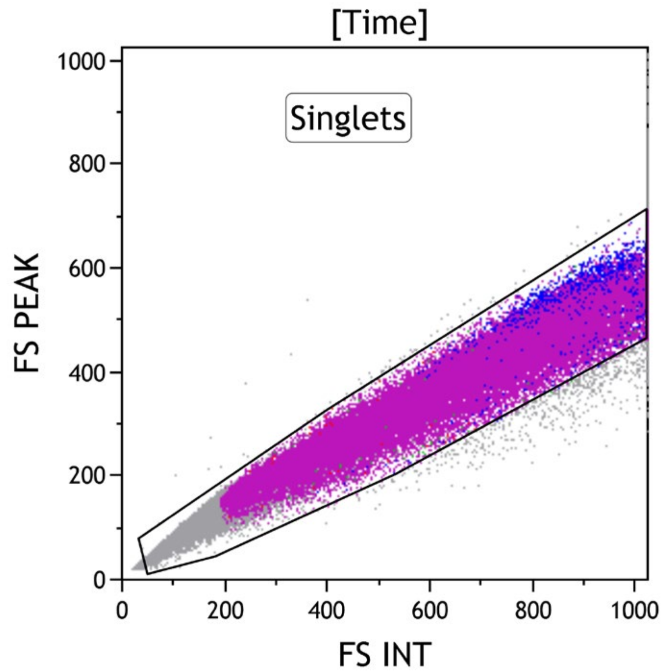


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

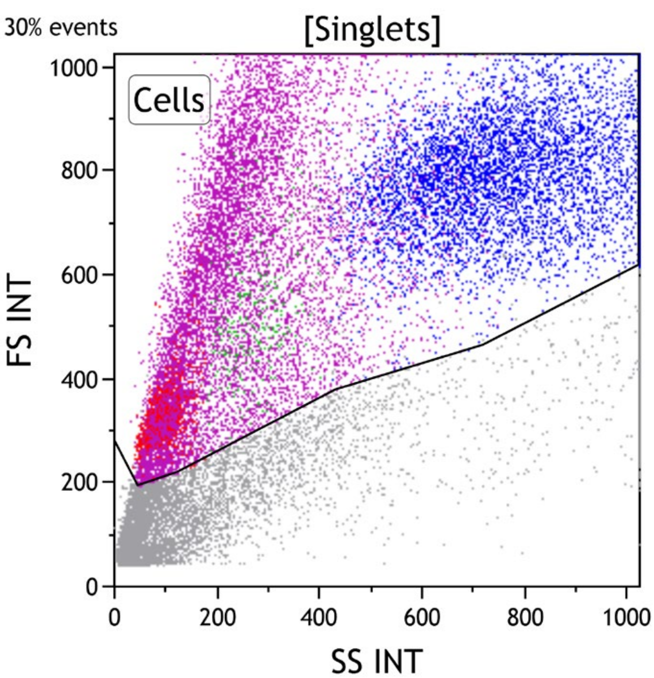


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. The aberrant population (purple) has increased forward and side scatter.

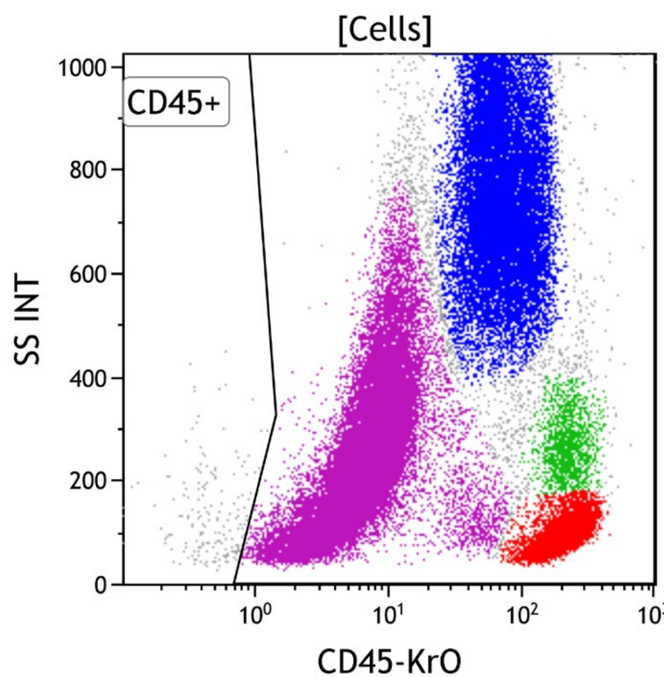


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray) usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells. The aberrant population (purple) has low level of CD45 expression.

M1 Cell Tube

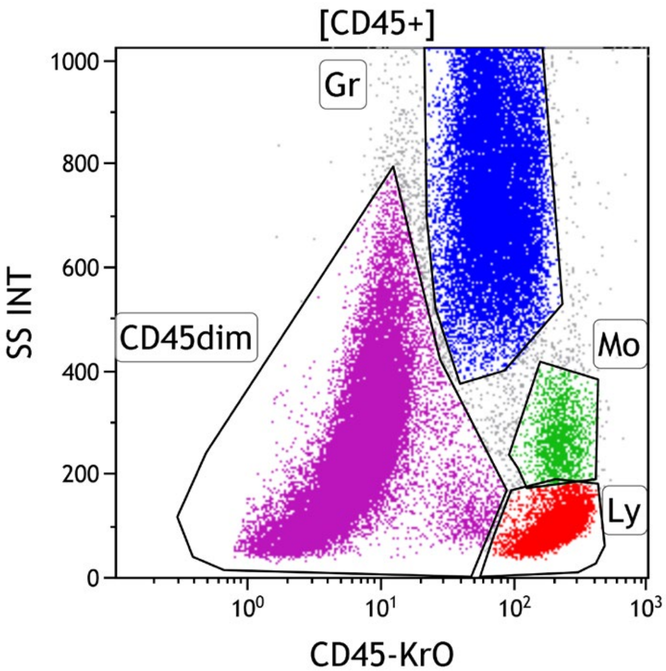


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The aberrant population (purple) has low level expression of CD45.

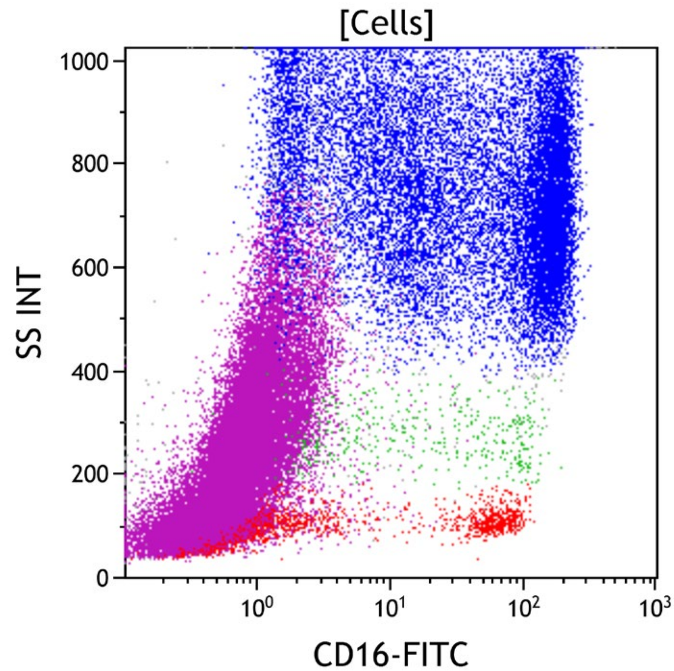


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The aberrant population (purple) is negative for CD16.

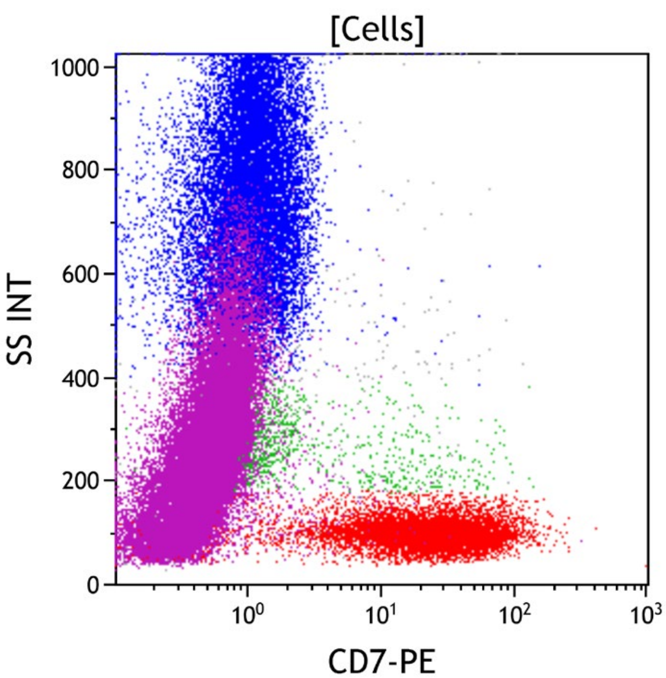


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors (purple). The aberrant population (purple) is negative for CD7.

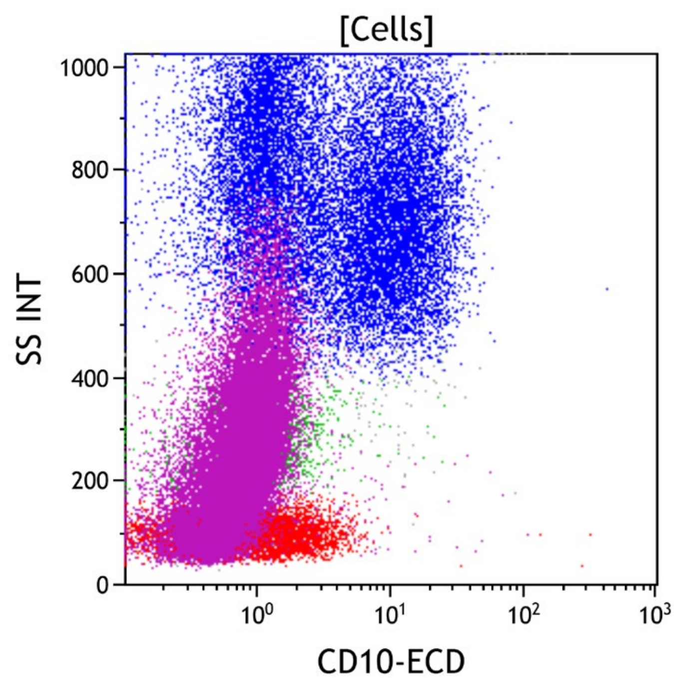


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple) is negative for CD10.

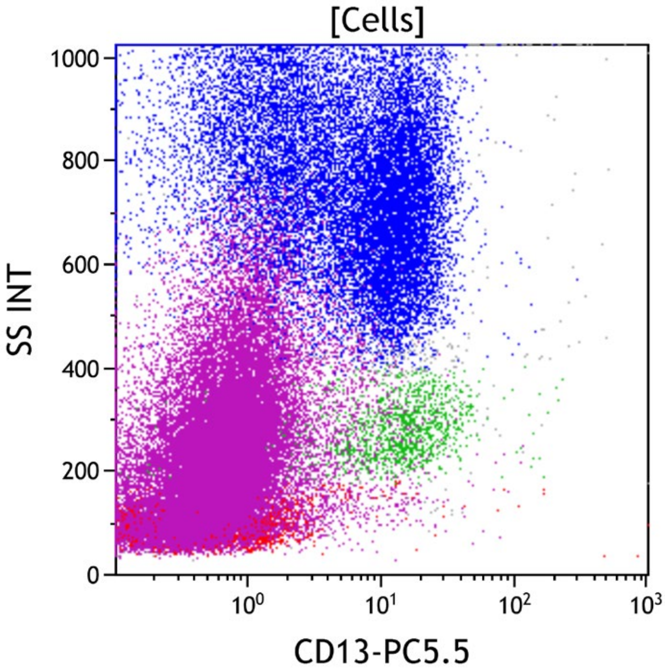


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple). The aberrant population (purple) is negative for CD13.

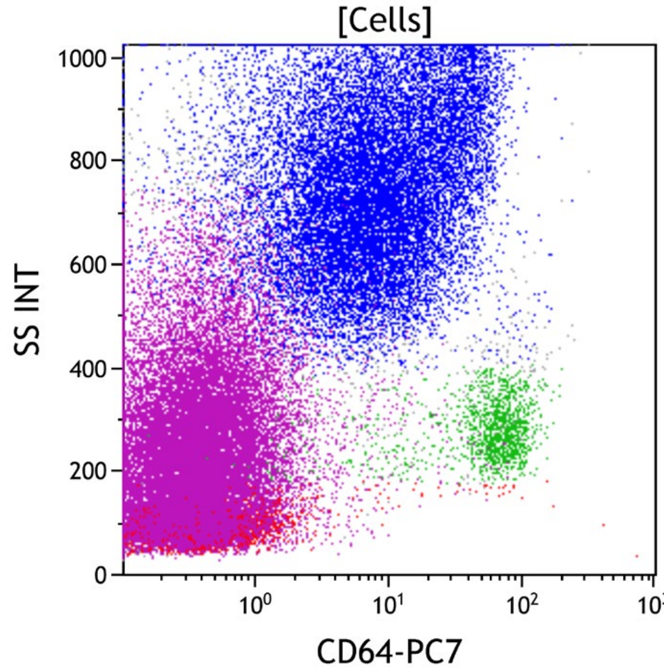


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The aberrant population (purple) is negative for CD64.

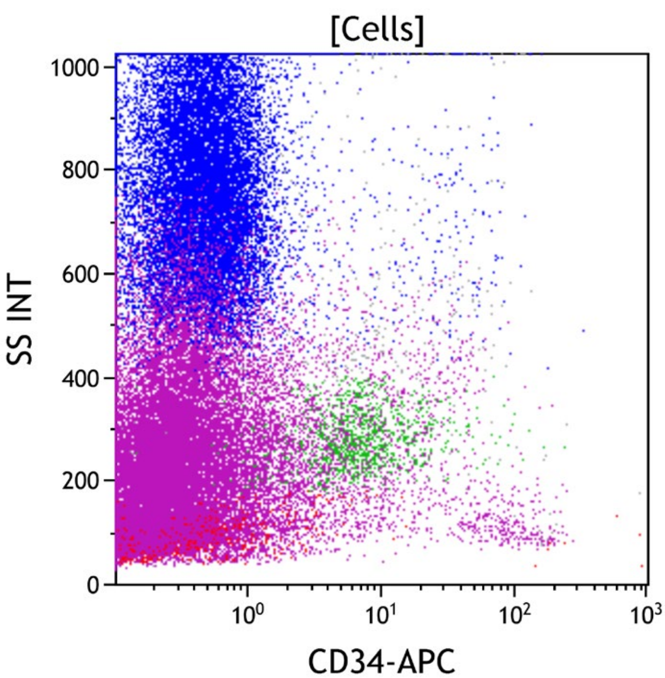


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes (blue), monocytes (green), and lymphocytes (Red) are negative for CD34. The aberrant population (purple) is negative for CD34.

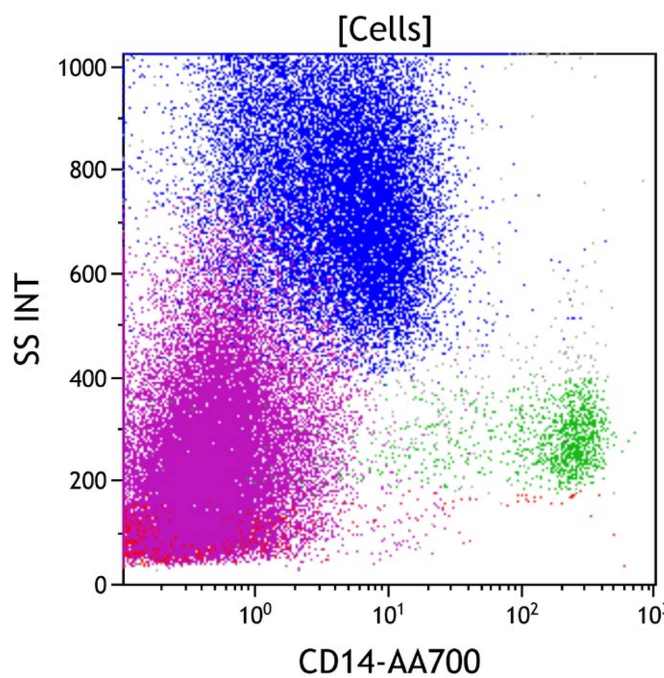


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The aberrant population (purple) is negative for CD14.

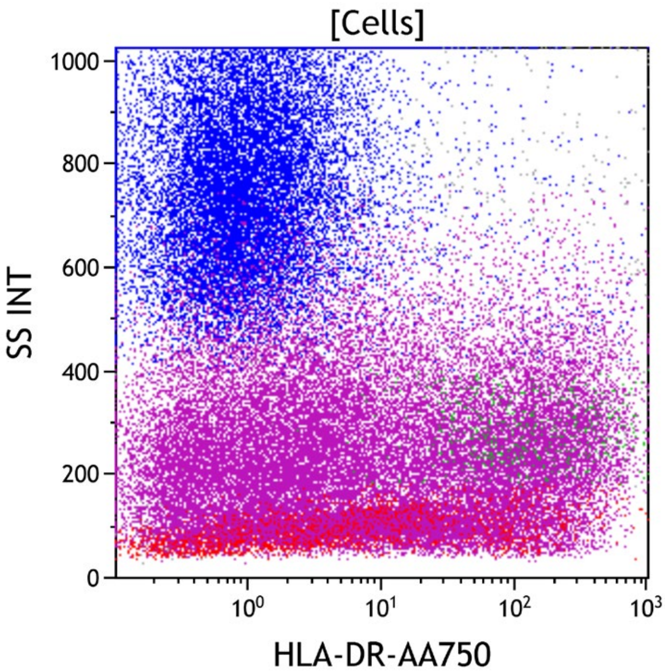


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red). The aberrant population (purple) has variable HLA-DR expression.

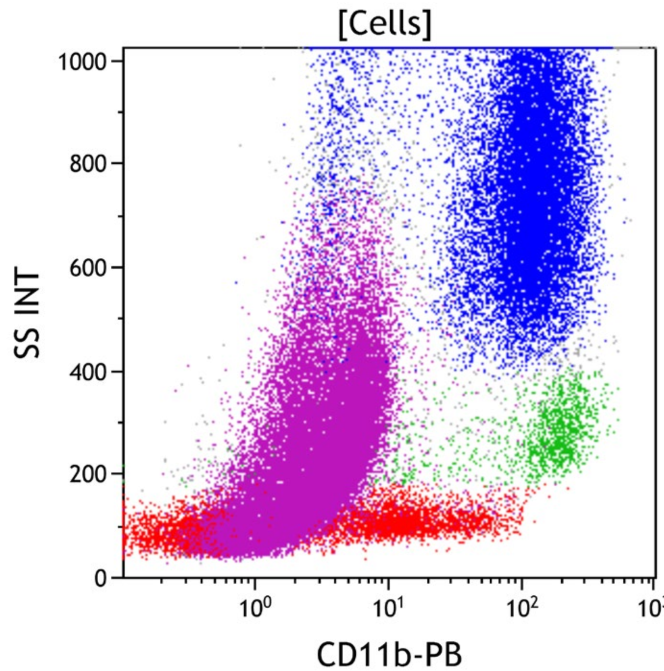


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The aberrant population (purple) is negative for CD11b.

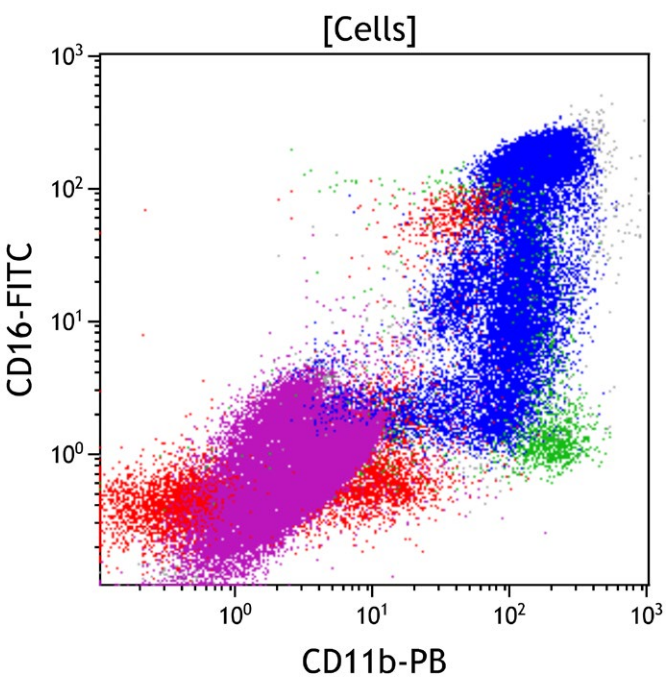


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

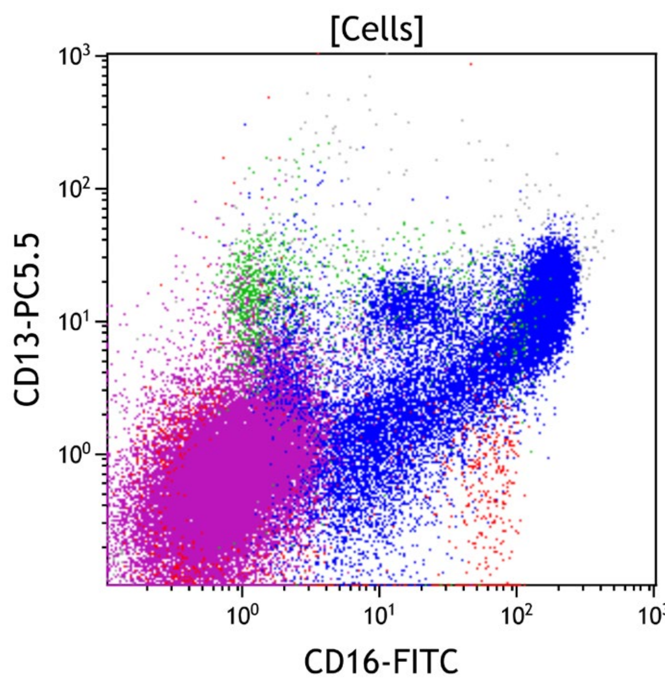


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, upper left) and lose CD13 as they mature to myelocytes (blue, lower left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue, upper right).

M1 Cell Tube

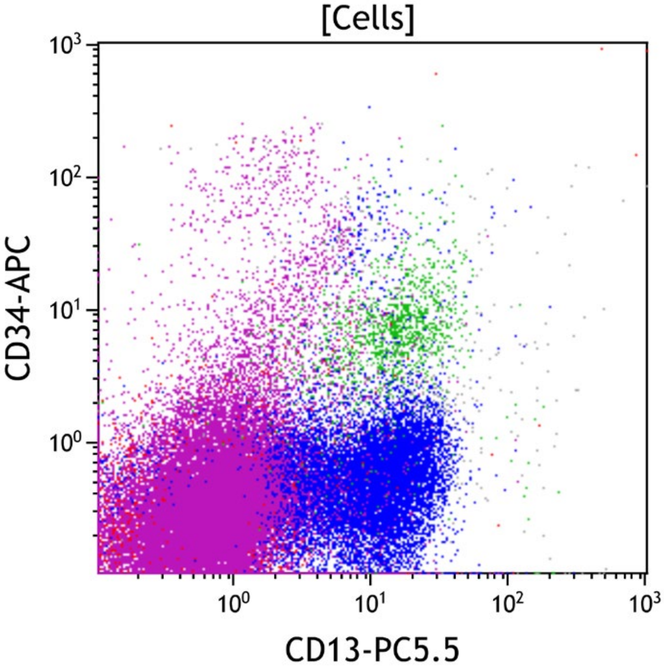


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphocytes (red).

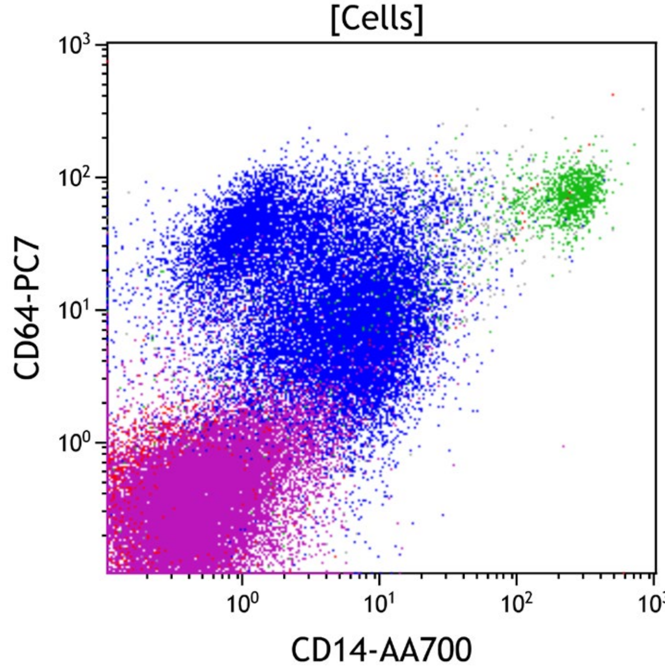


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (green, upper left) and progressively acquire CD14 during maturation to mature monocytes while retaining high level CD64 (green, upper right). Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left).

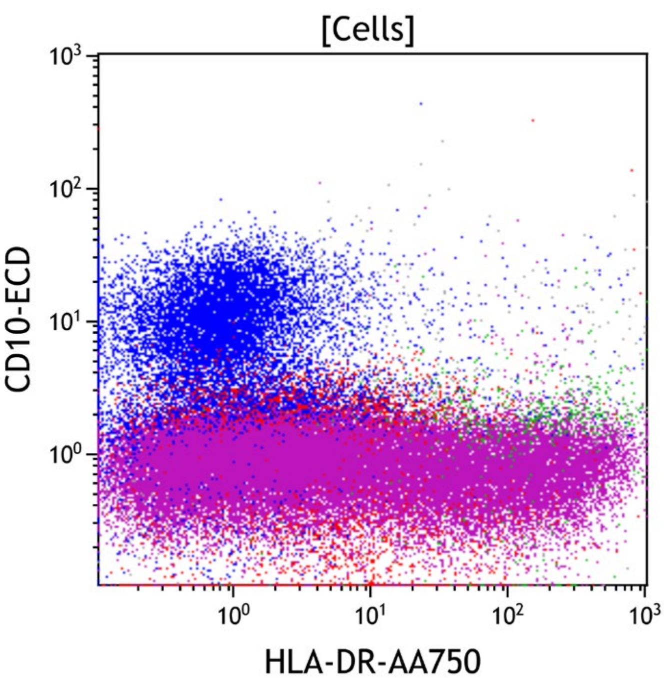


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR. The aberrant population (purple) has variable HLA-DR expression.

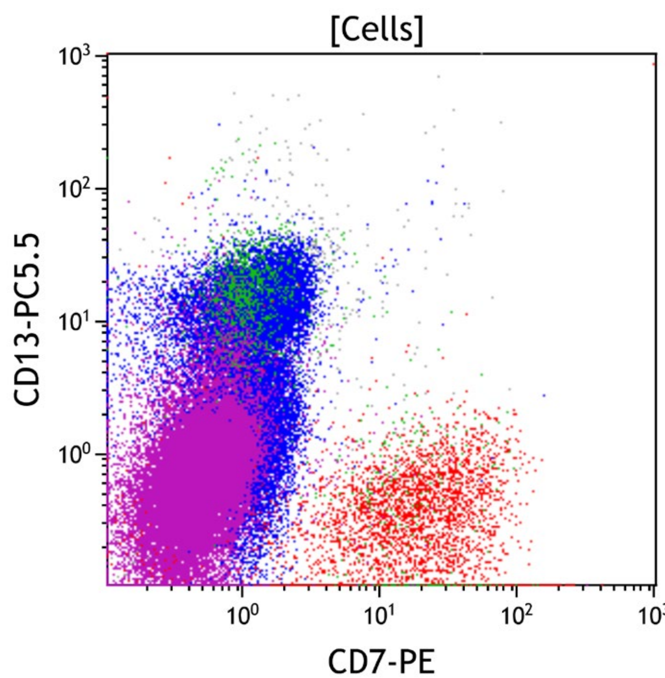


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

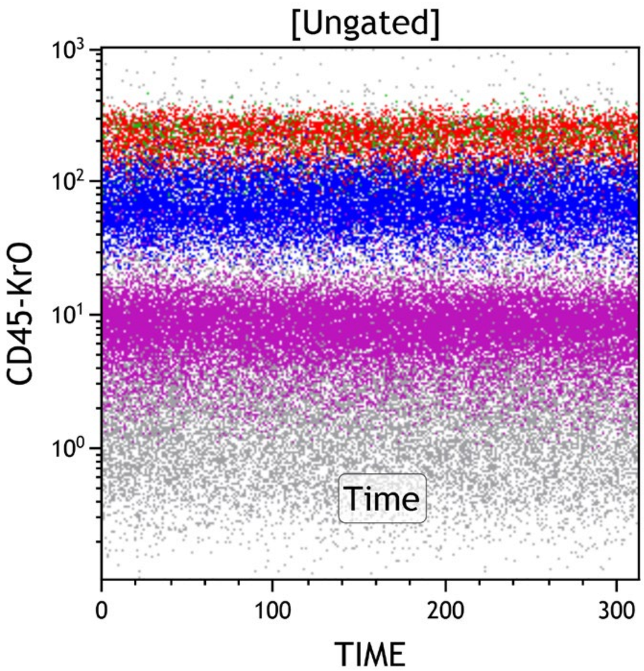


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

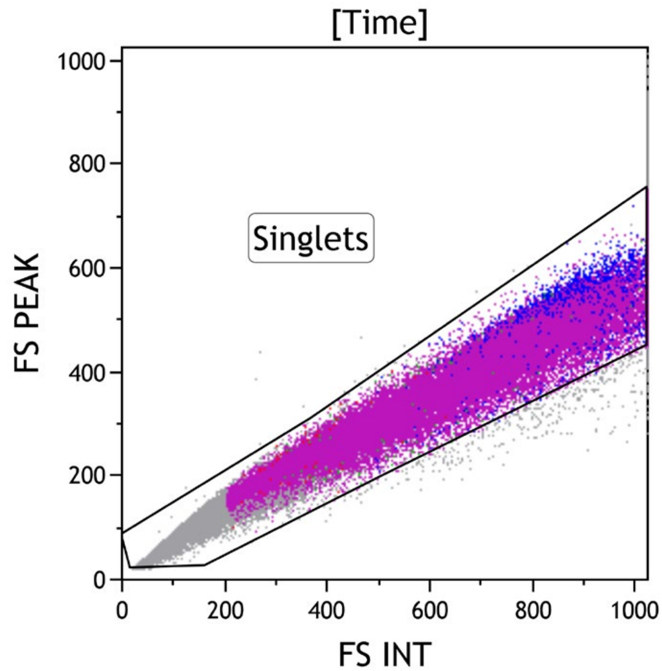


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

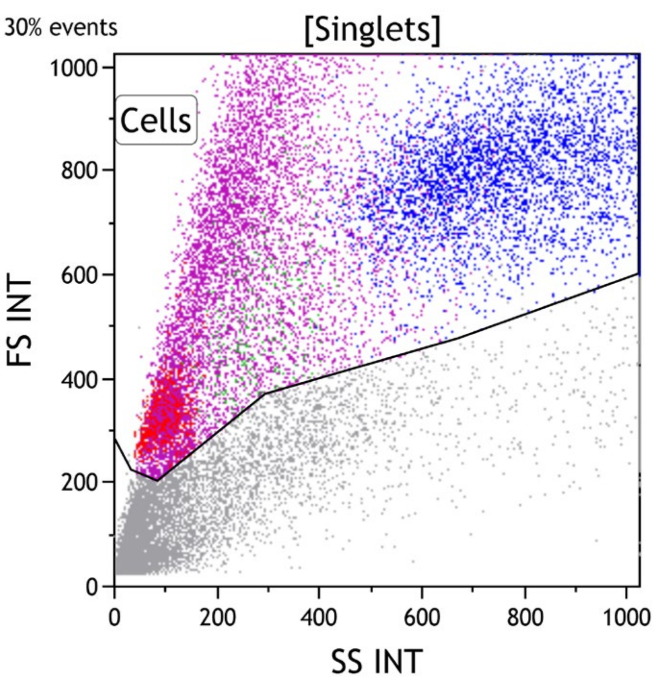


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. The aberrant population (purple) has increased forward and side scatter.

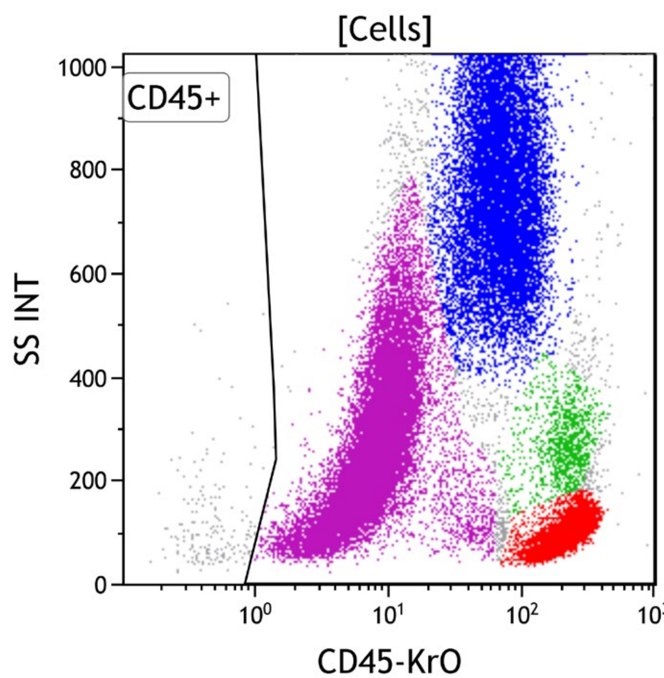


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells. The aberrant population (purple) has low level of CD45 expression.

M2 Cell Tube

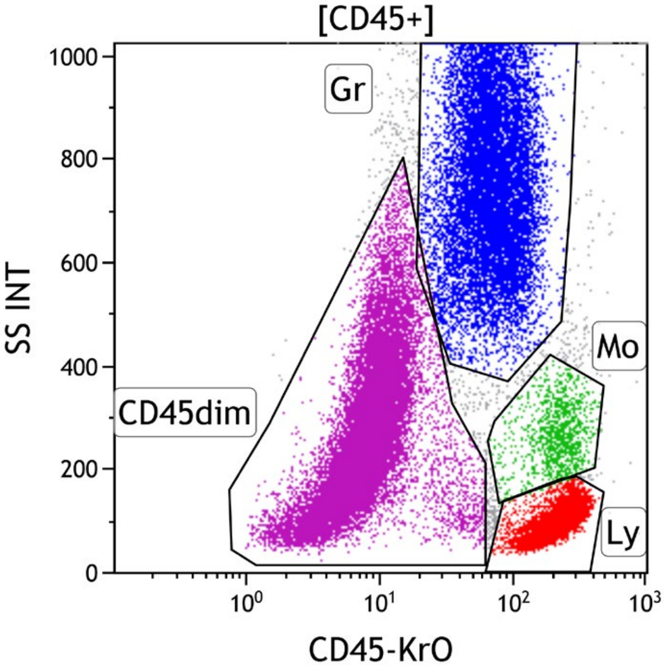


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The aberrant population (purple) has low level expression of CD45.

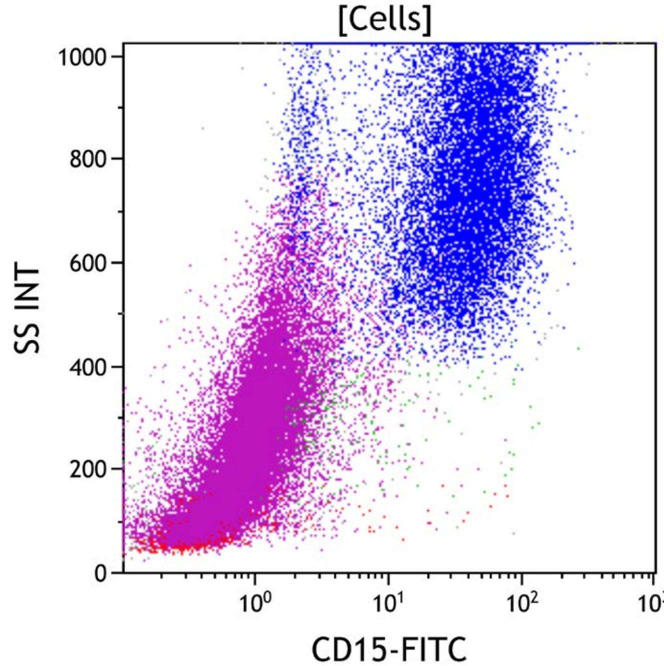


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The aberrant population (purple) is negative for CD15.

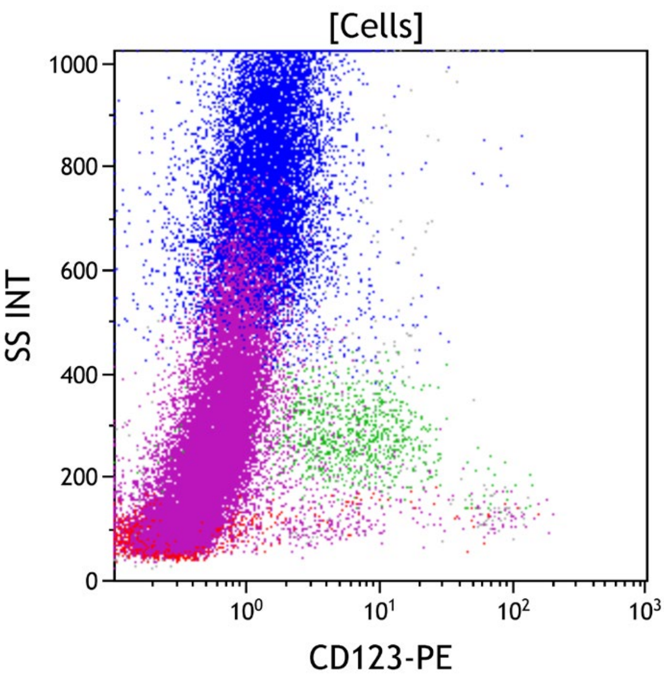


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple, lower right) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green). The aberrant population (purple) is negative for CD123.

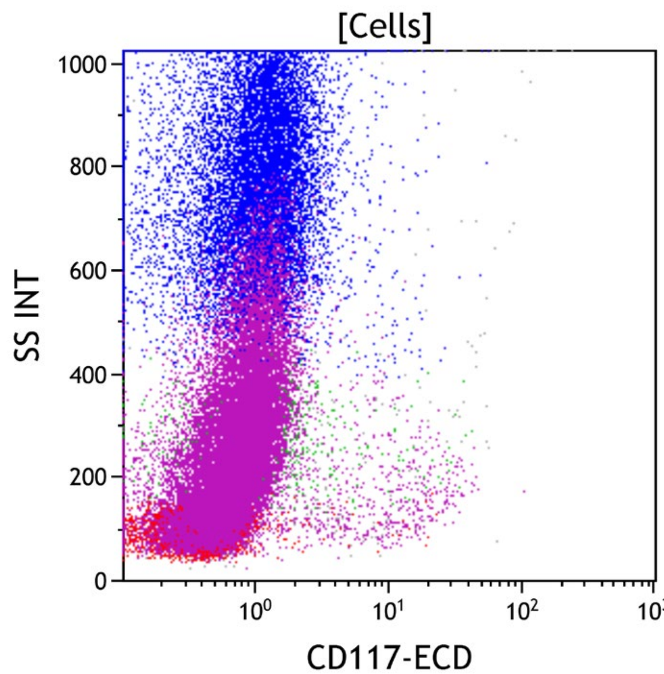


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors (purple), early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The aberrant population (purple) is negative for CD117.

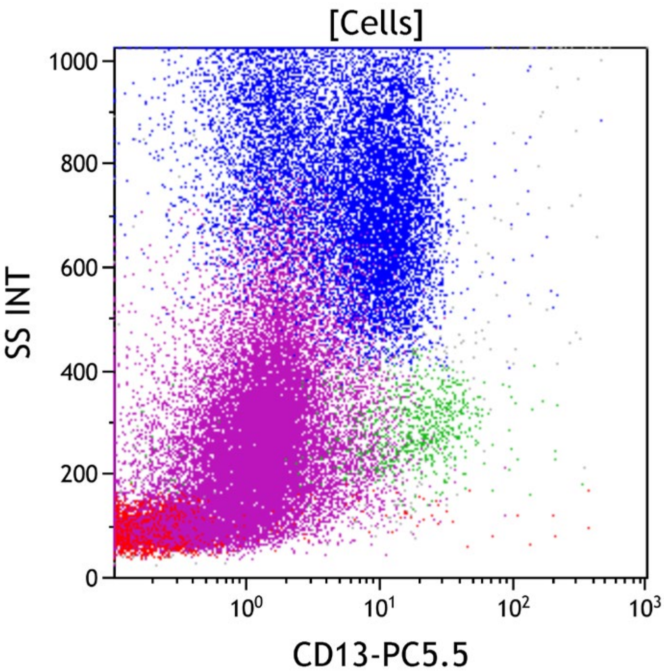


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple). The aberrant population (purple) is negative for CD13.

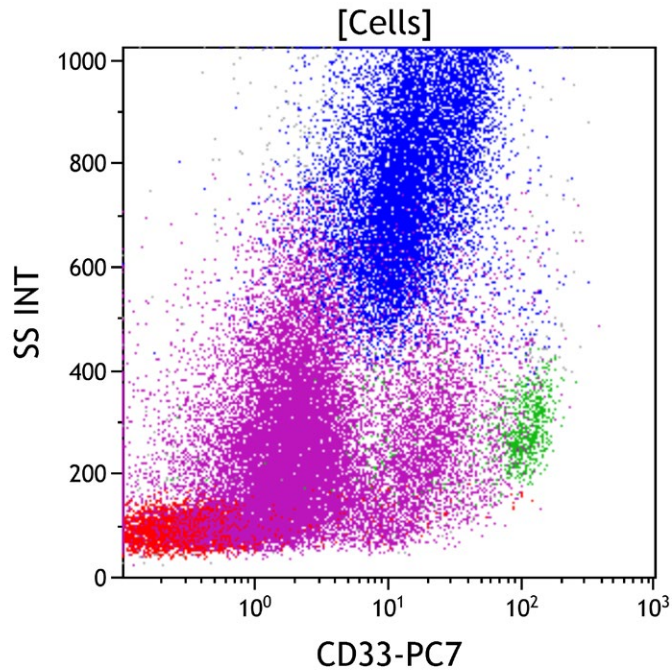


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors (purple). The aberrant population (purple) is positive for CD33 on a subset.

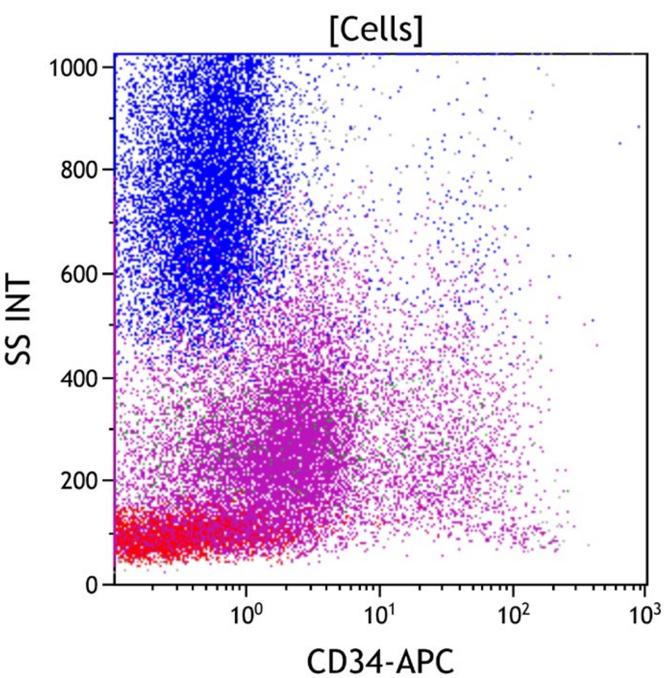


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45 dim gate (purple). The apparent variable CD34 positivity on the aberrant population (purple) is a compensation artifact due to the extremely high level of CD38.

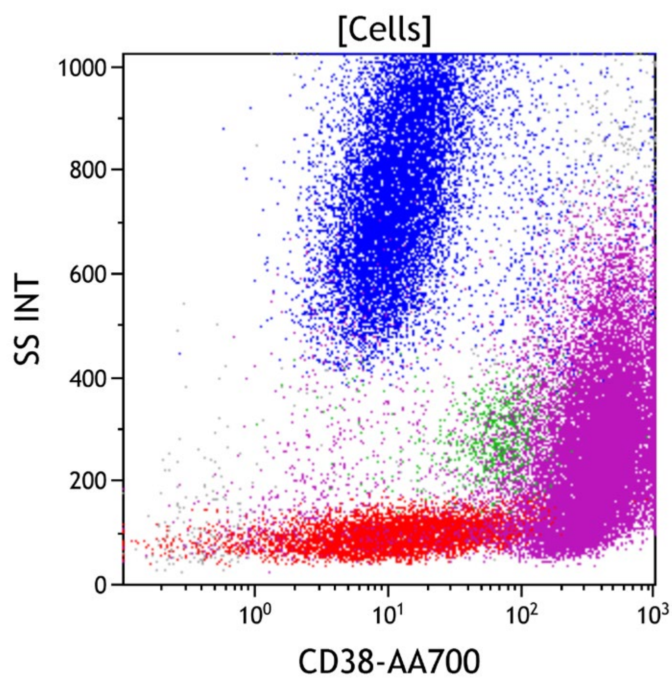


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The aberrant population (purple) has extremely bright CD38 expression that extends beyond the visible scale.

Table of Contents > Neoplastic Process of B-cell Origin > Case #9: Plasma Cell Myeloma

M2 Cell Tube

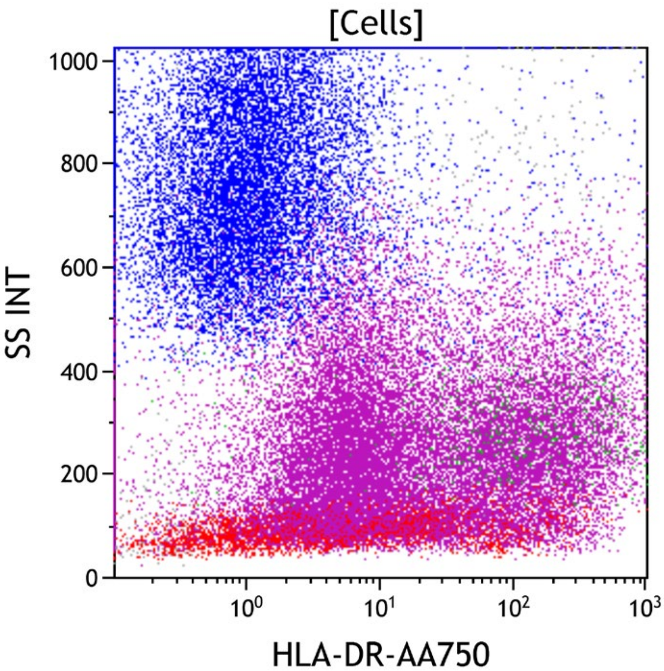


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red). The aberrant population (purple) has variable HLA-DR.

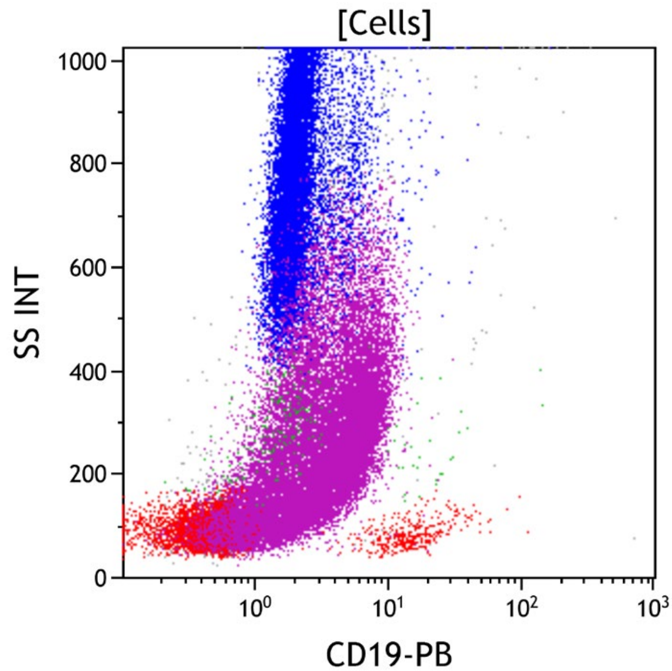


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) has low level of CD19 expression.

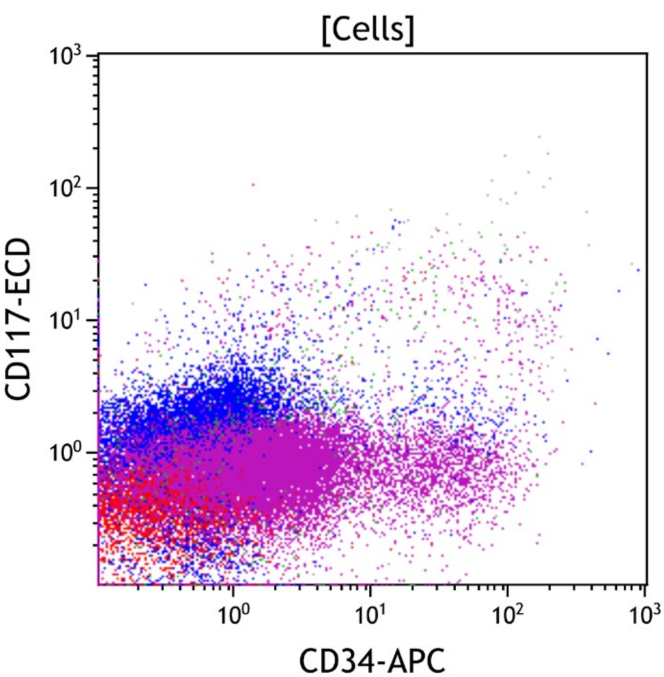


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (purple), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors. The apparent variable CD34 positivity on the aberrant population (purple) is a compensation artifact due to the extremely high level of CD38.

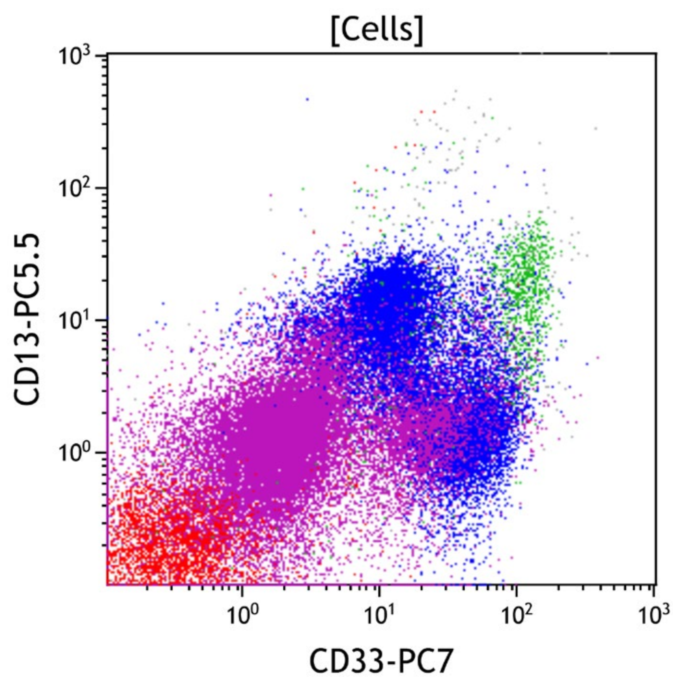


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue, lower middle) than more mature granulocytes (blue, upper left). Lymphocytes largely do not express either CD13 or CD33 (red).

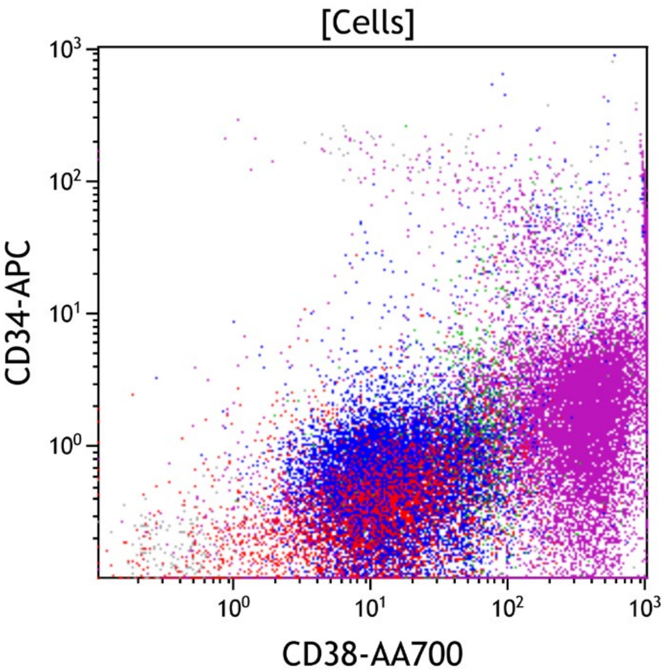


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple). The aberrant population (purple) has extremely bright CD38 expression. The apparent variable CD34 positivity on the aberrant population is a compensation artifact due to the extremely high level of CD38 that extends beyond the visible scale.

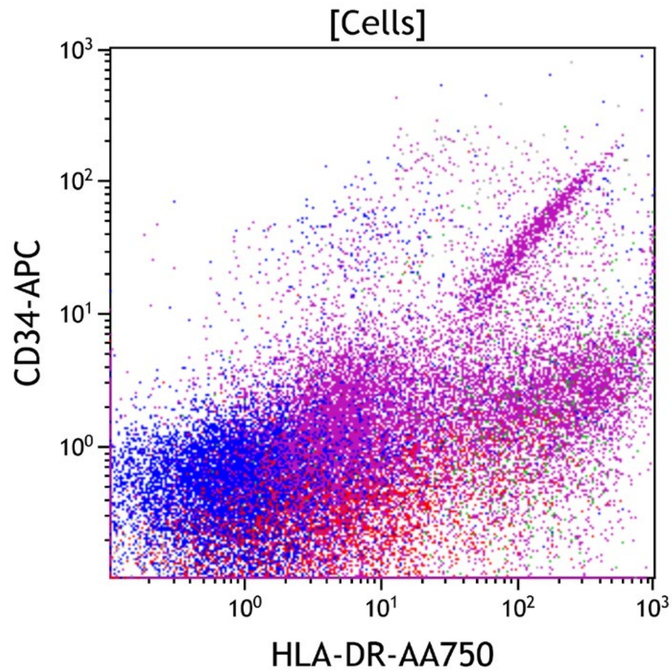


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The aberrant population (purple) has variable HLA-DR.

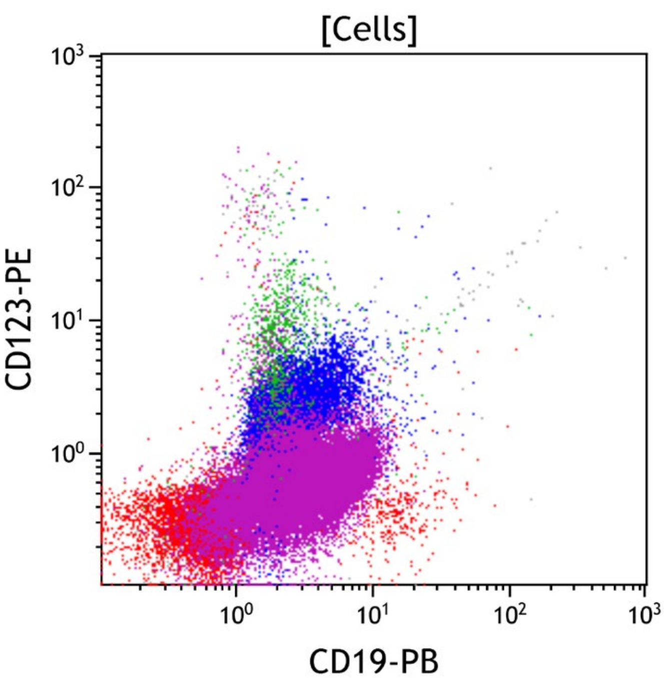


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (purple) has low level of CD19 expression.

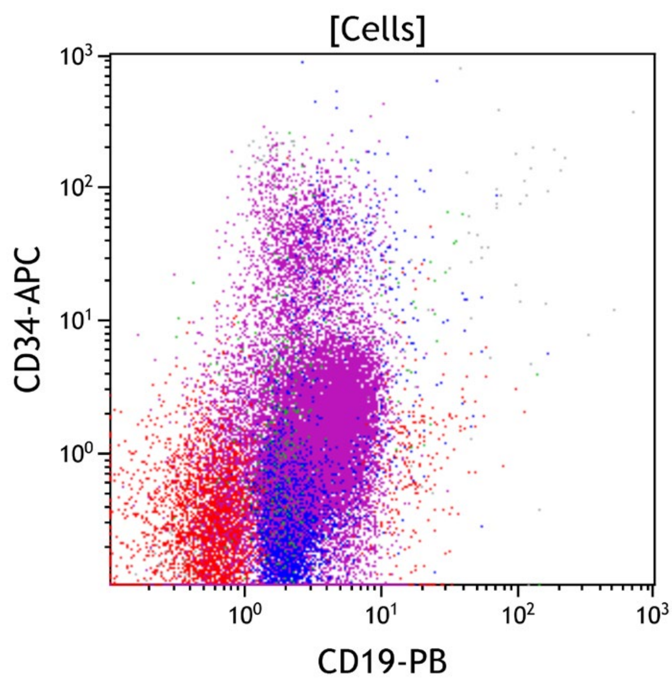


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. The aberrant population (purple) has low level of CD19 expression.

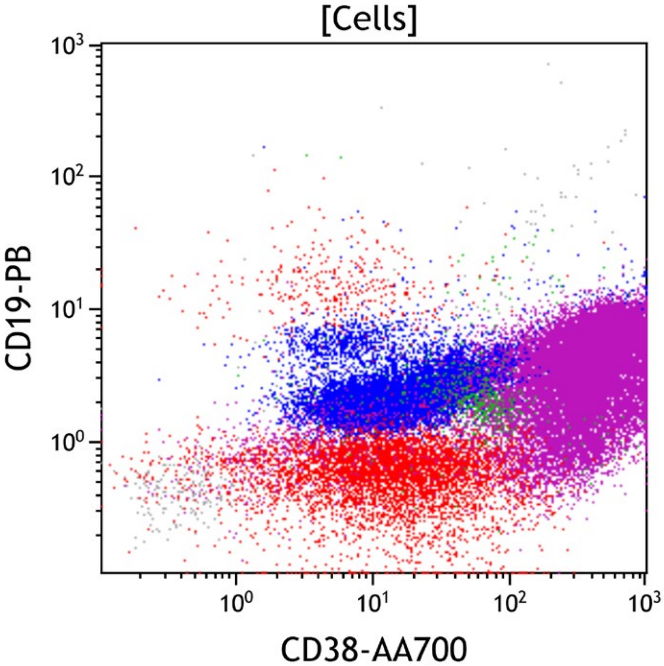


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Mature CD19 positive B cells show intermediate CD38 expression (red, upper middle). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (purple) show extremely high CD38 expression that is largely off scale with low CD19.

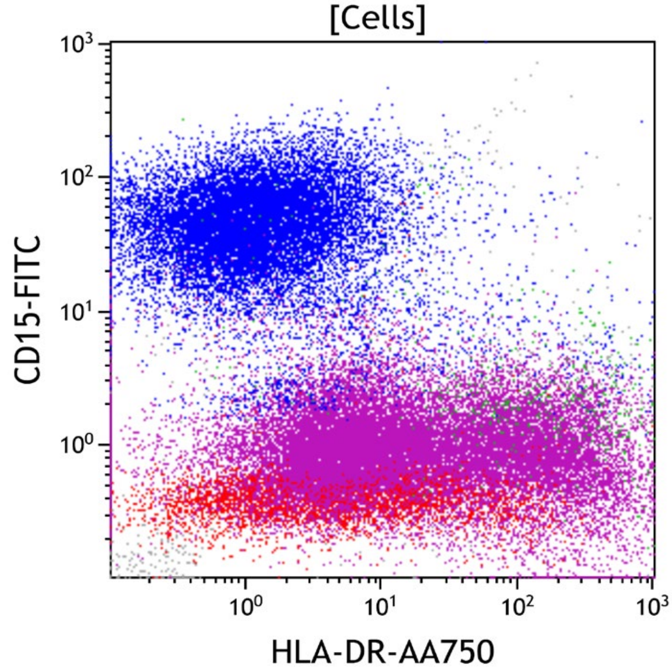


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors (purple) express HLA-DR but only transiently express CD15. The aberrant population (purple) has variable HLA-DR.

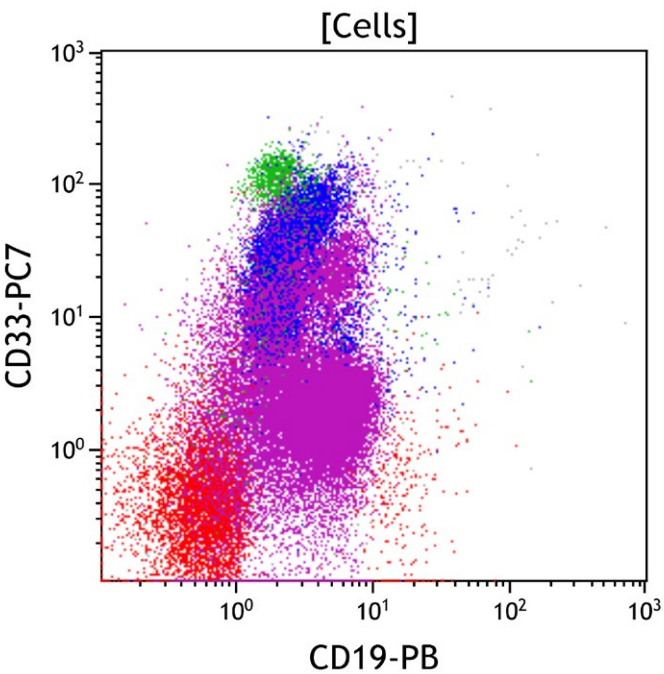


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells (red, lower right) normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (purple) has low level of CD19 expression.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased forward and side scatter, low CD19, CD20 (small subset), bright CD38, low CD45, variable HLA-DR without CD5, CD10, CD56, surface light chains or other T or myeloid markers. The immunophenotype of the aberrant population is consistent with a plasma cell neoplasm and could be consistent with plasmacytoma or plasma cell myeloma. Compared with normal plasma cells, the decreased CD19 and CD45 expression is aberrant. Morphology shows 67% atypical plasma cells, consistent with a diagnosis of either mutliple myeloma or plasmacytoma depending on the clinical context. Correlation with clinical, morphologic and laboratory data is required for definitive subclassification of this plasma cell neoplasm, and that additional immunophenotyping such as cytoplasmic light chain expression may be warranted.

Case #10: Follicular Lymphoma

Clinical Vignette

37 year-old female presents with lymphocytosis, bone marrow aspirate is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

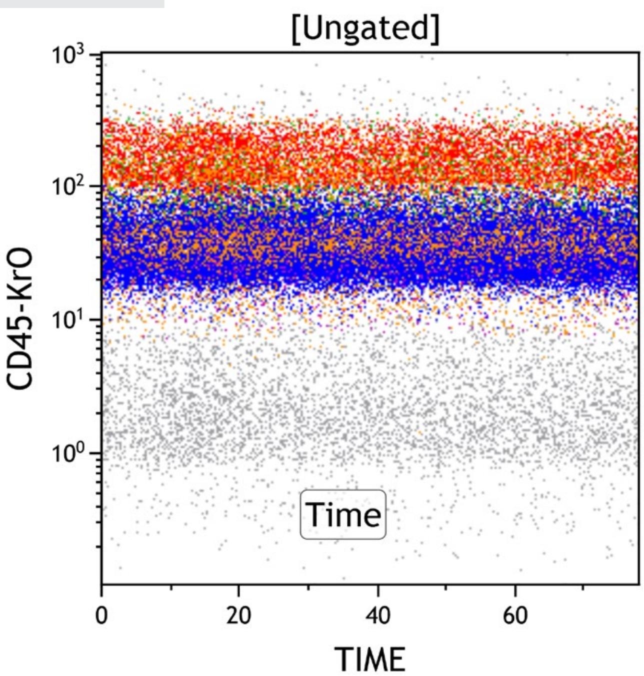


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate

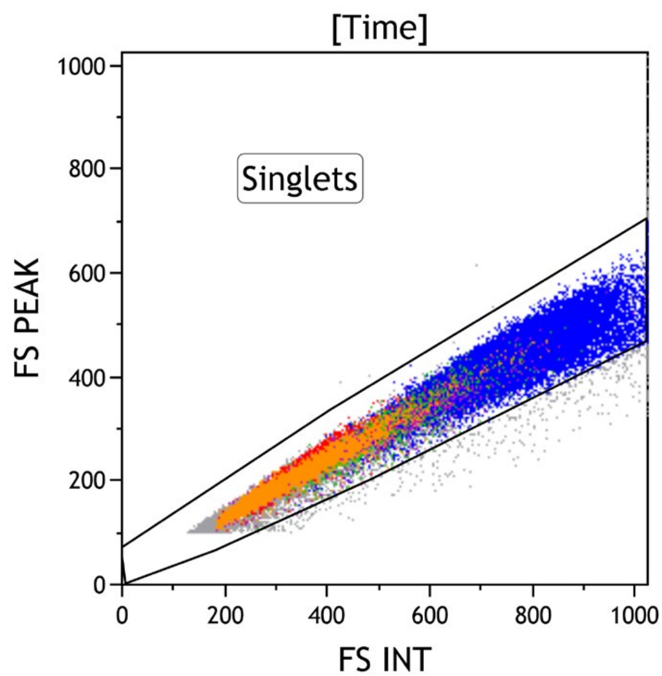


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events shows a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

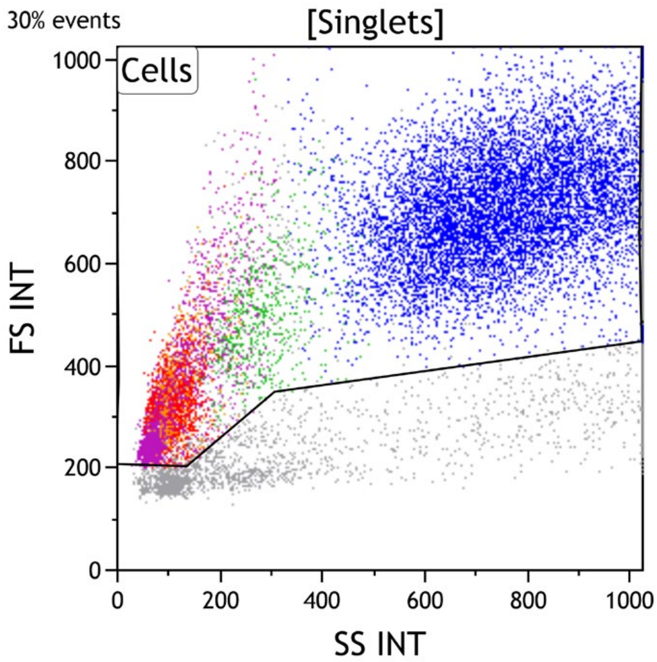


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

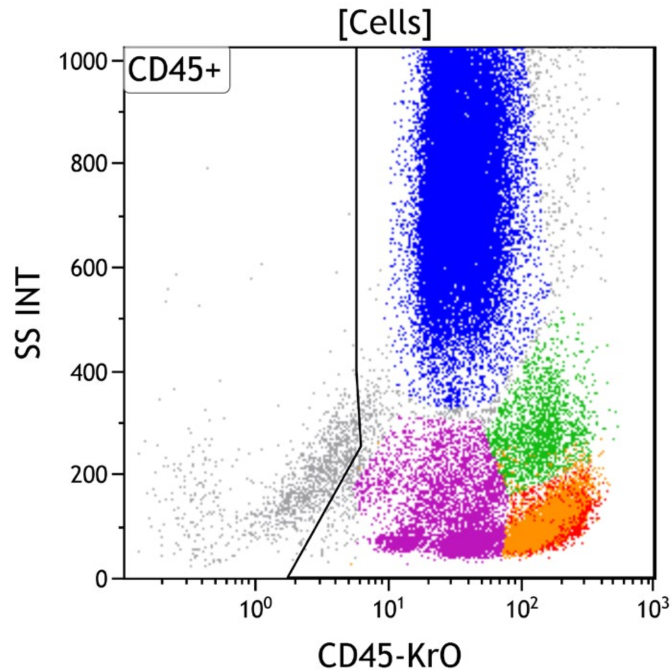


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

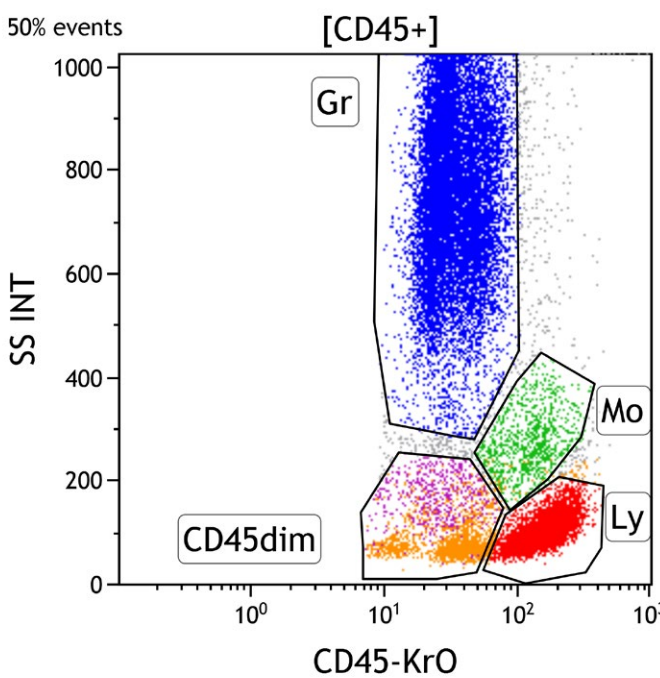


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple/orange) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

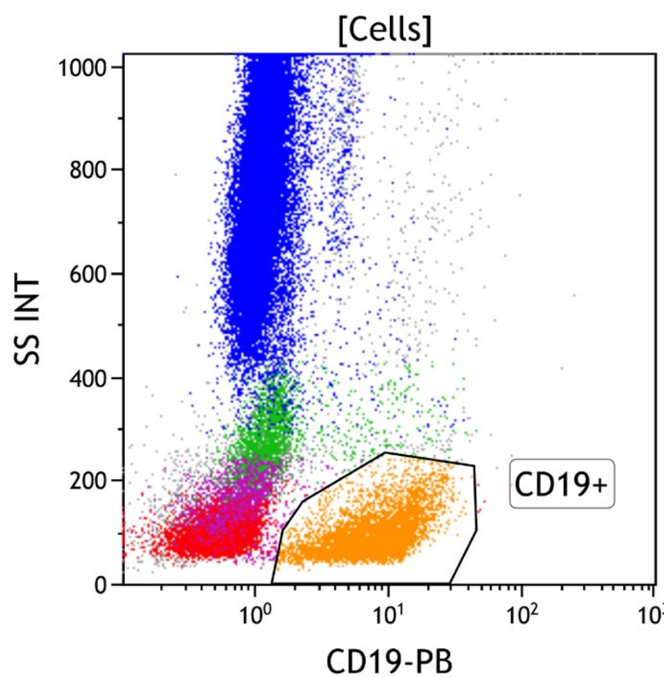


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The mature B cells in the lymphocyte gate (orange) are proportionally increased.

Table of Contents > Neoplastic Process of B-cell Origin > Case #10: Follicular Lymphoma

B Cell Tube

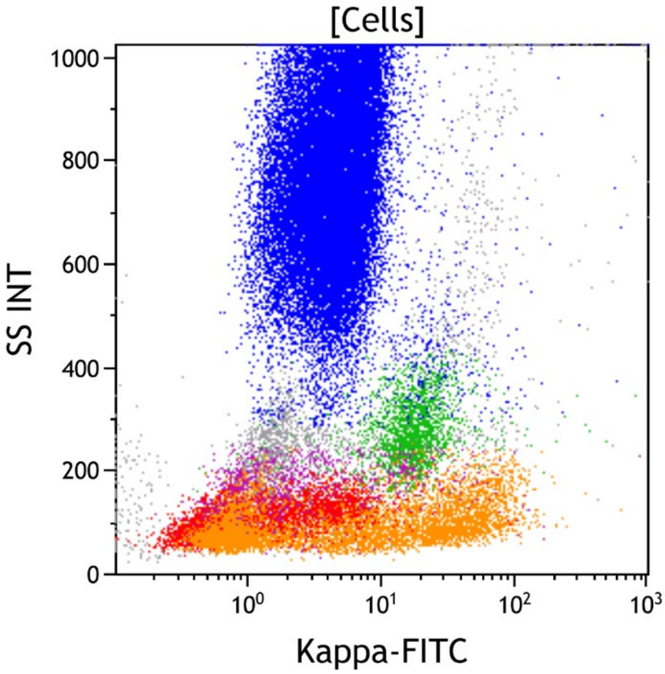


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The mature B cells (orange) show preferential expression of kappa light chains.

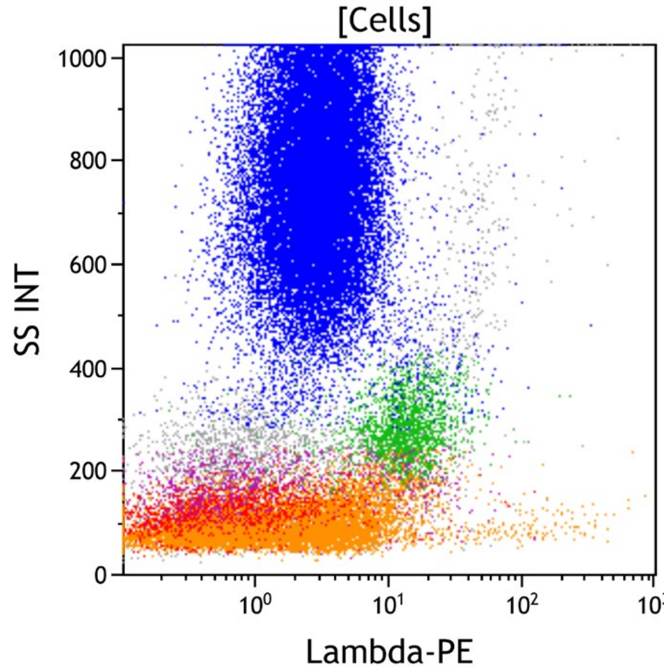


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (yellow) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The mature B cells (orange) show proportionately reduced expression of lambda light chains.

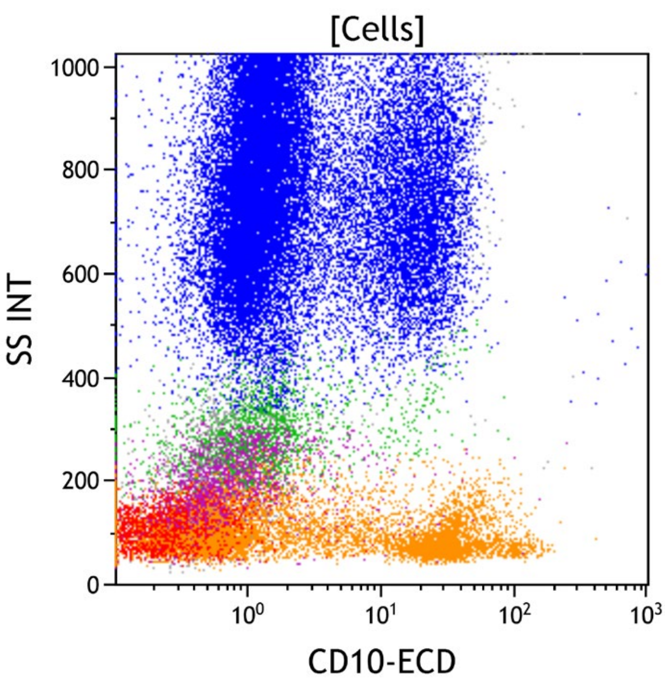


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. A subset of the mature B cells (orange) show expression of CD10.

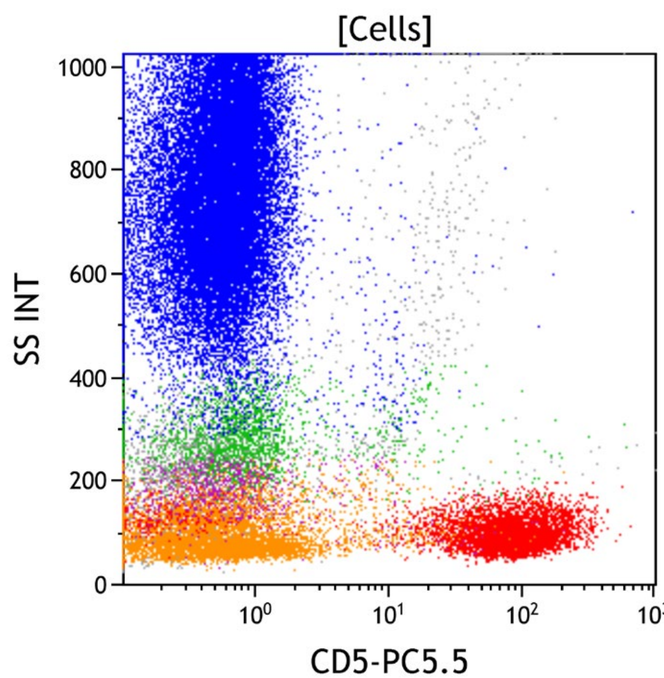


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. Only a small subset of the mature B cells show expression of CD5.

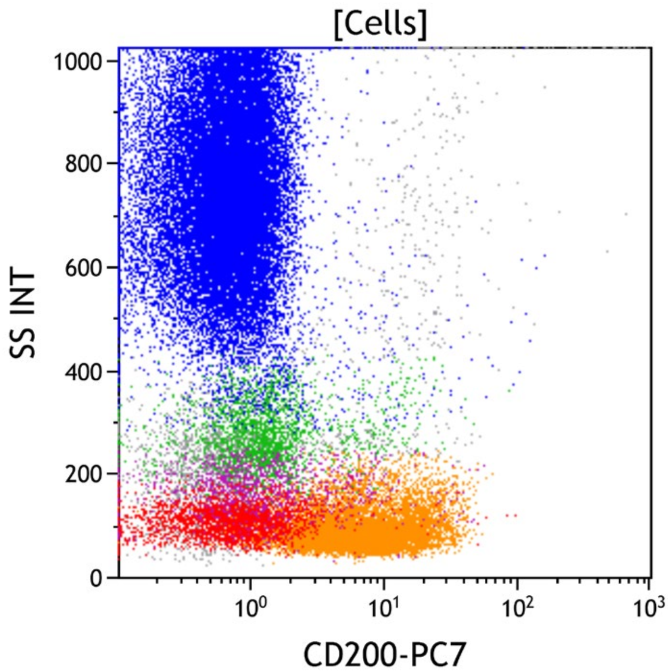


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The mature B cells are positive for CD200.

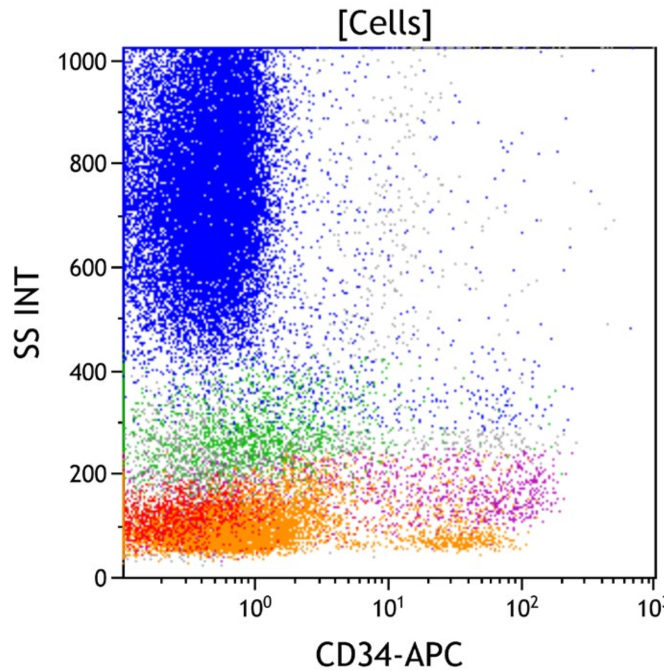


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The mature B cells lack expression of CD34.

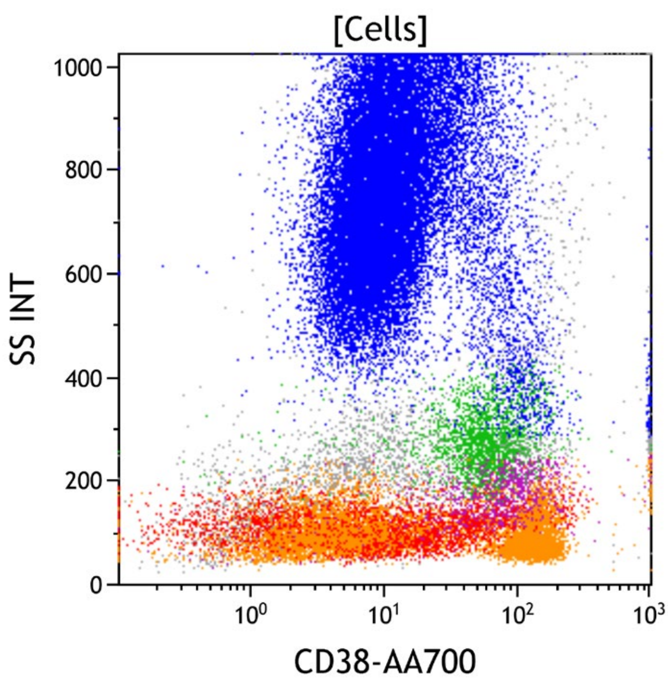


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The mature B cells show low to absent expression of CD38.

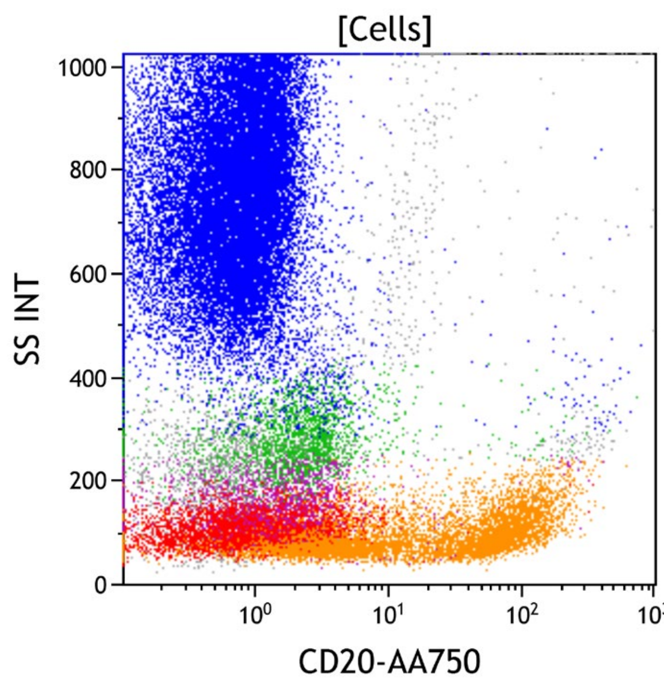


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (yellow) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The mature B cells express CD20 at a normal level.

B Cell Tube

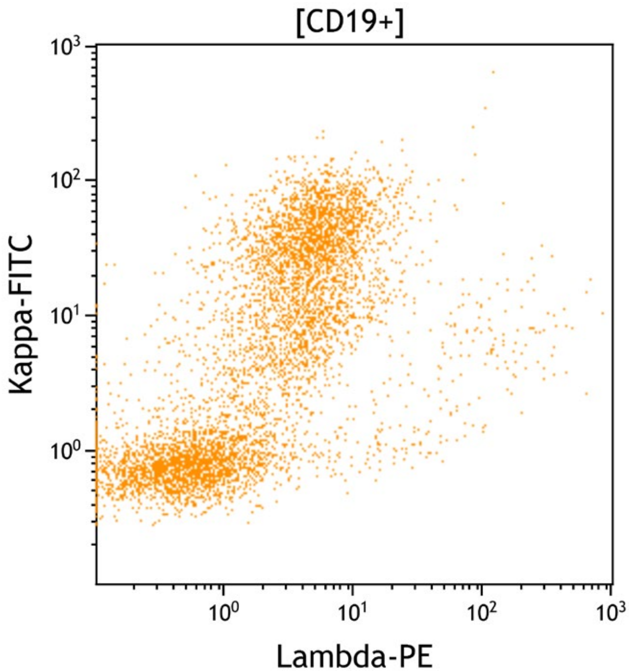


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. In this case, the kappa to lambda ratio on the mature B cells (orange) suggests an expanded B-cell population with restricted kappa light chain expression at a normal level of intensity. This finding indicates a clonal B cell population, and suggests the presence of a B cell lymphoproliferative disorder. However, clonal B cells may also be seen in some reactive conditions, so clonality does not necessarily equate with neoplasia and the presence of other immunophenotypic abnormalities should be sought to provide improved specificity for the diagnosis of neoplasia.

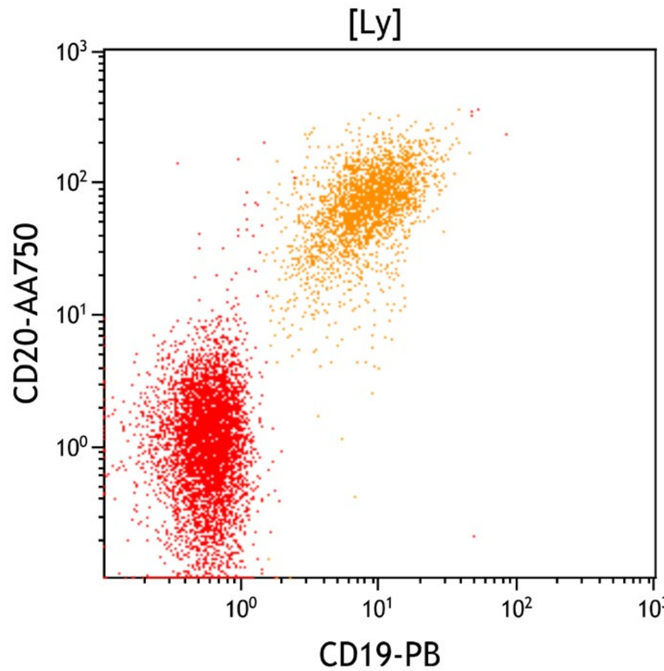


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. The clonal B cells (orange) express CD19 at a variably decreased level of intensity compared with normal mature B cells, but express CD20 at a near normal level.

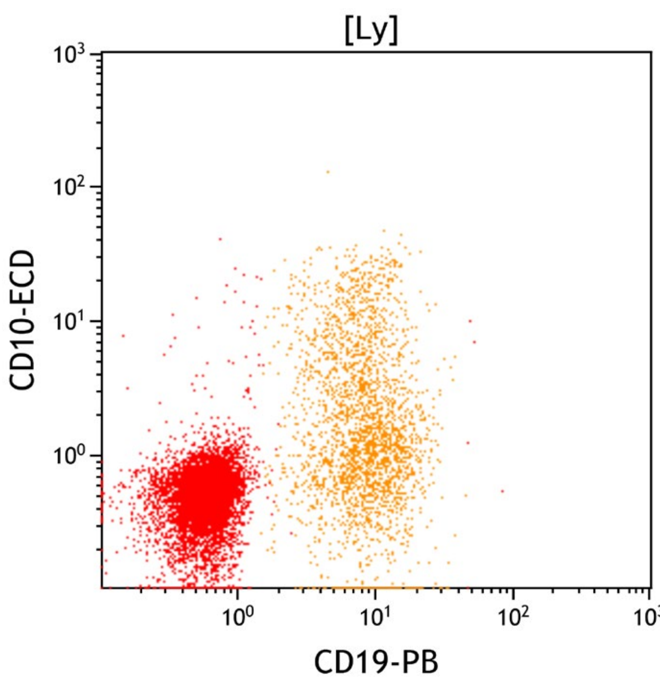


Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45dim gate. The clonal mature B cells (orange) have dim and variable CD10 expression, an immunophenotype associated with germinal center B cells. The variable expression of CD10 is not normal for germinal center B cells.

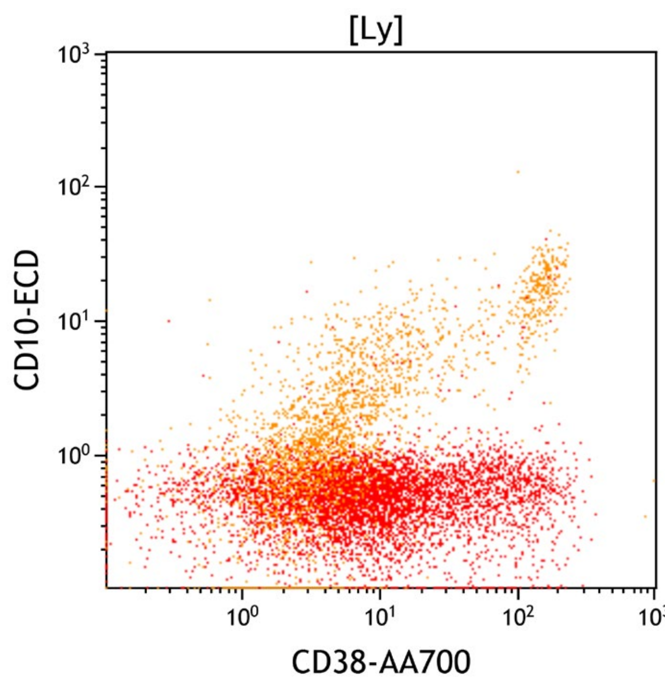


Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The subset of CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. The clonal mature B cells (orange) express variably decreased CD38 in comparison with normal germinal center B cells, and at a level lower than that of the immature B cells.

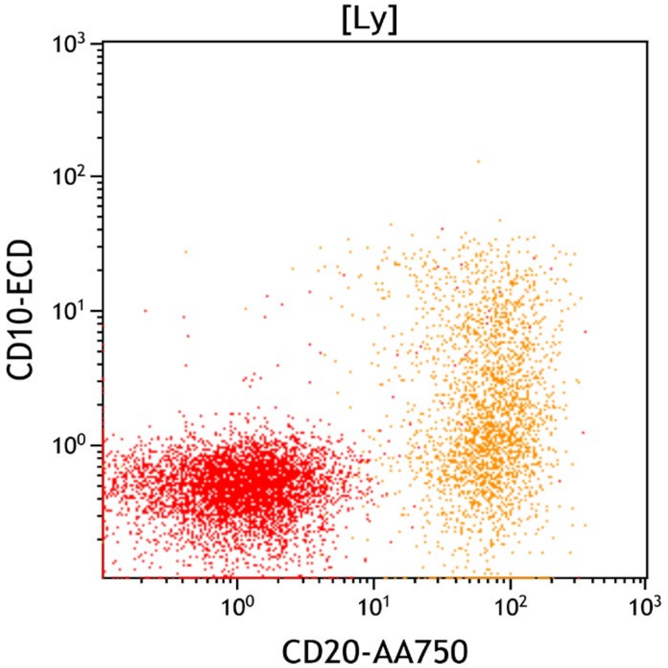


Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). The mature B cells (orange) uniformly express high-level CD20. The few B cells with the highest CD10 expression variably express CD20 and represent late stage immature B cells in the mature lymphocyte gate. The clonal mature B cells express CD20 and variably dim CD10, an immunophenotype usually associated with germinal center B cells.

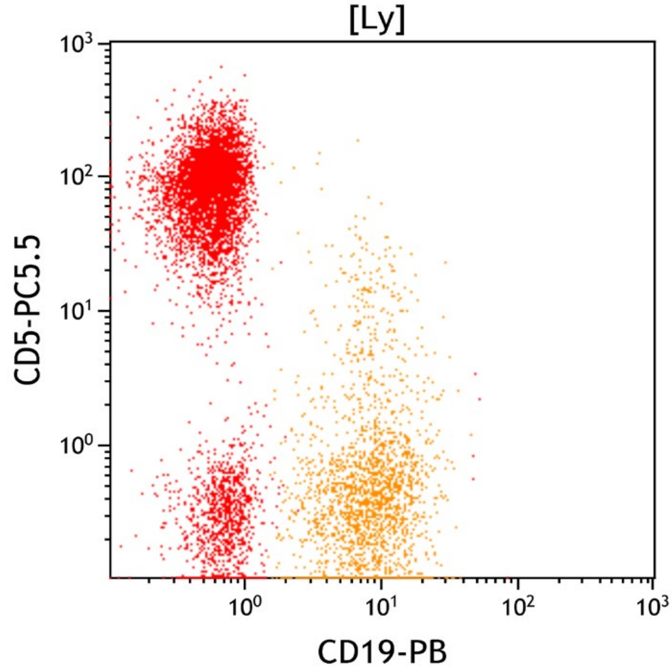


Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal mature B cells (orange) do not express significant CD5.

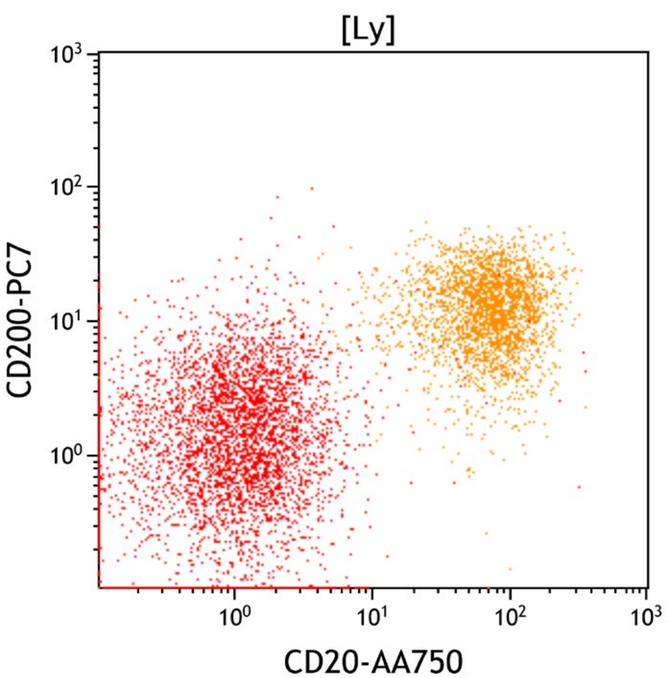


Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The clonal mature B cells (orange) coexpress CD20 and CD200 at normal levels.

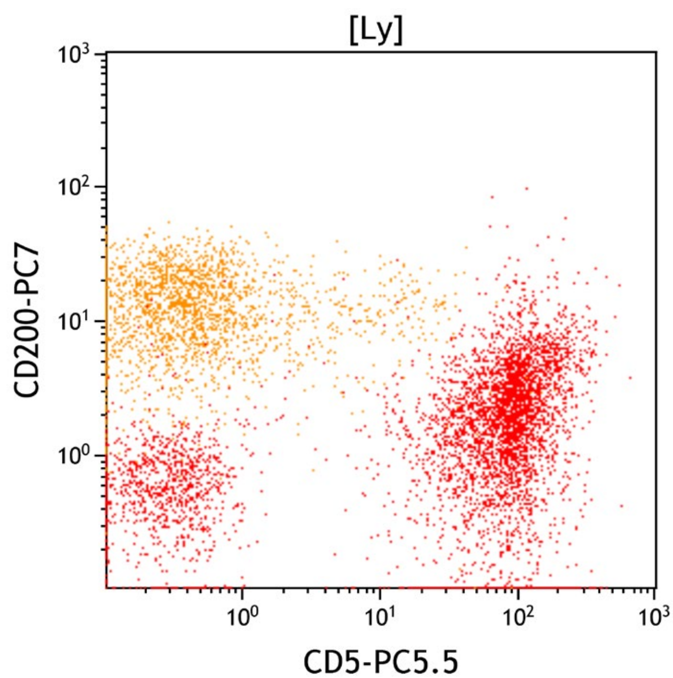


Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The clonal mature B cells in this case (orange) express CD200 but not significant CD5.

T Cell Tube

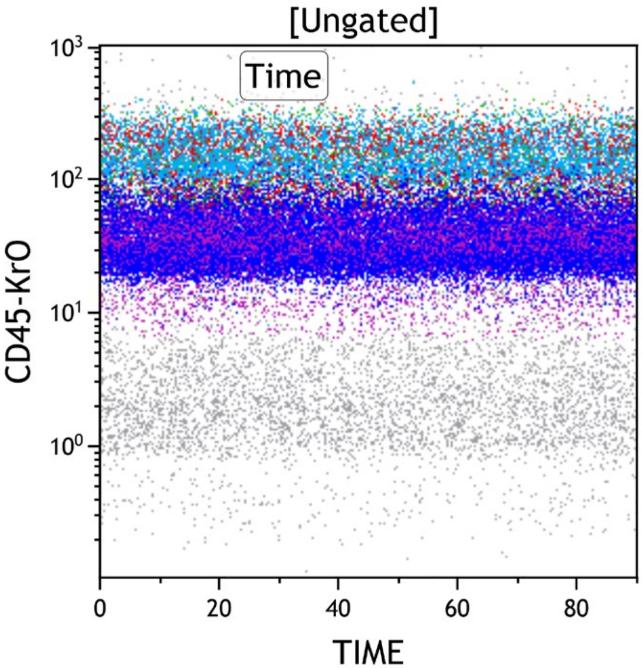


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

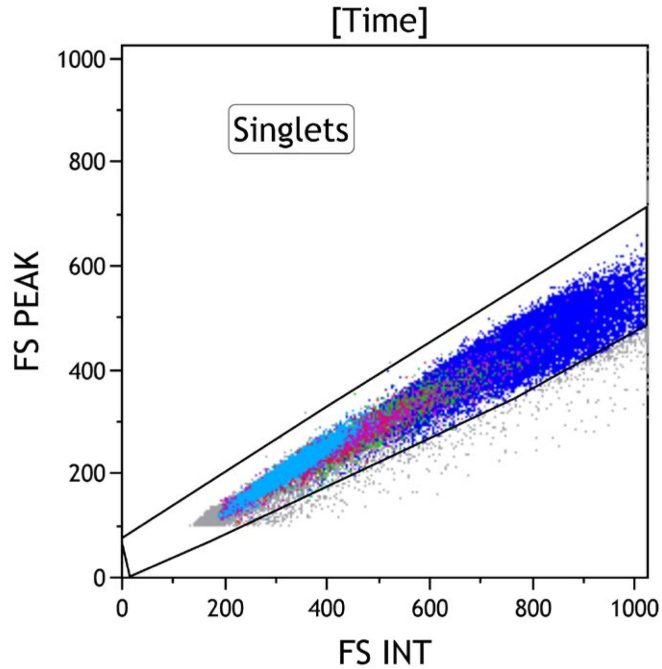


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events shows a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

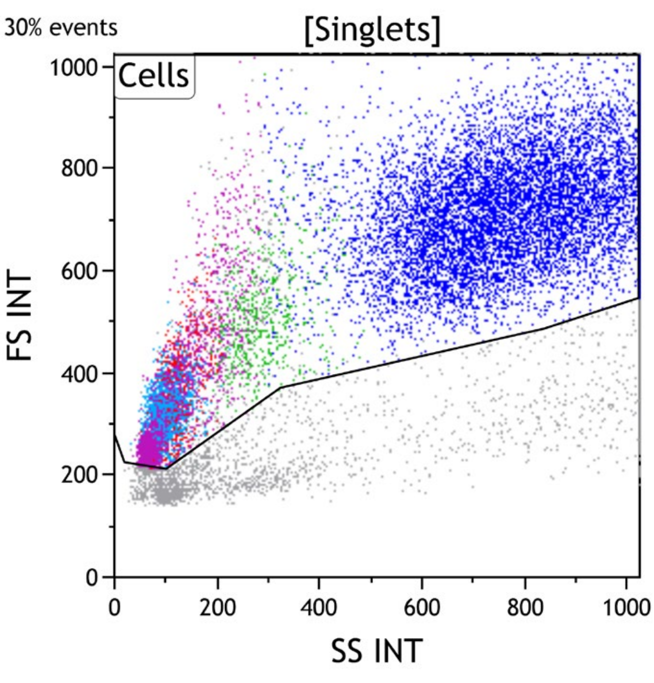


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

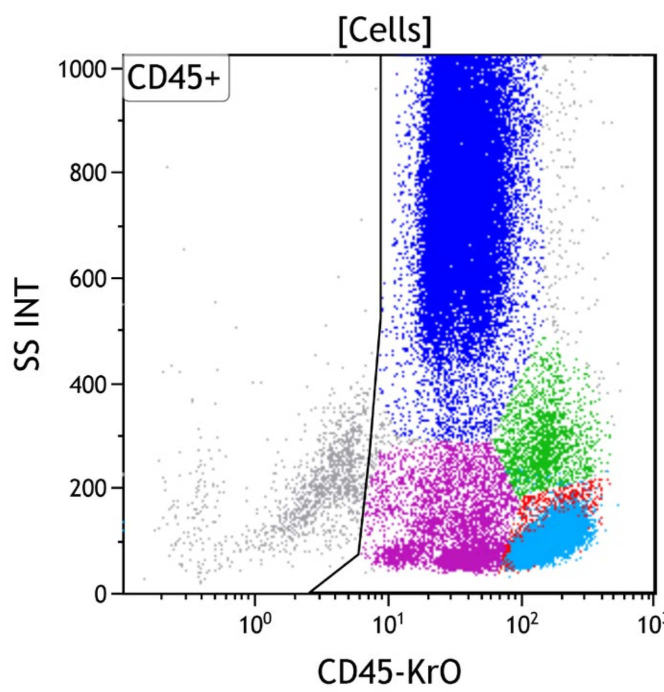


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

T Cell Tube

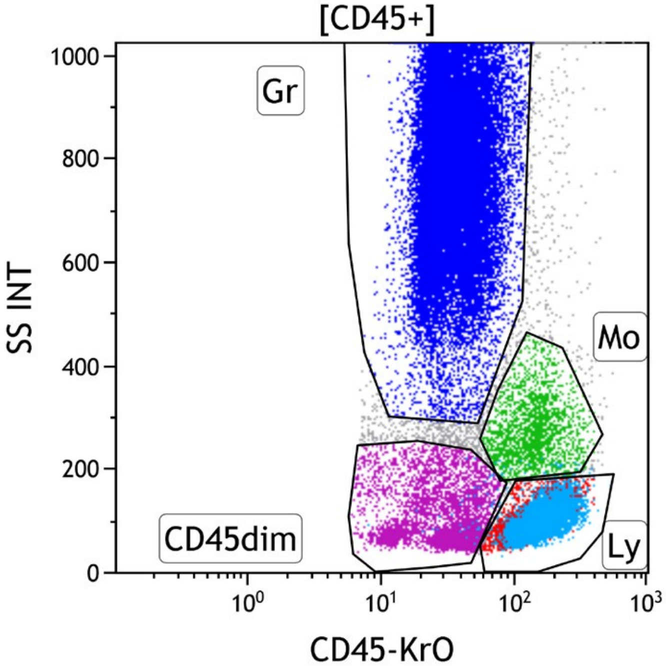


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

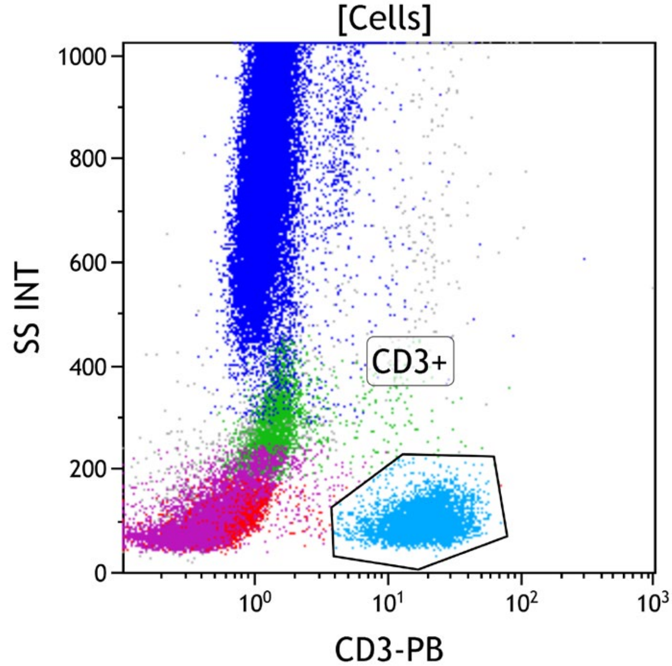


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

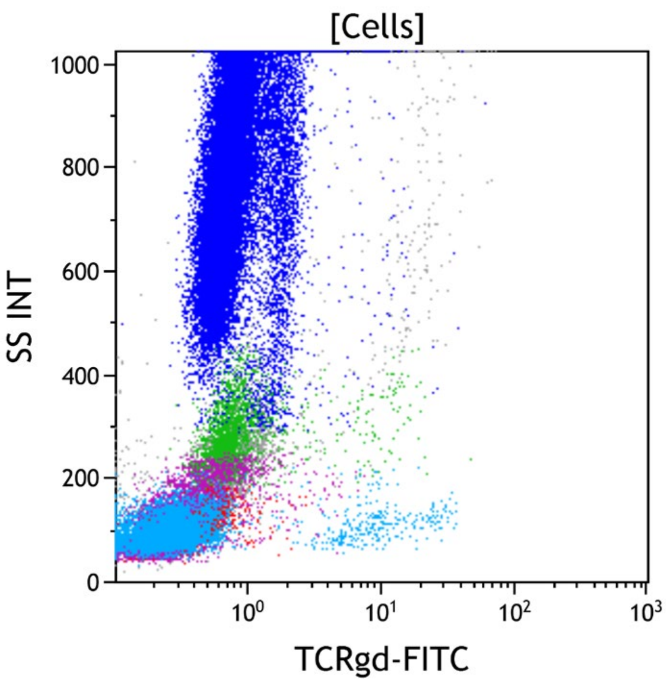


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

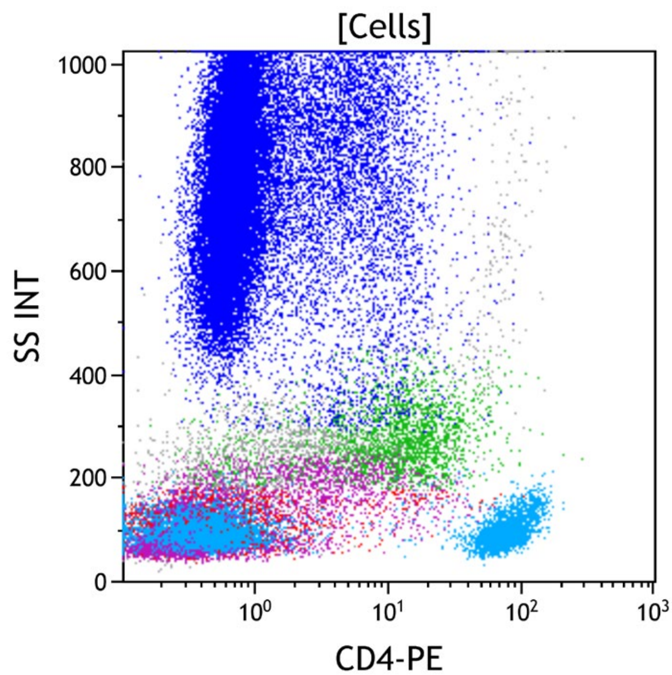


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

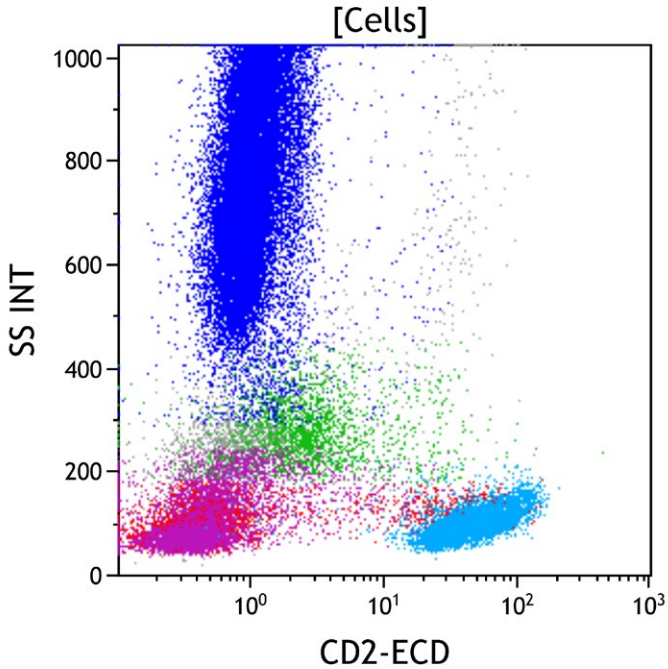


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

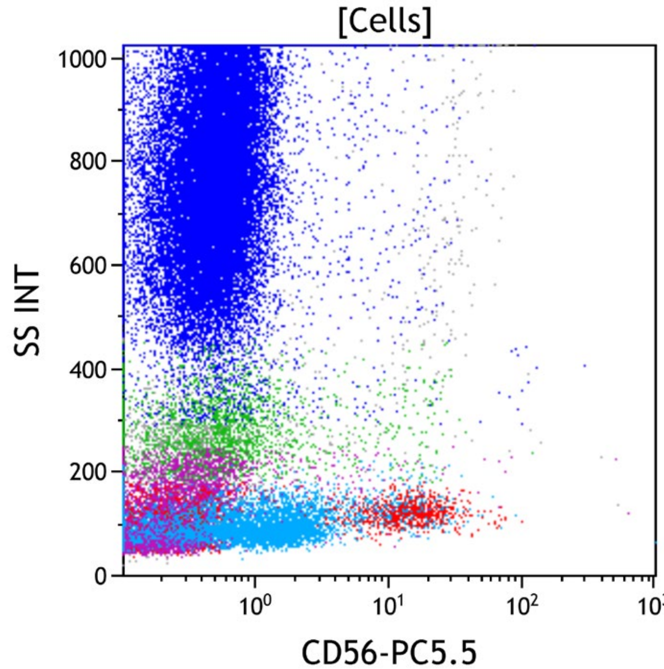


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

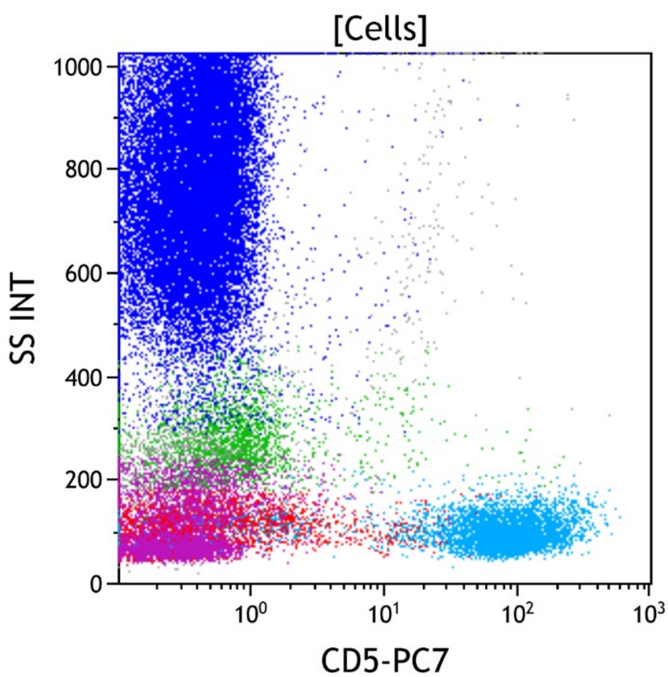


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

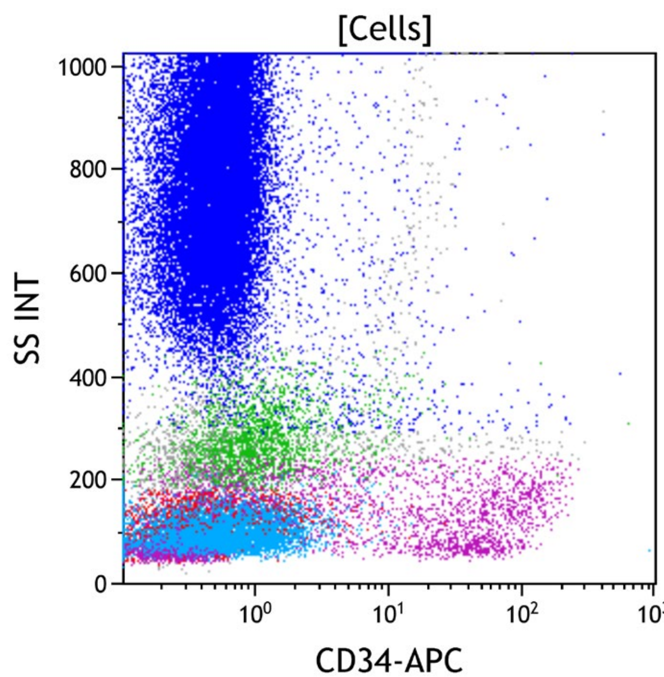


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

T Cell Tube

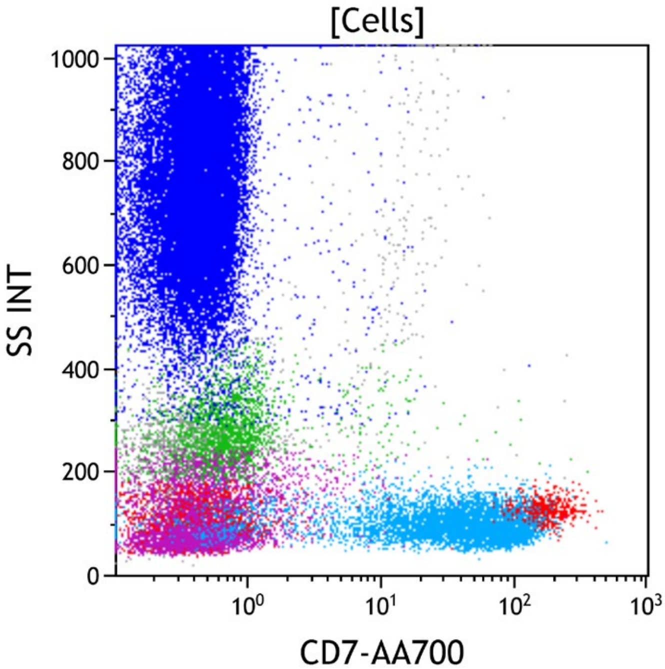


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage-committed CD34 positive progenitors.

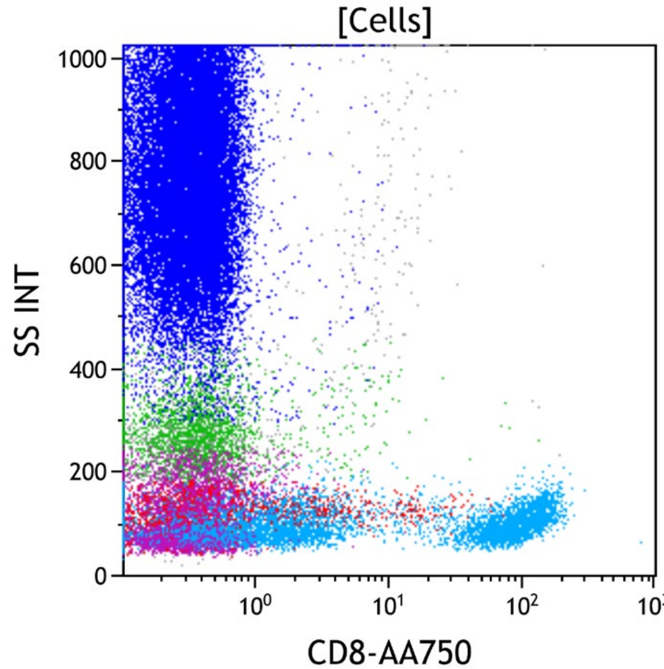


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

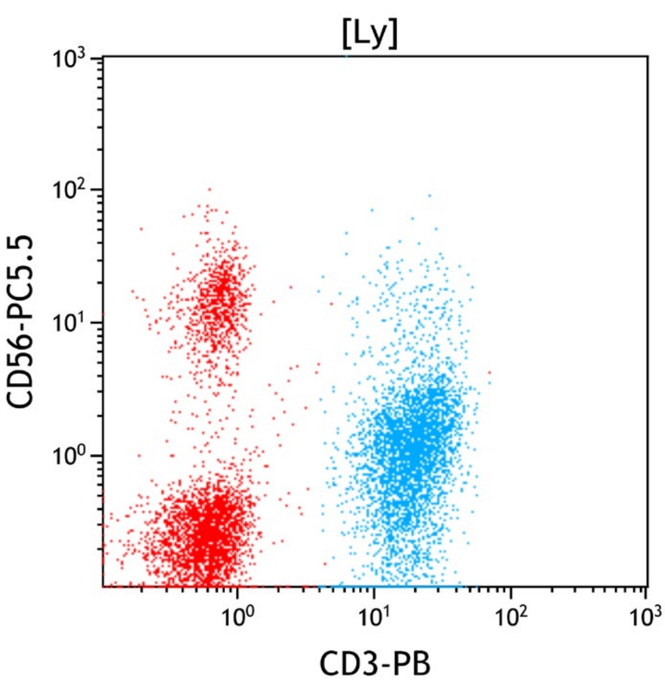


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

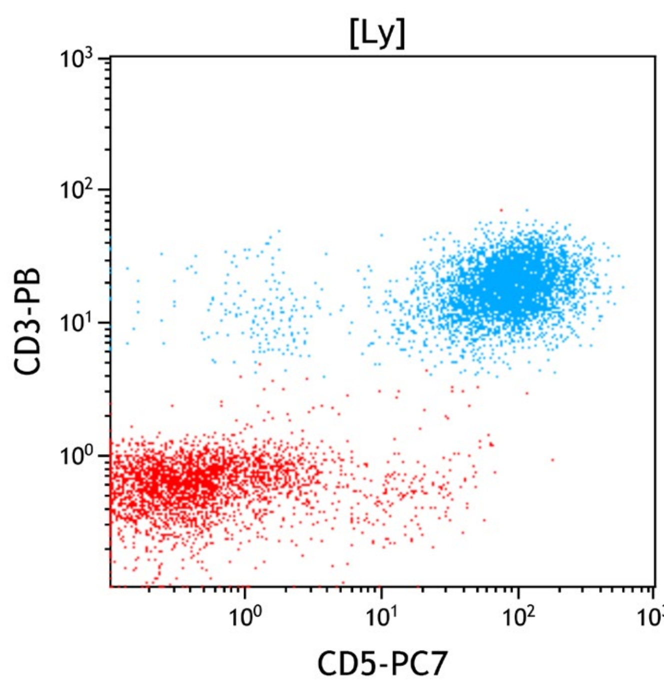


Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red).

T Cell Tube

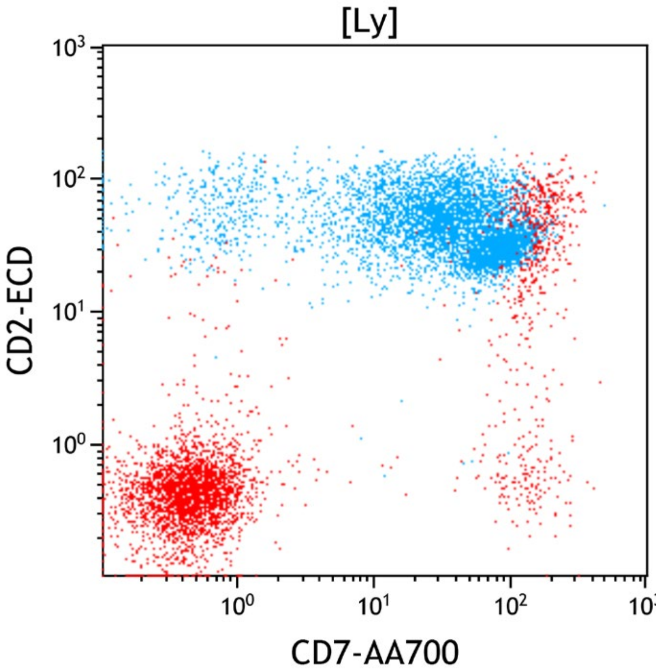


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

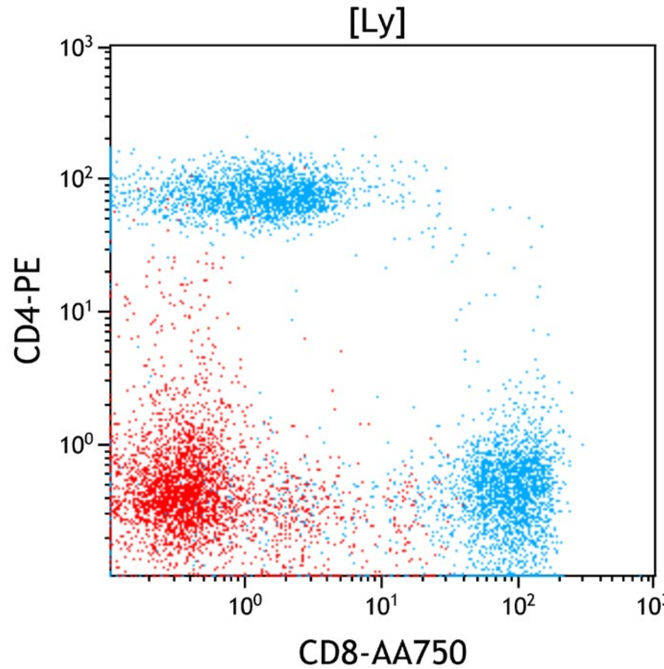


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

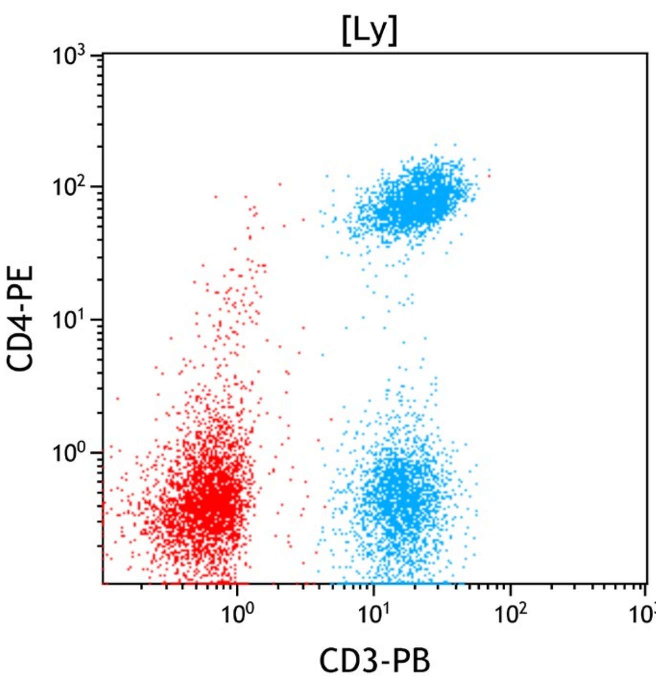


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

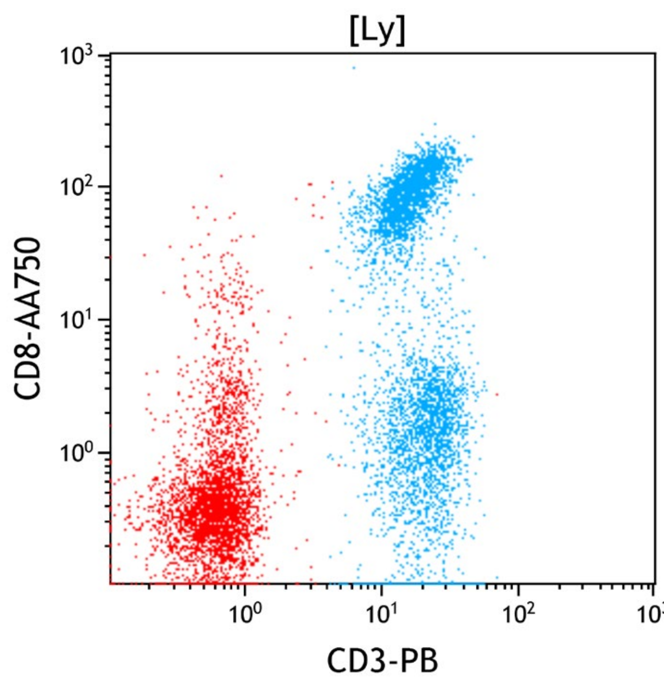


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

T Cell Tube

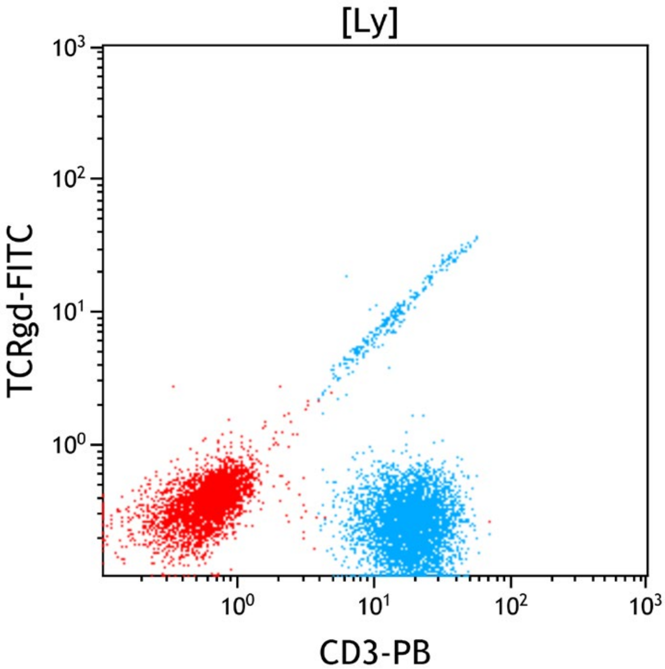


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly) A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

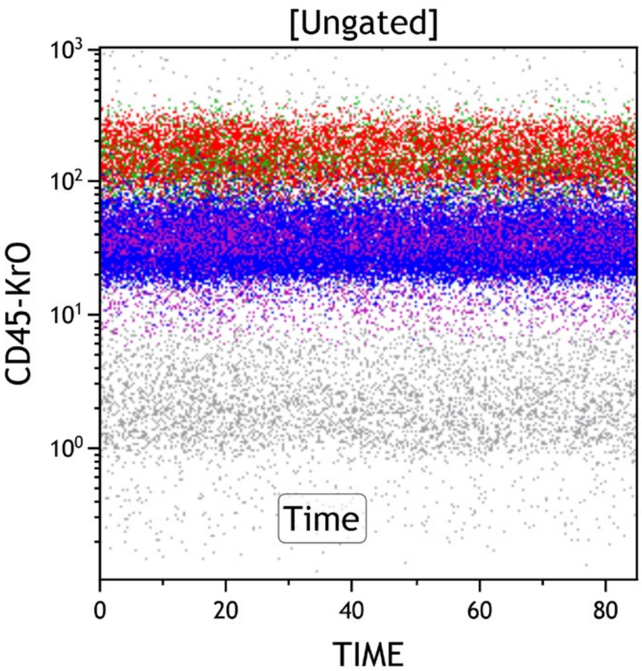


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

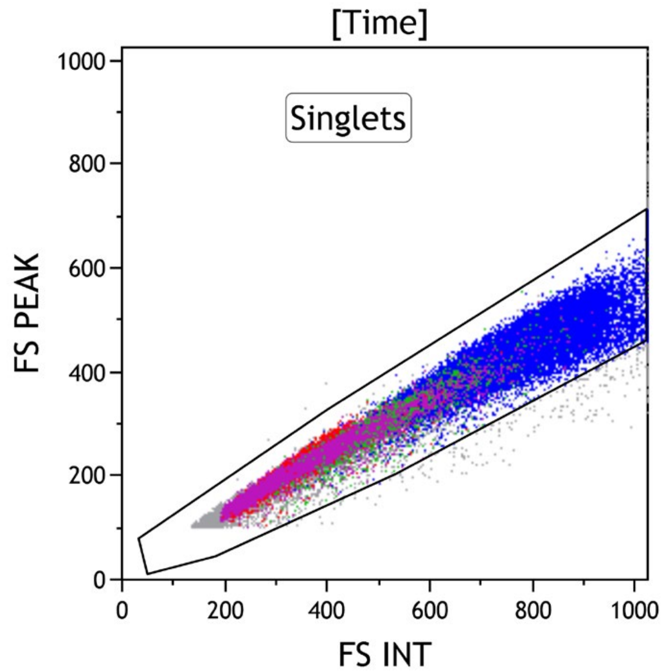


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events shows a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

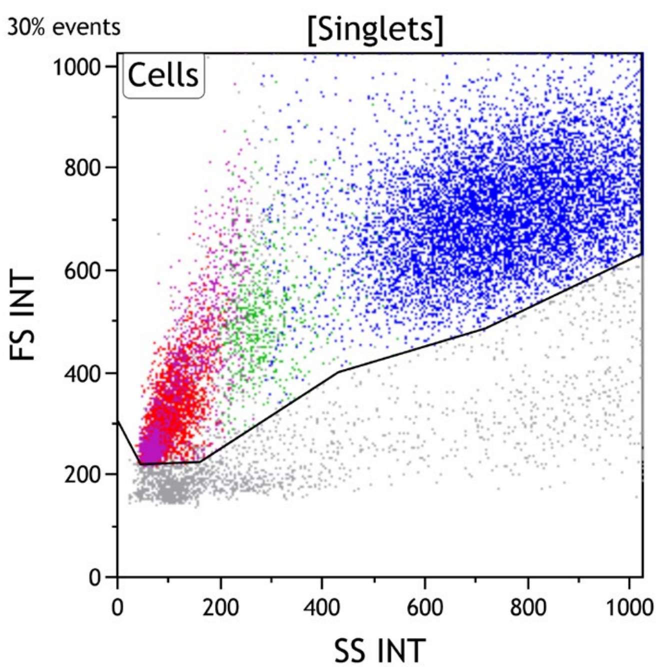


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

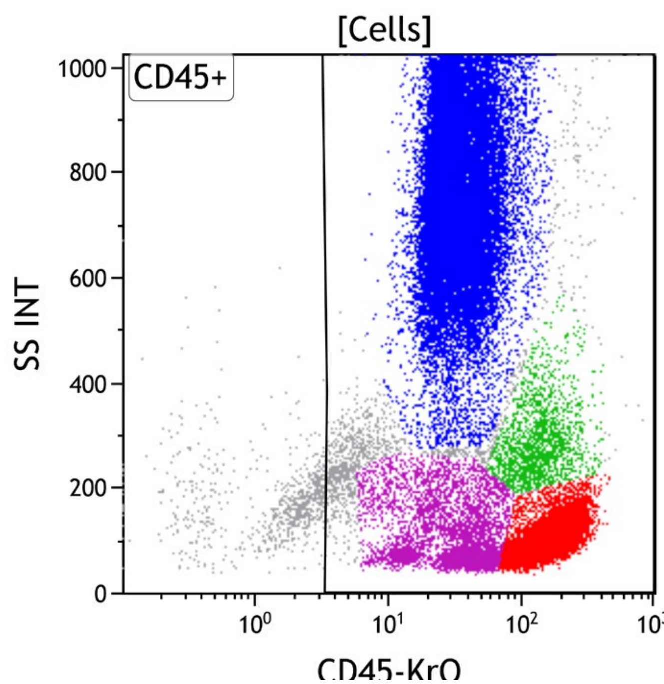


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

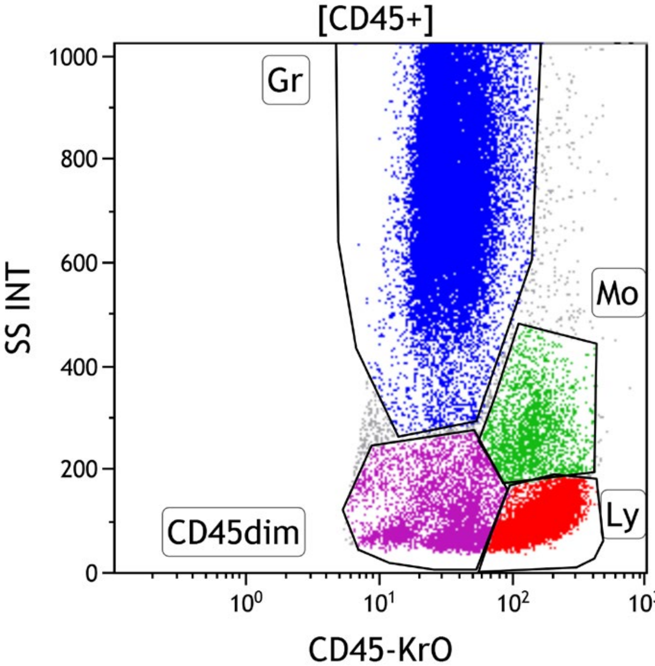


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

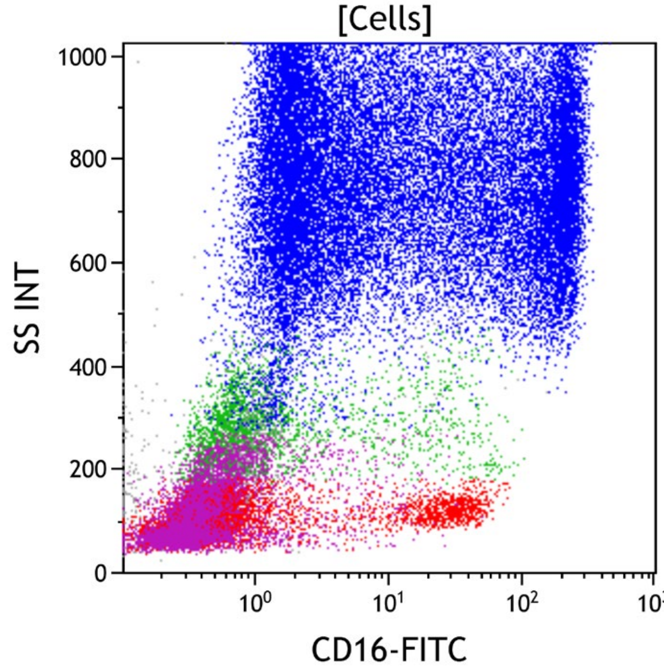


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

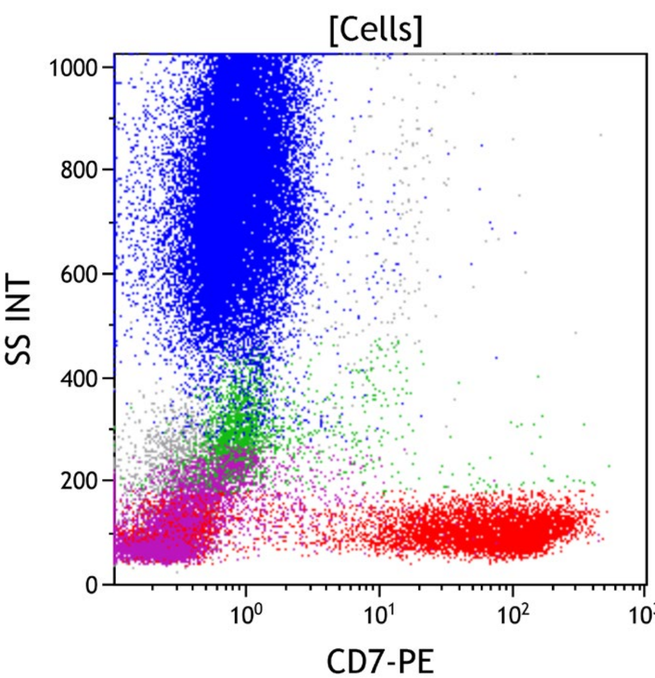


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage-committed progenitors (purple).

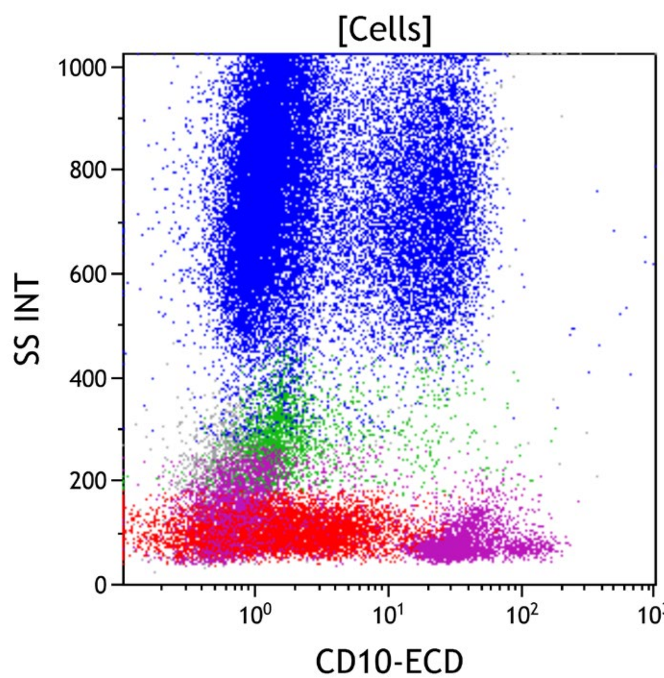


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show variable dim expression of CD10.

Table of Contents > Neoplastic Process of B-cell Origin > Case #10: Follicular Lymphoma

M1 Cell Tube

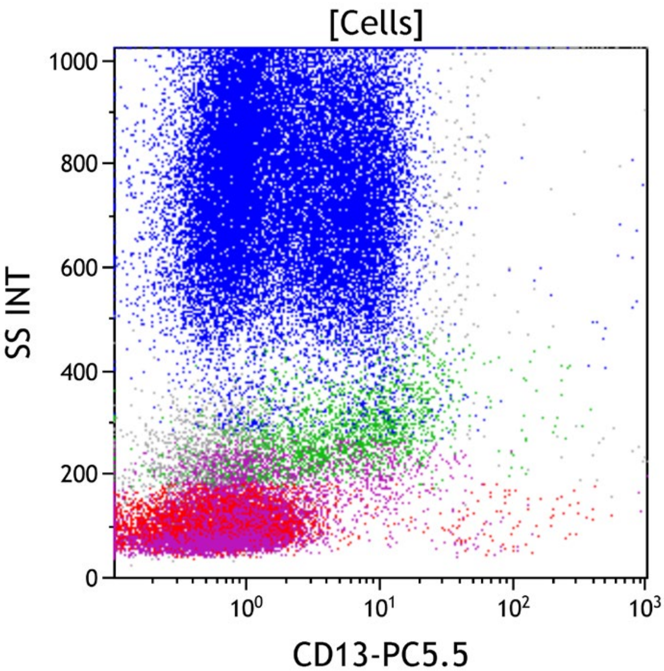


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

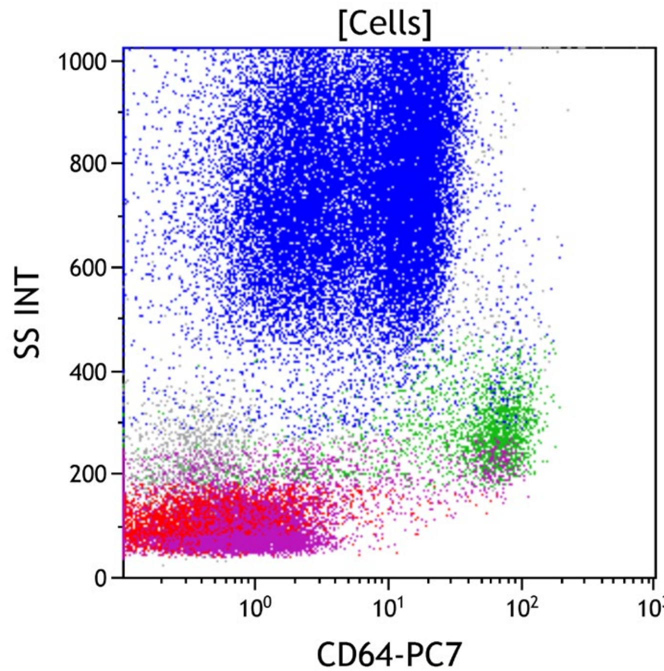


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors.

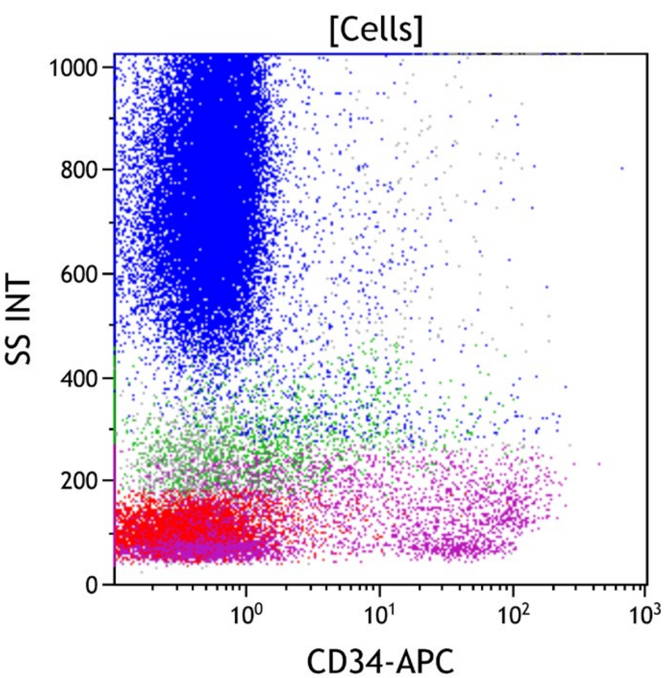


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

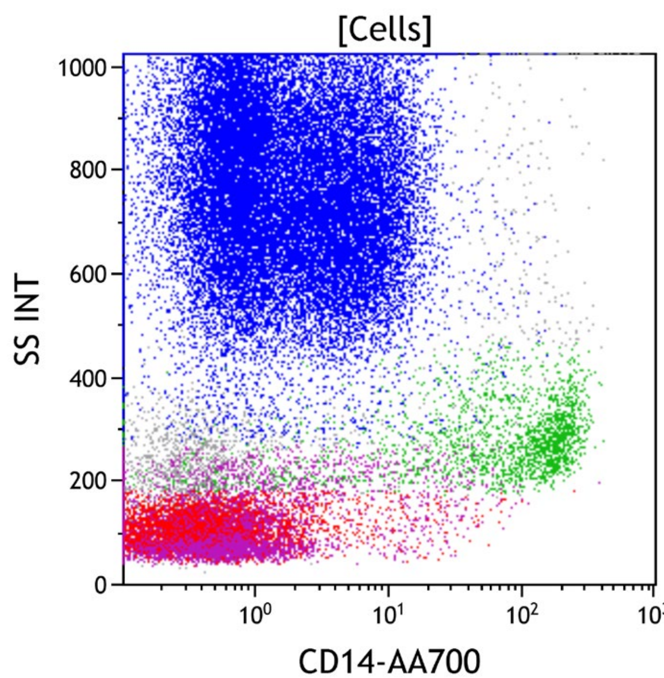


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

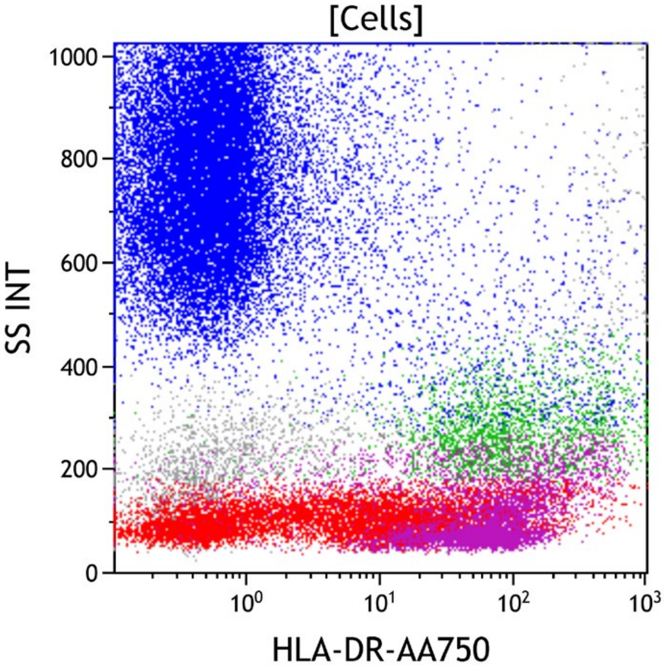


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

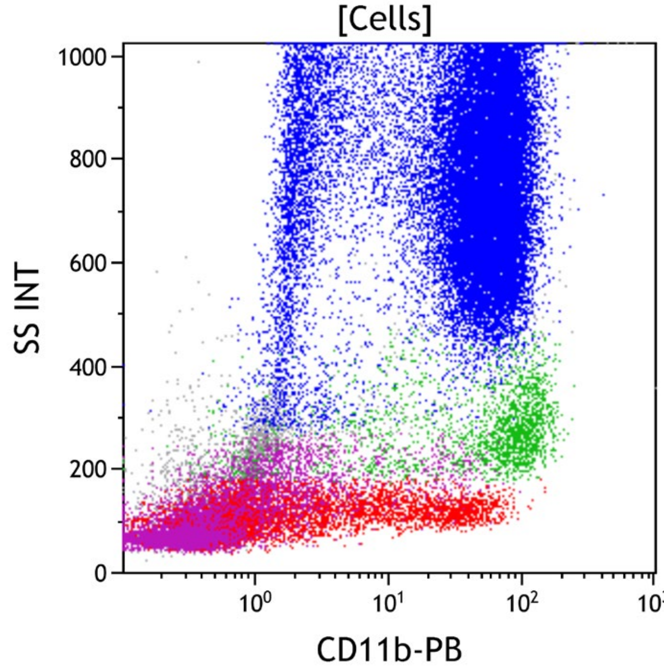


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

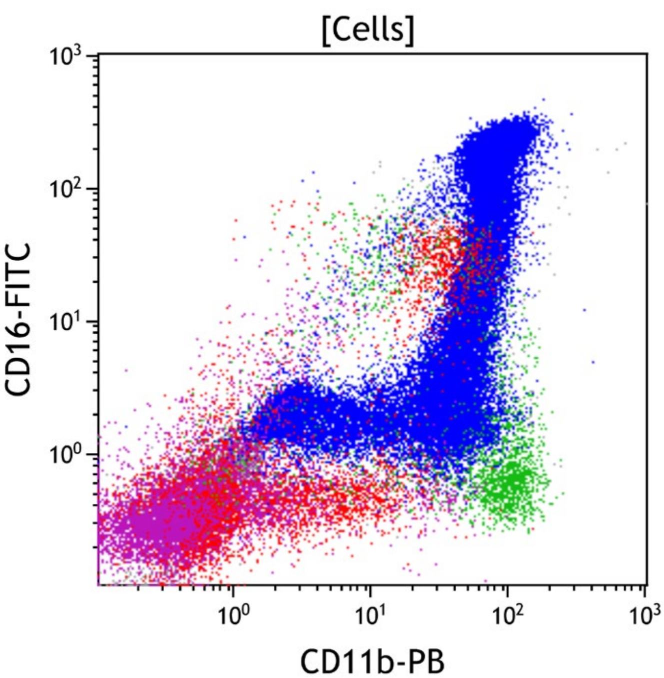


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue bottom left) and acquire CD11b as they mature toward myelocytes (blue bottom right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

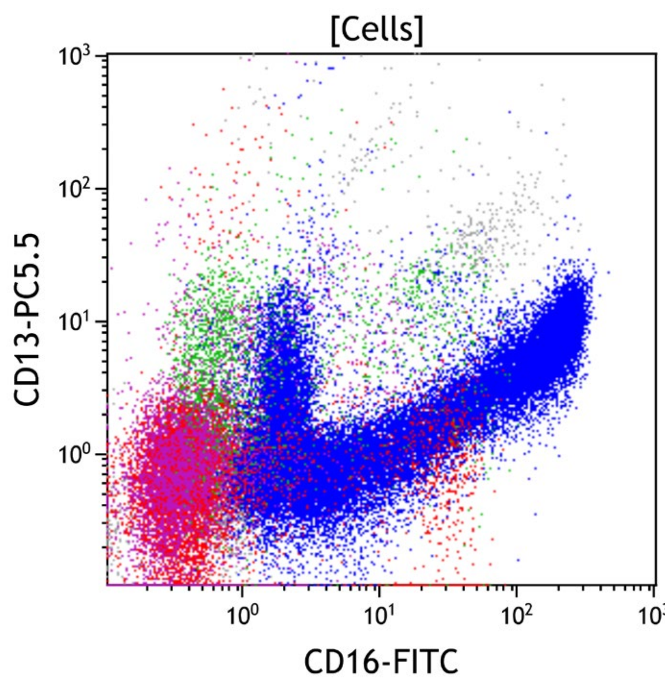


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue left) and lose CD13 as they mature to myelocytes (blue bottom left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue top right).

Table of Contents > Neoplastic Process of B-cell Origin > Case #10: Follicular Lymphoma

M1 Cell Tube

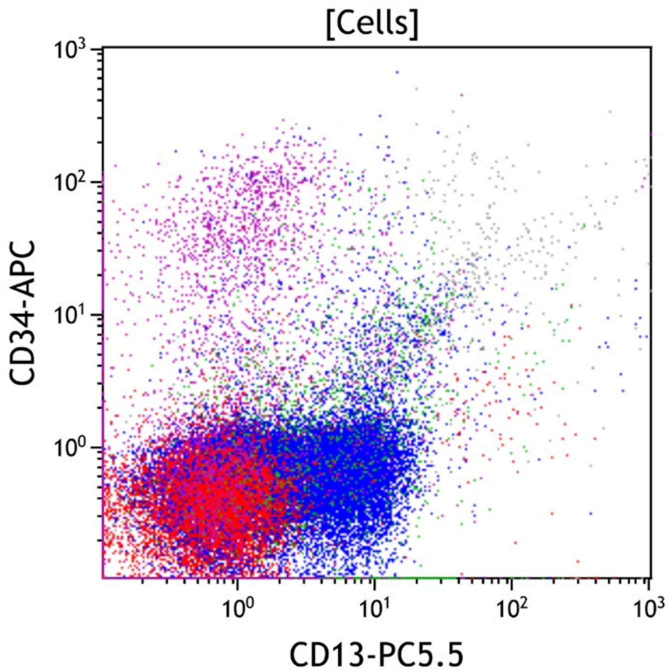


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34+ B cell progenitors (purple) or mature lymphoid cells (red).

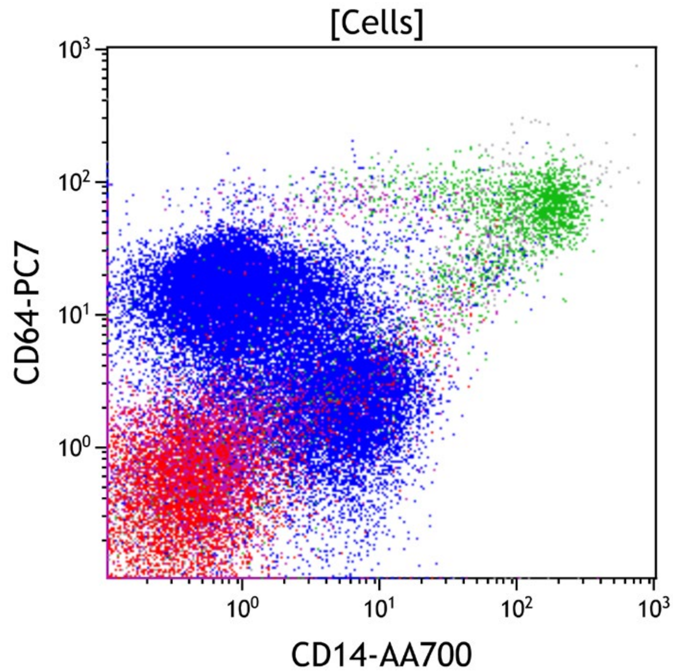


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (blue top left) and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64 (green). Immature granulocytes express moderate CD64 without CD14 (blue left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue bottom).

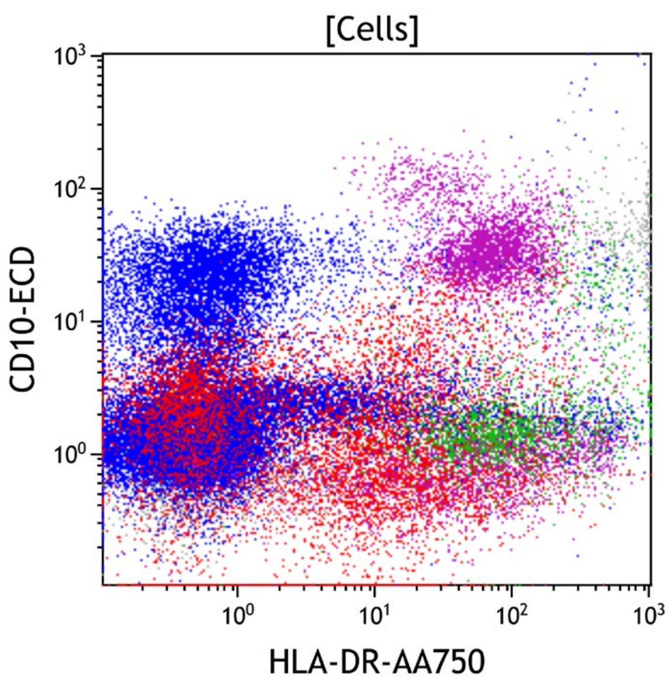


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple). A subset of the mature lymphocytes (red) express variably decreased HLA-DR with variably dim CD10, consistent with the clonal B cell population seen in the B cell tube.

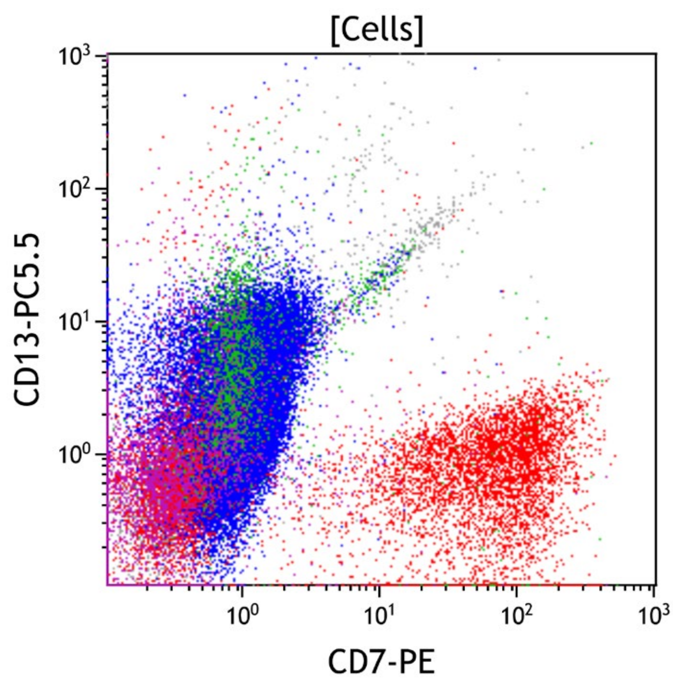


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

Table of Contents > Neoplastic Process of B-cell Origin > Case #10: Follicular Lymphoma

M2 Cell Tube

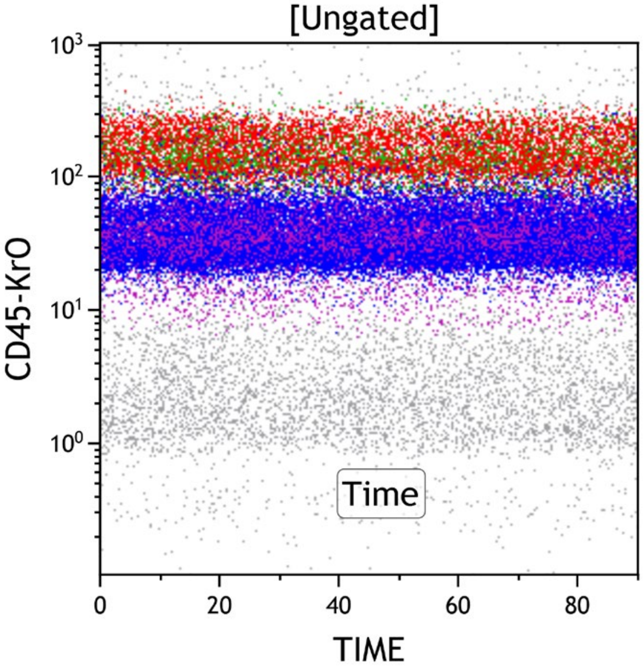


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

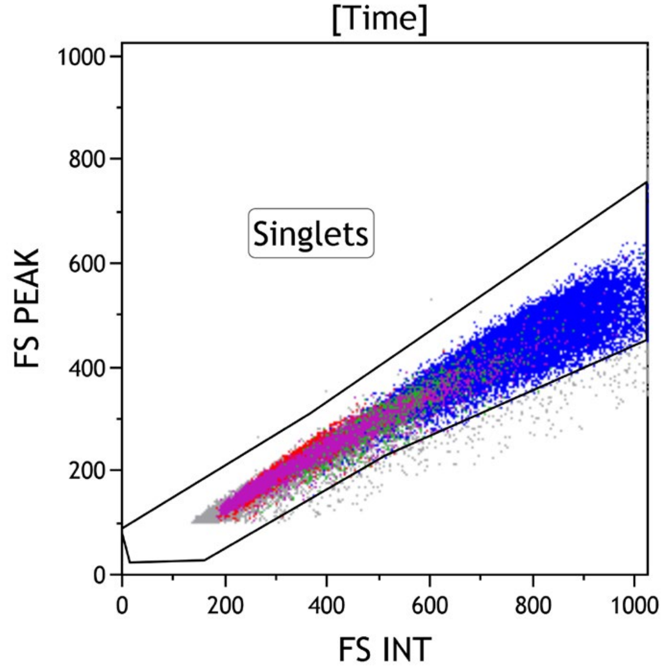


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events shows a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

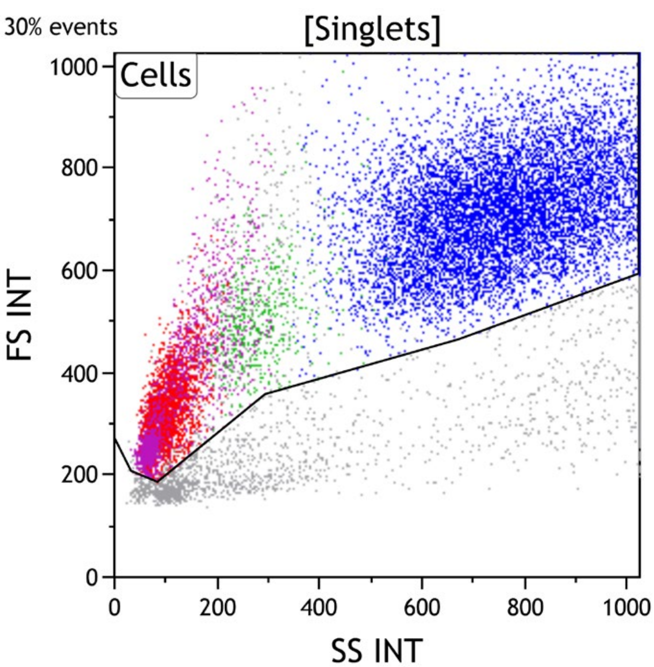


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

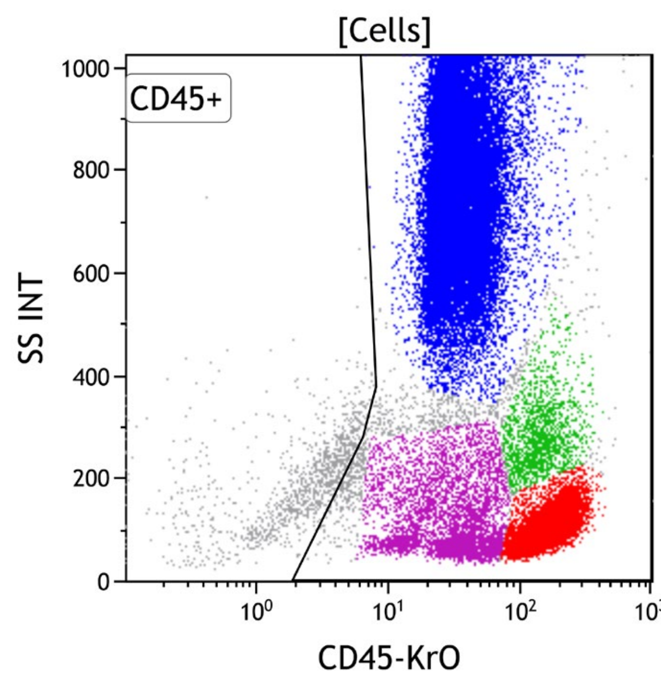


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

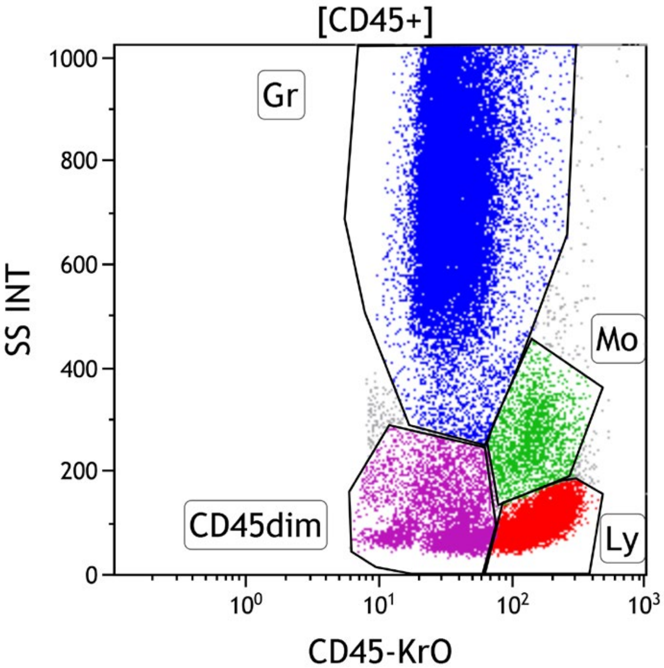


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

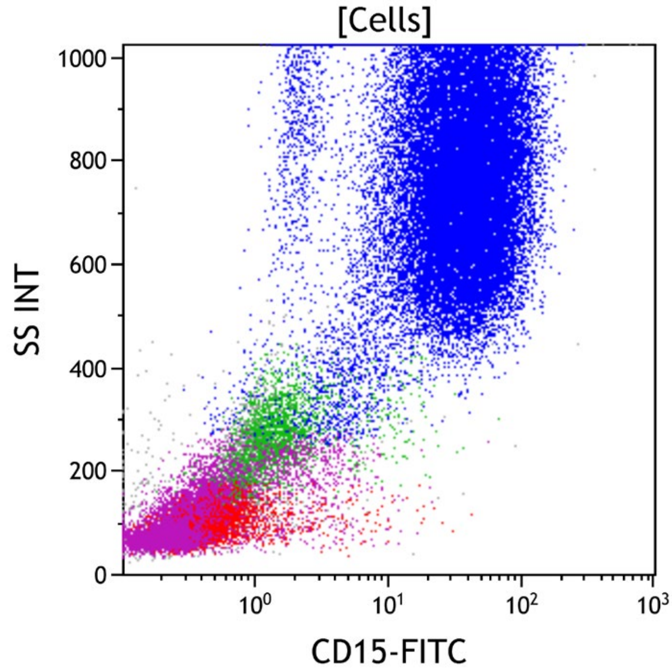


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

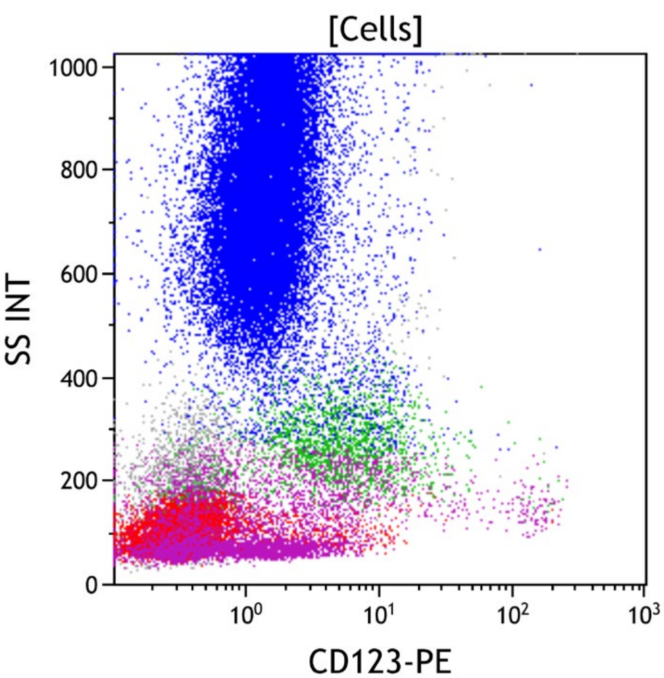


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34+ myeloid progenitors (purple) and monocytes (green).

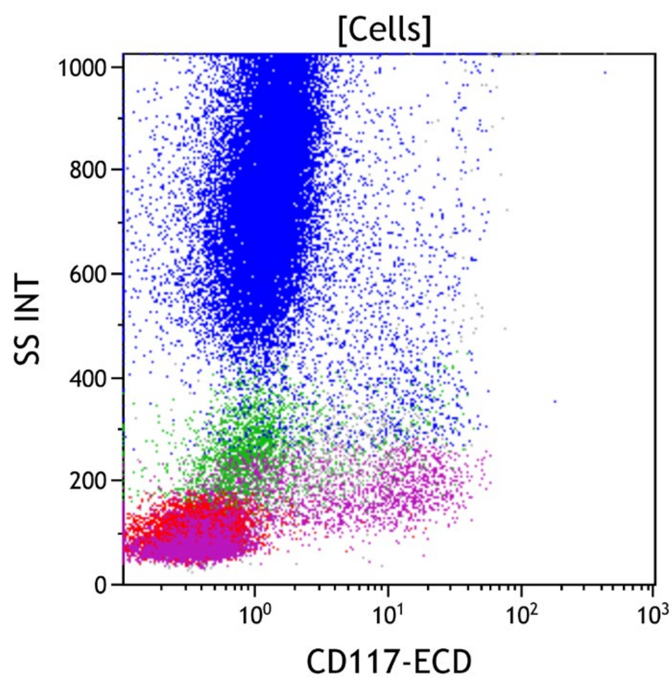


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

M2 Cell Tube

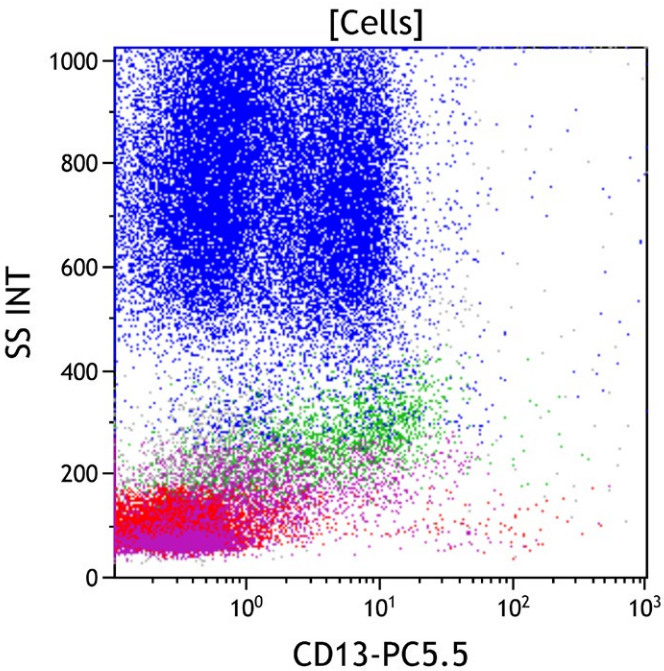


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

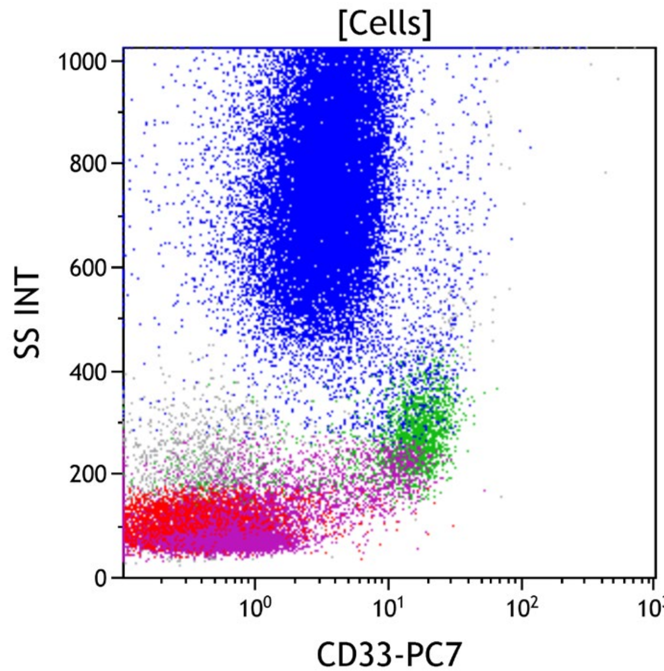


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34+ myeloid progenitors (purple).

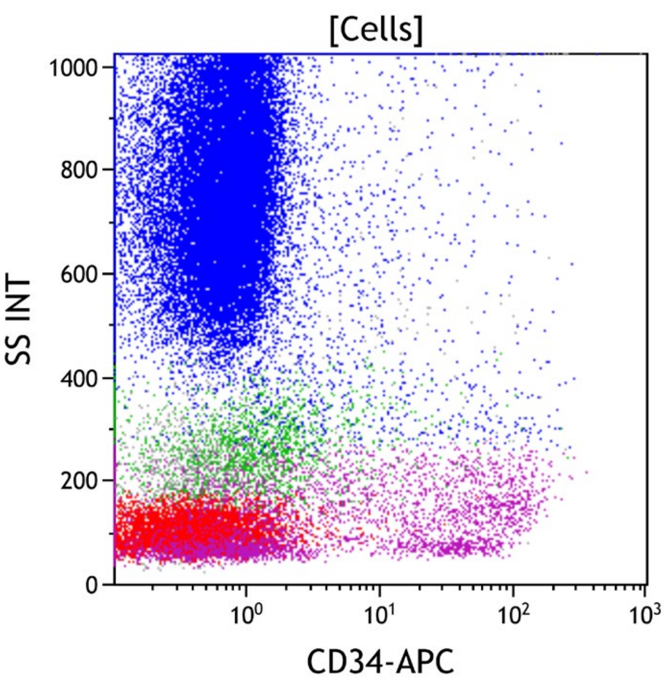


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

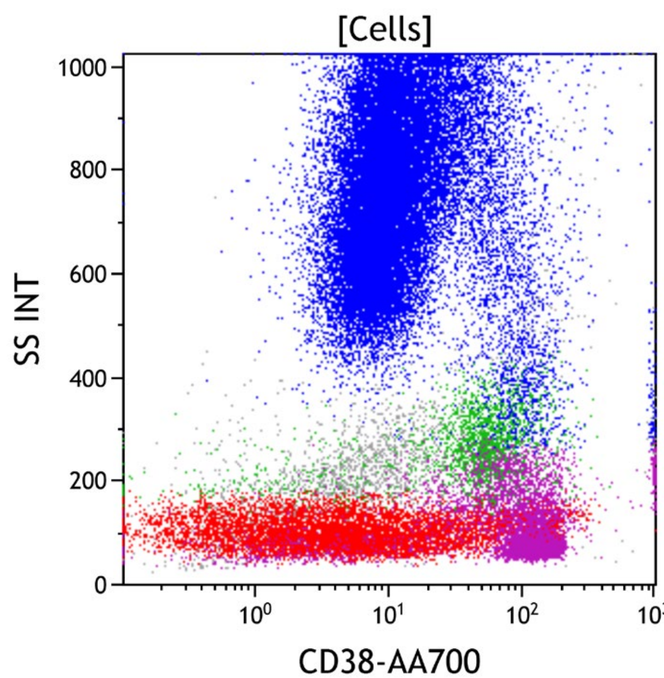


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level.

M2 Cell Tube

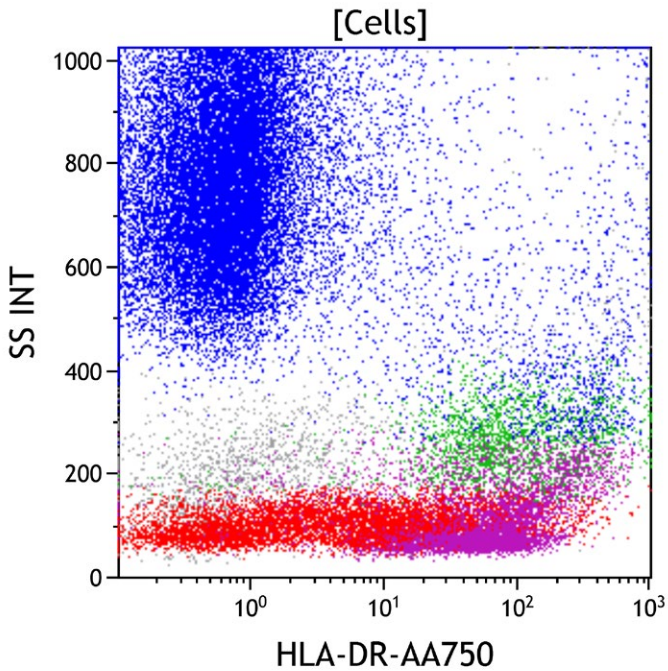


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

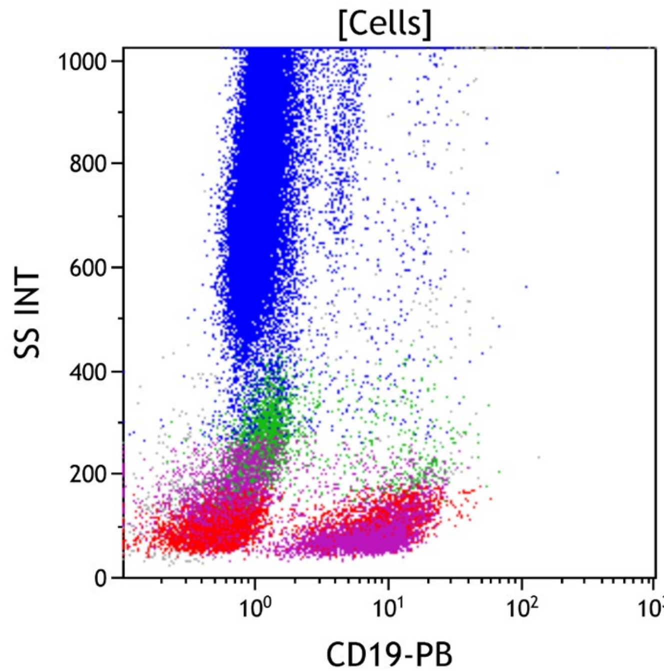


Figure 14: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The mature B cell population (red) is relatively expanded compared with normal.

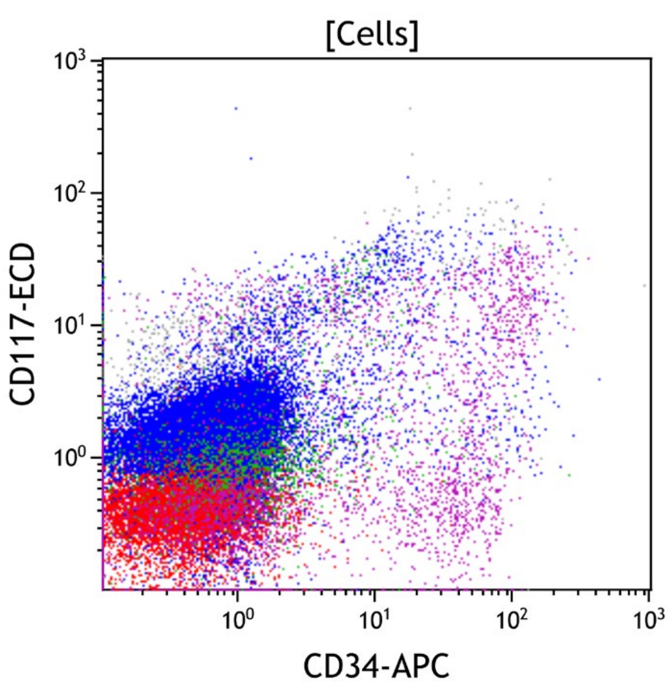


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (purple, upper right), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (purple, lower right).

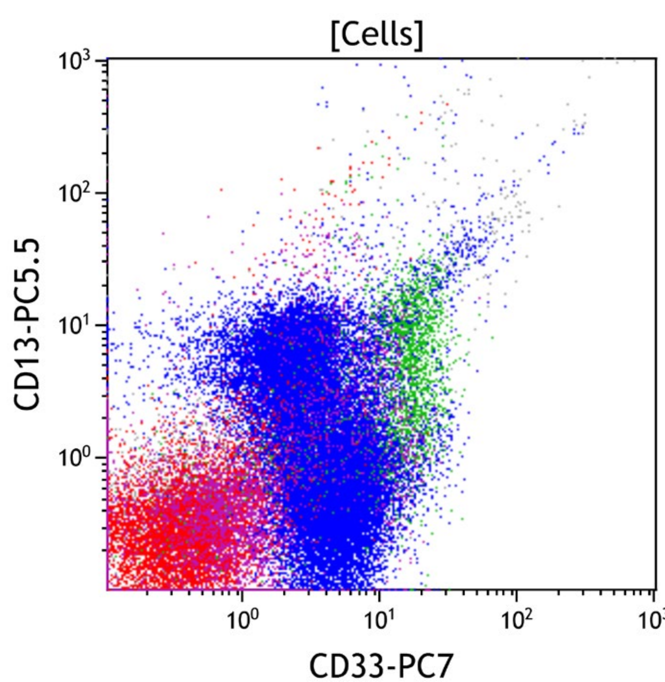


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34+ progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red).

M2 Cell Tube

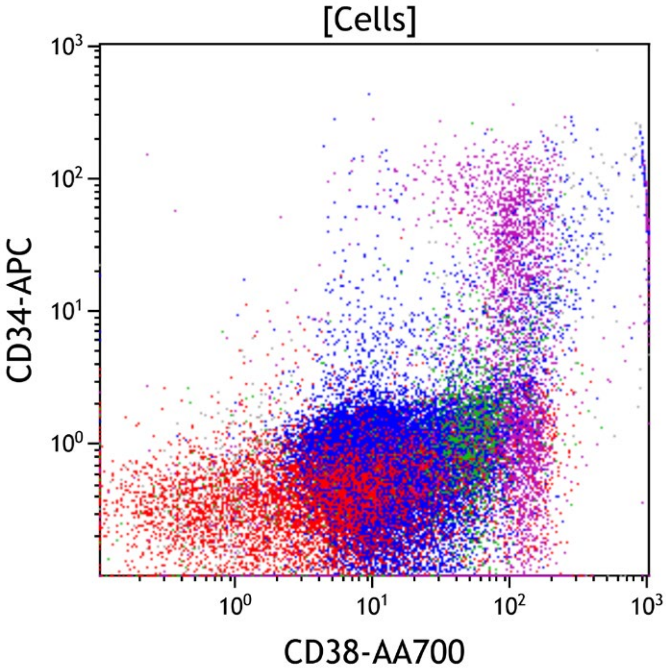


Figure 17. This CD38 vs CD34 dot plots shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable CD34 expression by plasma cells (purple extreme right) is a compensation artifact due to the extremely high level of CD38 that extends beyond the visible scale.

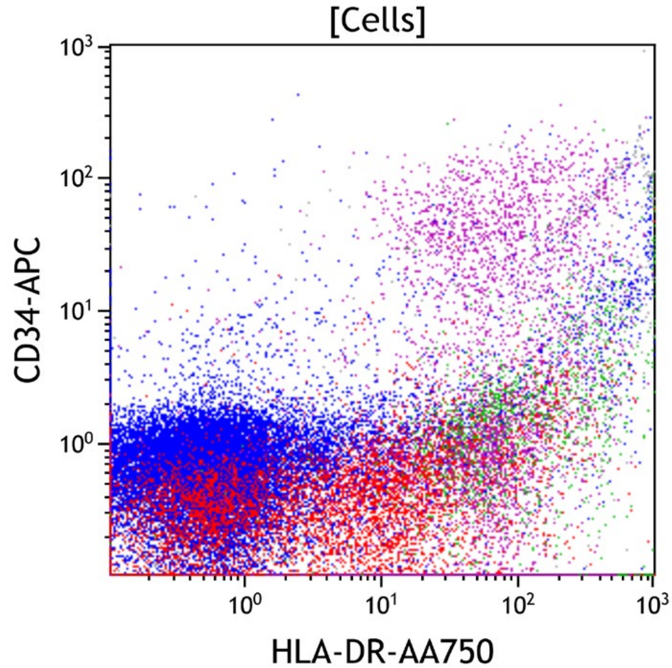


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes (purple right). The mature B cells (red CD19+) do not express CD33.

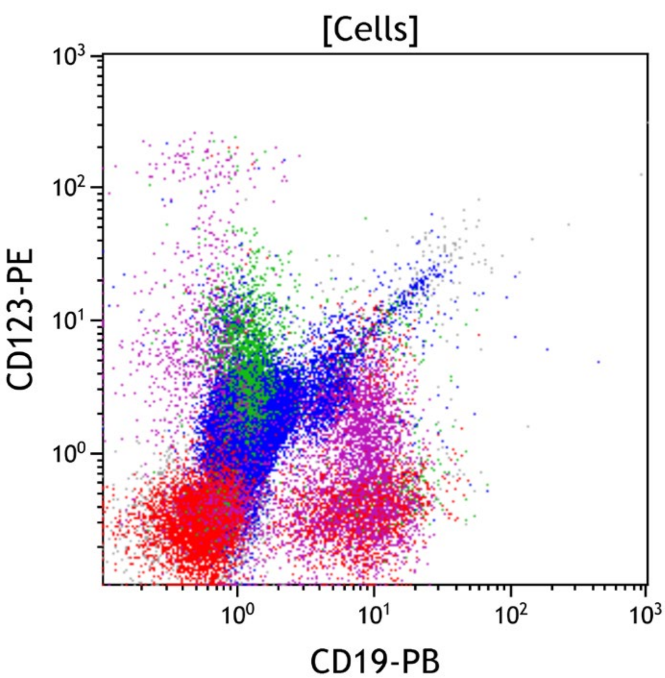


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not expression CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

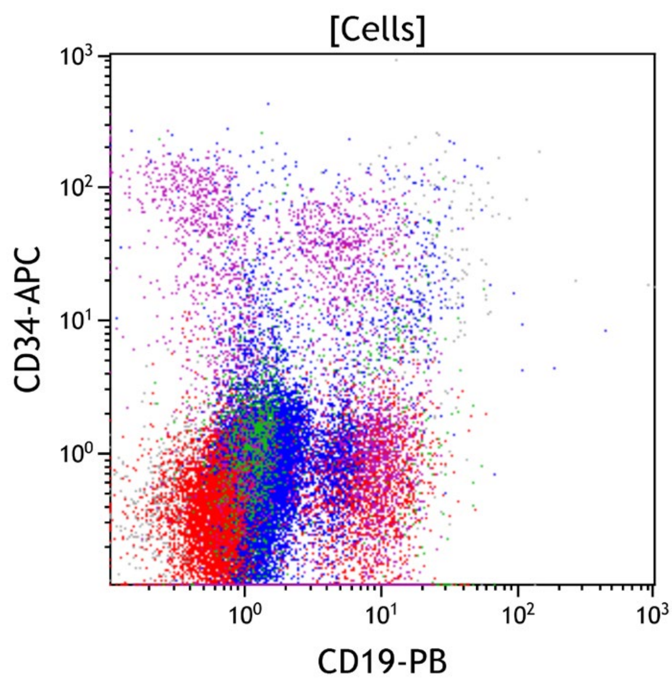


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19. The mature B cells (red CD19+) do not express CD34.

M2 Cell Tube

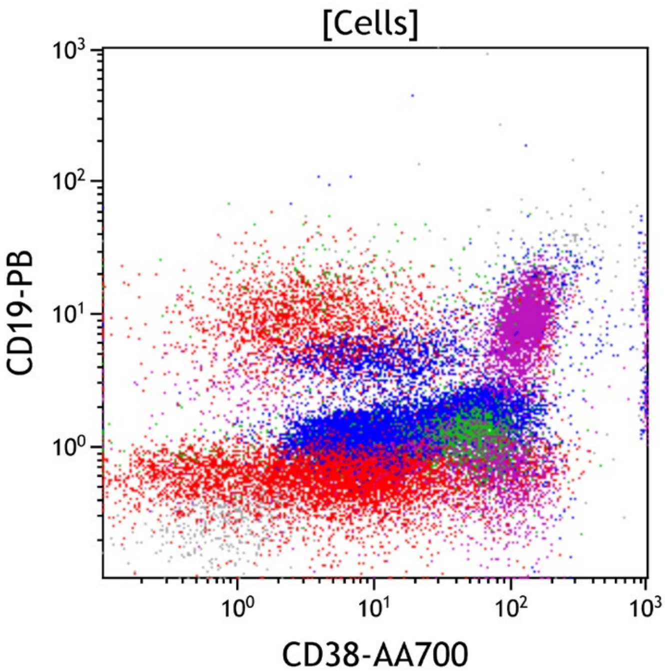


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression of CD38 (red left). Plasma cells show extremely high CD38 expression that is largely off scale (purple extreme right), but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The mature B cells (red CD19+) show variable dim expression of CD38.

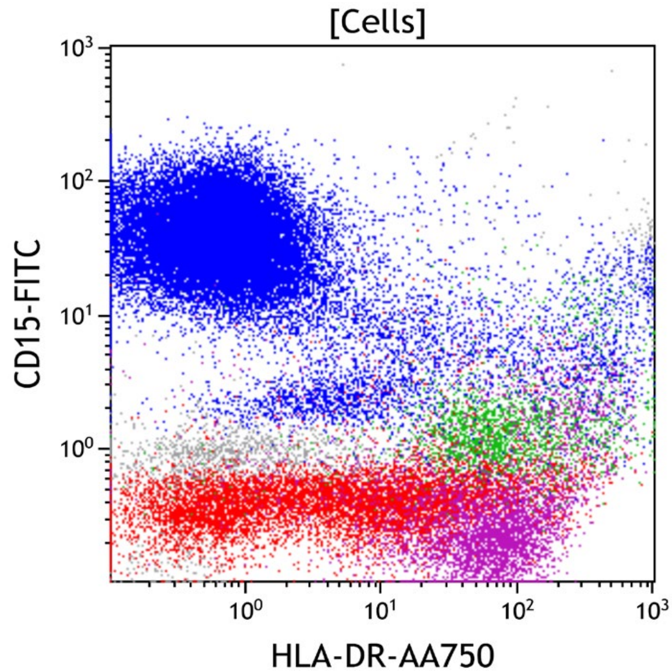


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34+ myeloid progenitors (purple) express HLA-DR but only transiently express CD15.

Results of Flow Cytometric Immunophenotyping

Flow cytometry detects an abnormal mature B cell population with restricted surface kappa light chain expression, consistent with a monoclonal B cell proliferation. The clonal B cell population has abnormal expression of CD10 (decreased), CD19 (variably decreased) and CD38 (decreased) in comparison with normal germinal center B cells with normal expression of CD20 and CD200 without significant CD5. This immunophenotype is consistent with B cell lymphoma of germinal center B cells, such as follicular lymphoma. Definitive diagnosis and classification requires correlation with clinical and morphologic findings.

The concurrent morphologic and immunohistochemical findings confirm the presence of lymphoid aggregates composed of B cells that are small in size and expressing Bcl-6 on a subset and Bcl-2, consistent with a low-grade follicular lymphoma.

Case #11: B cell lymphoma of germinal center origin

Clinical Vignette

This 55-year-old male presents with anemia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

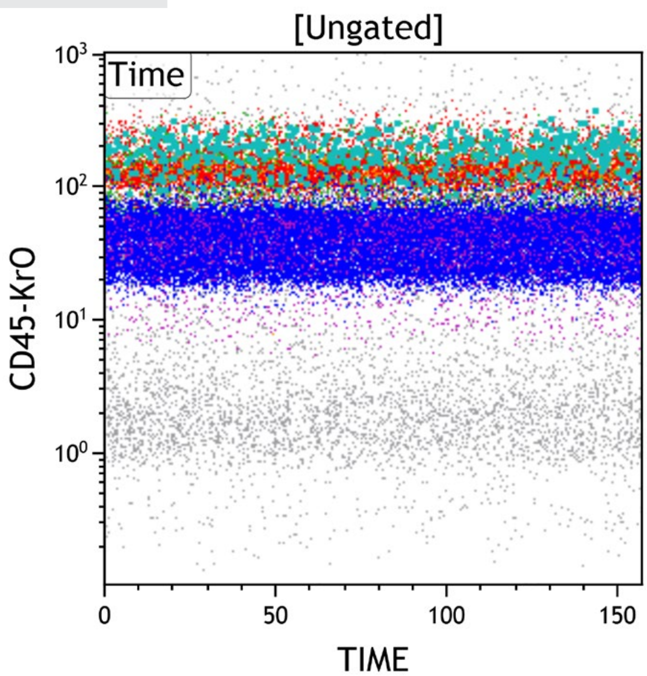


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

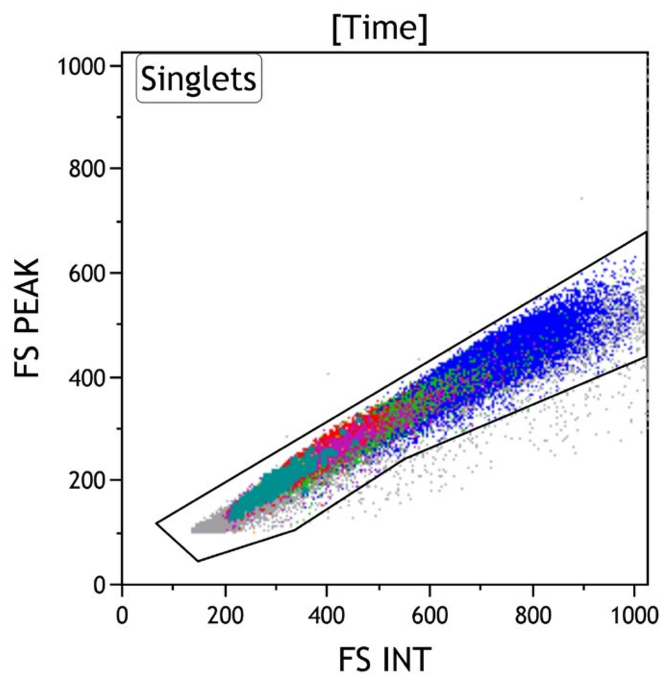


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

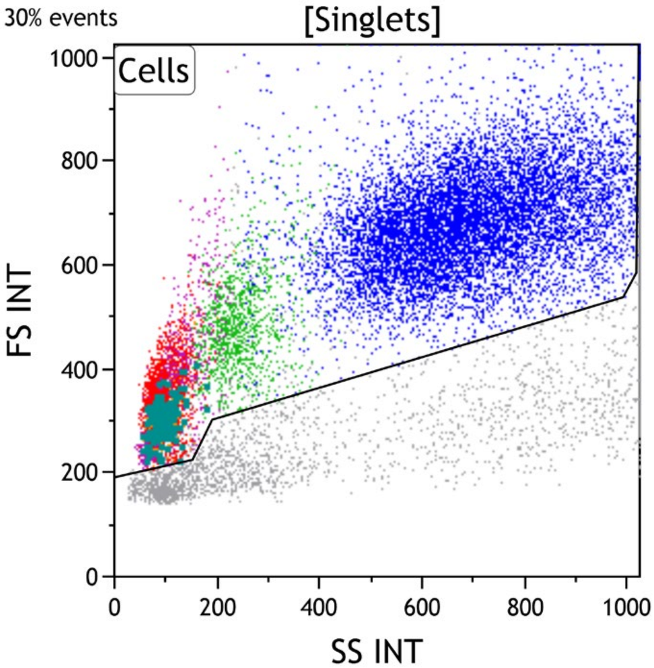


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

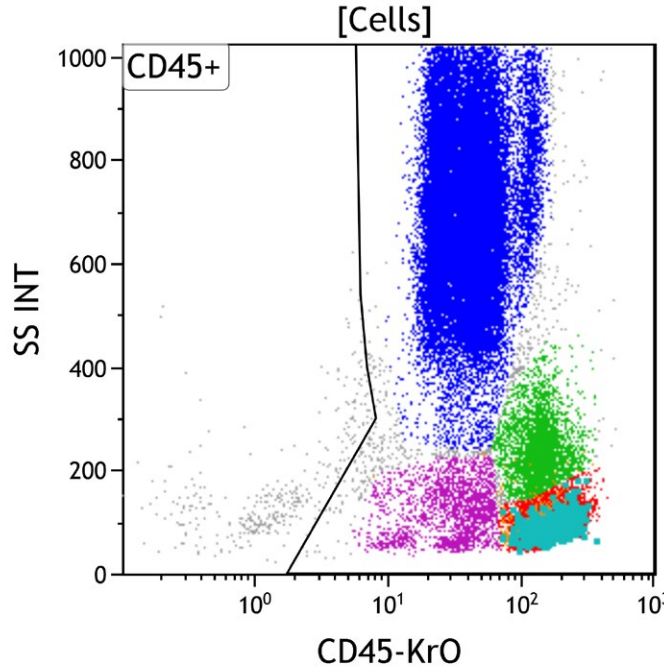


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

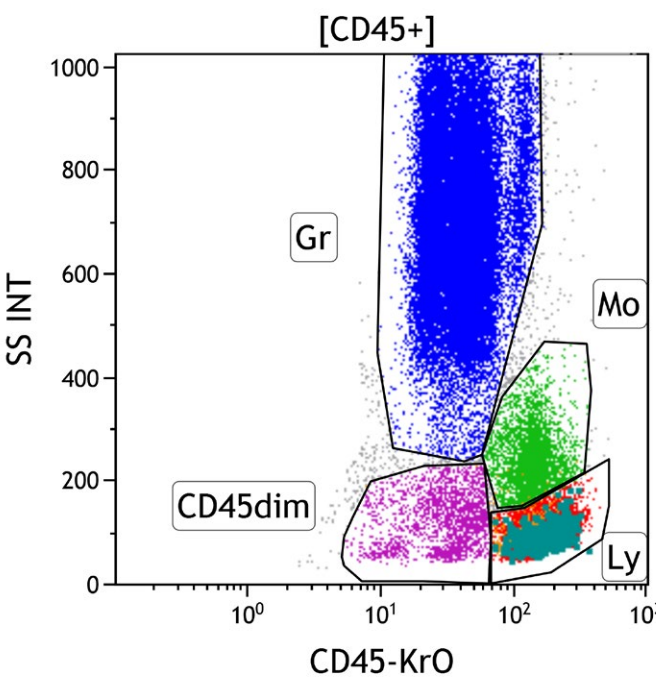


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

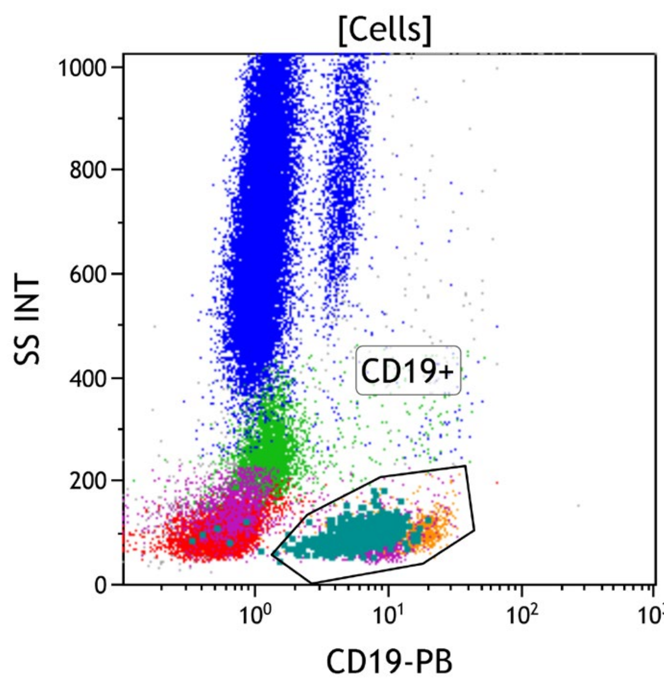


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note the aberrant population of B cells having low CD19 expression (teal).

B Cell Tube

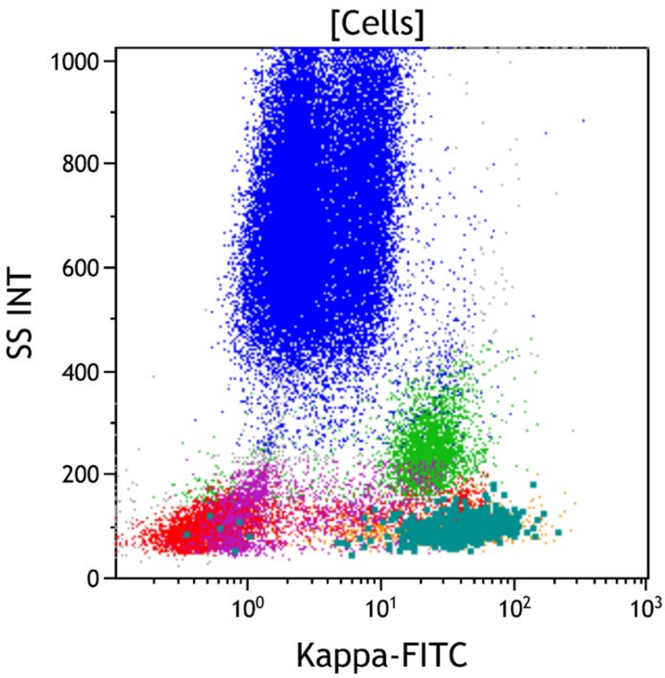


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The aberrant population (teal) expressed only kappa light chains.

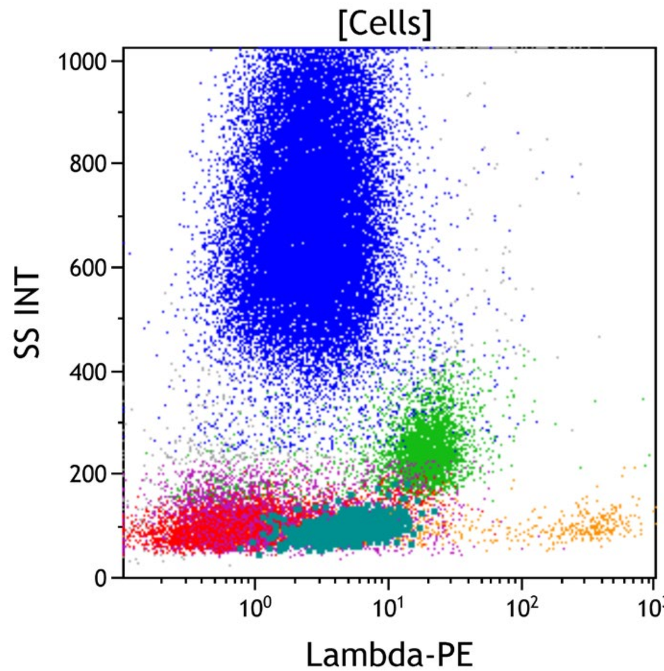


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells (orange) are shown on the right side of the plot. The aberrant population (teal) does not express lambda light chain.

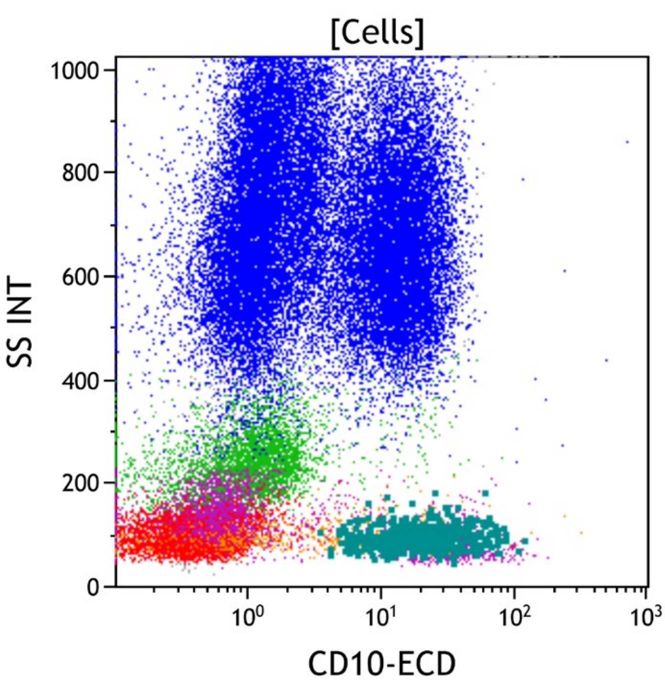


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple, lower right), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (teal) is positive for CD10.

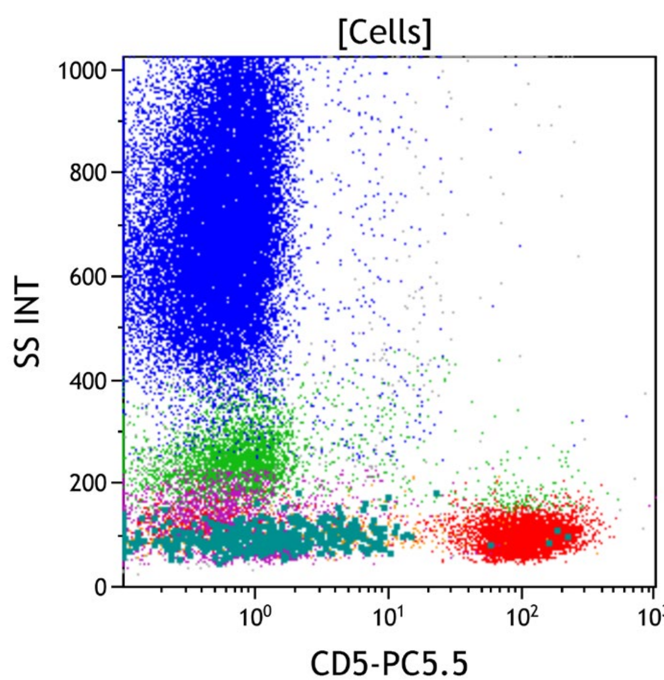


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The aberrant population (teal) is negative for CD5.

B Cell Tube

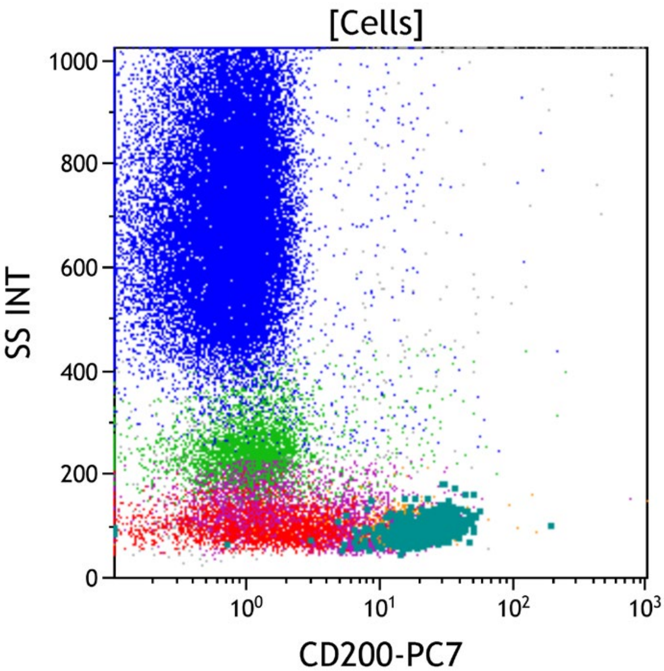


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The aberrant population (teal) is positive for CD200.

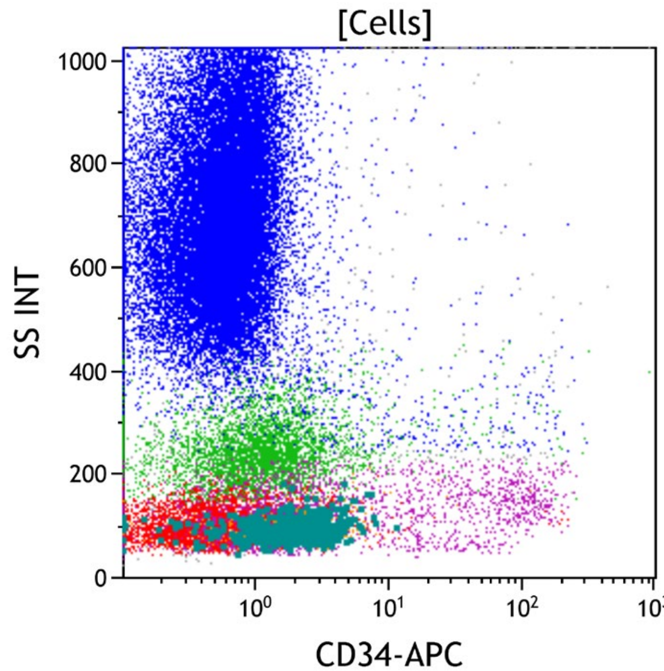


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The aberrant population (teal) is negative for CD34.

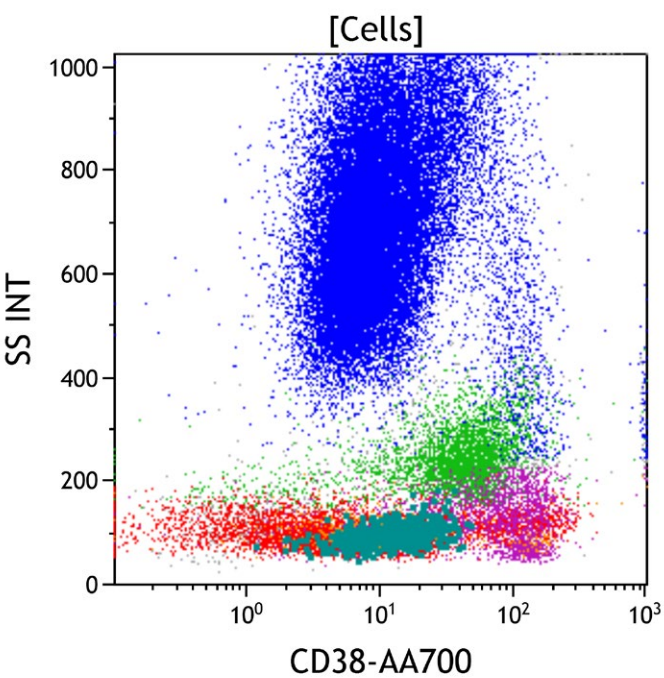


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The aberrant population (teal) displays intermediate CD38 expression.

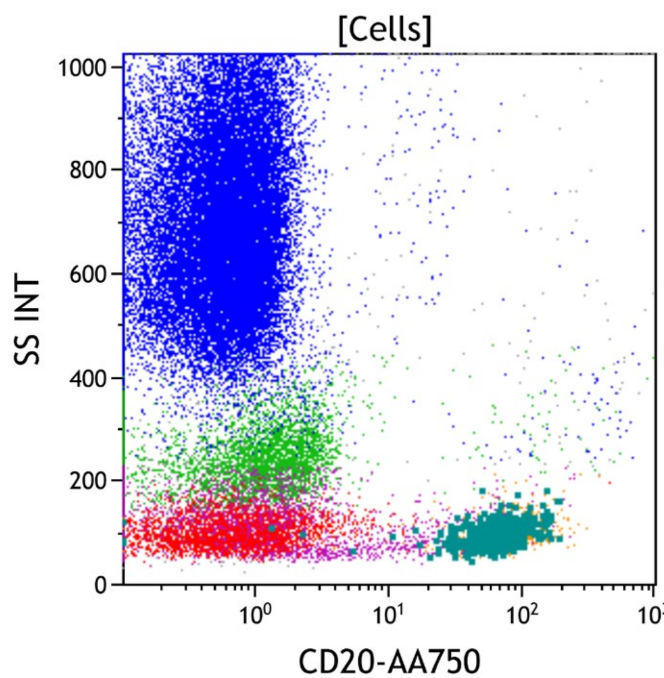


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The aberrant population (teal) is positive for CD20.

B Cell Tube

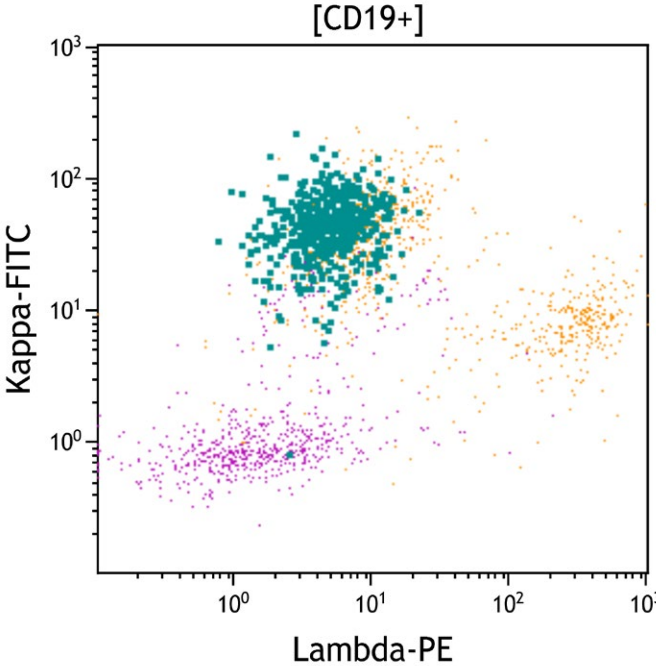


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The normal mature B cells (orange) are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. The aberrant population (teal) expresses only surface kappa light chains.

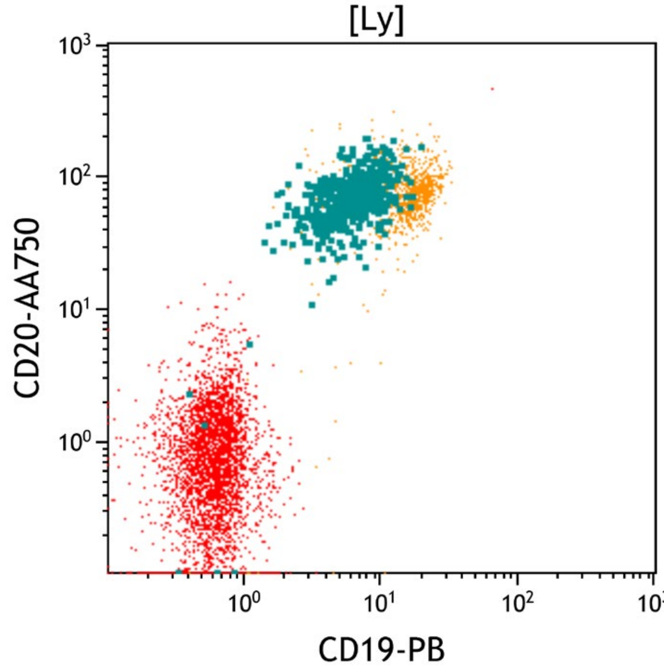


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. The aberrant population (teal) displays decreased CD19 expression compared with the higher level seen on normal mature B cells (orange).

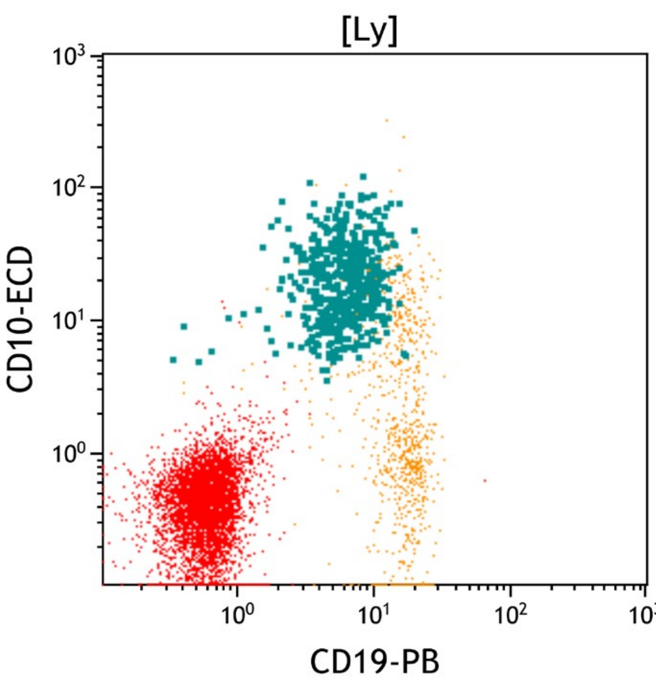


Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange and teal) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. The aberrant population (teal) is positive for CD10 and expresses CD19 at a lower level than that seen on normal B cells (orange).

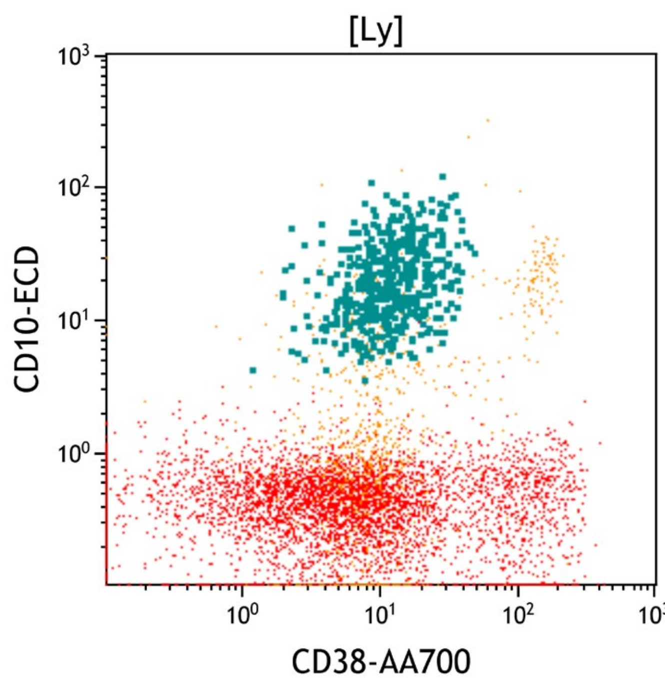


Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells, and at a low level on germinal center B cells. Most mature B cells display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The CD10 positive and CD38 positive cells (orange, upper right) represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. The aberrant population (teal) is positive for CD10 and CD38.

B Cell Tube

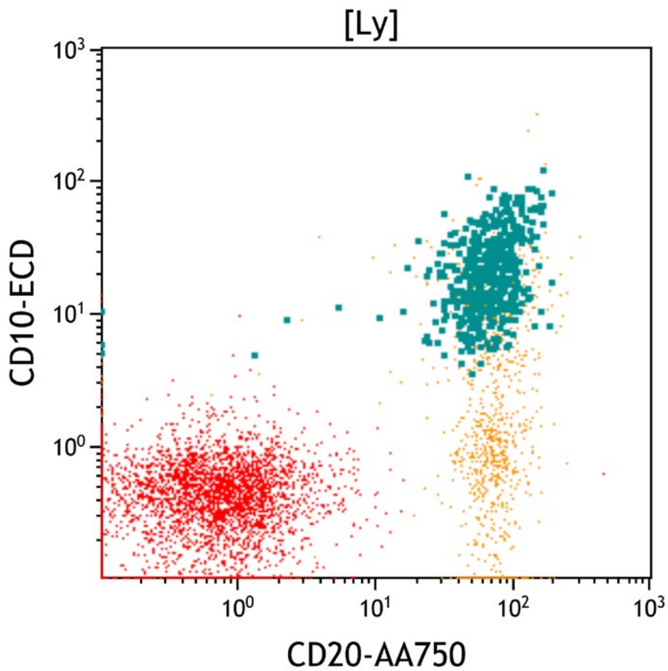


Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange, lower right) uniformly express high level CD20. The aberrant population (teal) is positive for CD10 and CD20. Compared with normal germinal center B cells, the level of CD10 is increased.

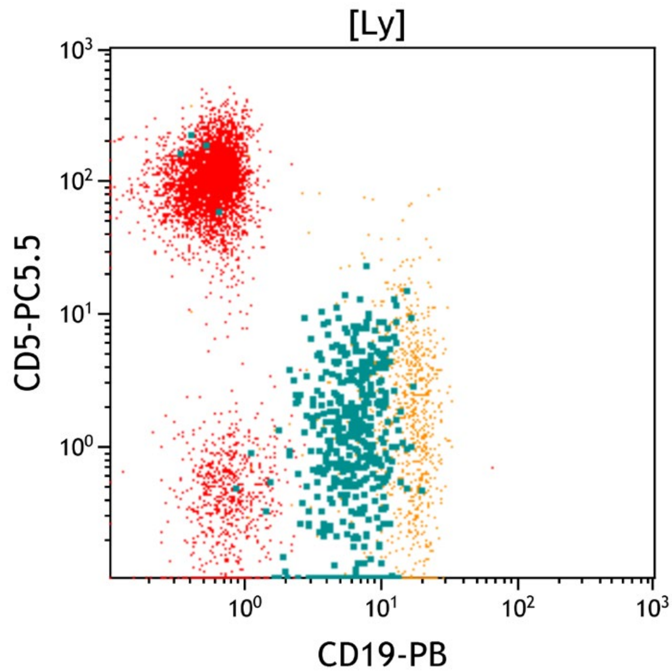


Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The aberrant population (teal) expresses CD19 at a lower level than that seen on normal B cells (orange) and is negative for CD5.

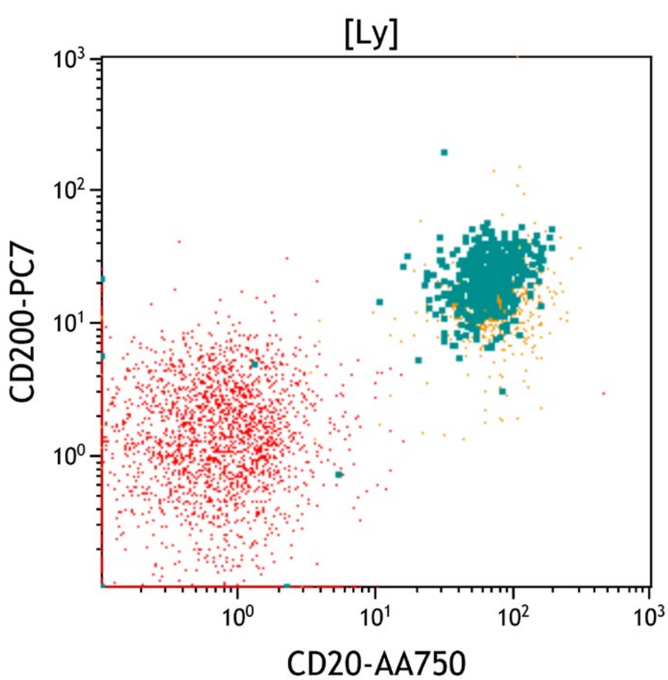


Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange). The aberrant population (teal) is positive for CD20 and CD200.

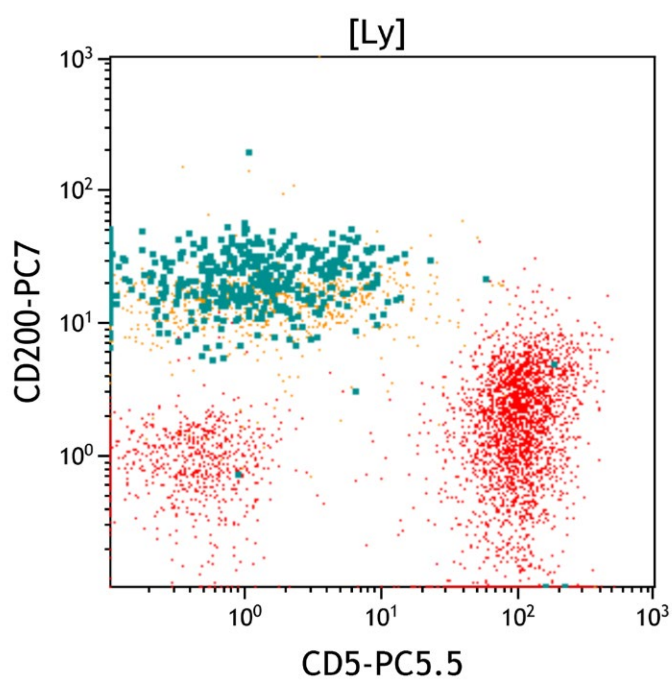


Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange)normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The aberrant population (teal) is positive for CD200 and negative for CD5.

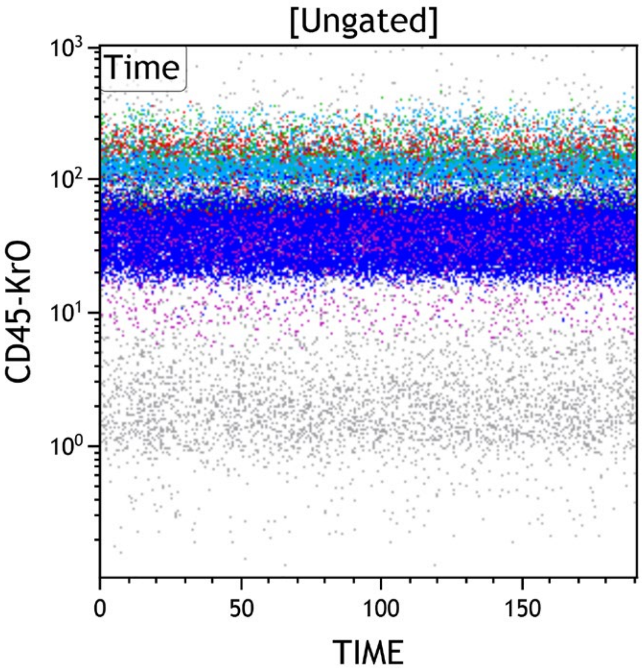


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

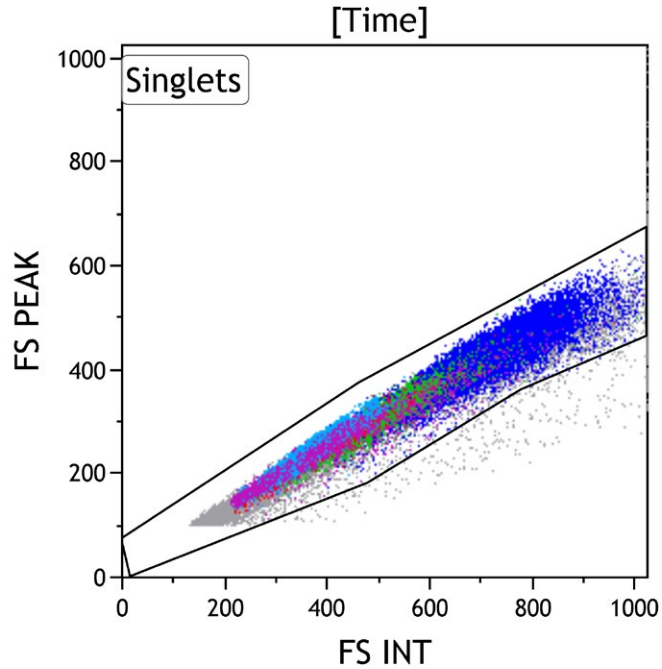


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

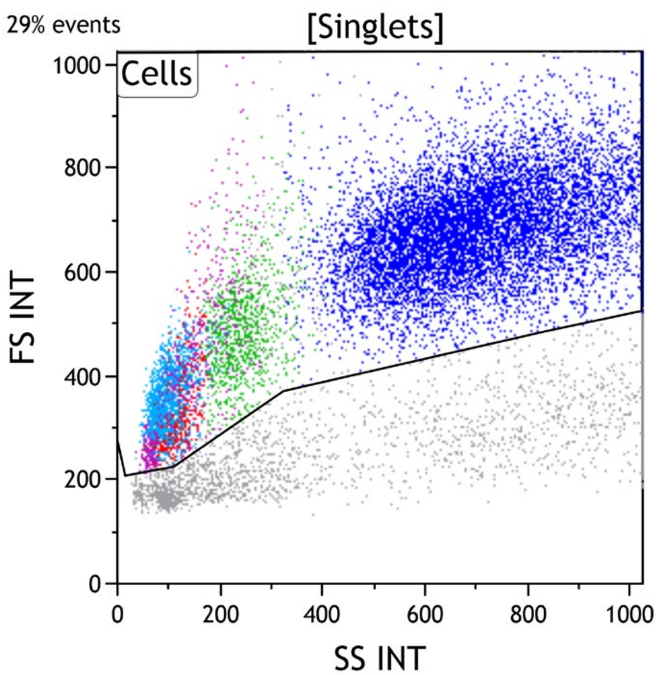


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

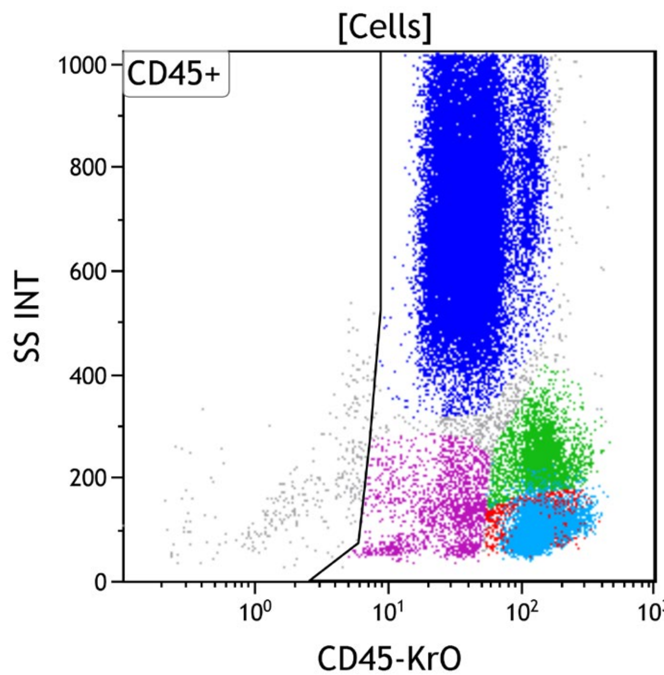


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

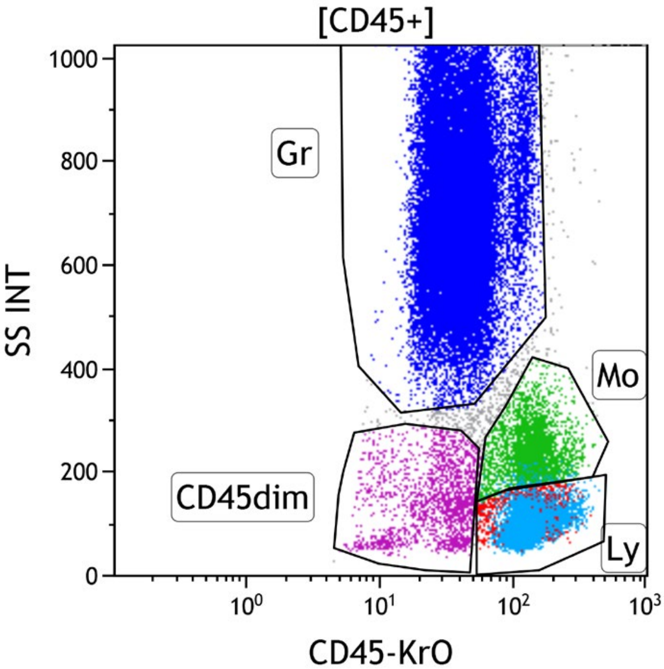


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

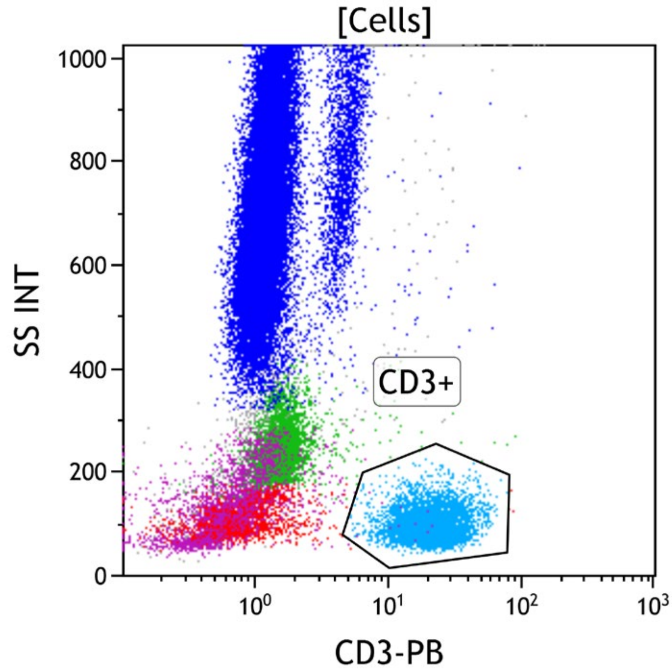


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

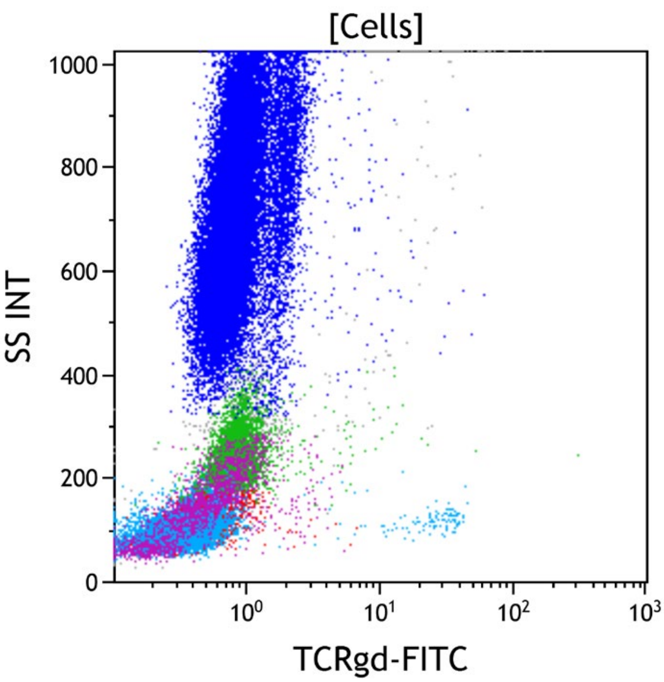


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua, lower right).

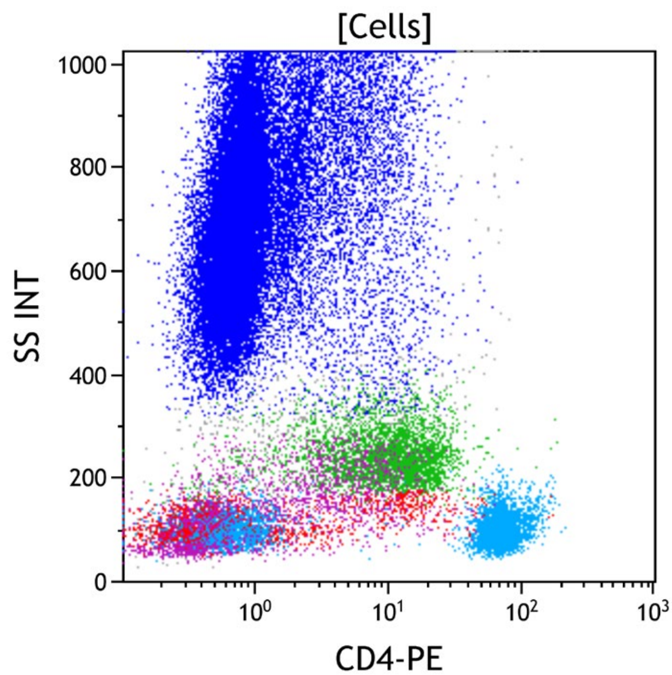


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua, lower right). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

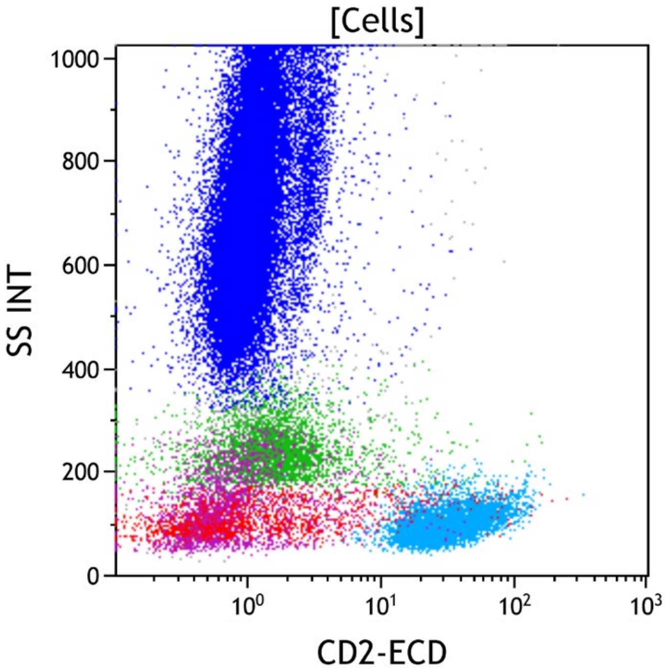


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

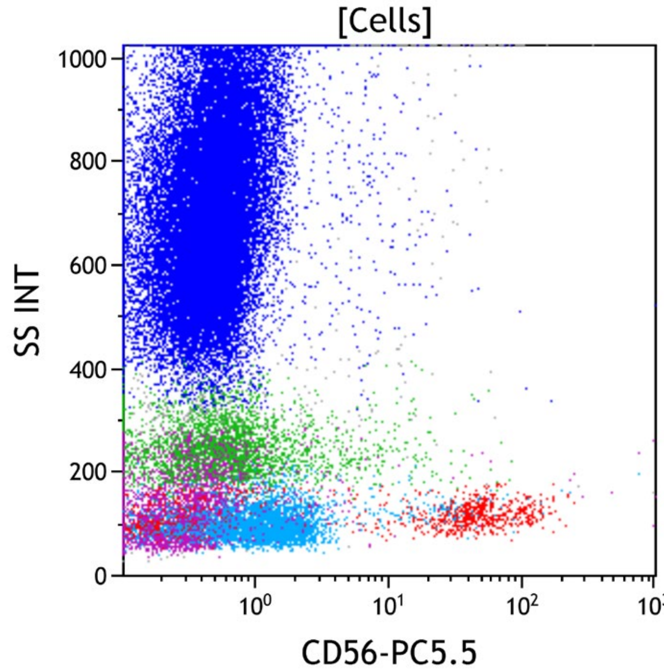


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red, lower right), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells. CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

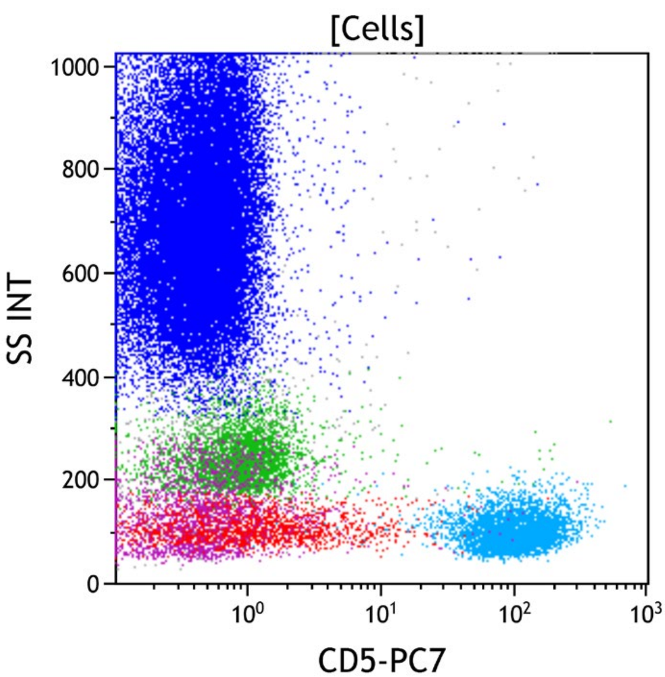


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

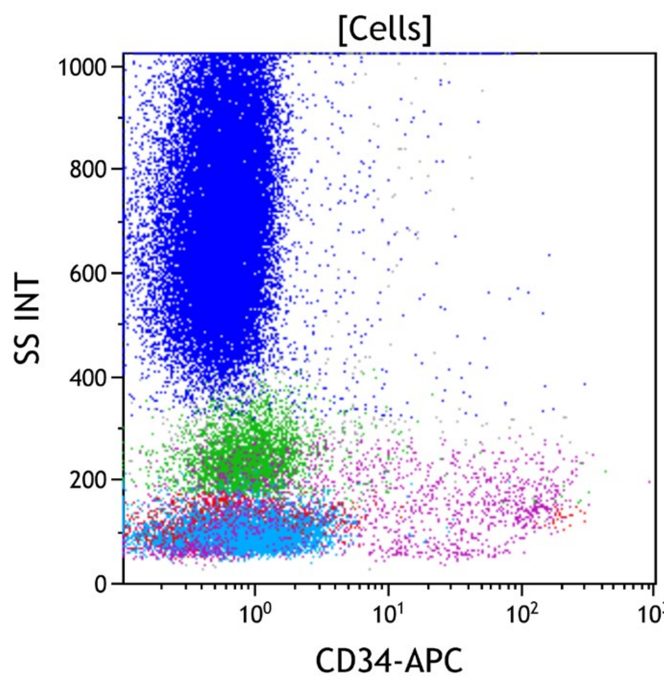


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

T Cell Tube

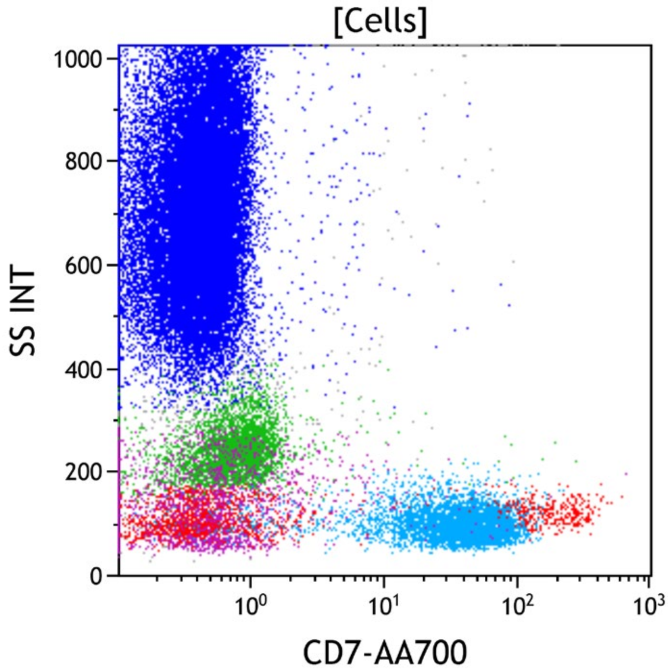


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

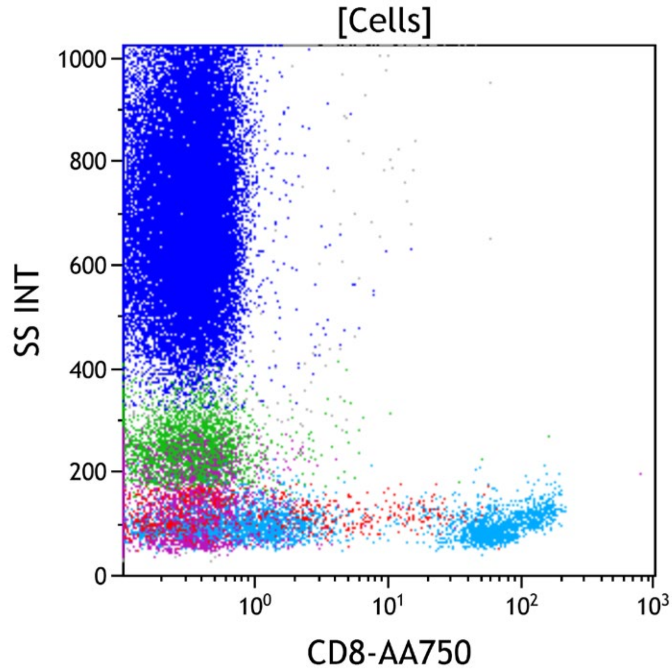


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua, lower right) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

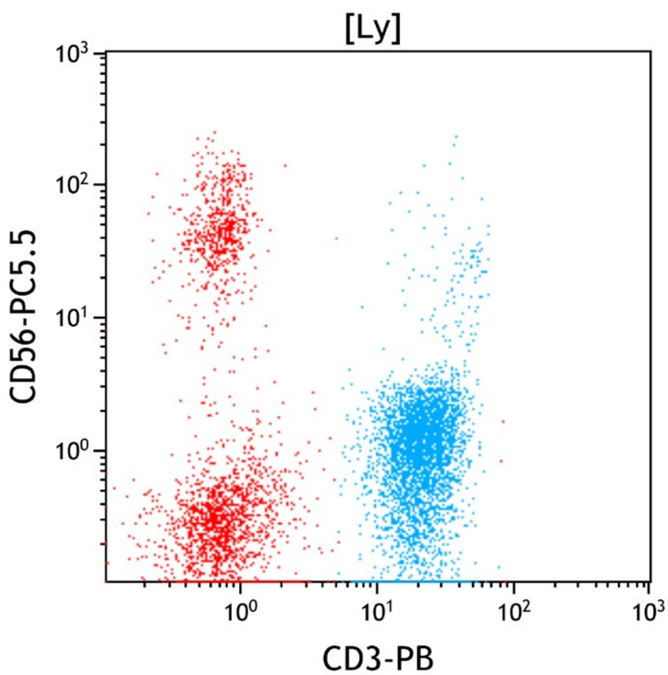


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells (aqua) are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells (aqua, upper right) also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

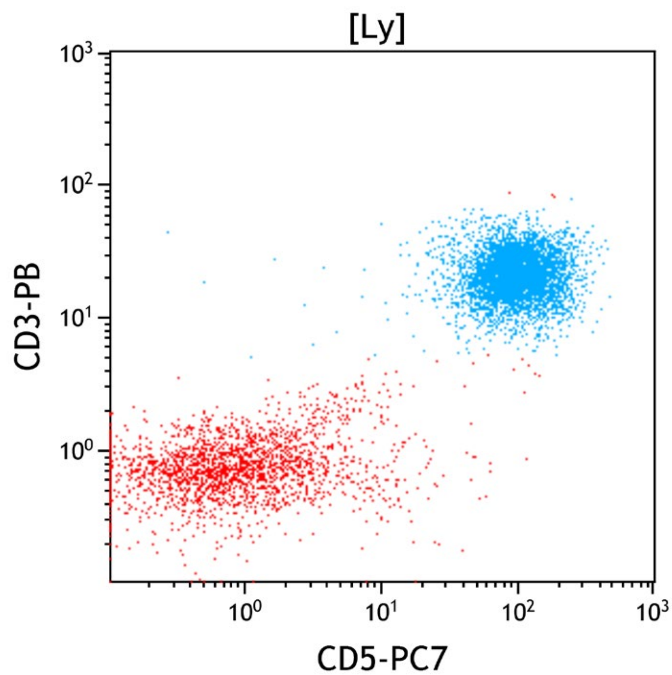


Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells and B cells (red).

T Cell Tube

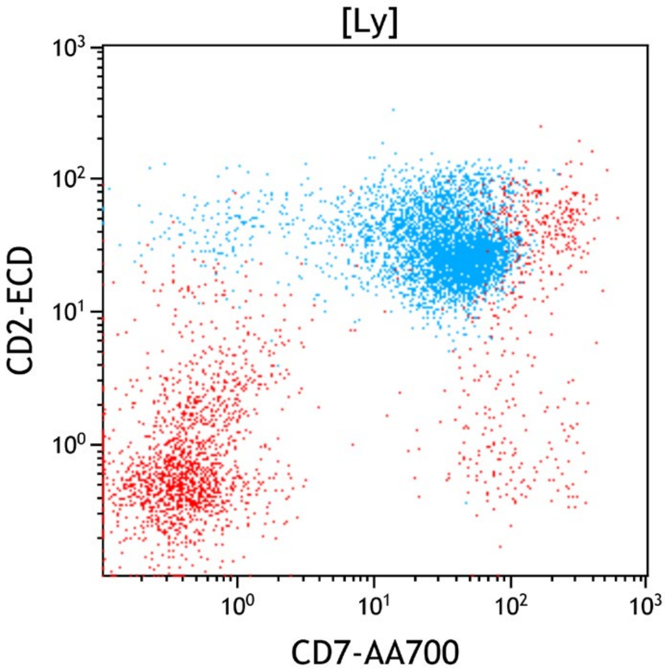


Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

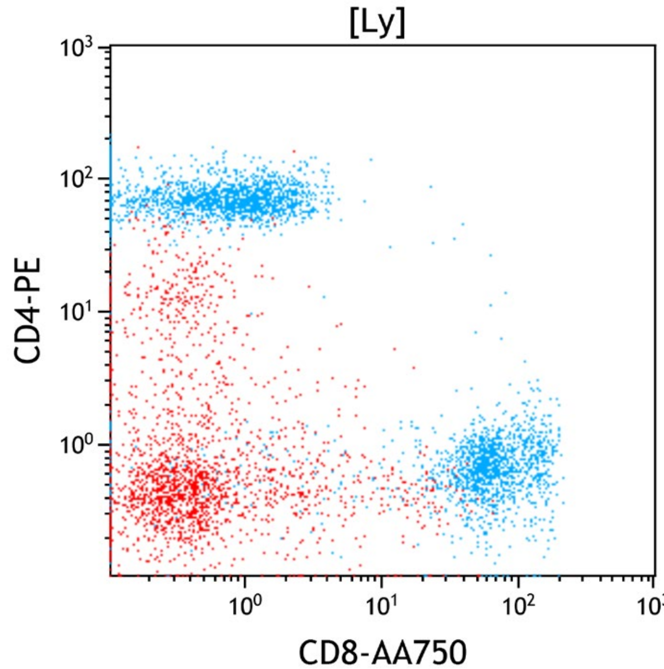


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, upper left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

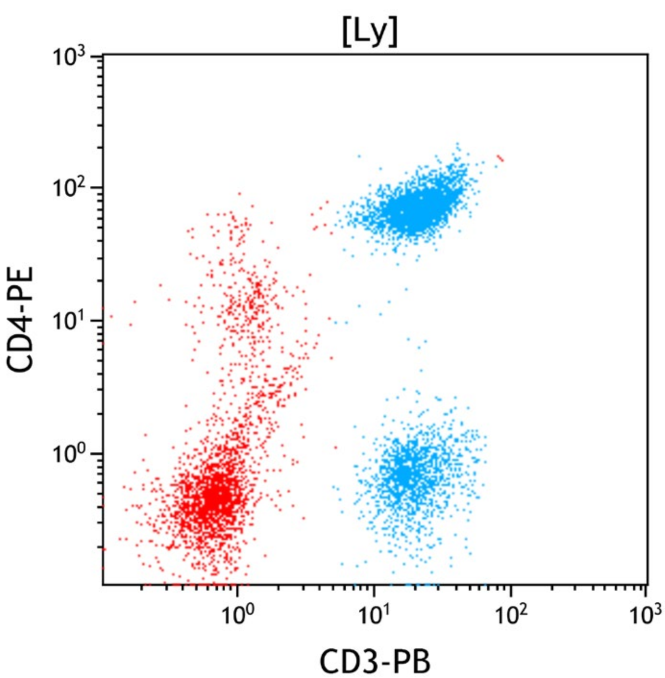


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes (red, upper left) and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

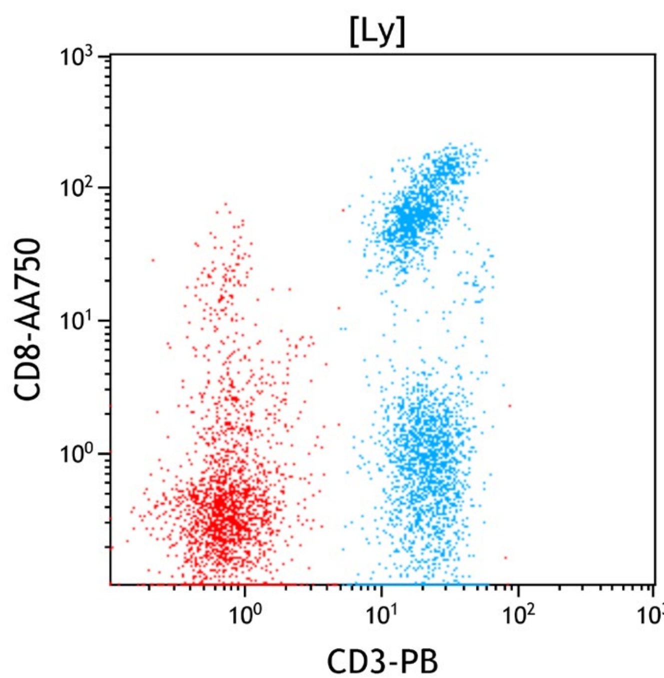


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

T Cell Tube

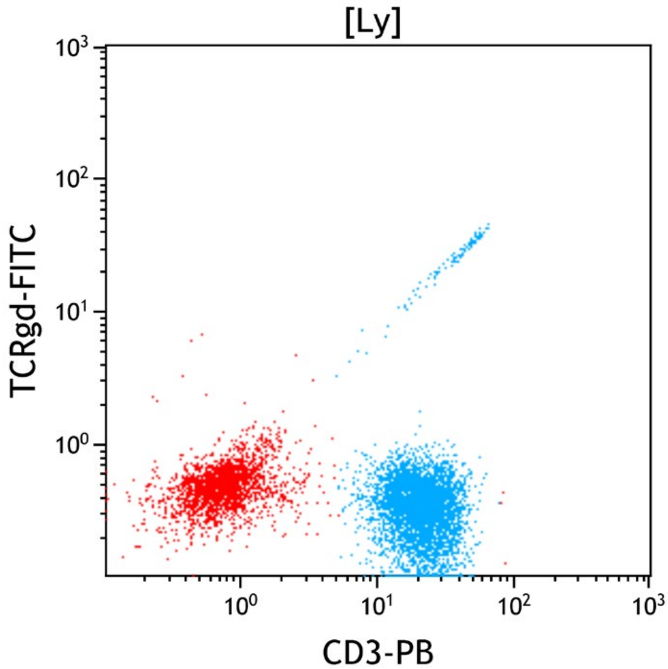
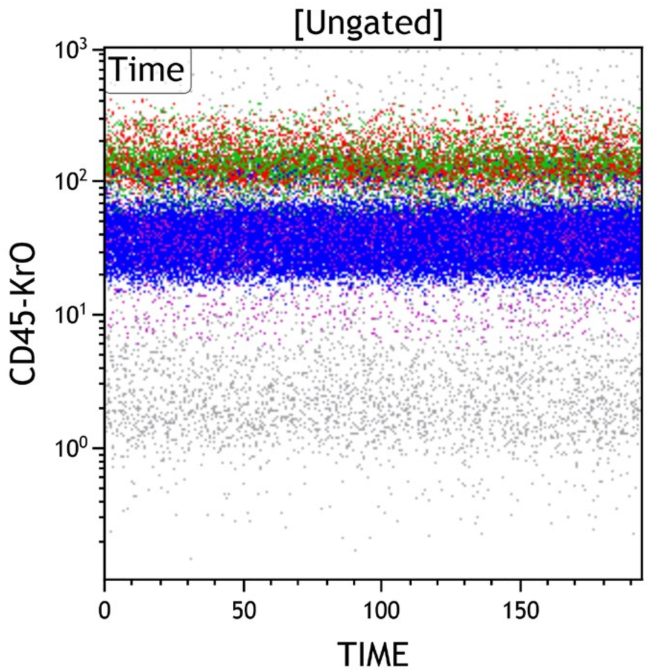


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube



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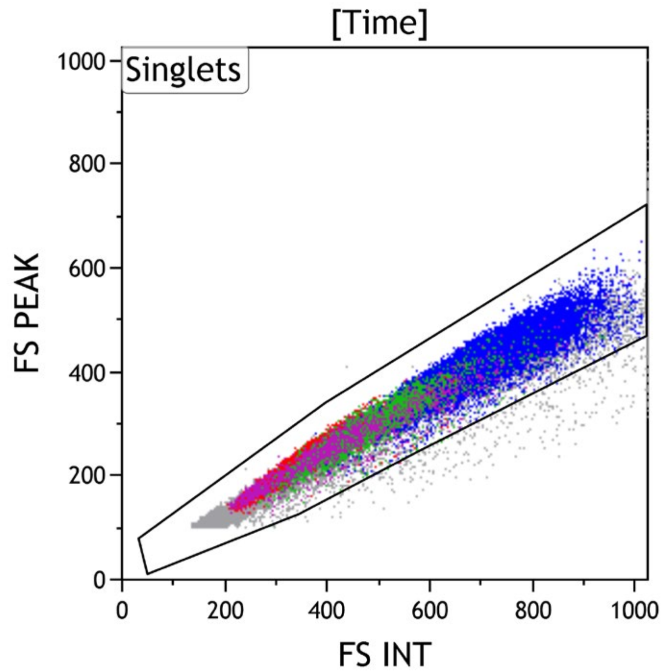

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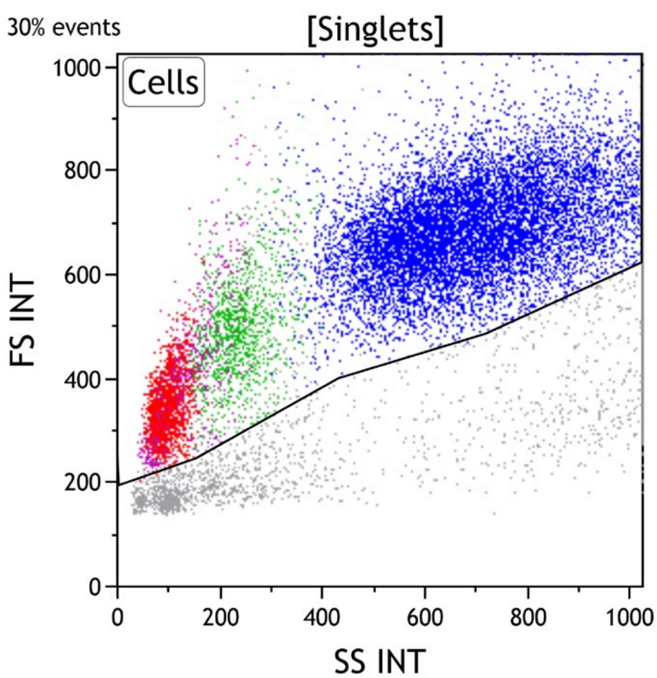


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

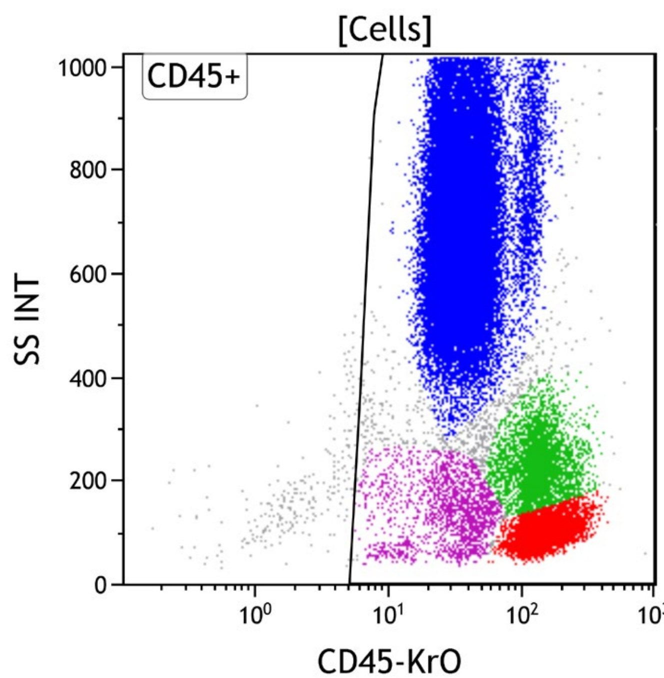
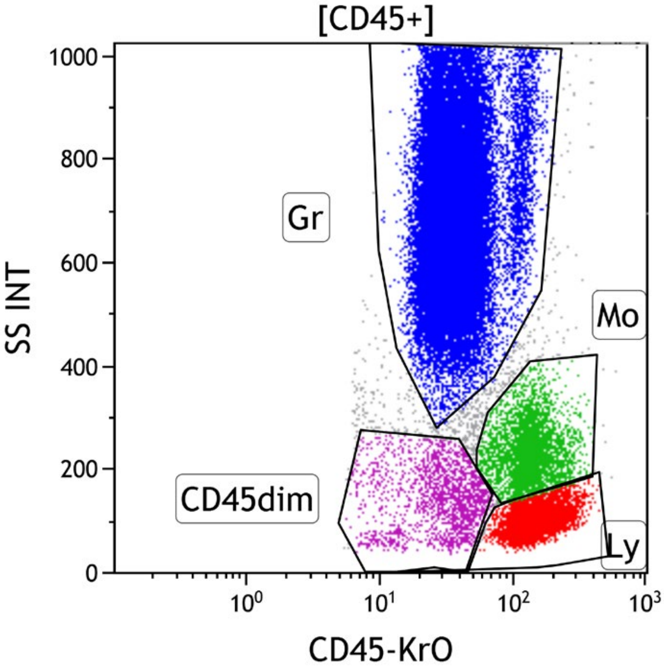


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

Table of Contents > Neoplastic Process of B-cell Origin > Case #11: B-cell lymphoma of germinal center origin



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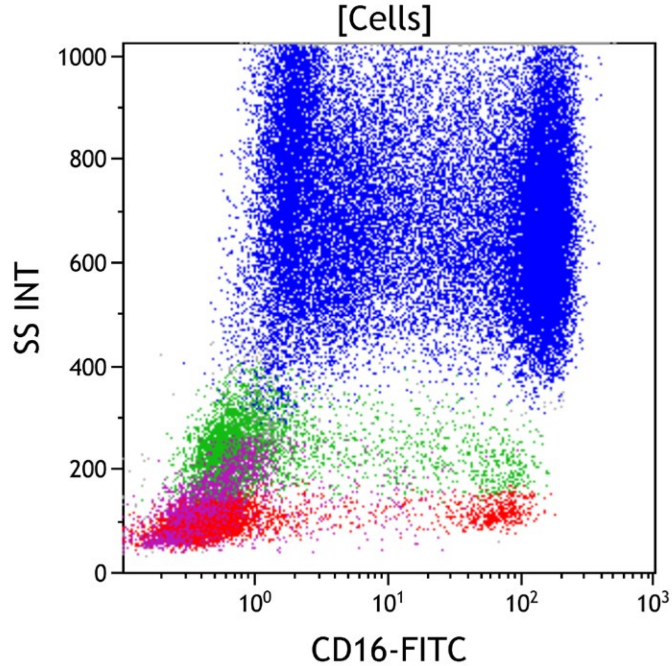

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

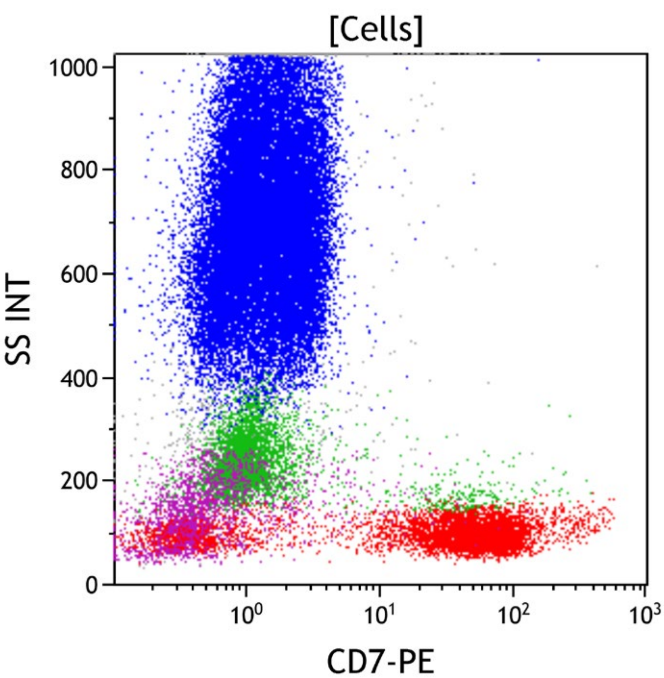

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

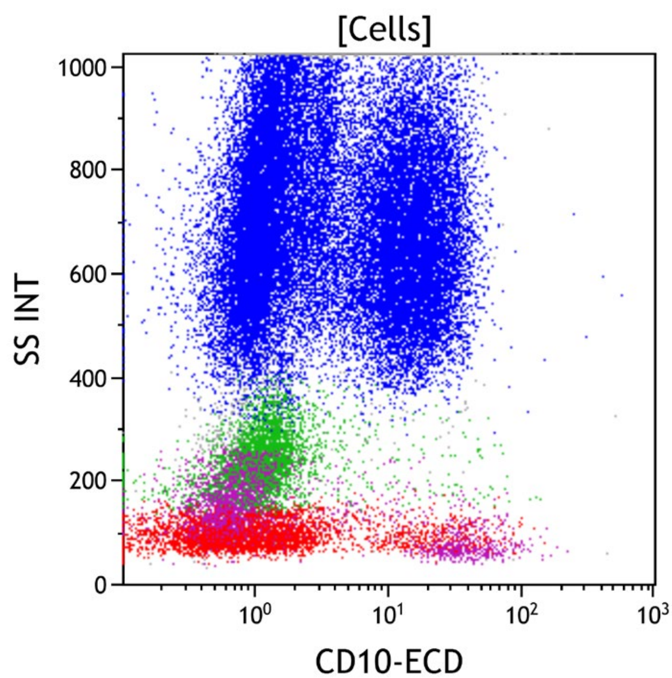

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple, lower right), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

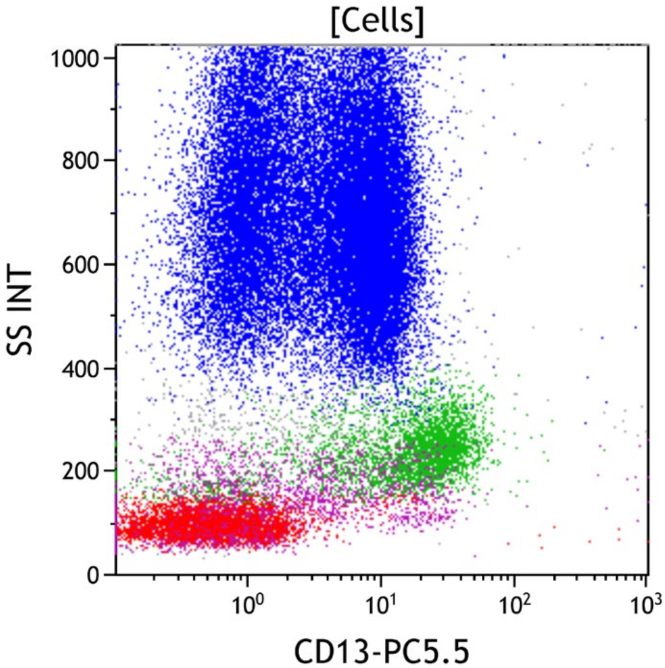

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

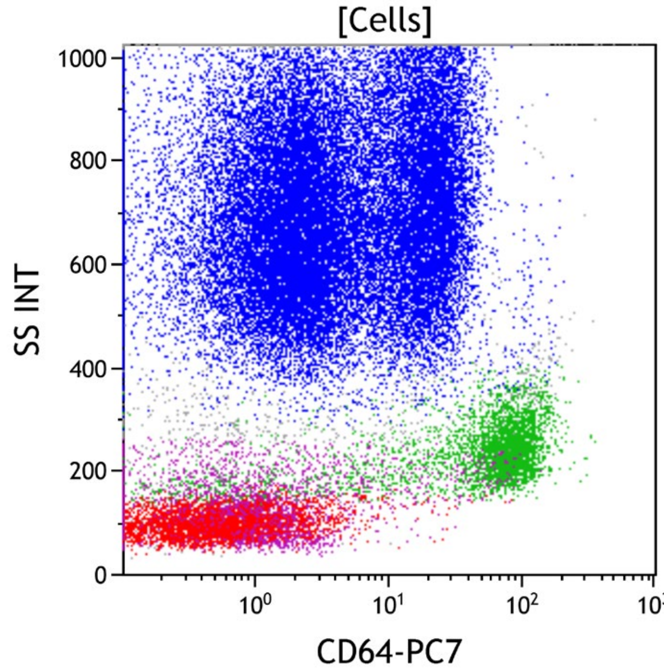

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells (red) or most CD34 positive progenitors.

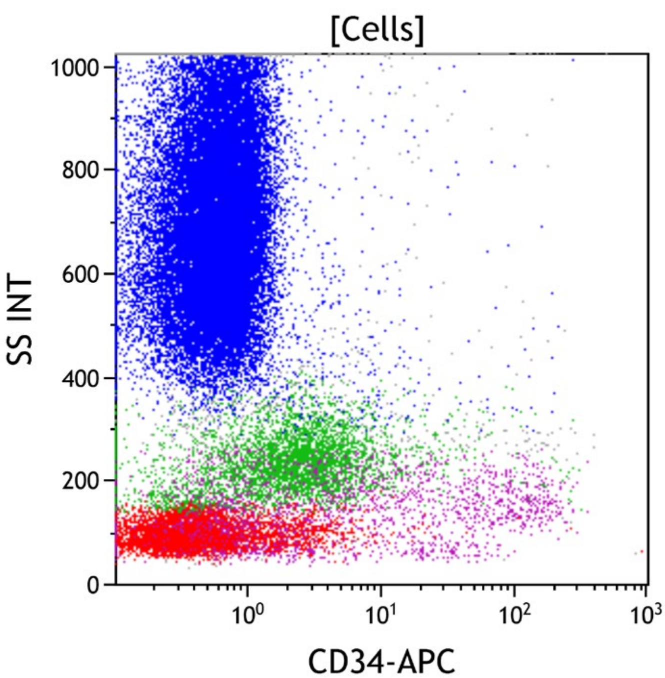

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34.

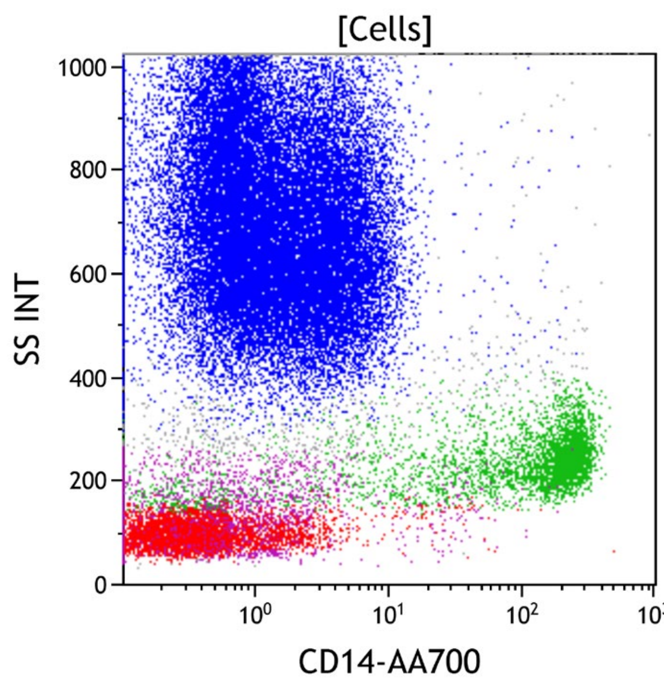

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes (blue) at a low level.

M1 Cell Tube

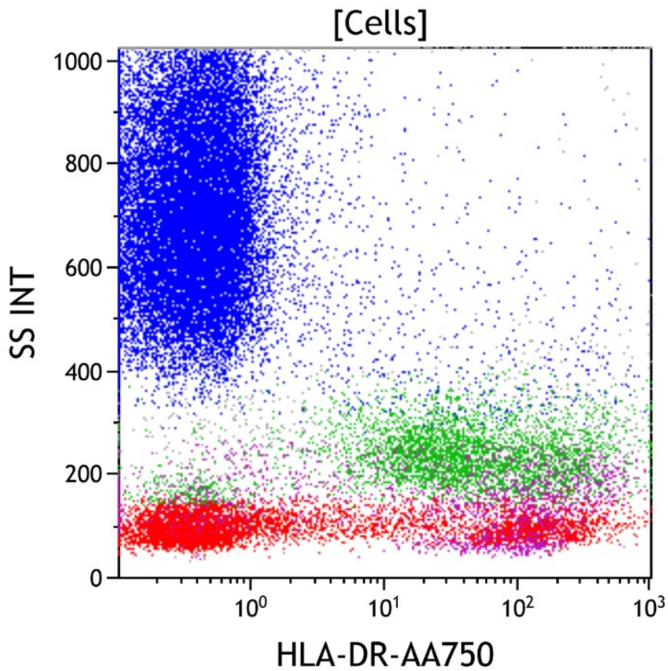

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red, lower right) and immature (purple) B cells, and activated T cells.

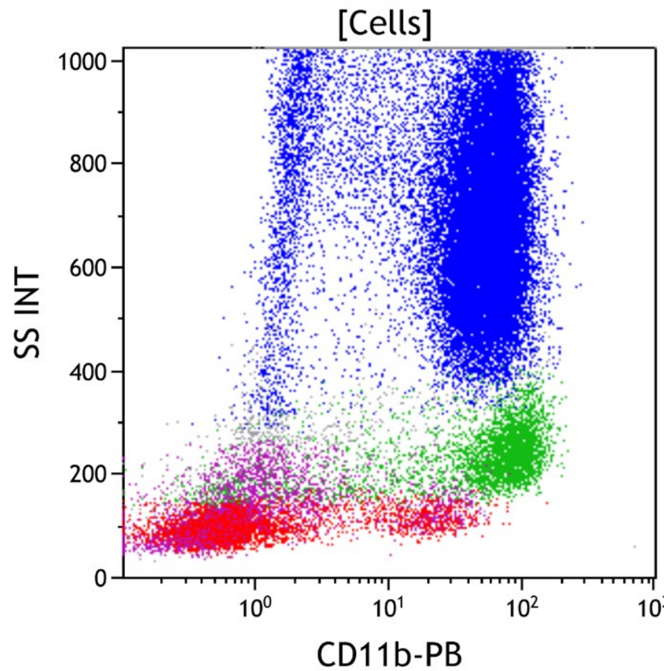

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils (purple, lower right).

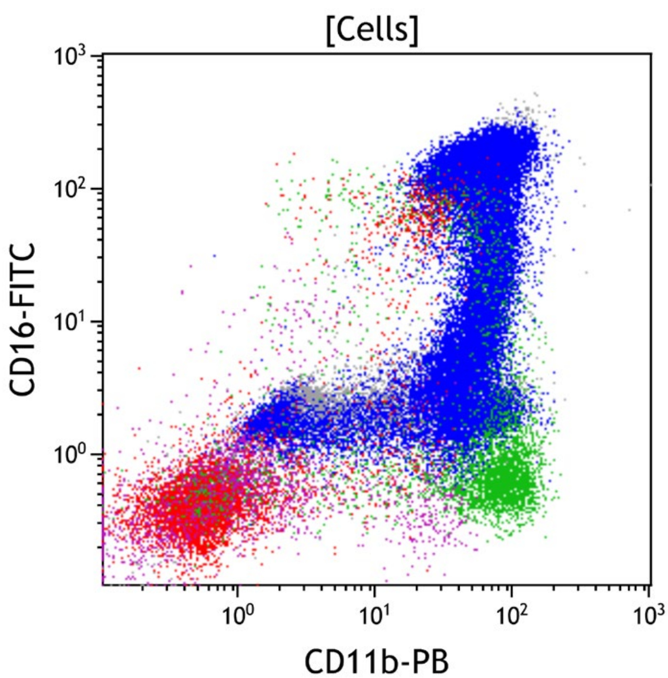

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (in green), immature and mature granulocytes (blue) and NK cells (red, upper right). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes (blue, upper right), where it is expressed at its highest level.

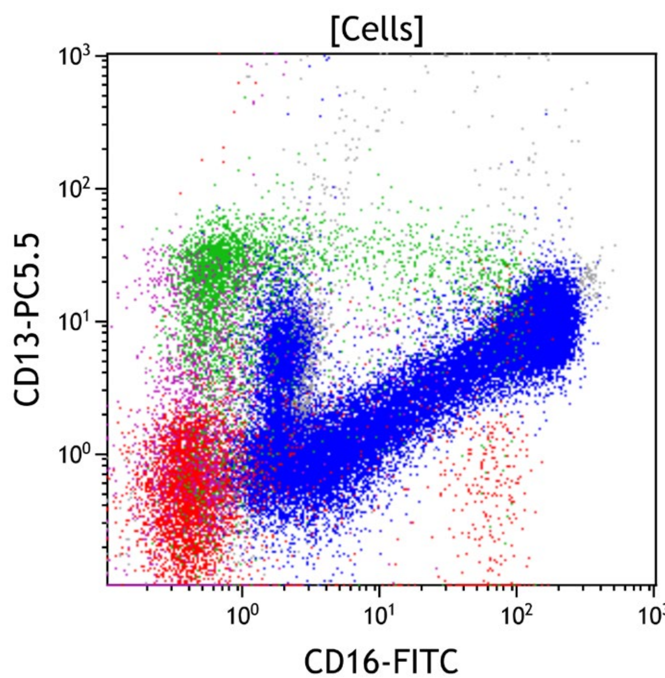

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, upper left) and lose CD13 as they mature to myelocytes (blue, lower left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue, upper right).

M1 Cell Tube

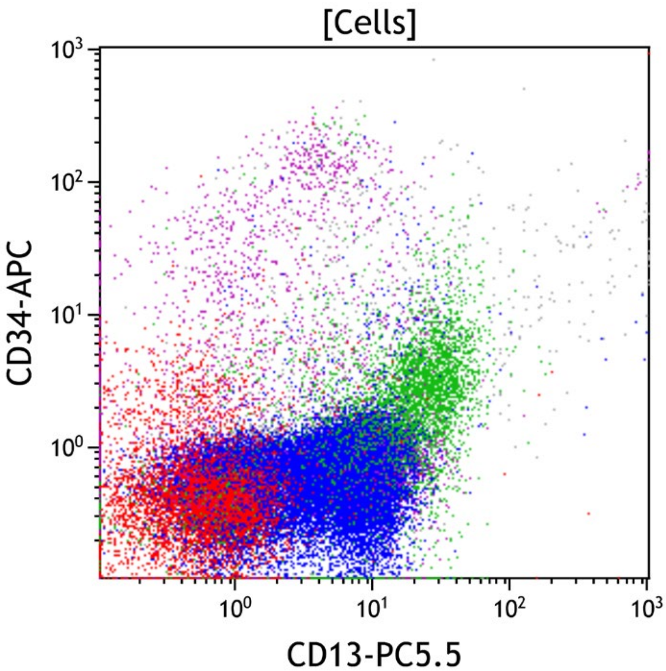

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells (red).

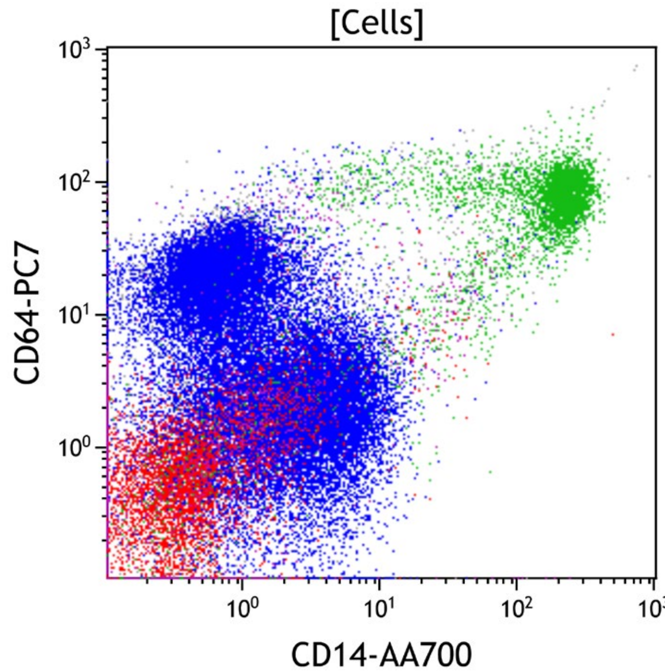

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (green, upper left) and progressively acquire CD14 during maturation to mature monocytes while retaining high level CD64 (green, upper right). Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left).

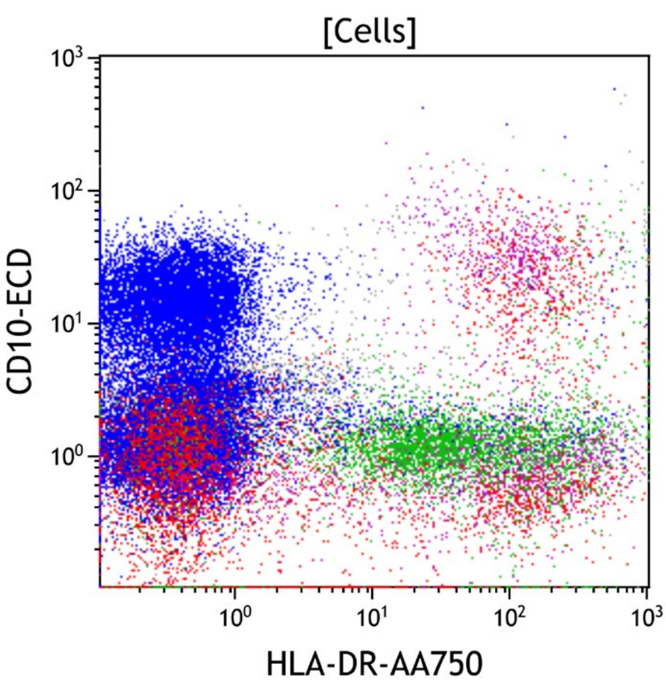

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells (Red, lower right), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple, upper right).

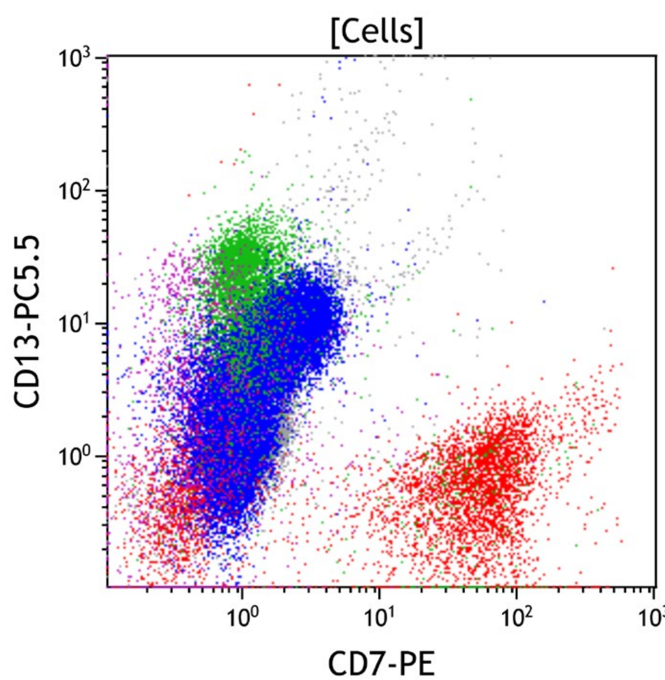

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

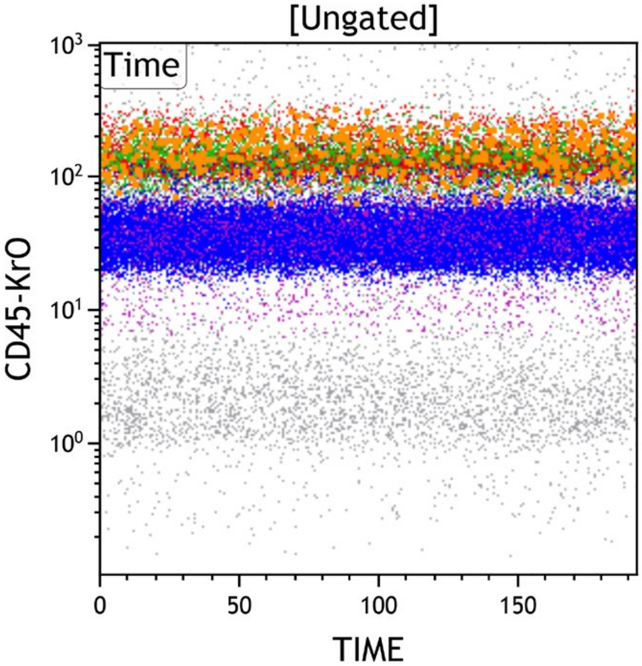

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

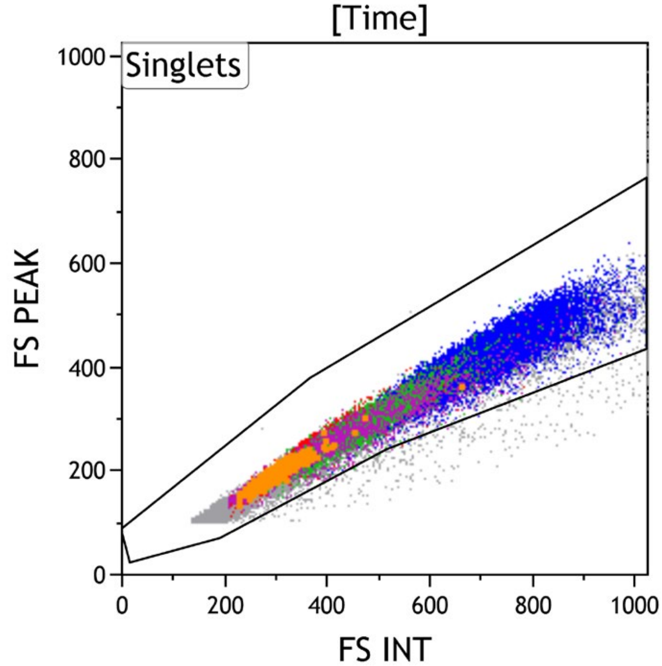

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

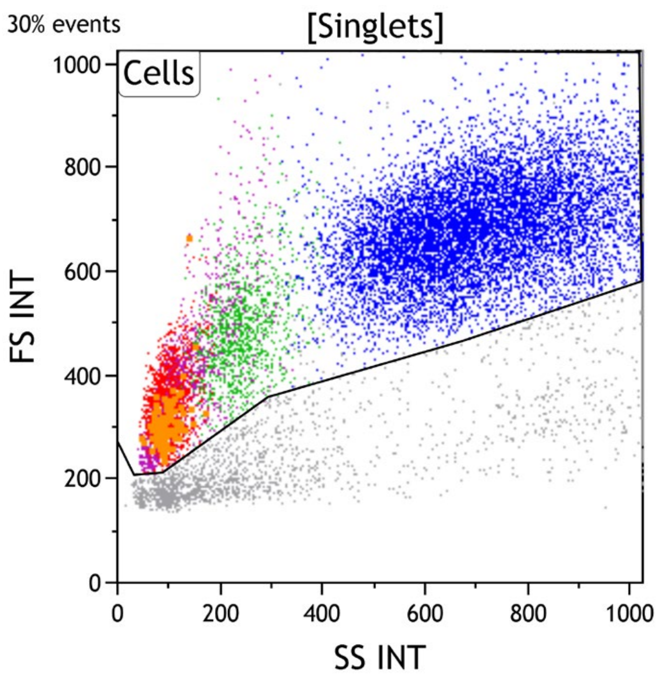

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

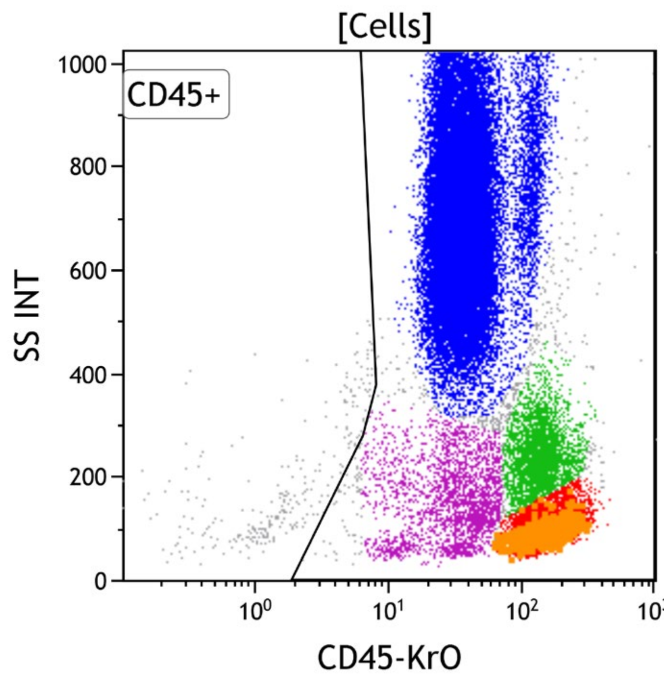

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

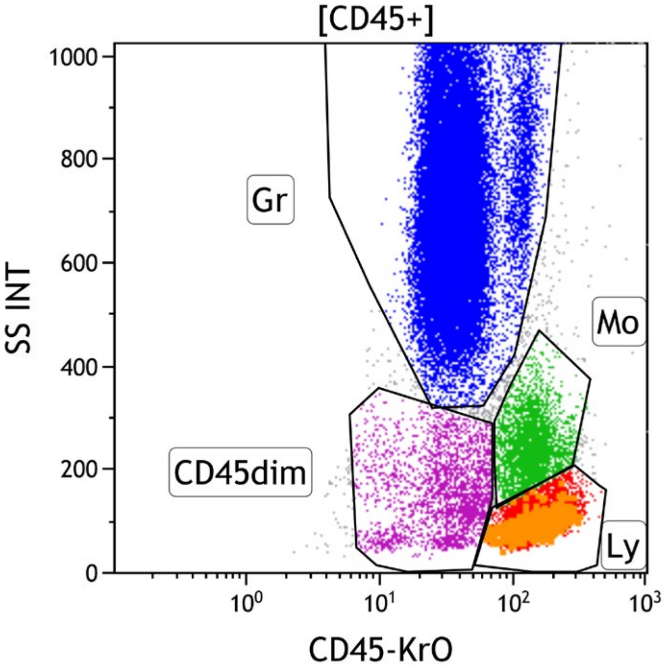

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

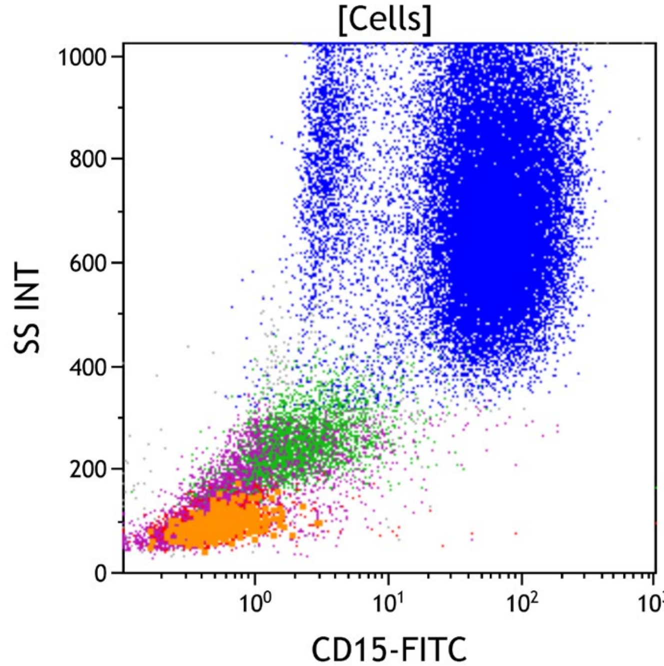

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The aberrant population (orange) is negative for CD15.

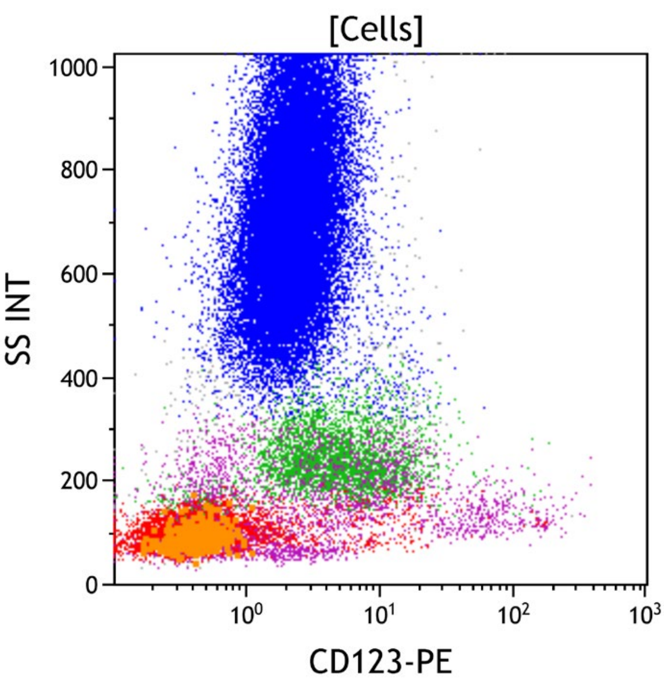

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors (purple, lower right) and monocytes (green). The aberrant population (orange) is negative for CD123.

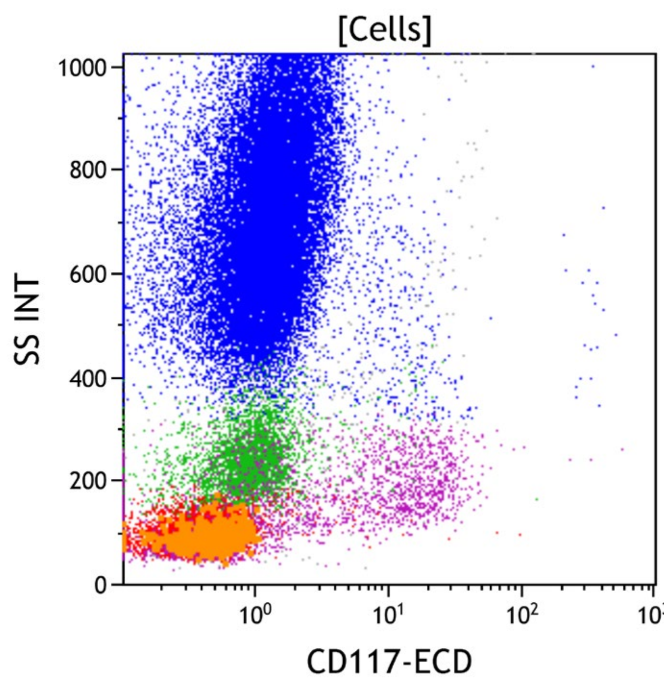

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors (purple), early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue, right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The aberrant population (orange) is negative for CD117.

M2 Cell Tube

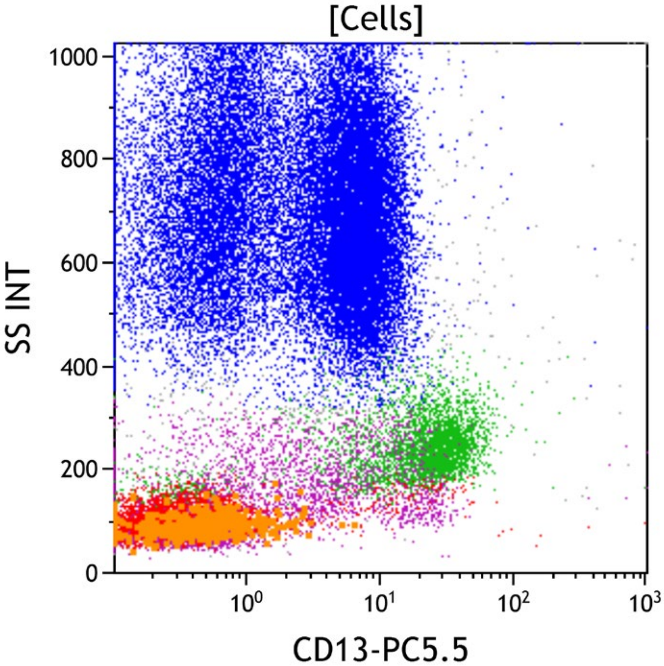

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple). The aberrant population (orange) is negative for CD13.

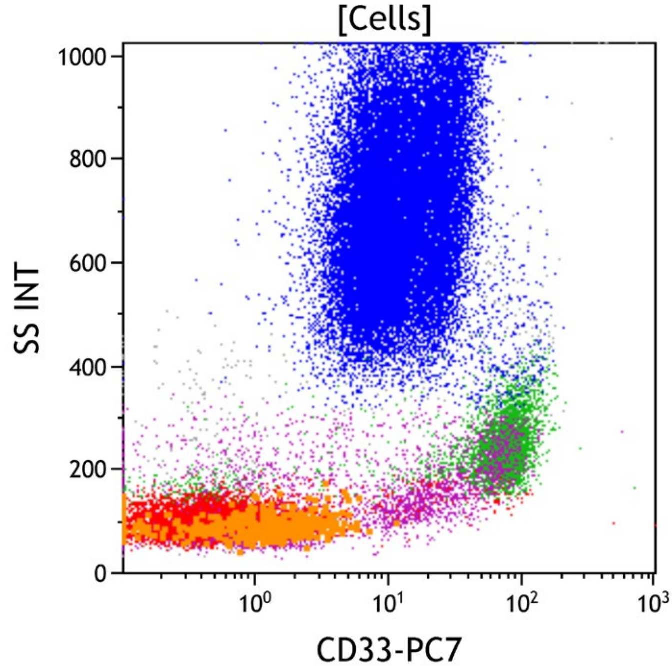

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors (purple). The aberrant population (orange) is negative for CD33.

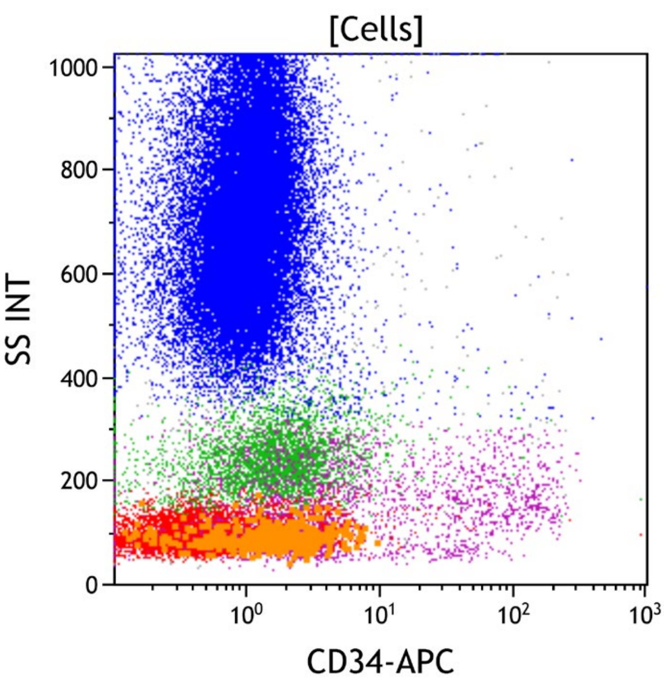

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45 dim gate (purple). The aberrant population (orange) is negative for CD34.

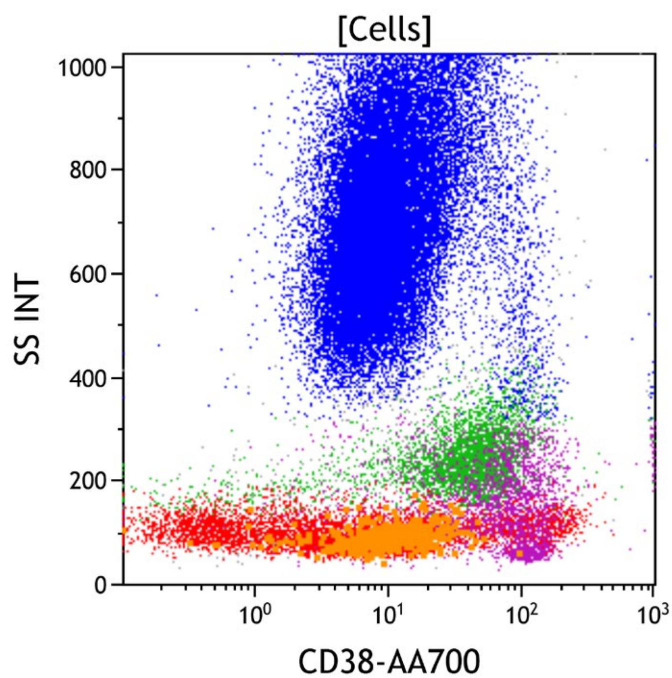

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The aberrant population (orange) displays intermediate CD38 expression.

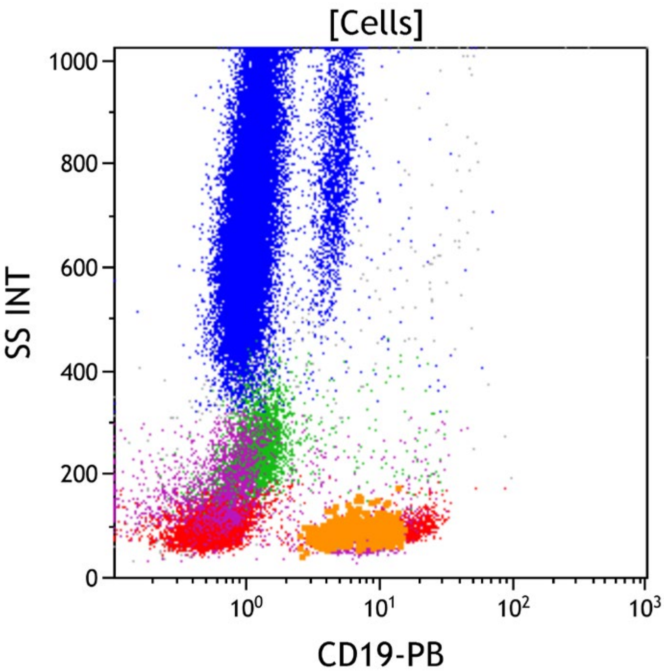

Figure 13: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (orange) is positive for CD19 at a lower level than that seen on normal B cells (red, lower middle).

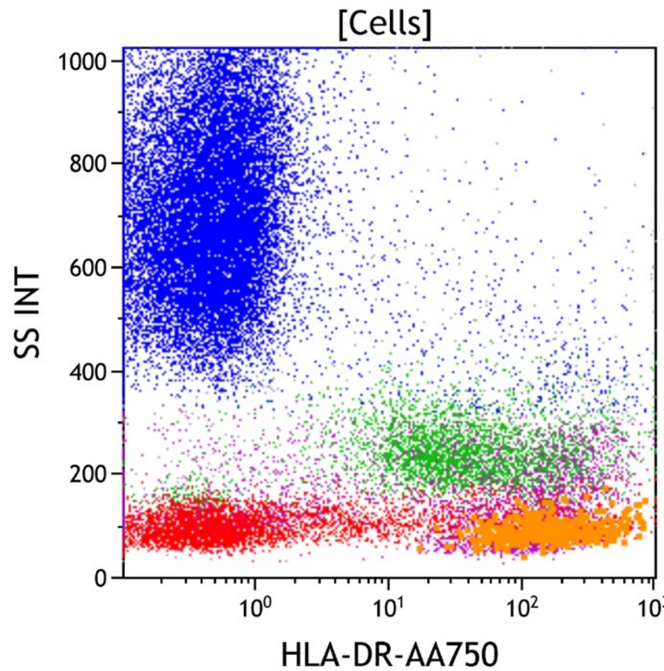

Figure 14: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature and immature B cells, and activated T cells (red, lower right). The aberrant population (orange) is positive for HLA-DR.

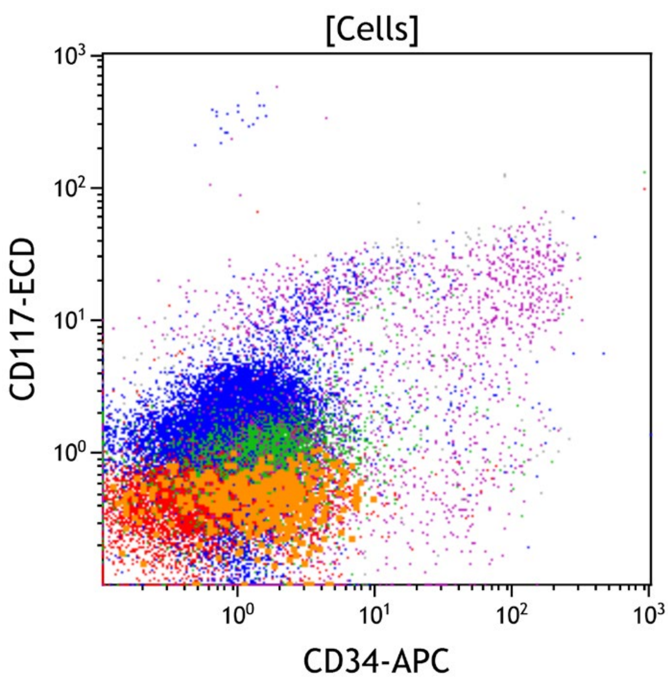

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (purple, upper right), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (purple, lower right). The aberrant population (orange) is negative for CD34 or CD117.

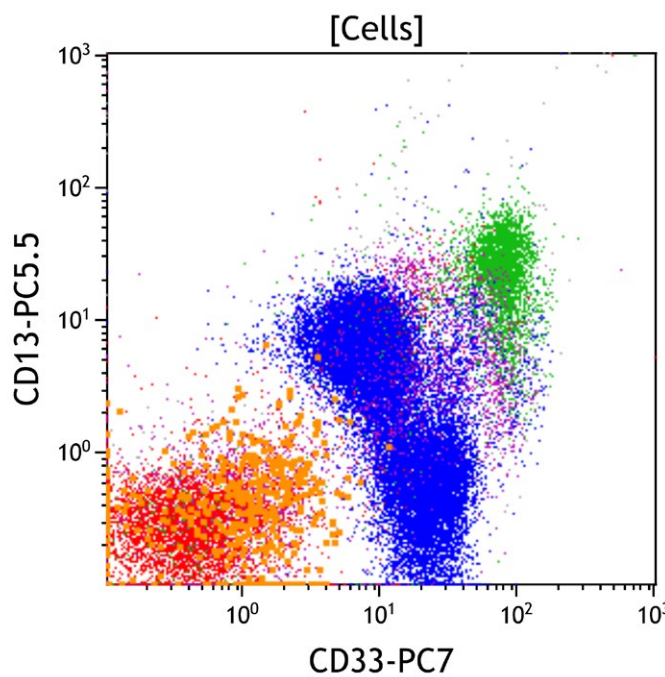

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue, bottom) than more mature granulocytes (blue, upper left). Lymphocytes largely do not express either CD13 or CD33 (red). The aberrant population (orange) is negative for CD13 or CD33.

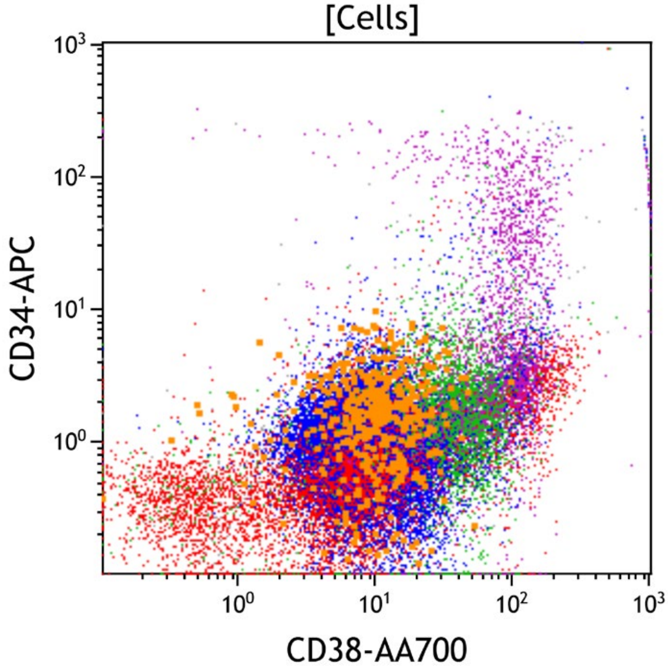

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple). The aberrant population (orange) displays intermediate CD38 expression and is negative for CD34.

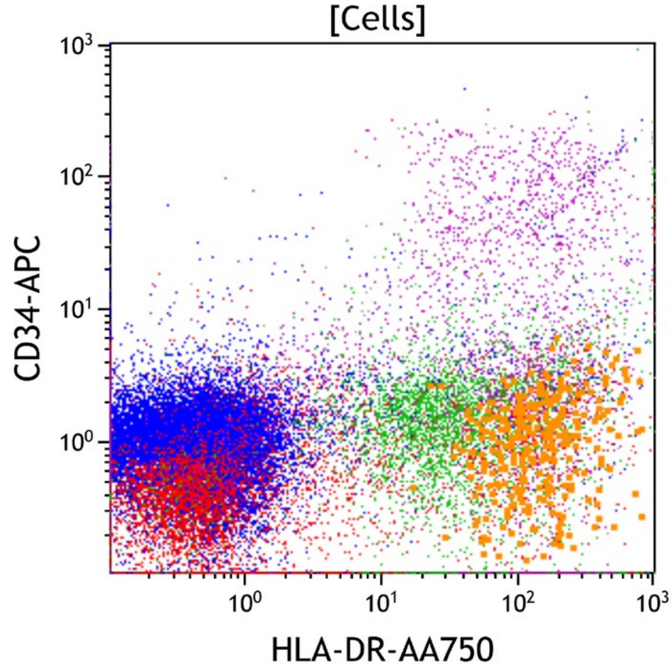

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes. The aberrant population (orange) is positive for HLA-DR and negative for CD34.

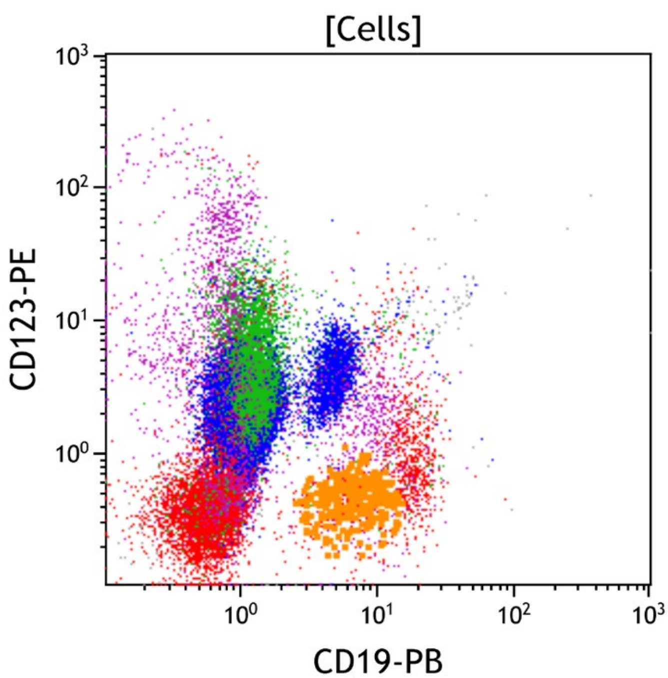

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors (purple). CD19 positive B cells normally do not express significant CD123. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (orange) is positive for CD19 at a lower level than that seen on normal B cells (red, lower middle) and negative for CD123.

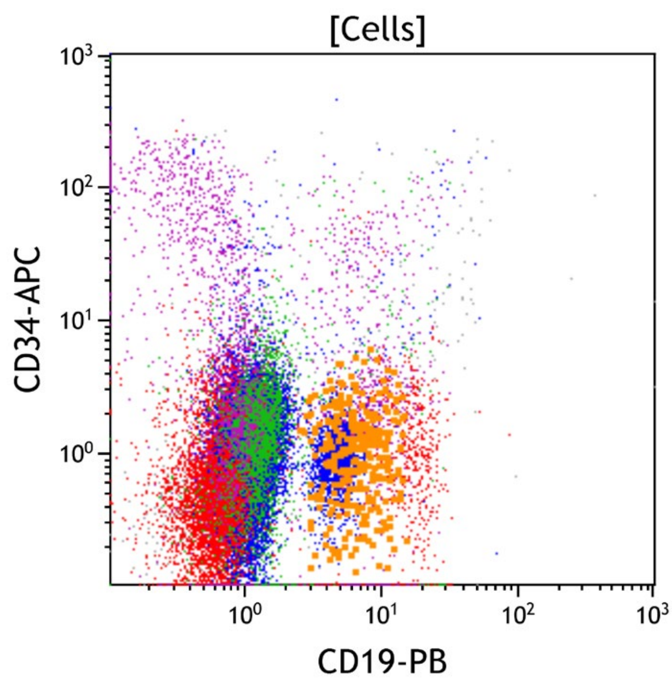

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors (purple, upper left) do not express CD19. The aberrant population (orange) is positive for CD19 at a lower level than that seen on normal B cells (red, lower middle) and negative for CD34.

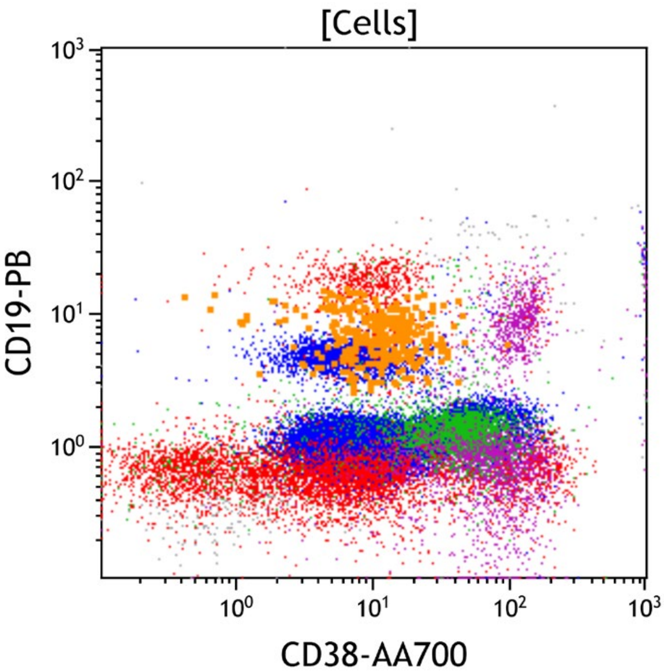

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Mature CD19 positive B cells show lower expression of CD38 (red, upper left). Plasma cells show extremely high CD38 expression that is largely off scale (purple, extreme right), but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (orange) is positive for CD19 at a lower level than that seen on normal B cells (red, upper middle) with intermediate CD38.

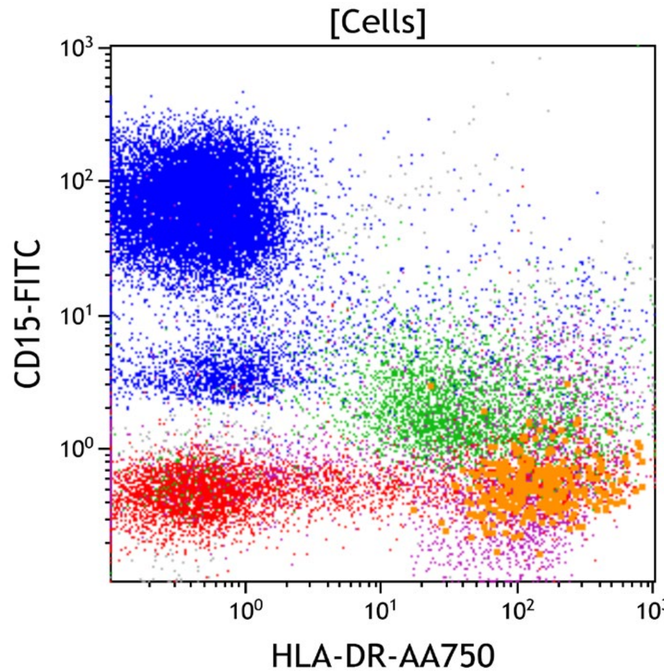

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors (purple) express HLA-DR but only transiently express CD15. The aberrant population (orange) is positive HLA-DR and negative for CD15.

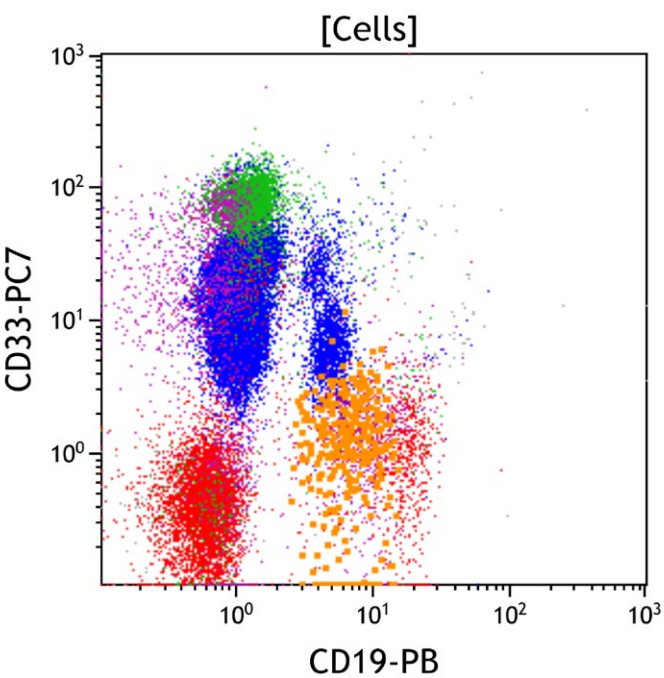

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple, lower right). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (orange) is positive for CD19 at a lower level than that seen on normal B cells (red, lower middle) and negative for CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate to bright CD10, intermediate CD19, bright CD20, intermediate CD38, bright CD45, intermediate CD200, and intermediate surface kappa light chain expression without CD5 or other T or myeloid markers. Compared with normal germinal center B cells, the increased expression of CD10, decreased CD19, and kappa light chain restriction are aberrant.

Taken together, the immunophenotype of the aberrant population is most consistent with B cell lymphoma of germinal center origin, such as follicular lymphoma or diffuse large B cell lymphoma. Definitive diagnosis and classification requires correlation with morphologic findings. Correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

Case #12: Mature B-cell lymphoma

Clinical Vignette

This 71-year-old male presents with leukopenia and organomegaly. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

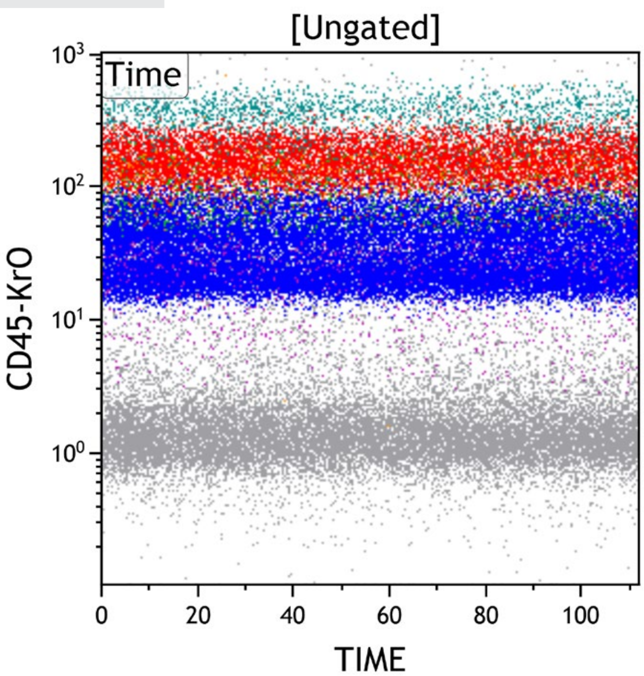

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

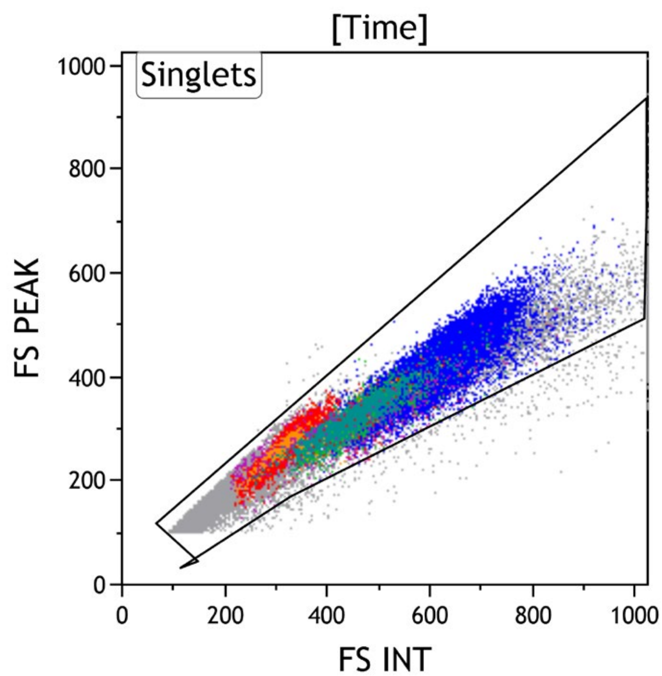

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

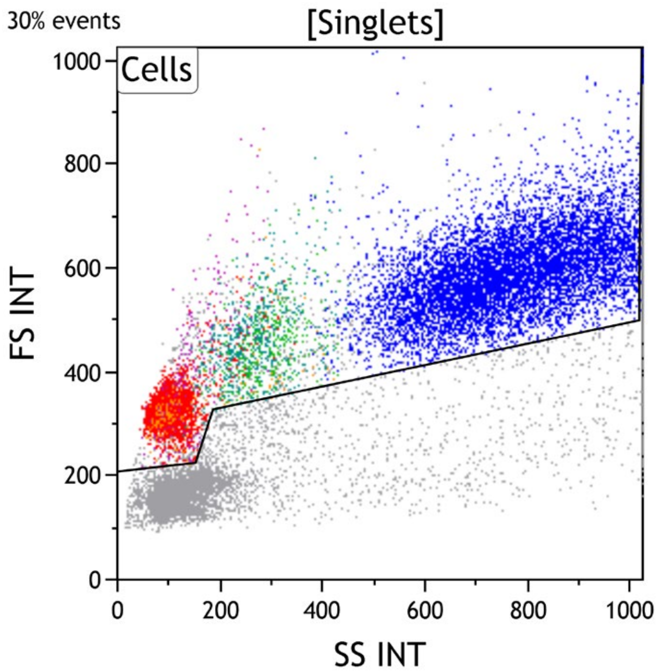

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. The aberrant population (teal) has increased forward and side scatter properties.

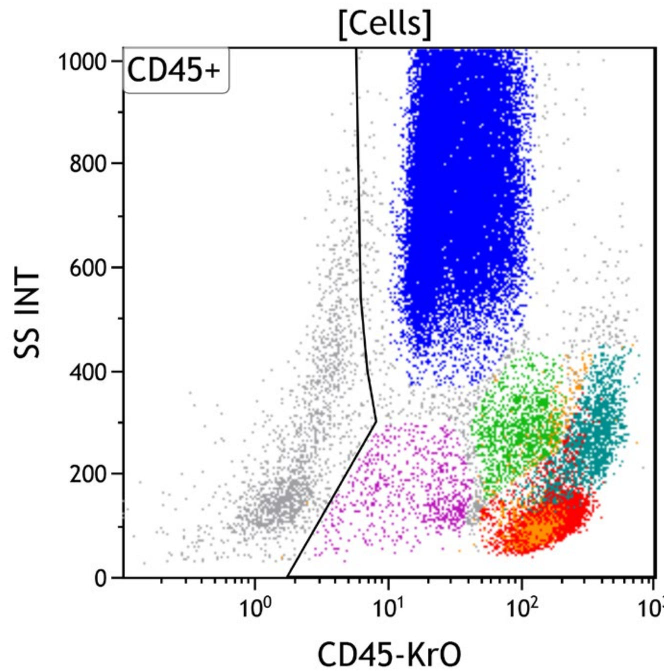

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells. The aberrant population (teal) expressed bright CD45.

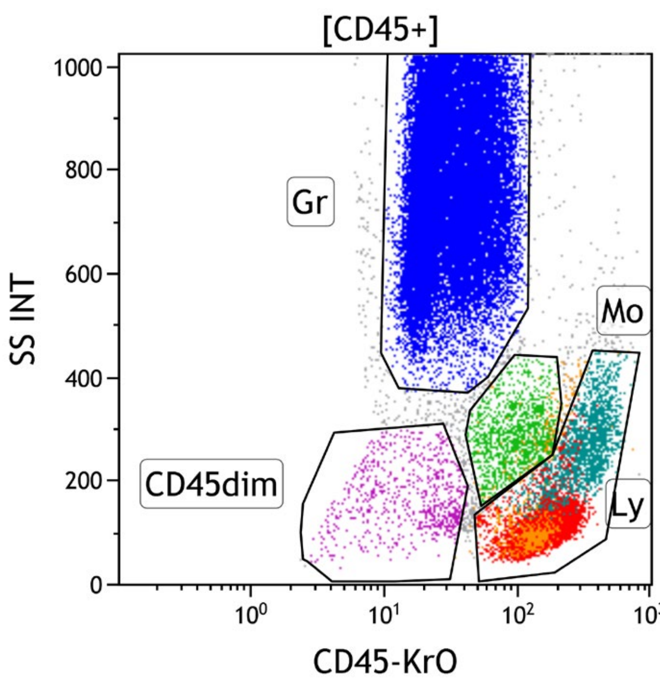

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red /orange/teal), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

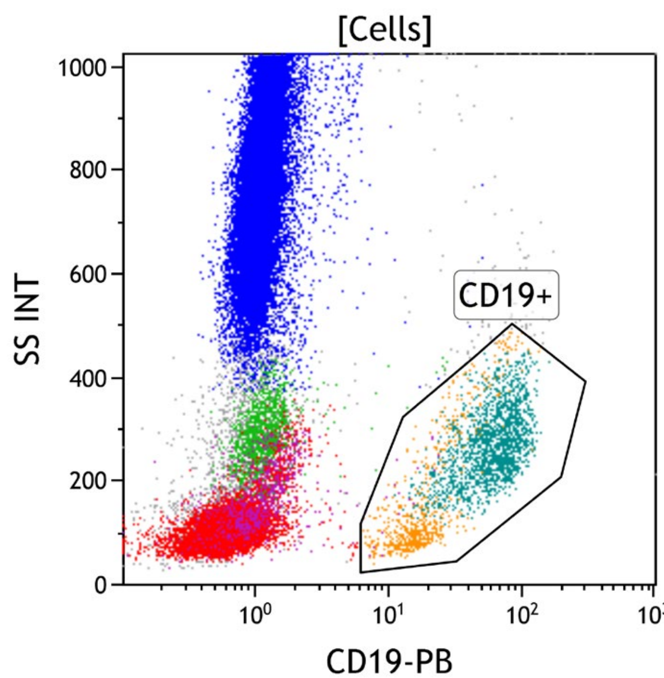

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. The aberrant B cells (teal) have increased side scatter and CD19 expression compared with that seen on normal B cells (orange).

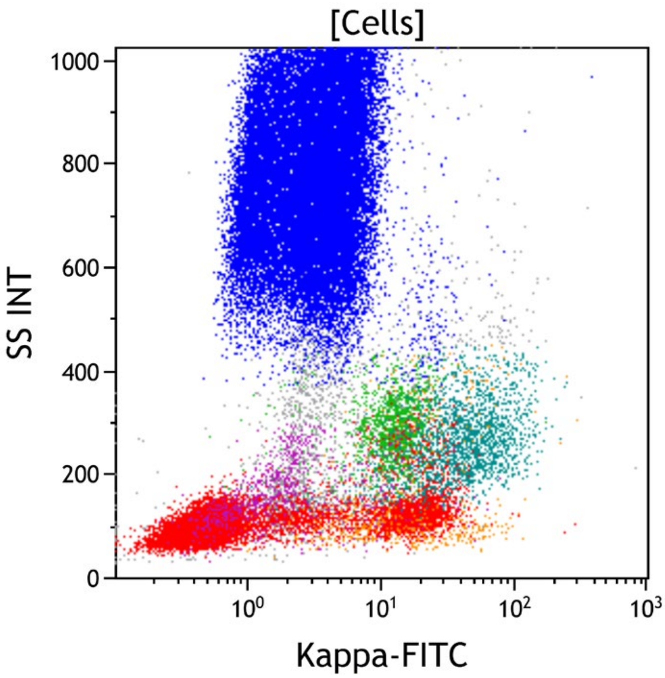

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The aberrant B cells (teal) express surface kappa light chain.

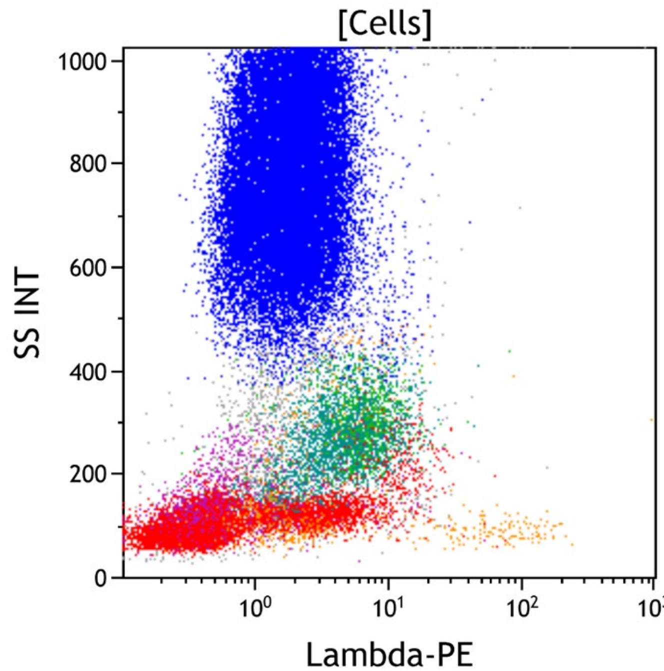

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The normal lambda light chain positive cells (orange) are shown on the right side of the plot. The aberrant B cells (teal) lack lambda light chain expression.

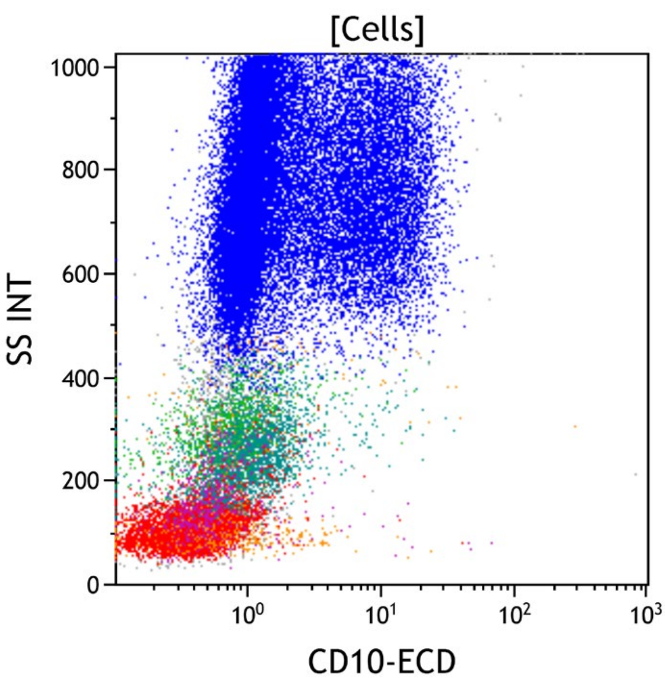

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple, lower right), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant B cell population (teal) is negative for CD10.

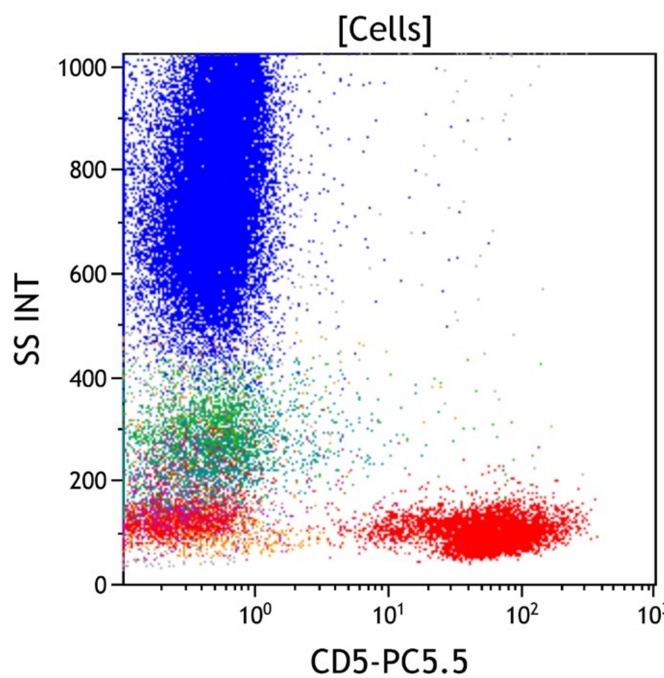

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The aberrant B cell population (teal) is negative for CD5.

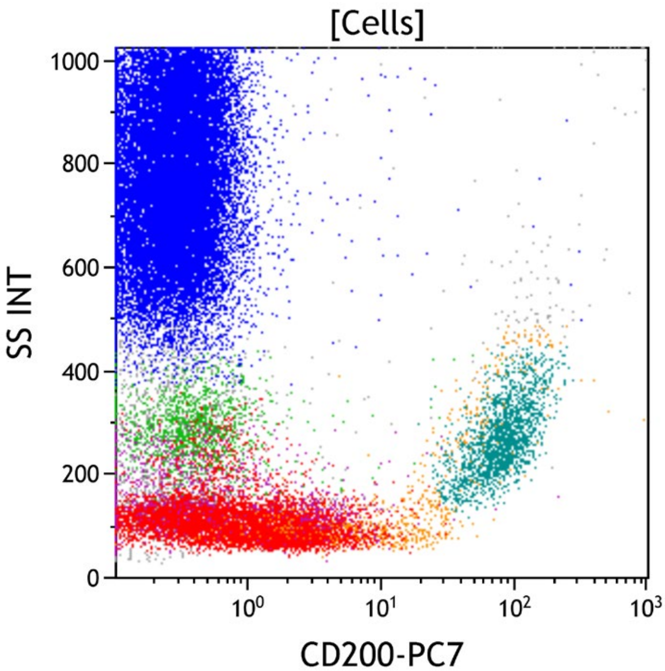

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The aberrant B cell population (teal) is positive for CD200 with increased side scatter.

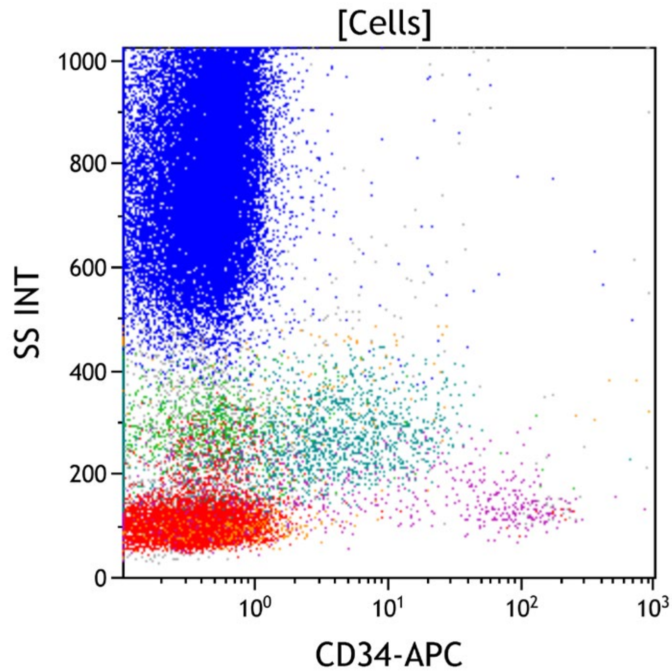

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The apparent CD34 positivity on the aberrant B cell population (teal) is likely due to bright CD20-APC-AA750 causing an increase in APC background.

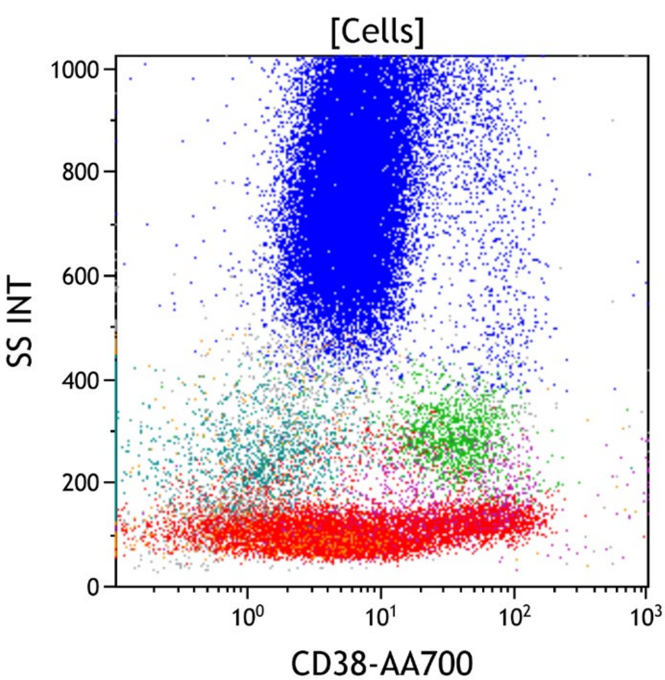

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The aberrant population (teal) displays low CD38 expression.

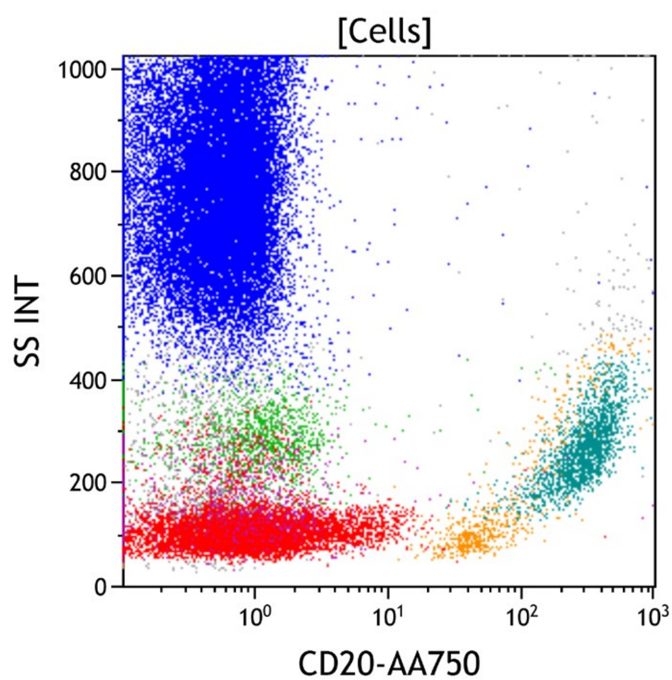

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The aberrant B cell population (teal) expresses CD20 at a higher level than that seen on normal B cells (orange).

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B Cell Tube

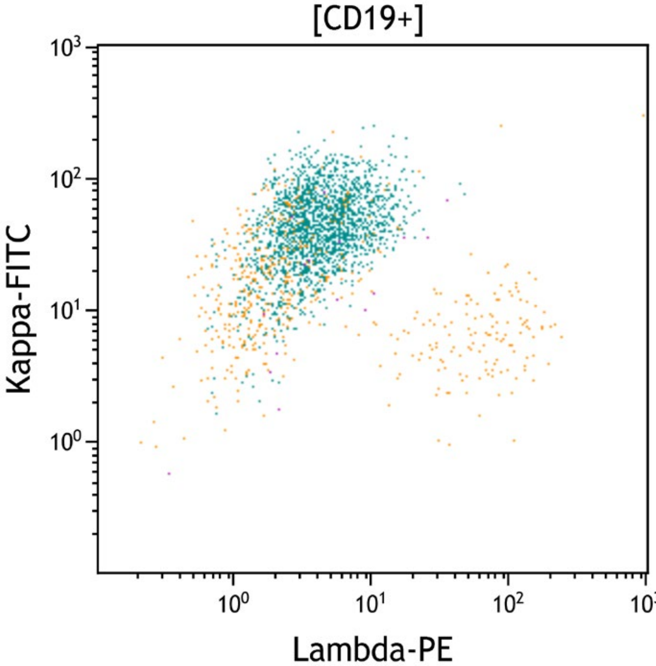

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. The aberrant B cell population (teal) predominantly has surface kappa light chain expression, indicating a clonal B cell population. A small population of polyclonal B cells (also in orange) with kappa and lambda light chain expression is present.

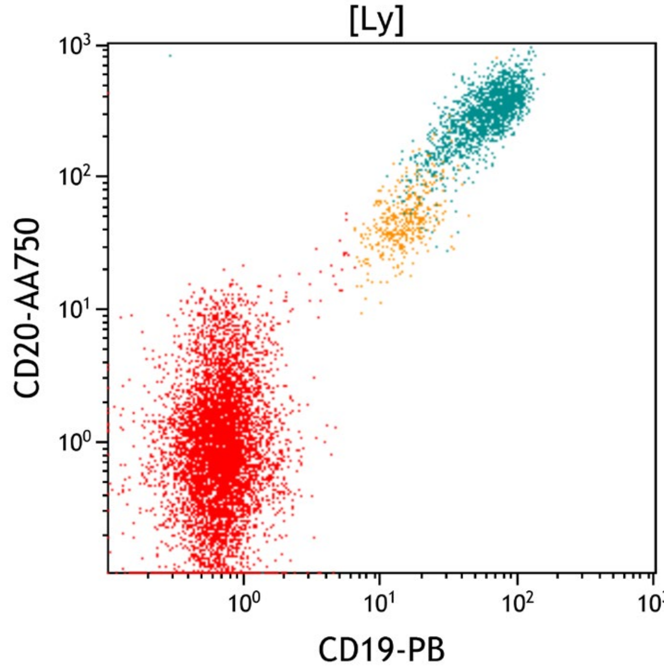

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. The aberrant B cell population (teal) displays increased CD19 and CD20 expression compared with the lower level seen on normal mature B cells (orange).

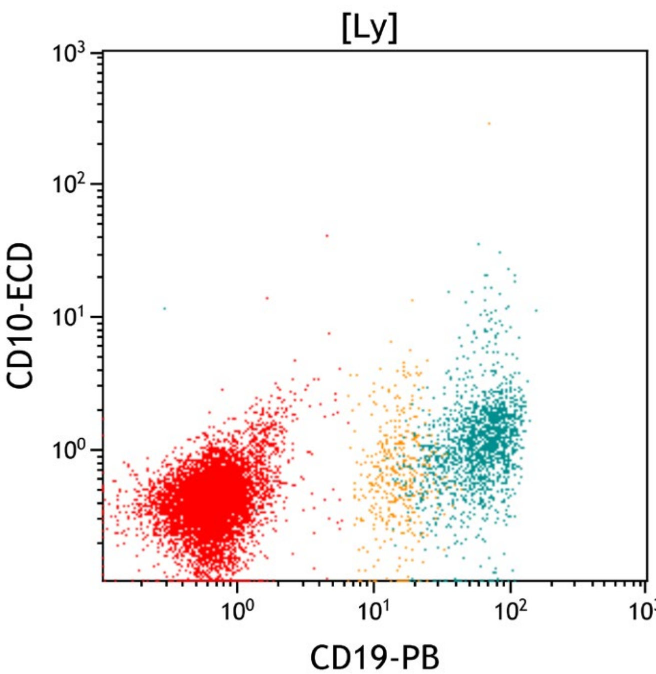

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate. The aberrant B cell population (teal) expresses CD19 at a higher level than that seen on normal B cells (orange) and is negative for CD10.

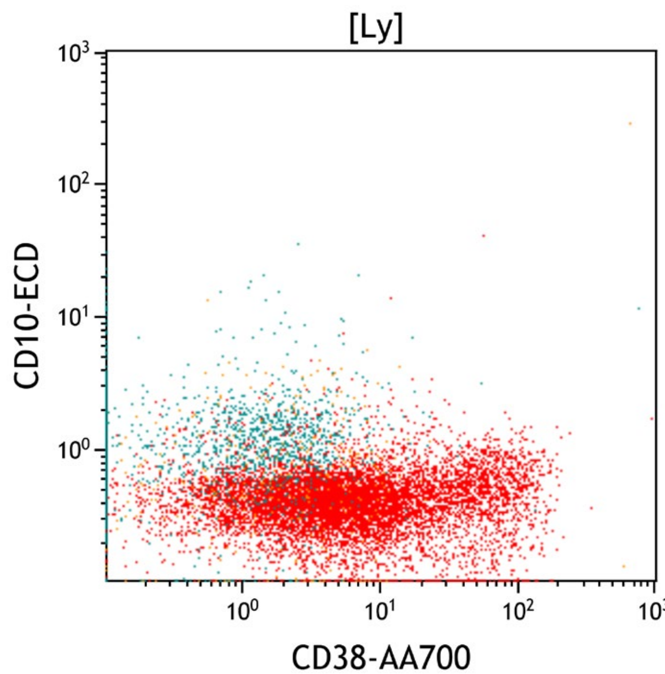

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38 expression dependent on activation state. The aberrant B cell population (teal) displays low CD38 expression and is negative for CD10.

B Cell Tube

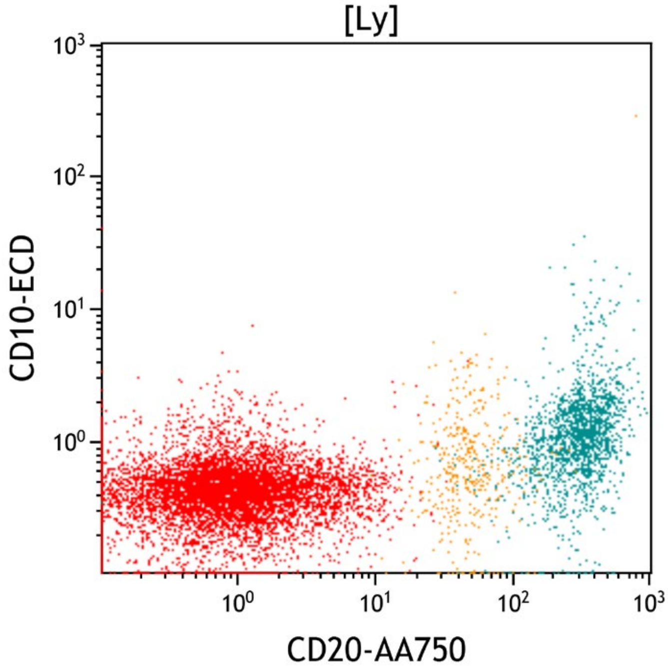

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The aberrant B cell population (teal) expresses CD20 at a higher level than that seen on normal B cells (orange) and is largely negative for CD10.

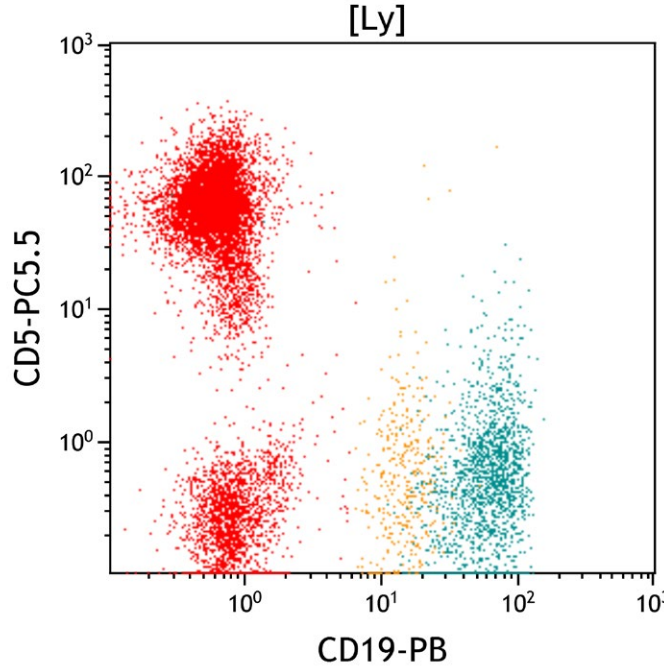

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper left), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The aberrant B cell population (teal) expresses CD19 at a higher level than that seen on normal B cells (orange) and is largely negative for CD5.

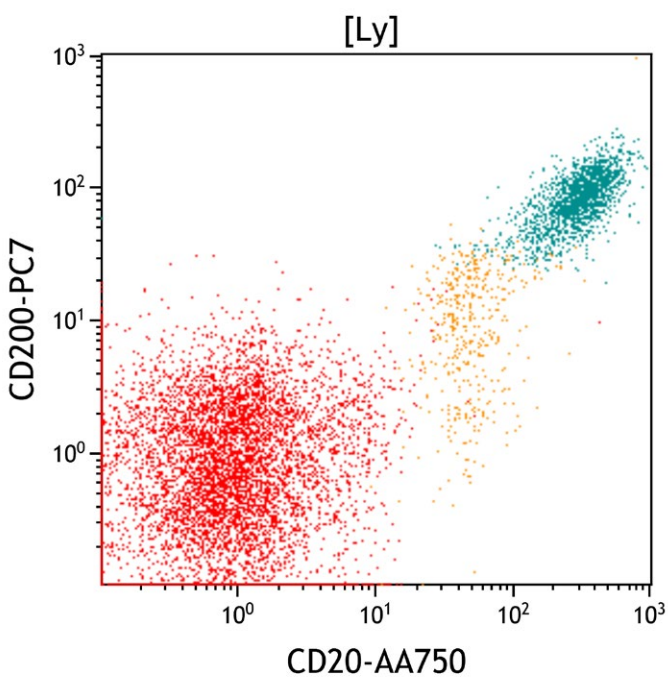

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The aberrant B cell population (teal) expresses CD20 and CD200 at higher levels than those seen on normal B cells (orange).

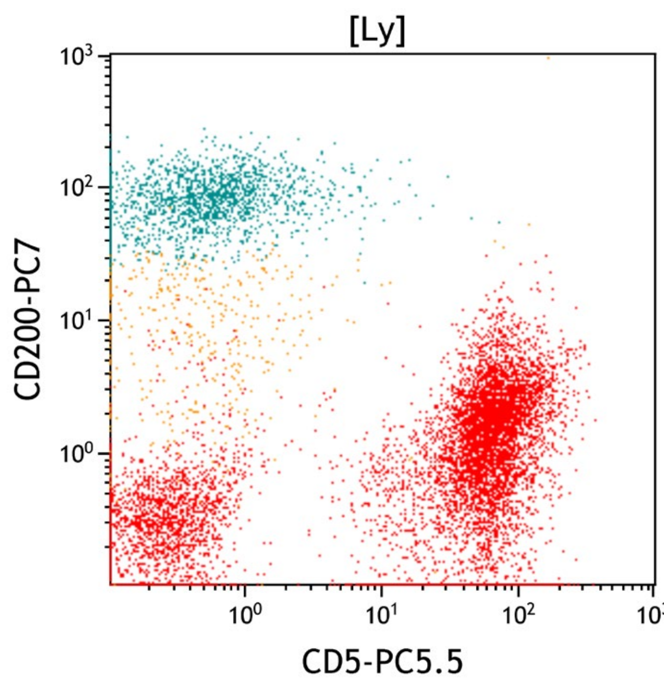

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange)normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The aberrant B cell population (teal) expresses CD200 at a higher level than that seen on normal B cells (orange) and is largely negative for CD5.

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T Cell Tube

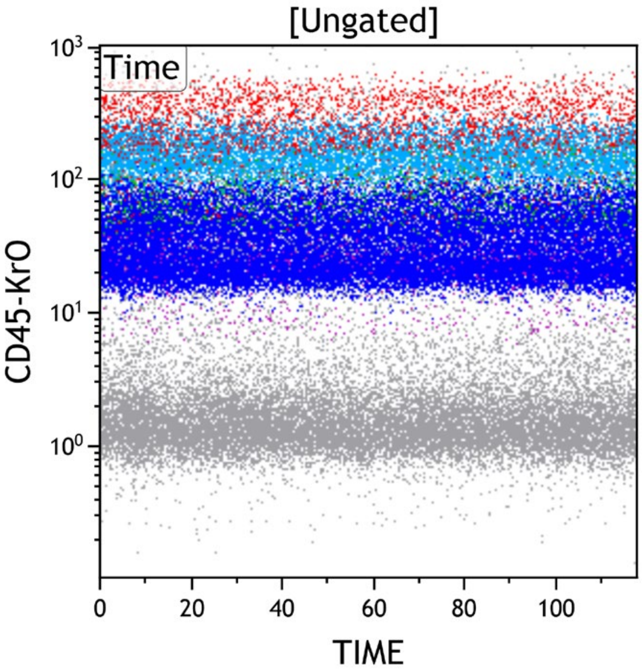

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

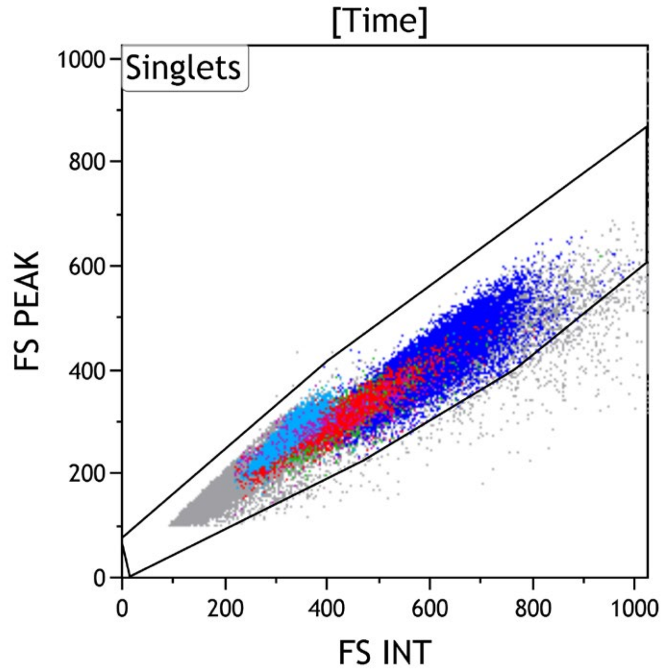

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

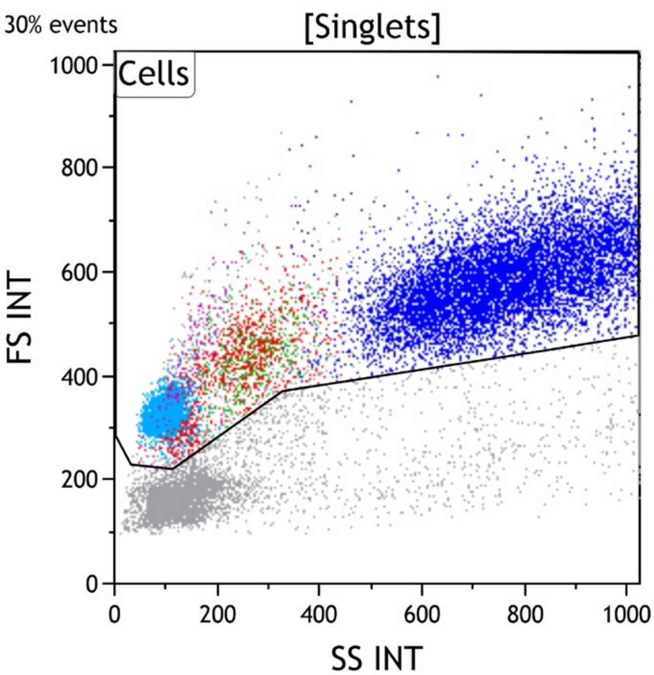

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are excluded in the Cells gate.

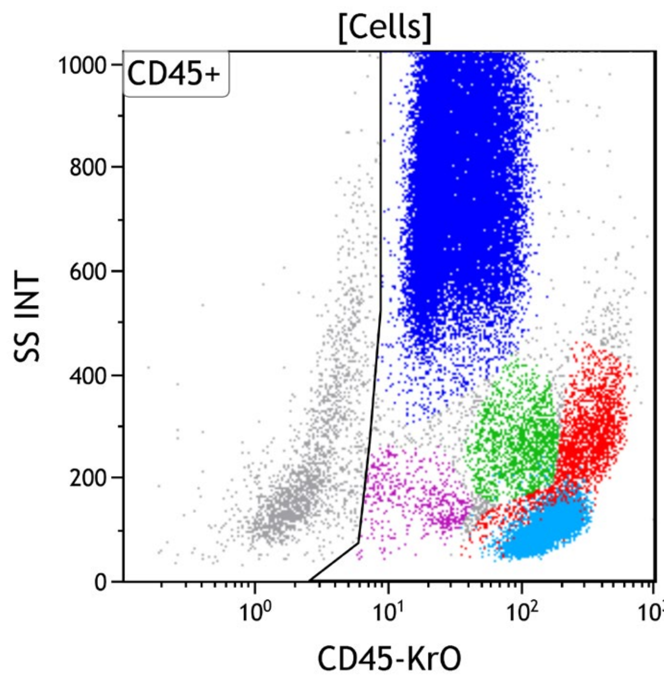

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

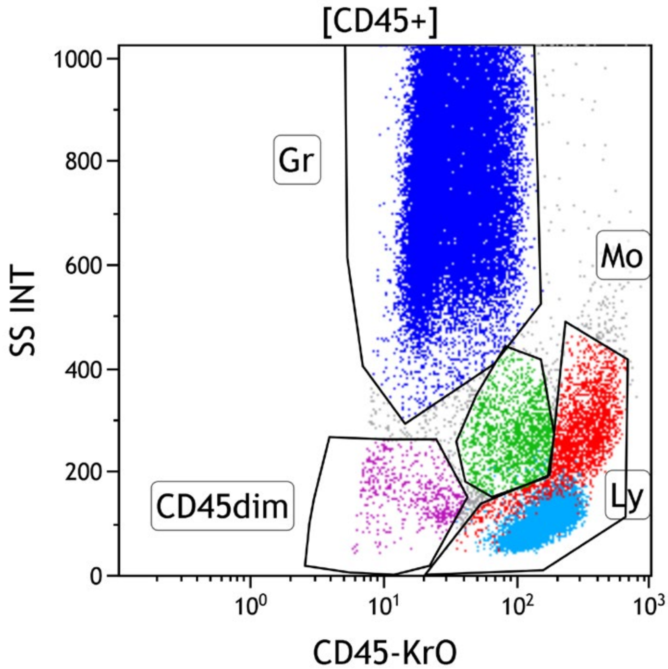

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

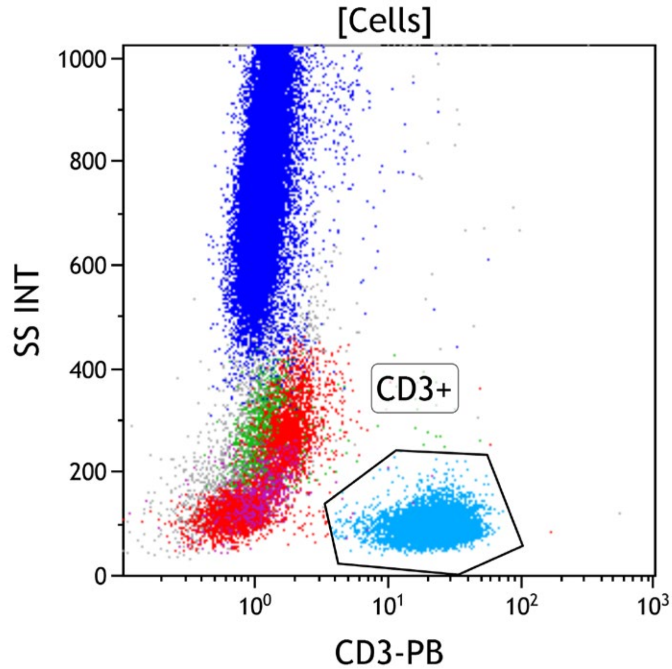

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

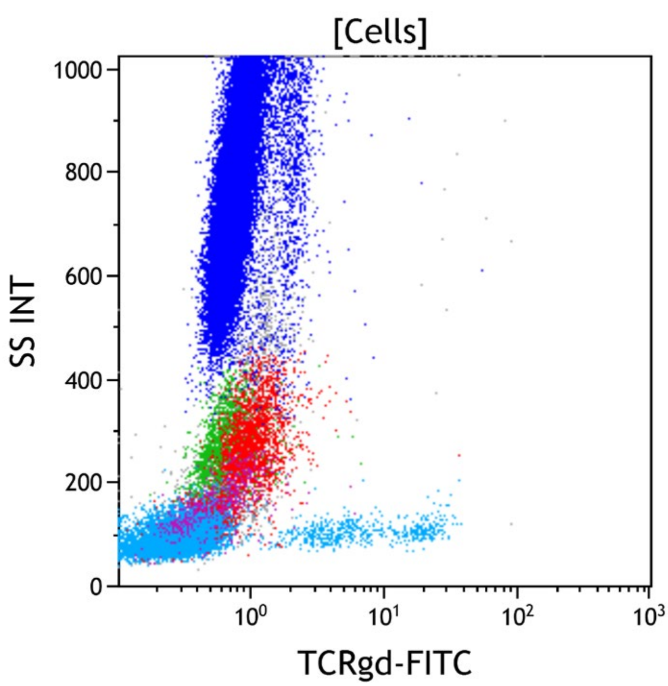

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua, lower right).

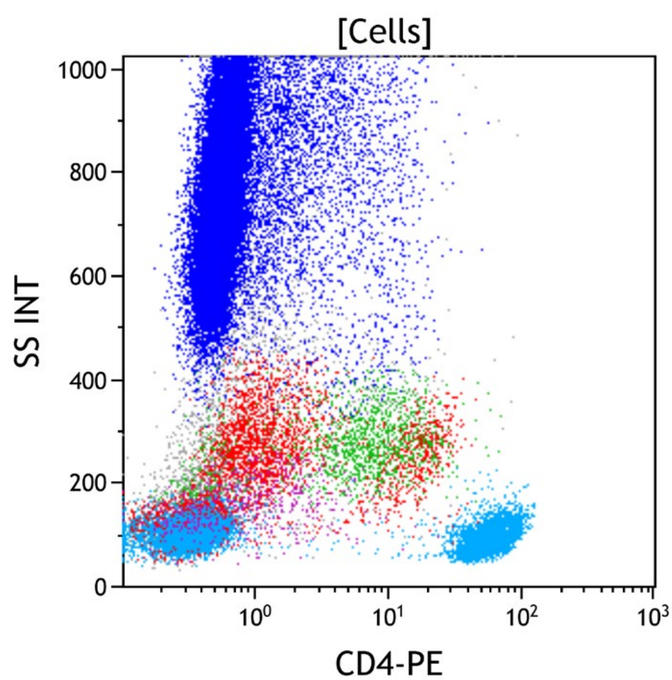

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua, lower right). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

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T Cell Tube

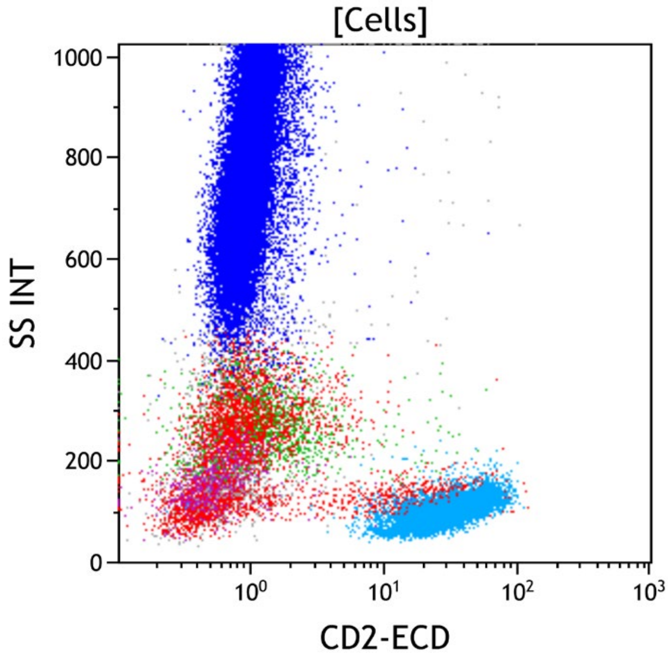

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

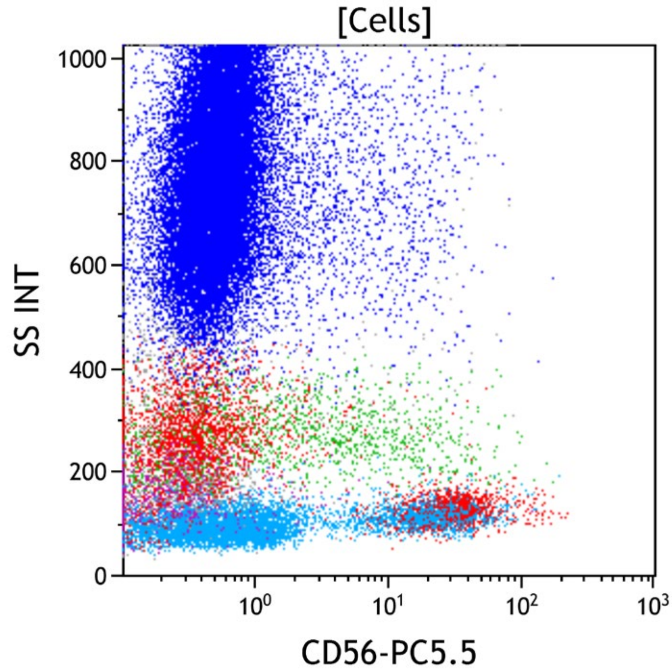

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red, lower right), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells. CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

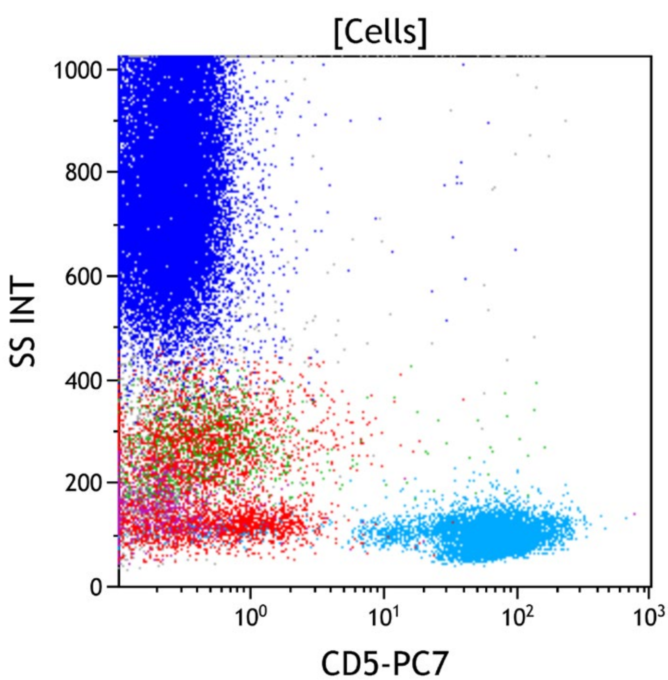

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

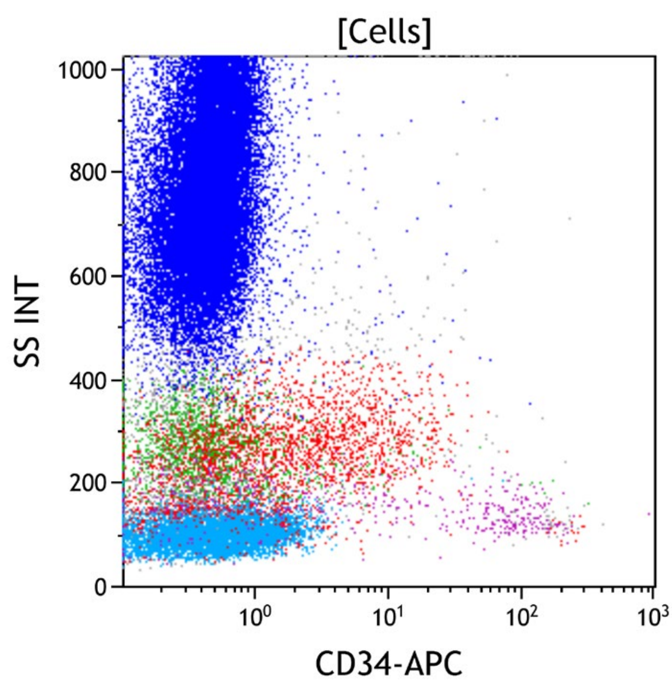

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

T Cell Tube

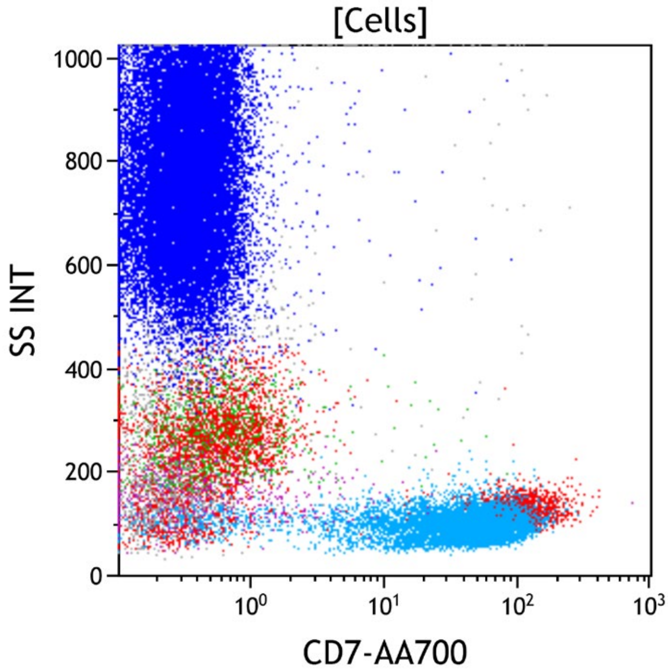

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

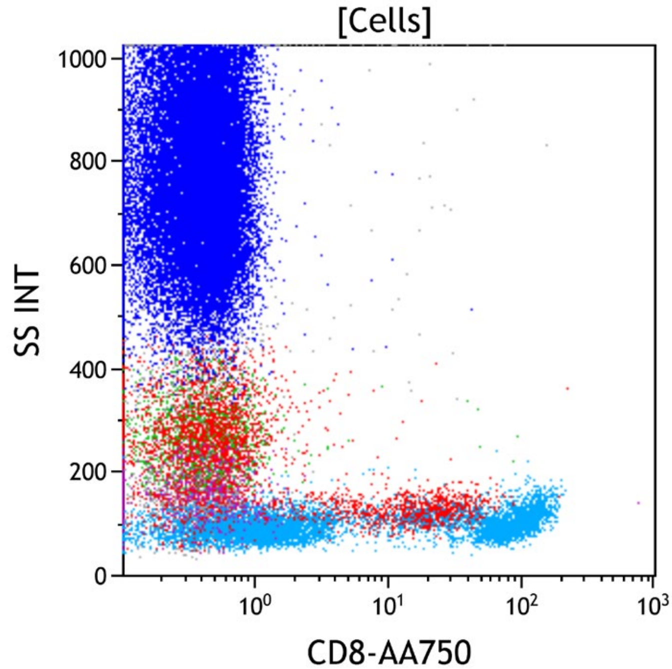

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua, lower right) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

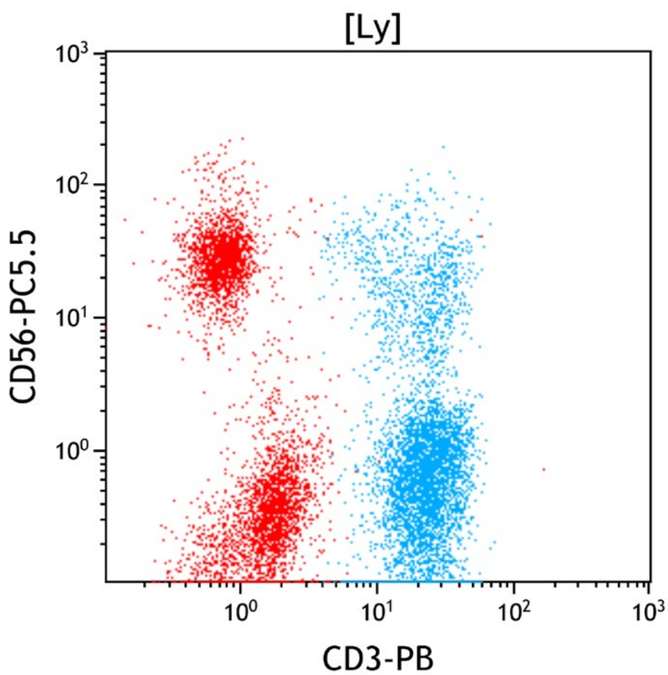

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells (aqua) are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells (aqua, upper right) also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

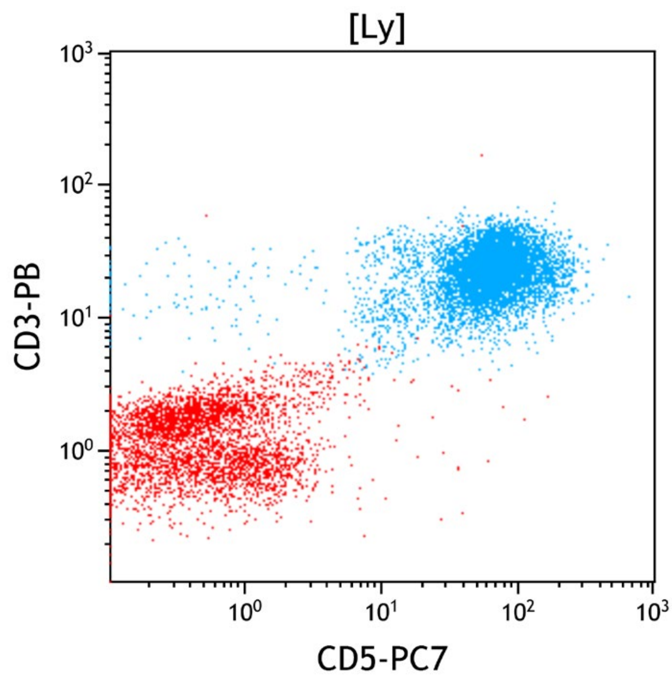

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells and B cells (red).

T Cell Tube

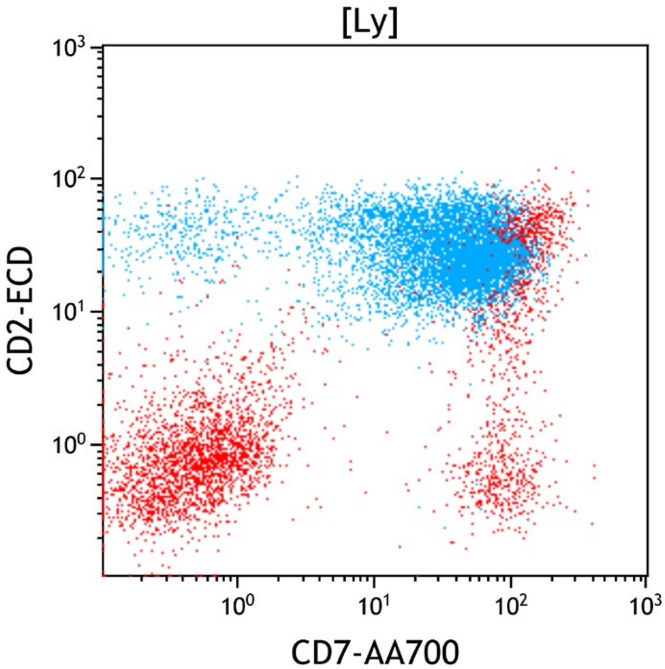

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

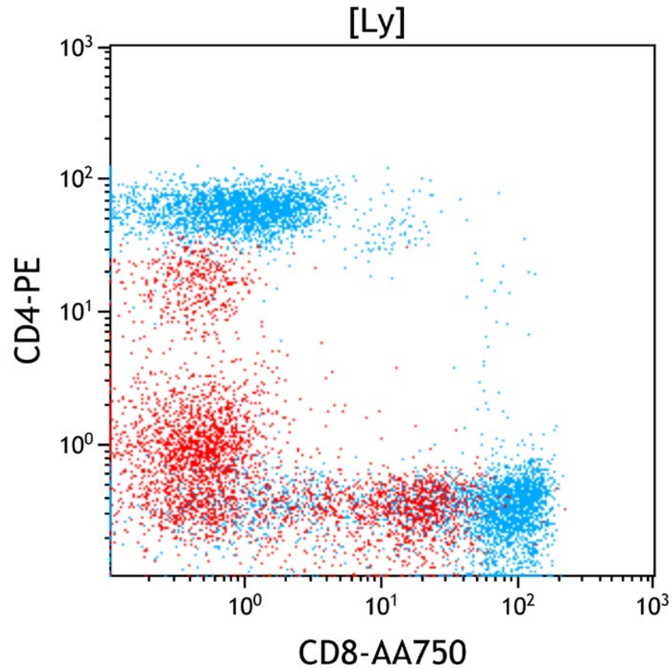

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, upper left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

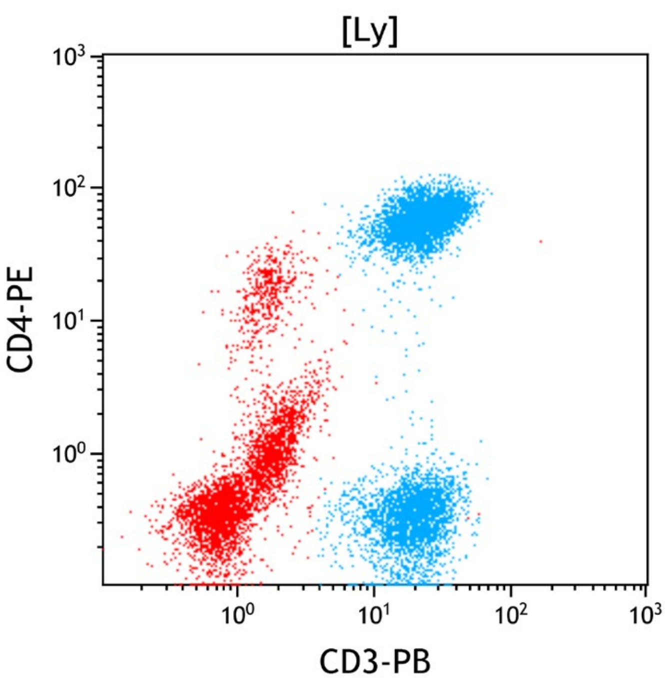

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes (red, upper left) and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

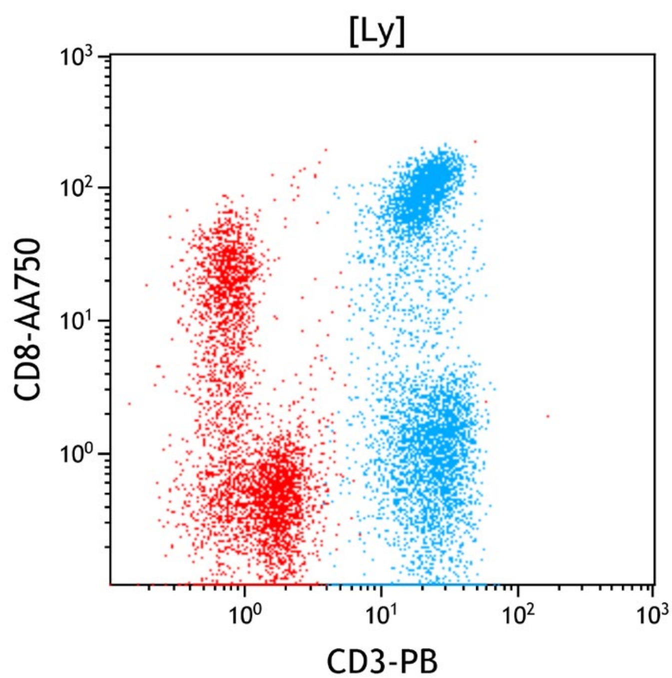

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

T Cell Tube

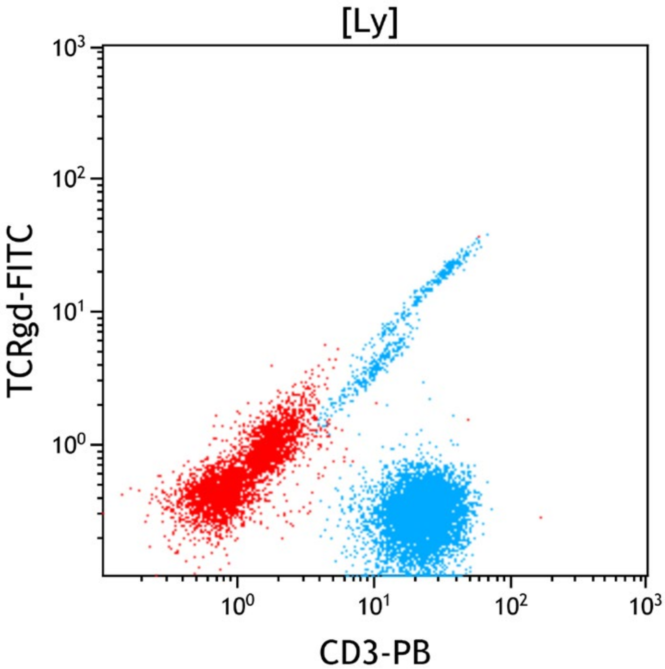

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly) . A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

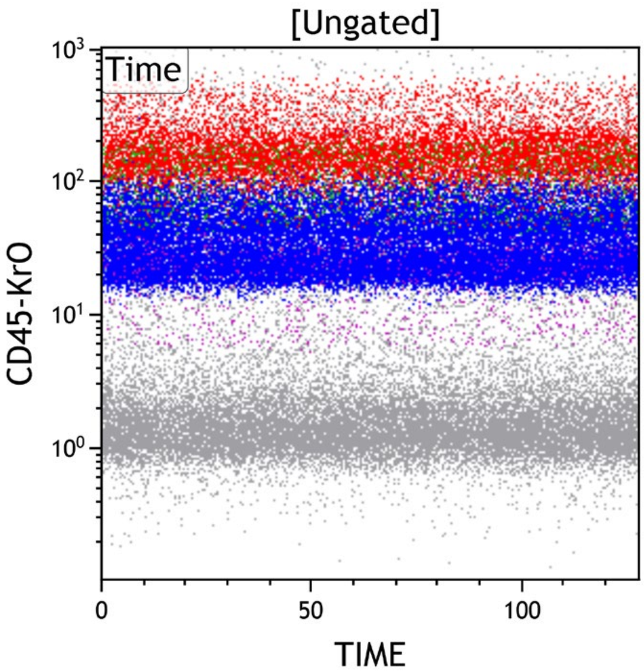

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

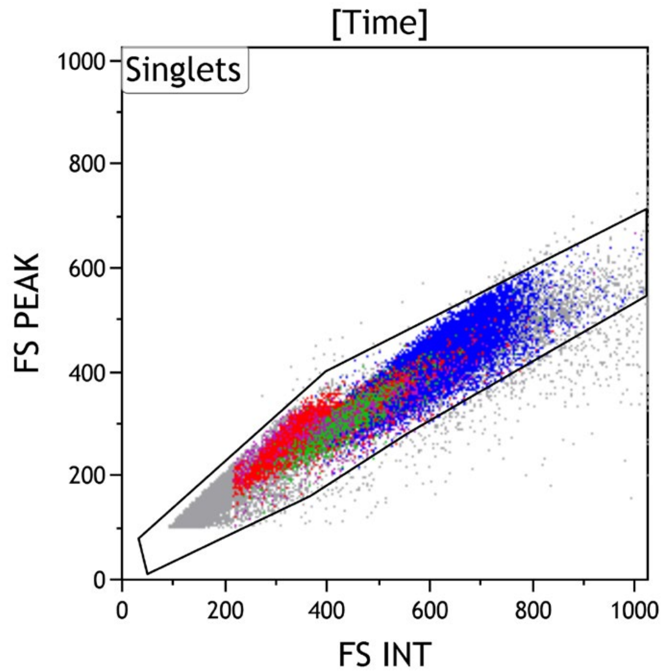

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

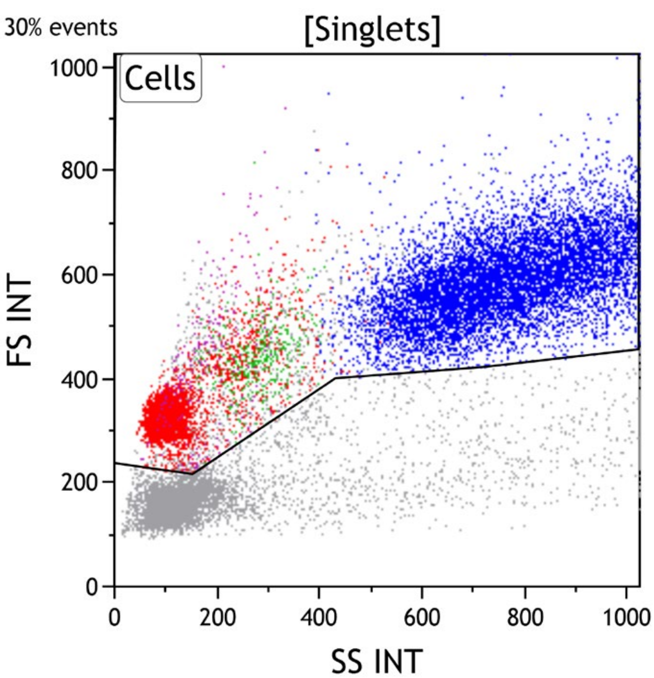

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

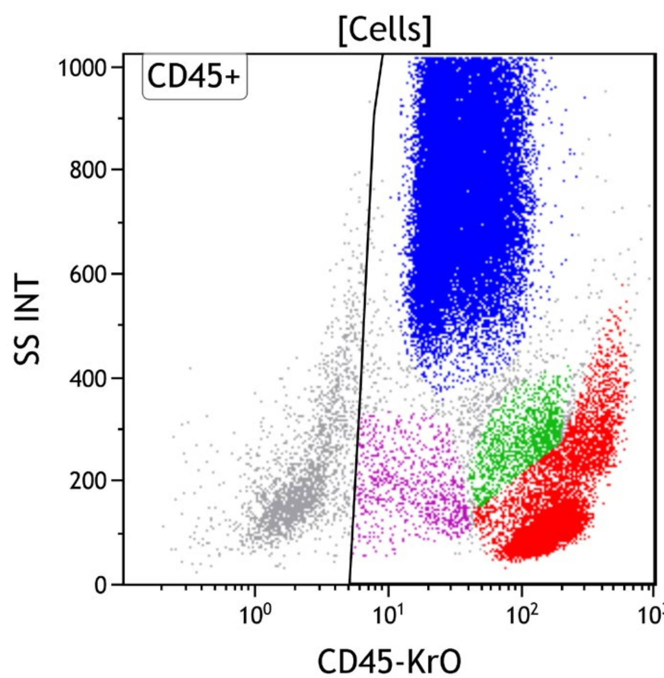

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

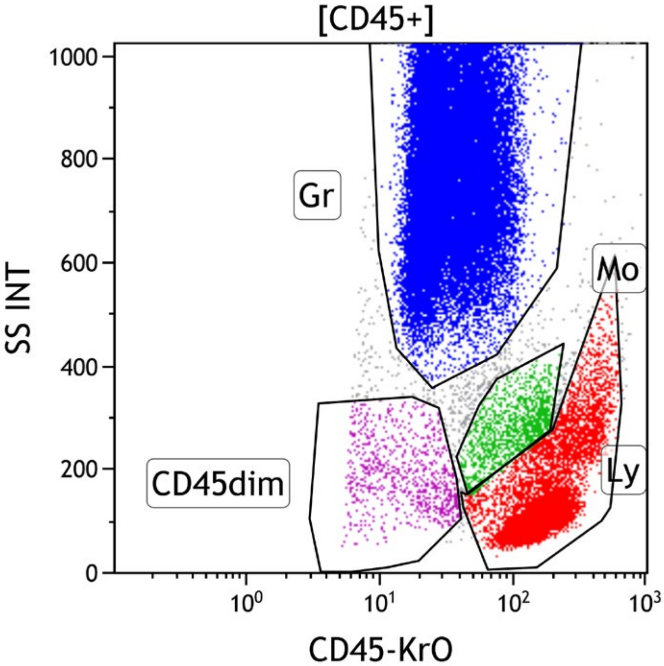

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

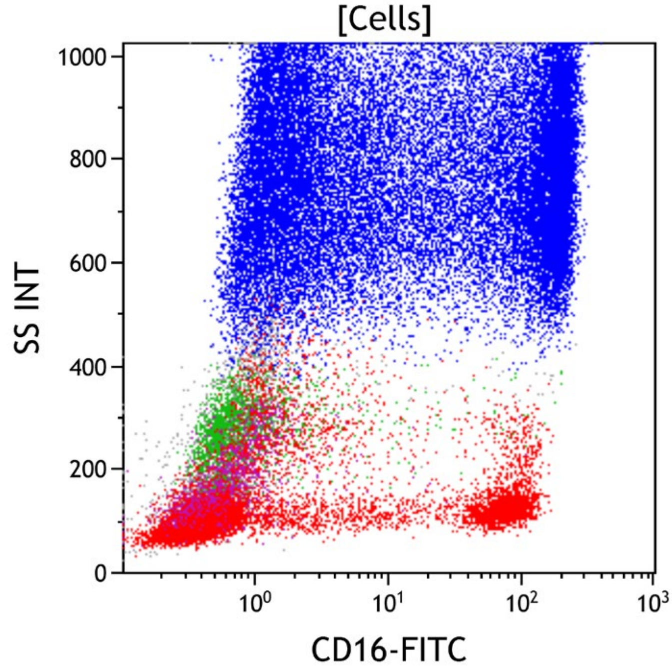

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes.

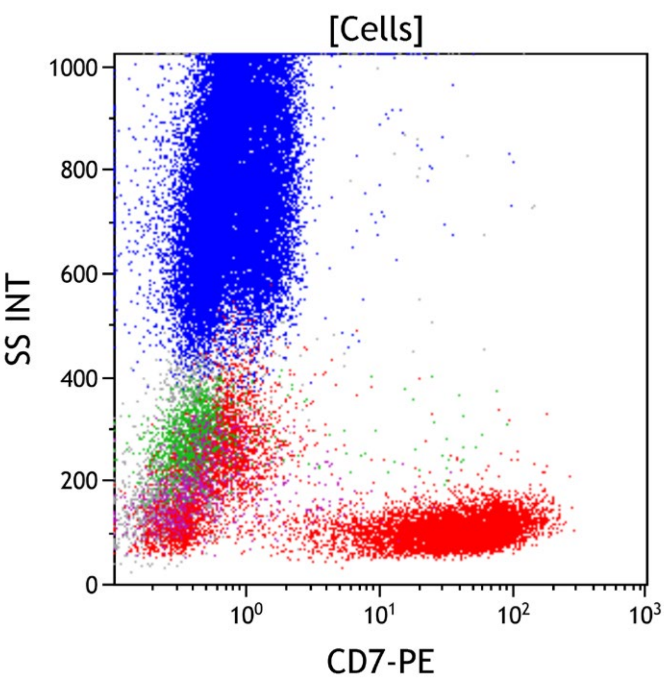

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

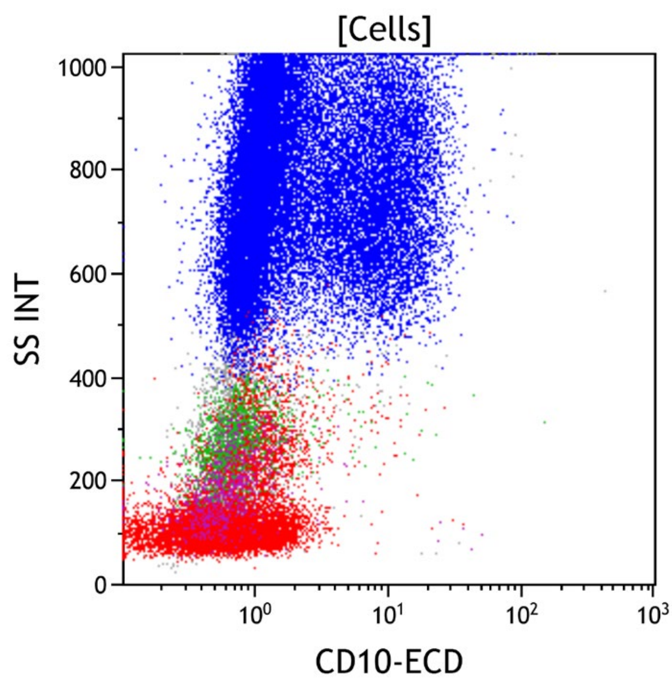

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple, lower right), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

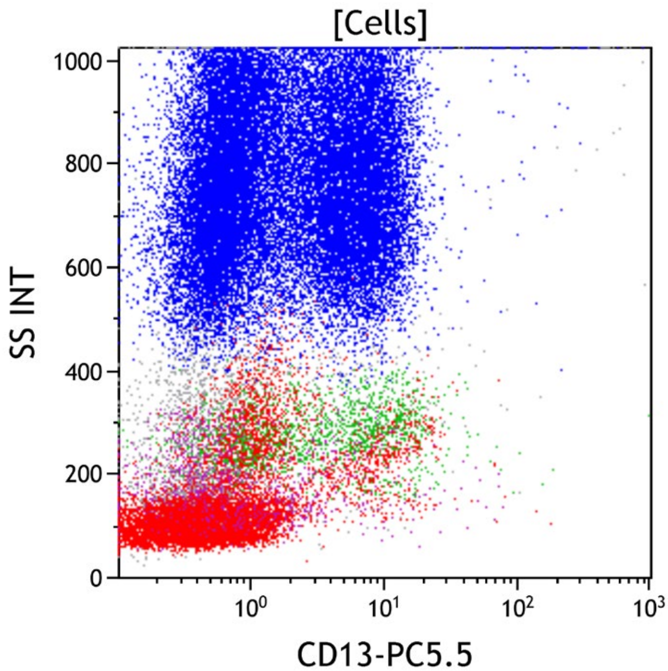

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

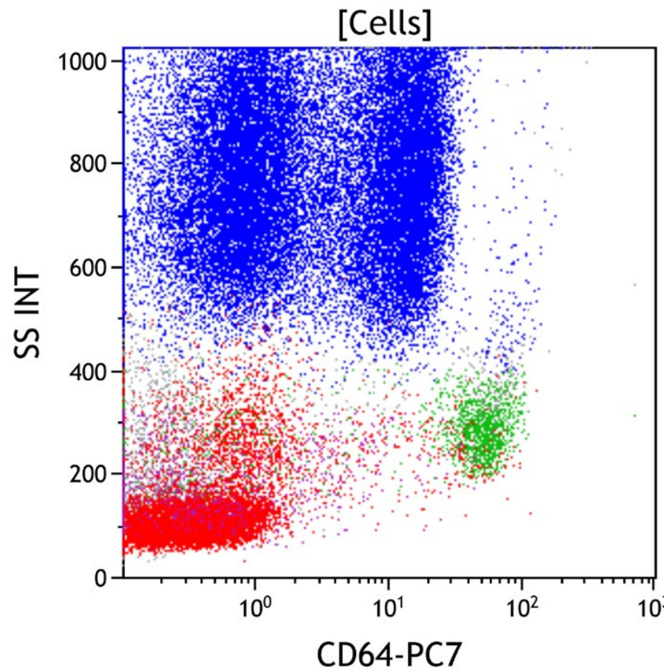

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells (red) or most CD34 positive progenitors.

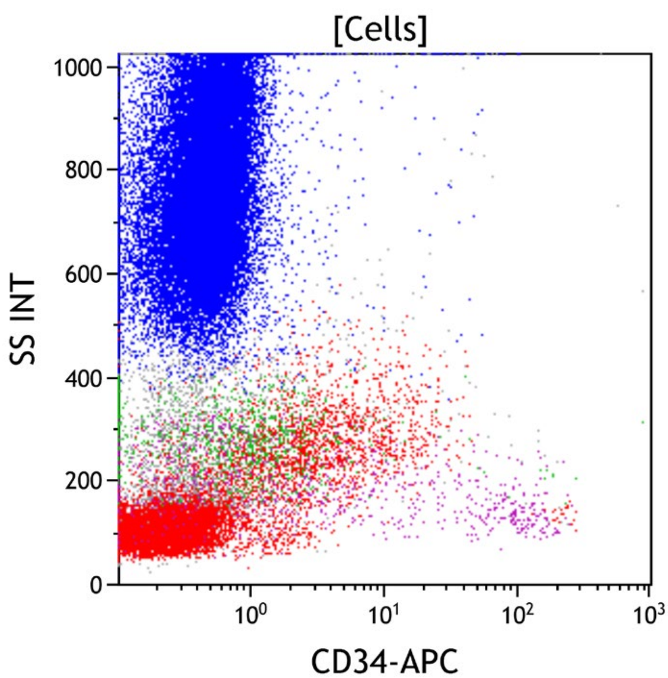

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34.

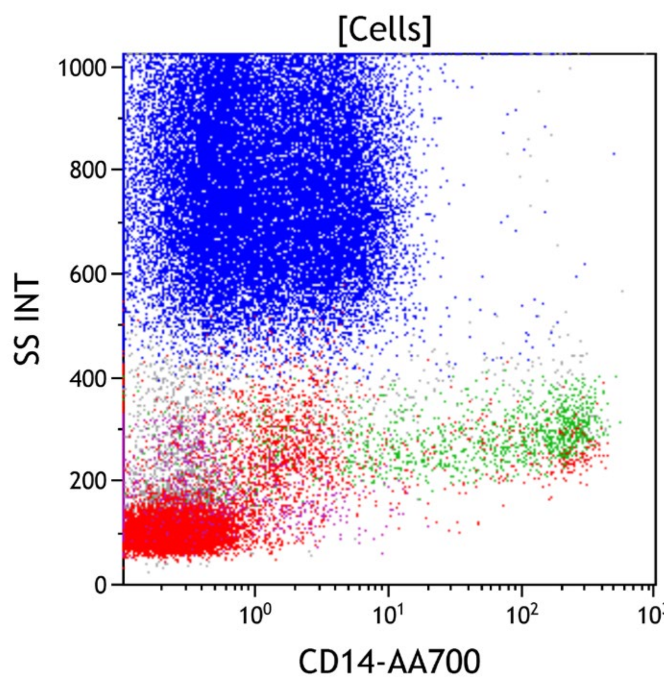

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes (blue) at a low level.

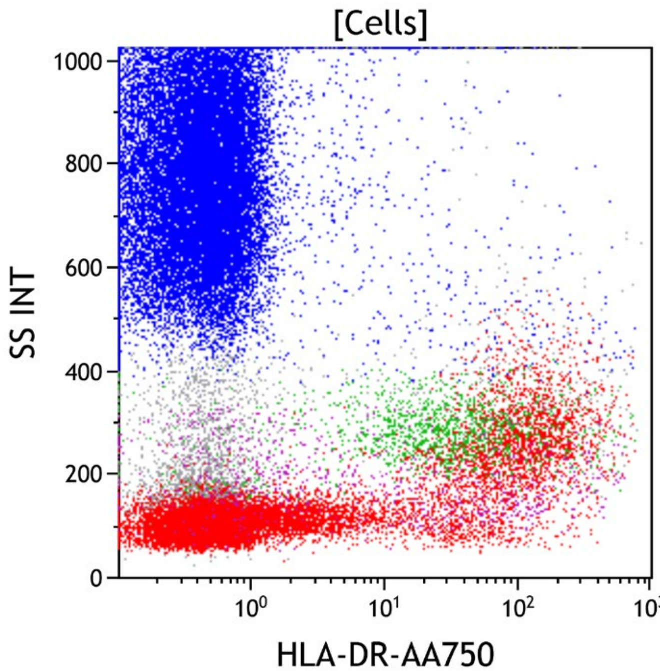

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red, lower right) and immature (purple) B cells, and activated T cells. Note the mature B cells have increased side scatter.

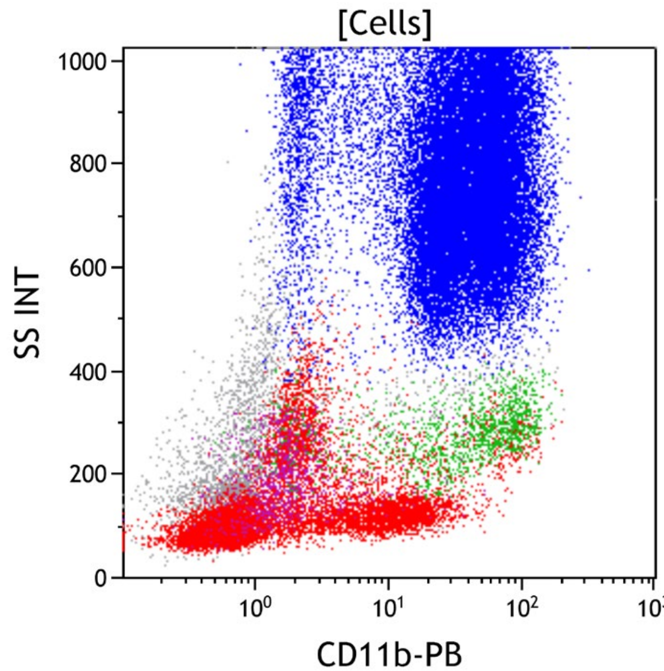

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils (purple, lower right).

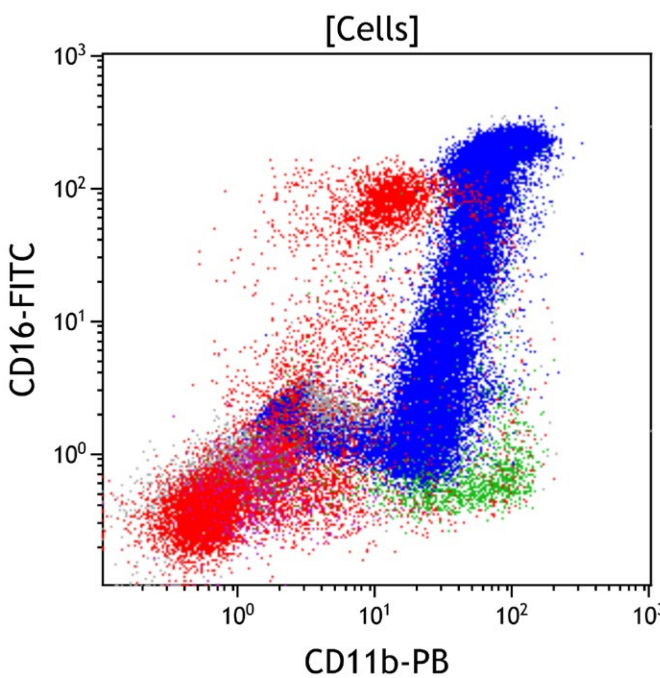

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (in green), immature and mature granulocytes (blue) and NK cells (red, upper right). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes (blue, upper right), where it is expressed at its highest level.

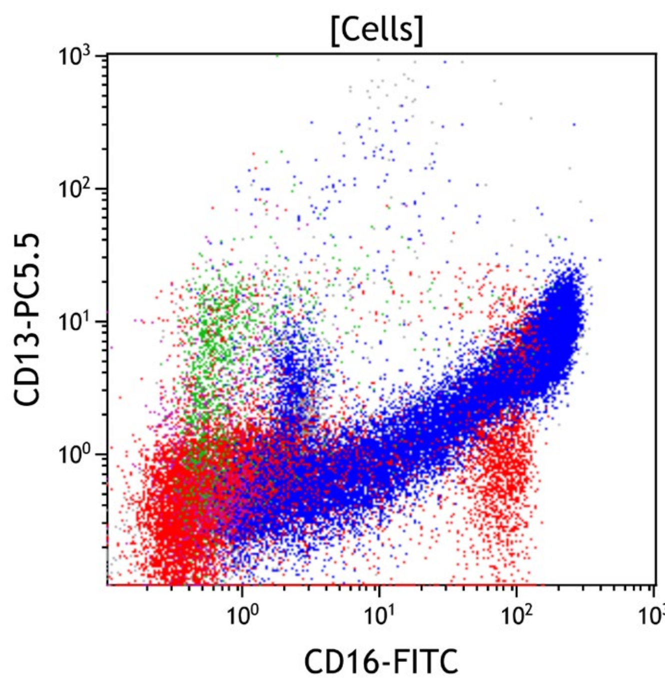

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, upper left) and lose CD13 as they mature to myelocytes (blue, lower left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue, upper right).

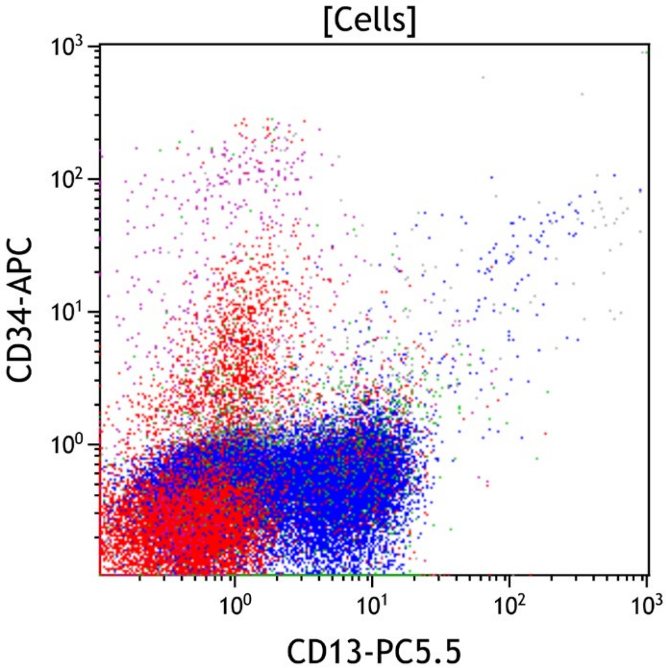

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphocytes (red).

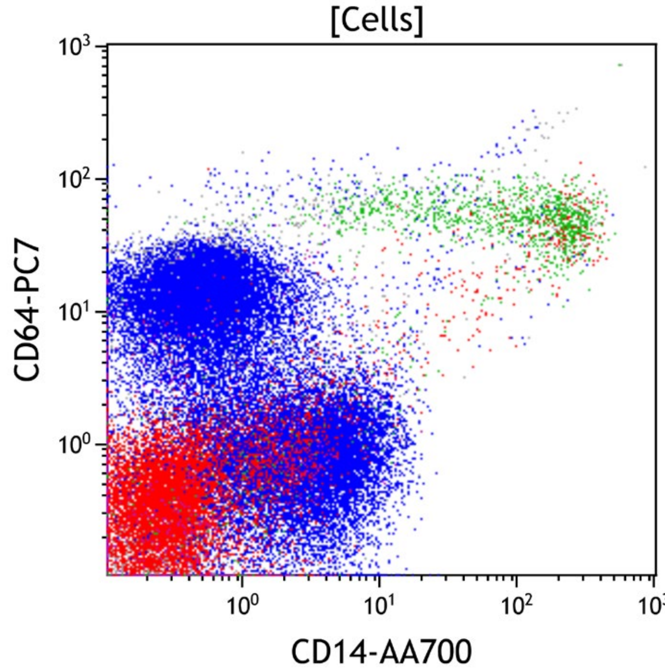

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (green, upper left) and progressively acquire CD14 during maturation to mature monocytes while retaining high level CD64 (green, upper right). Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left).

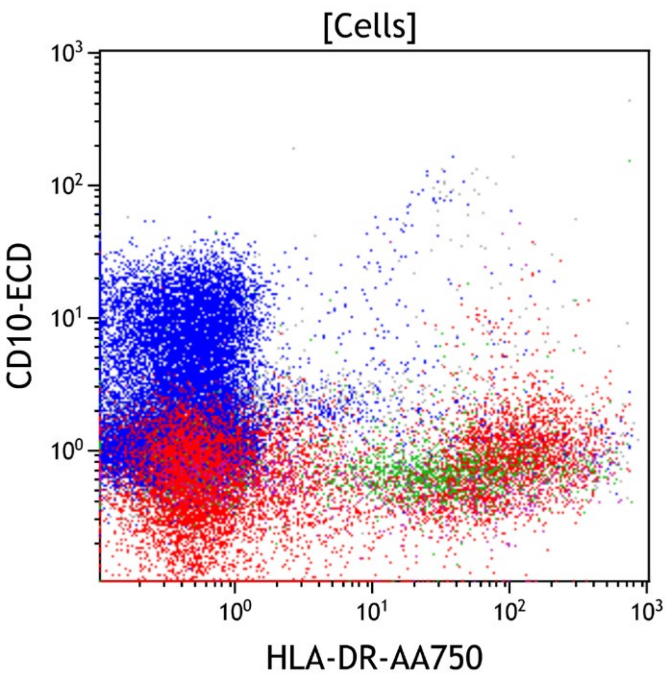

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells (Red, lower right), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells.

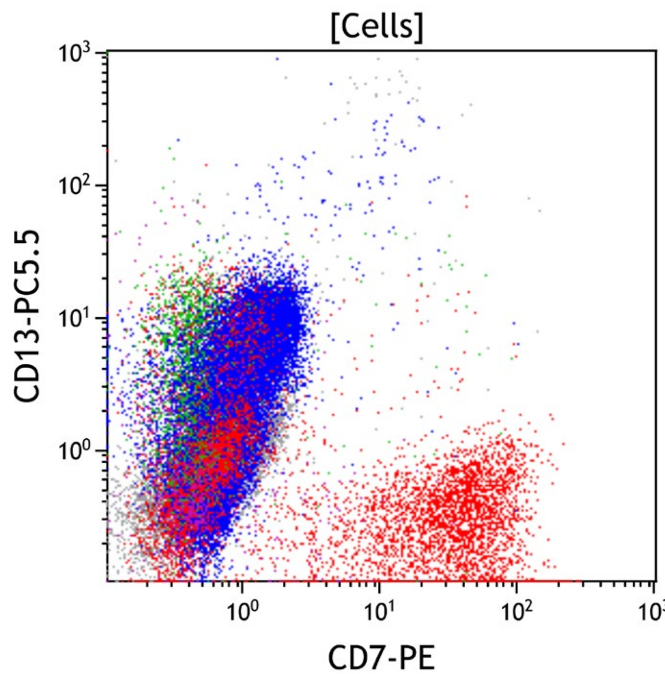

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

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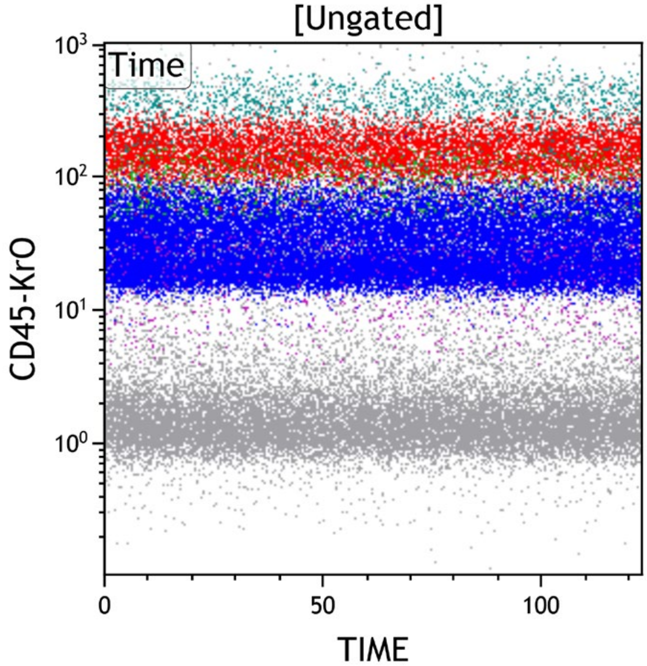

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

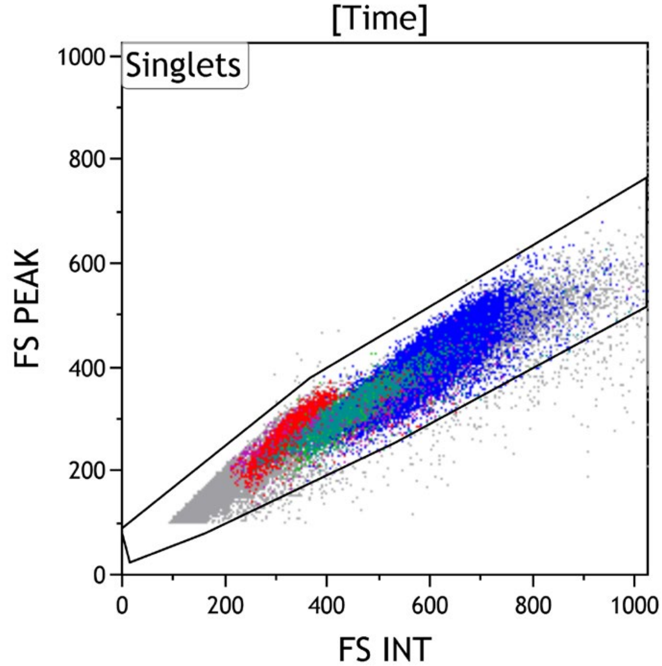

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

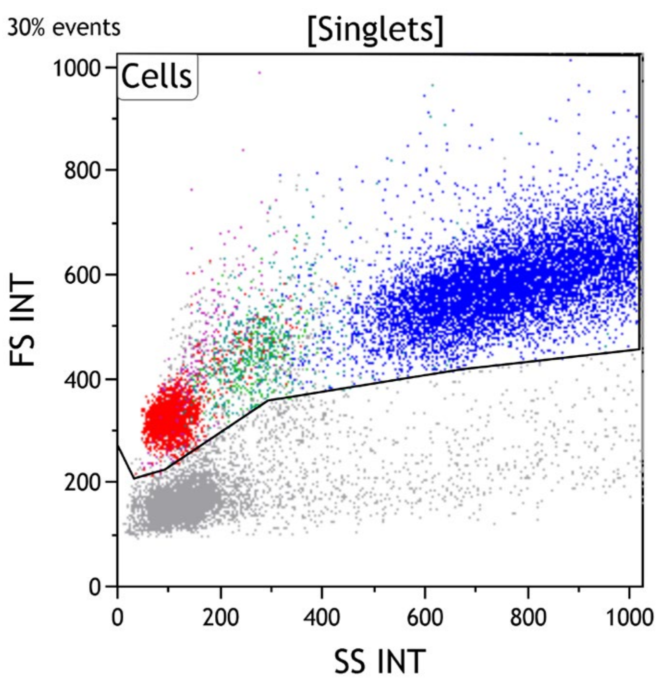

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

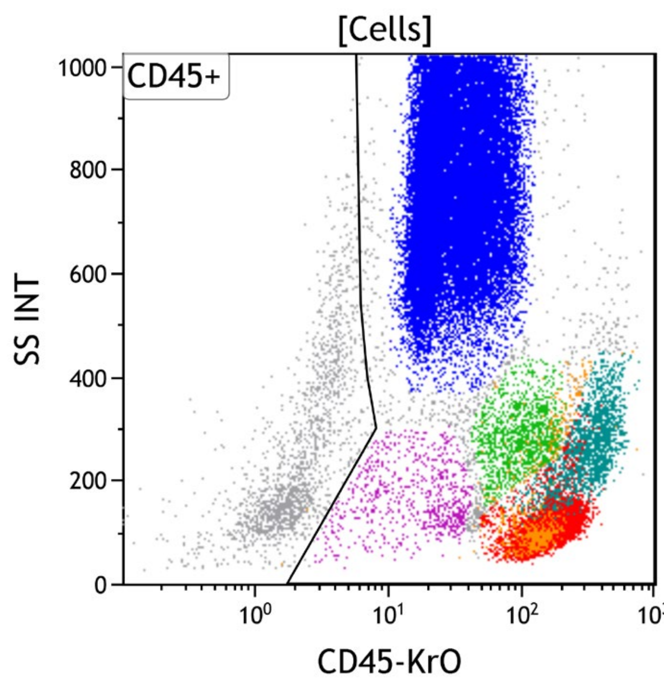

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells. The aberrant population (teal) expresses bright CD45.

M2 Cell Tube

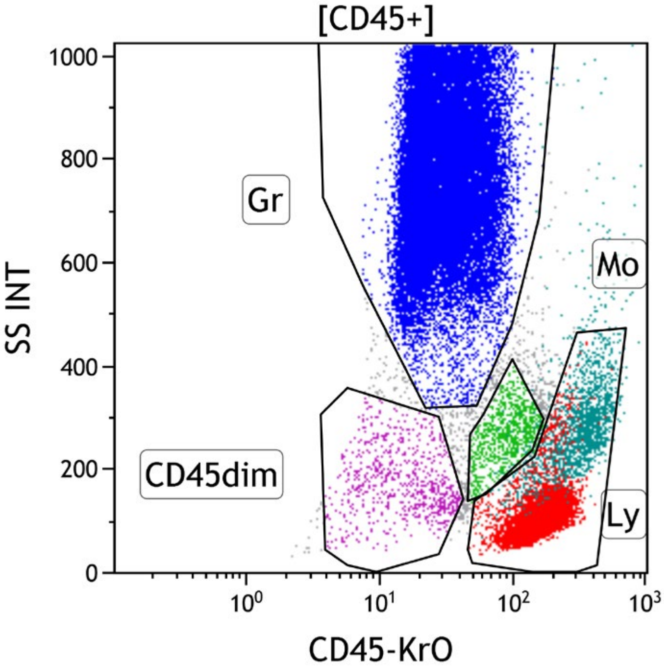

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/teal), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

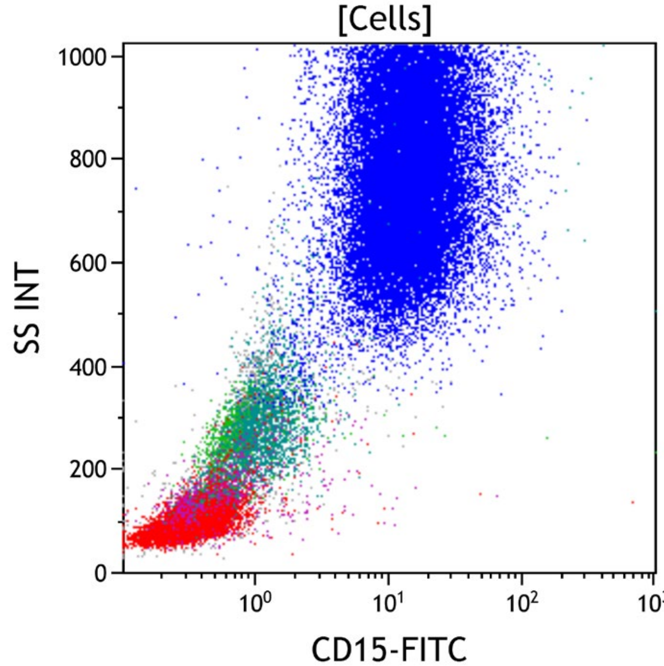

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The aberrant population (teal) is negative for CD15.

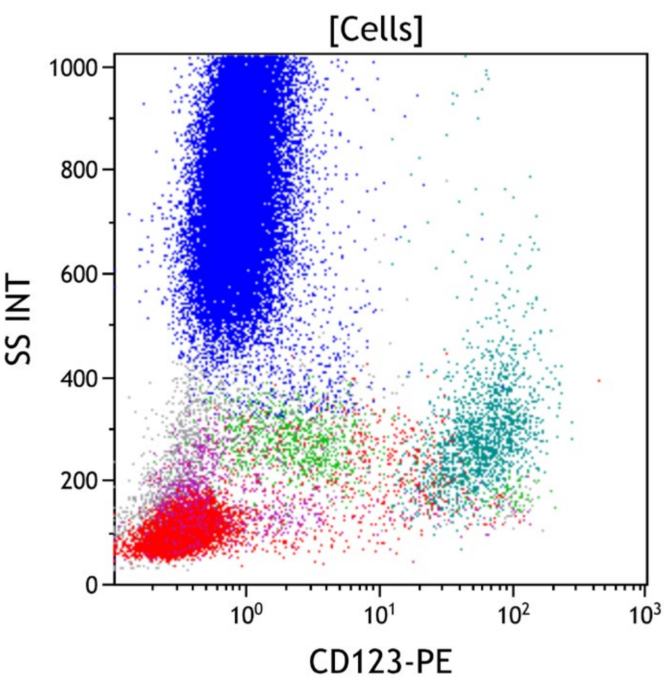

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors (purple, lower right) and monocytes (green). The aberrant population in the lymphocyte gate (teal) has high side scatter and bright CD123 expression.

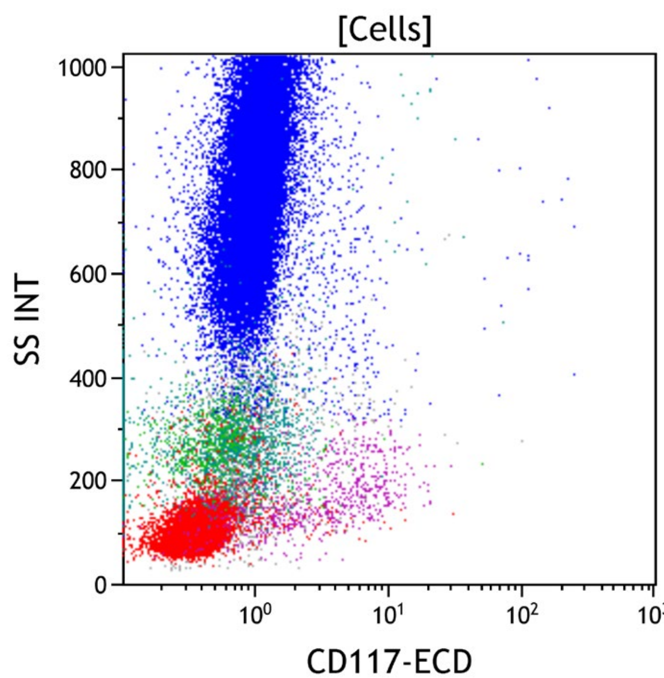

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors (purple), early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue, right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The aberrant population (teal) is negative for CD117.

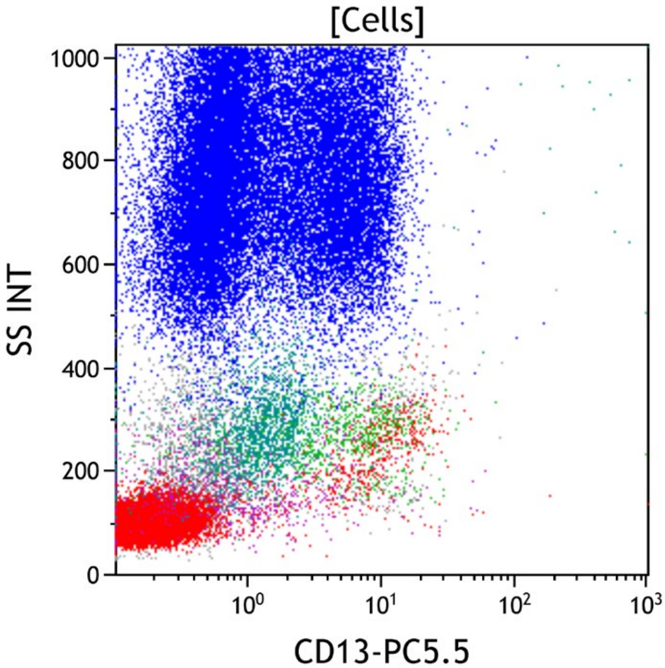

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple). The aberrant population (teal) displays low expression of CD13.

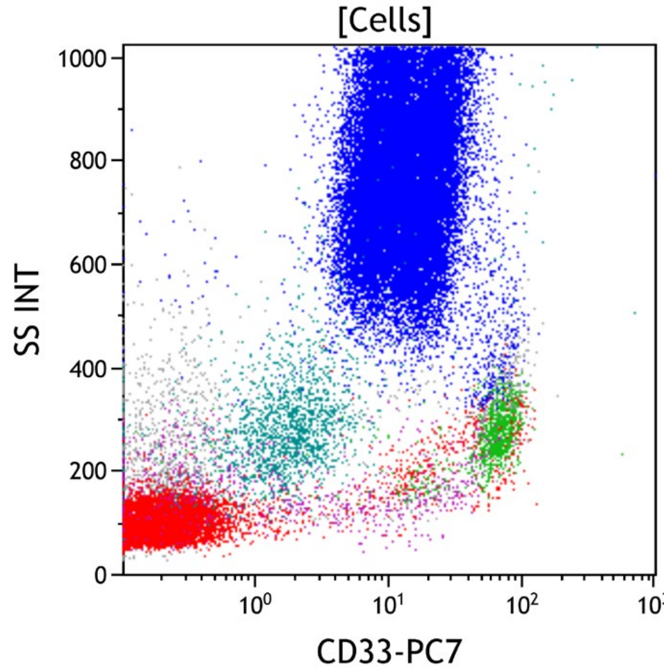

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors (purple). The aberrant population (teal) displays low expression of CD33.

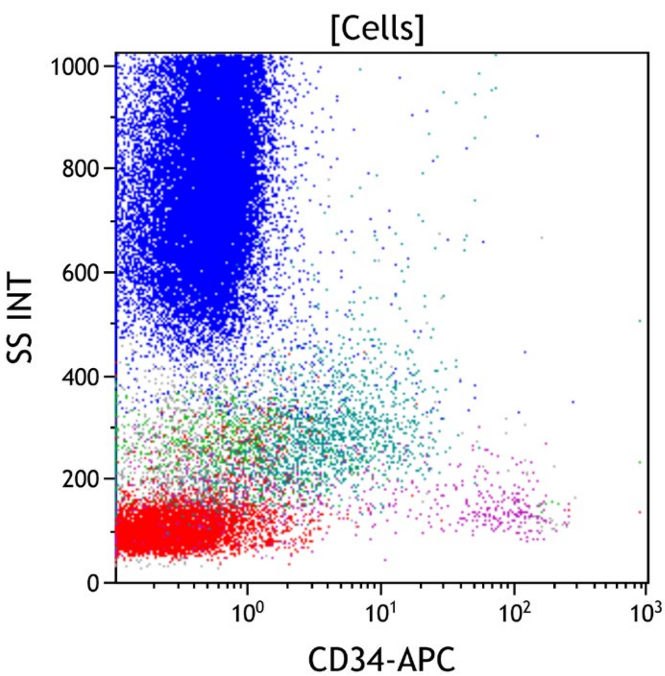

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45 dim gate (purple). The aberrant population (teal) is negative for CD34; the apparent CD34 positivity is likely due to bright CD45 causing an increase in APC background.

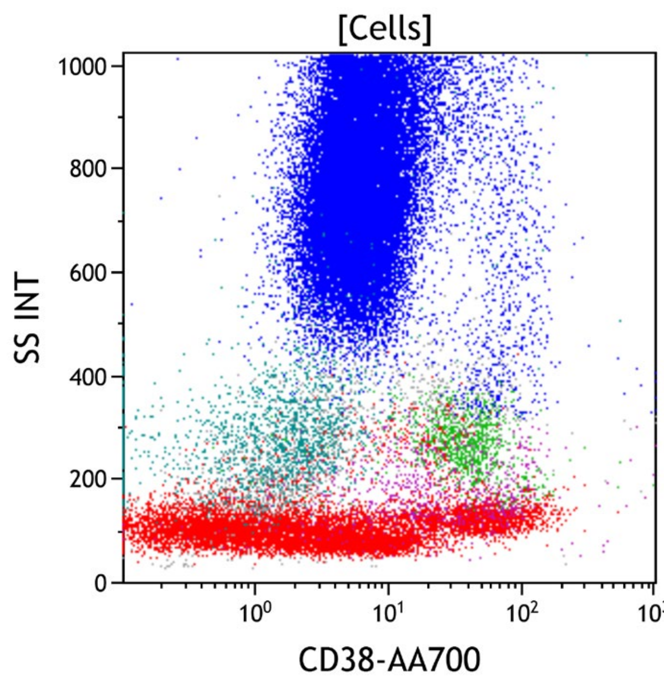

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The aberrant population (teal) displays low CD38 expression.

M2 Cell Tube

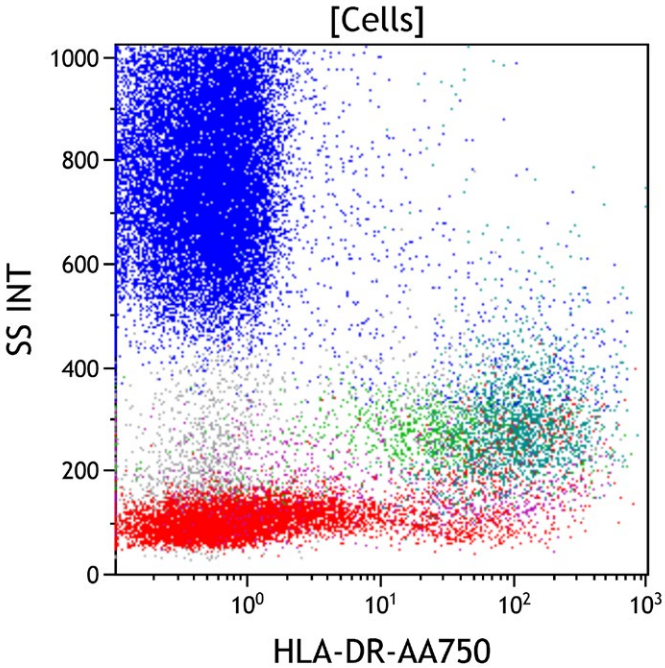

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red, lower right). The aberrant population (teal) is positive for HLA-DR.

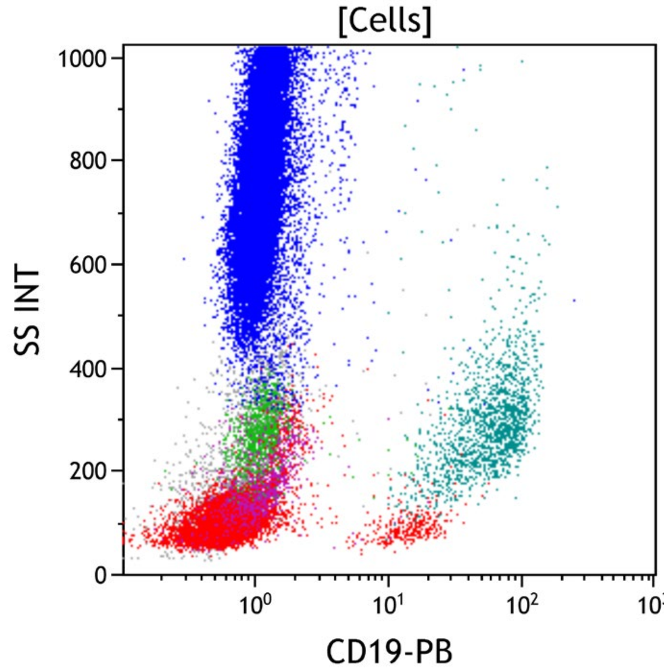

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (teal) is of B cell lineage with high side scatter and bright CD19 expression.

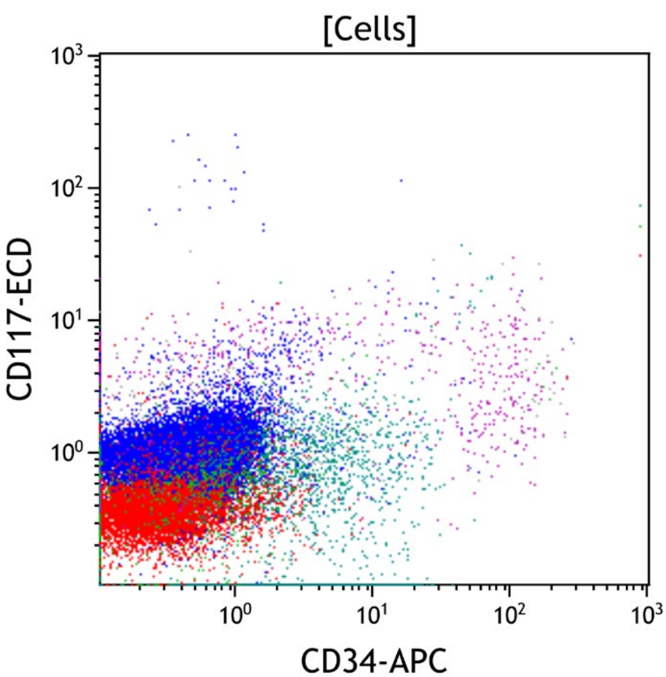

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (purple), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors. The aberrant population (teal) does not express CD34 or CD117.

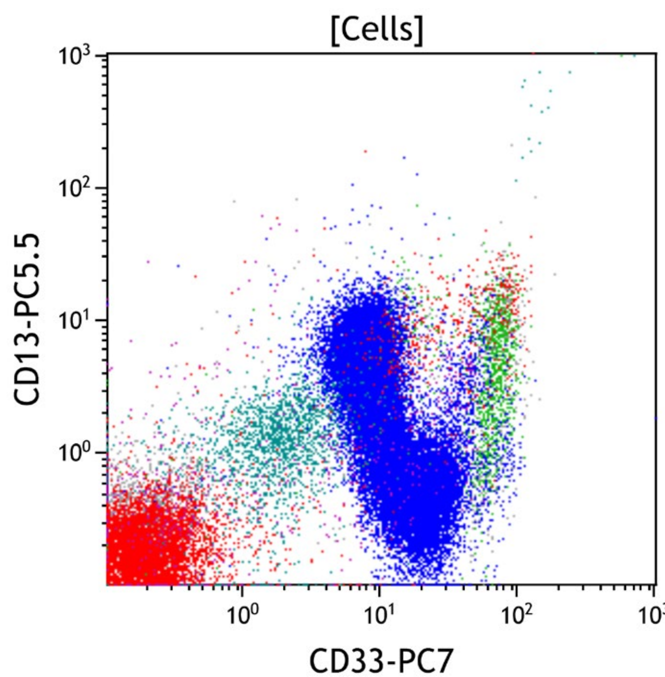

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue, bottom) than more mature granulocytes (blue, upper left). Lymphocytes largely do not express either CD13 or CD33 (red). The aberrant population (teal) displays low CD13 and CD33 expression.

M2 Cell Tube

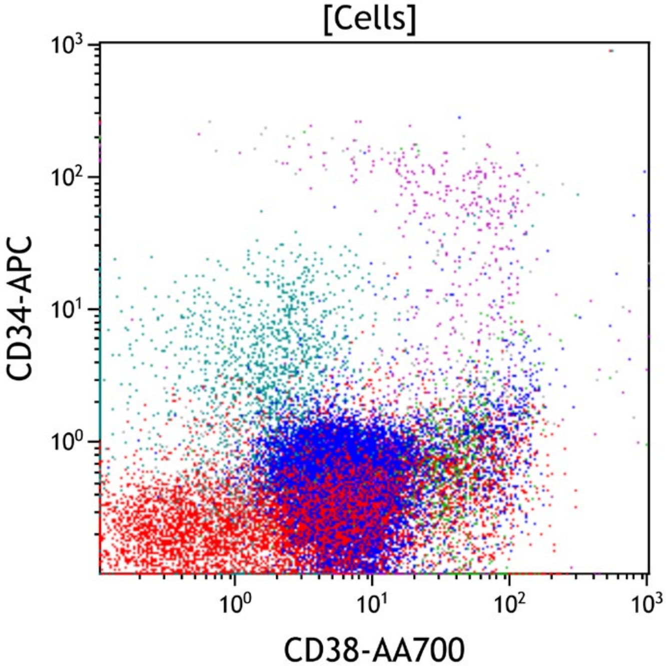

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple).

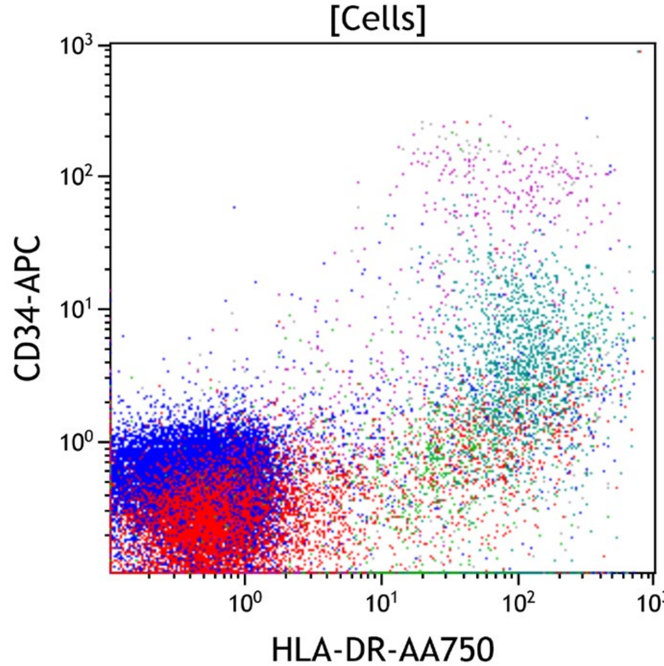

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes. The aberrant population (teal) is positive for HLA-DR.

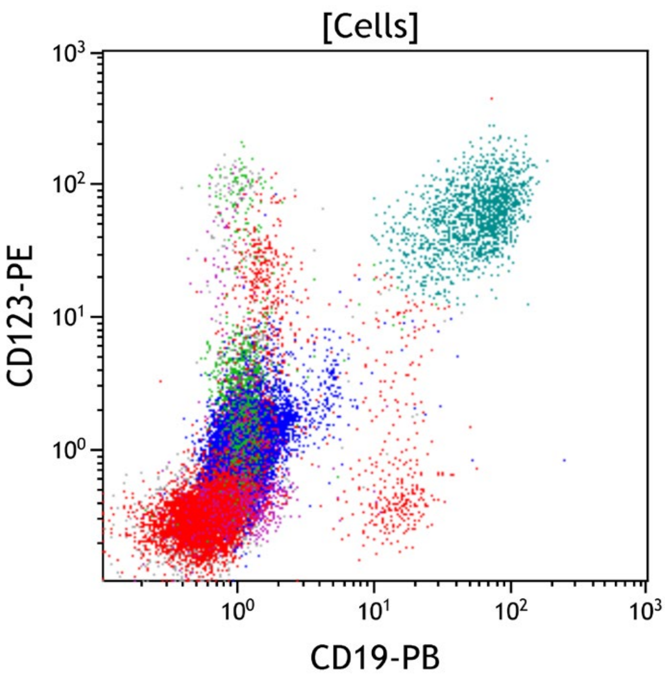

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors (purple). CD19 positive B cells normally do not express significant CD123. The aberrant population (teal) displays bright CD19 and CD123 expression.

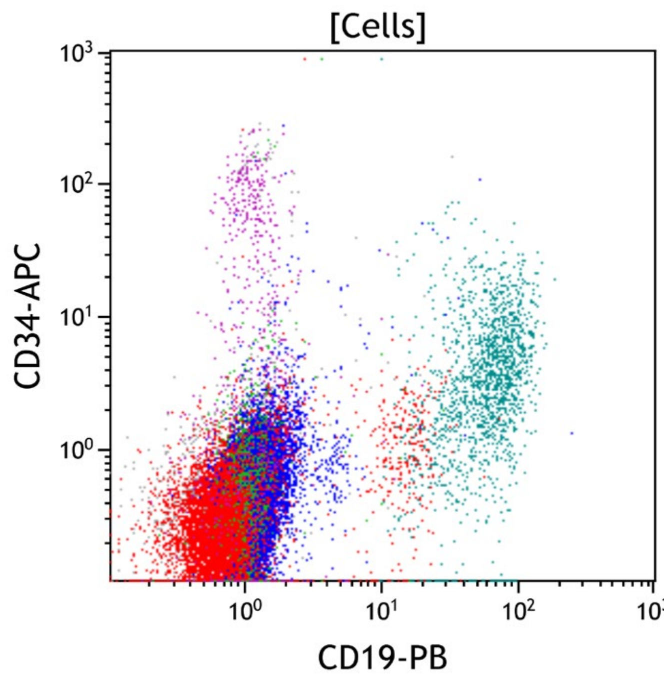

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors (purple, upper left) do not express CD19. The aberrant population (teal) expresses CD19 at a higher level than that seen on normal B cells (red, lower right).

M2 Cell Tube

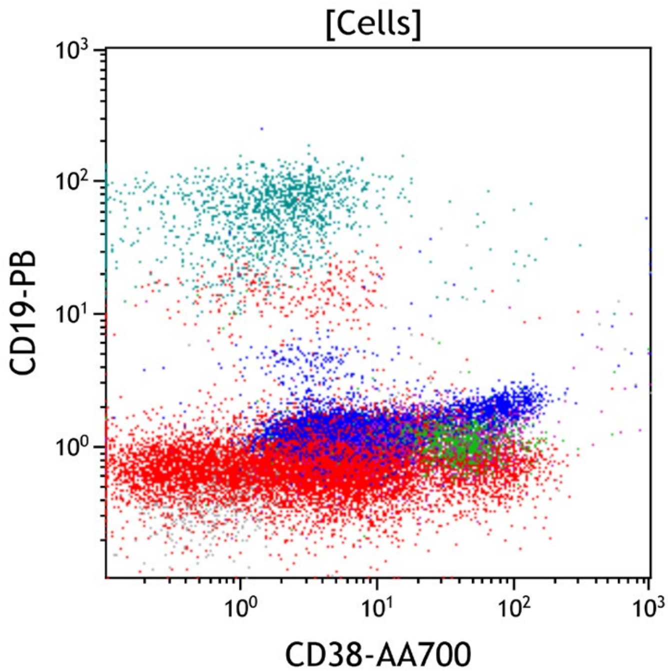

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (teal and red, upper left) show lower expression of CD38. The aberrant population (teal) expresses CD19 at a higher level than that seen on normal B cells (red, upper left).

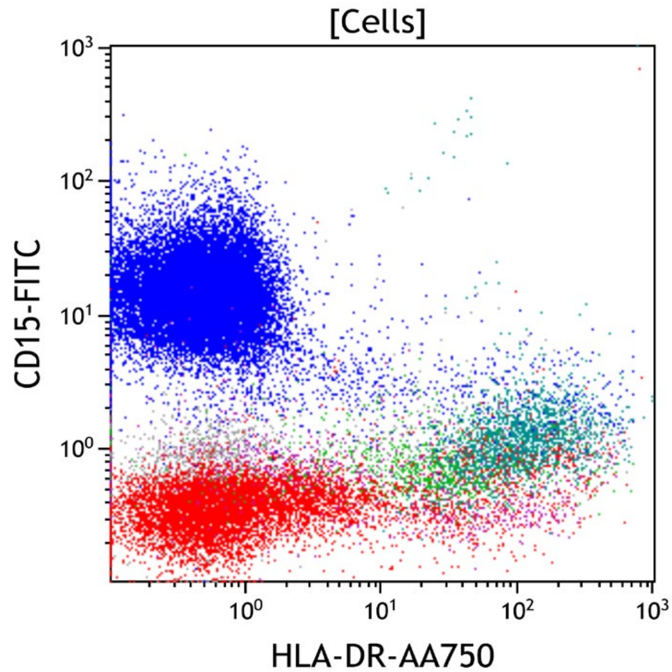

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors (purple) express HLA-DR but only transiently express CD15. The aberrant population (teal) is positive for HLA-DR.

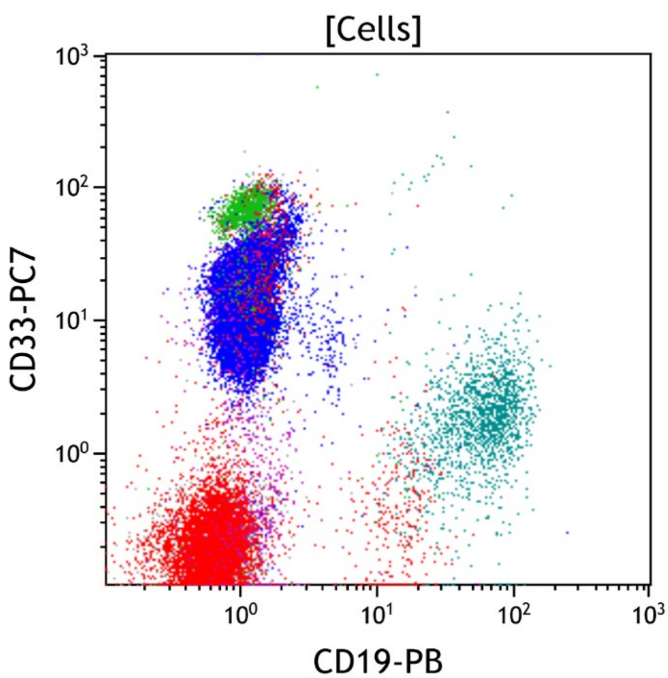

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple, lower right). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The aberrant population (teal) is positive for CD19 and dimly positive for CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased forward and side scatter and expression of bright CD19, bright CD20, low CD38, bright CD45, bright CD123, bright CD200, and intermediate to bright surface kappa light chain expression without CD5, CD10, or other T or myeloid markers. Compared with normal B cells, the increased forward and side scatter, expression of CD19 and CD20 at a high level, expression of CD123, and kappa light chain restriction are aberrant.

Taken together, the immunophenotype of the aberrant population is consistent with mature B cell lymphoma. While the increased forward and side scatter, increased expression of CD19 and CD20, and expression of CD123 raise the possibility of hairy cell leukemia, additional immunophenotyping for CD11c, CD25, and CD103 is required for definitive diagnosis. Correlation with clinical and laboratory data and morphologic findings is recommended.

Case #13: Mature B-cell lymphoma

Clinical Vignette

This 73-year-old male presents with thrombocytopenia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

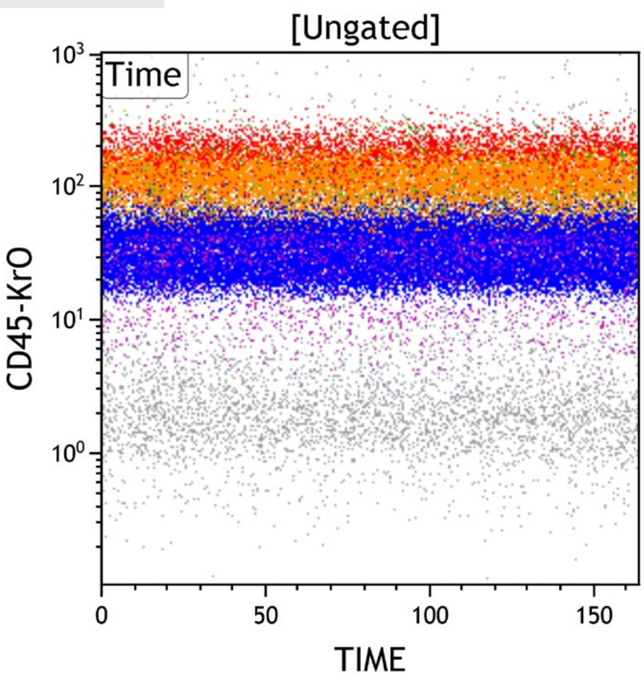

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

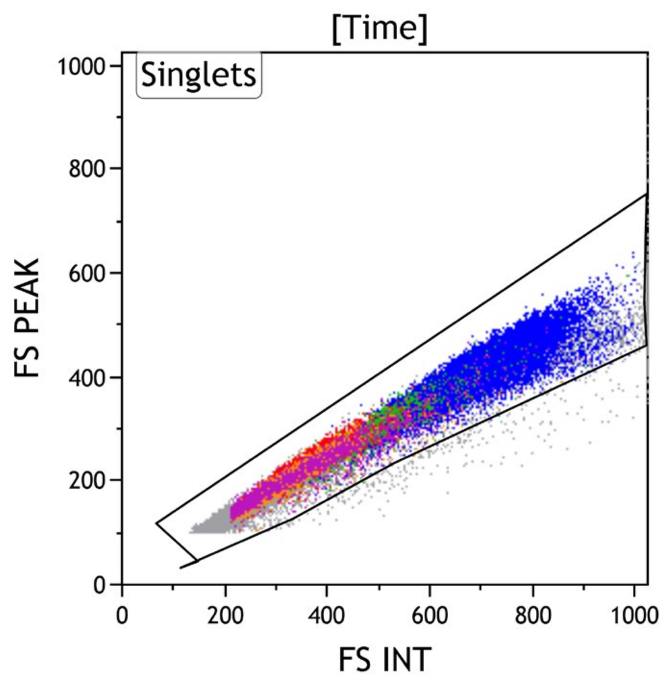

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

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B Cell Tube

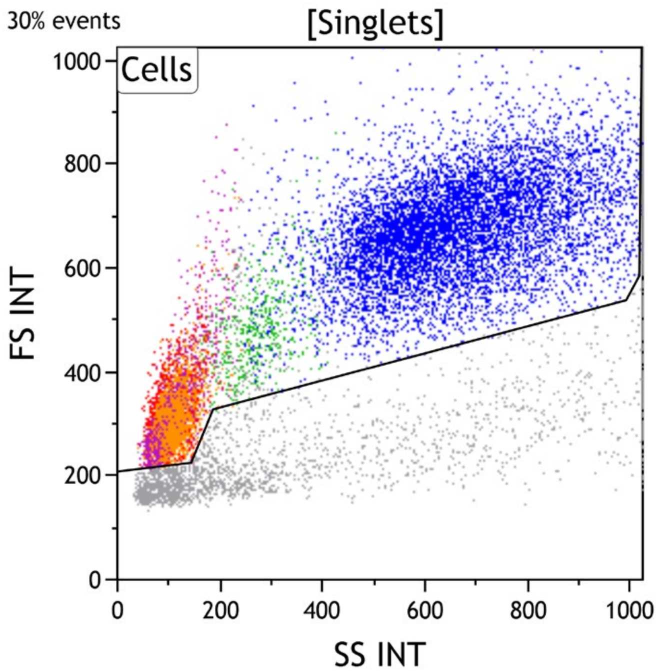

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

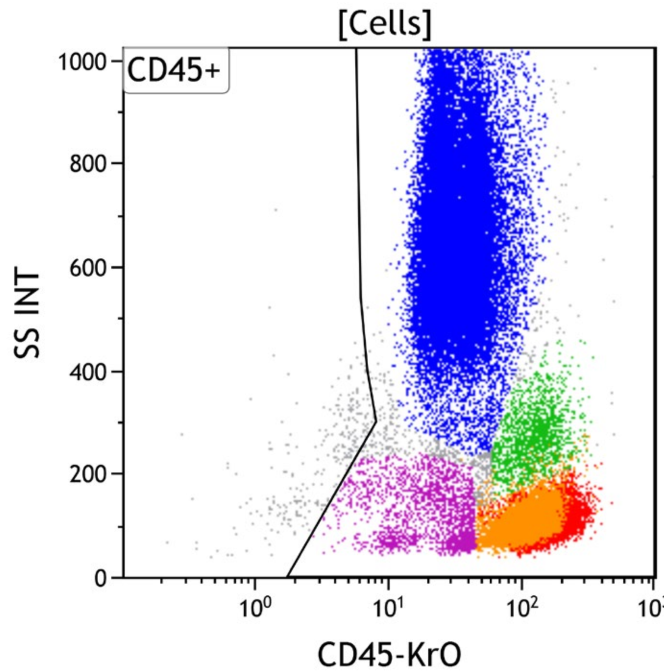

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

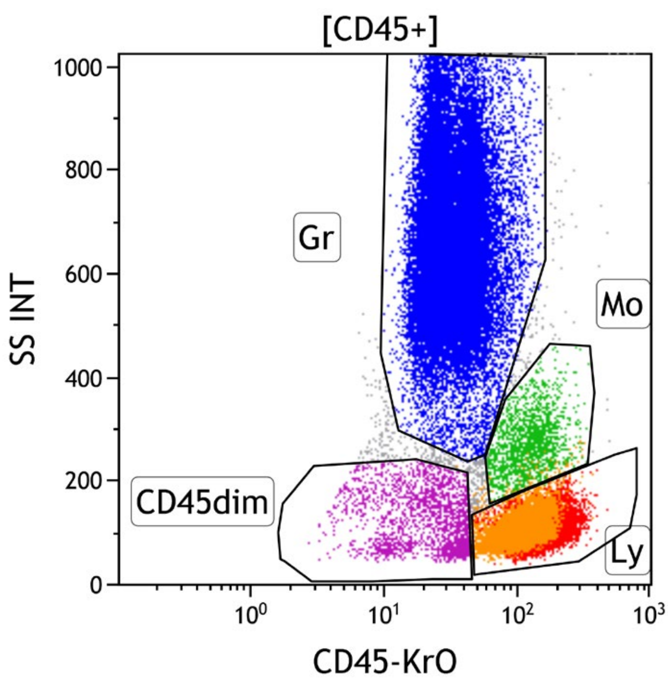

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis.

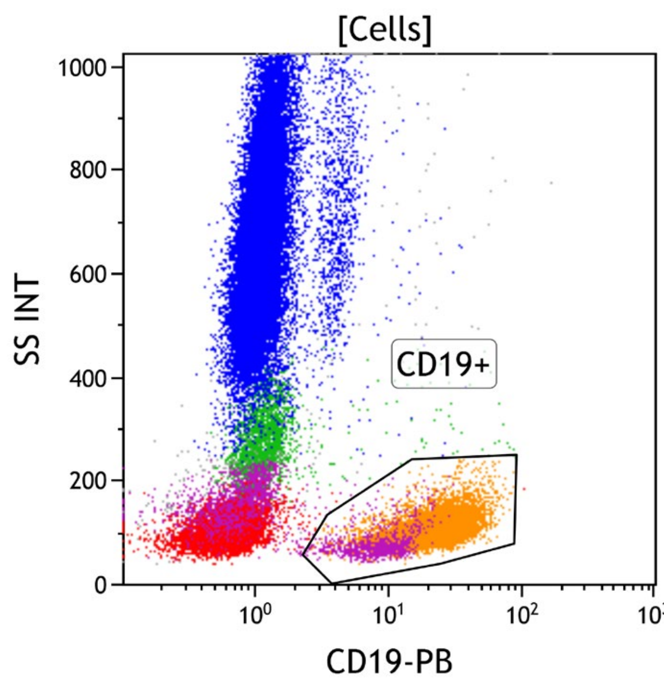

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note the proportionally increased CD19 positive B cells in this sample.

B Cell Tube

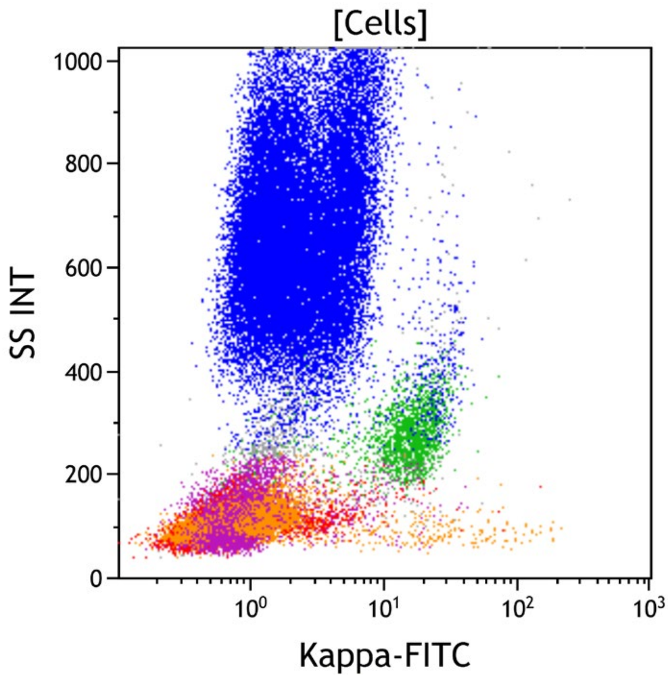

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) lack kappa light chain expression.

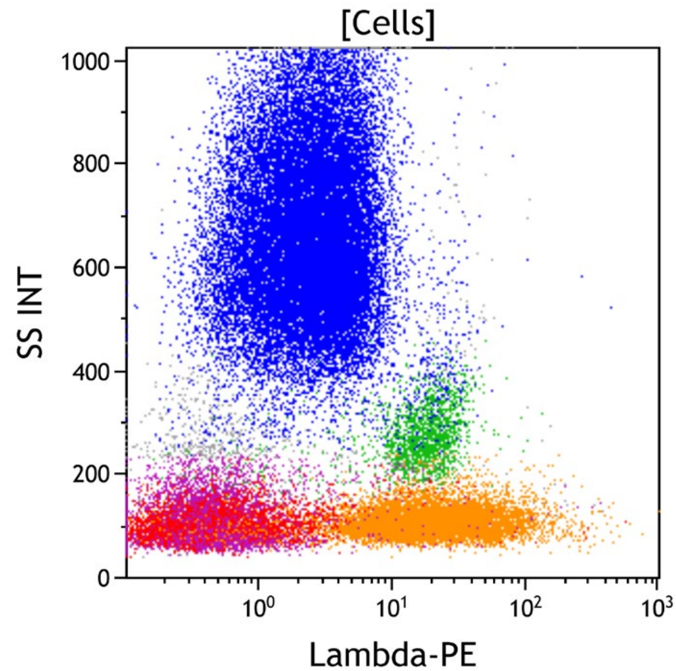

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The majority of the CD19 positive cells (orange) have surface lambda light chain expression.

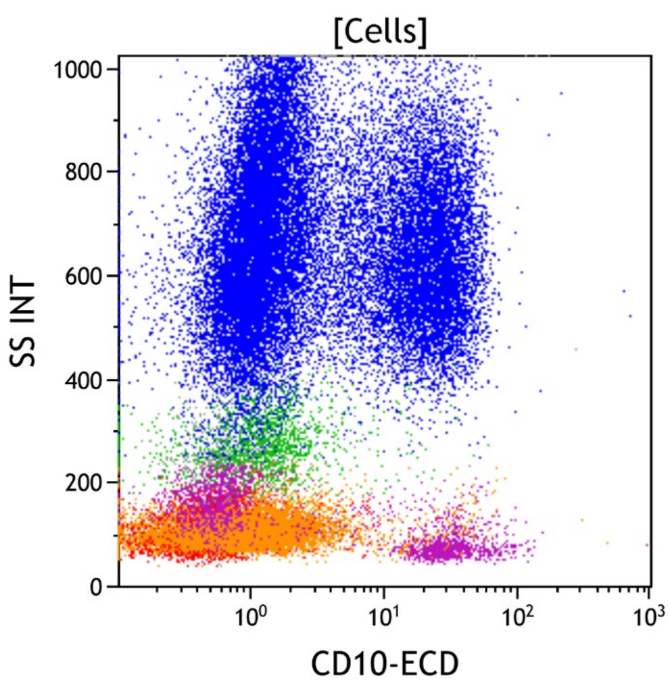

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange) is negative for CD10.

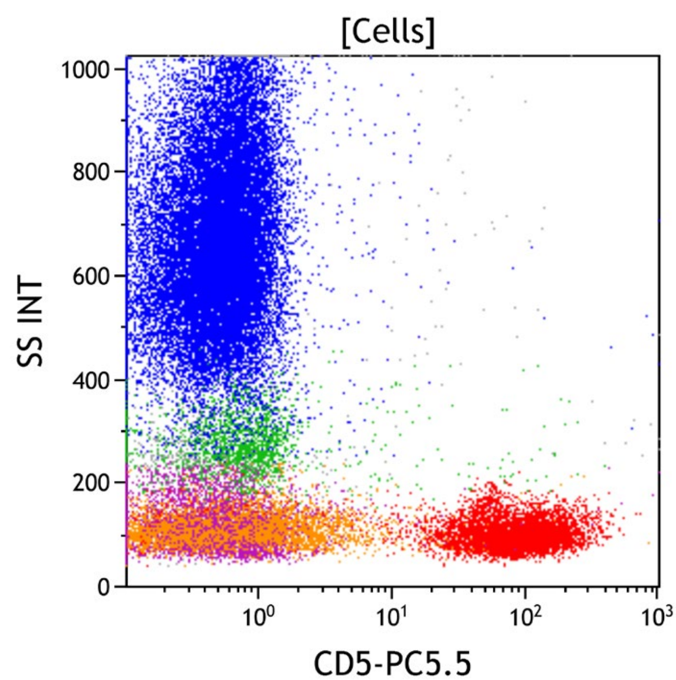

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The CD19 positive population (orange) is negative for CD5.

B Cell Tube

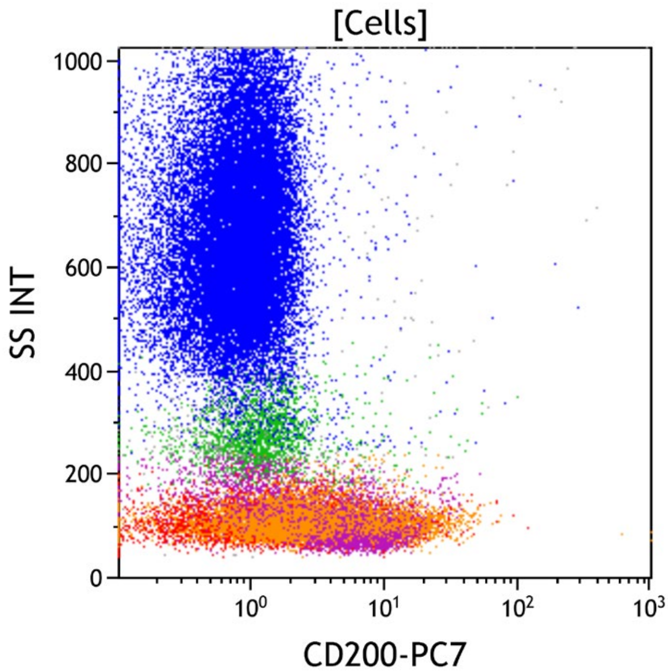

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The CD19 positive population (orange) has variable CD200 expression.

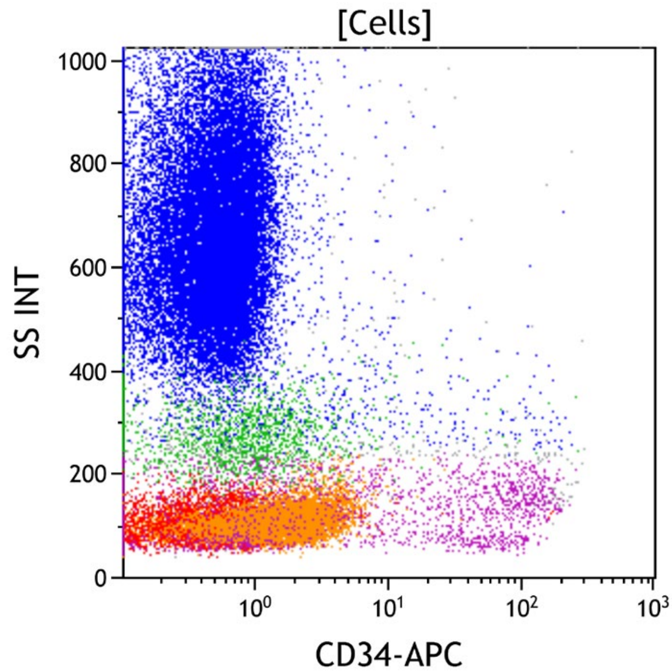

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The CD19 positive population (orange) is negative for CD34.

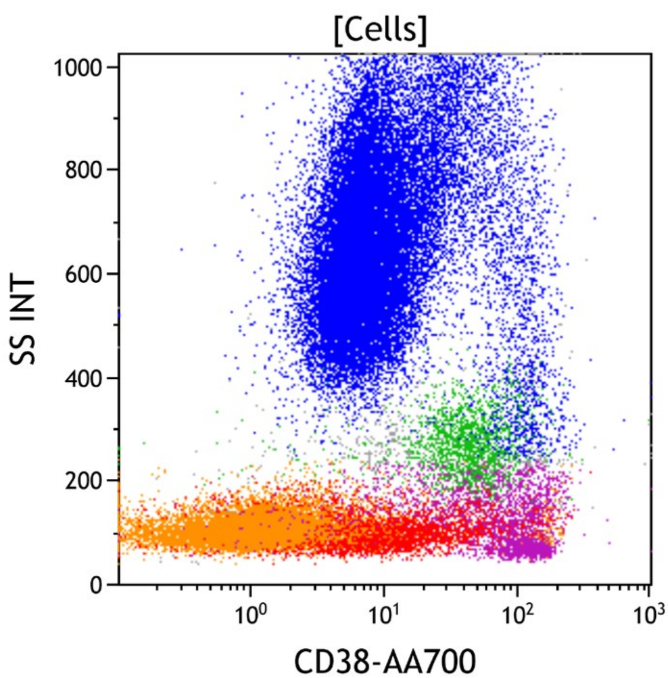

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red). The CD19 positive population (orange) displays low to absent CD38 expression.

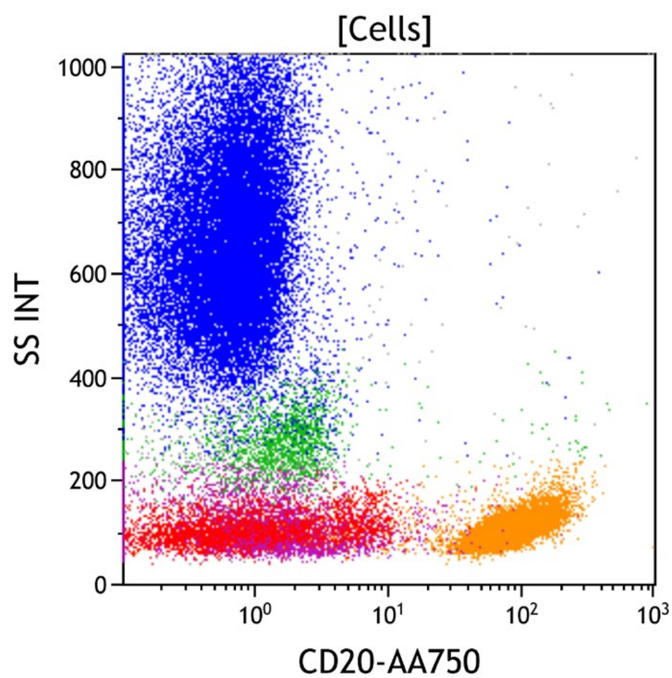

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The CD19 positive population (orange) is positive for CD20.

B Cell Tube

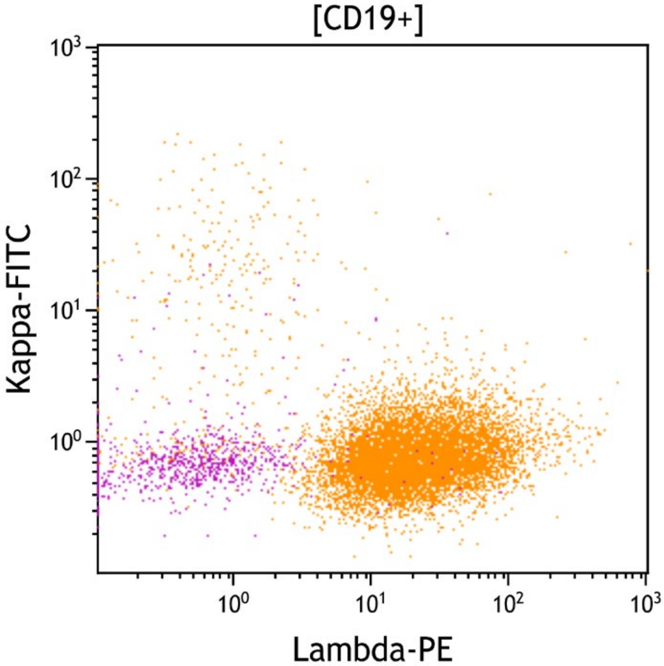

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. The CD19 positive cells (orange) predominantly have surface lambda light chain expression, indicating a clonal B cell population. A small population of polyclonal B cells (also in orange) with kappa and lambda light chain expression is present.

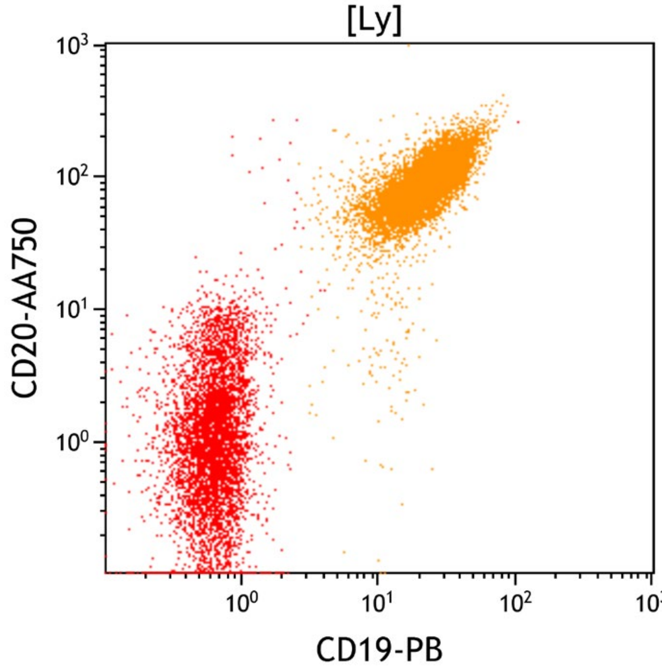

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cells (orange) is positive for CD19 and CD20.

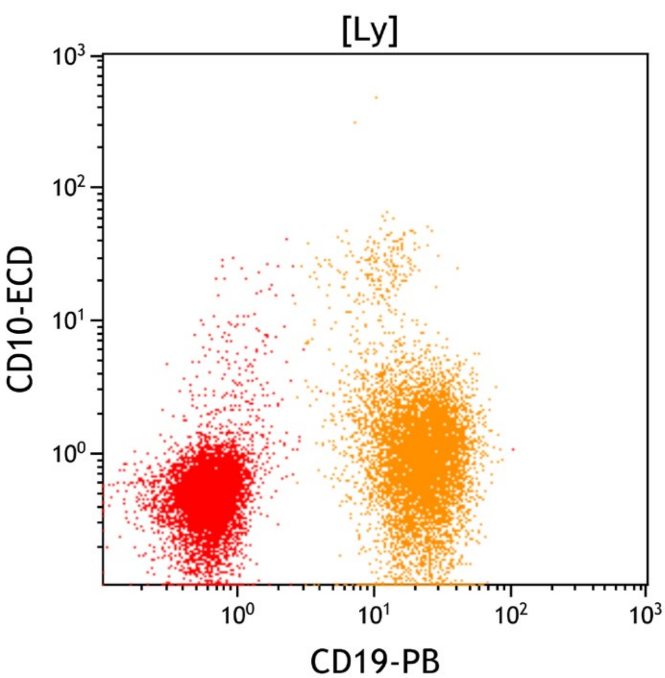

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. The few CD10 positive B cells (orange, upper middle) represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. The CD19 positive clonal B cell population (orange, lower middle) is largely negative for CD10.

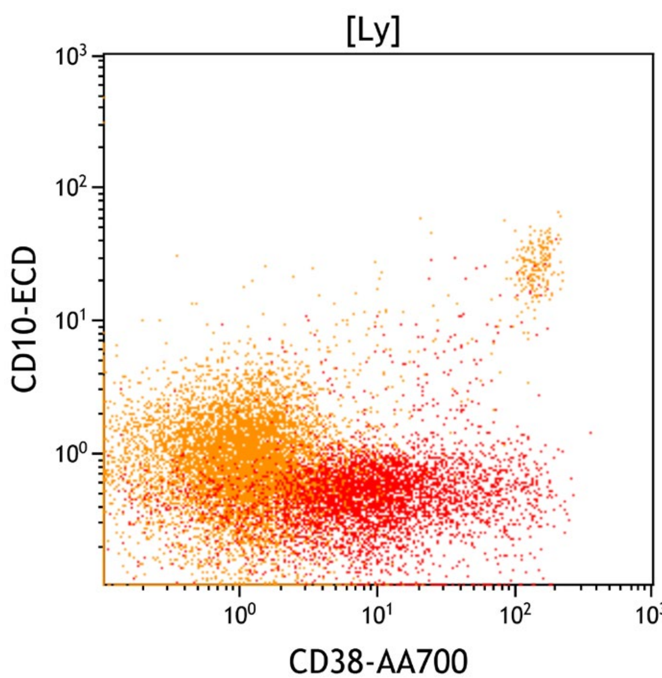

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells display low to absent CD38 expression. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 and CD38 positive cells (orange, upper right) represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. The clonal B cell population (orange, lower left) displays low to absent CD38 expression and is negative for CD10.

B Cell Tube

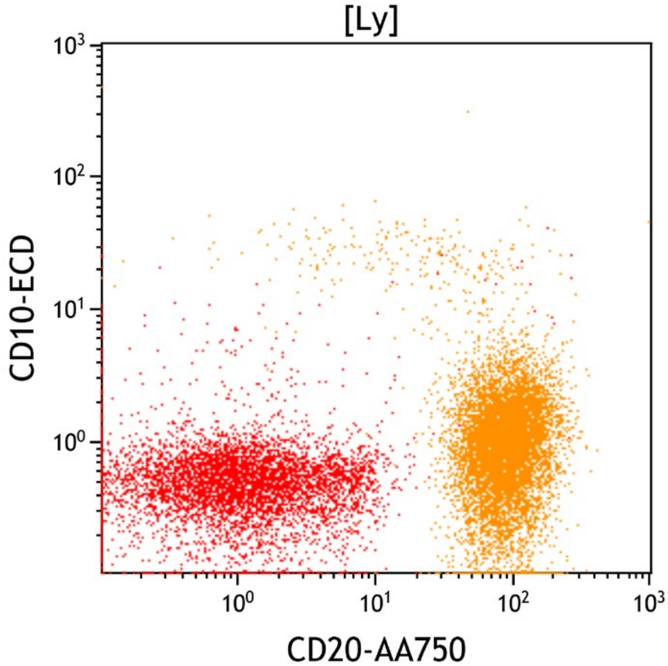

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 (orange, upper middle) represent late stage immature B cells. The clonal B cell population (orange, lower right) is positive for CD20 and negative for CD10.

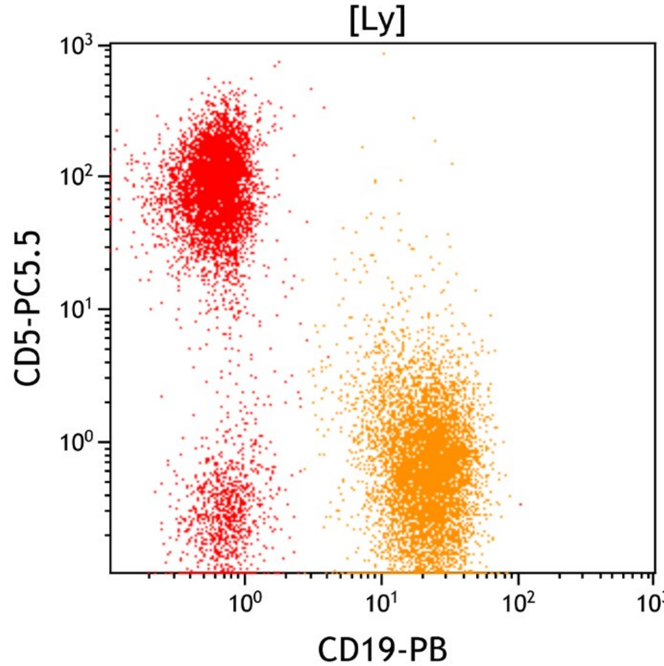

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) is positive for CD19 and negative for CD5.

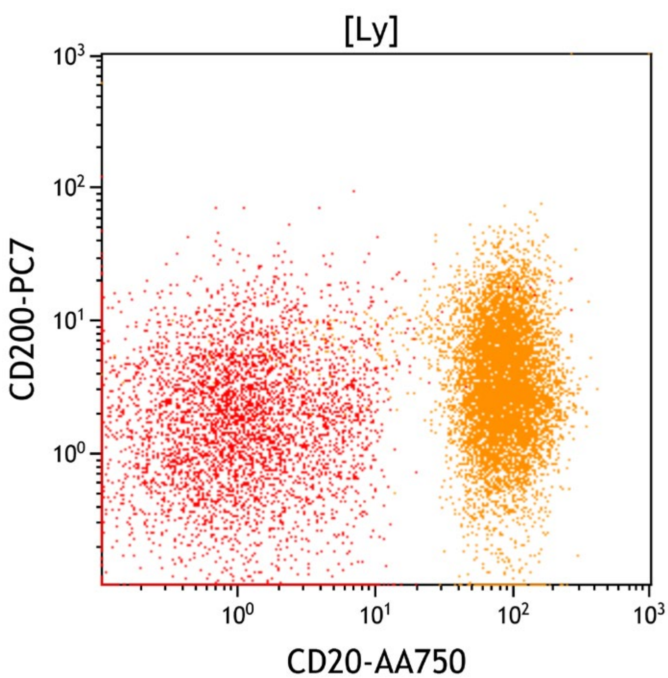

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to intermediate level. The clonal B cell population (orange) is positive for CD20 and expresses CD200 at a low level.

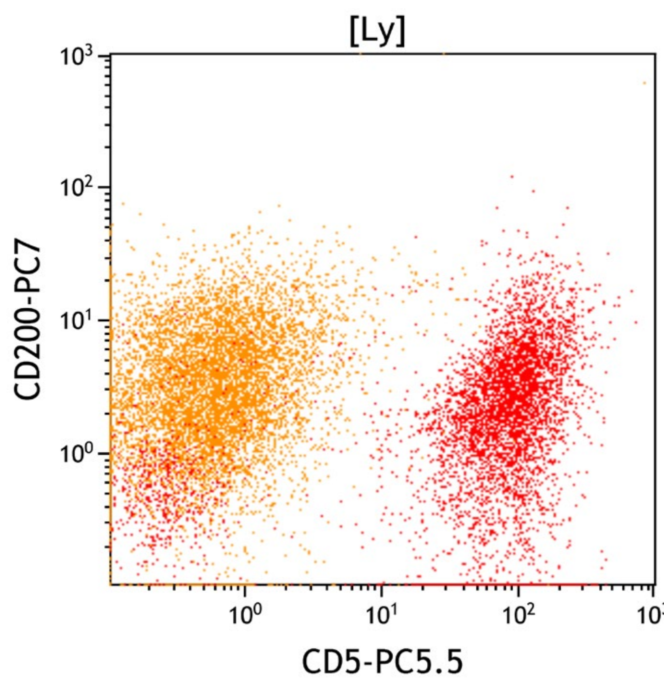

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The clonal B cell population (orange) expresses CD200 at a low level and is negative for CD5.

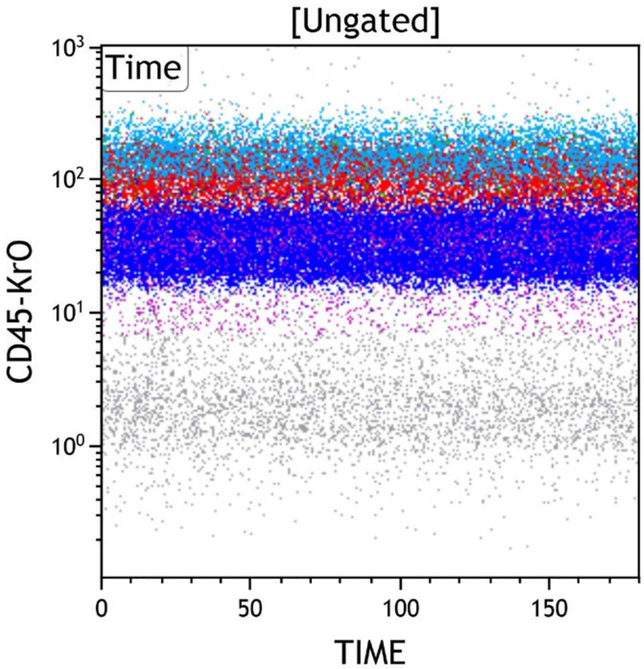

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

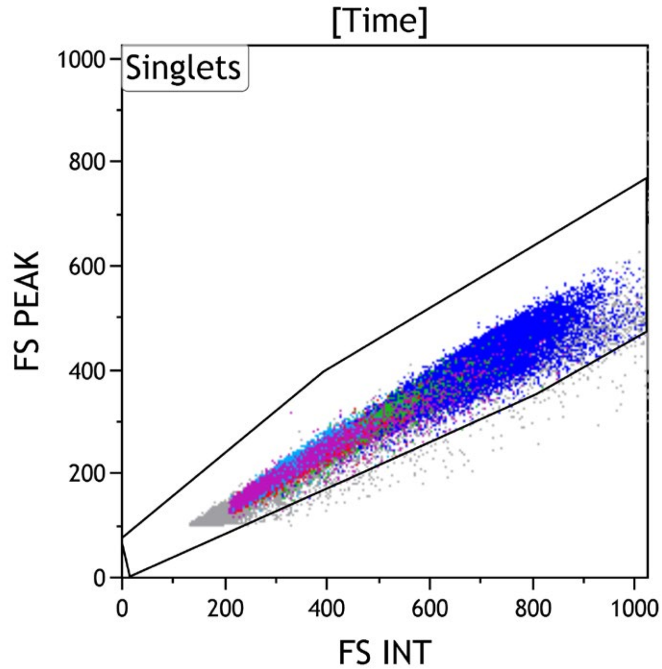

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

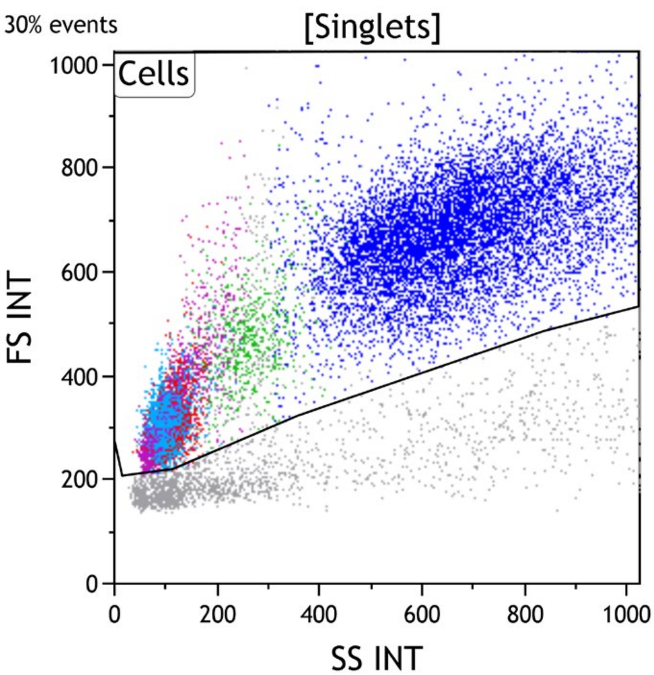

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

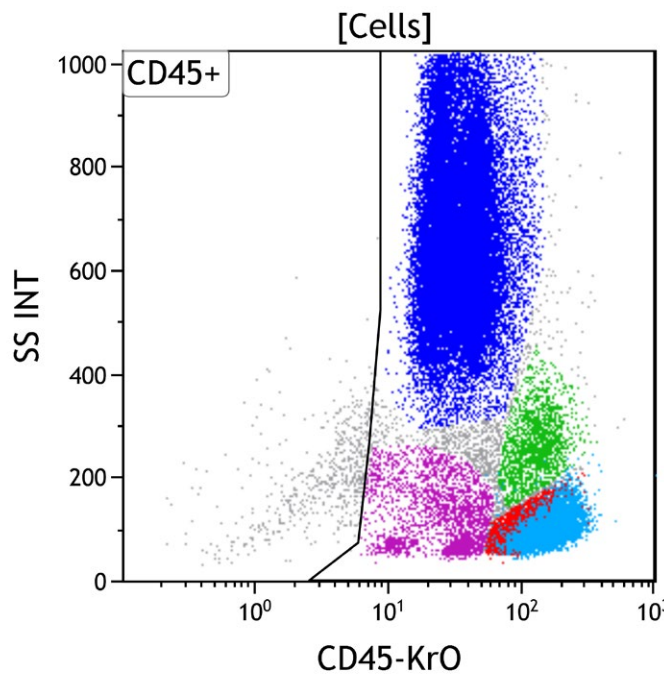

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

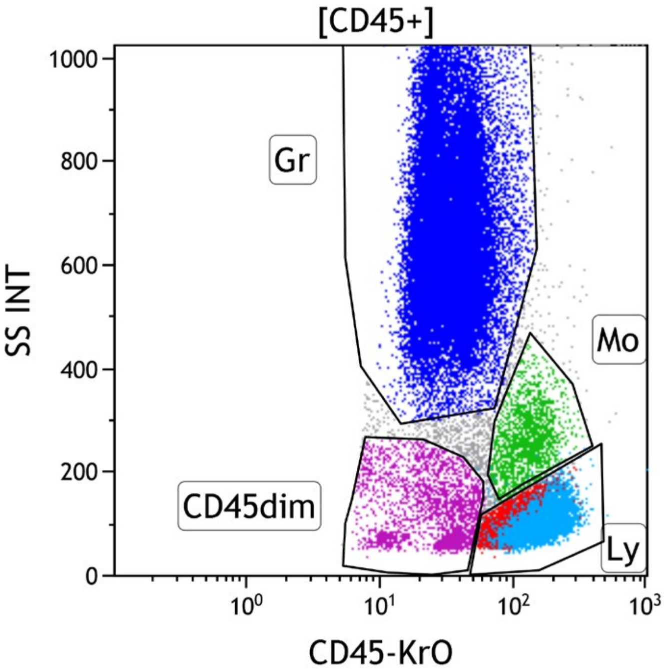

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

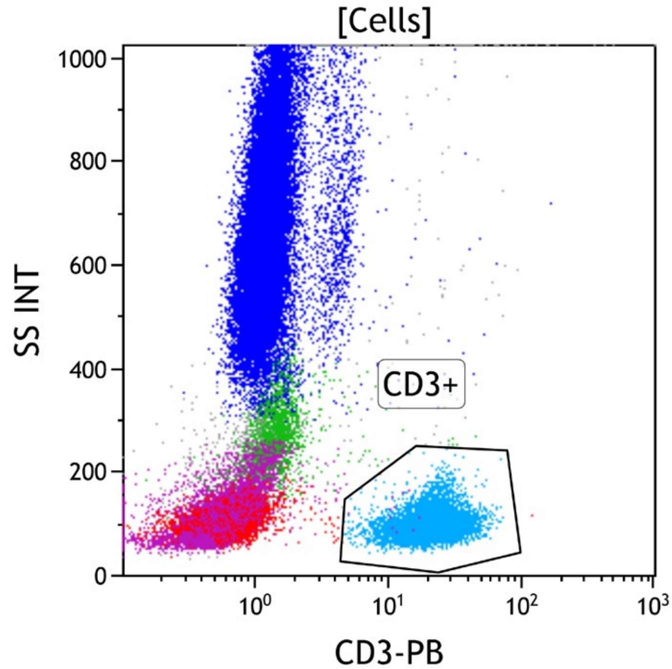

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

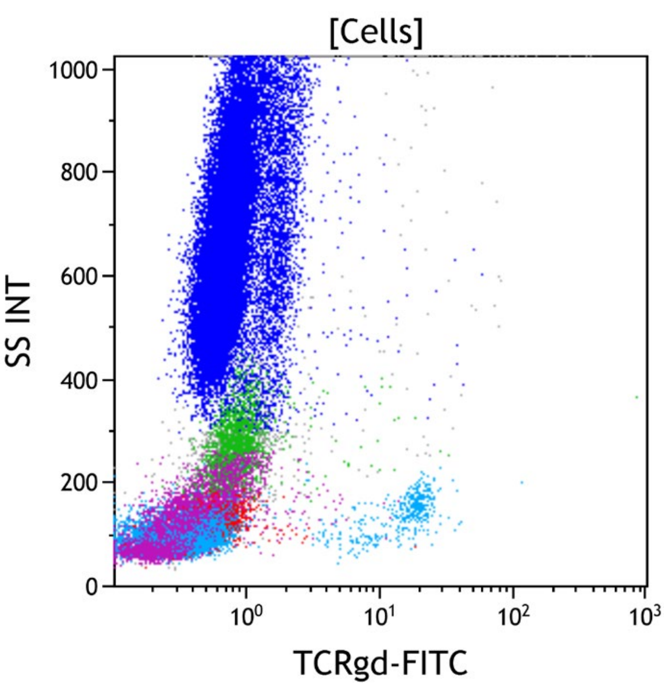

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua, lower right).

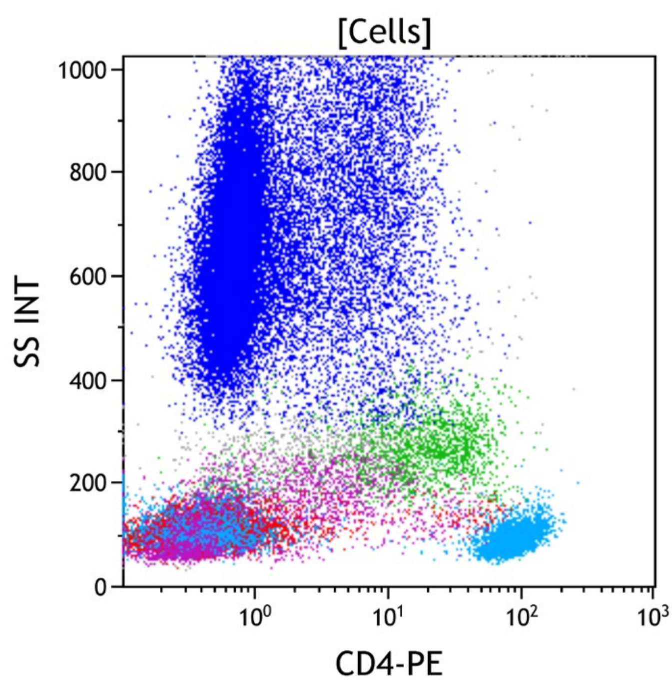

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

T Cell Tube

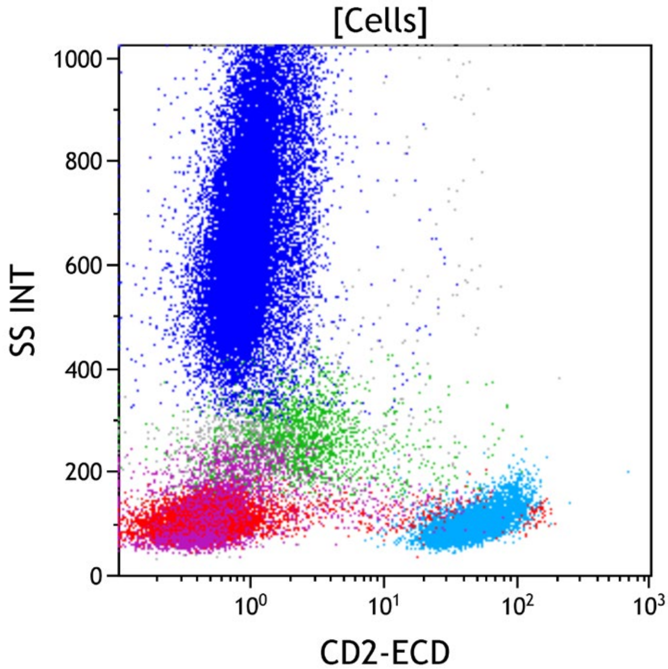

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower right) and at a low level on monocytes (green).

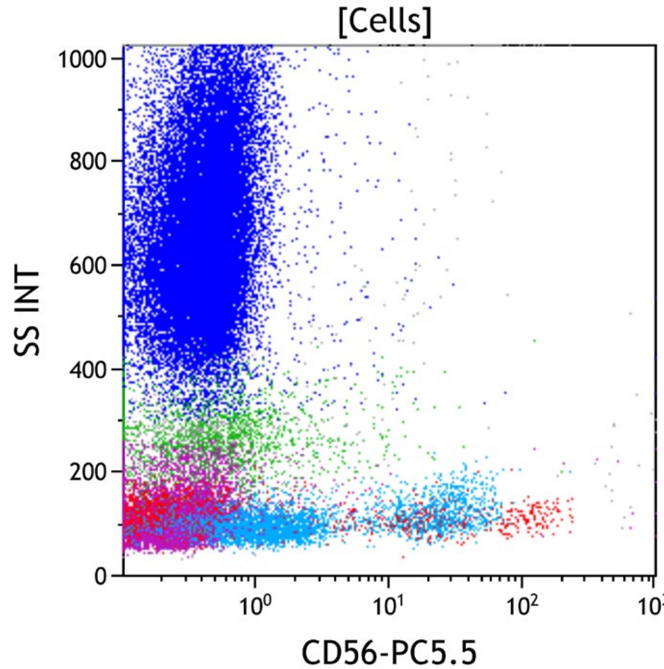

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red, lower right), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions.

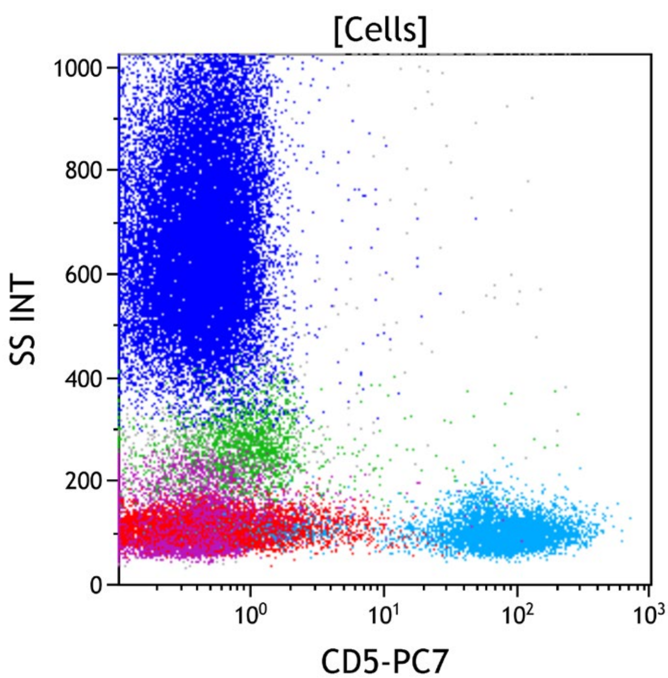

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

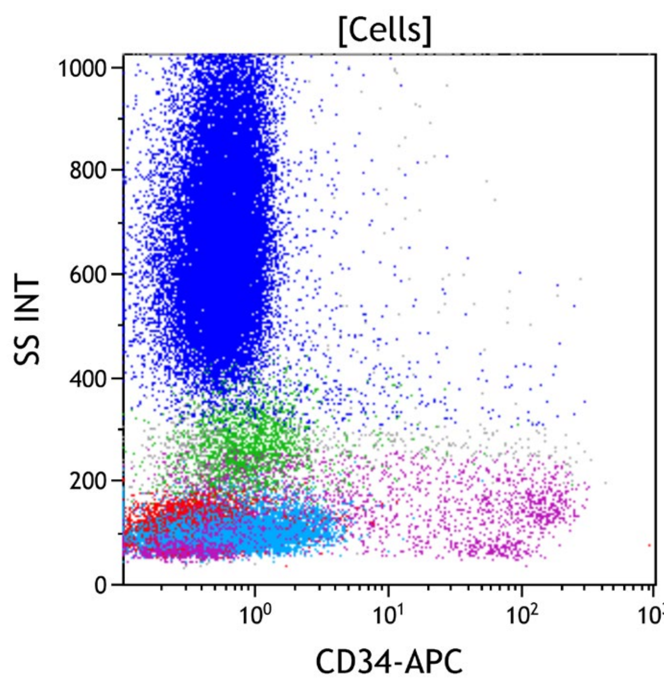

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The mature granulocytes (blue), monocytes (green), and lymphocytes (aqua and red) are negative for CD34.

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T Cell Tube

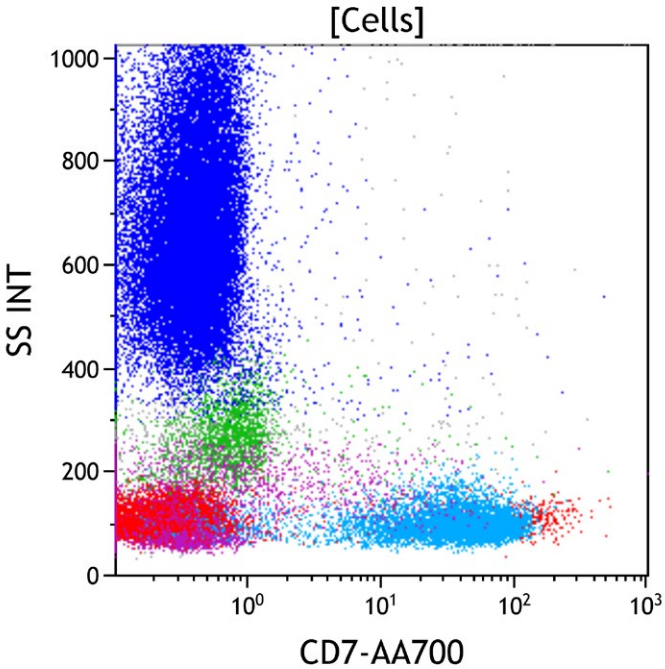

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

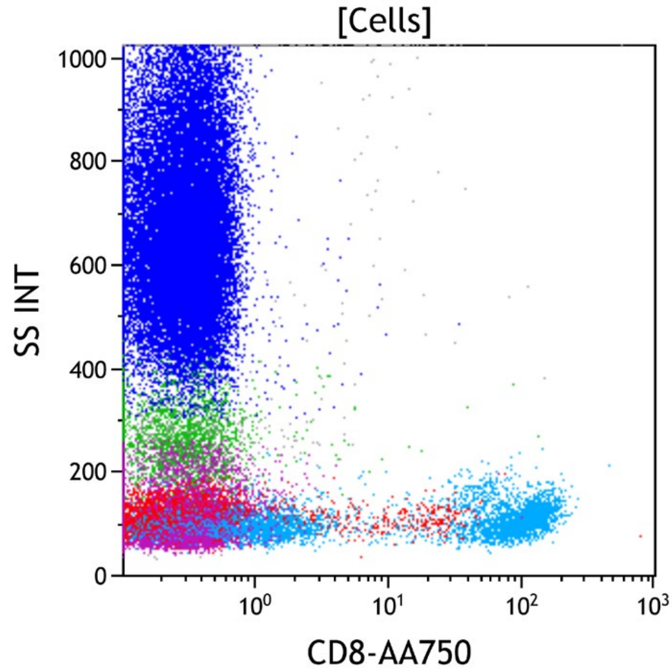

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

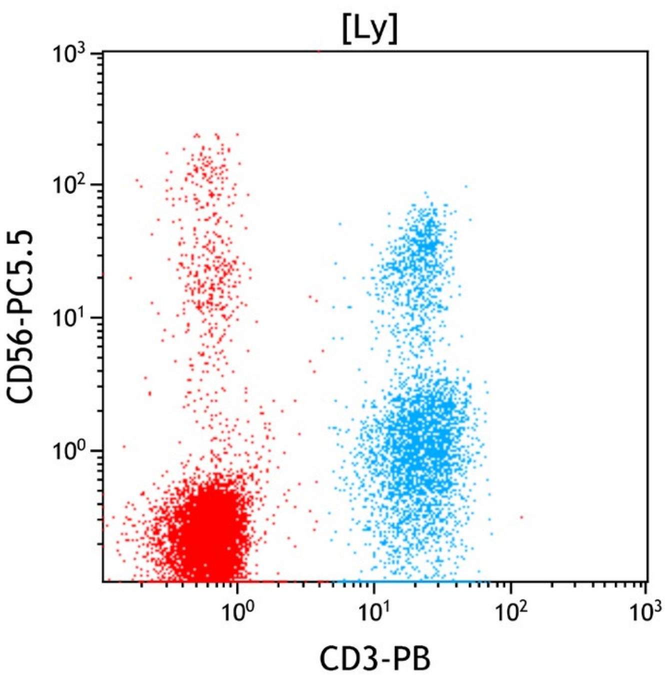

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

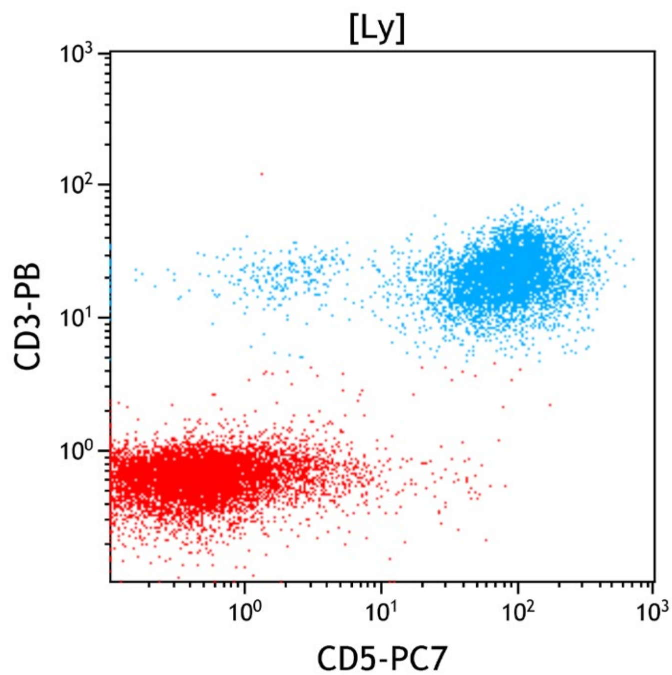

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells and B cells.

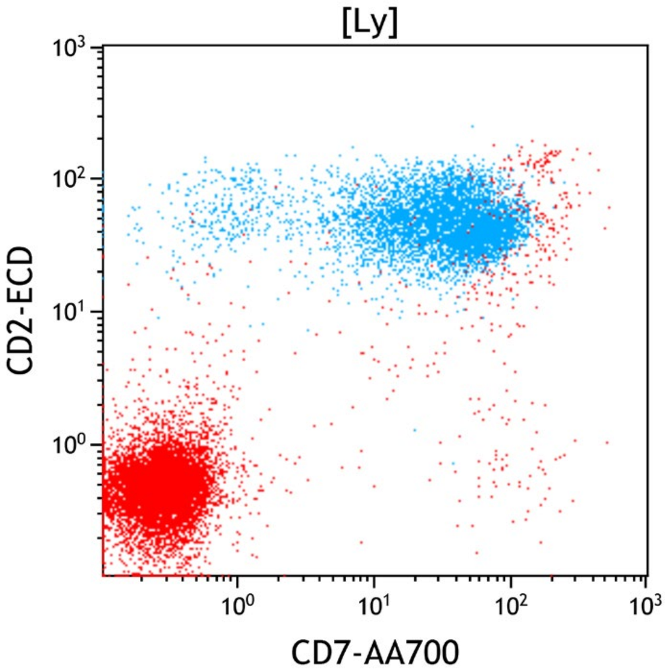

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

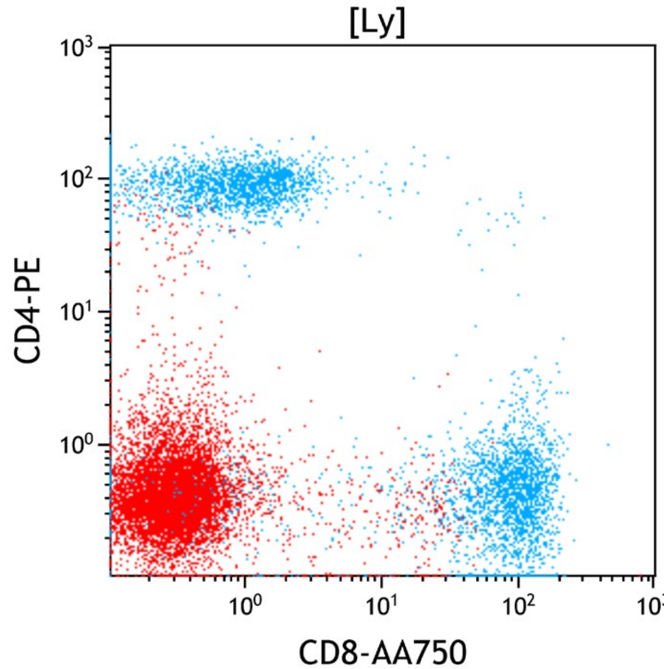

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells.

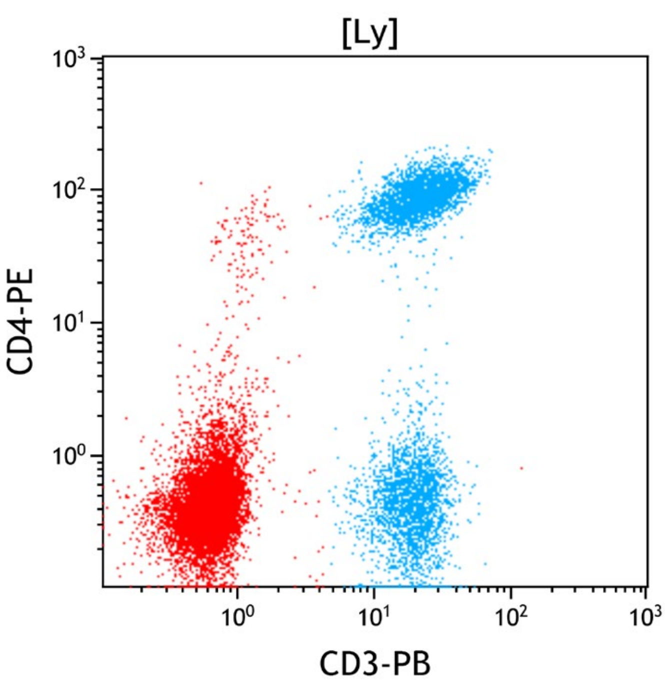

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

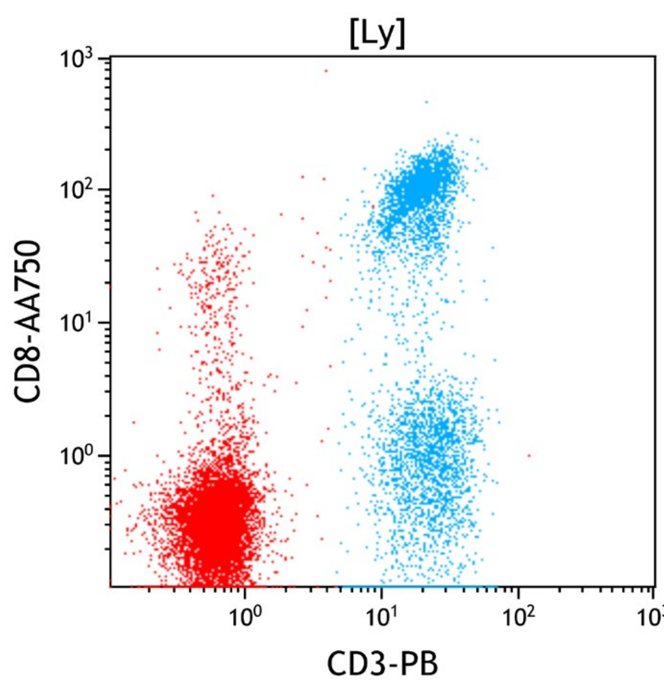

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

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T Cell Tube

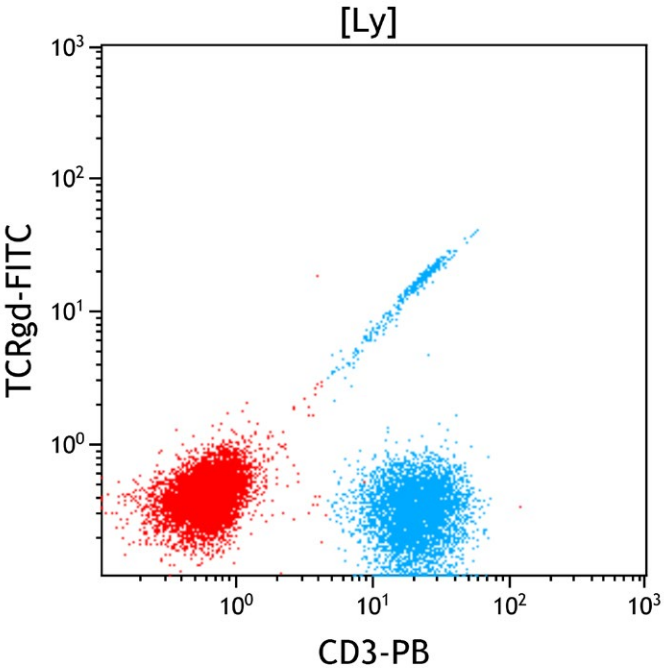

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

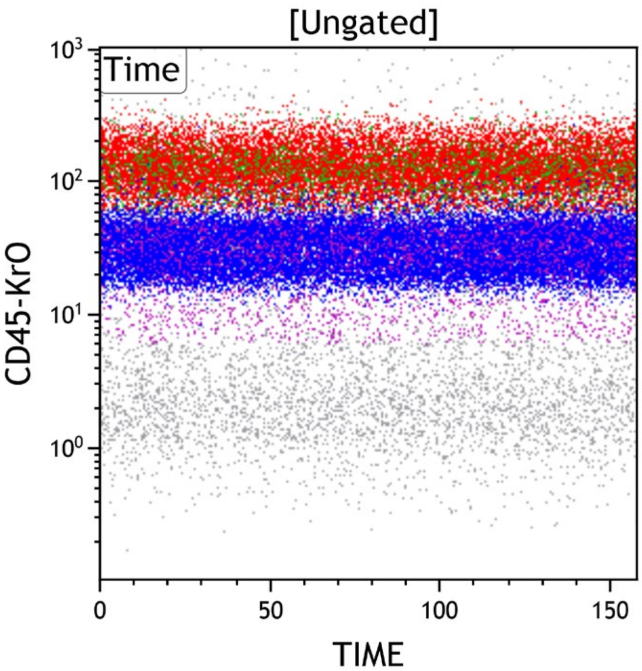

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

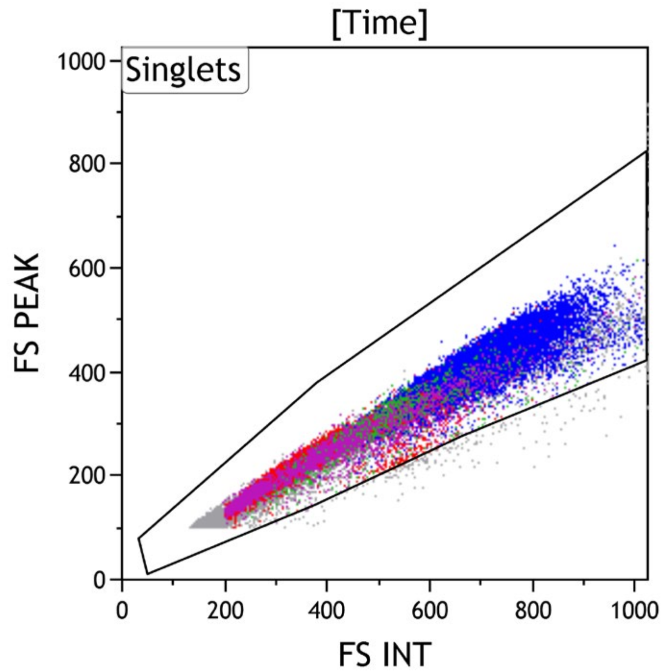

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

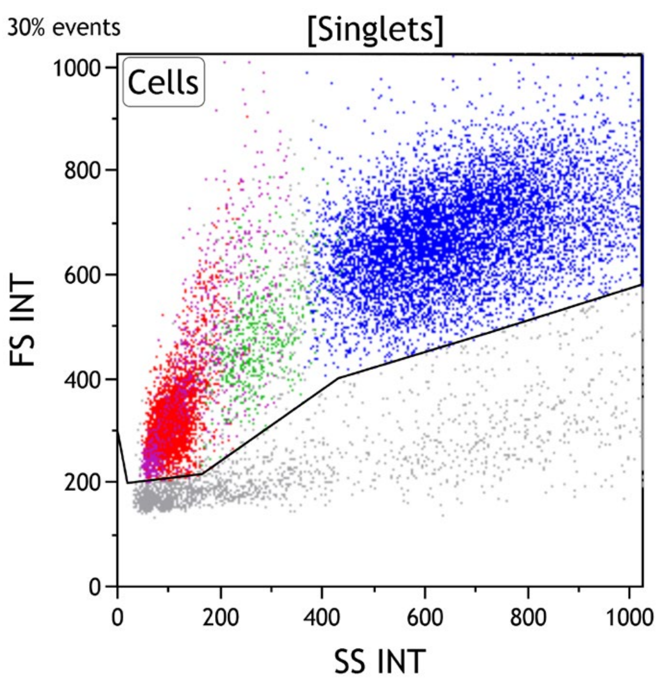

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

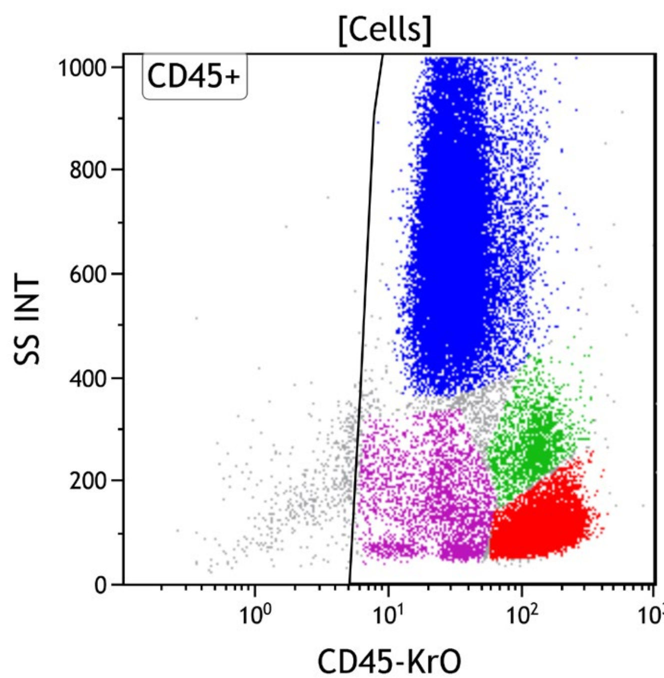

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

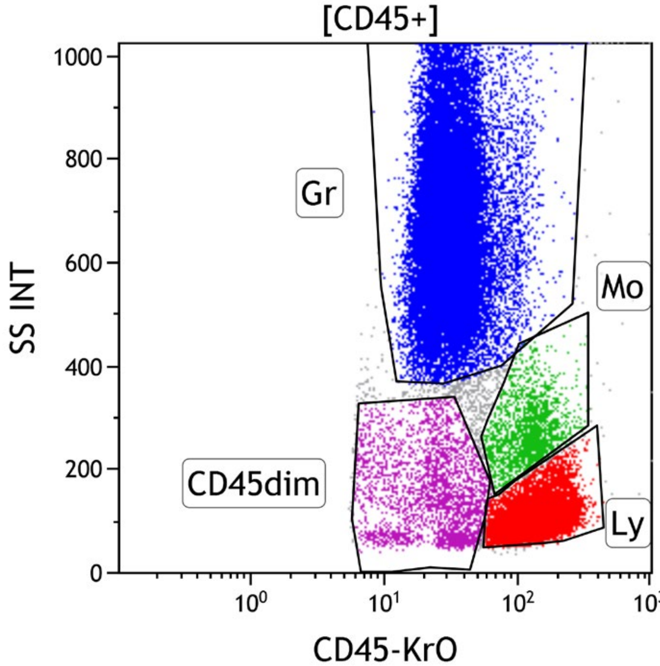

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

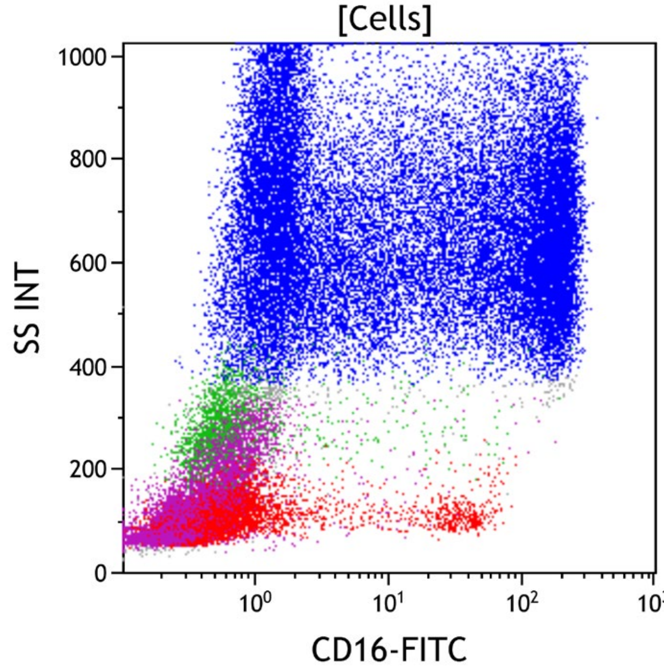

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

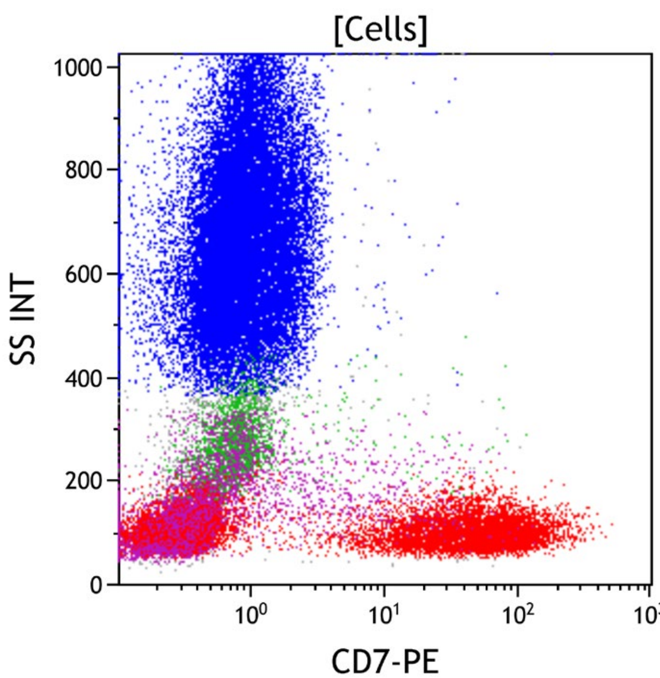

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors.

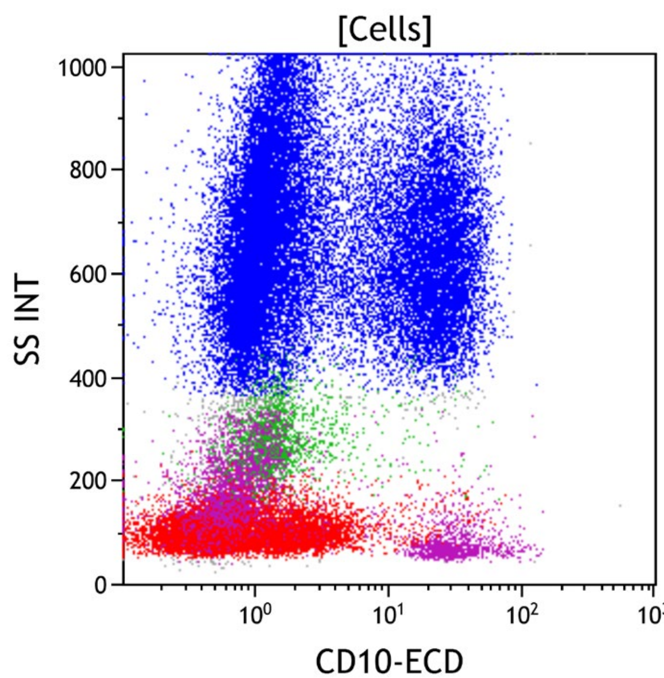

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

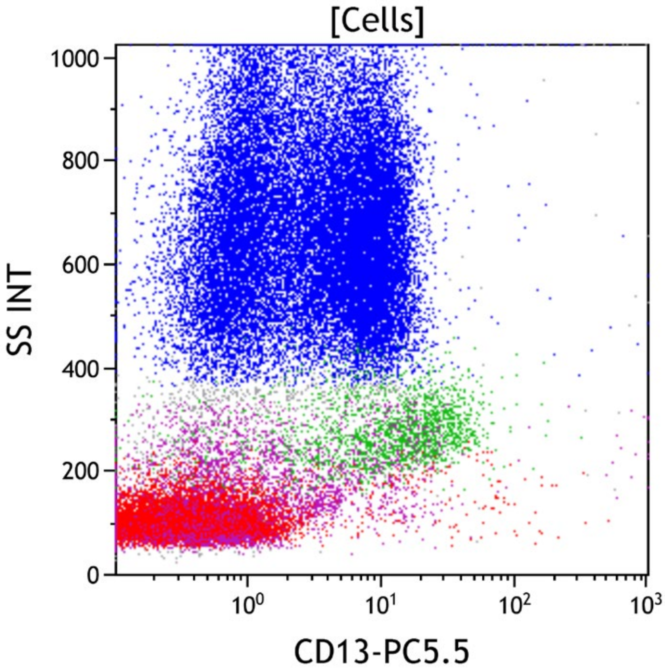

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

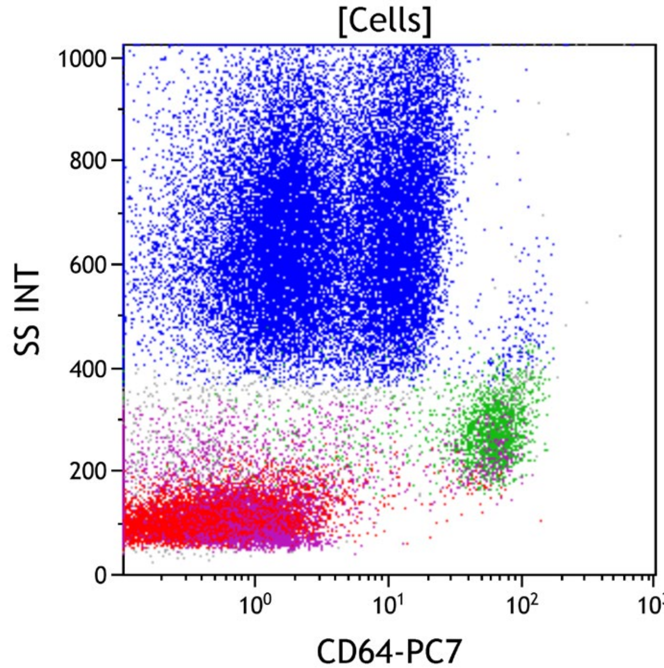

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors.

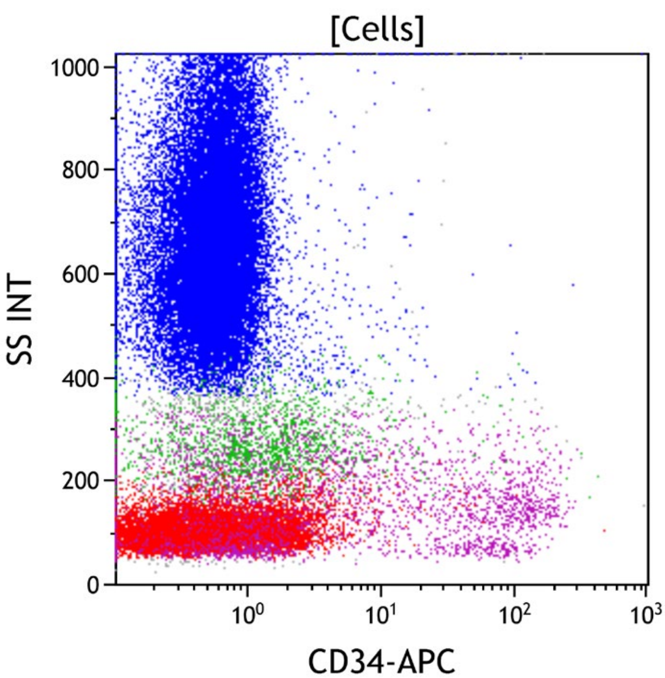

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34.

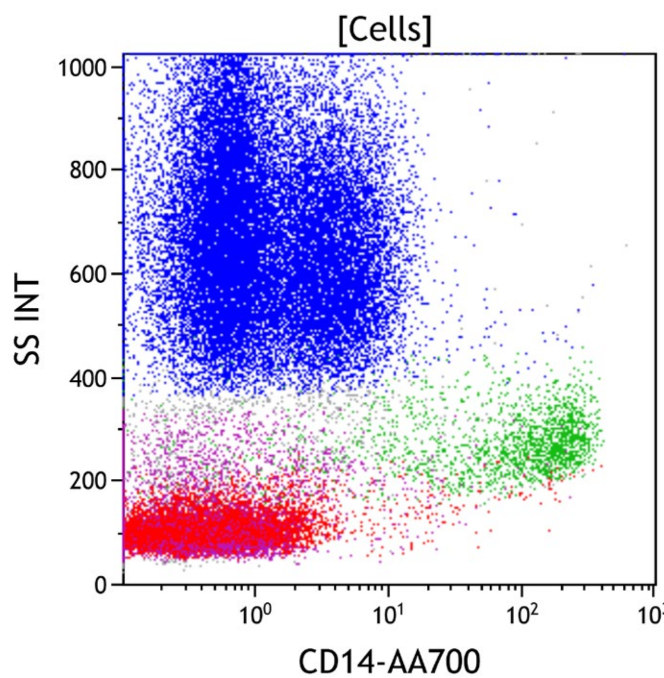

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

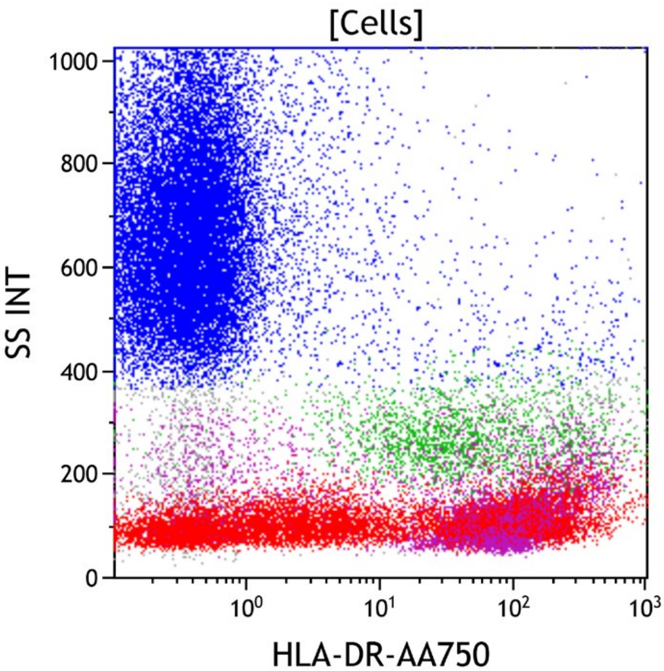

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

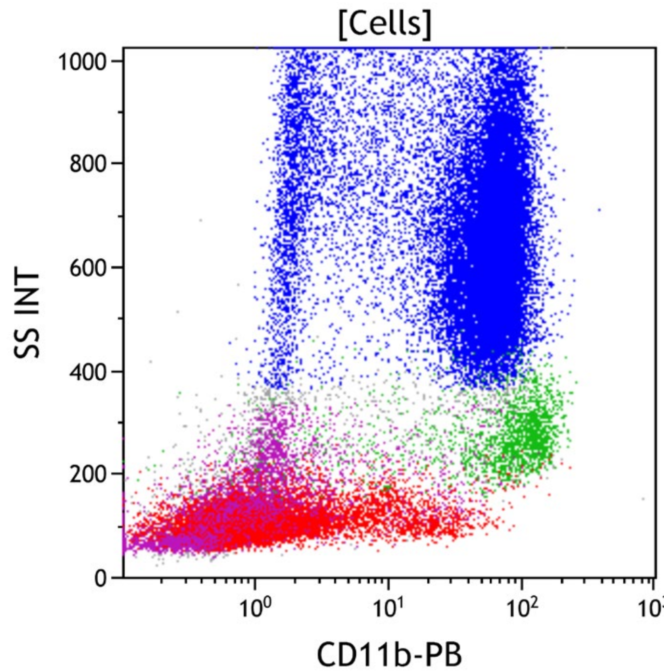

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils (purple).

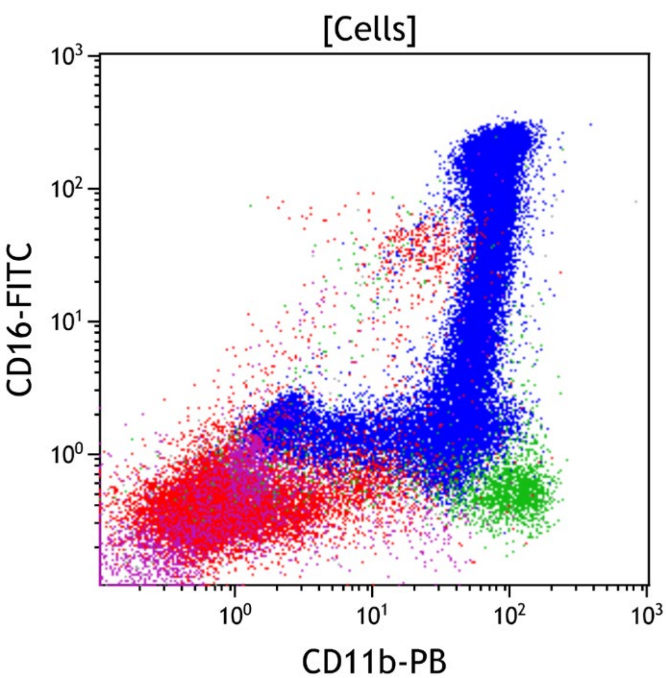

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red, upper right). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower right). CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

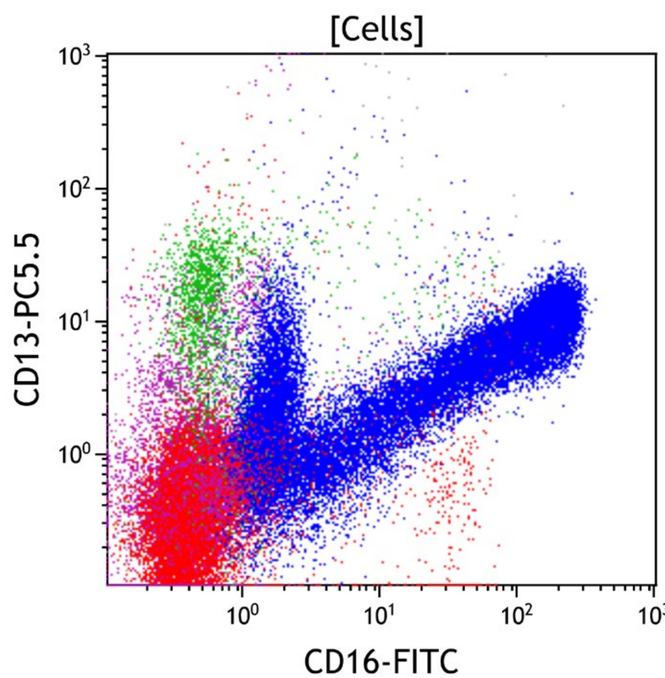

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red, lower right). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, upper left) and lose CD13 as they mature to myelocytes (blue, lower left). Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue, upper right).

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M1 Cell Tube

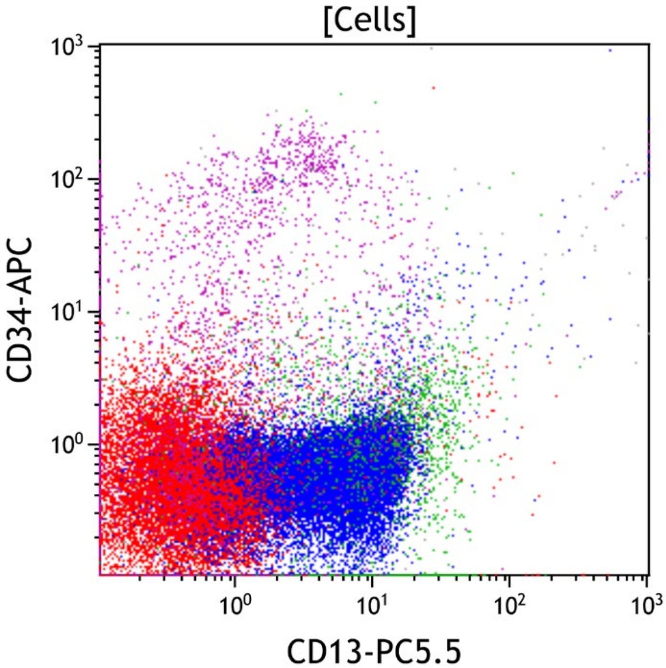

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphocytes (red).

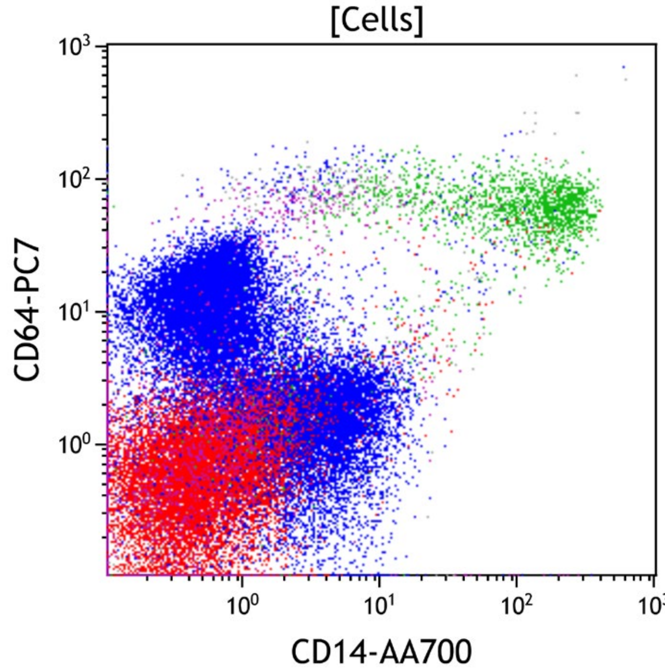

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 green, upper left) and progressively acquire CD14 during maturation to mature monocytes while retaining high level CD64 (green, upper right). Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left).

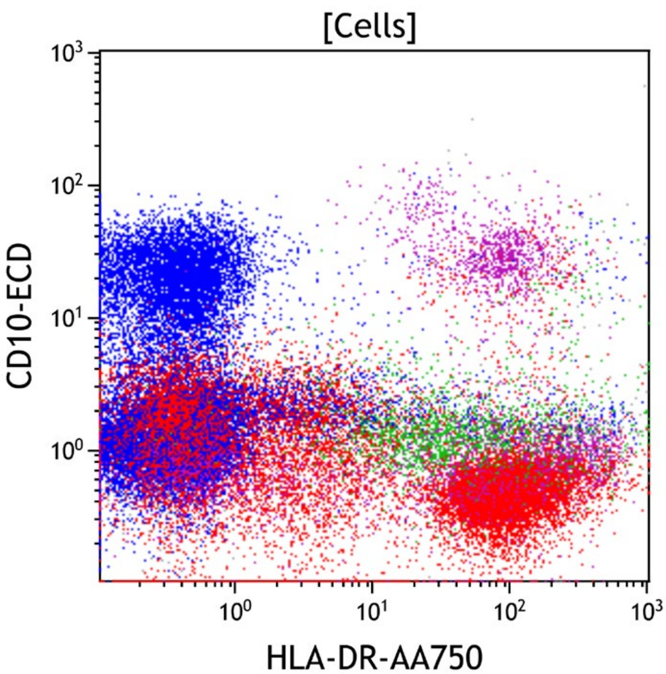

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

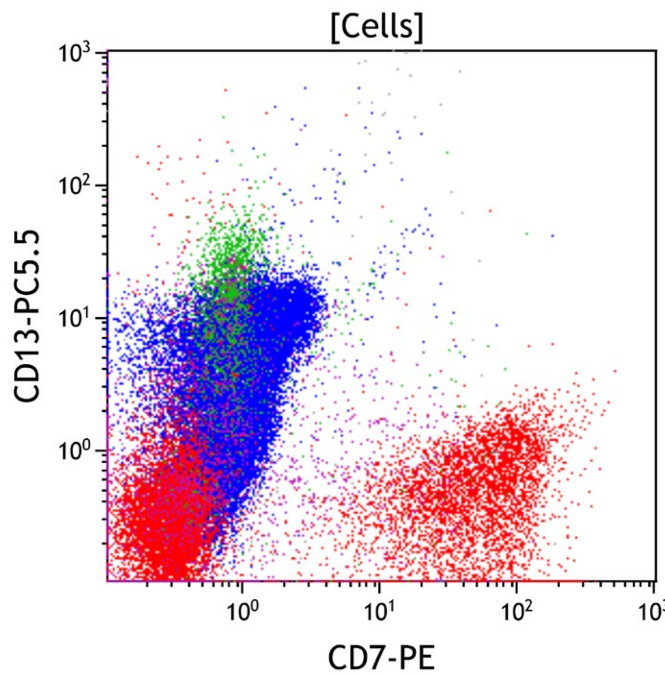

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

M2 Cell Tube

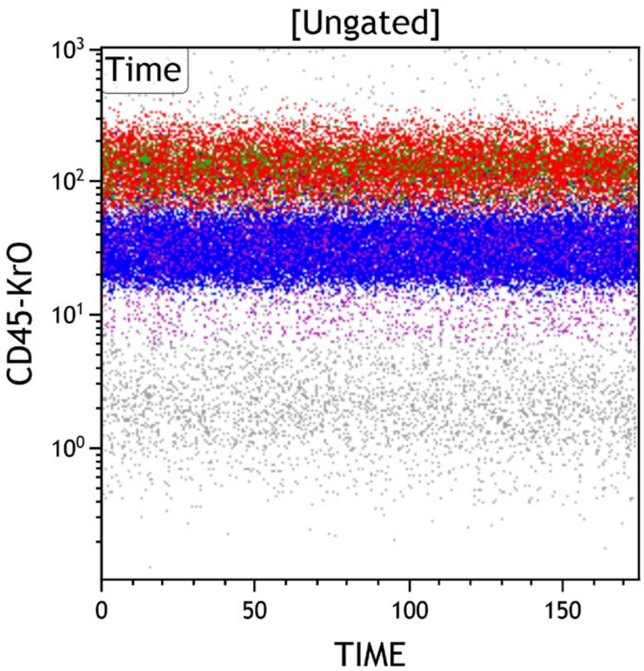

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

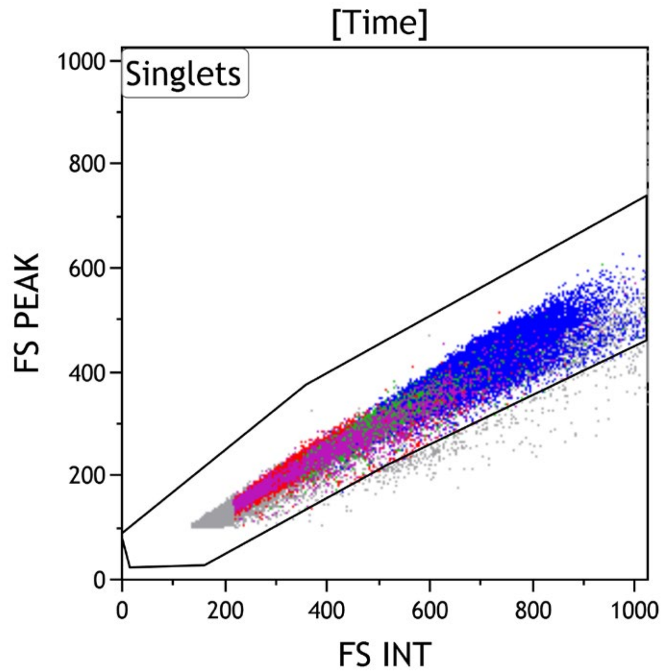

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

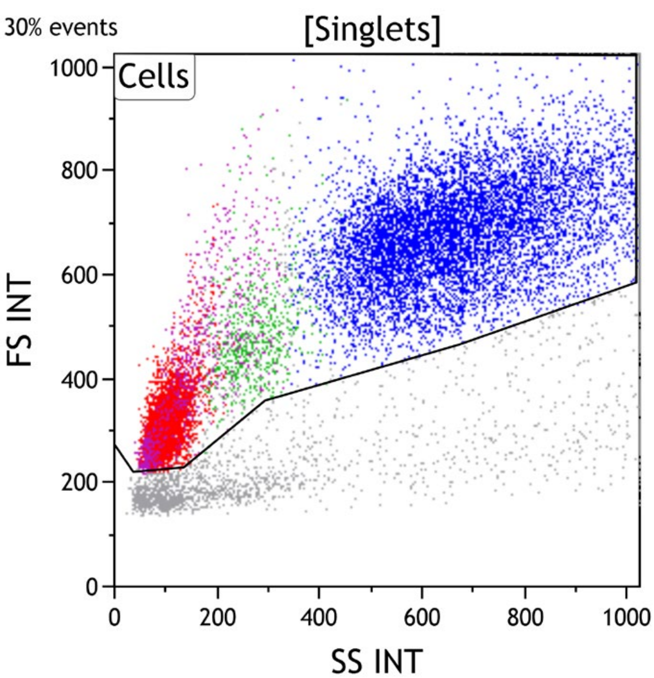

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

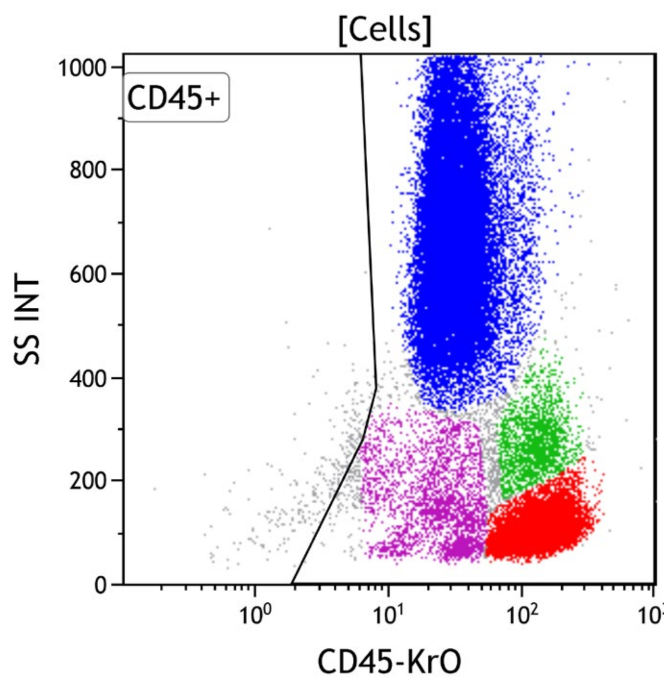

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

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M2 Cell Tube

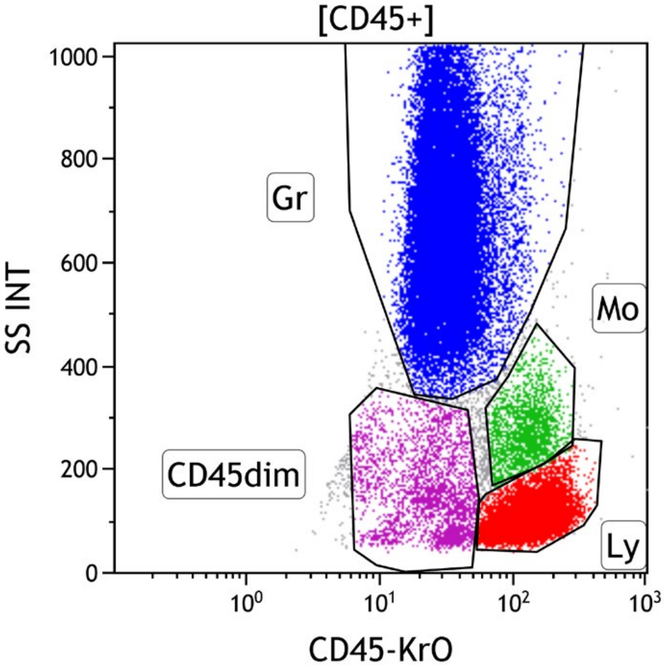


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis.

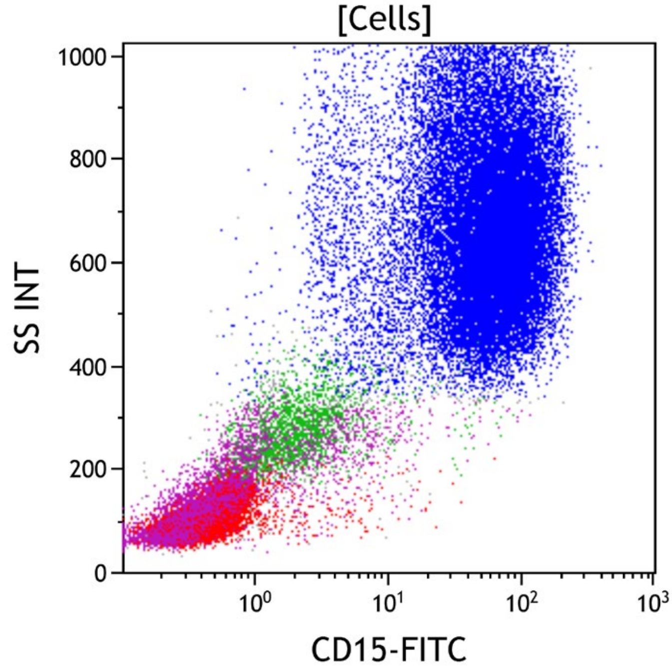


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

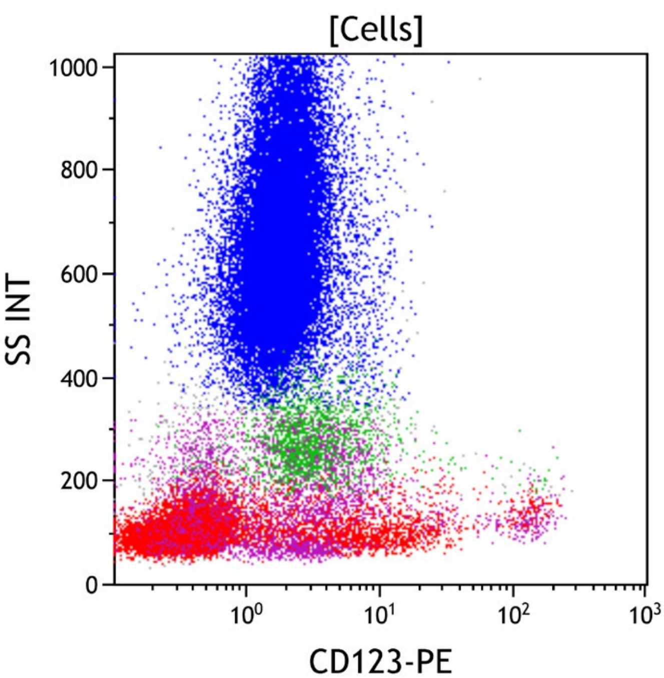


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple, lower right) and at a lower level on CD34 positive myeloid progenitors (purple, lower left) and monocytes (green).

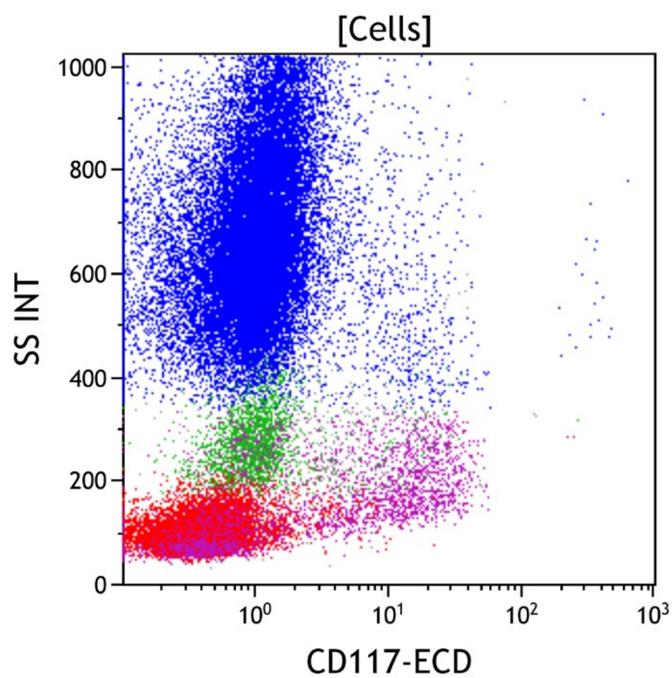


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors (purple), early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

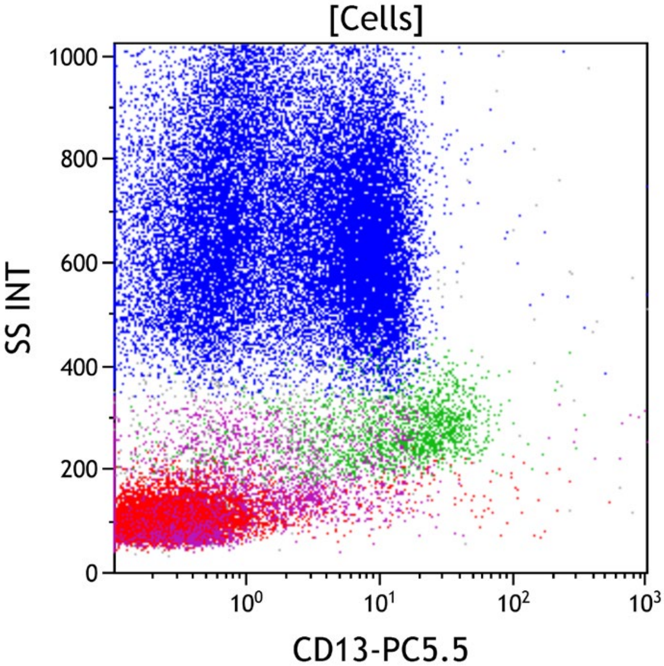


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple).

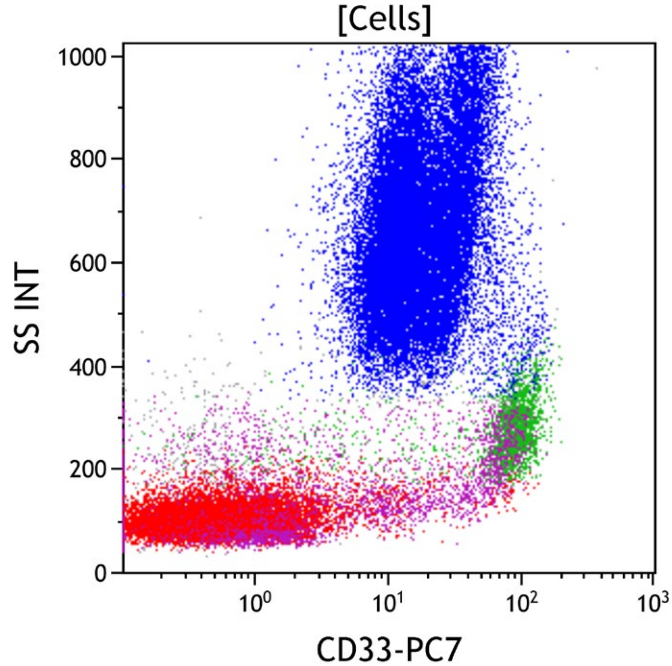


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors (purple).

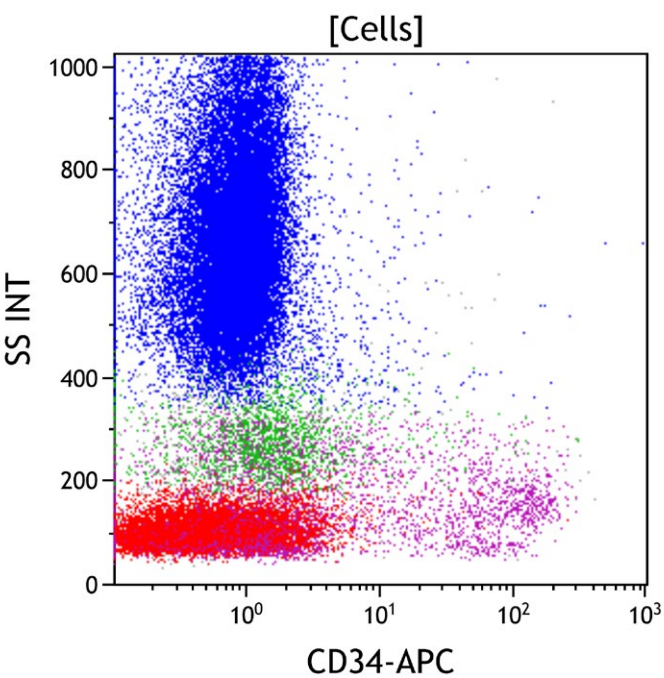


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45 dim gate (purple).

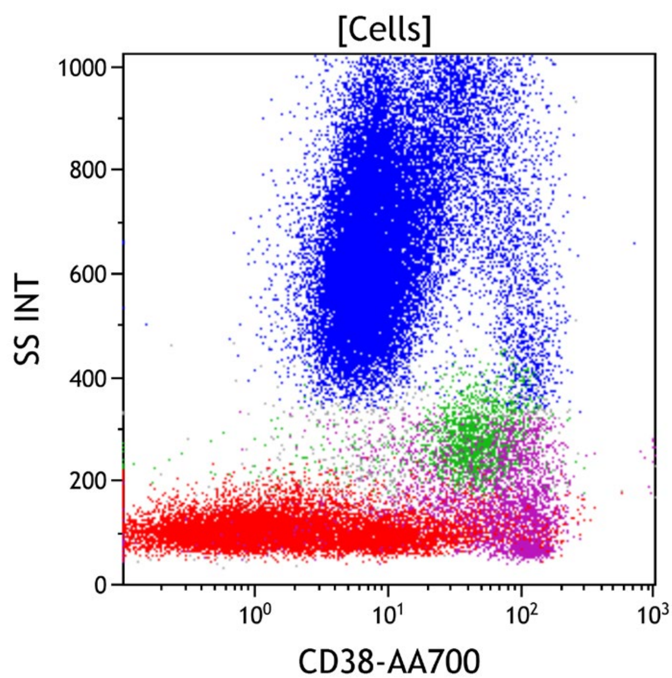
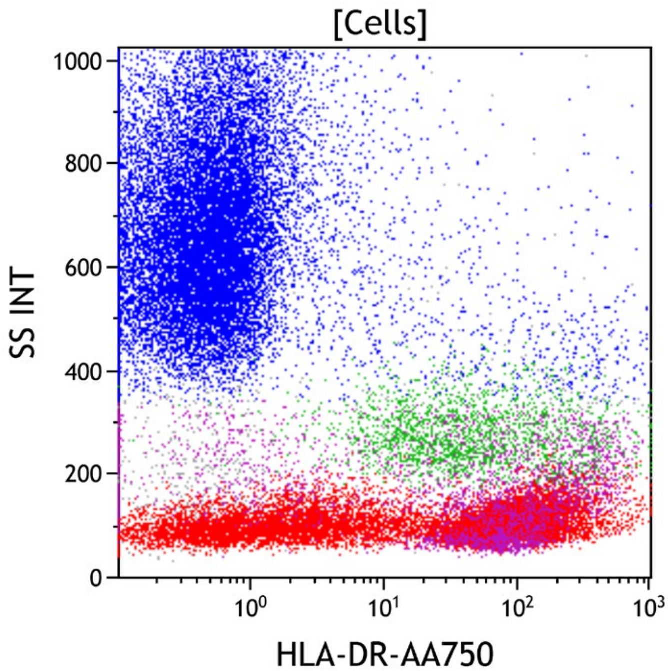


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple) and on monocytes (green), and at a variable level on activated mature lymphocytes (red).

M2 Cell Tube



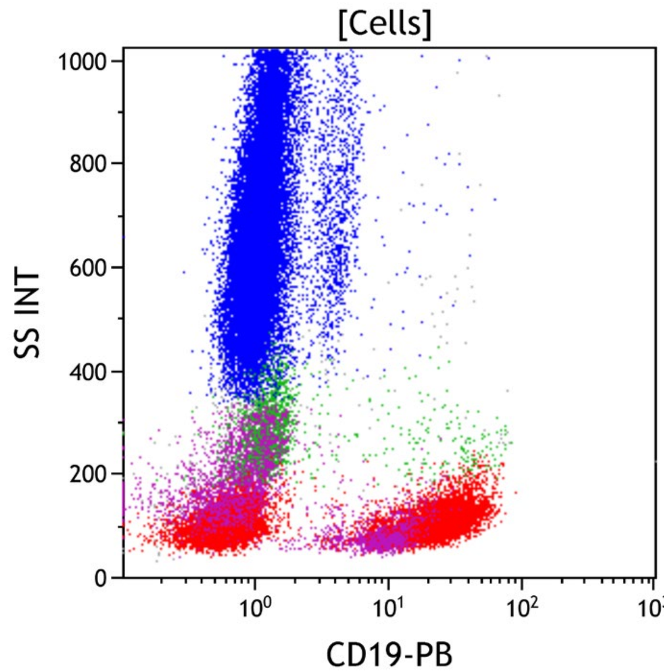
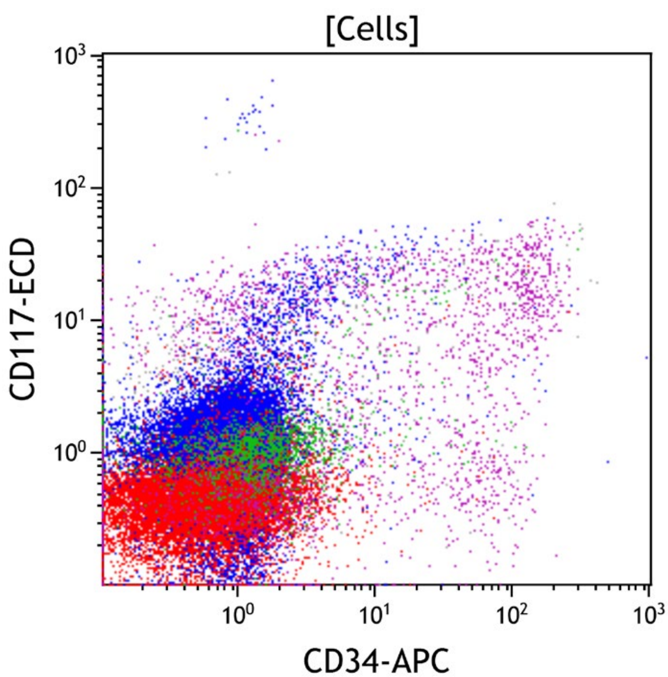


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells (red, lower right), as well as most plasma cells. These cells typically have low to moderate side scatter.



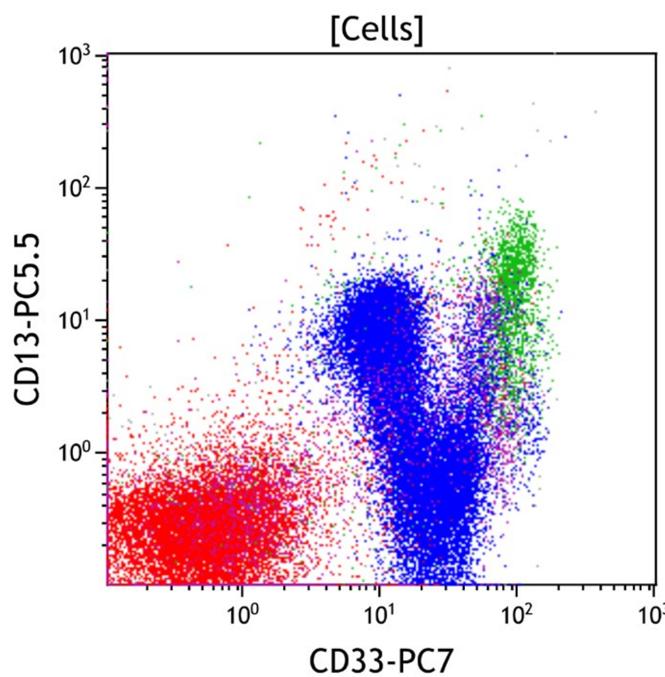


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (purple, upper right), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (purple, lower right).

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue, bottom) than more mature granulocytes (blue, upper left). Lymphocytes largely do not express either CD13 or CD33 (red).

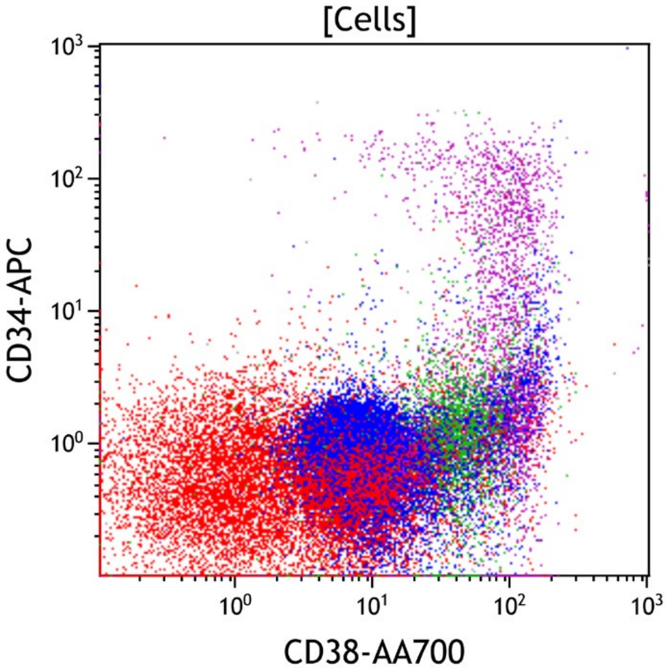


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple).

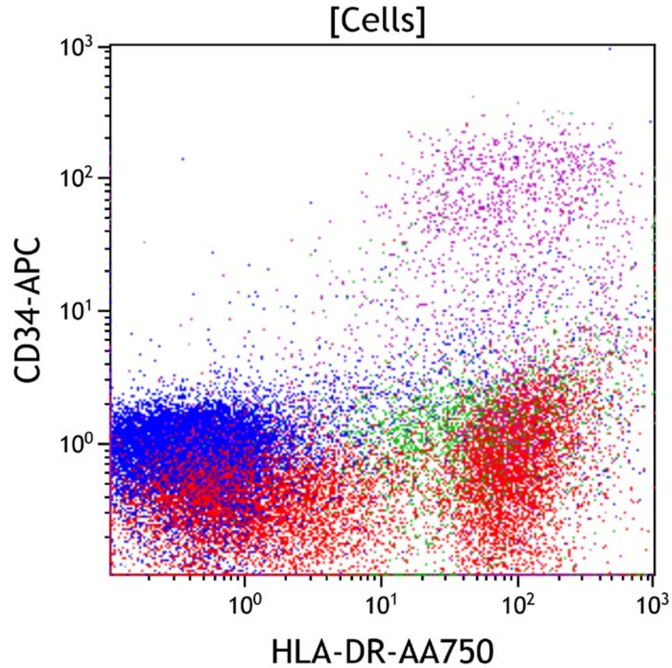


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes.

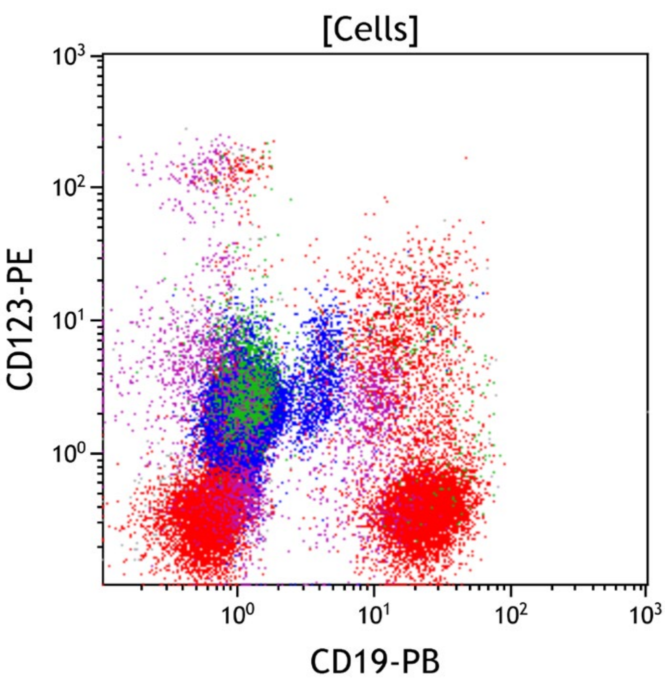


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors (purple). CD19 positive B cells (red, lower middle) normally do not express significant CD123. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

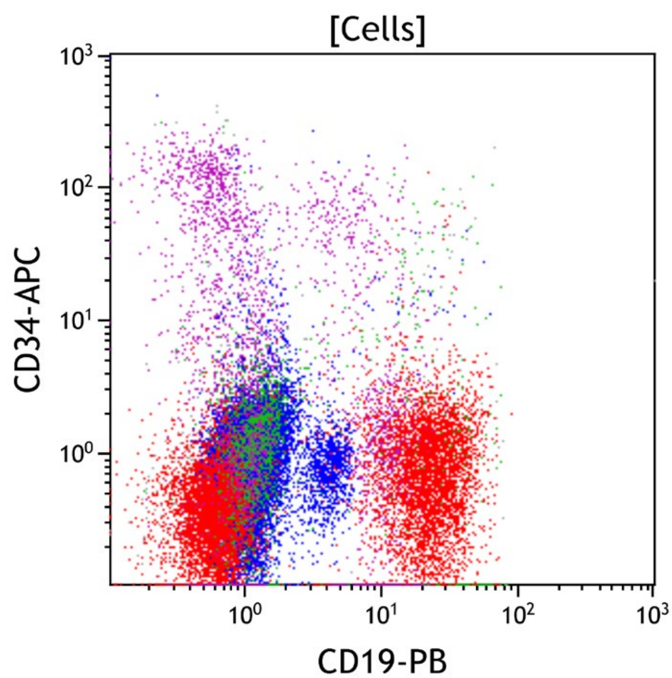


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors (purple). Mature CD19 positive B cells (red, lower middle) do not express CD34.

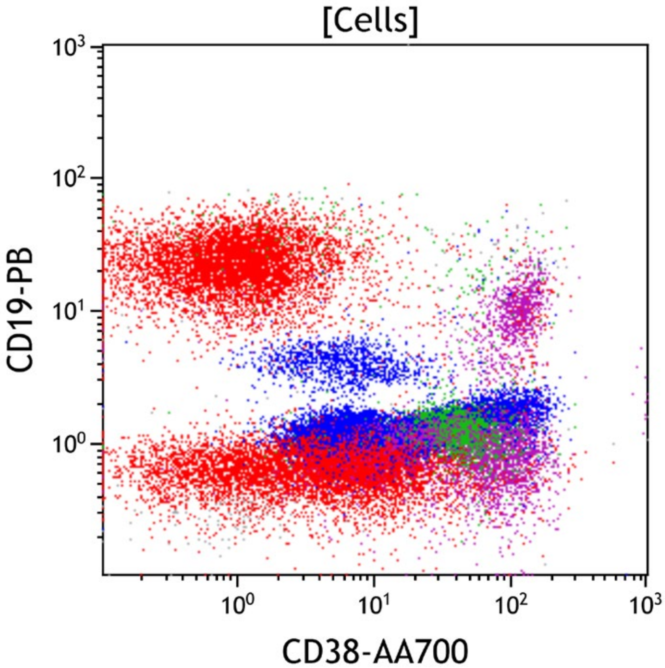


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Mature CD19 positive B cells show low to absent expression of CD38 (red, upper left). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

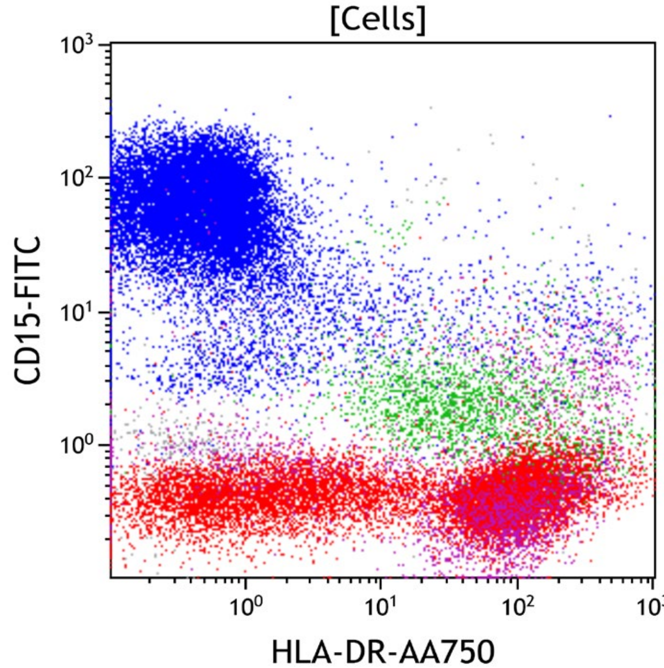


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired.

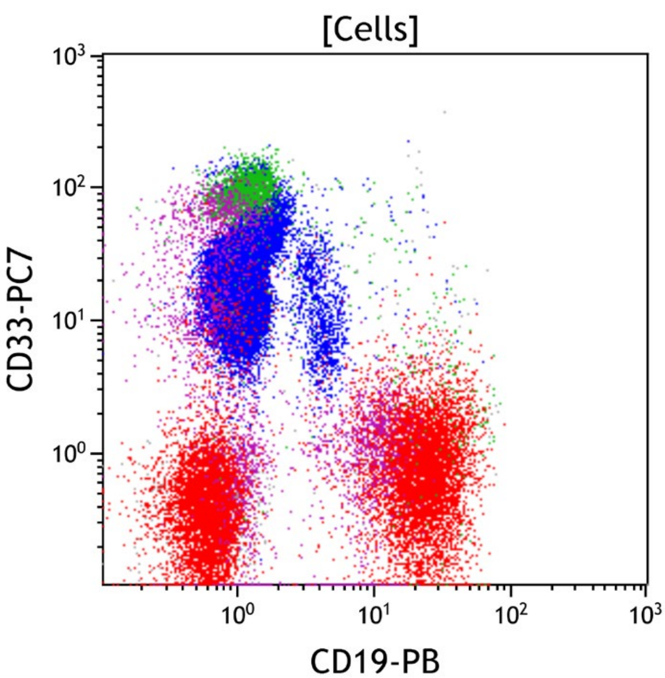


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red, lower right). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate CD19, bright CD20, low to absent CD38, bright CD45, low CD200, and surface lambda light chain restriction without CD5, CD10, or other T or myeloid markers. Compared with normal B cells, the lambda light chain restriction is aberrant.

Taken together, the immunophenotype of the aberrant population is consistent with mature B lymphoma, but does not allow specific subclassification. The diagnostic considerations include monoclonal B cell lymphocytosis, lymphoplasmacytic lymphoma, and marginal zone lymphoma. Correlation with clinical, morphologic and laboratory data is required for definitive diagnosis and subclassifcation, and that additional immunophenotyping may be warranted.

The concurrent morphologic and immunohistochemical findings confirm the presence of non-paratrabecular lymphoid aggregates composed of B cells that are small in size with increased clear cytoplasm, consistent with involvement by marginal zone lymphoma.

NEOPLASTIC PROCESS OF T-CELL ORIGIN

T cell and NK cell neoplasms also include acute lymphoblastic and mature lymphoid neoplasms. They are relatively uncommon, but many of them are among the most aggressive of all lymphoid neoplasms. Some, however, have a more prolonged clinical course. Immunophenotypically, these neoplasms often show aberrant expression or loss of T cell markers that aid in the differential diagnosis. Additionally, they may be associated with a viral infection. Epstein-Barr virus (EBV) is most often associated with NK cell leukemias and extranodal NK/T cell lymphomas. Human T cell leukemia virus (HTLV-1) is etiologically linked to adult T cell leukemia/lymphoma. Besides morphologic, immunophenotypic, and genetic characteristics, clinical features play an important part in the definition of these diseases.

T LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

Case #14: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

Clinical Vignette

This 20-year-old male presents with tissue mass. A lymph node biopsy sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

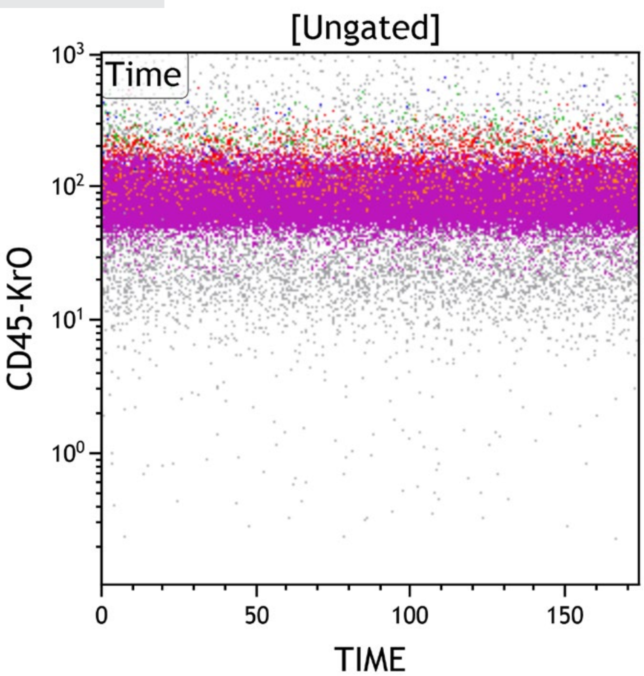


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

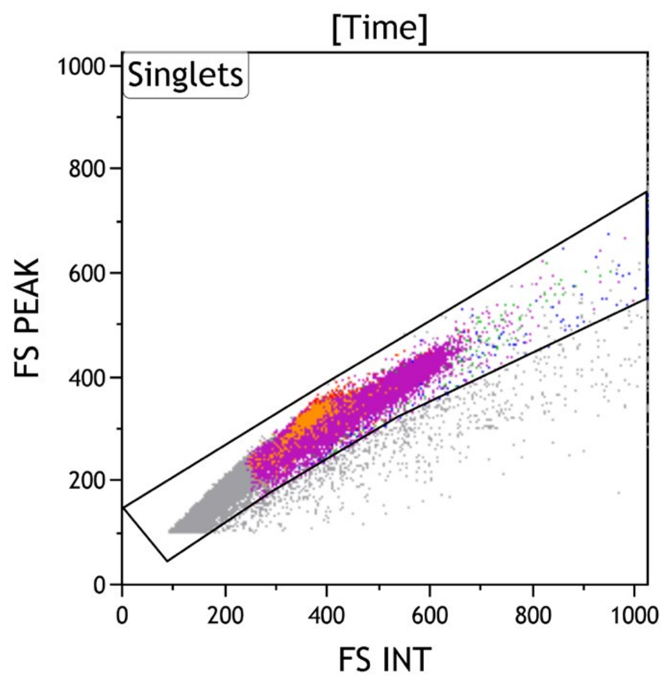


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

Table of Contents > Neoplastic Process of T-cell Origin > Case #14: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

B Cell Tube

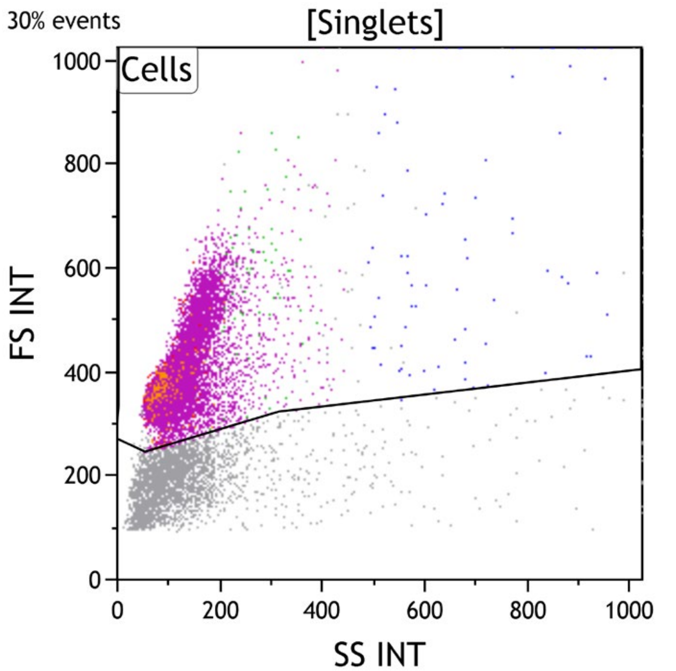


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

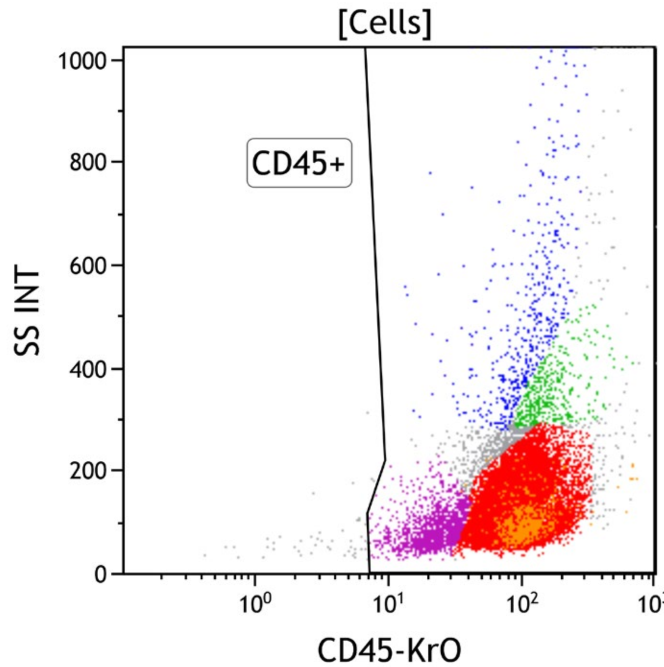


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

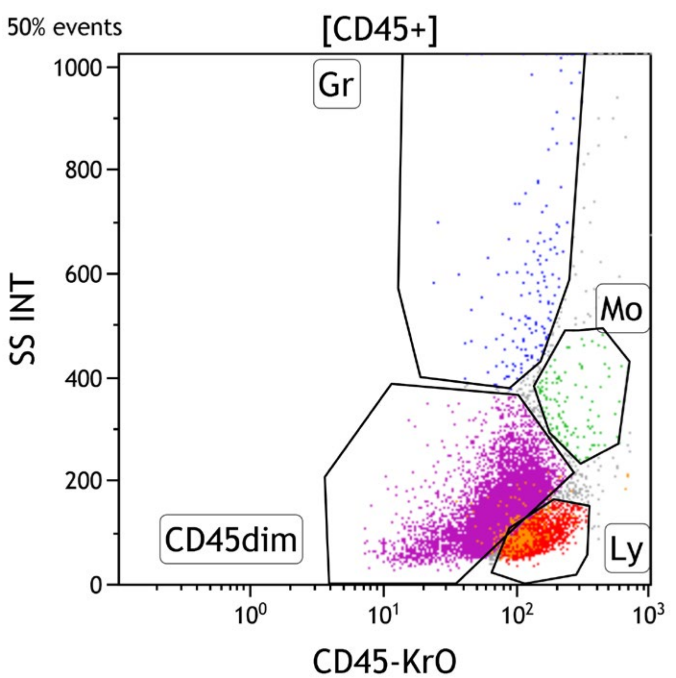


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orage), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. The progenitor population (purple) is expanded.

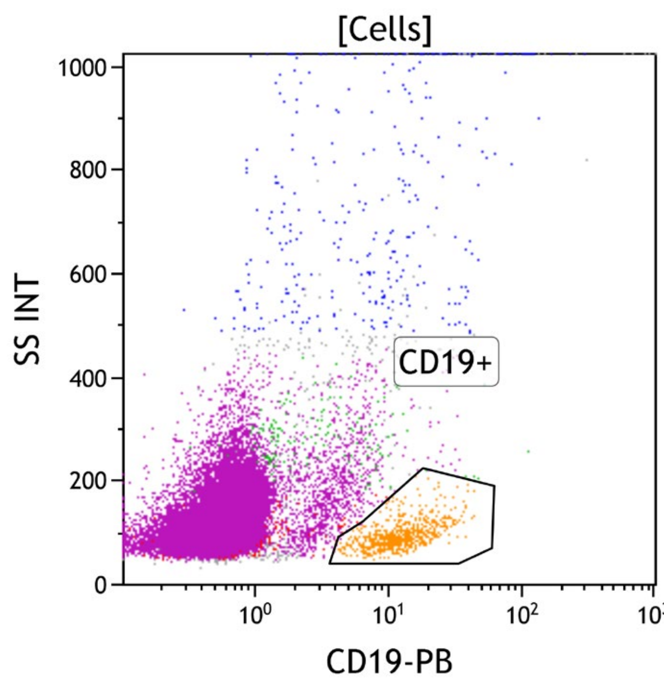


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express CD19.

B Cell Tube

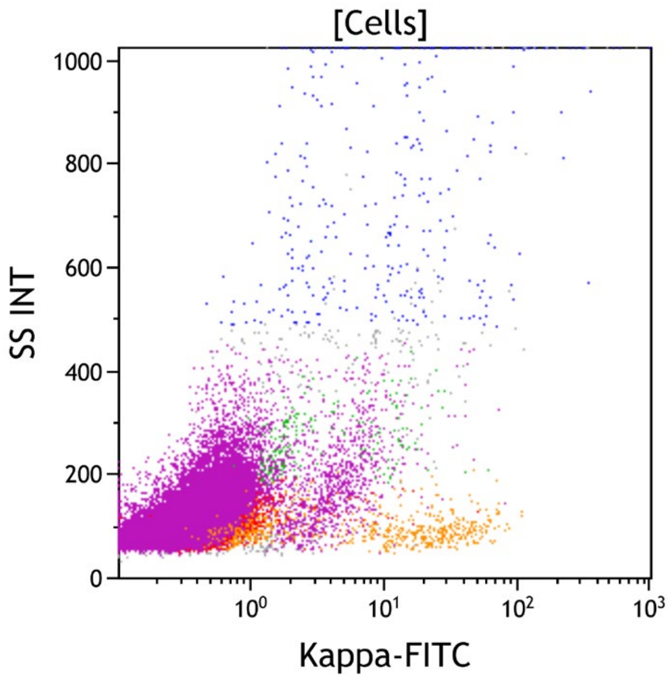


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. Progenitors (purple) do not express kappa.

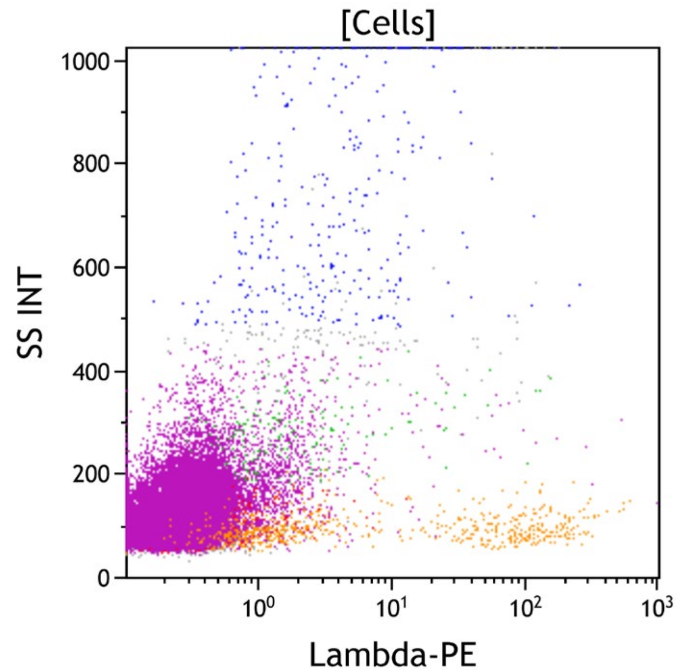


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. Progenitors (purple) do not express lambda.

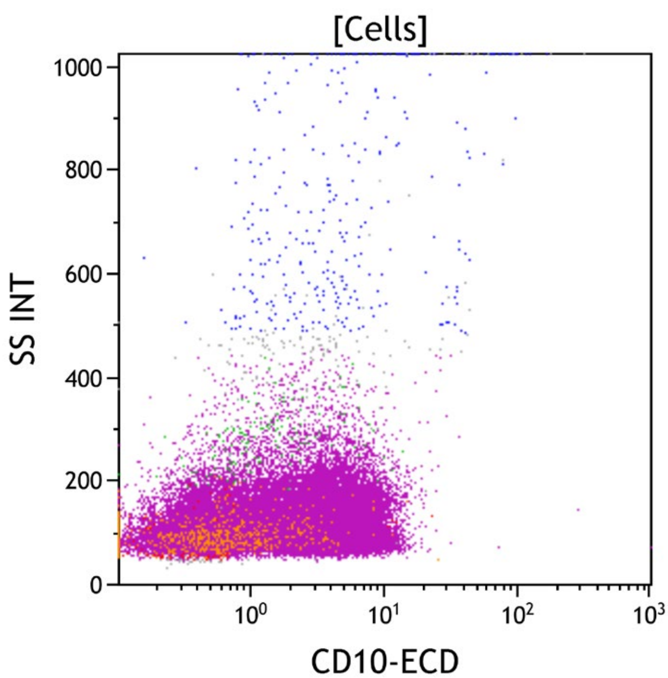


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Progenitors (purple) express partial CD10.

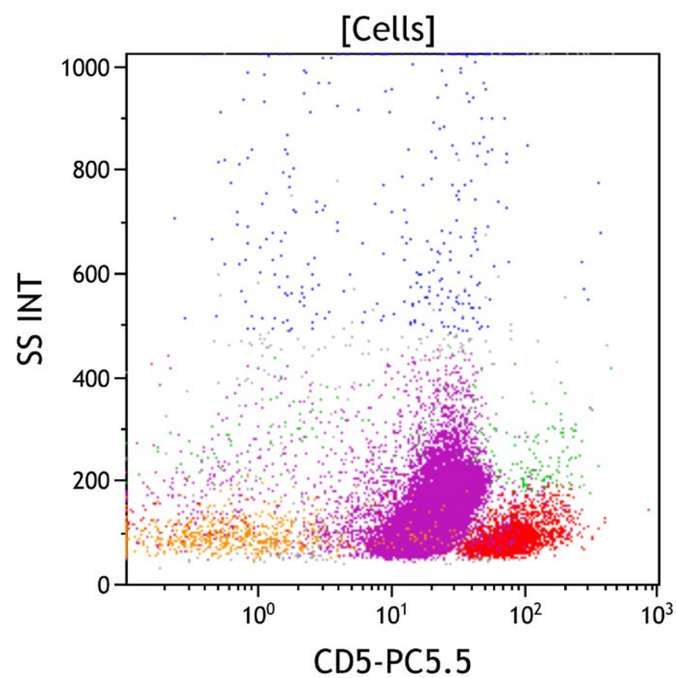


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. Progenitors (purple) express CD5 at a level lower than mature T cells (red).

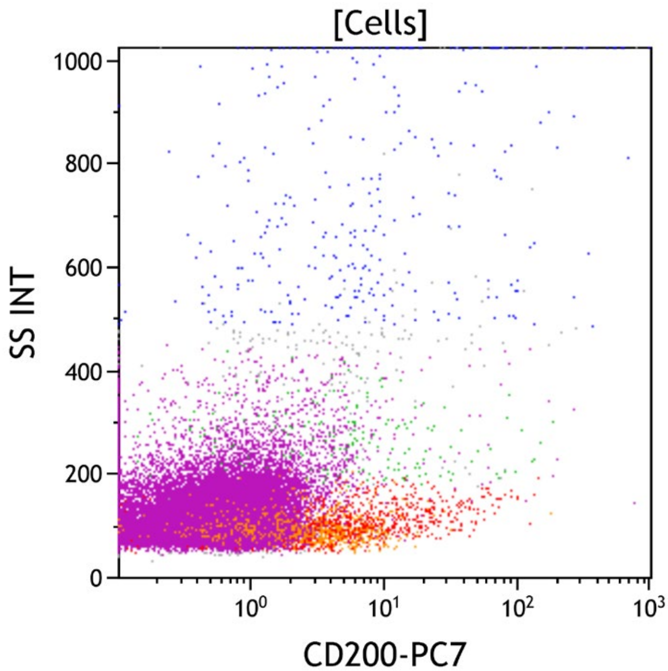


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). Progenitors (purple) do not express CD200.

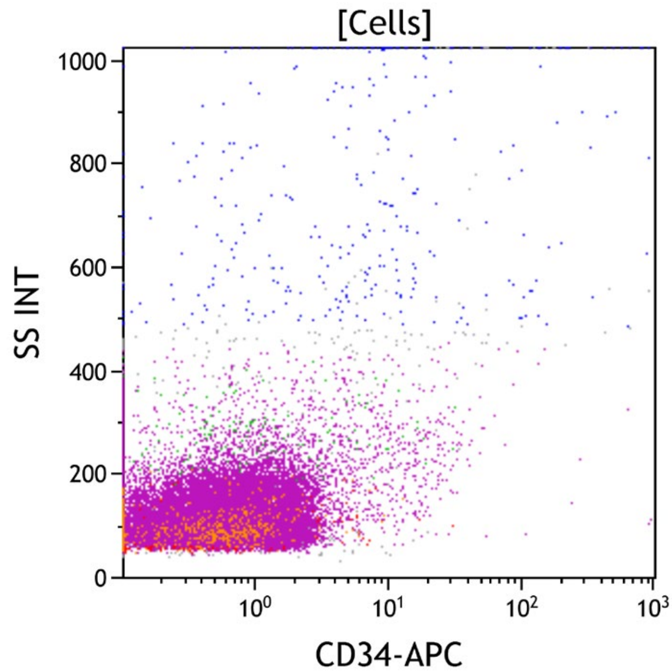


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Progenitors (purple) do not express CD34.

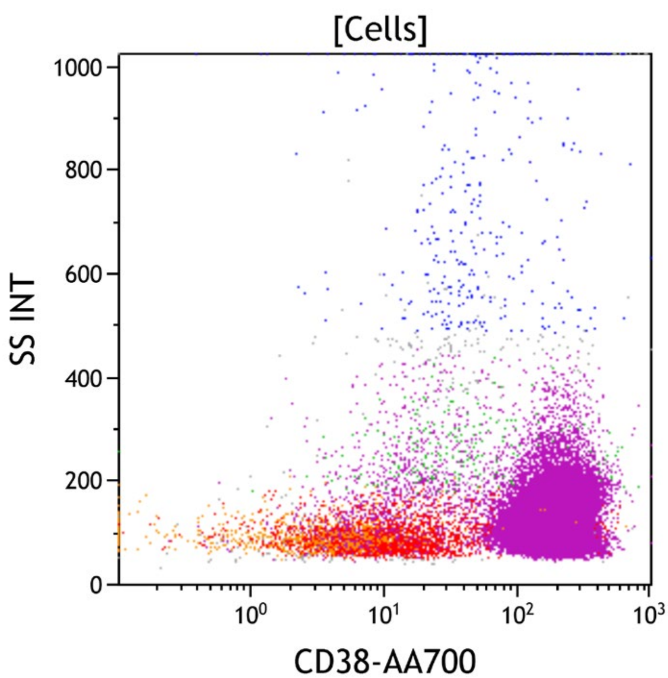


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent levels. Progenitors (purple) strongly express CD38.

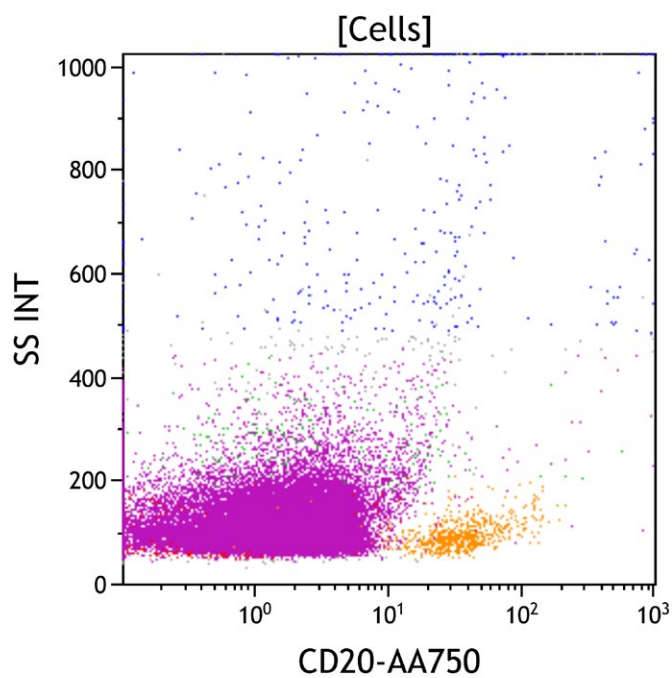


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. Progenitors (purple) do not express CD20, the apparent low-level positivity being increased is due to compensation background from the bright CD38.

B Cell Tube

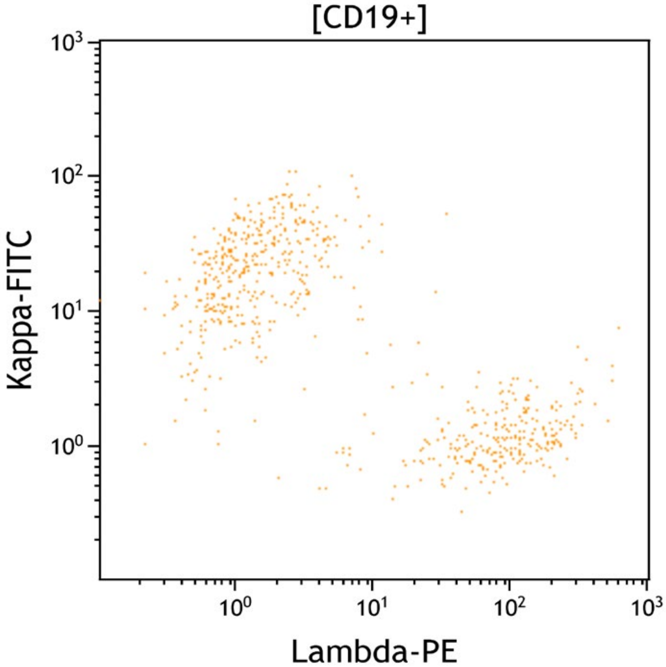


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

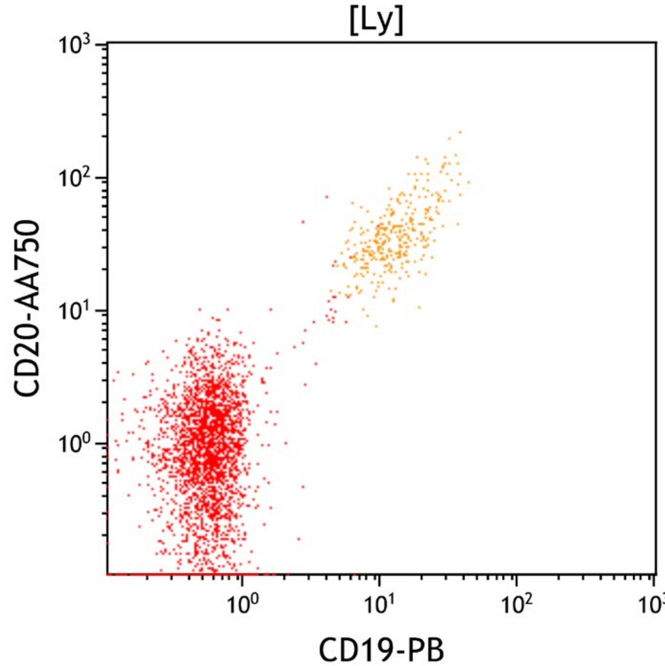


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

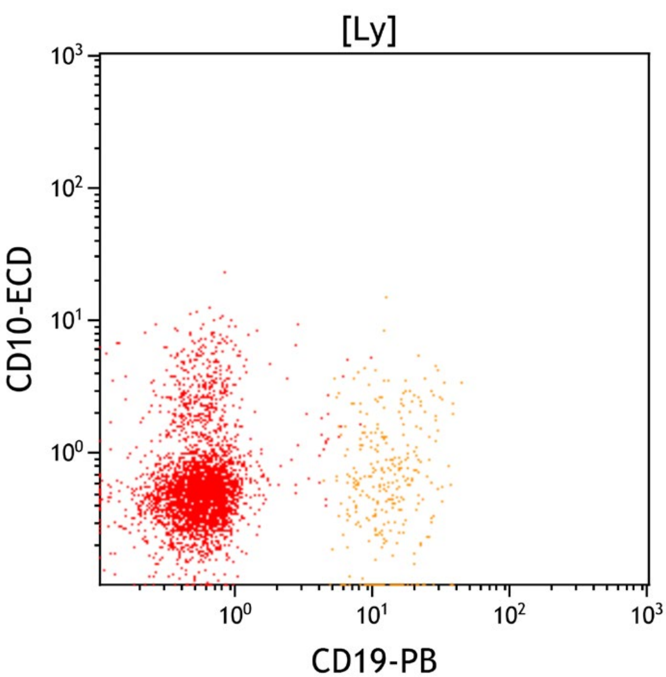


Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate. This case displays extensive overlap among gates. The CD10 dim staining (red, upper left) is due to overlap of the progenitors in the Lymphocyte gate (Ly).

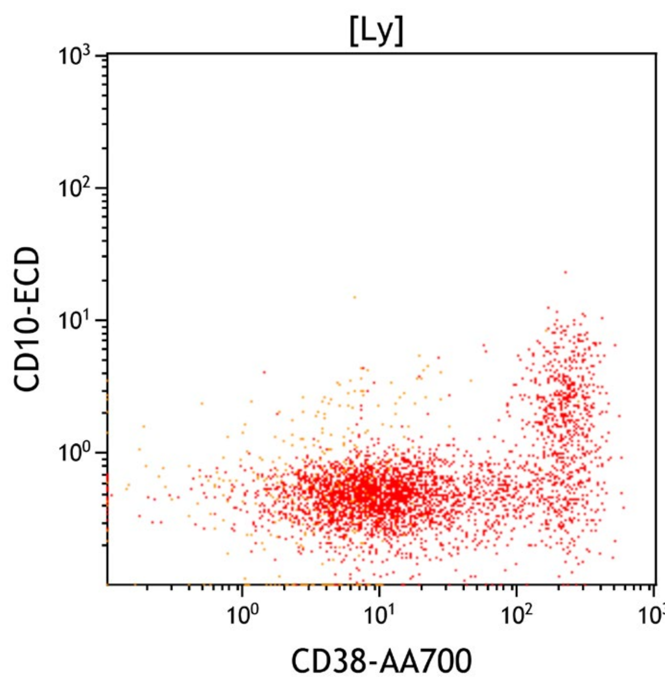


Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

B Cell Tube

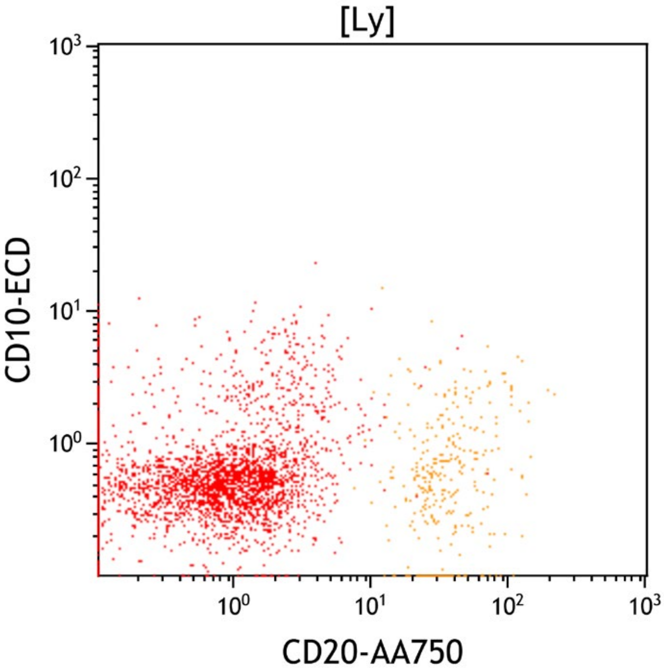


Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells. This case displays extensive overlap among gates. The CD10 dim staining (red, upper left) is due to overlap of the progenitors in the Lymphocyte gate (Ly).

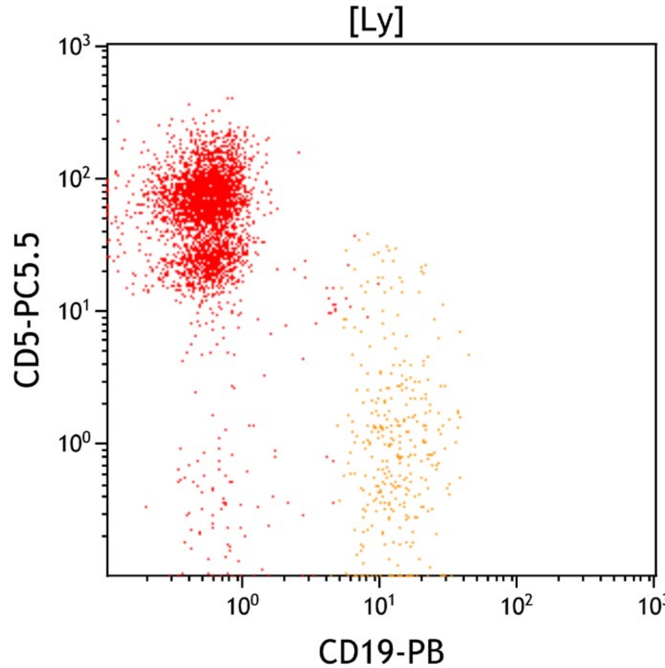


Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells. This case displays extensive overlap among gates. The intermediate CD5 staining (red, middle left) is due to overlap of the progenitors in the Lymphocyte gate (Ly).

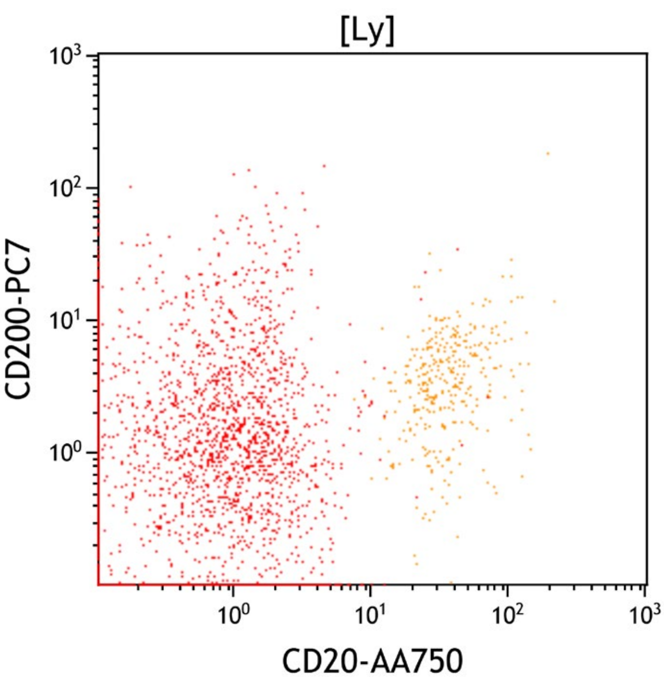


Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

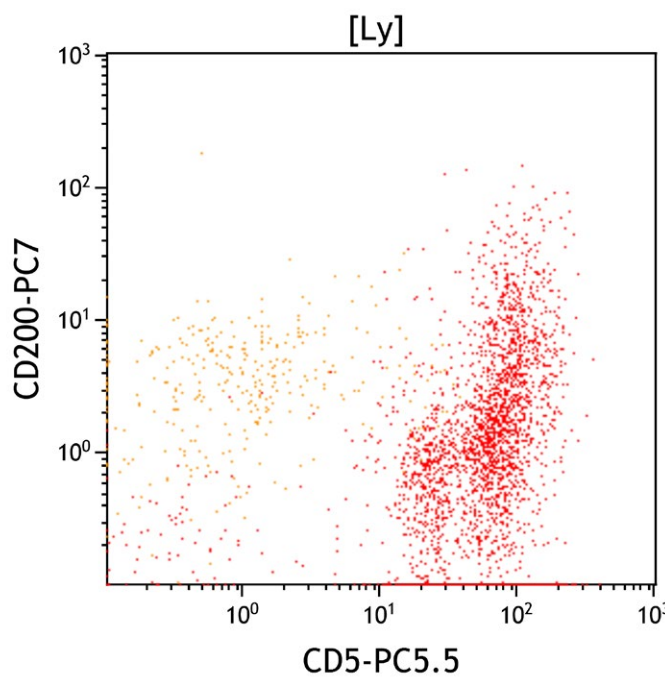


Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. This case displays extensive overlap among gates. The intermediate CD5 staining is due to overlap of the progenitors in the Lymphocyte gate (Ly).

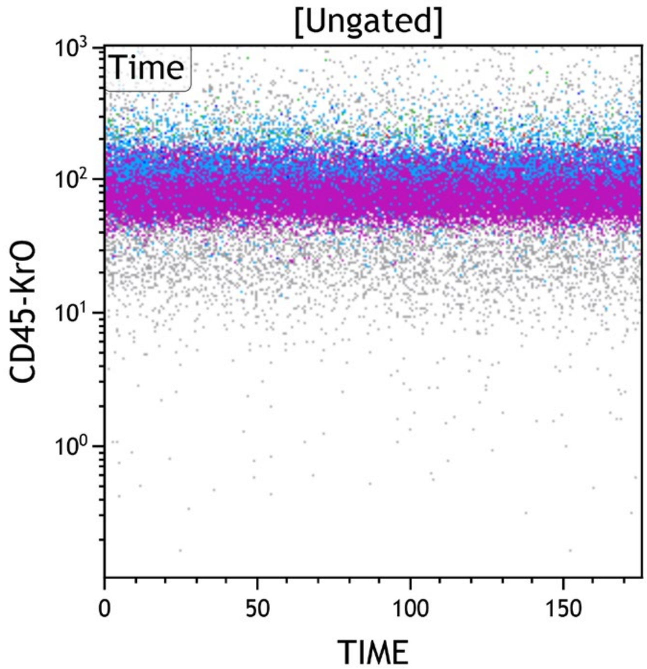


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

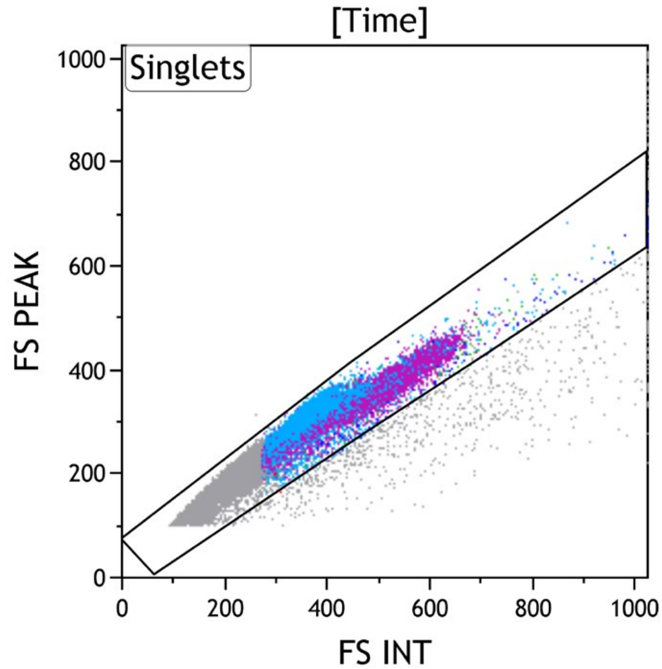


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

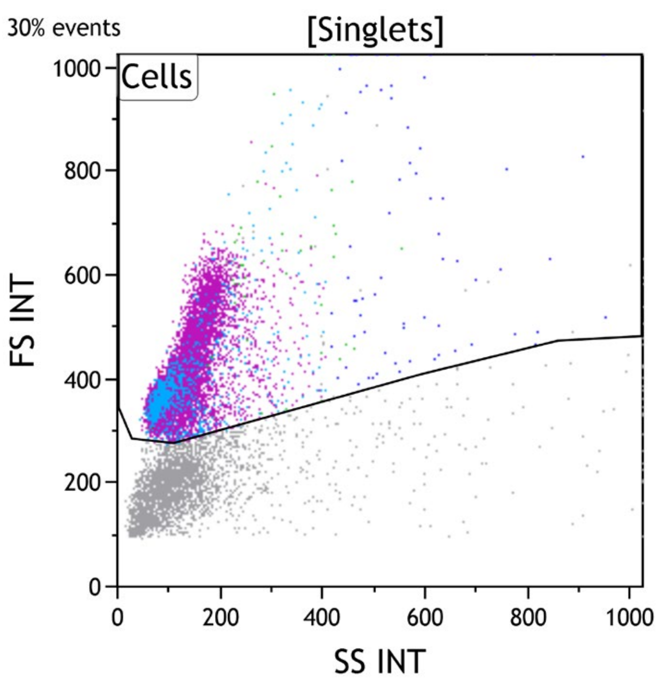


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

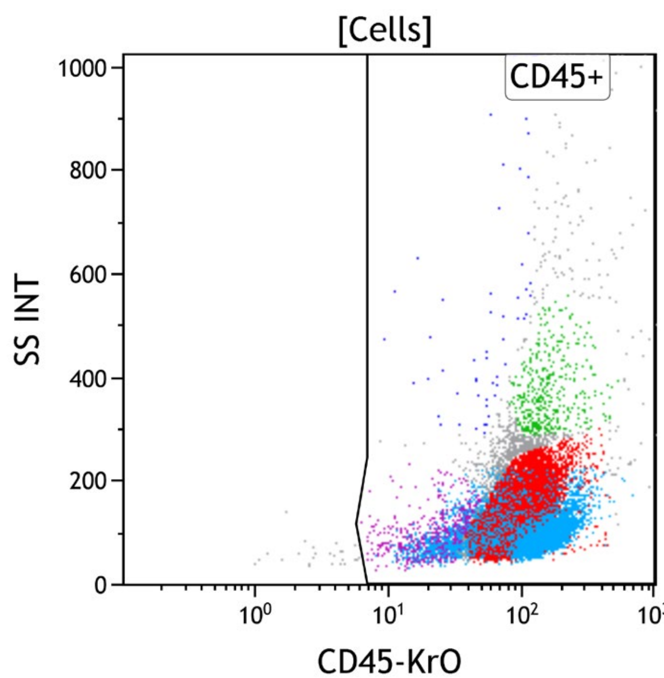


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

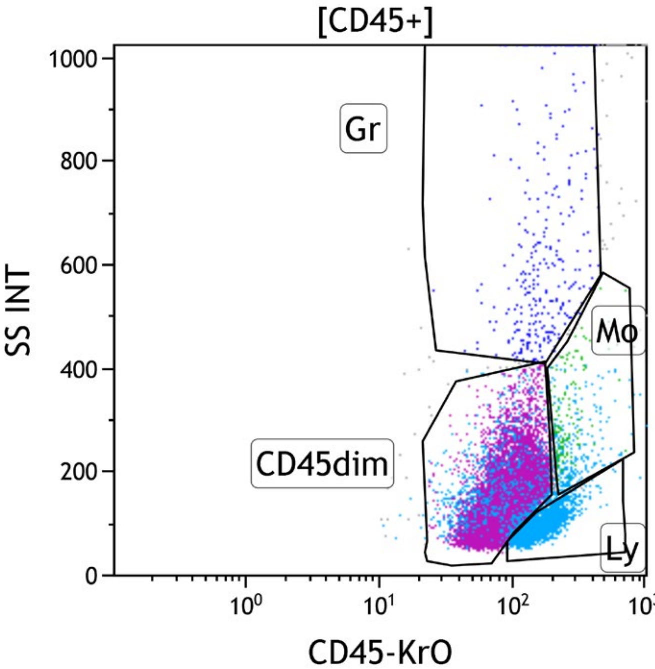


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, aqua/red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The progenitor population (purple) is expanded.

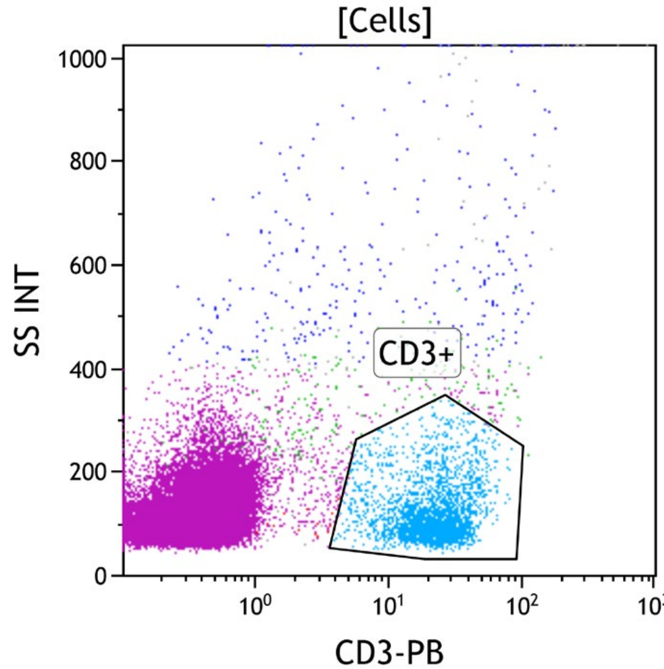


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express surface CD3.

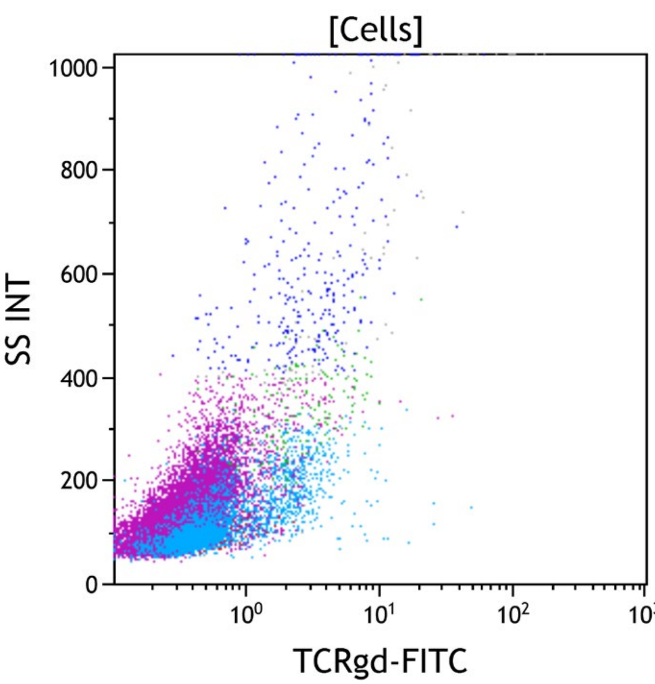


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). Progenitors (purple) do not express TCRγδ.

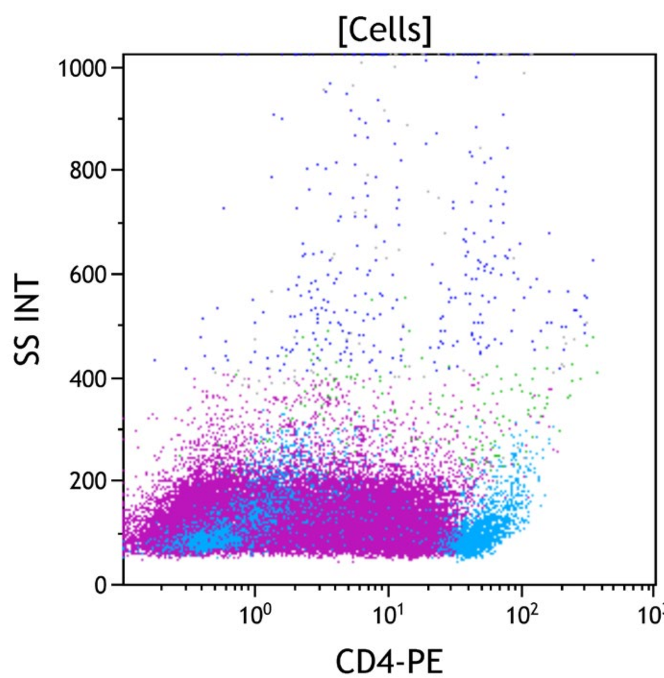


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. Progenitors (purple) variably express CD4.

T Cell Tube

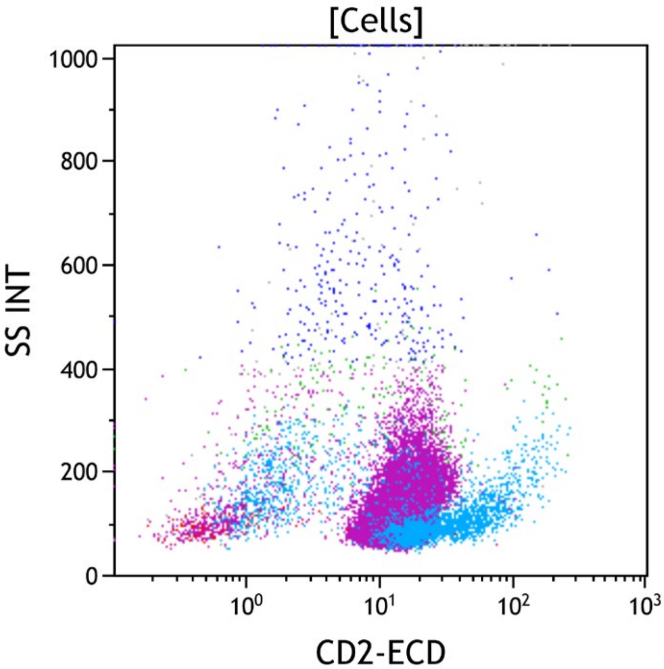


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). Progenitors (purple) do express intermediate CD2.

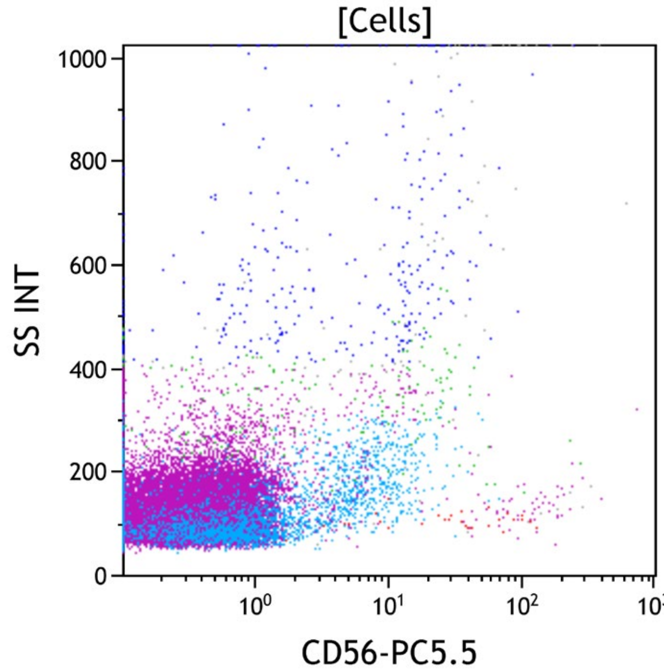


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. Progenitors (purple) do not express CD56.

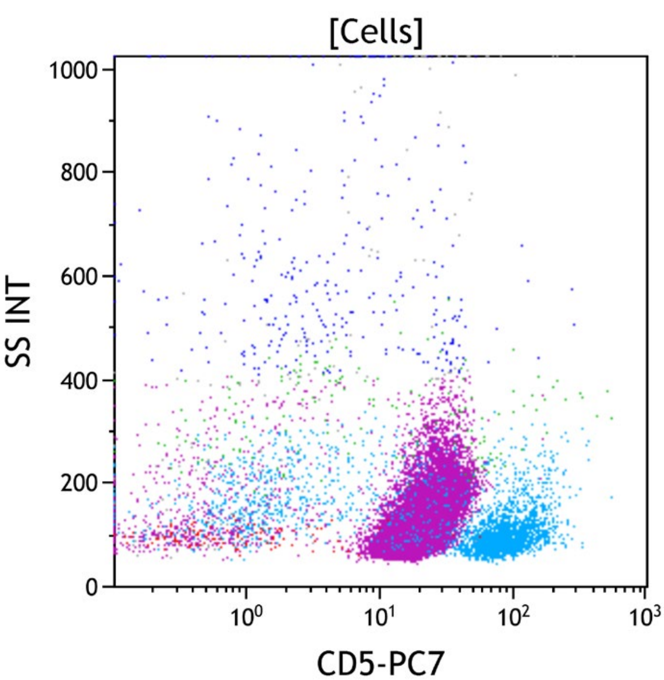


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Progenitors (purple) express CD5 at a level lower than mature T cells (aqua).

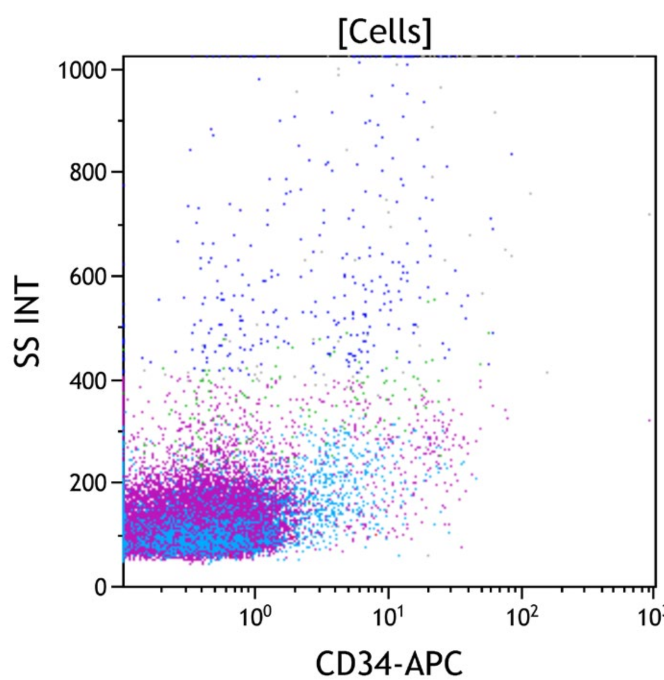


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Progenitors (purple) do not express CD34.

T Cell Tube

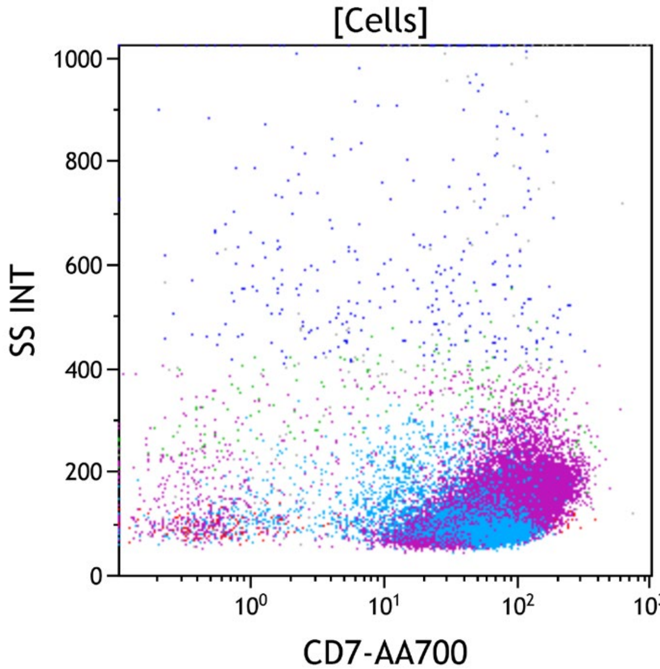


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. Progenitors (purple) express bright CD7.

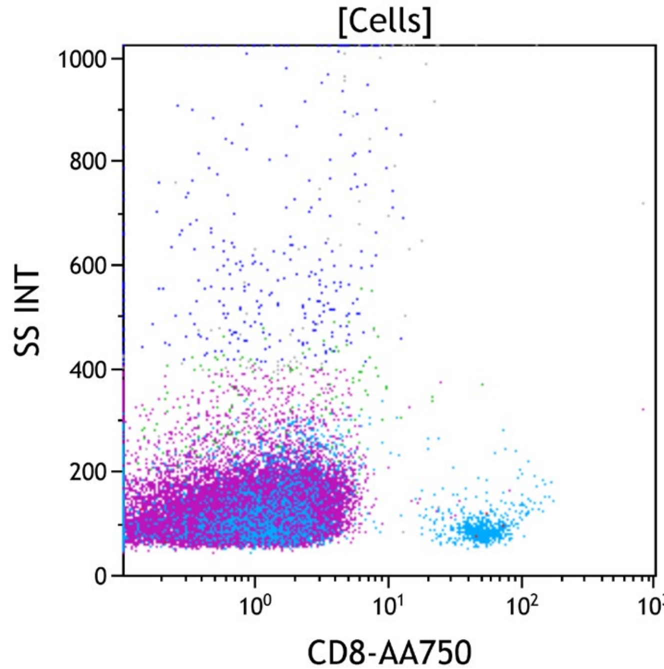


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. Progenitors (purple) do not express CD8.

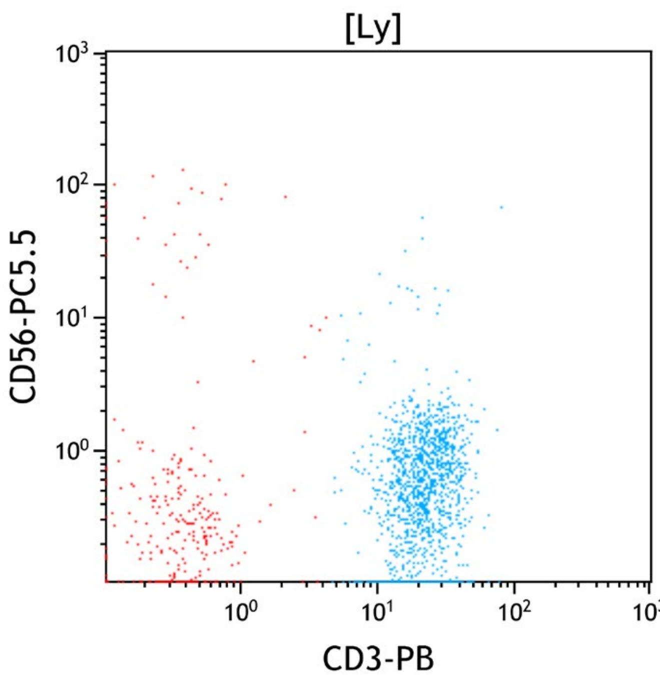


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

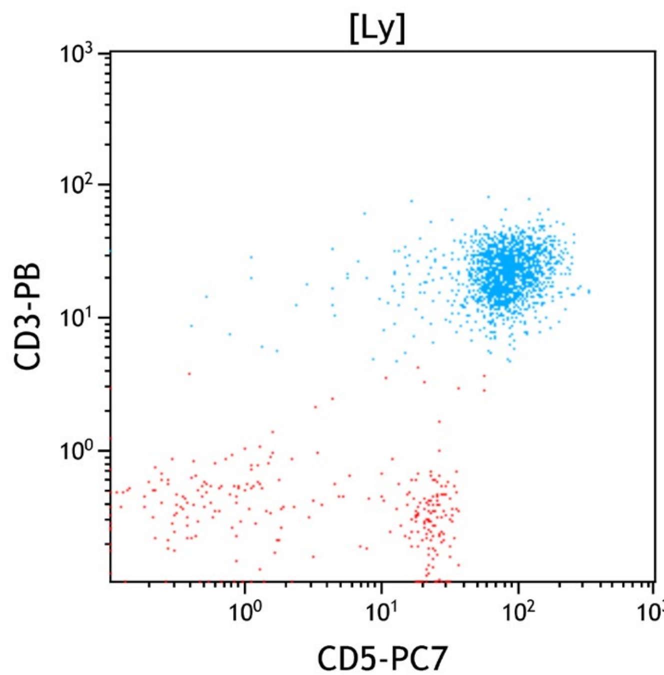


Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

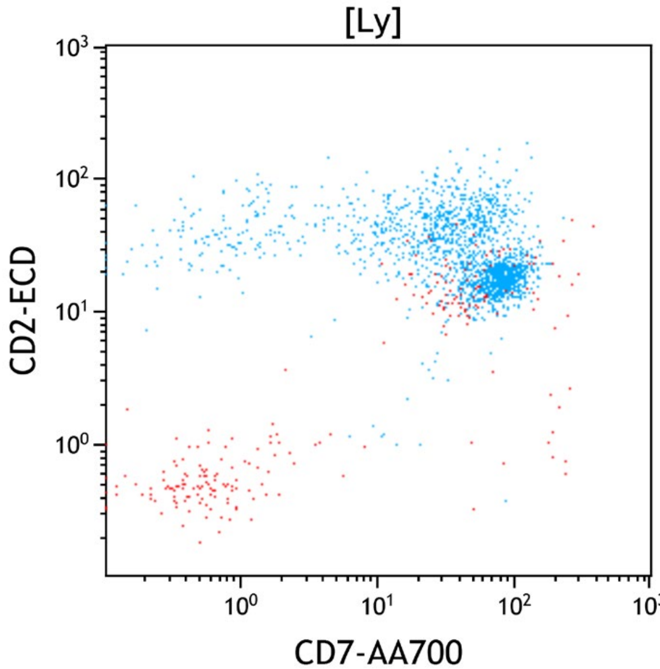


Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

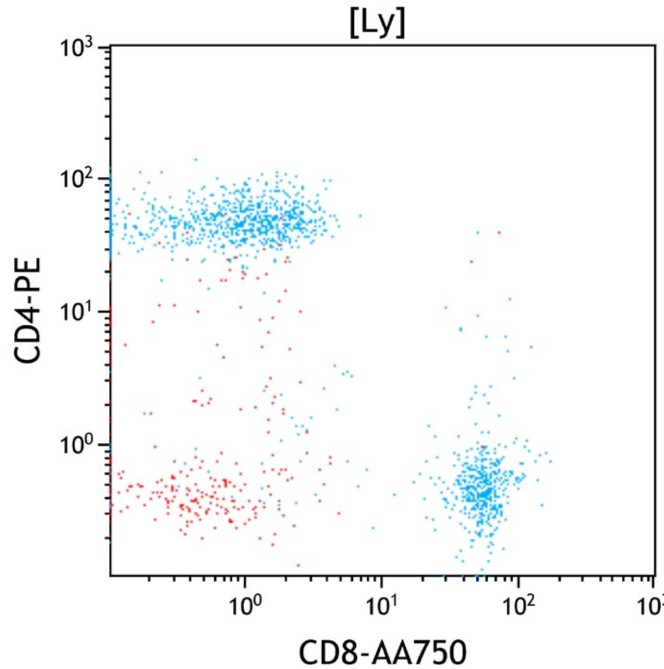


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

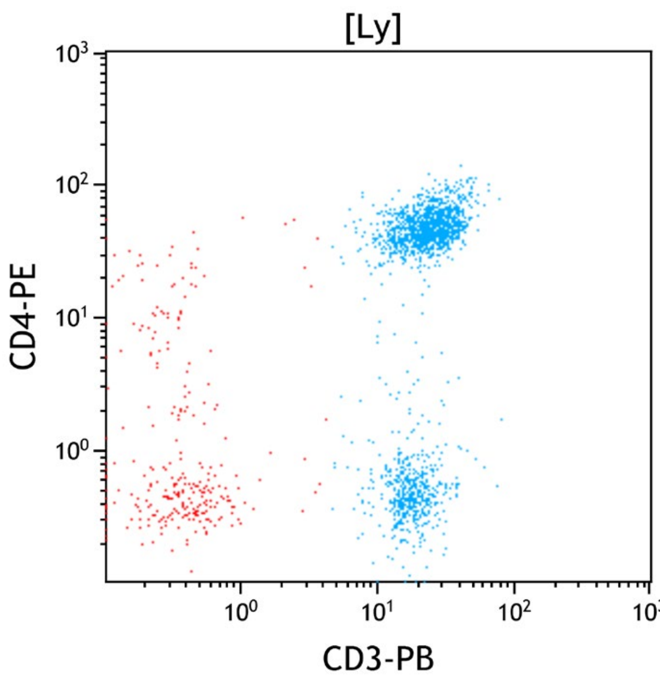


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

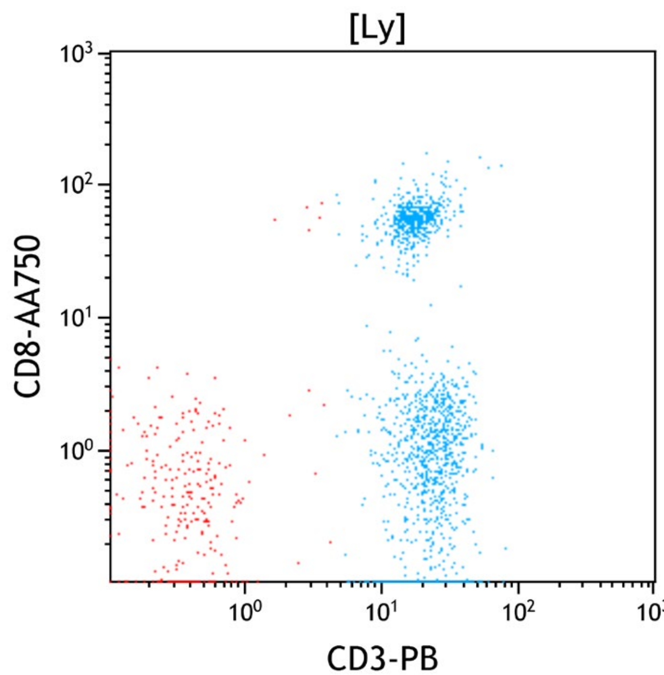


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 without CD3.

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T Cell Tube

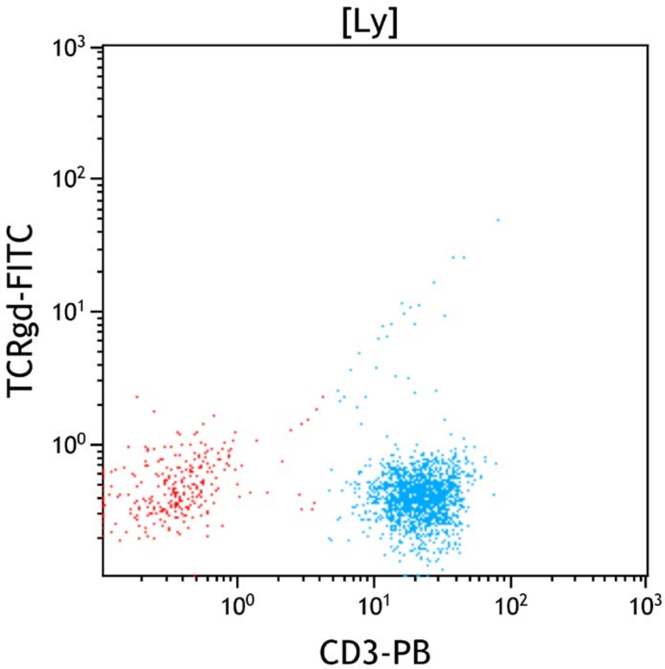


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCRγδ is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

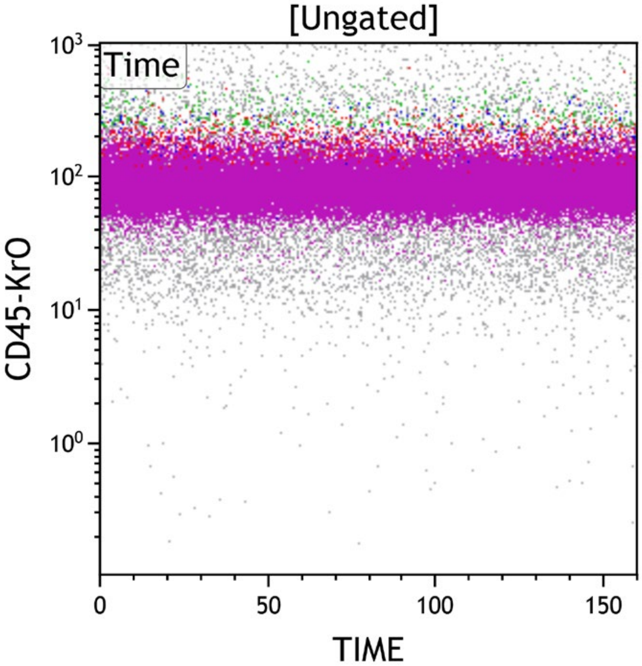


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

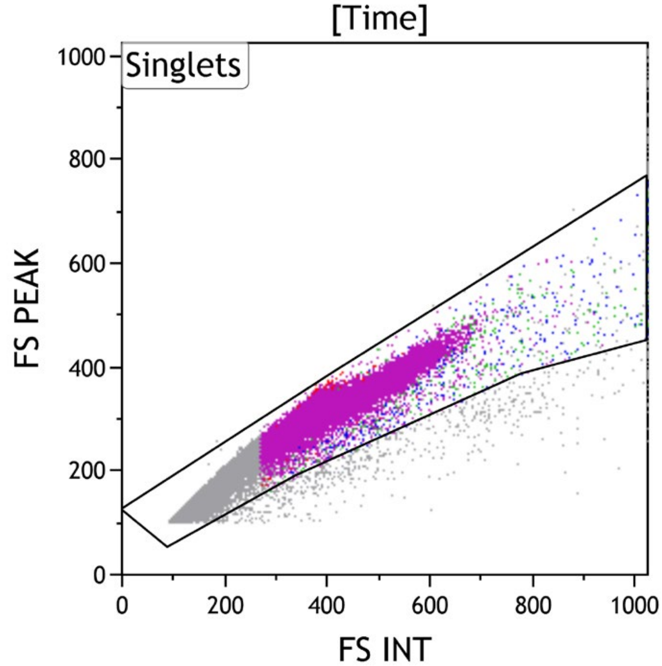


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

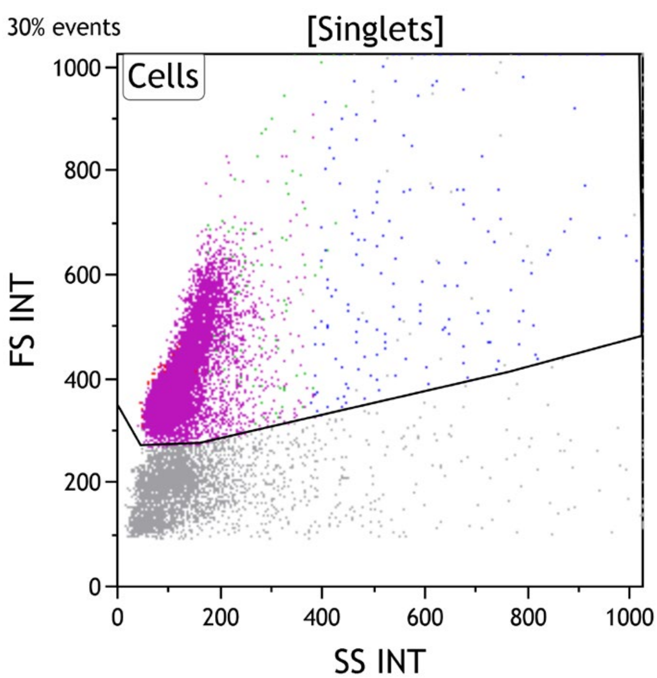


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

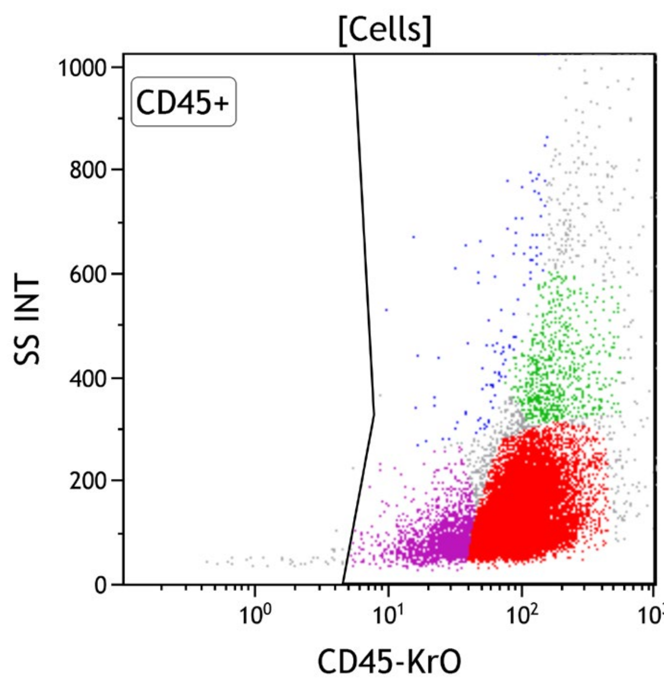


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

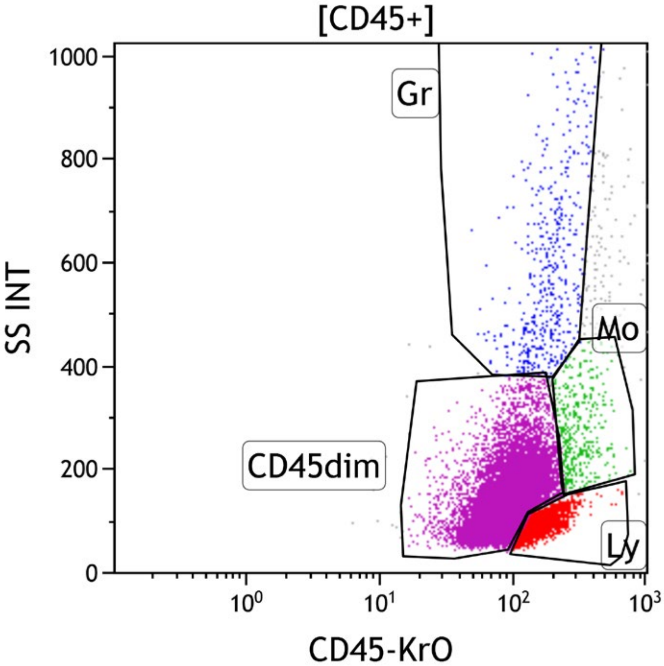


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The progenitor population (purple) is expanded.

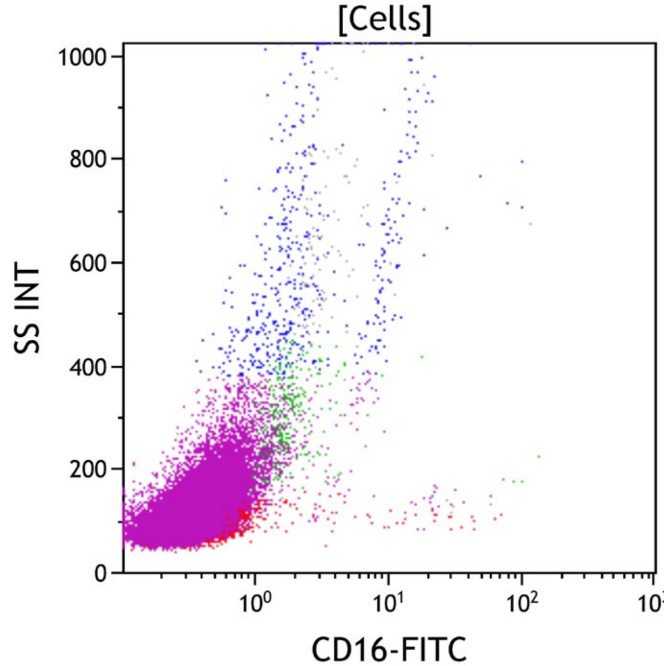


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). Progenitors (purple) do not express CD16.

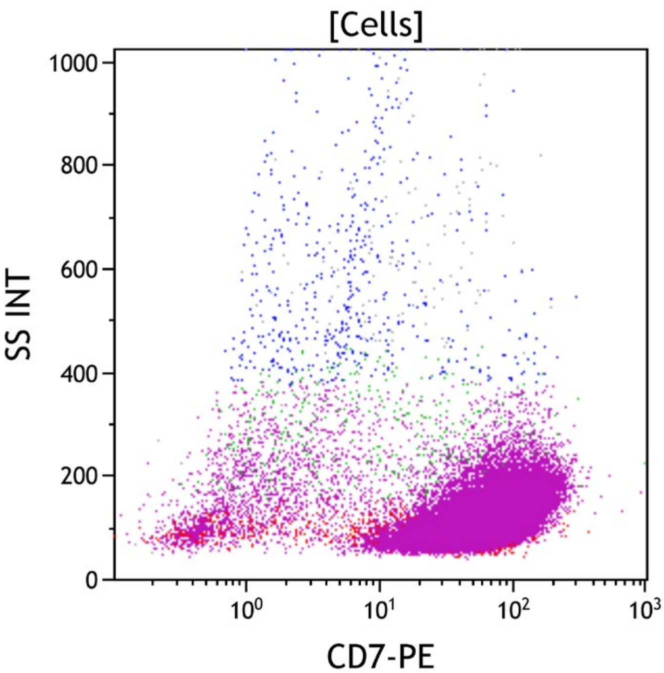


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors Progenitors (purple) express bright CD7.

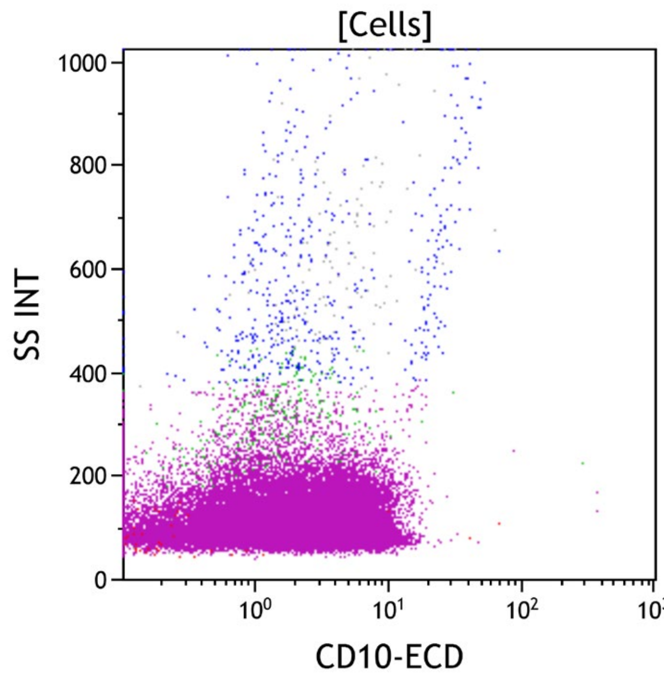


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. Progenitors (purple) express variable CD10.

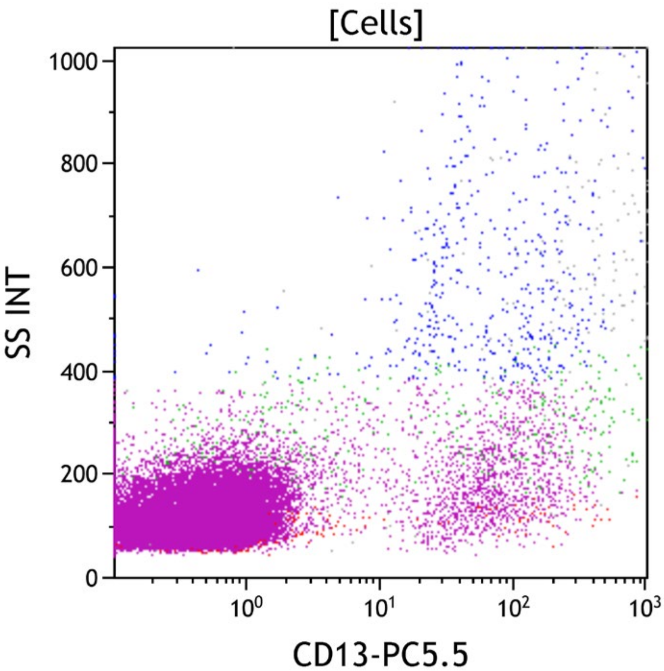


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. Progenitors (purple) do not express CD13.

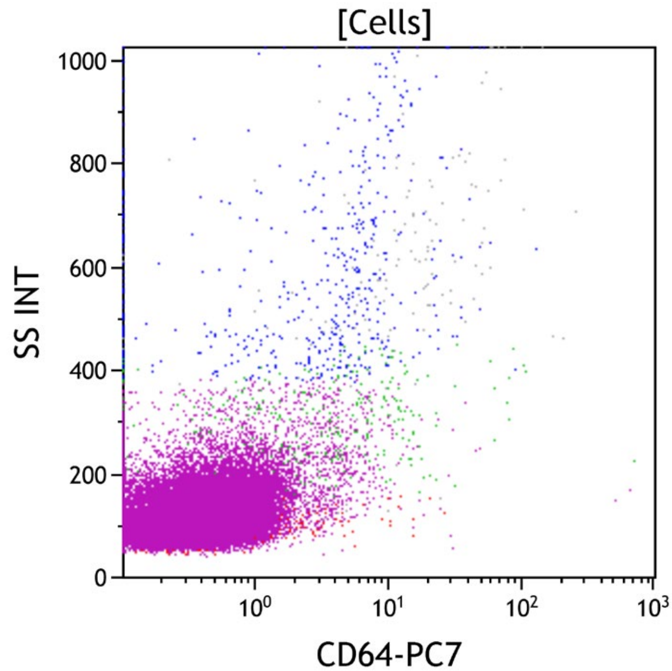


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. Progenitors (purple) do not express CD64.

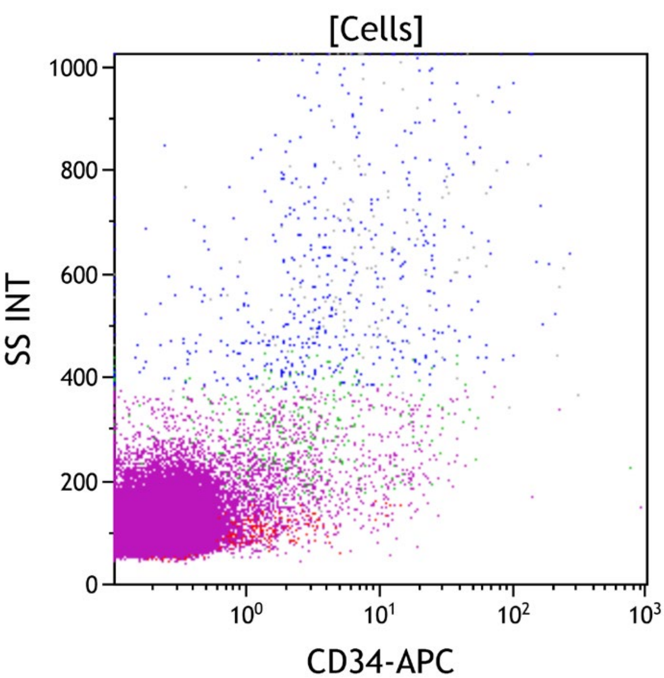


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. Progenitors (purple) do not express CD34.

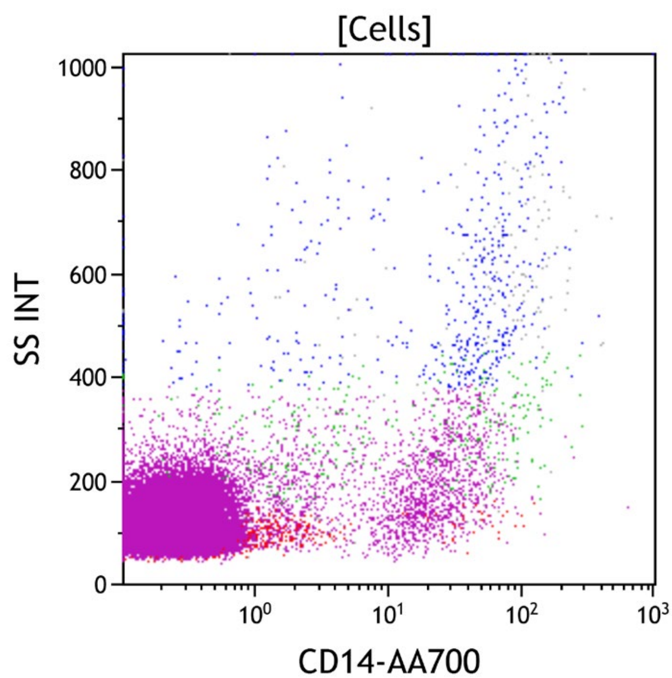


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Progenitors (purple) do not express CD14.

M1 Cell Tube

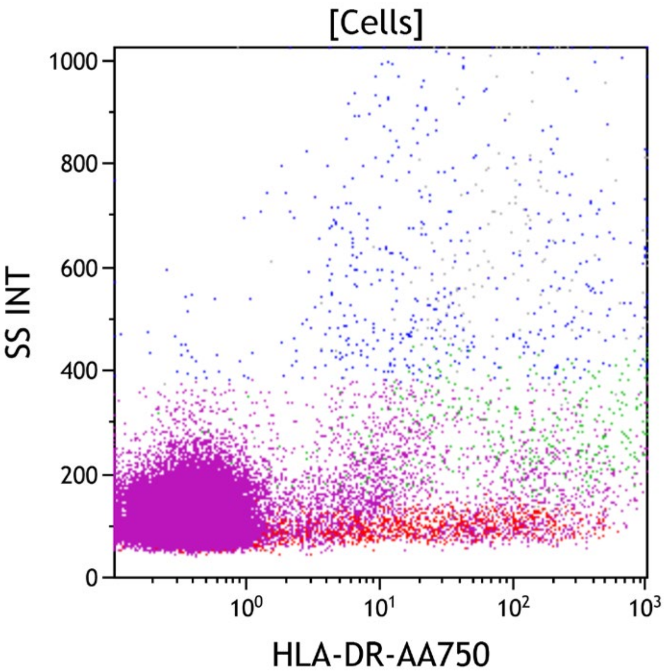


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). Progenitors (purple) do not express HLA-DR.

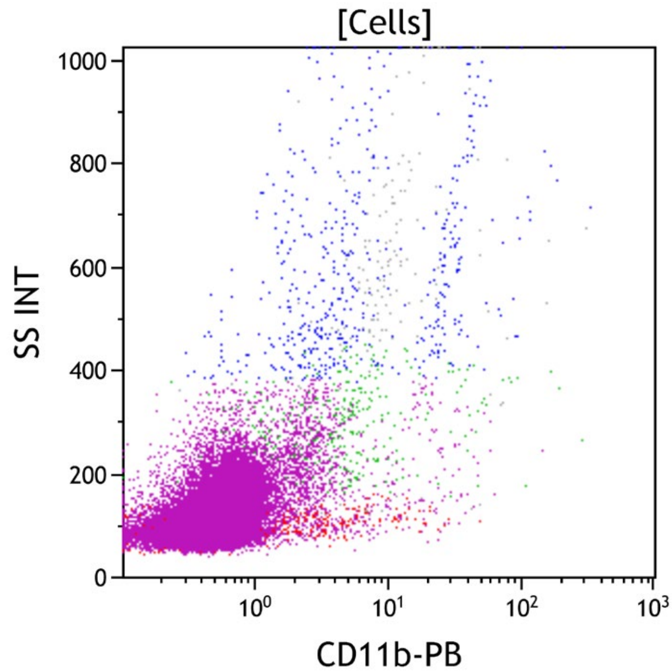


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. Progenitors (purple) do not express CD11b.

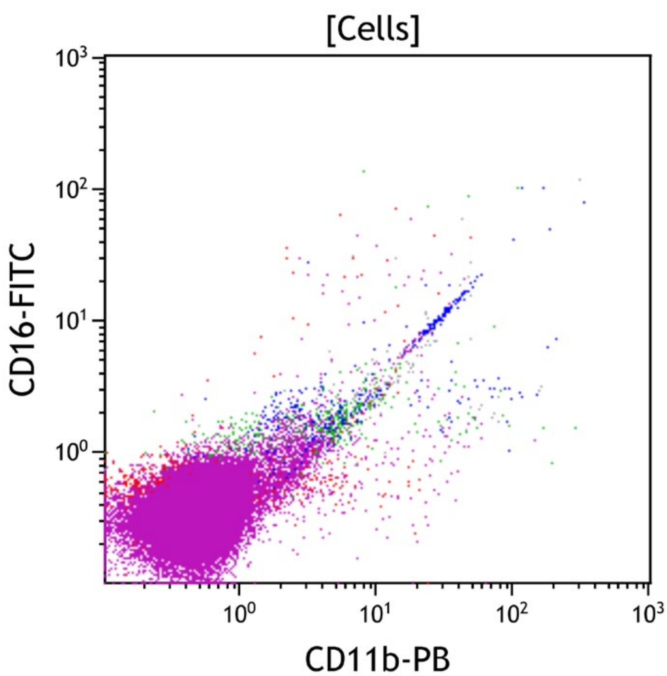


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. Progenitors (purple) do not express CD11b or CD16.

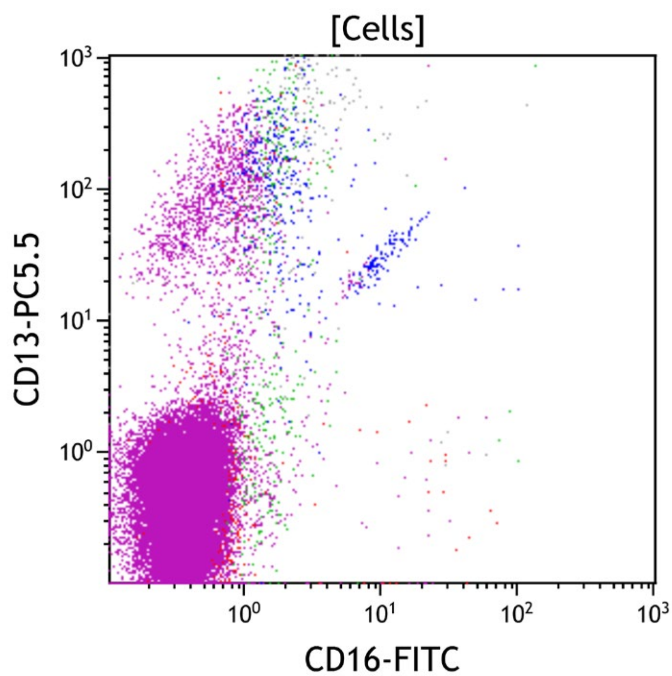


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. Progenitors (purple) do not express CD16 or CD13.

M1 Cell Tube

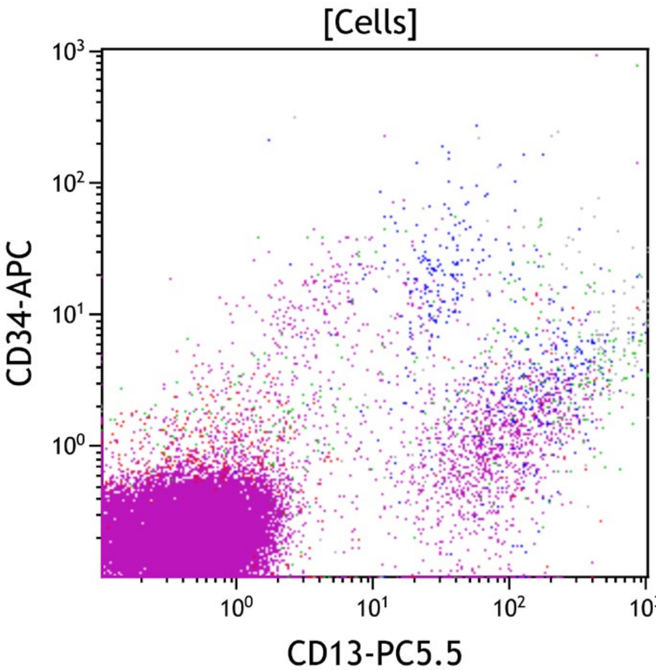


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells. Progenitors (purple) do not express CD13 or CD34.

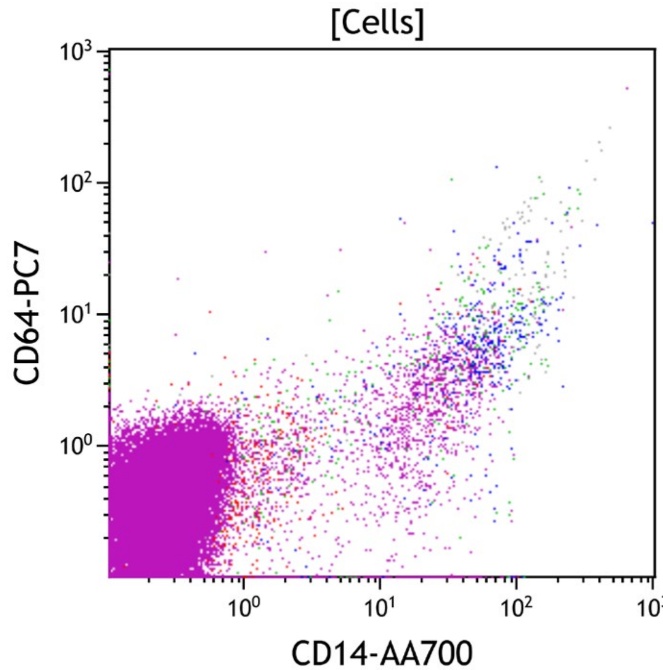


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. Progenitors (purple) do not express CD14 or CD64.

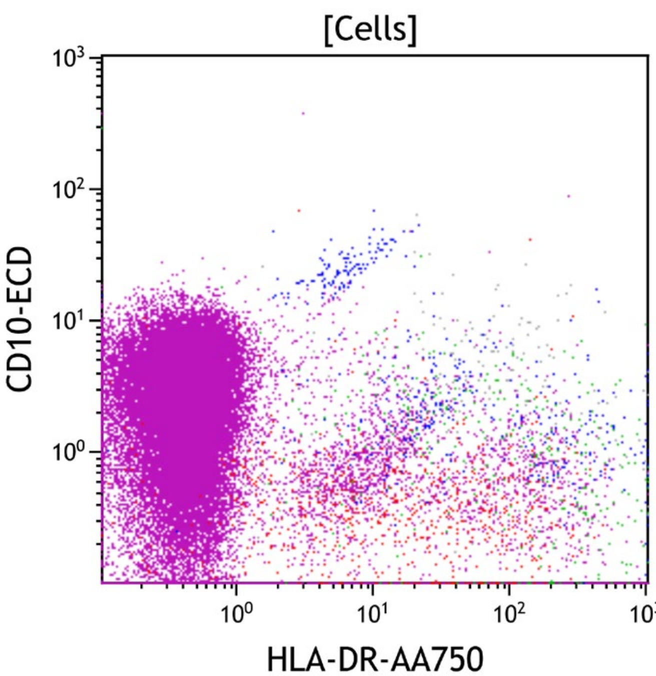


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue) and immature B cells. Immature B cells express both CD10 and HLA-DR. The abnormal progenitors (purple) express variable CD10 without HLA-DR.

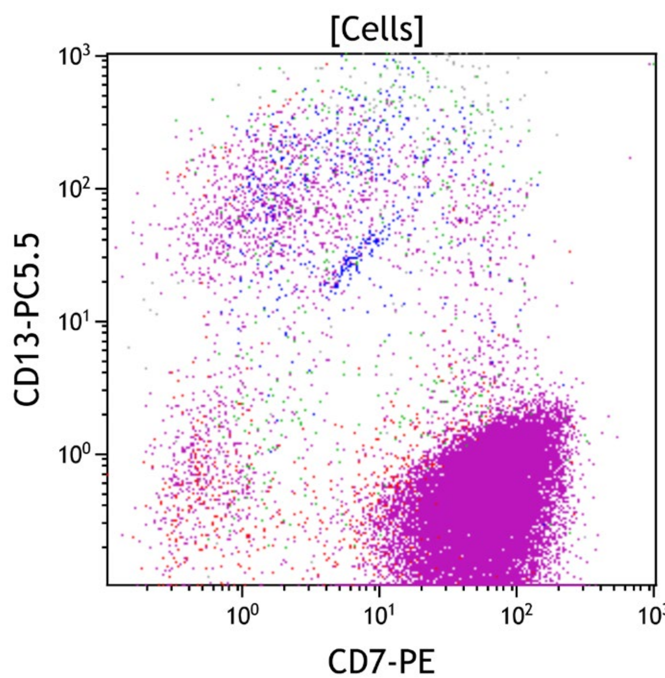


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The abnormal progenitors (purple) express bright CD7 without CD13.

M2 Cell Tube

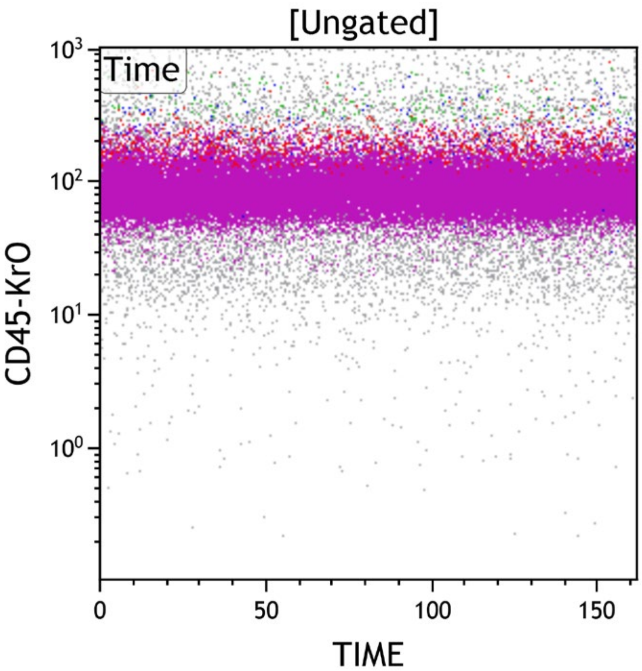


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

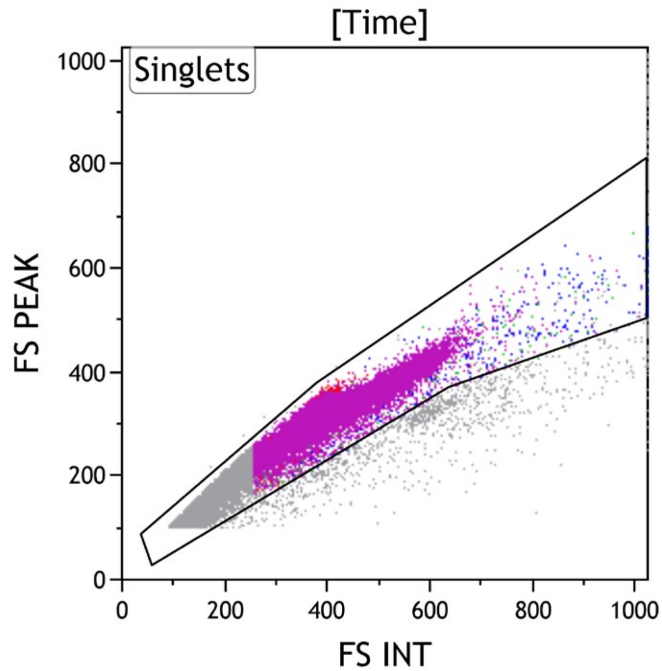


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

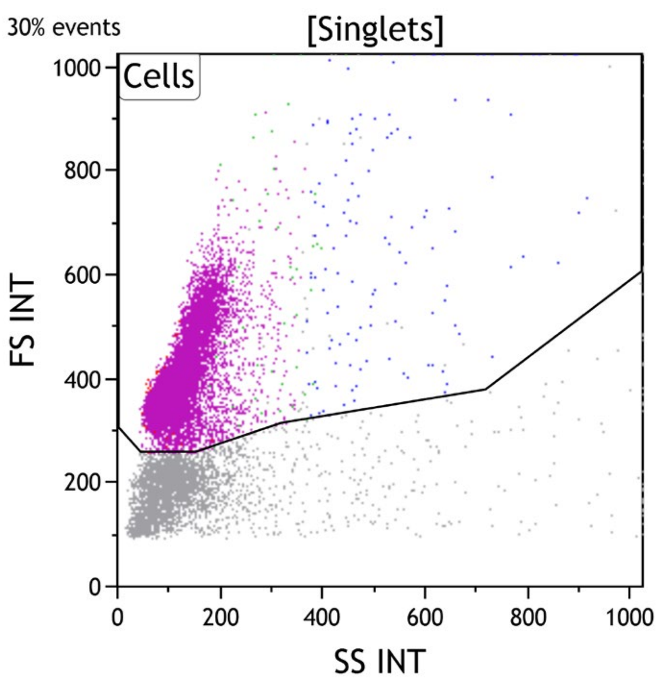


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

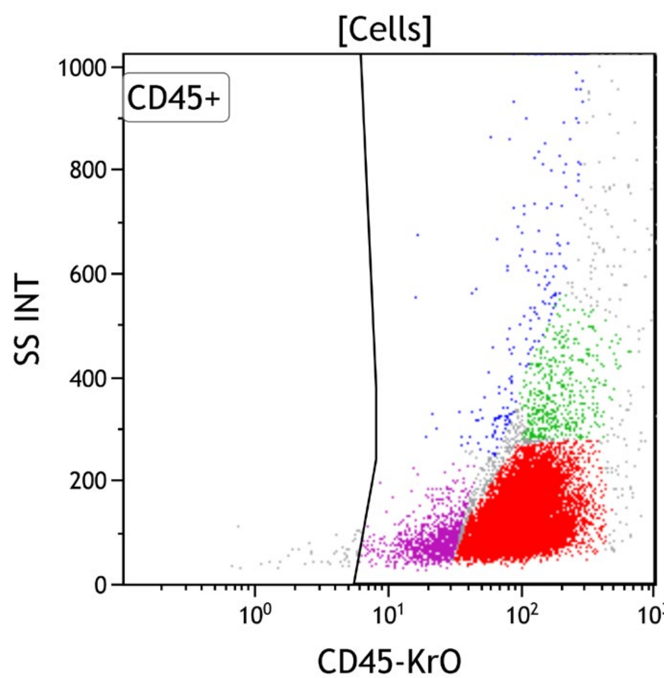


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

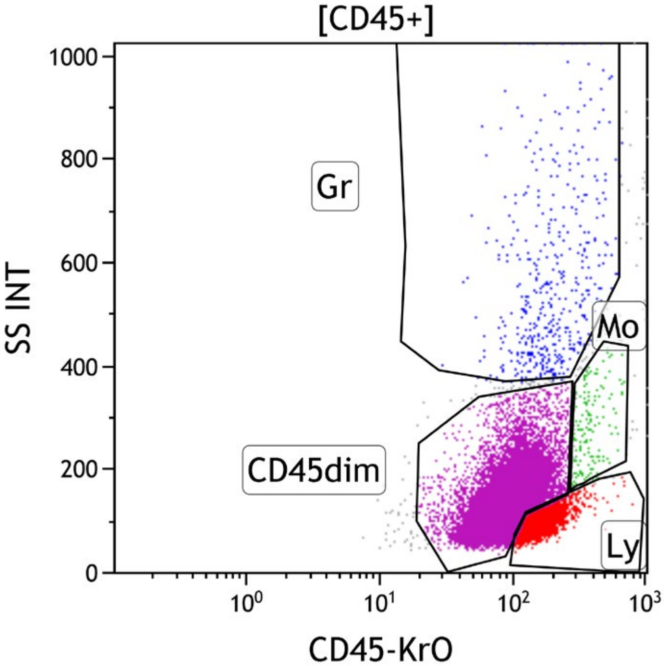


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Blasts are increased. The progenitor population (purple) is expanded.

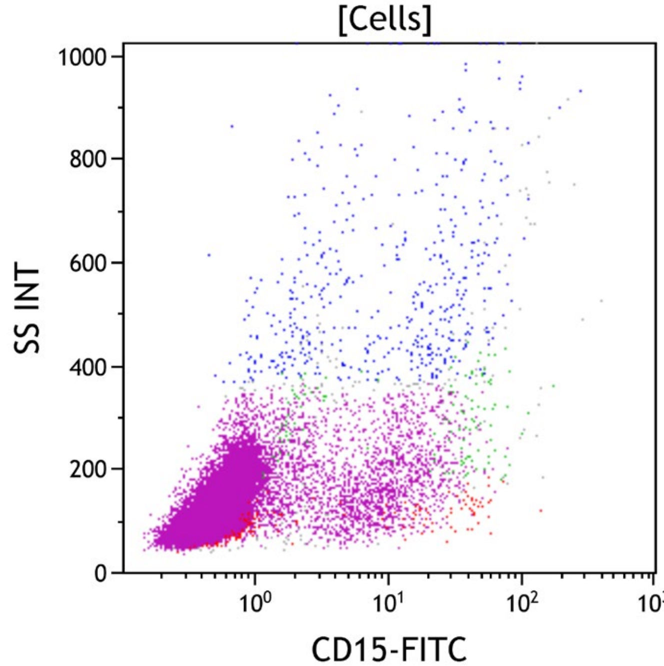


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes. Progenitors (purple) do not express CD15.

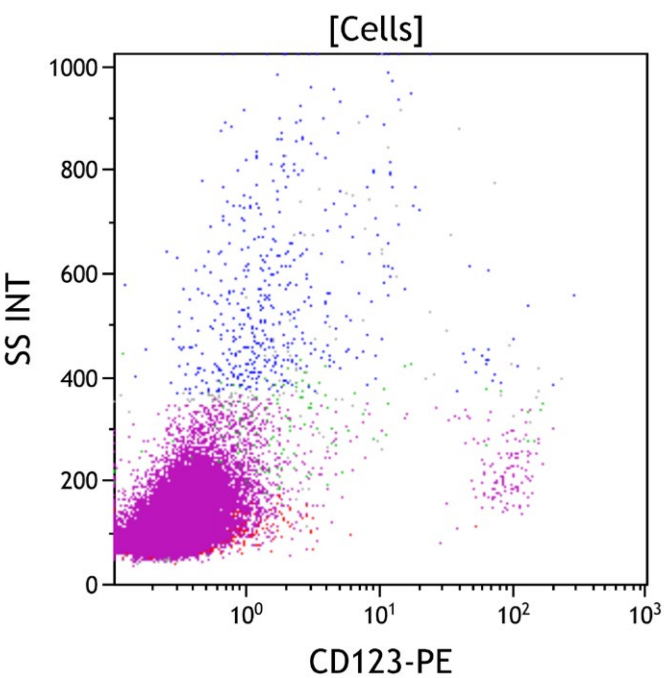


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. Progenitors (purple) do not express CD123.

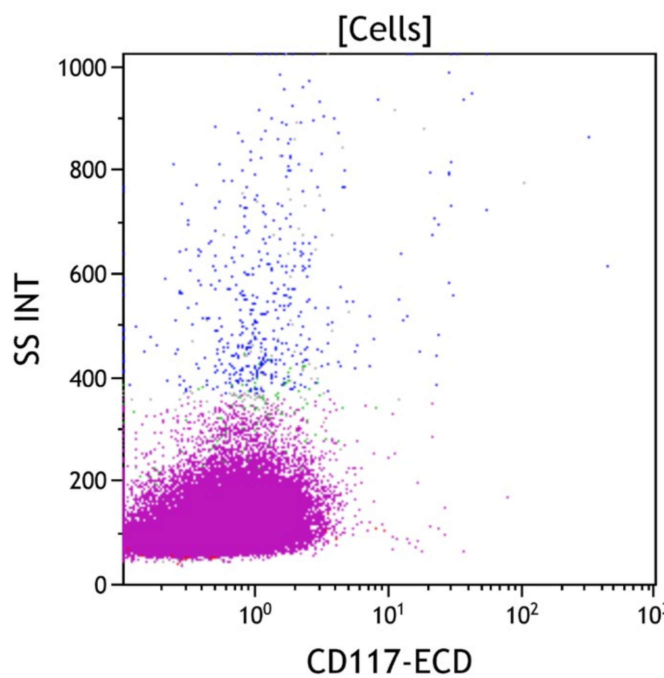


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Progenitors (purple) do not express CD117.

M2 Cell Tube

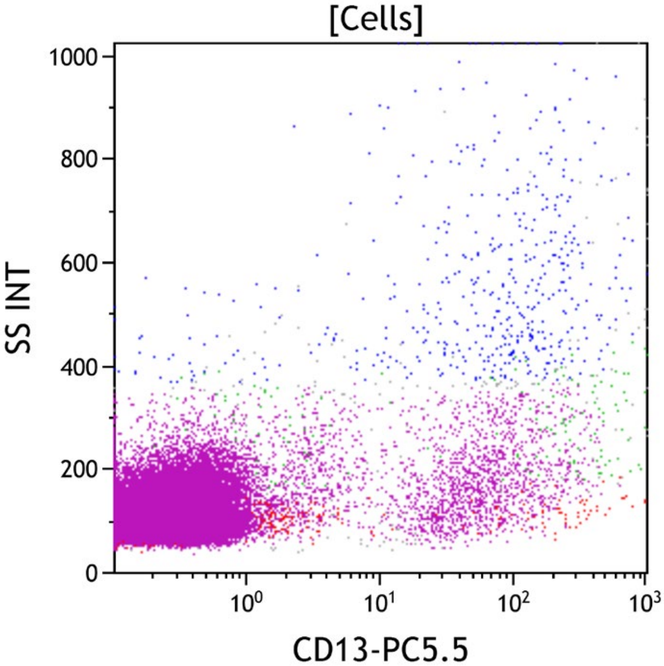


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. Progenitors (purple) do not express CD13.

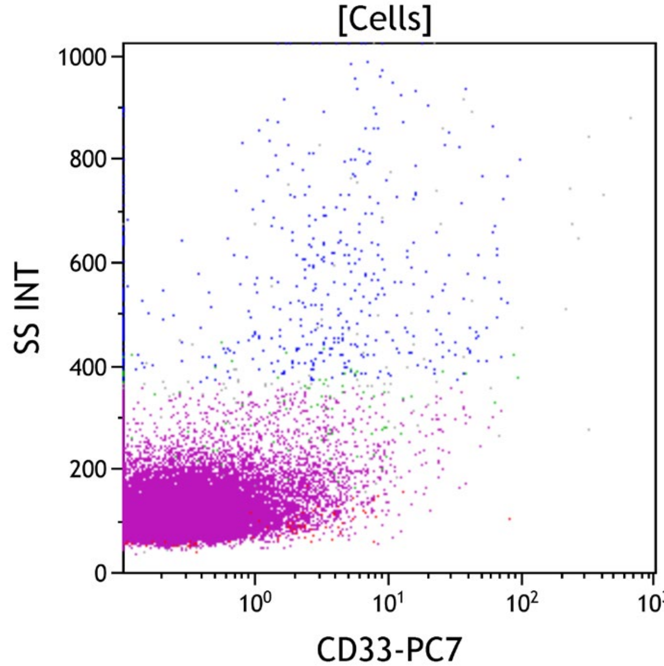


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes, and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. Progenitors (purple) do not express CD33.

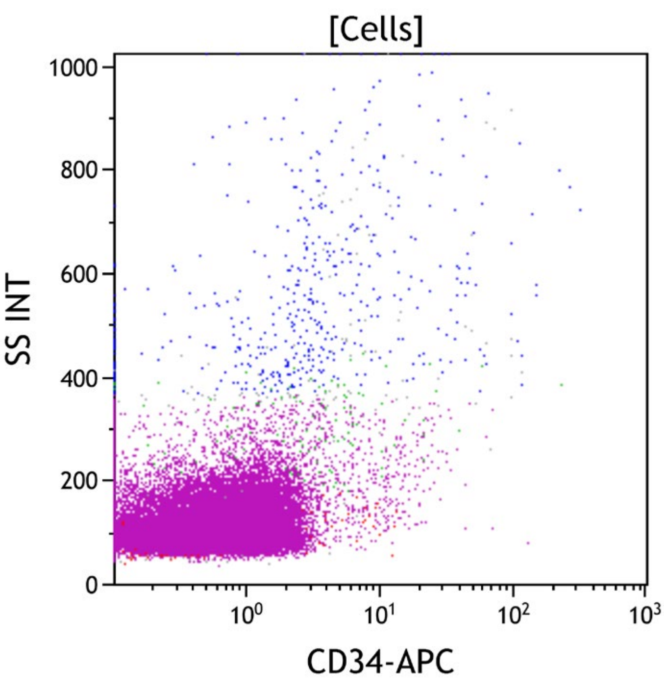


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. Progenitors (purple) do not express CD34.

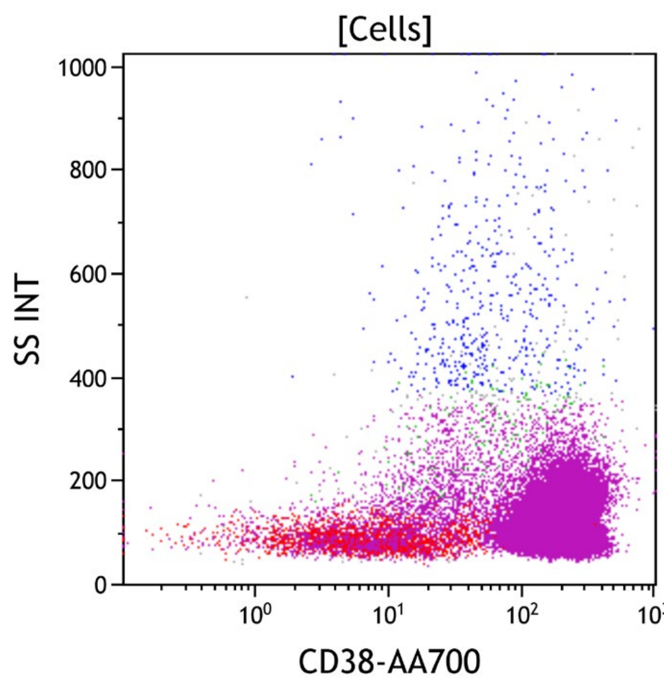


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Progenitors (purple) express bright CD38.

M2 Cell Tube

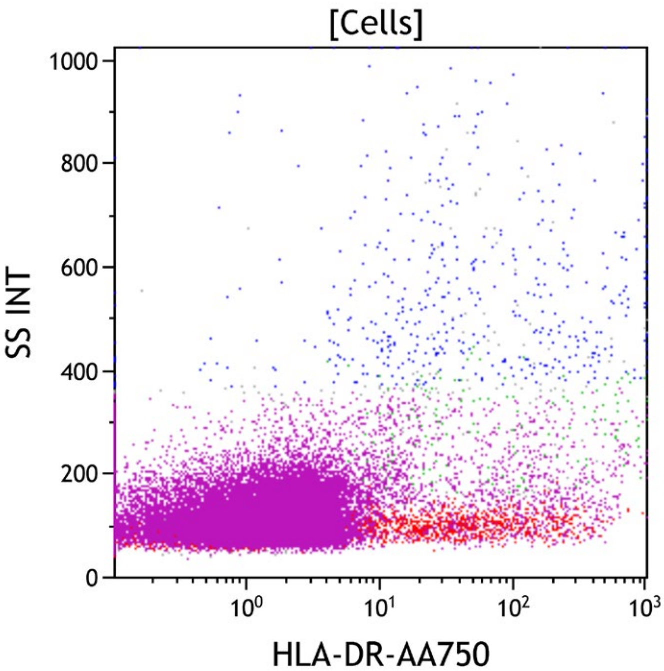


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. Progenitors (purple) do not express HLA-DR.

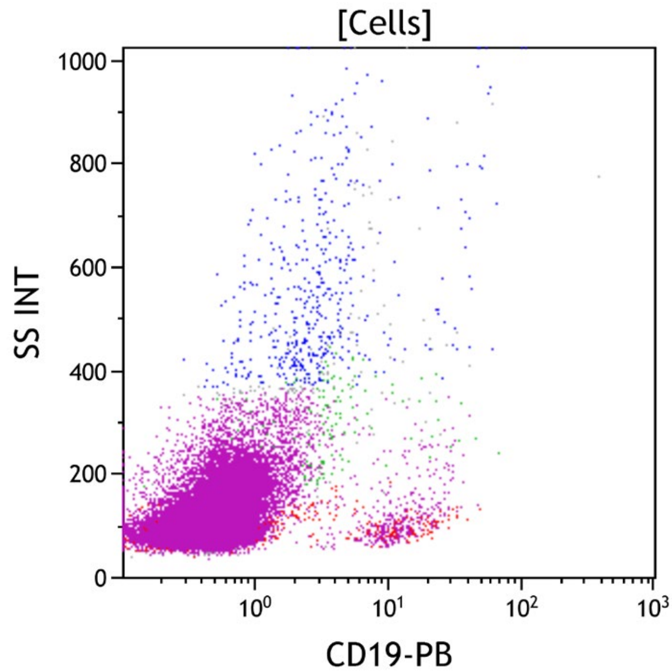


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express CD19.

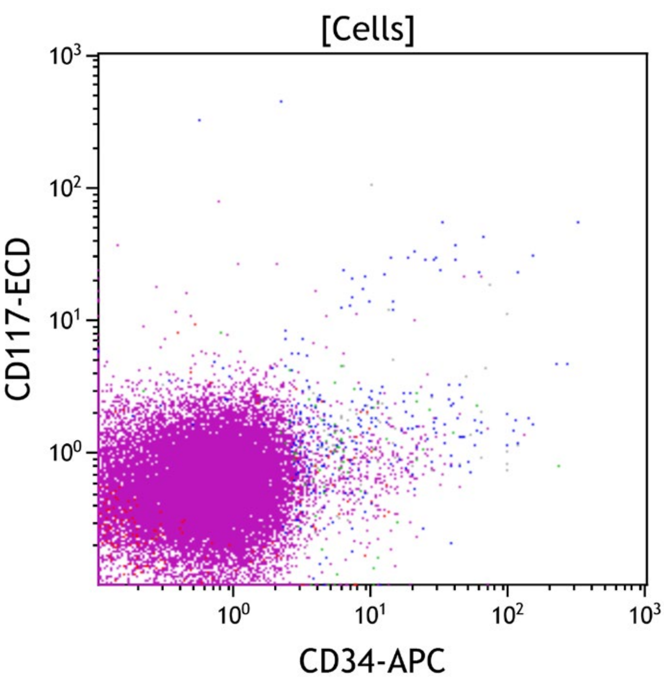


Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The progenitors (purple) do not express CD34 or CD117.

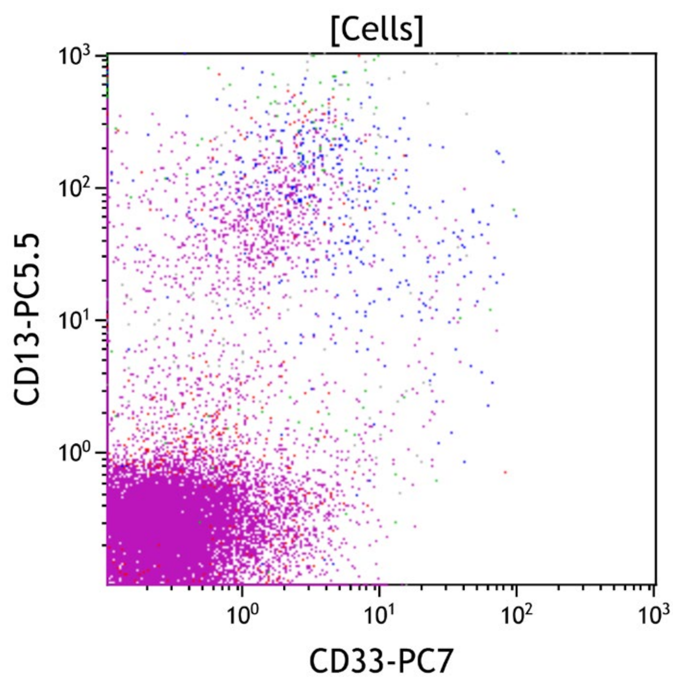


Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red). The progenitors (purple) do not express CD13 or CD33.

M2 Cell Tube

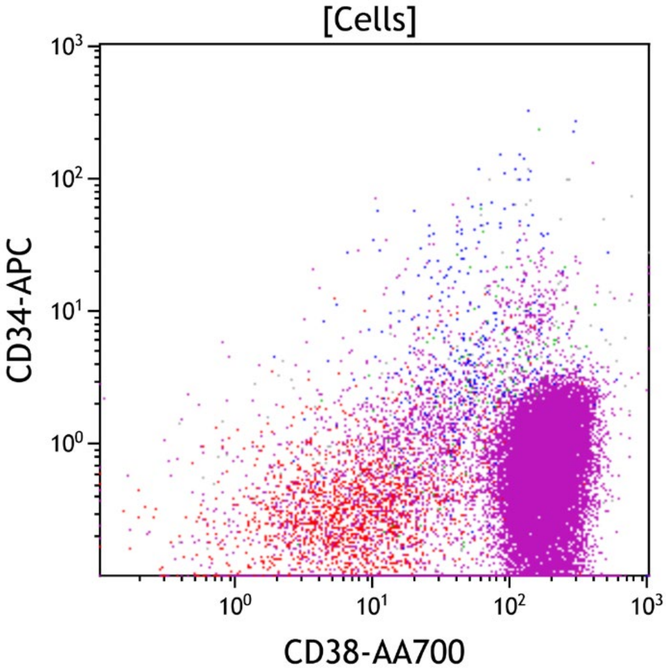


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 Progenitors (purple) express bright CD38 without CD34.

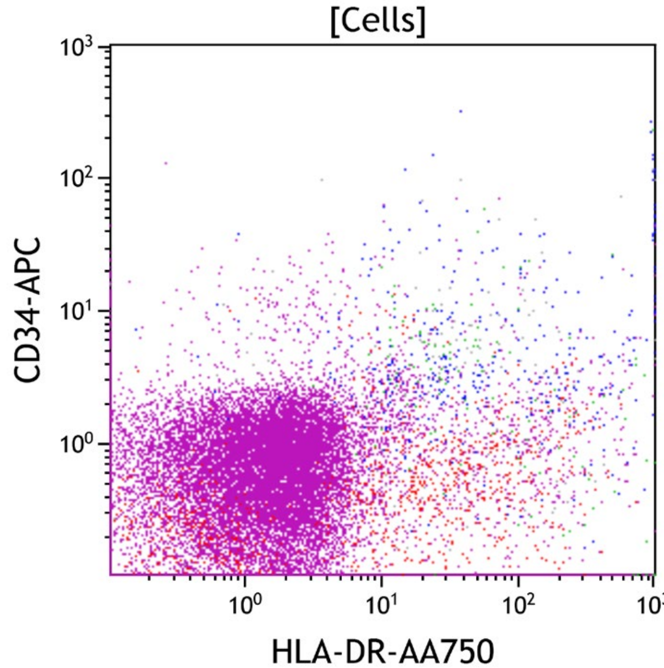


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. Progenitors (purple) do not express HLA-DR or CD34.

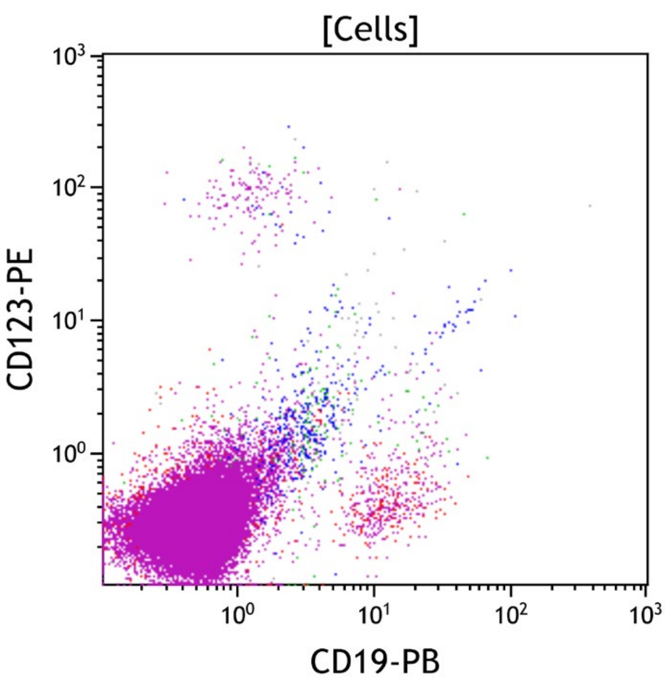


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) do not express CD19 or CD123.

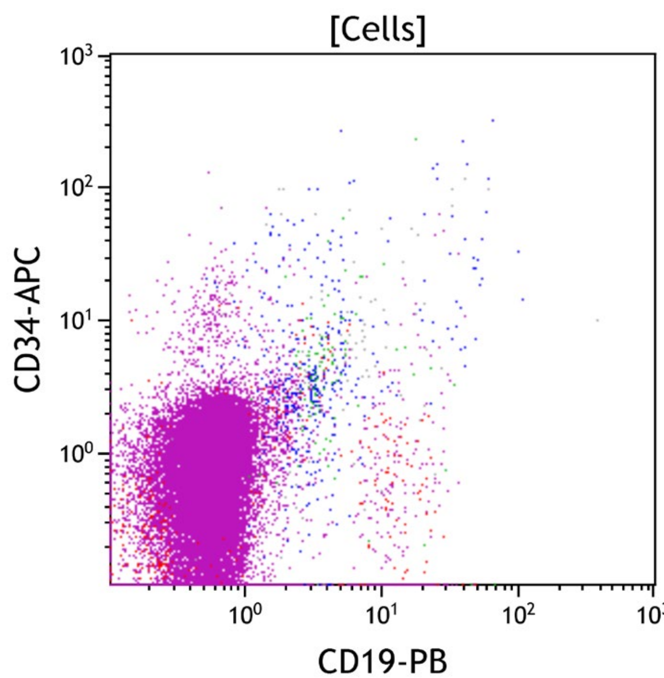


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. Progenitors (purple) do not express CD19 or CD34.

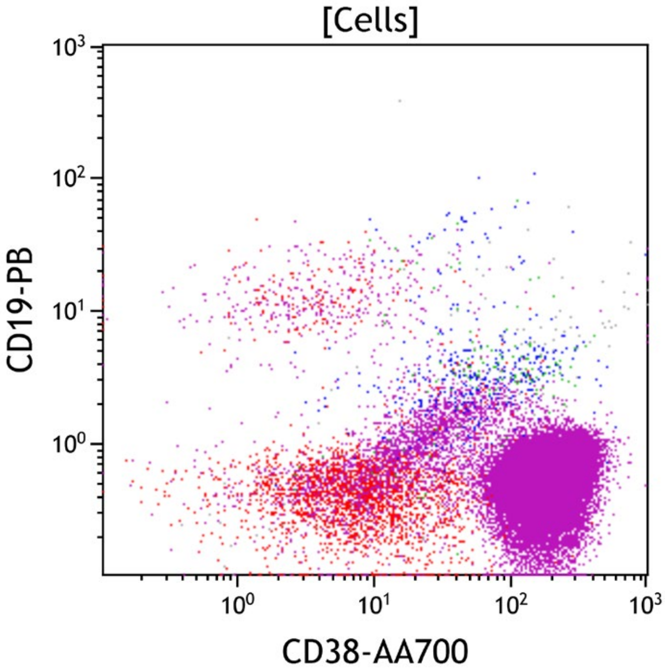


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38 (red left). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) express bright CD38 without CD19.

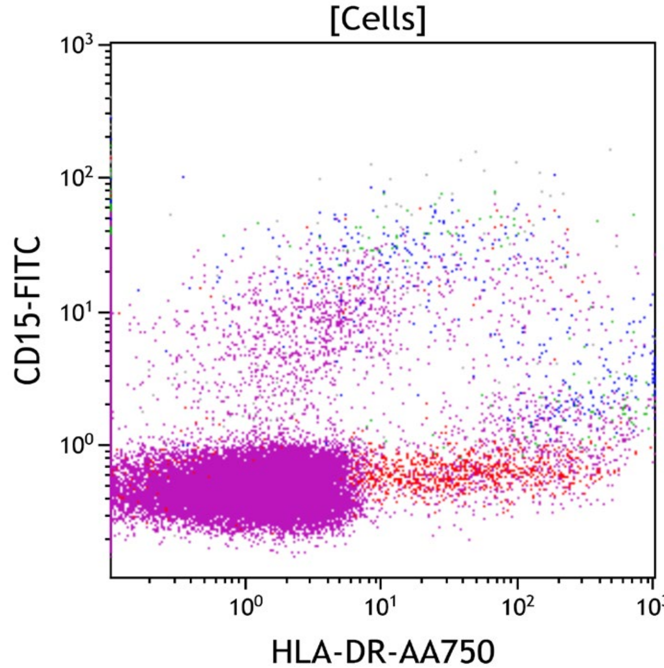


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR, except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. Progenitors (purple) do not express HLA-DR or CD15.

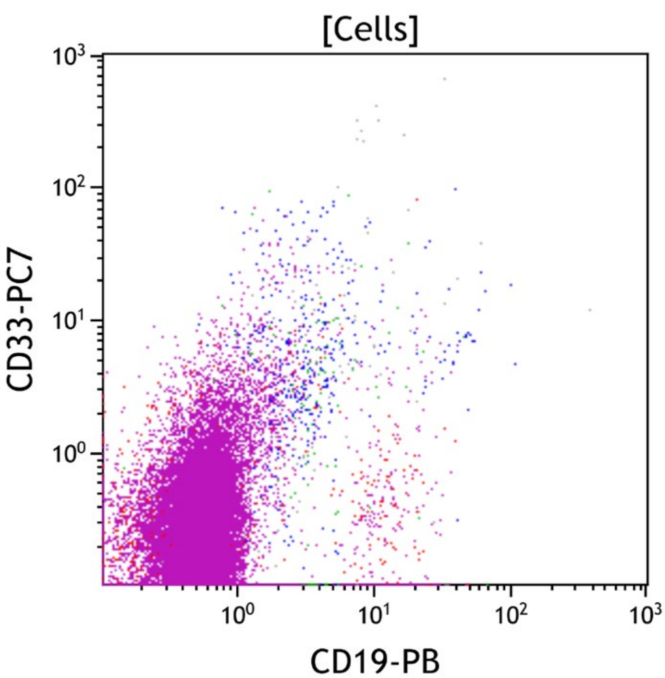


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) do not express CD19 or CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate CD2, variable CD4, intermediate CD5, bright CD7, dim CD10, bright CD38, intermediate CD45 and are negative for surface CD3, CD8, CD19, CD34, CD56, CD117 and other B cell or myeloid antigens. Compared with normal immature T cells, the expression of CD4 and CD10 without CD8 and the bright CD38 is abnormal.

The immunophenotype of the abnormal population is in keeping with abnormal immature T cells, i.e. T-lymphoblasts. The findings are consistent with a diagnosis of T–lymphoblastic leukemia/lymphoma. Additional testing for cytoplasmic CD3 and TdT could be performed to confirm T cell lineage and immaturity, respectively.

Case #15: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

Clinical Vignette

This 32-year-old male presents with circulating blasts on peripheral blood smear. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

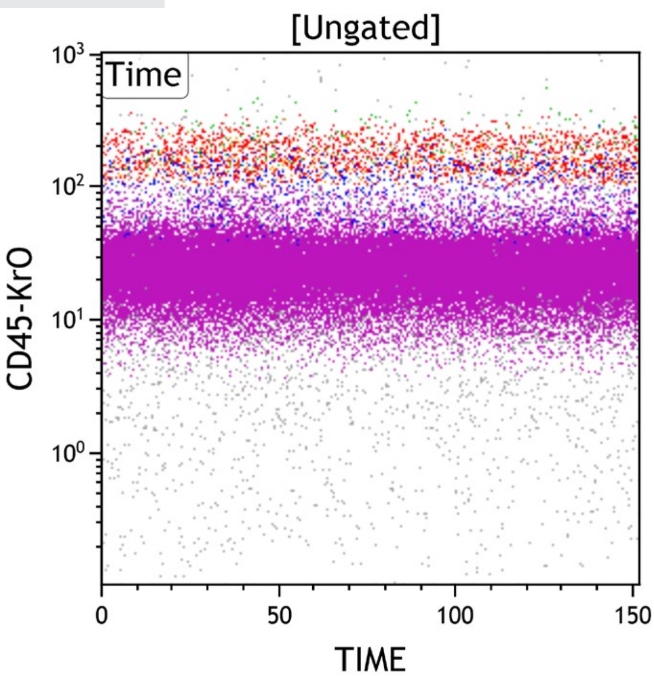


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

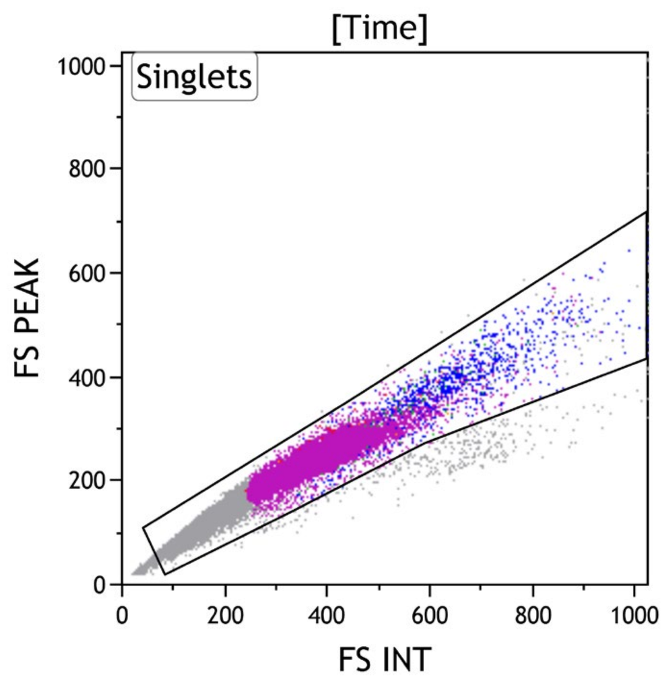


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

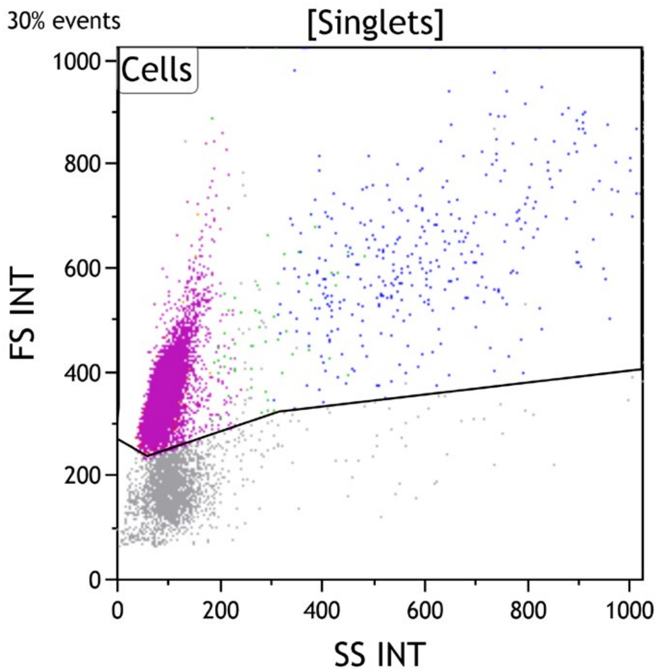


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

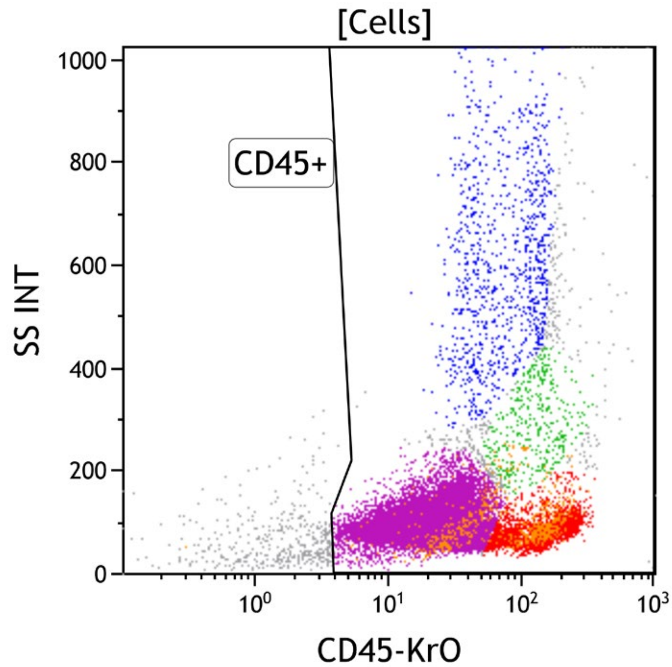


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

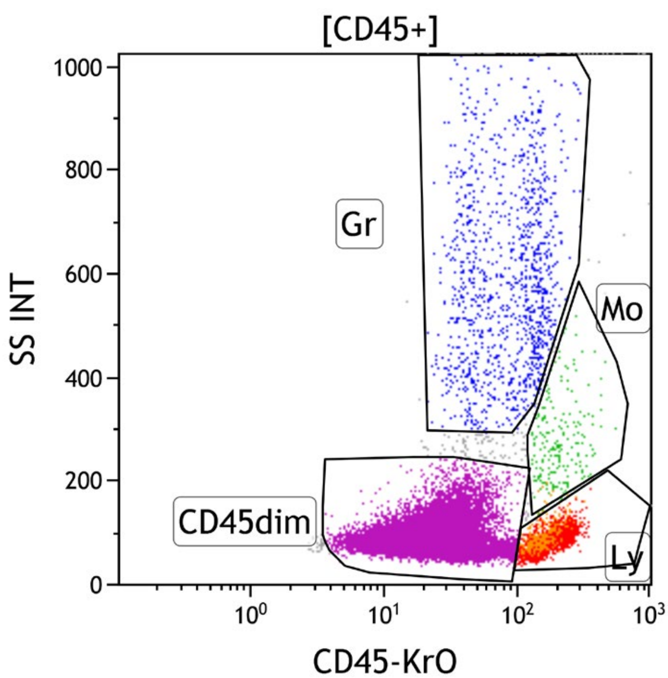


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Progenitors (purple) are expanded.

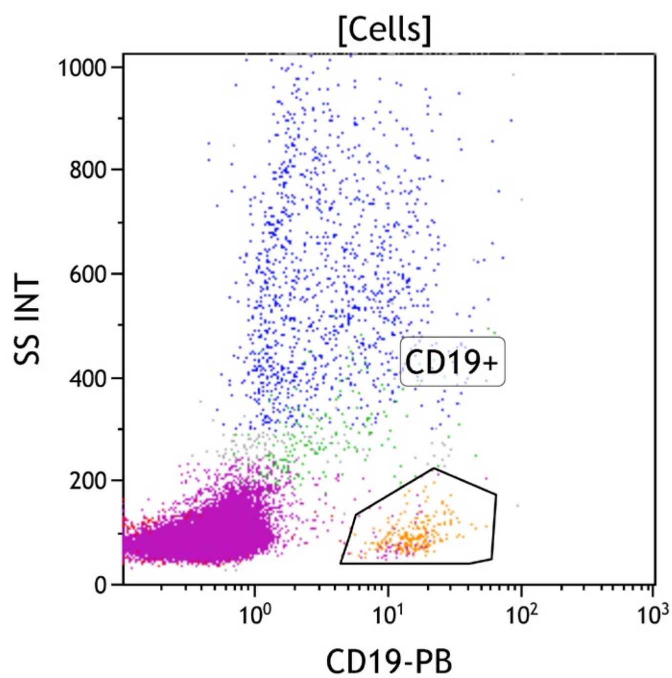


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express CD19.

B Cell Tube

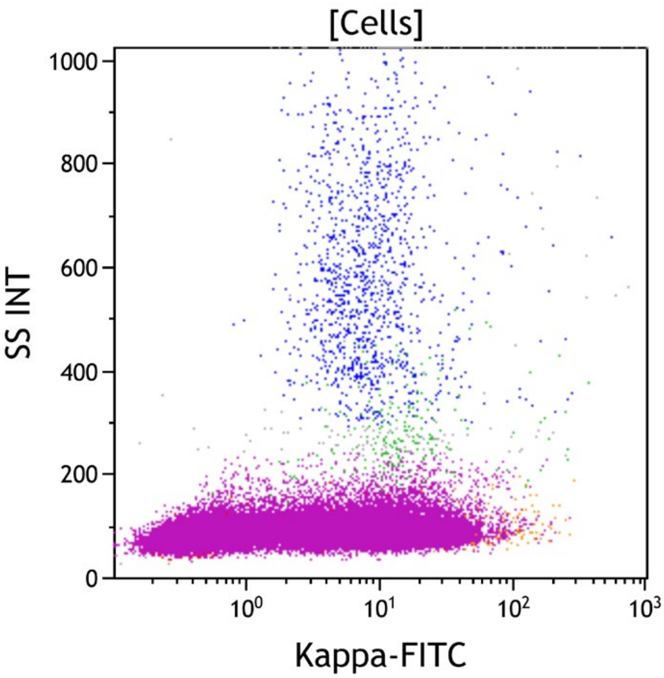


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. Progenitors (purple) show higher non-specific binding of light chain antibodies.

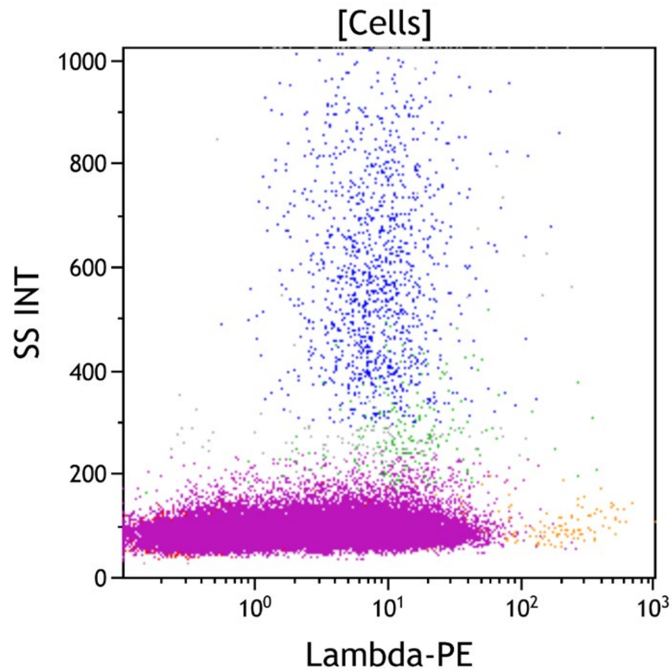


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. Progenitors (purple) show higher non-specific binding of light chain antibodies.

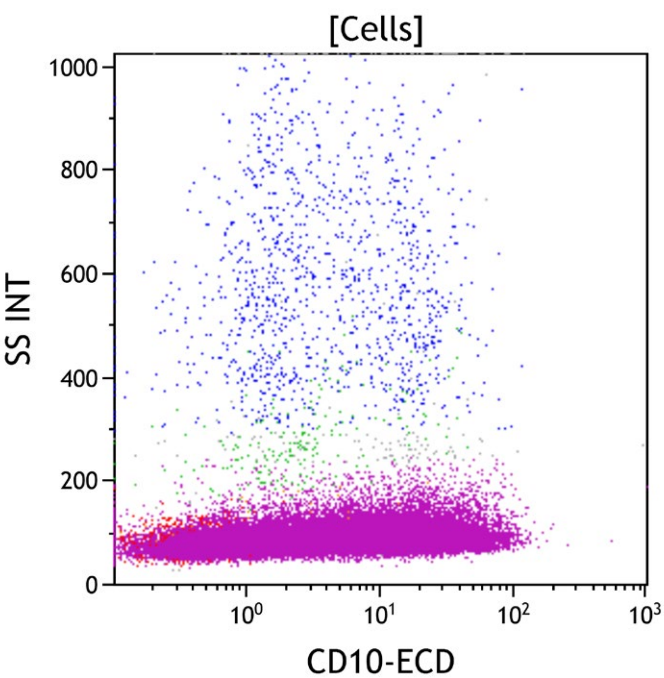


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B or T cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Progenitors (purple) variably express CD10.

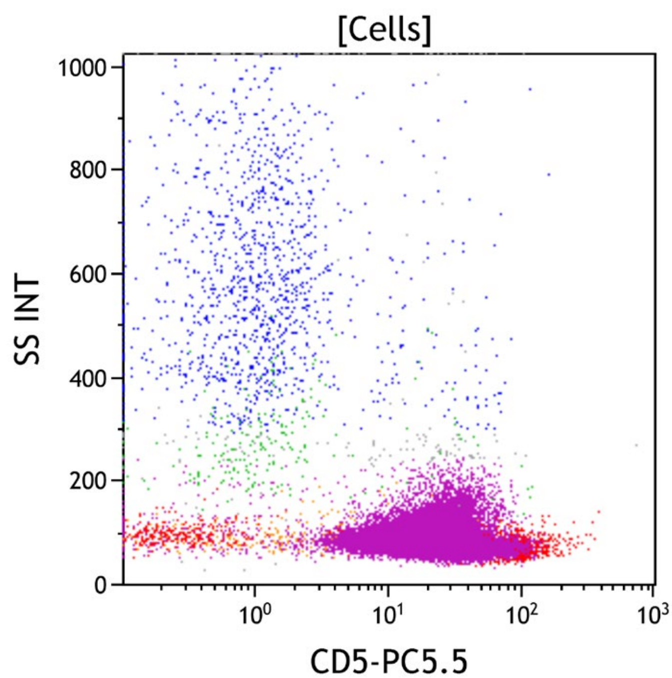


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. Progenitors (purple) express CD5 at a level slightly lower than mature T cells (red).

B Cell Tube

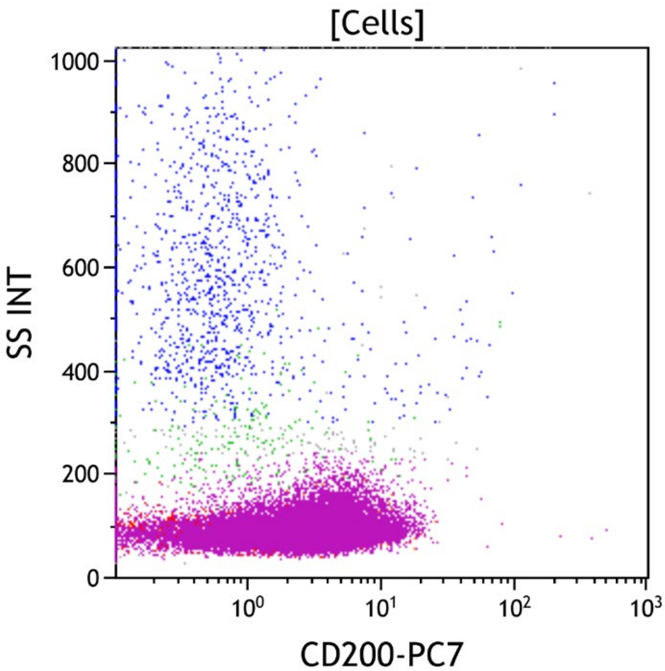


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). Progenitors (purple) variably express CD200.

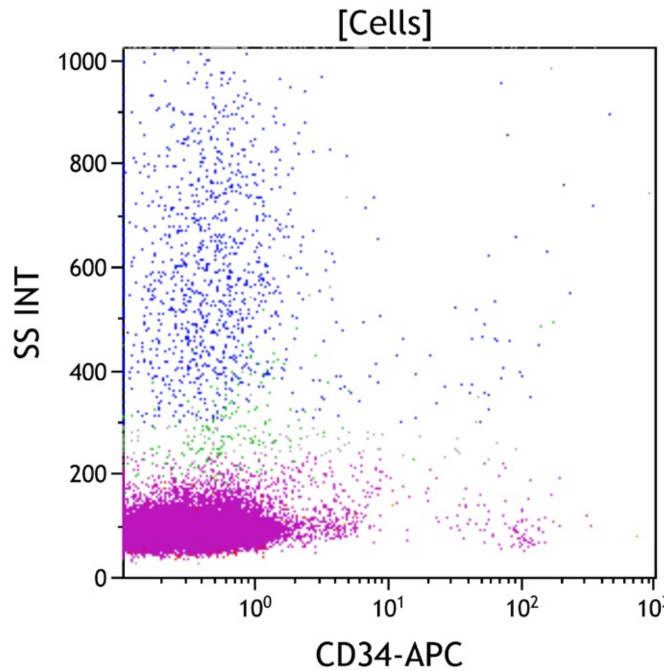


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Progenitors (purple) do not express CD34.

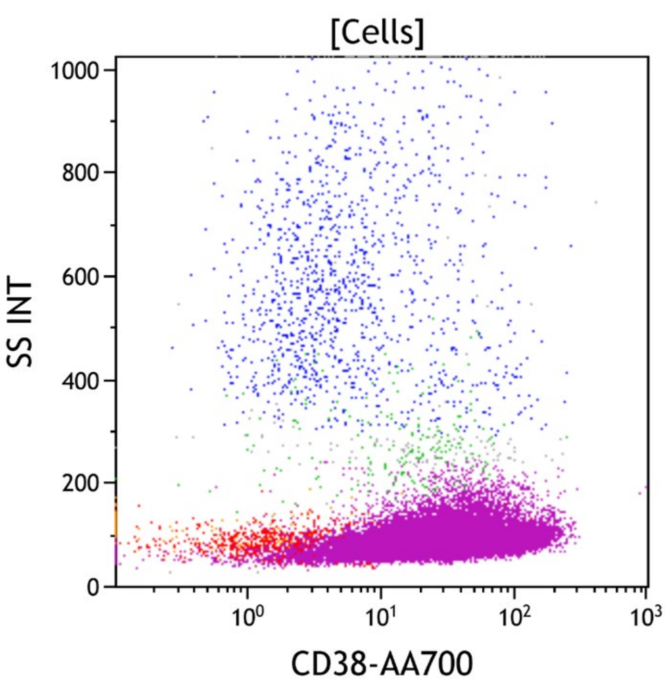


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). Progenitors (purple) express bright CD38.

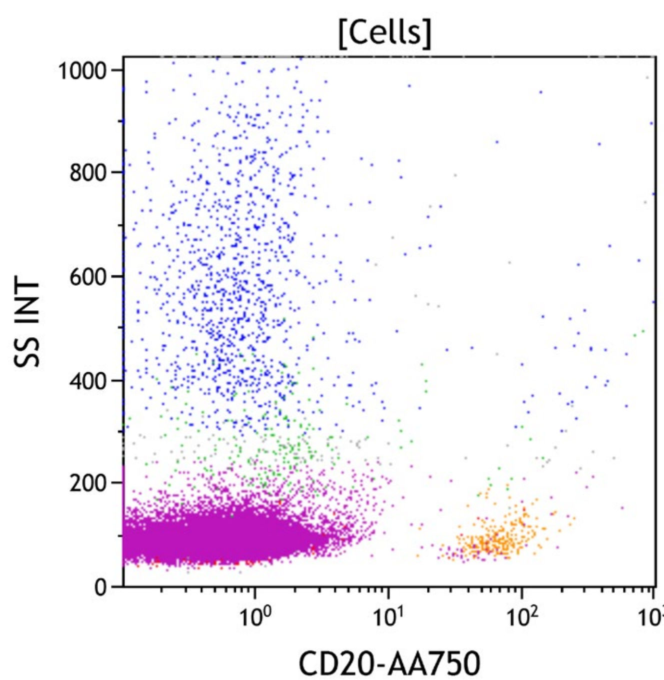


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. Progenitors (purple) do not express CD20.

B Cell Tube

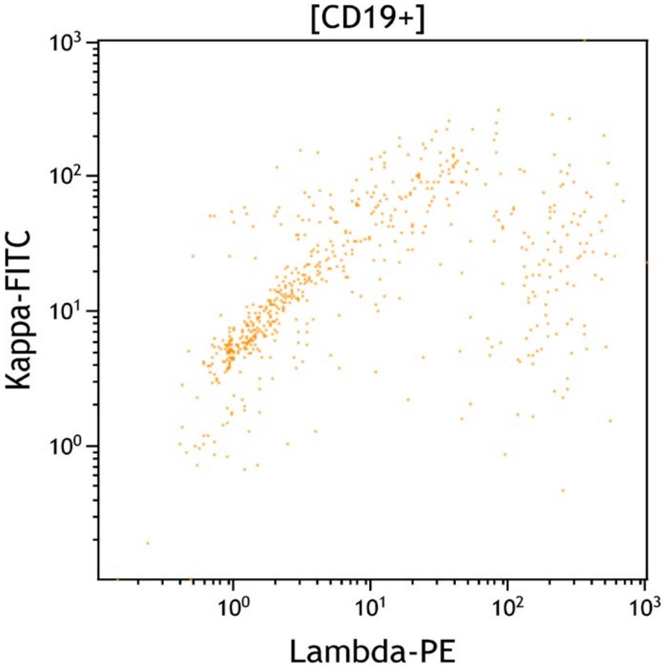


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

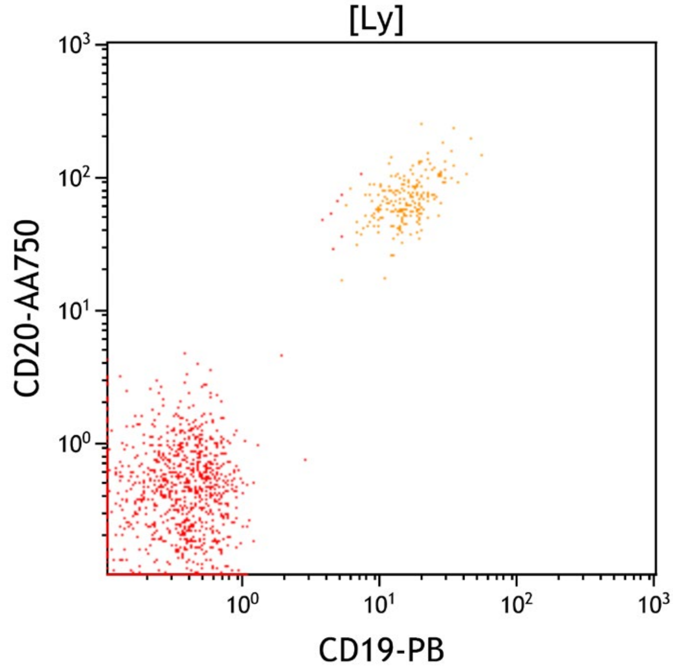


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate. Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

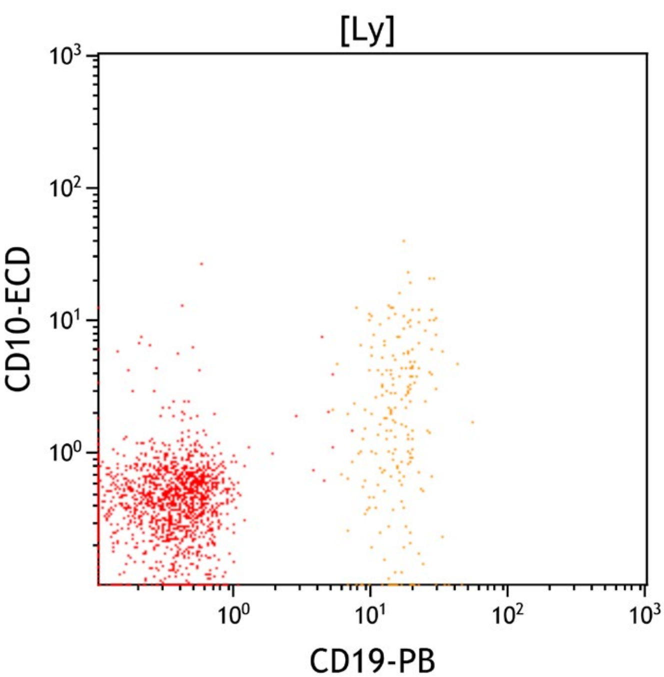


Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate. B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

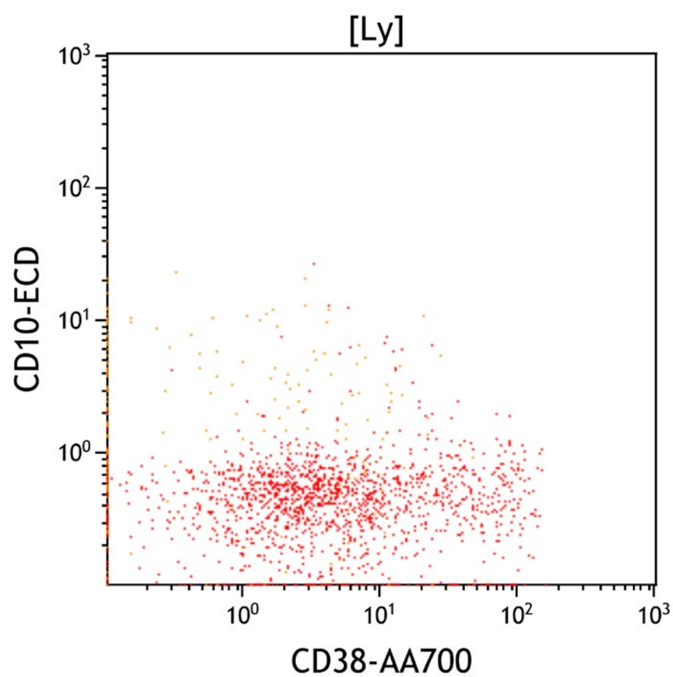


Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

B Cell Tube

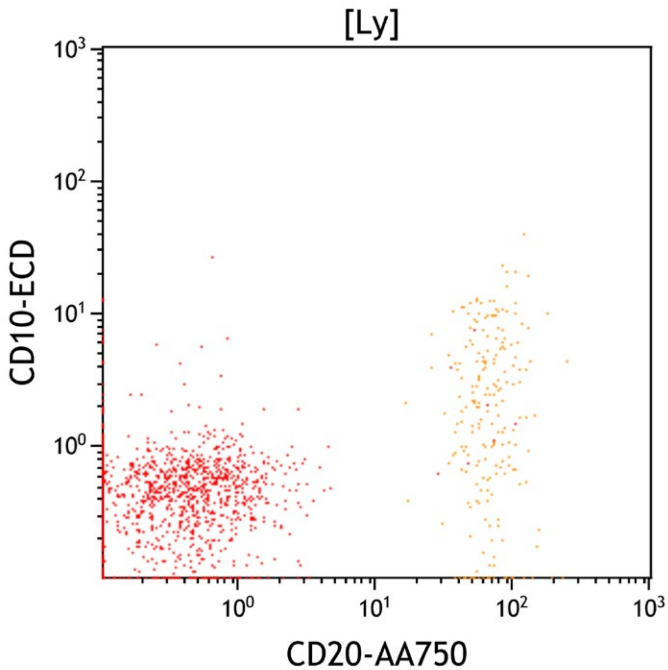


Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate. Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

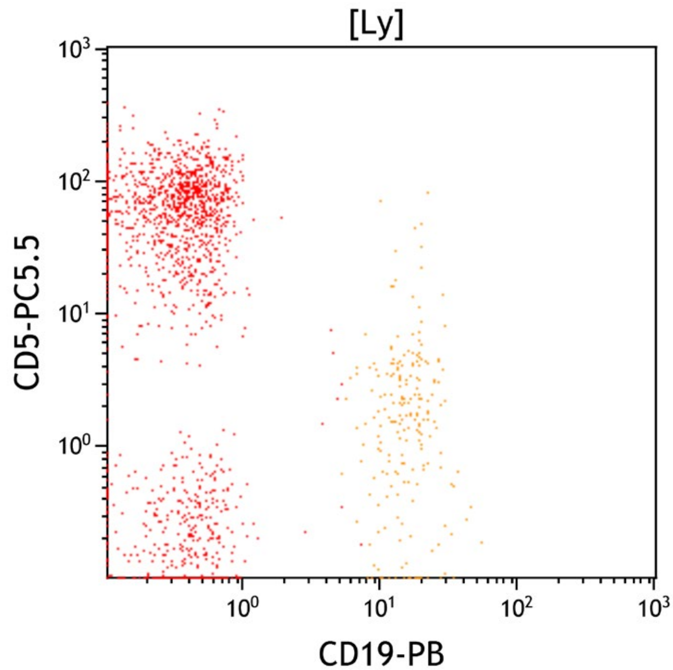


Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate. CD5 is expressed on T cells (red, upper left), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

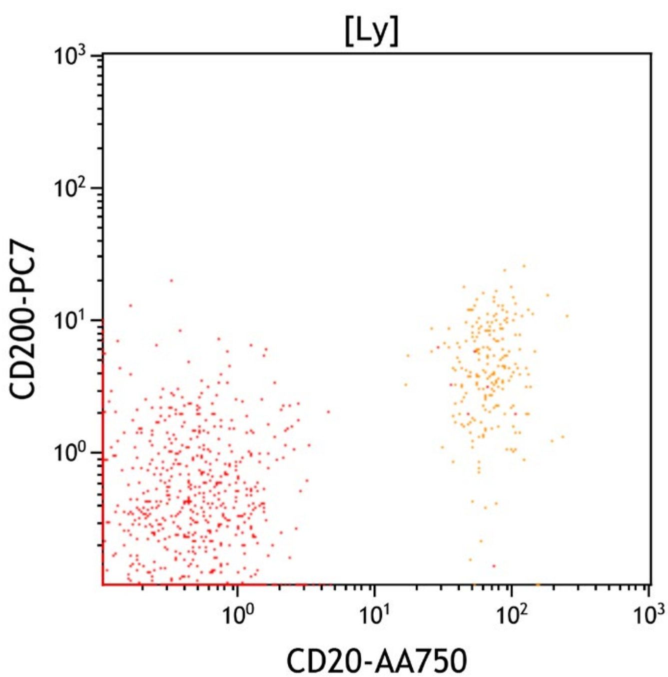


Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (orange).

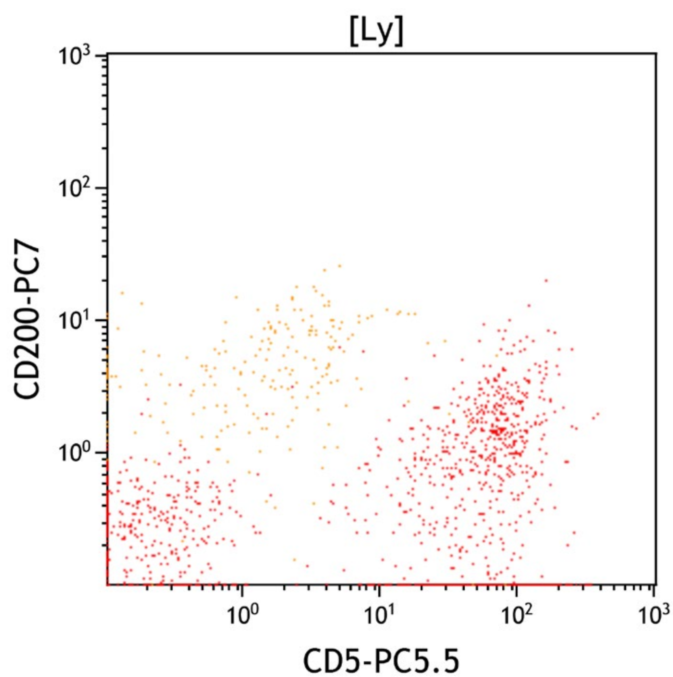


Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate. Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

T Cell Tube

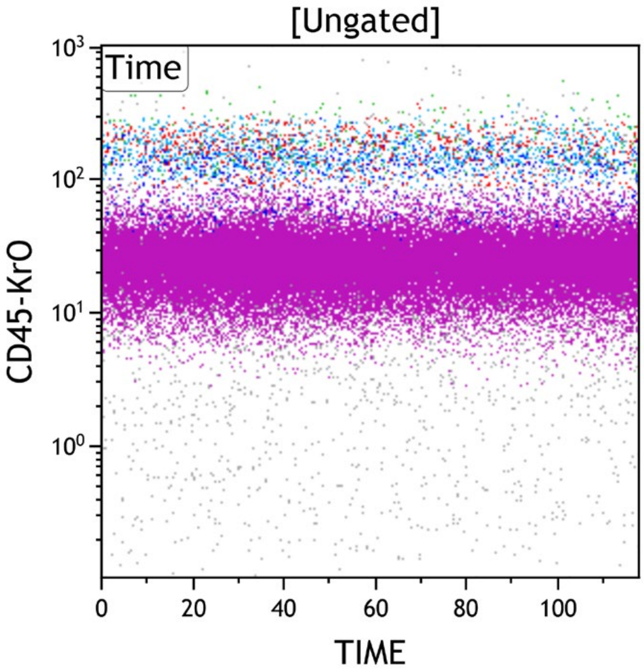


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

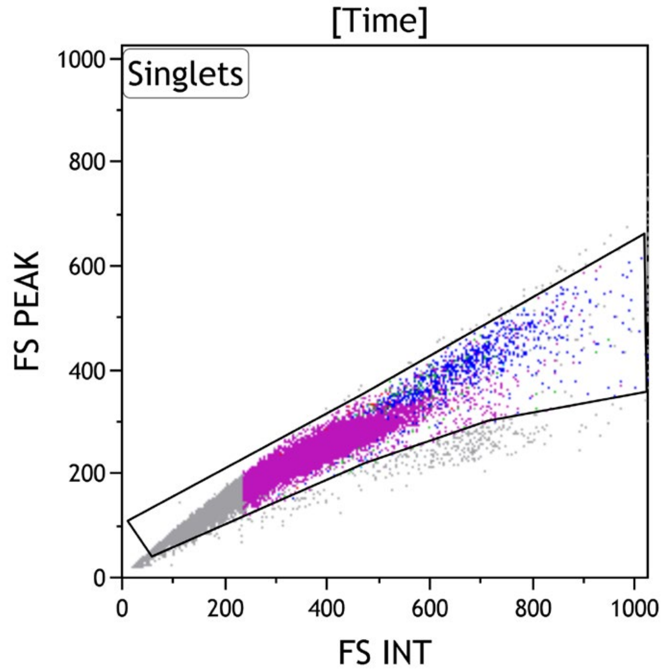


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

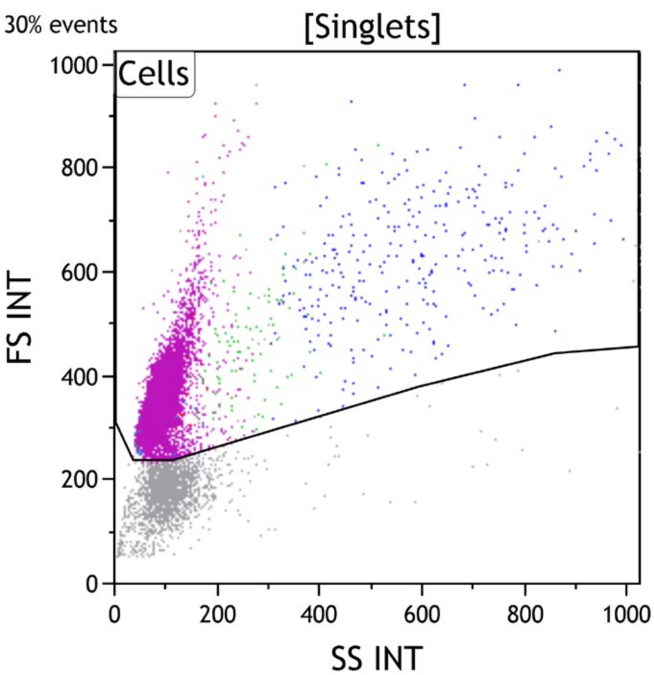


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

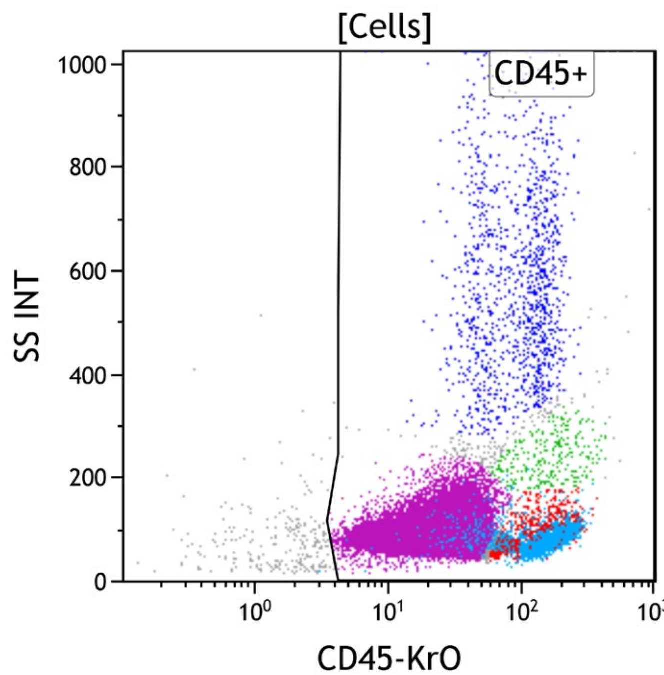


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

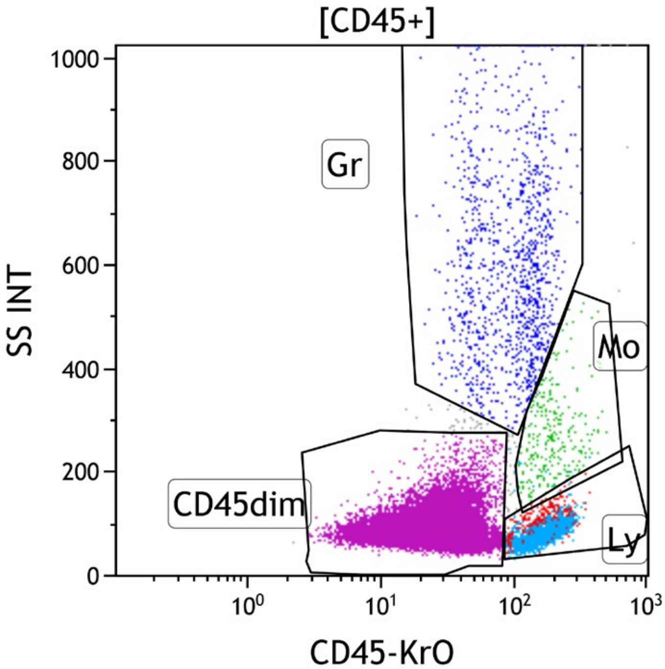


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The progenitor population (purple) is expanded.

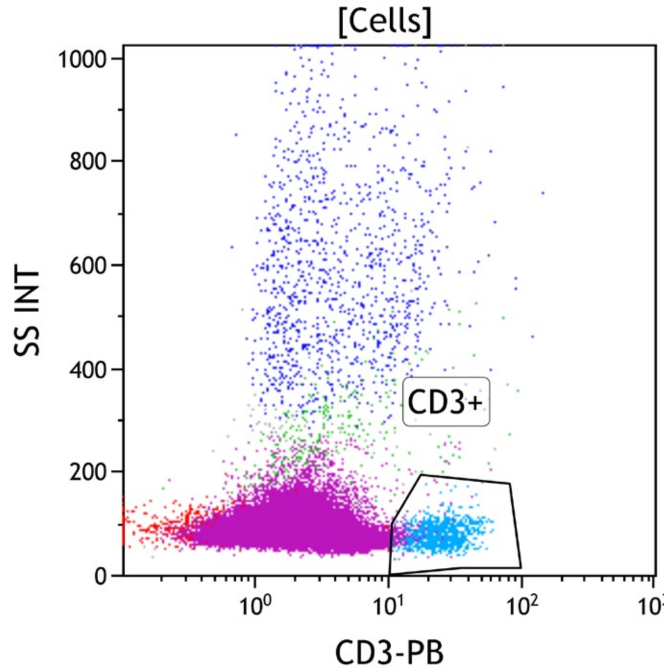


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. Progenitors (purple) are negative for surface CD3.

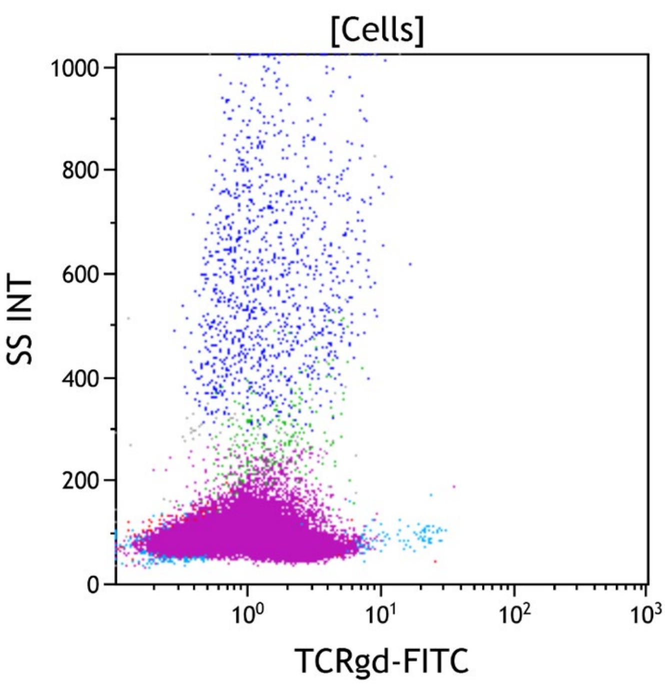


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). Progenitors (purple) do not express TCRγδ.

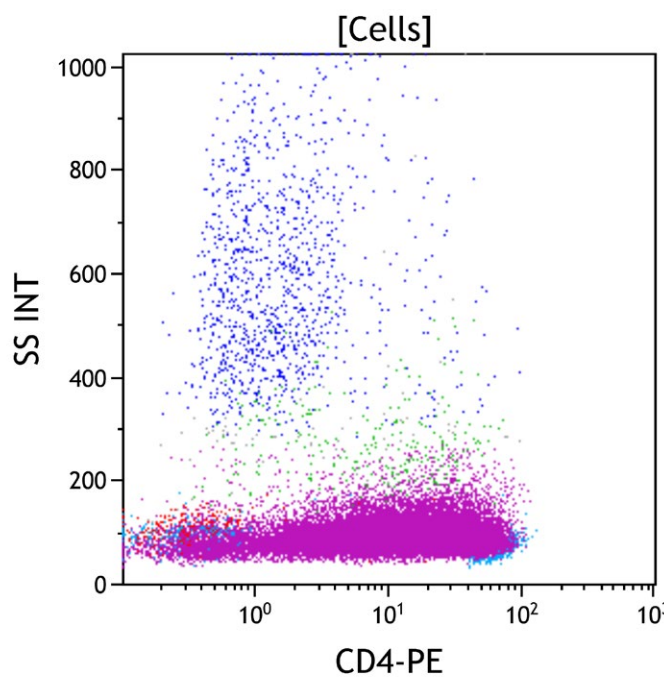


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. Progenitors (purple) express variable CD4.

T Cell Tube

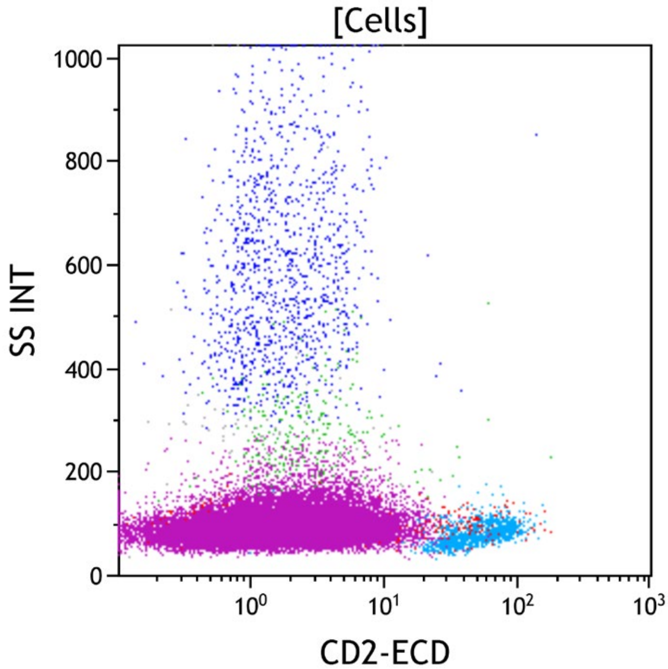


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). Progenitors (purple) express dim CD2.

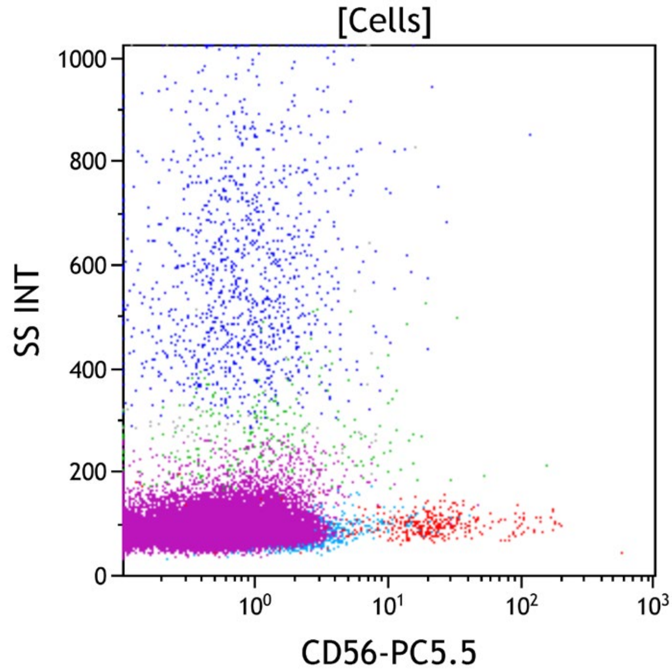


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells in both reactive and neoplastic conditions. Progenitors (purple) do not express CD56.

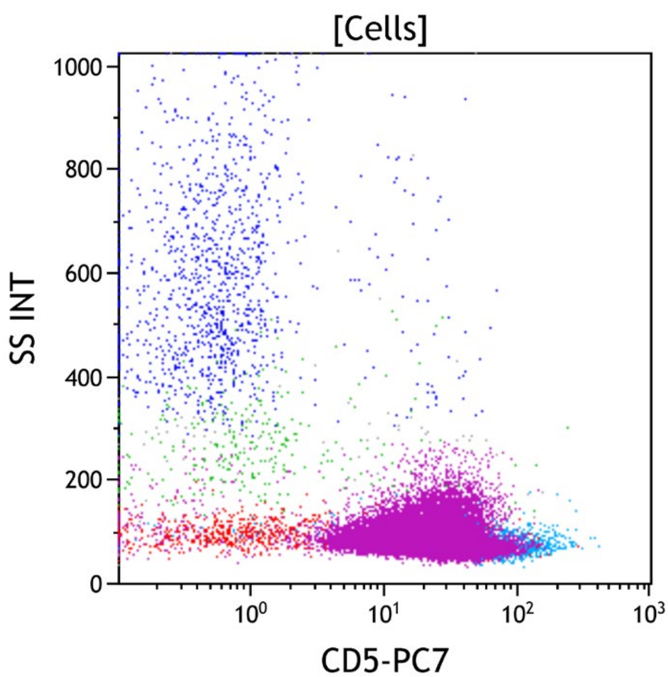


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Progenitors (purple) express CD5 at a level slightly lower than mature T cells (aqua).

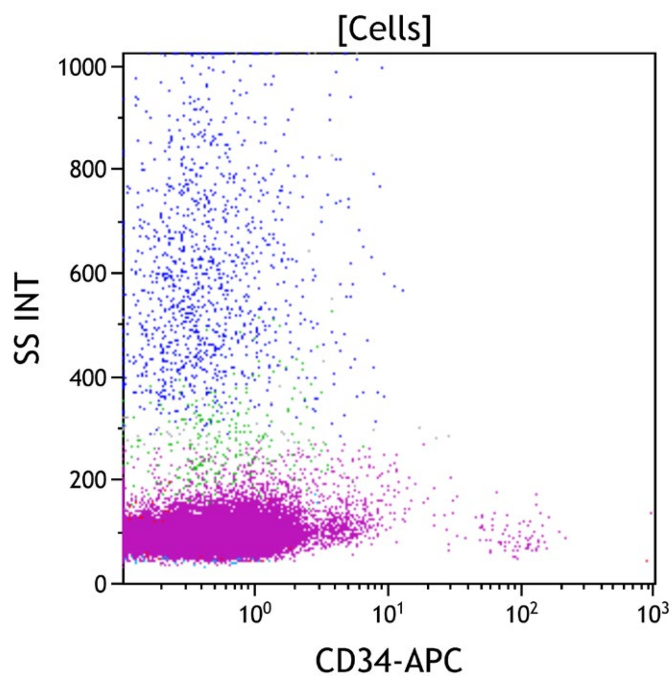


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Progenitors (purple) do not express CD34.

T Cell Tube

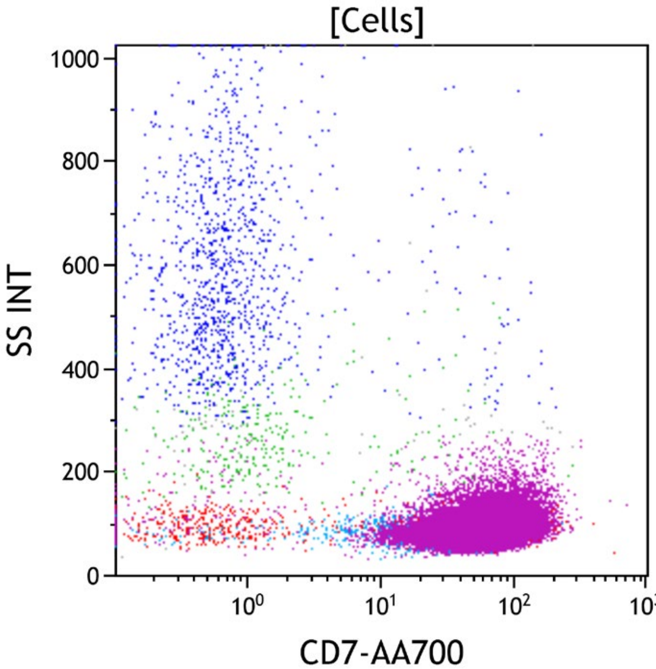


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. Progenitors (purple) express bright CD7.

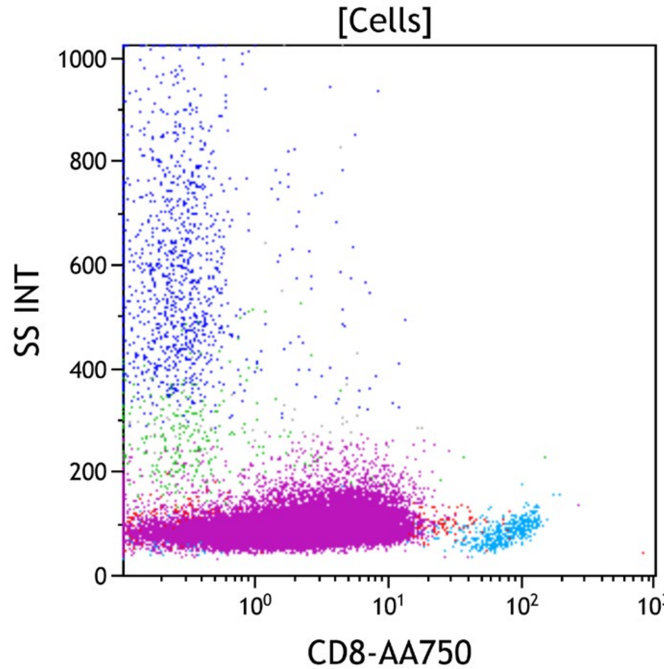


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. Progenitors (purple) express variable CD8.

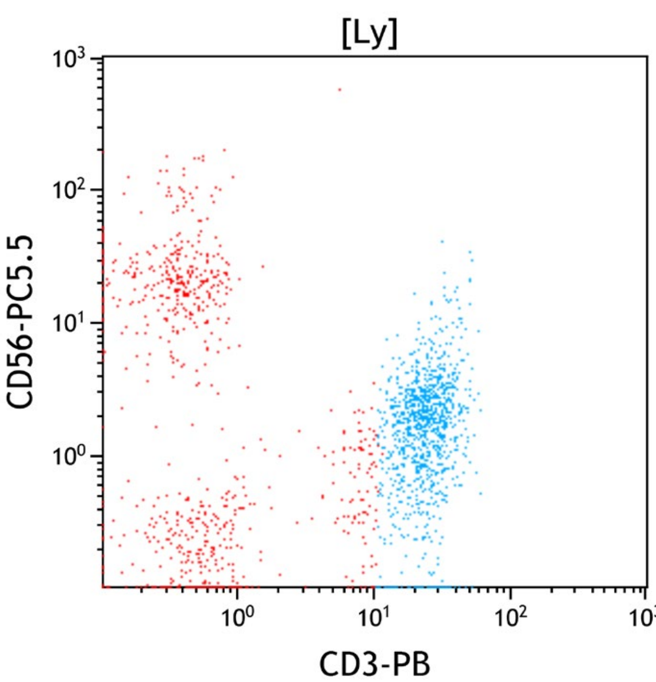


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

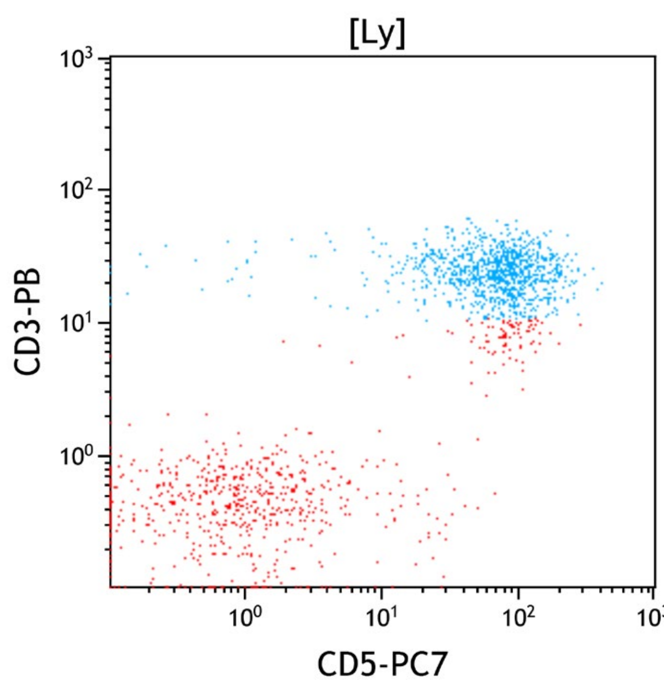


Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

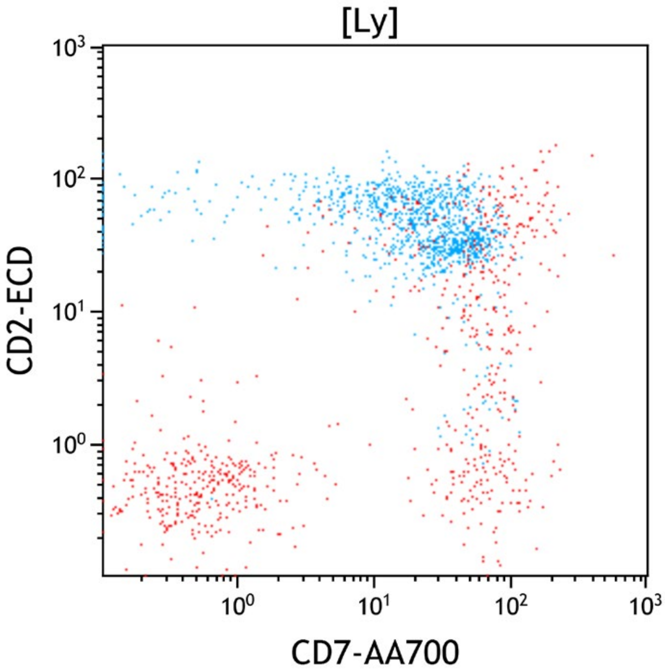


Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

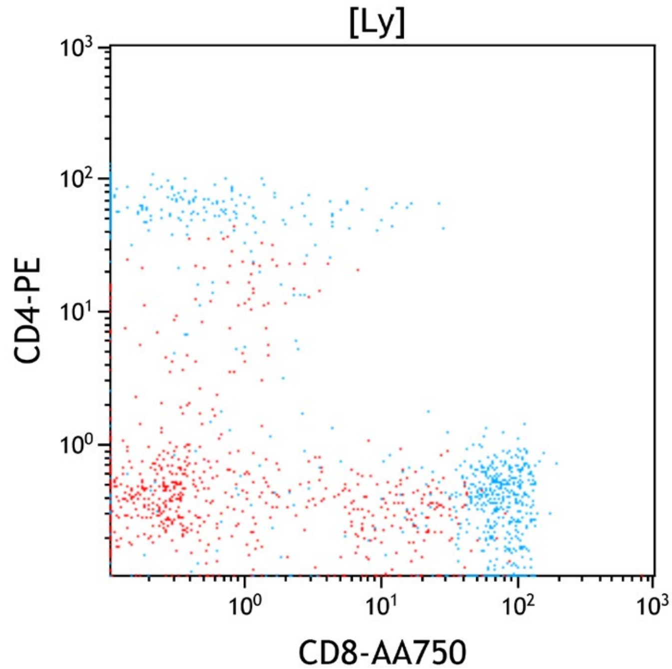


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

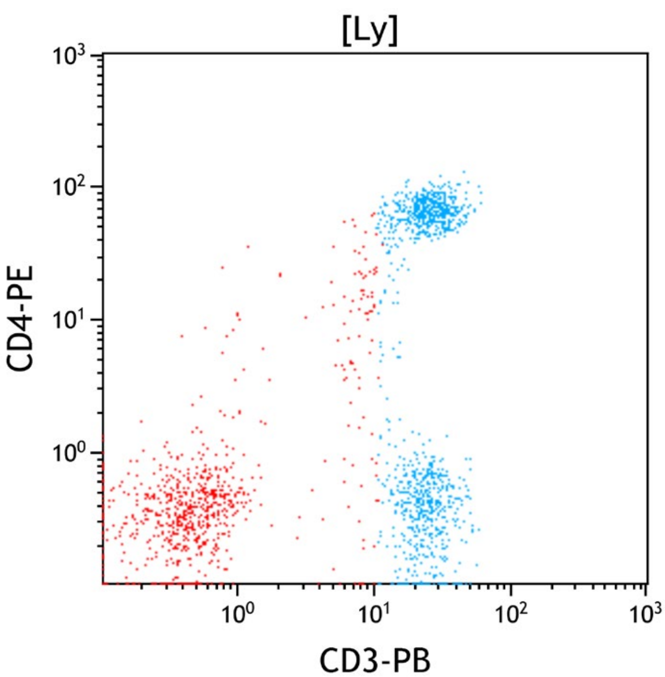


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

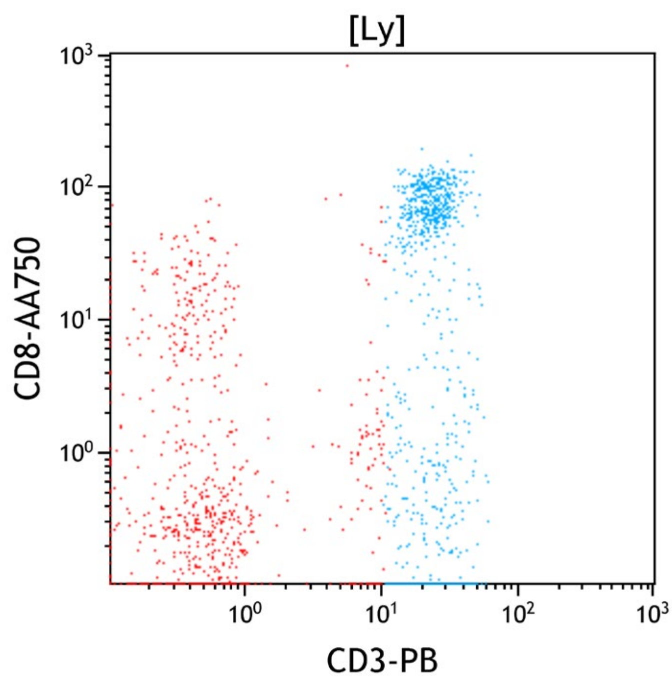


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 without CD3.

T Cell Tube

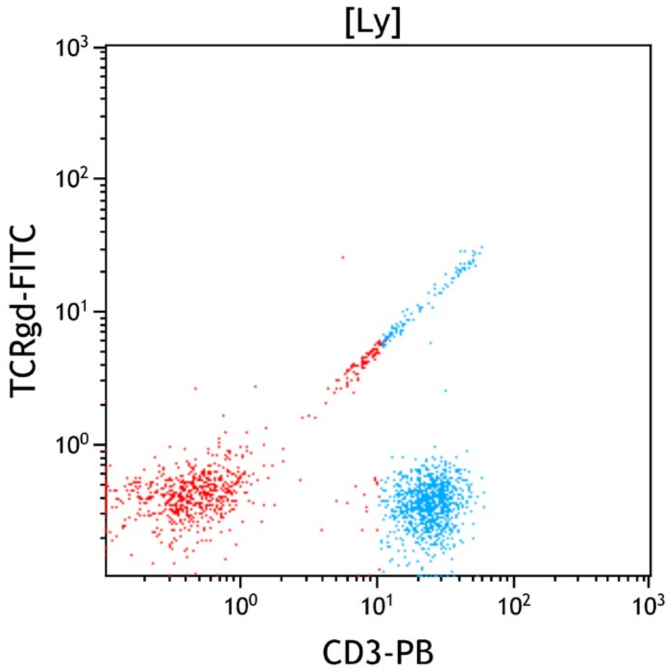


Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

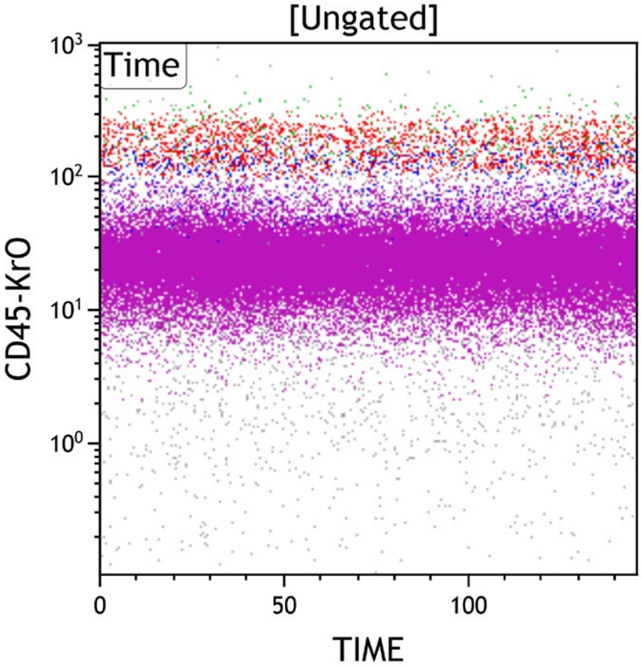


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

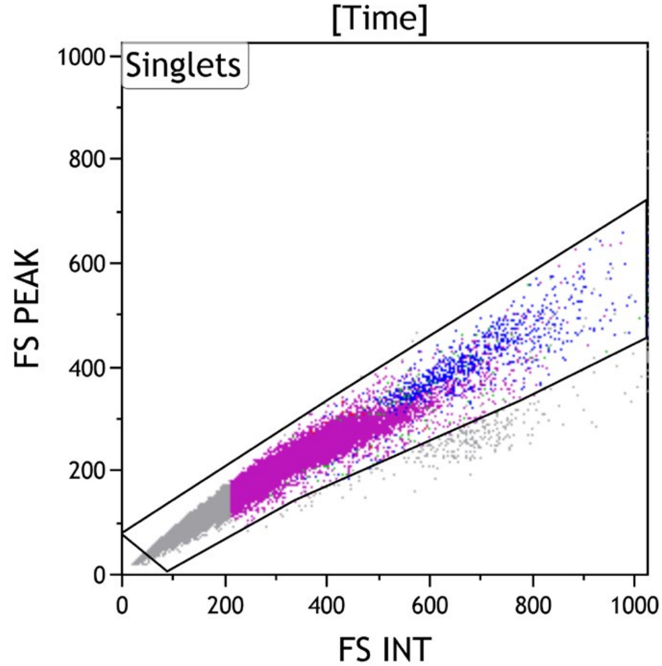


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

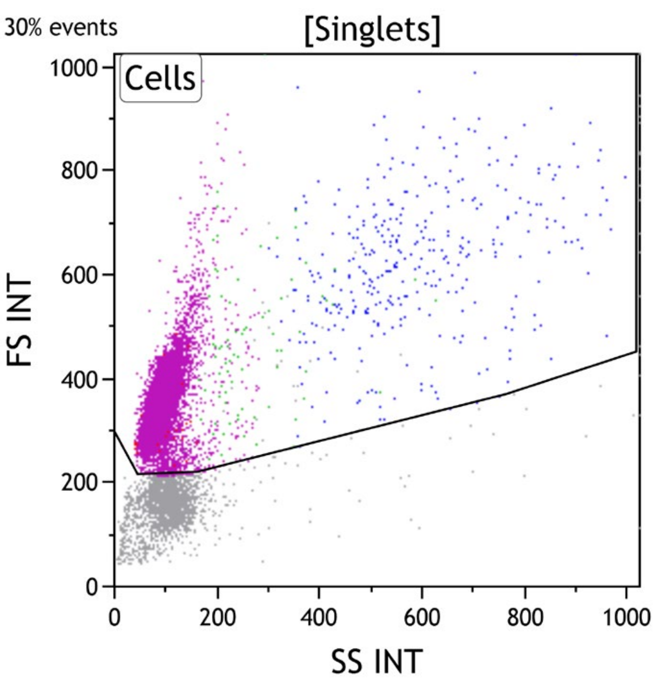


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

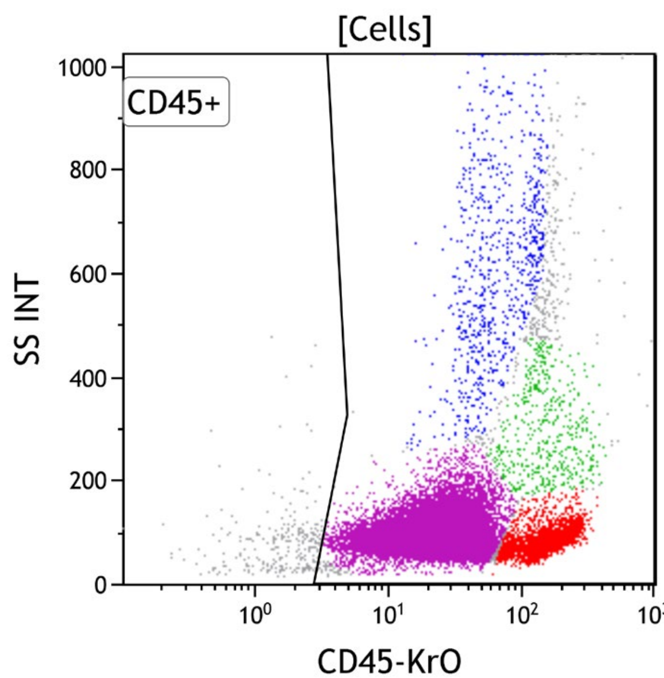


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

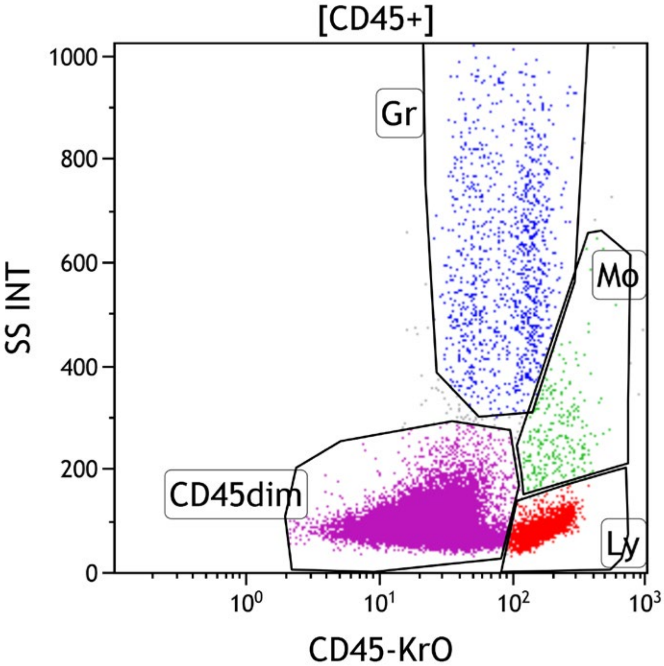


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The progenitor population (purple) is expanded.

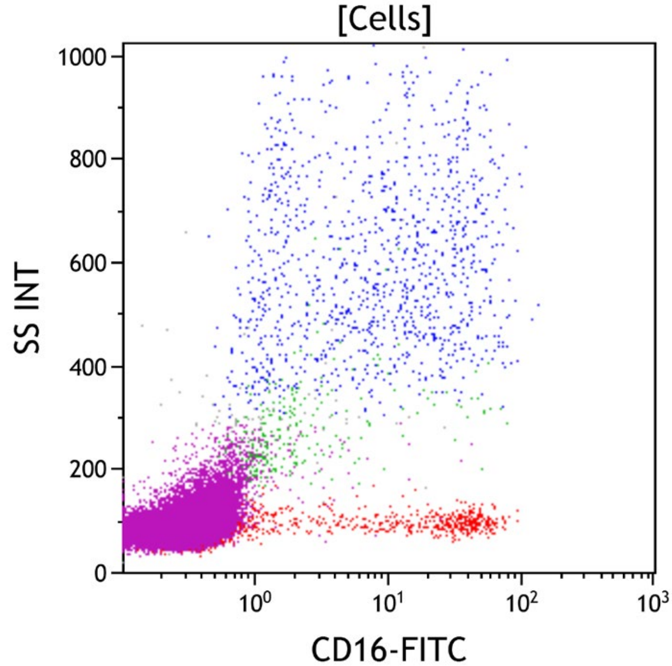


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). Progenitors (purple) do not express CD16.

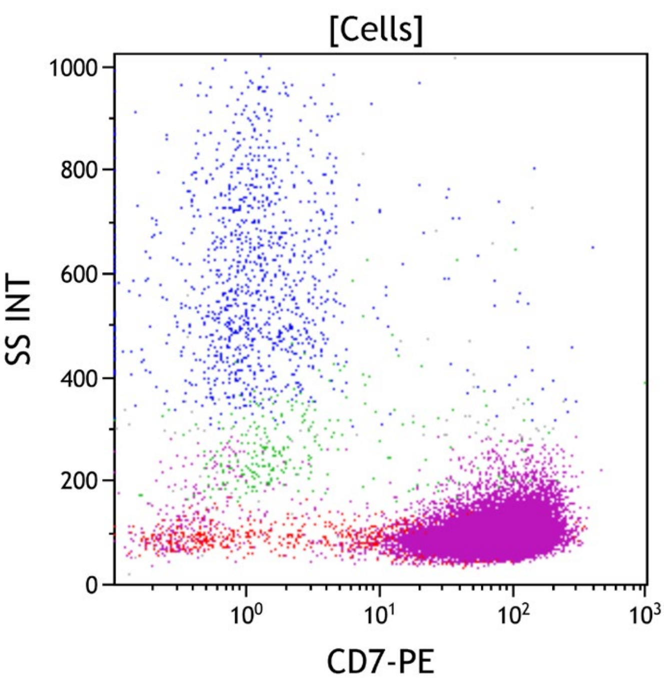


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells. It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. Progenitors (purple) express bright CD7.

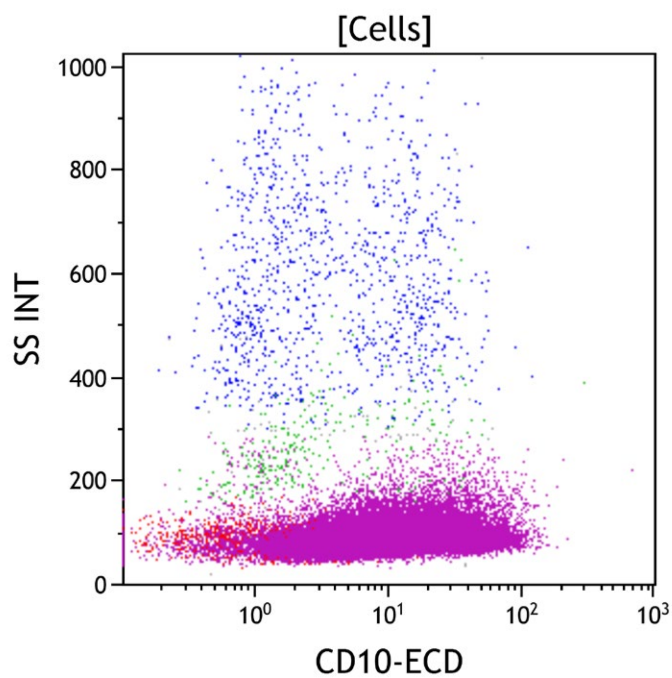


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Progenitors (purple) express variable CD10.

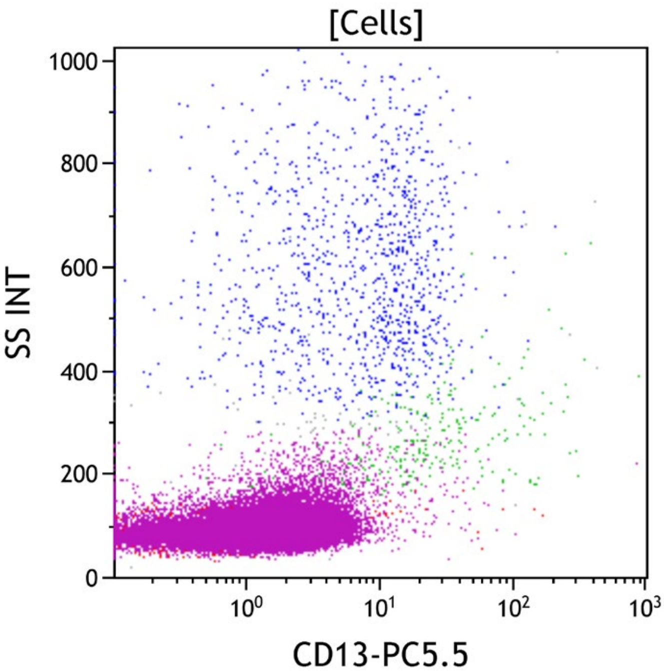


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. Progenitors (purple) do not express CD13, the apparent low level positivity is due to increased compensation background from the bright CD7 and variable CD10.

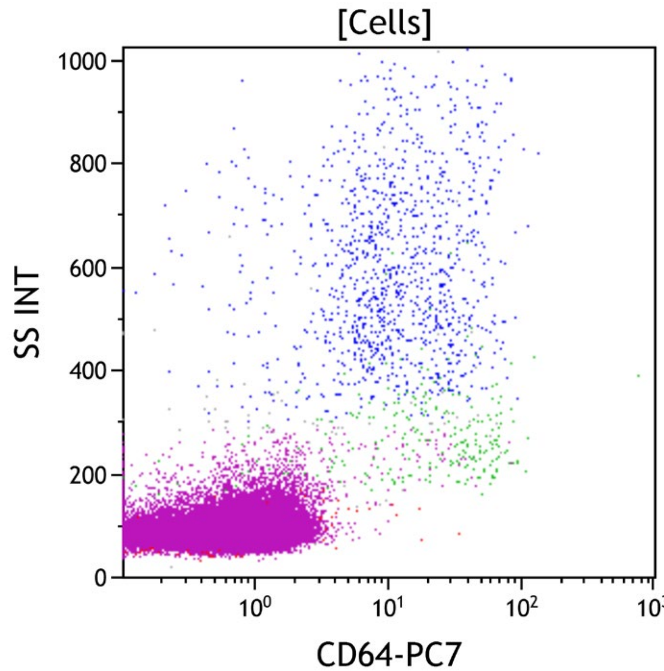


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. Progenitors (purple) do not express CD64.

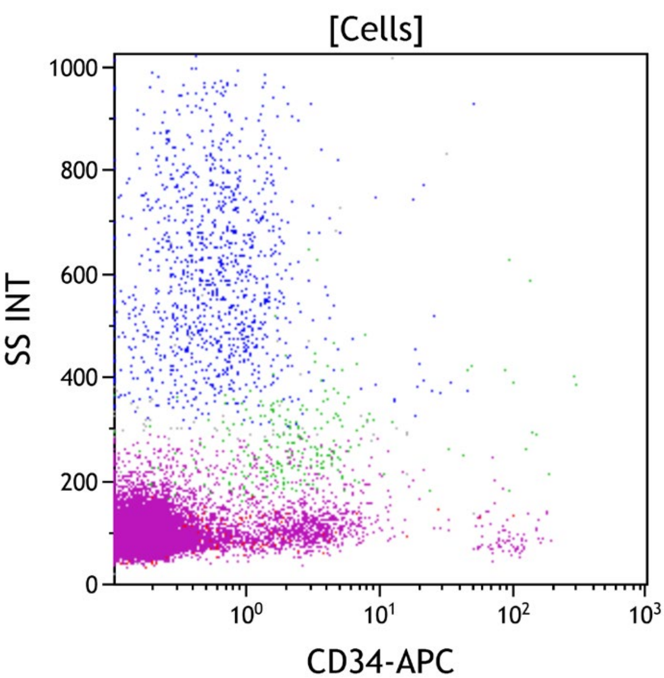


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. Progenitors (purple) do not express significant CD34.

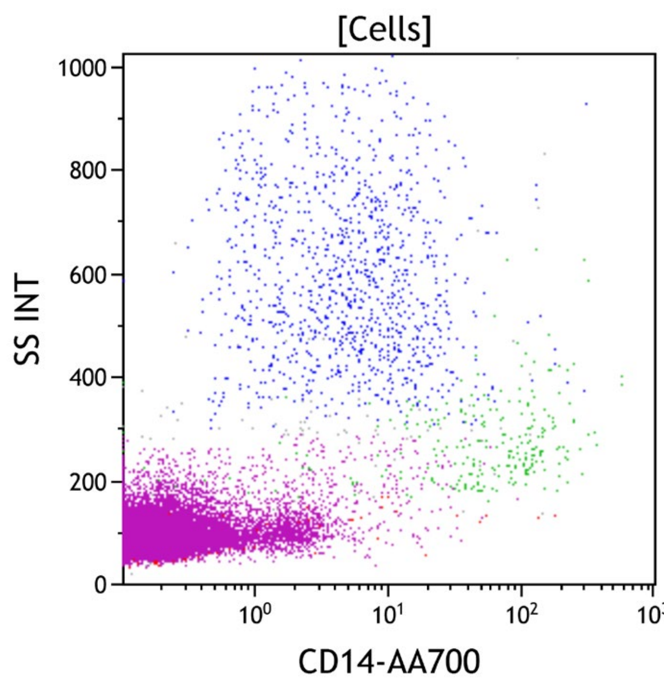


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Progenitors (purple) do not express CD14.

Table of Contents > Neoplastic Process of T-cell Origin > Case #15: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

M1 Cell Tube

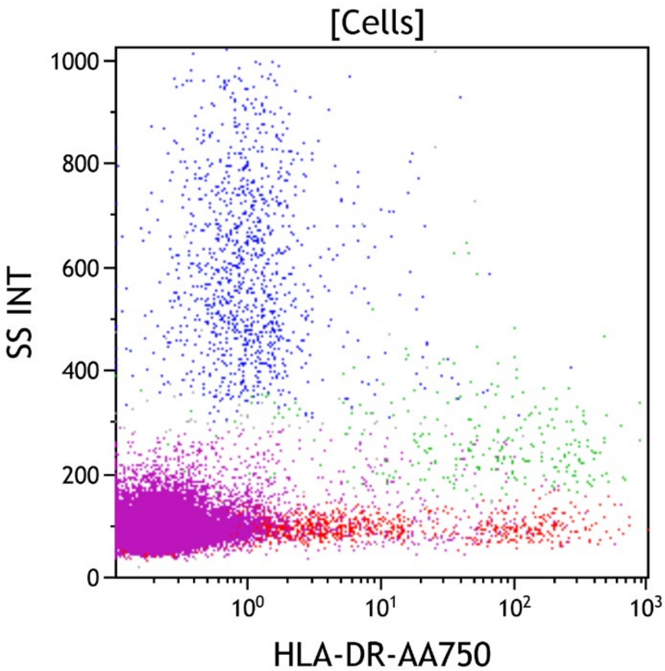


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. Progenitors (purple) do not express HLA-DR.

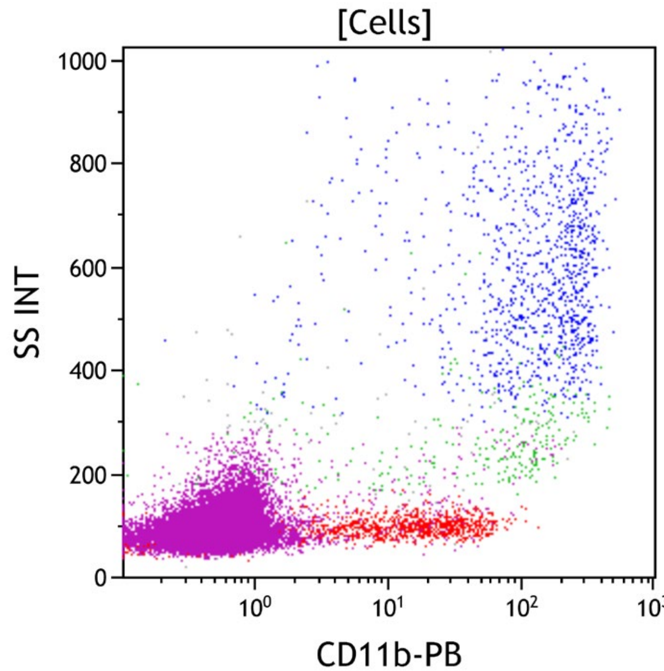


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. Progenitors (purple) do not express CD11b.

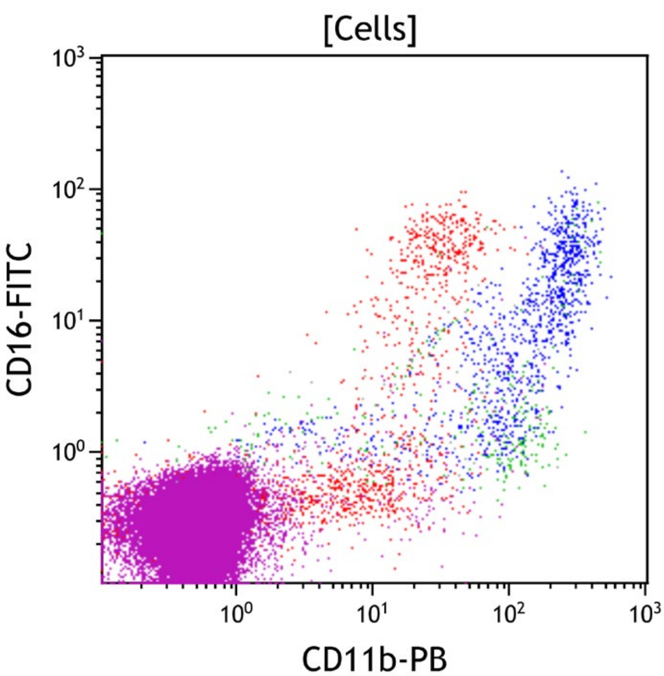


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. Progenitors (purple) do not express CD11b or CD16.

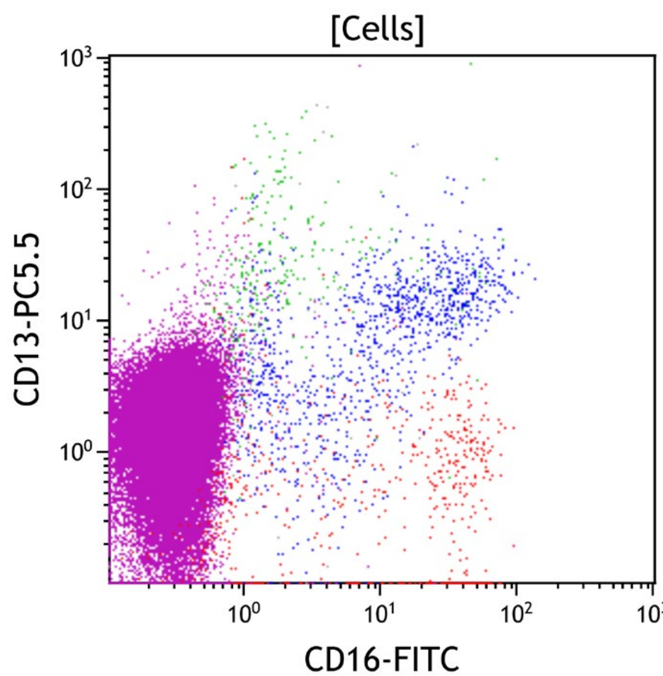


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue top right). Progenitors (purple) do not express CD16 or CD13.

Table of Contents > Neoplastic Process of T-cell Origin > Case #15: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

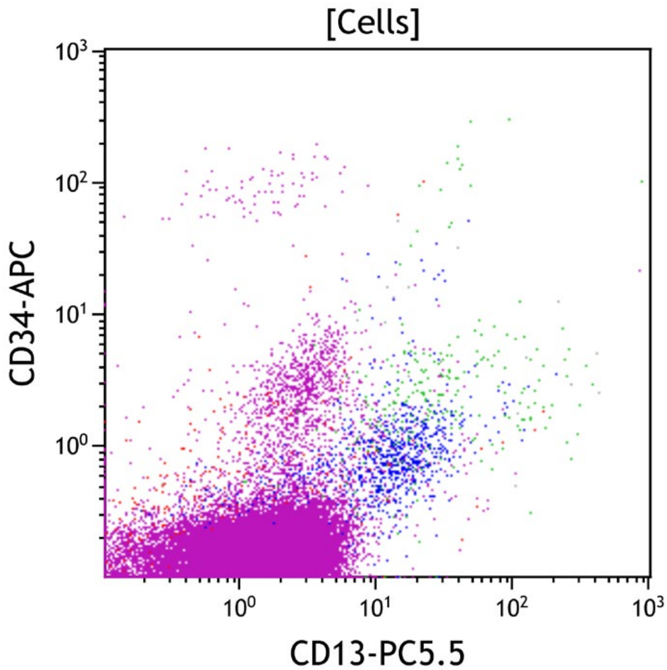


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells. Progenitors (purple) do not express CD34.

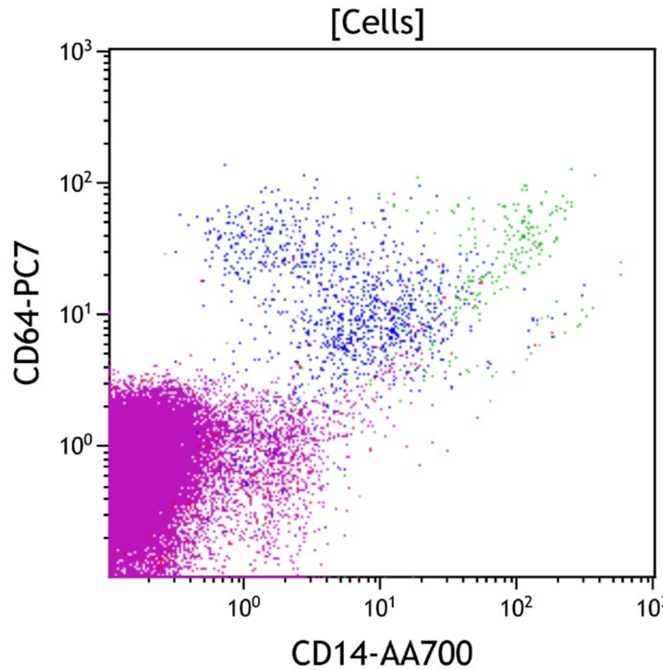


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64 (green). Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. Progenitors (purple) do not express CD14 or CD64.

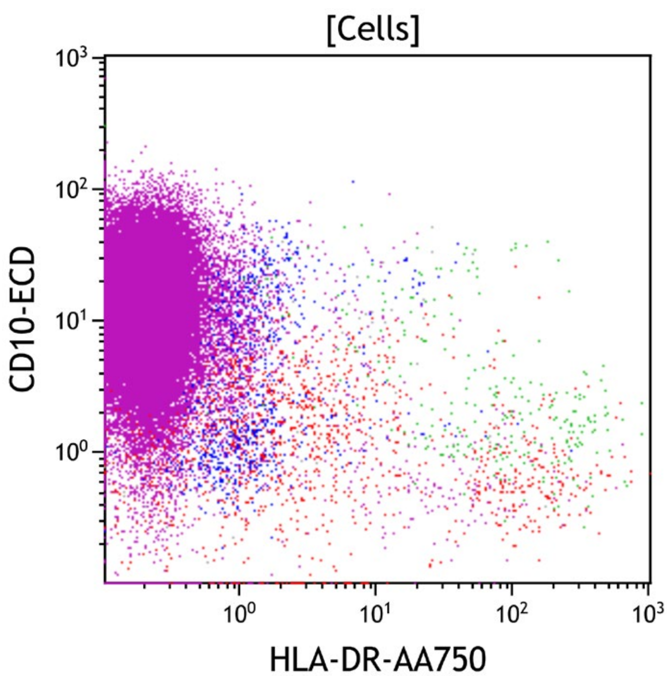


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes and immature B cells. Immature B cells express both CD10 and HLA-DR. Progenitors (purple) express CD10 but not HLA-DR.

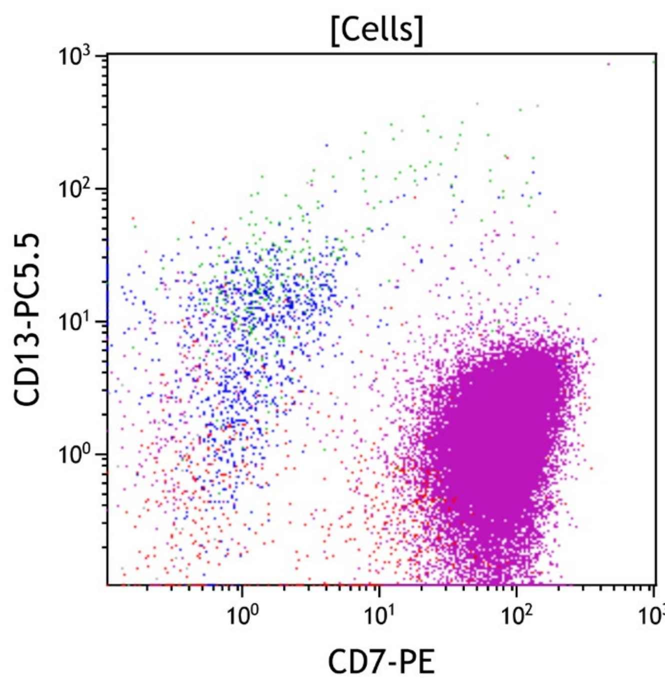


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. Progenitors (purple) express bright CD7 but not CD13.

M2 Cell Tube

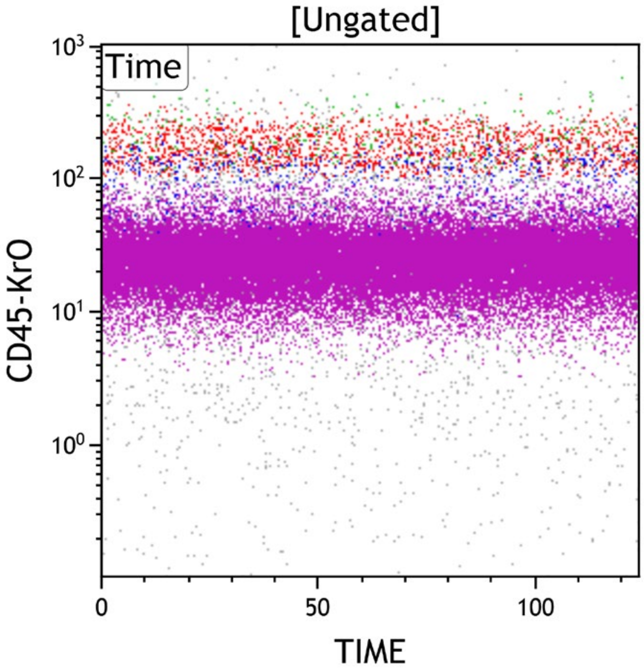
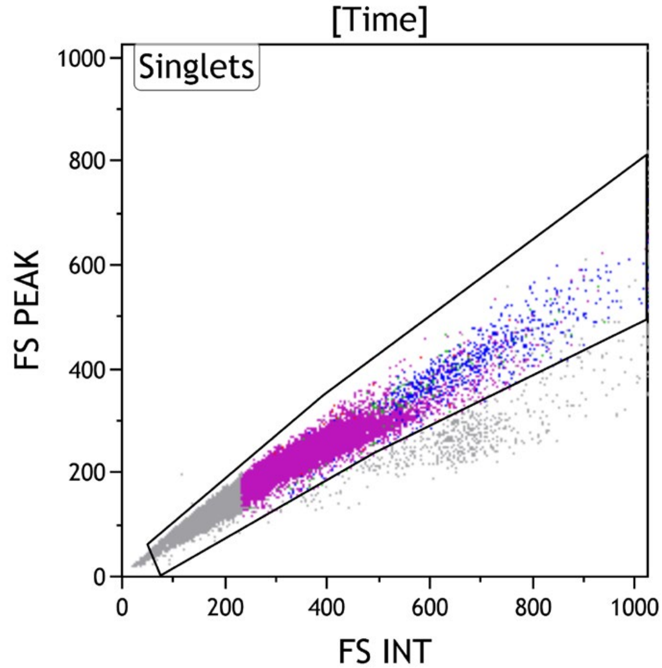


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.



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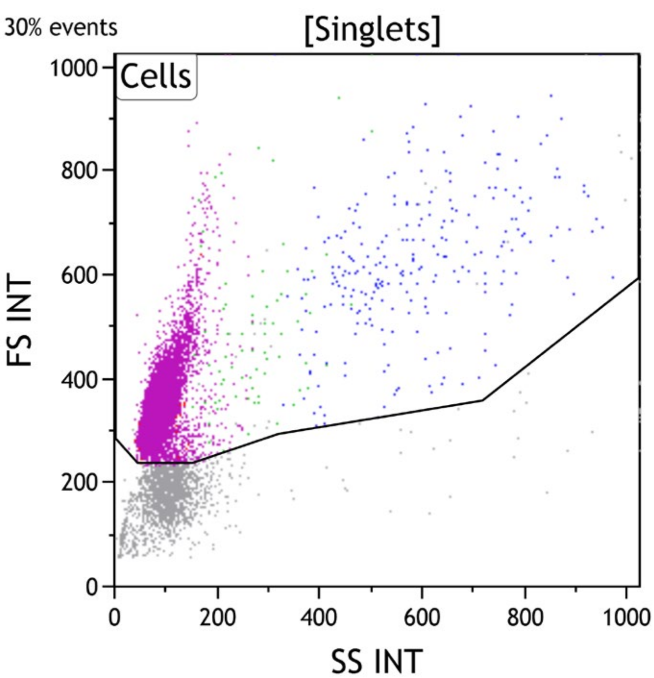

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

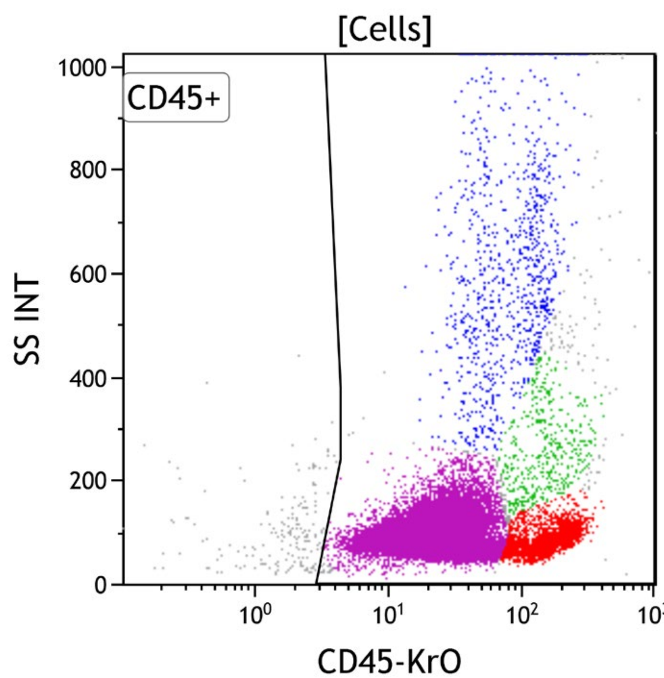

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

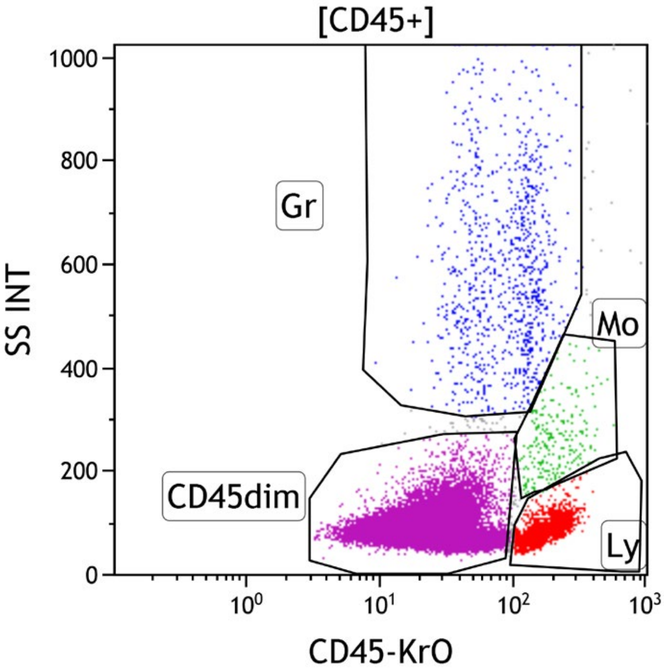

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. The progenitor population (purple) is expanded.

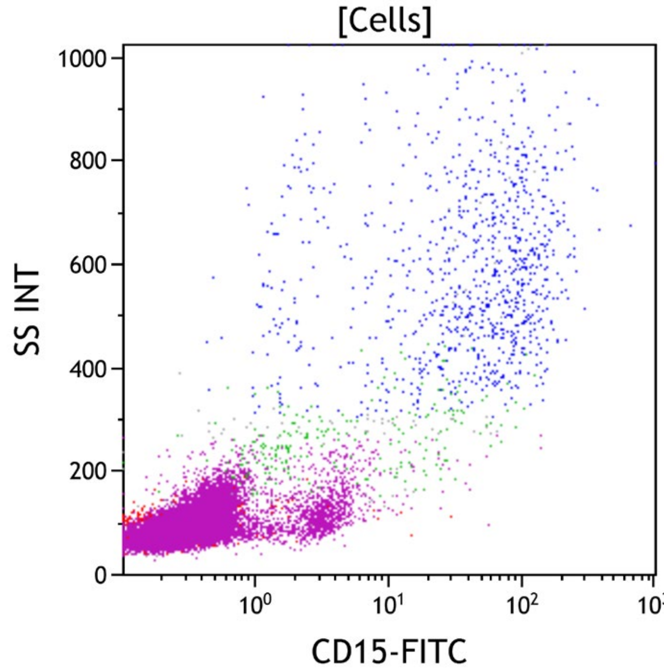

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). Progenitors (purple) do not express CD15.

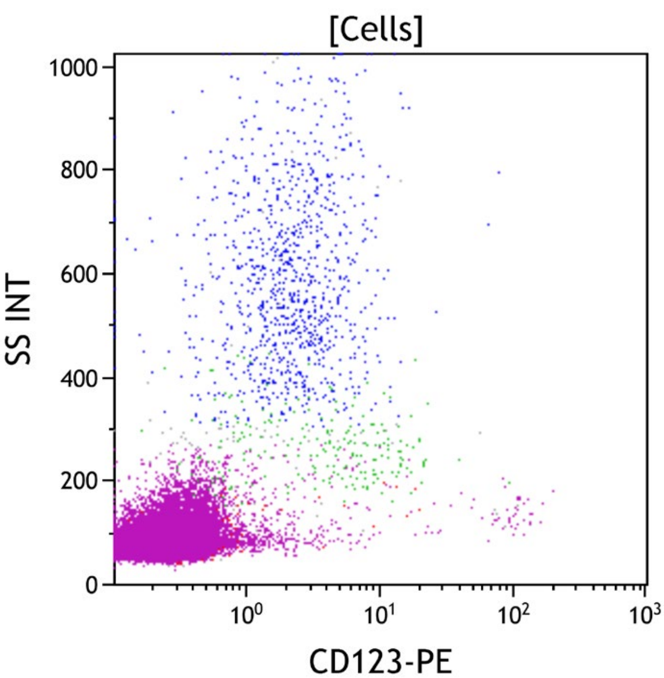

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). Progenitors (purple) do not express CD123.

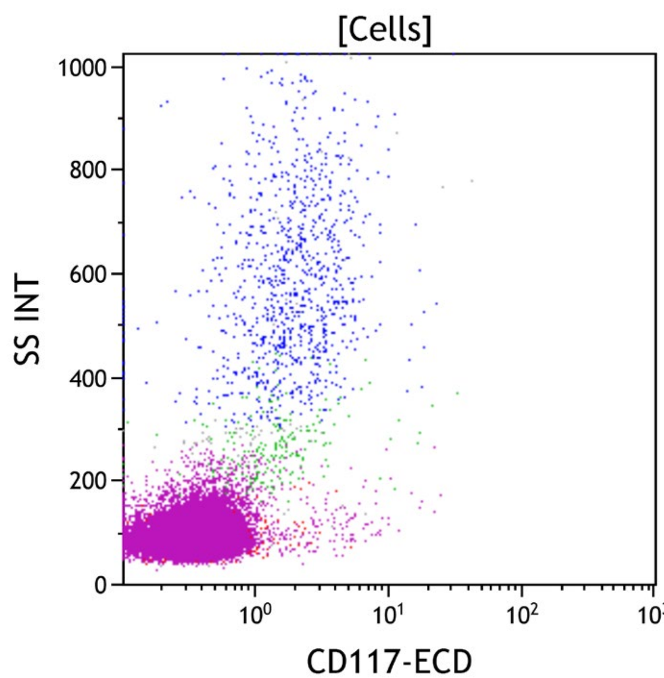

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Progenitors (purple) do not express CD117.

M2 Cell Tube

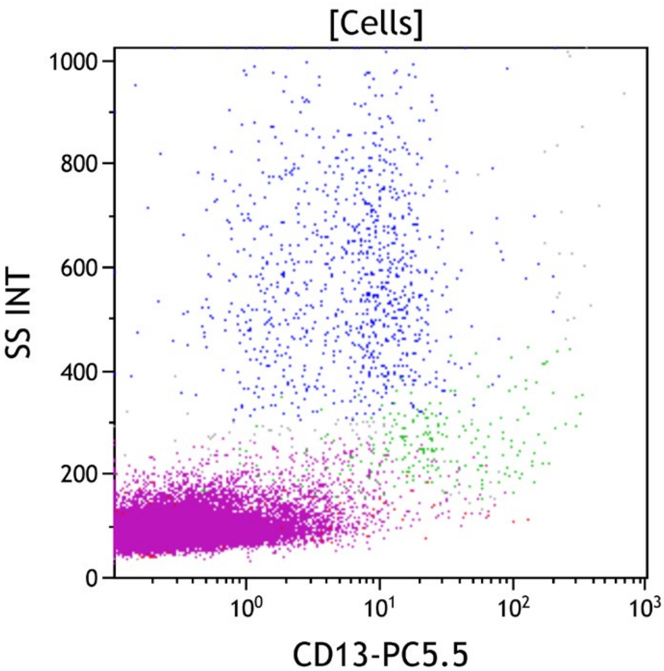

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. Progenitors (purple) do not express CD13.

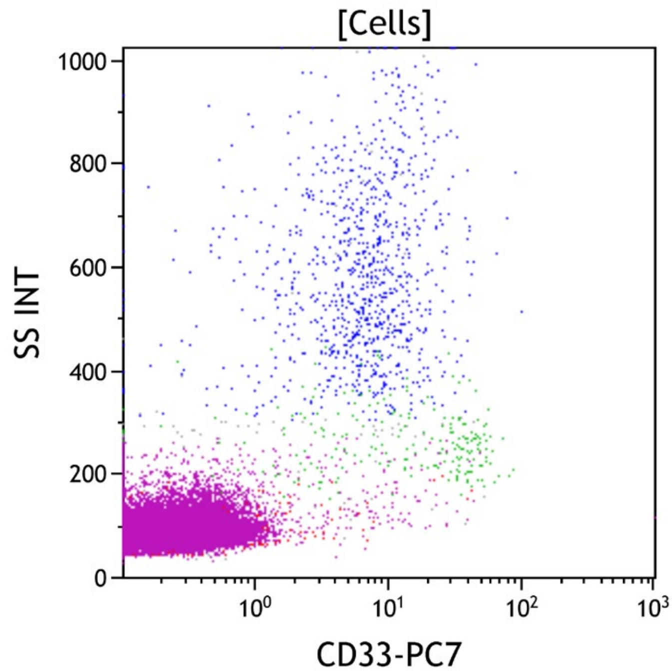

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. Progenitors (purple) do not express CD33.

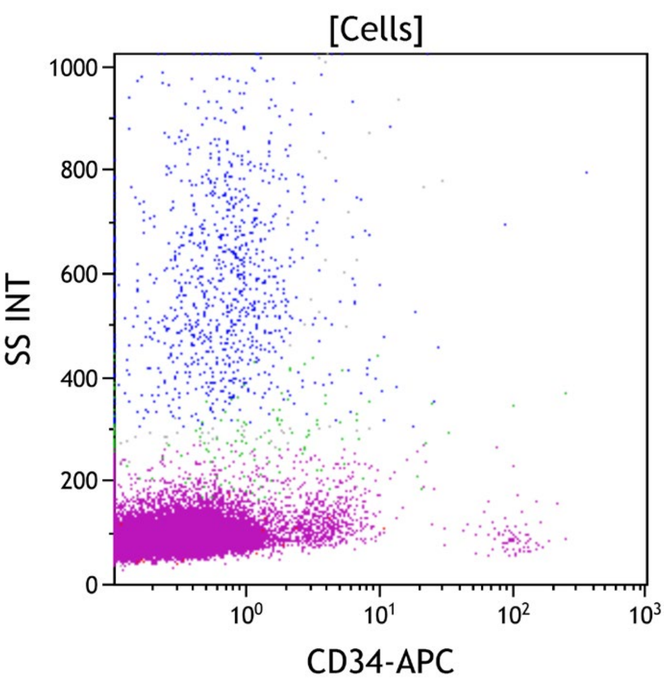

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate .Progenitors (purple) do not express CD34.

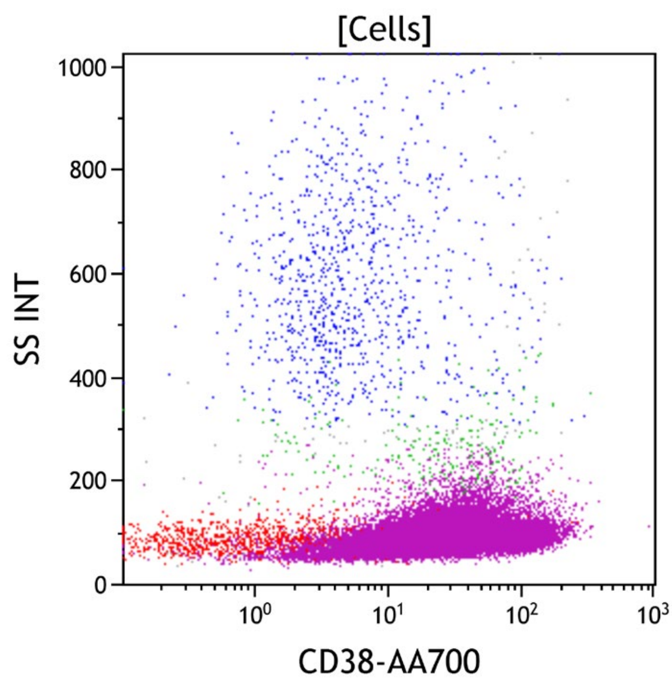

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Progenitors (purple) express bright CD38.

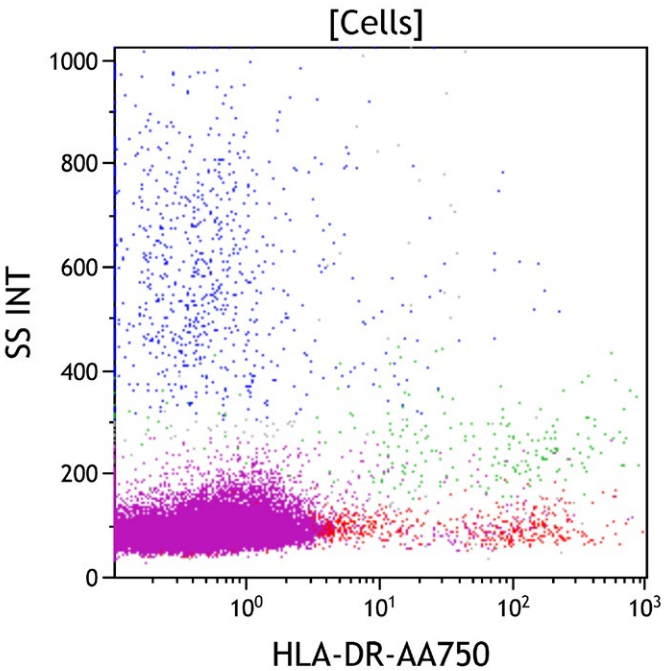

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). Progenitors (purple) do not express HLA-DR.

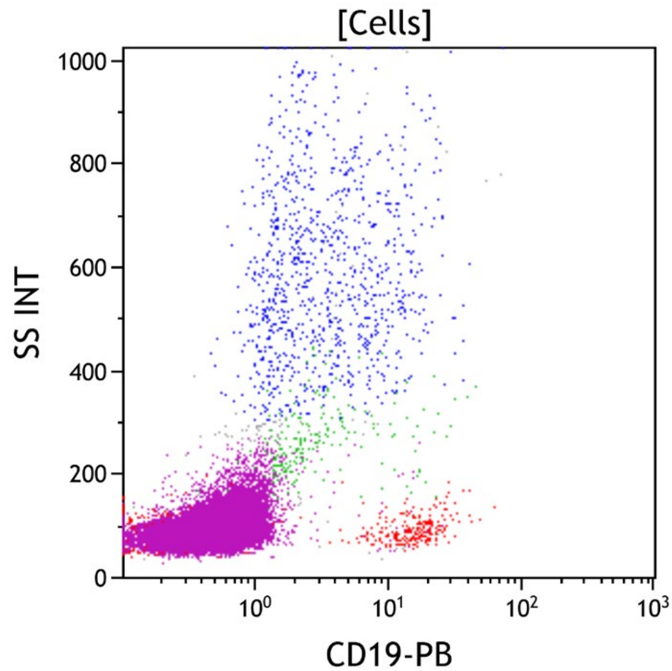

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express CD19.

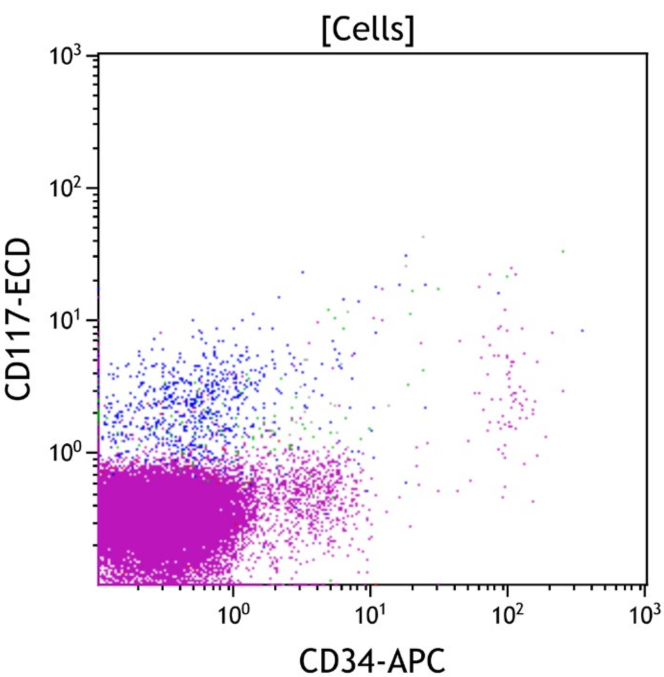

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts (upper right in purple), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (lower right in purple). Progenitors (purple) do not express CD34 or CD117.

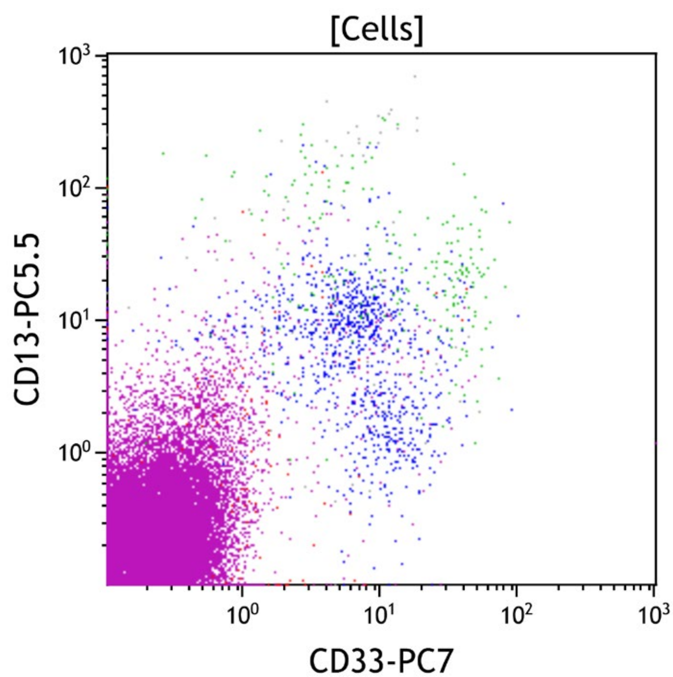

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33. Progenitors (purple) do not express CD13 or CD33.

M2 Cell Tube

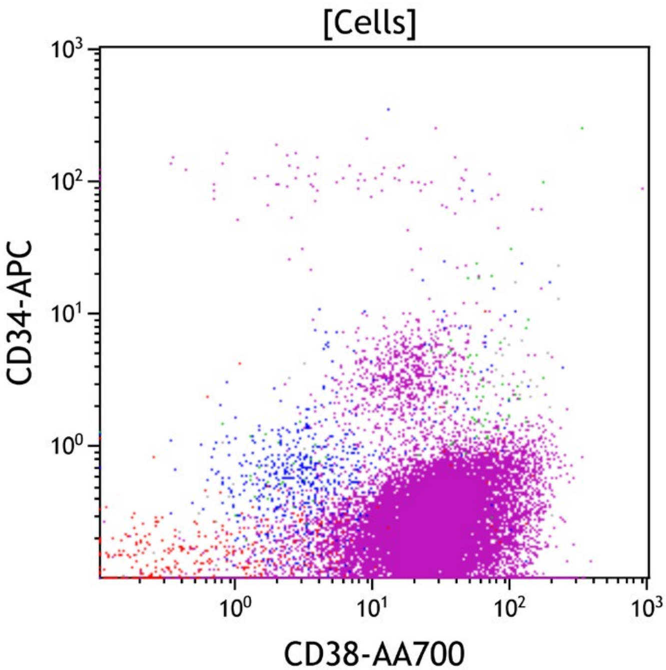

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38. Progenitors (purple) express bright CD38 without CD34.

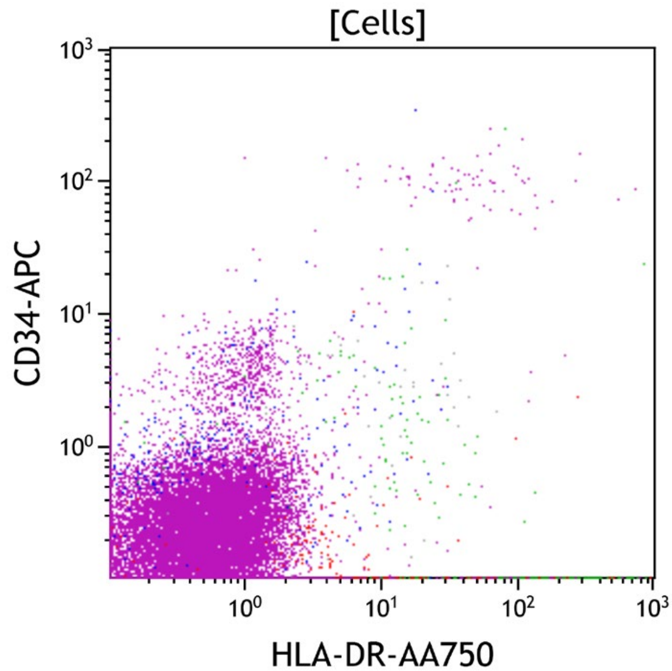

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. Progenitors (purple) do not express HLA-DR or CD34.

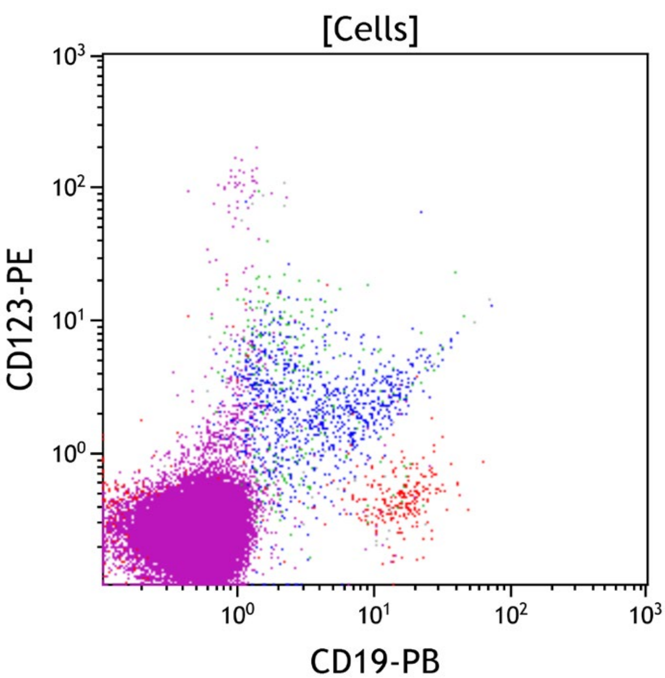

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) do not express CD19 or CD123.

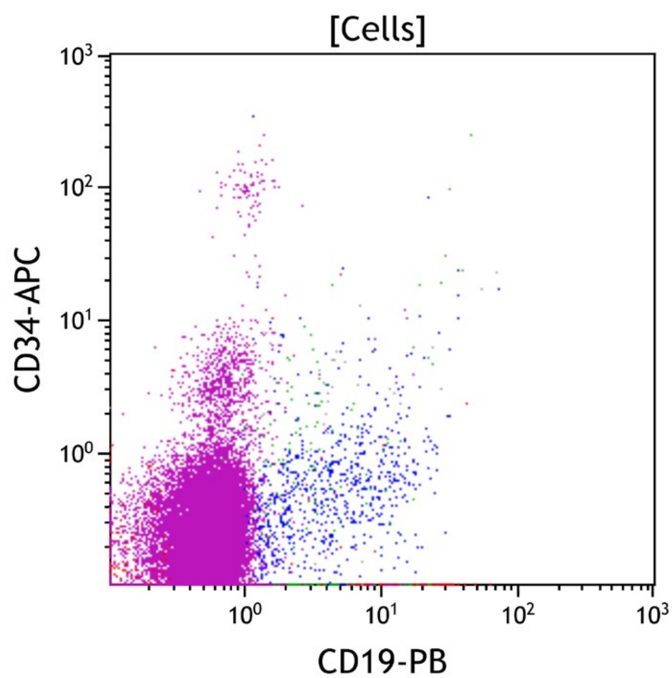

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. Progenitors (purple) do not express CD19 or CD34.

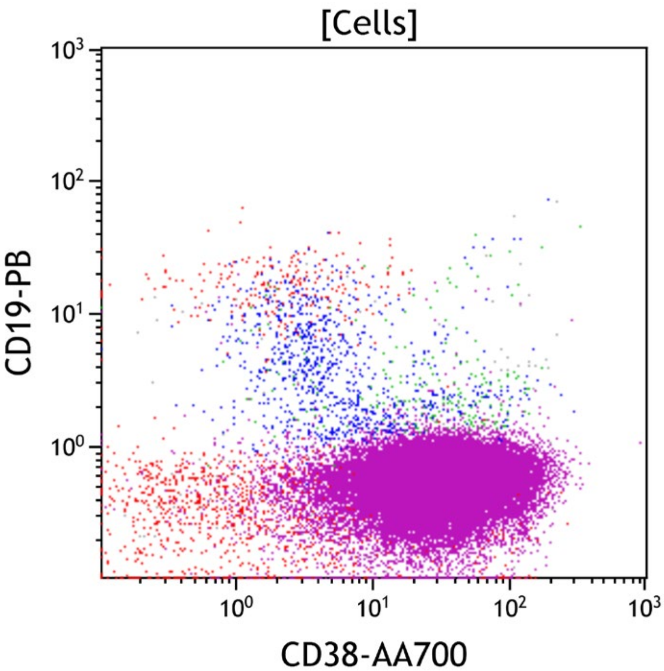

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38 (red left). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) express bright CD38 without CD19.

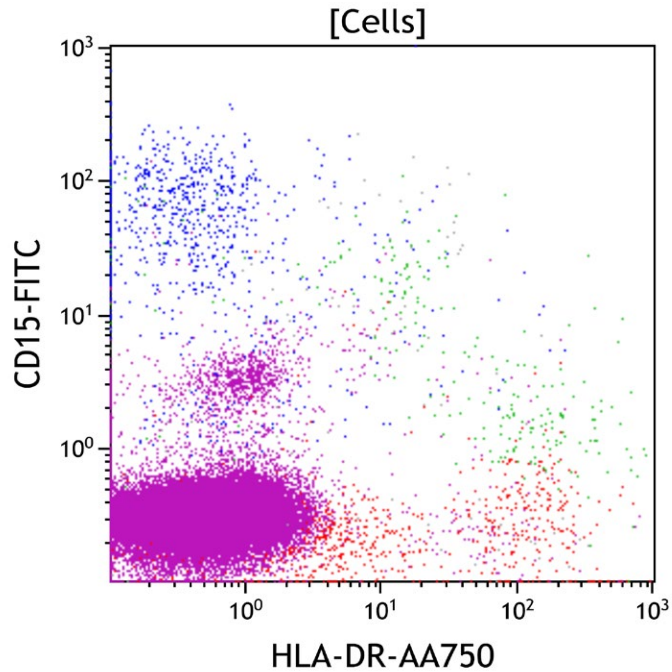

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. Progenitors (purple) do not express HLA-DR or CD15.

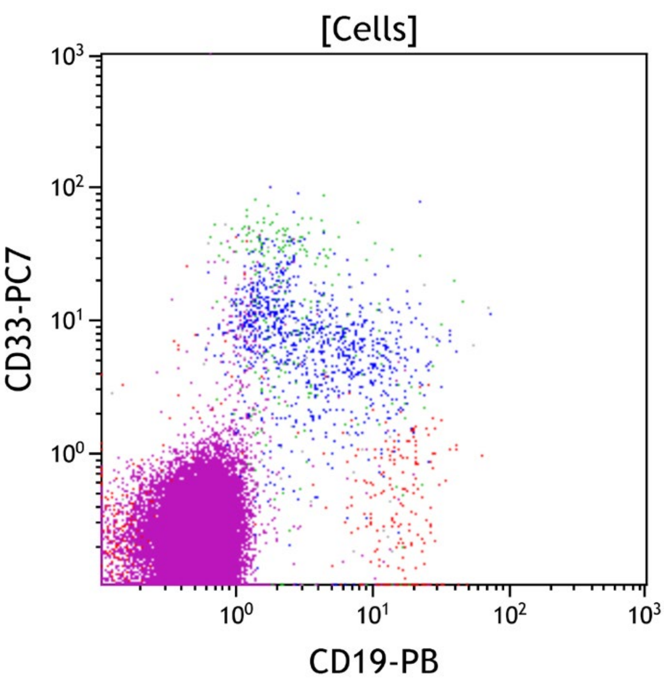

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) do not express CD19 or CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of dim CD2, dim CD4, intermediate CD5, bright CD7, variable CD10, bright CD38 and dim CD45 without expression of CD3, CD34, CD56, CD117, HLA-DR or other B cell or myeloid antigens. Compared with normal immature T cells, the decreased CD2 and dim CD45 are aberrant.

The immunophenotype of the population is in keeping with abnormal immature T cells, i.e. T-lymphoblasts. The finding supports a diagnosis of T –lymphoblastic leukemia/lymphoma. Additional Karyotype result shows a t(4;11) and molecular genetic testing a mutation of NOTCH1, these findings are seen in a subset of T lymphoblastic leukemia/lymphoma. Additional testing for cytoplasmic CD3 and TdT could be performed to confirm T cell lineage and immaturity, respectively.

Case #16: T–Cell Lymphoproliferative Disorder

Clinical Vignette

This 66-year-old female presents with atypical cells on peripheral blood smear. A peripheral whole blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

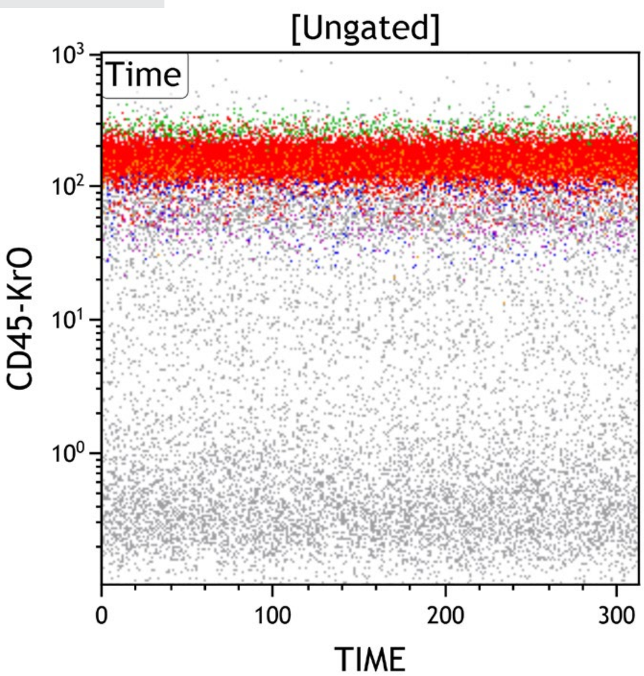

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

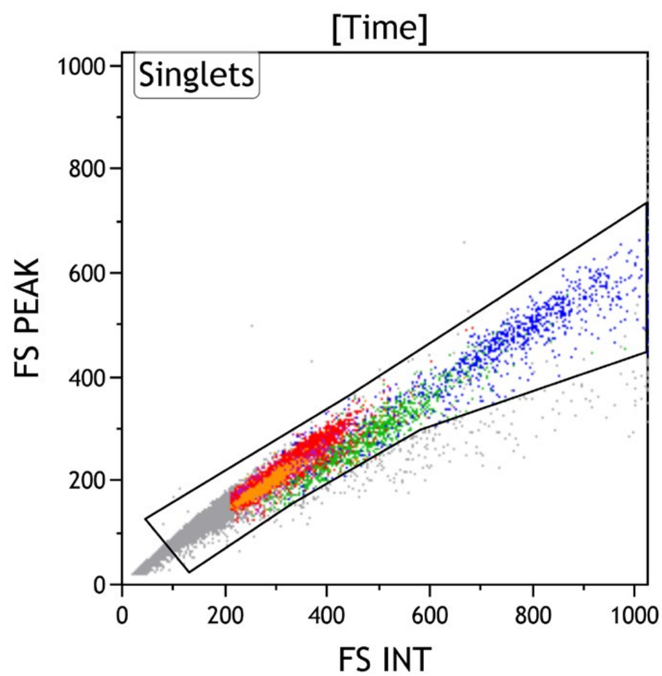

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

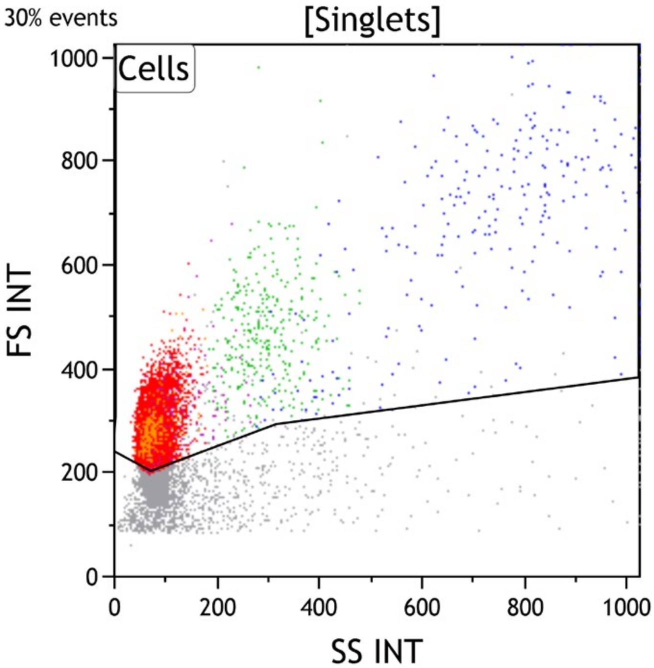

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

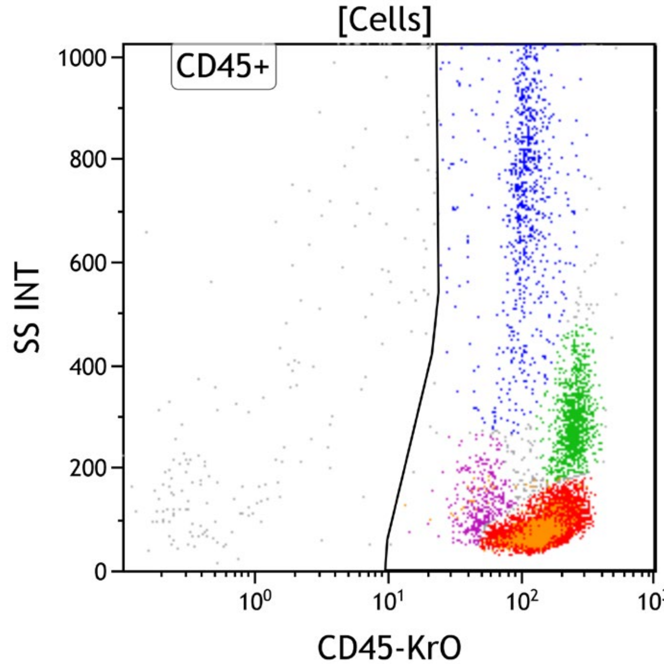

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

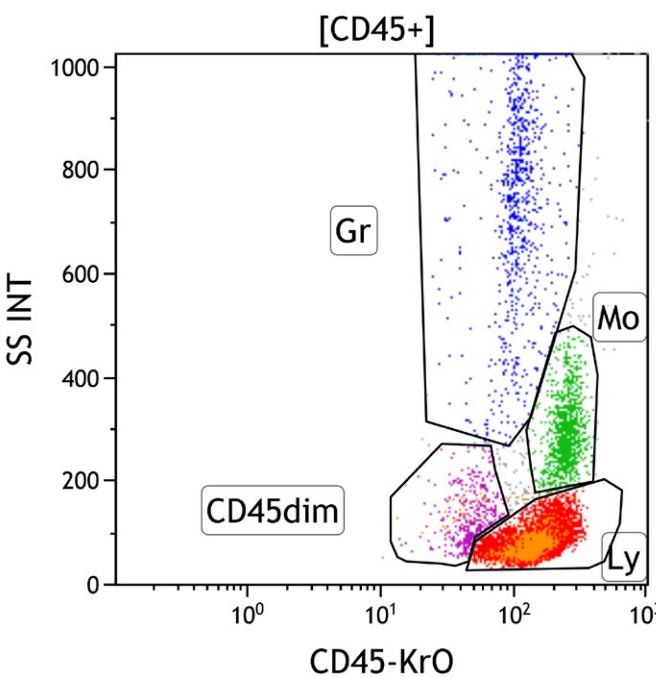

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note the relatively increased number of lymphocytes (red).

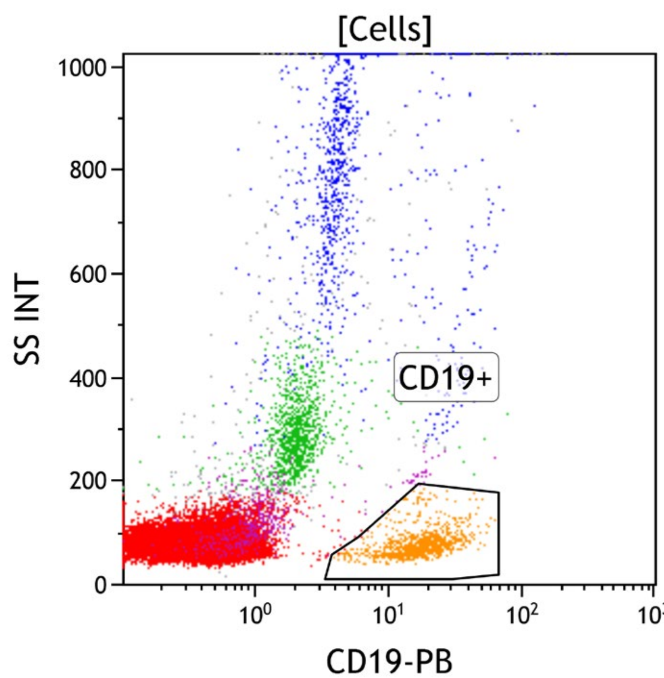

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Most lymphocytes (red) do not express CD19.

B Cell Tube

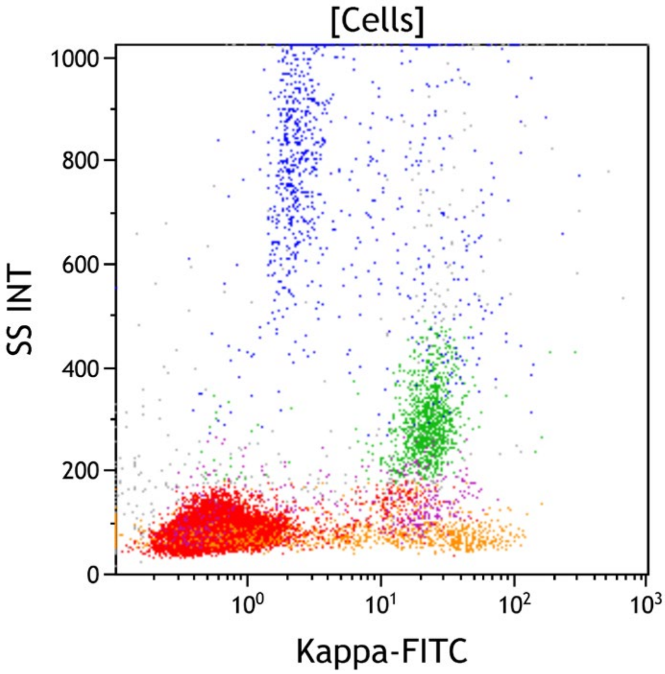

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. Most lymphocytes (red) do not express kappa.

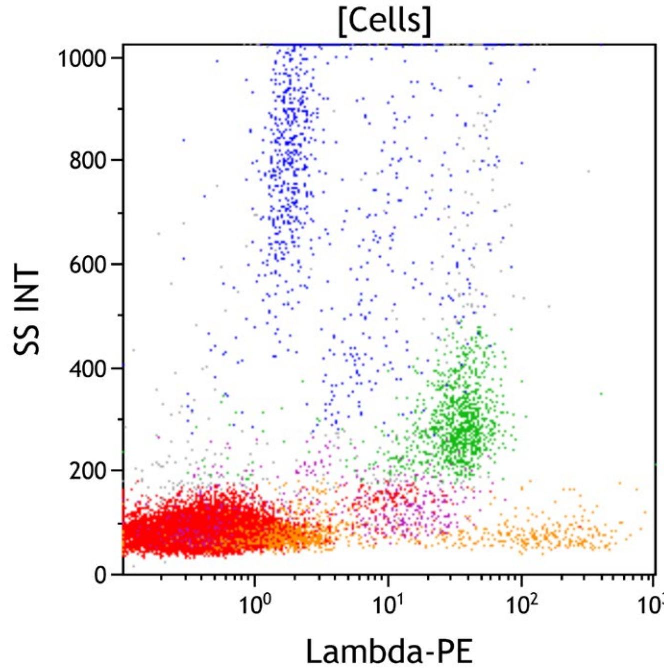

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. Most lymphocytes do not express lambda.

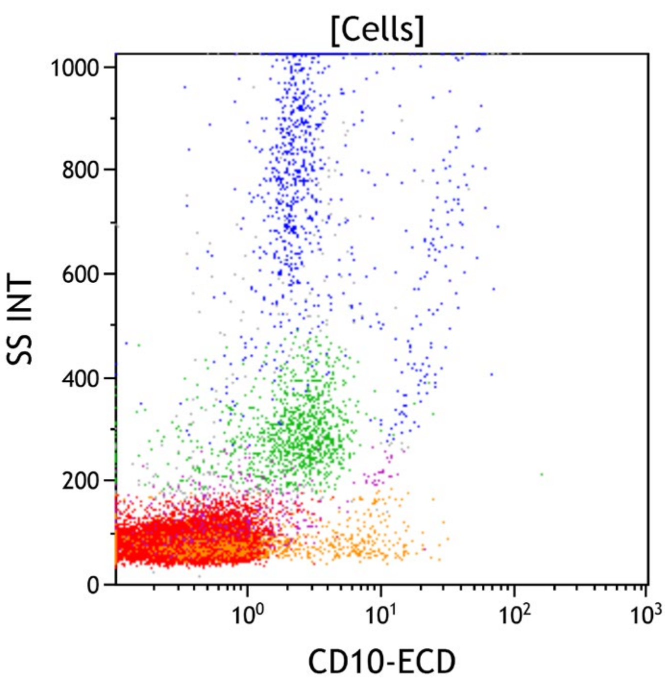

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Most lymphocytes (red) do not express CD10.

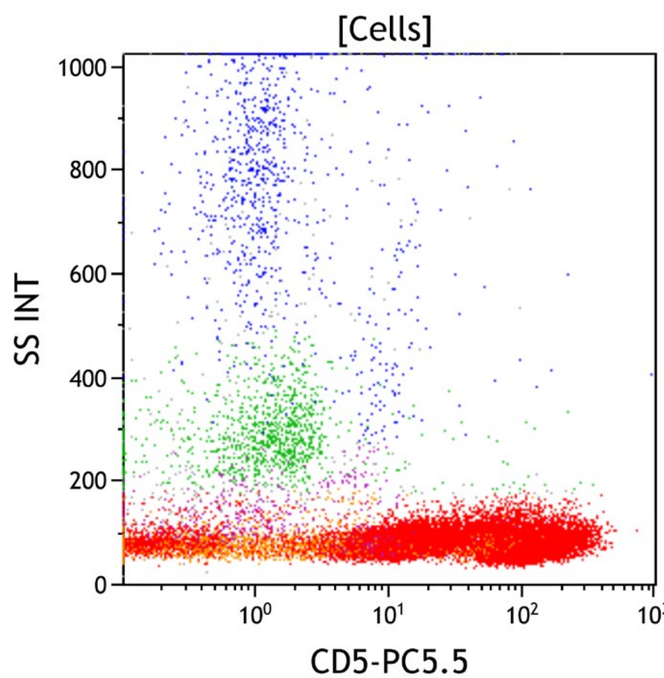

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. Most lymphocytes (red) variably express CD5.

B Cell Tube

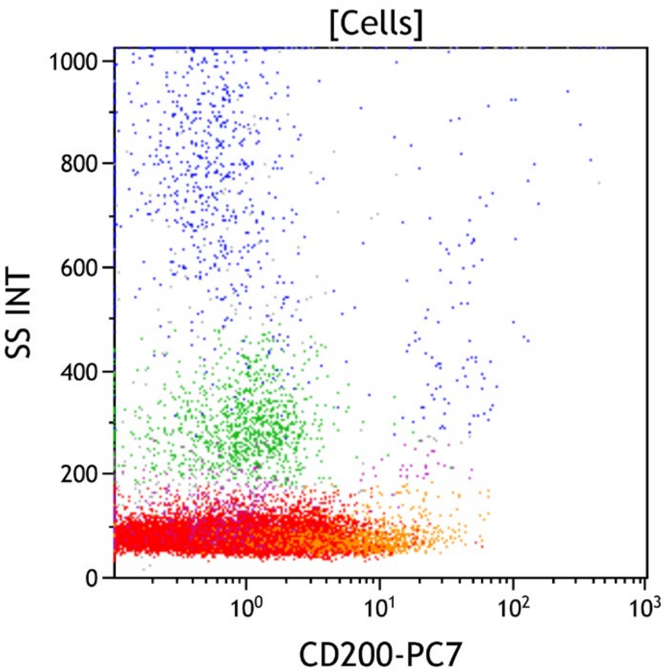

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). Most lymphocytes (red) do not express CD200.

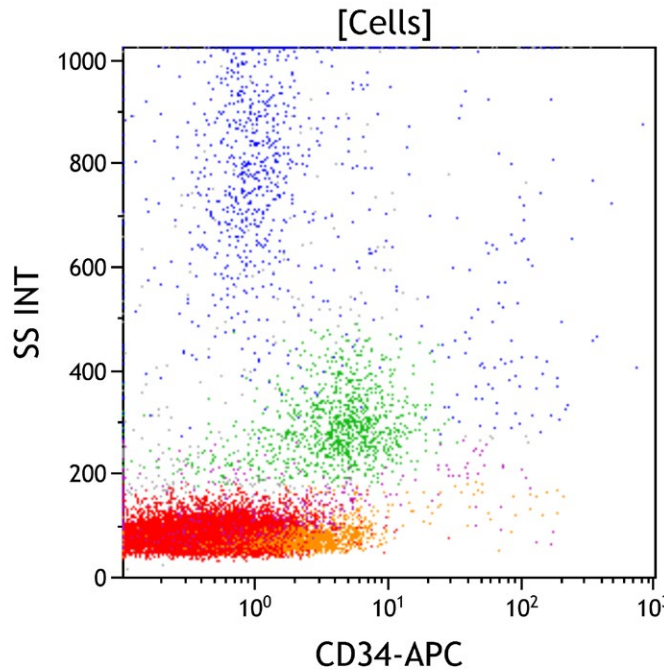

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Most lymphocytes (red) do not express CD34.

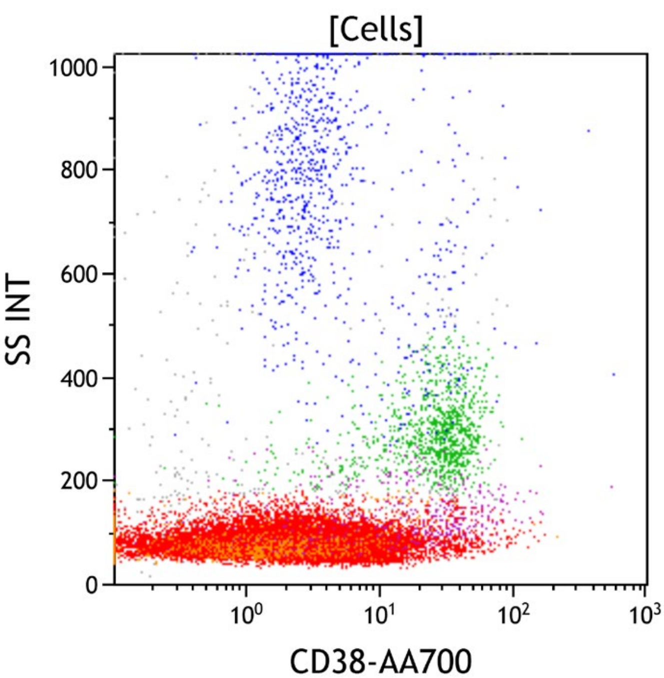

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Most lymphocytes (red) express dim CD38.

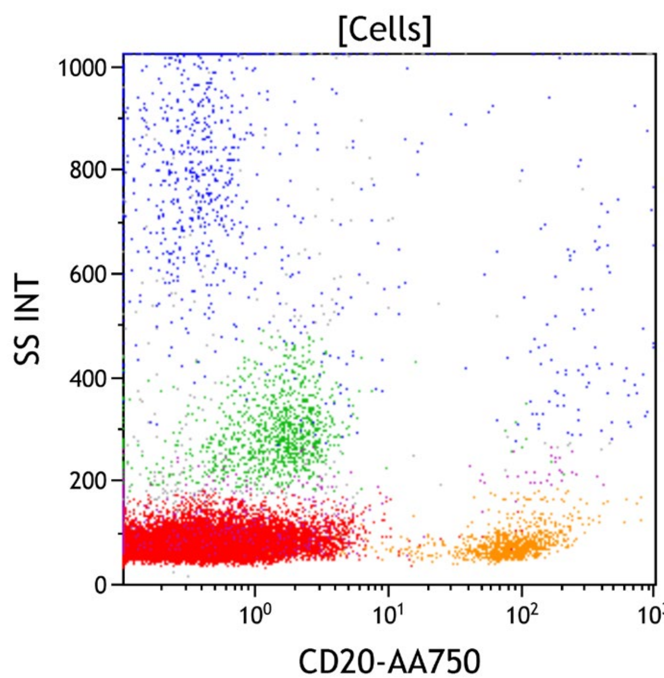

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. Most lymphocytes (red) do not express CD20.

B Cell Tube

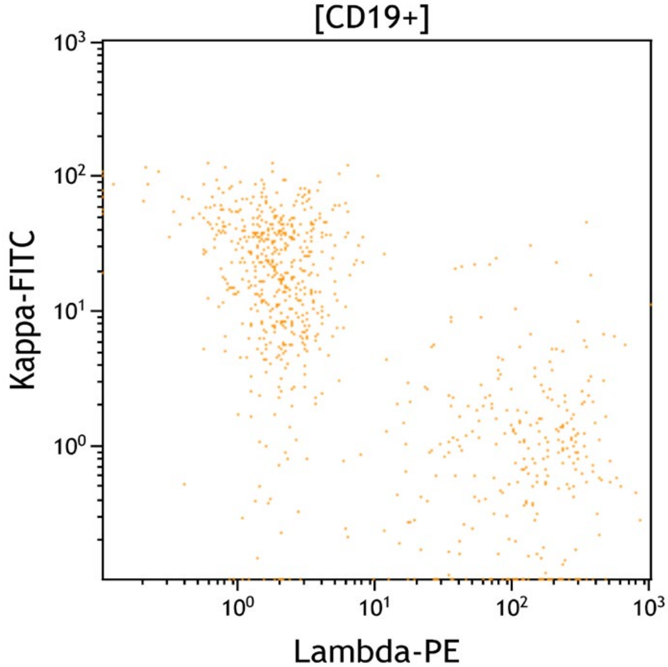

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

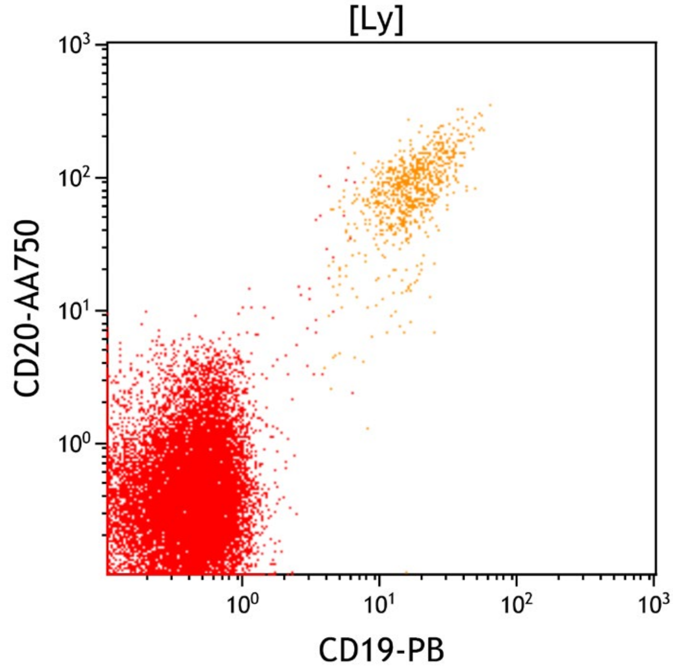

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

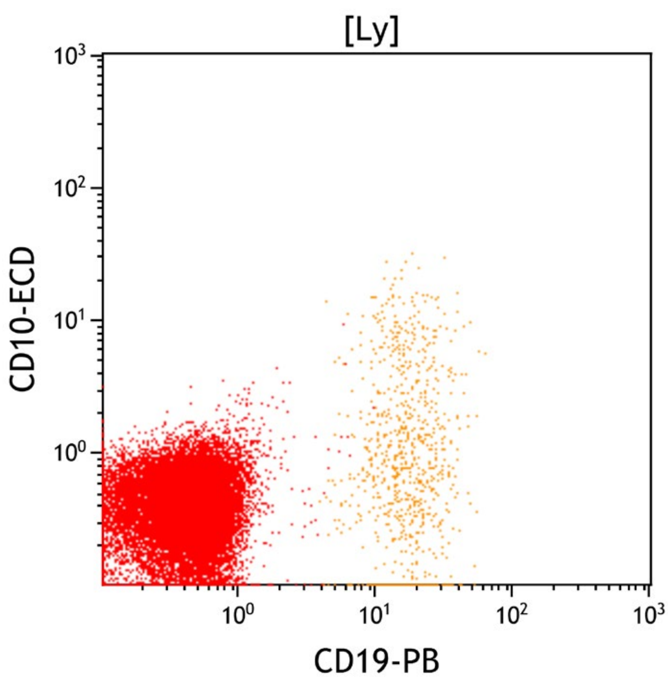

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

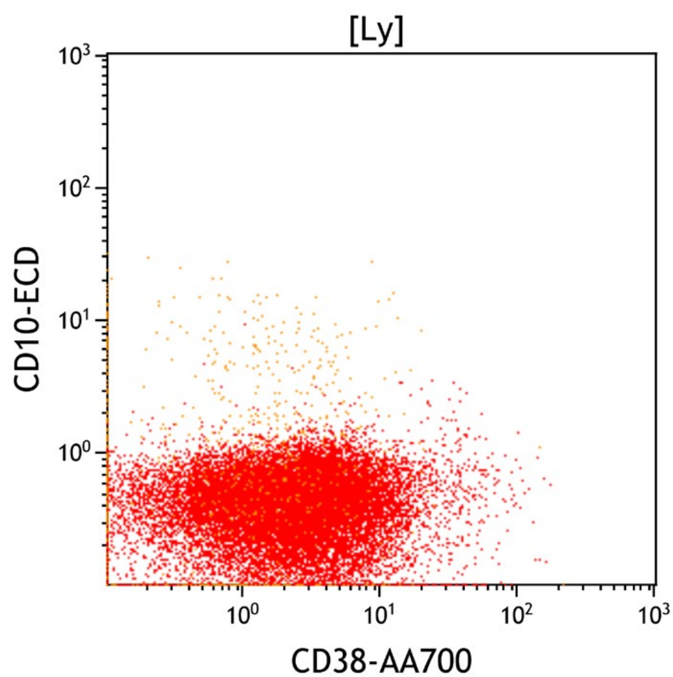

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. Most lymphocytes express dim CD38 but not CD10.

B Cell Tube

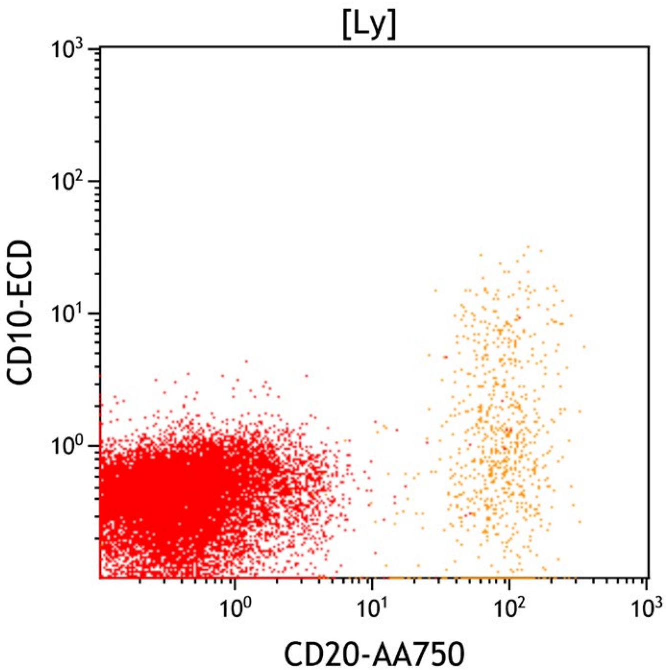

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

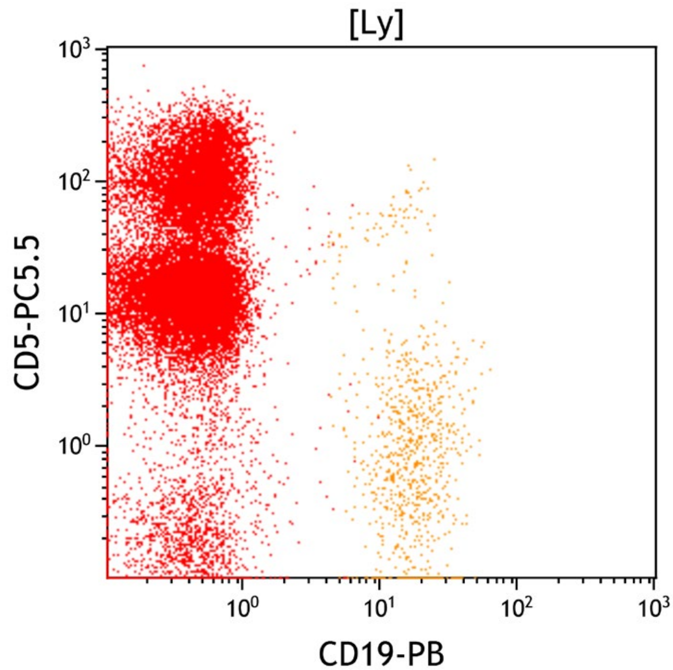

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells. Most lymphocytes express CD5, but two discrete subpopulations with different levels of CD5 are present, the lower level of CD5 being abnormal for mature T cells.

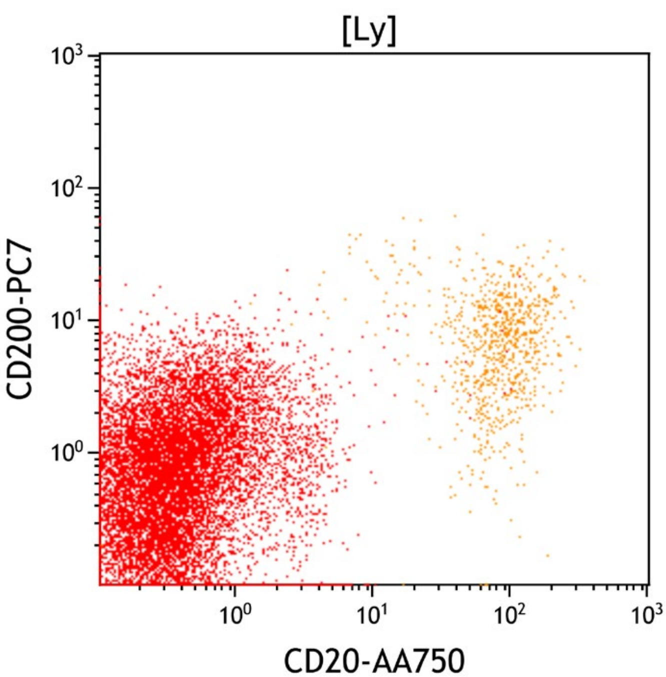

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

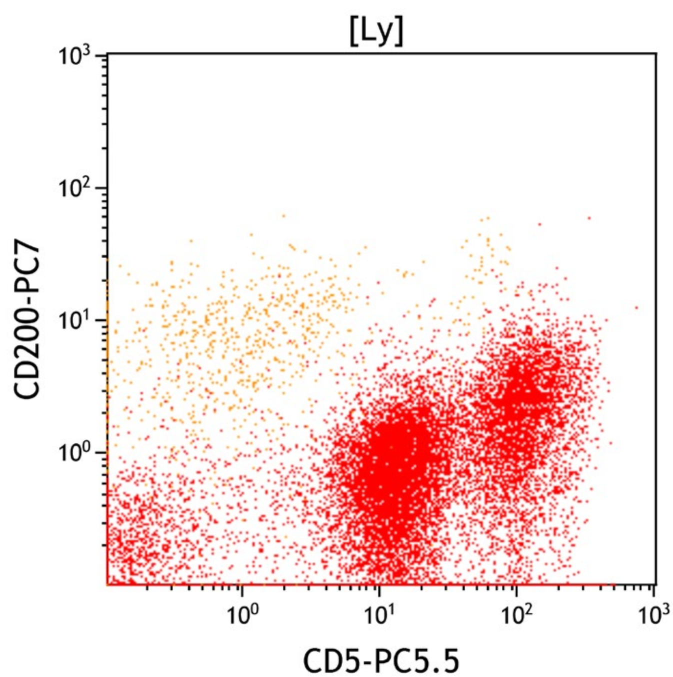

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. Most lymphocytes express CD5, but two discrete subpopulations with different levels of CD5 are present, the lower level of CD5 being abnormal for mature T cells.

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T Cell Tube

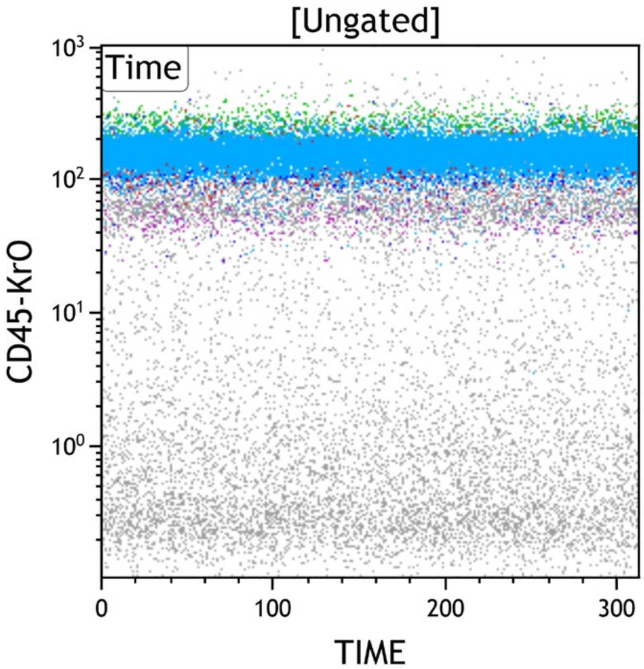

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

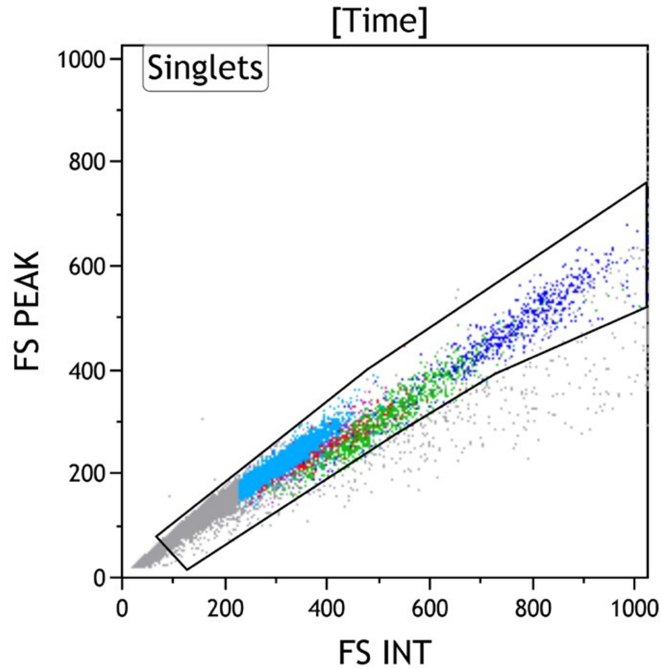

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

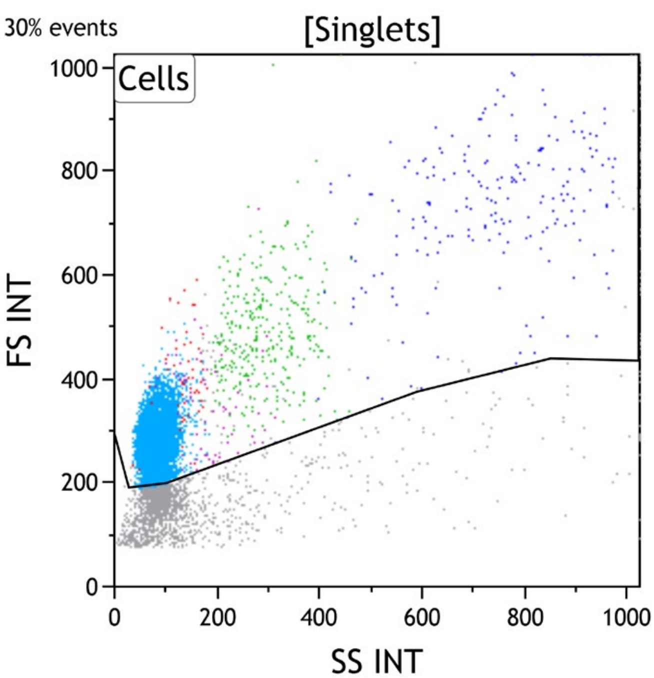

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

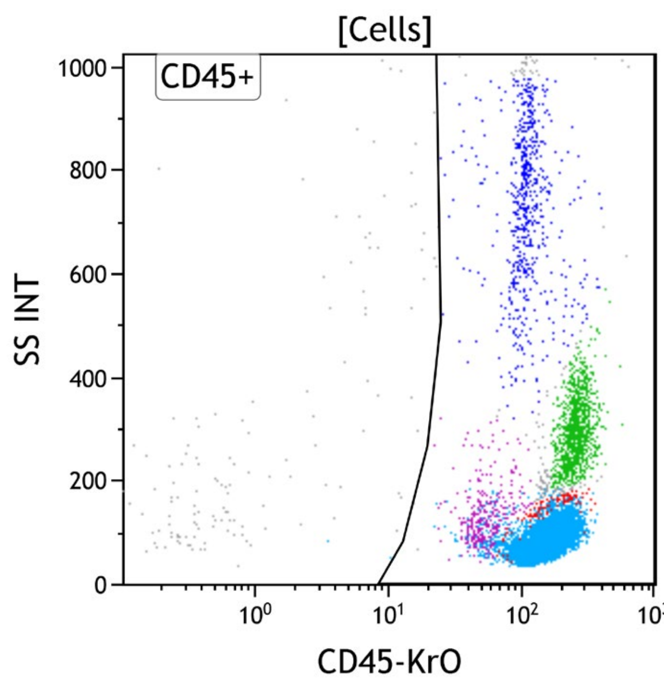

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

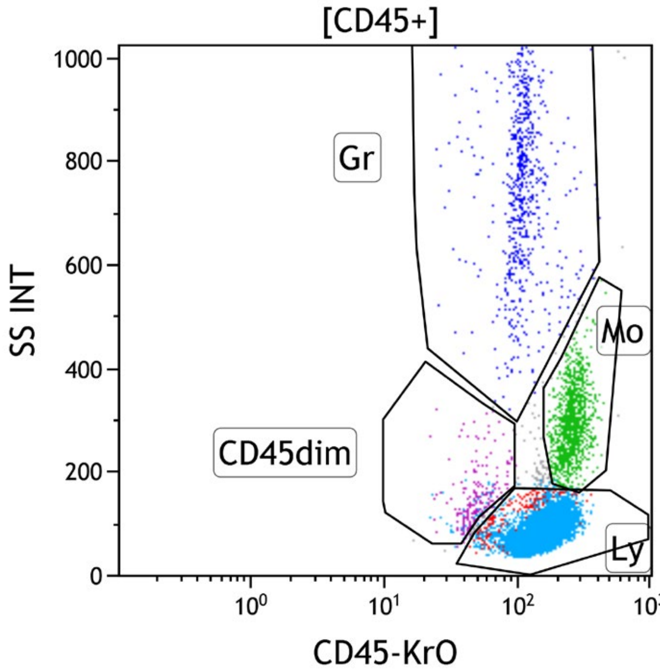

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased number of lymphocytes (red/aqua).

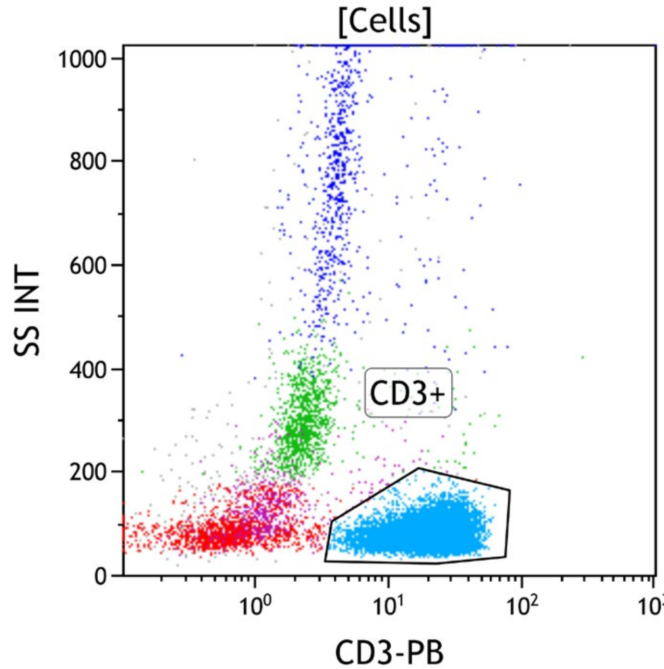

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. Most lymphocytes are CD3 positive T cells (aqua).

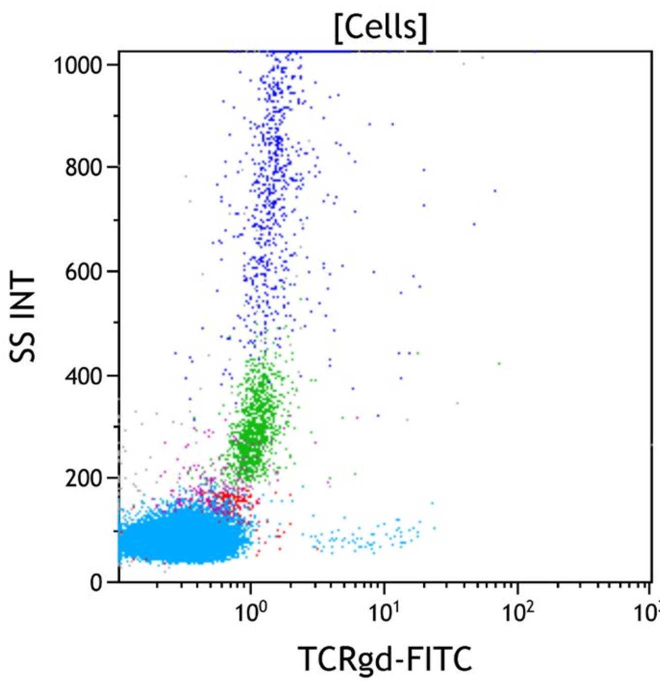

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). Most T cells do not express gamma/delta T cell receptor.

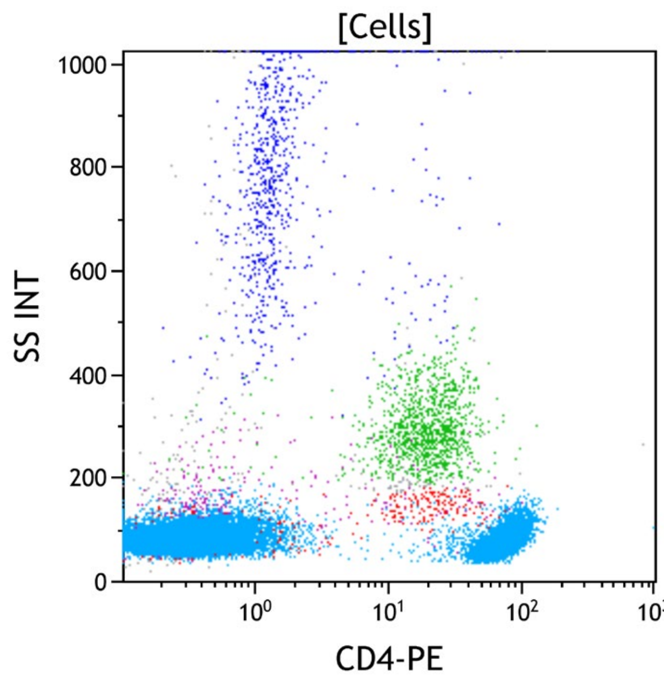

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. A small subset of T cells expresses CD4 (aqua).

T Cell Tube

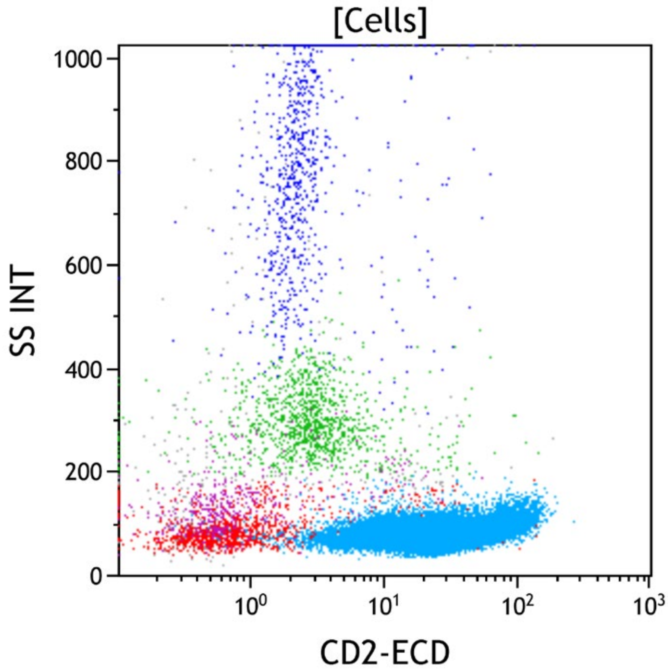

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). A subset of T cells has variably reduced CD2 expression (aqua).

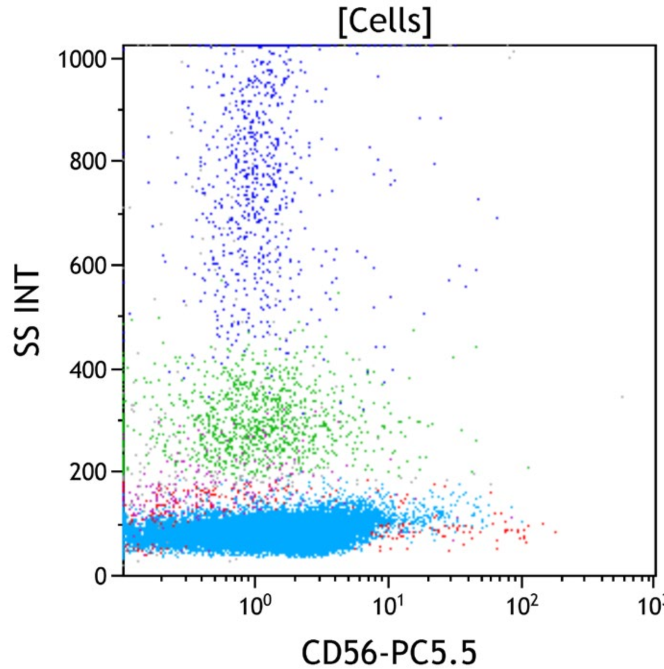

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. CD56 is expressed at a low level on a subset of T cells (aqua).

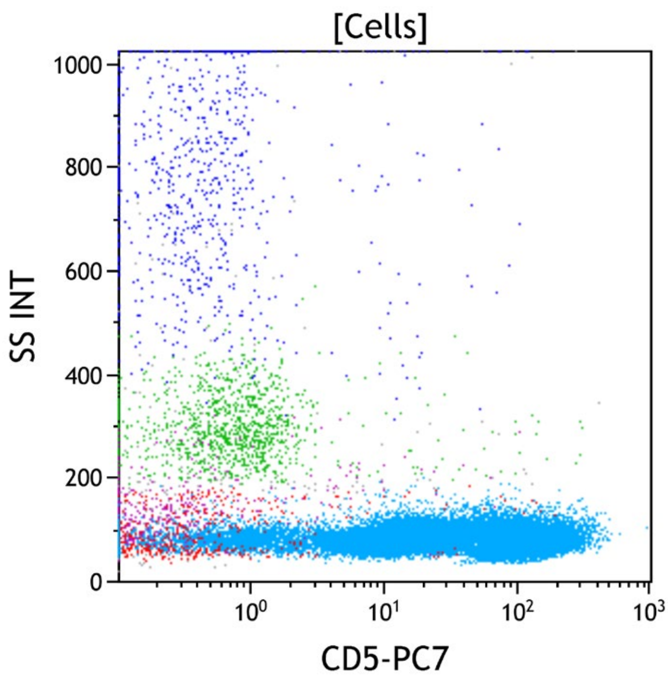

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. A subset of T cells (aqua) has variably decreased CD5 expression.

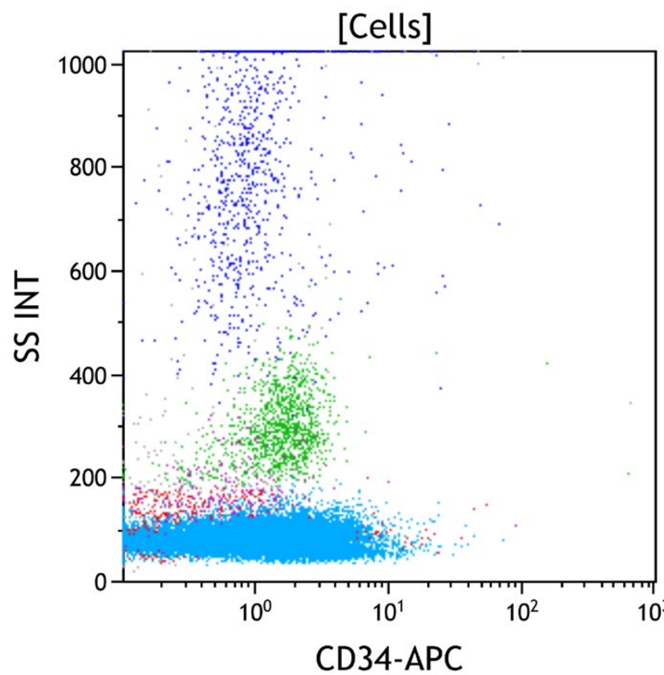

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. No significant CD34 expression on T cells (aqua).

T Cell Tube

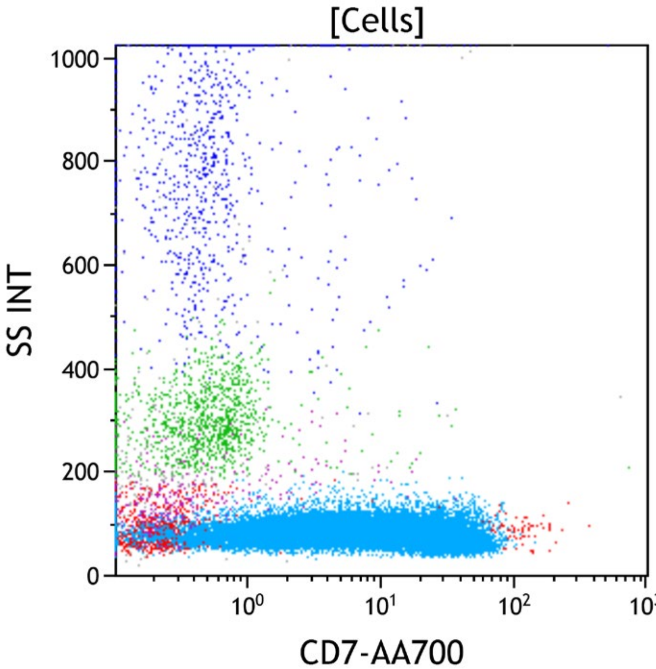

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. CD7 expression is variably decreased on T cells (aqua).

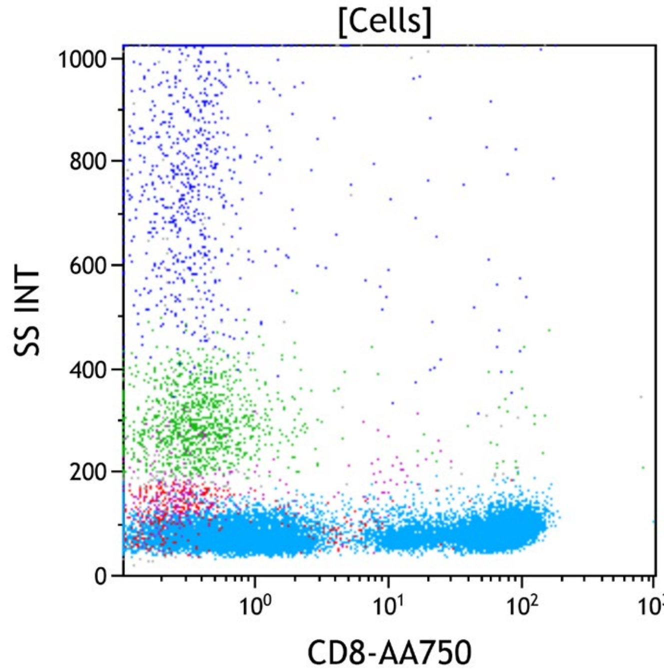

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. A major subset of T cells (aqua) expresses CD8.

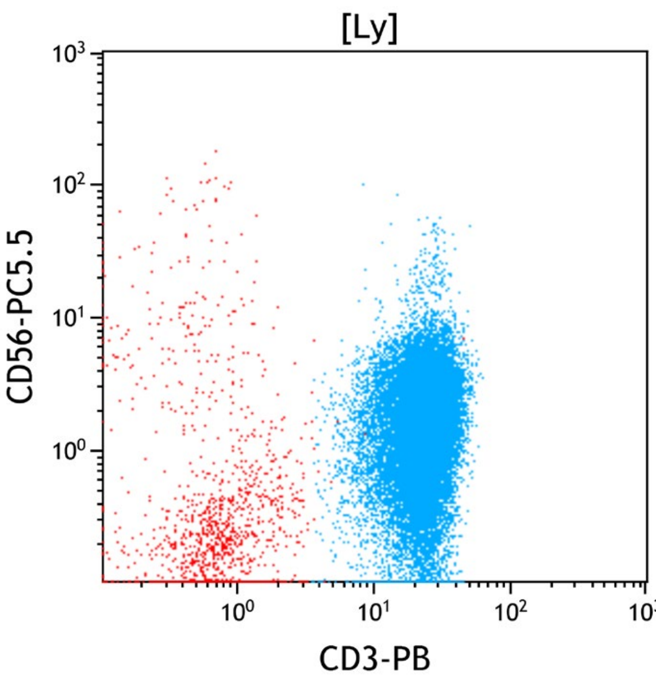

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells. CD56 is expressed at a low level on a subset of T cells.

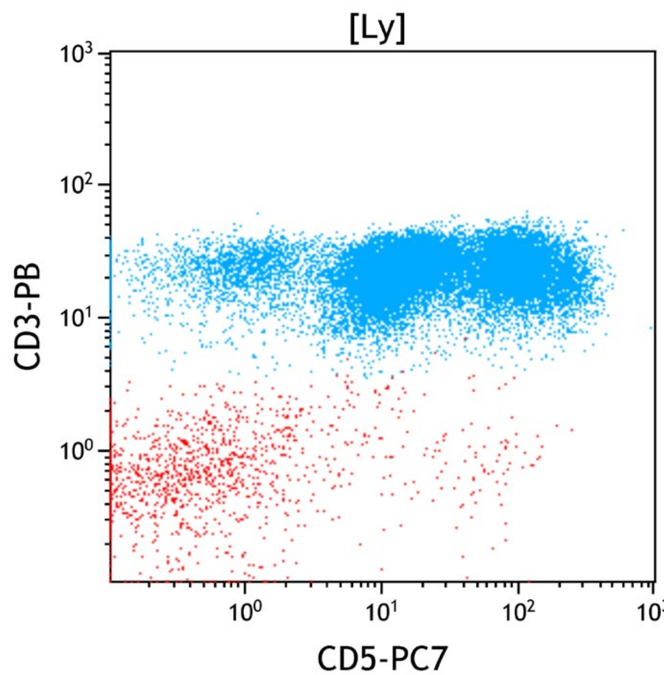

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red). CD5 expression is variably decreased on a subset of T cells.

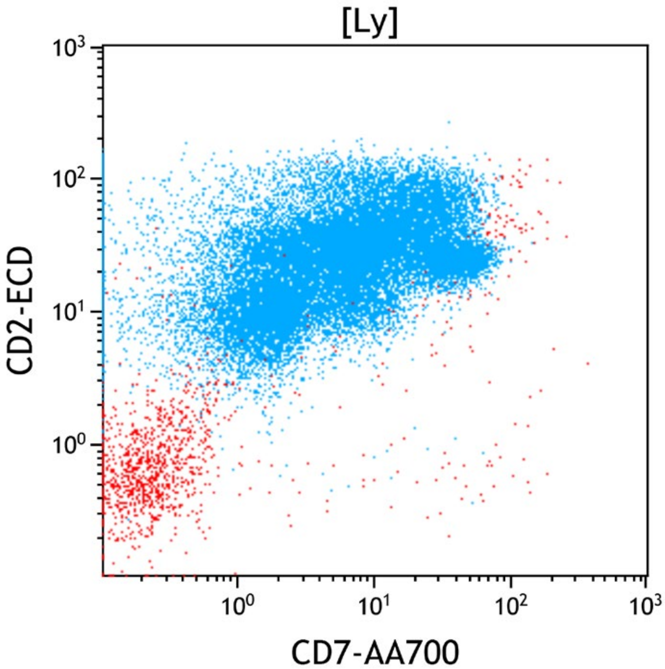

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red). CD2 and CD7 are variably decreased on a subset of T cells.

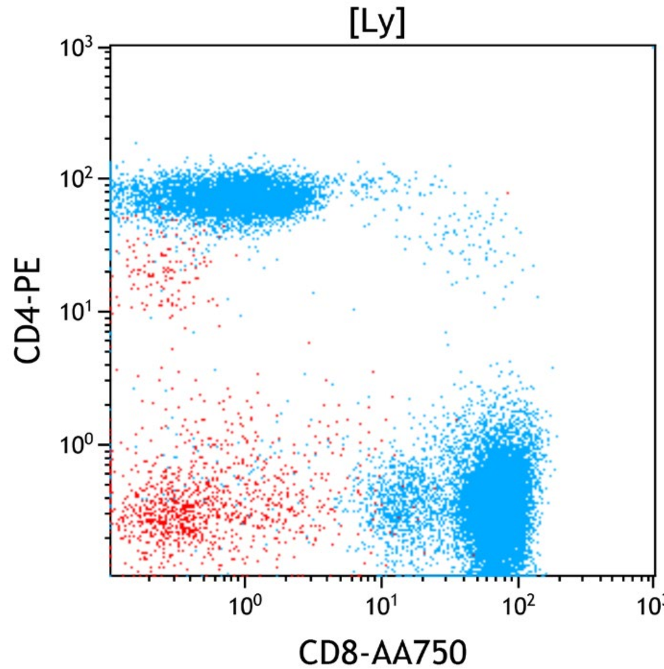

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification. CD8-positive T-cells are proportionally increased.

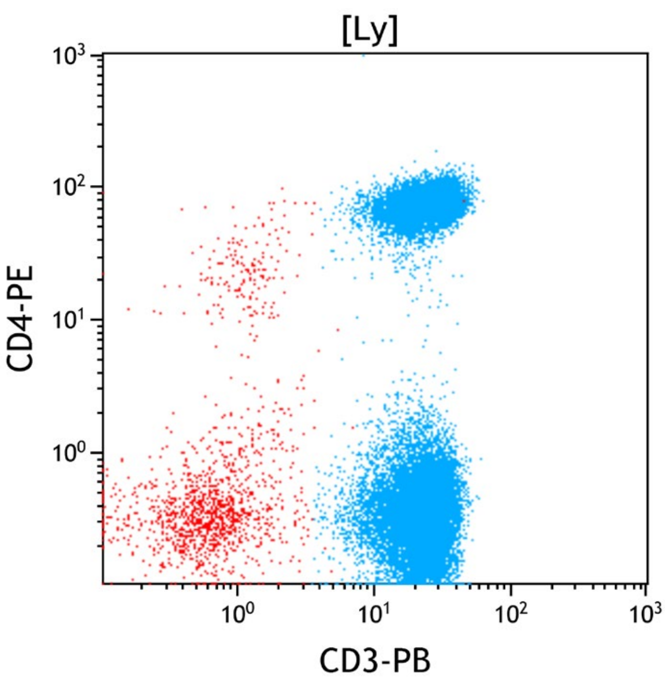

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

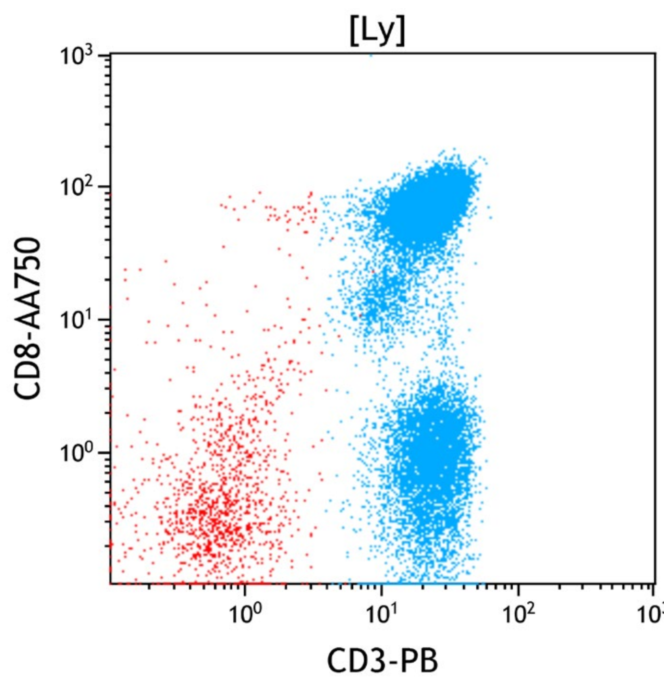

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3. CD8-positive T-cells are relatively increased.

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T Cell Tube

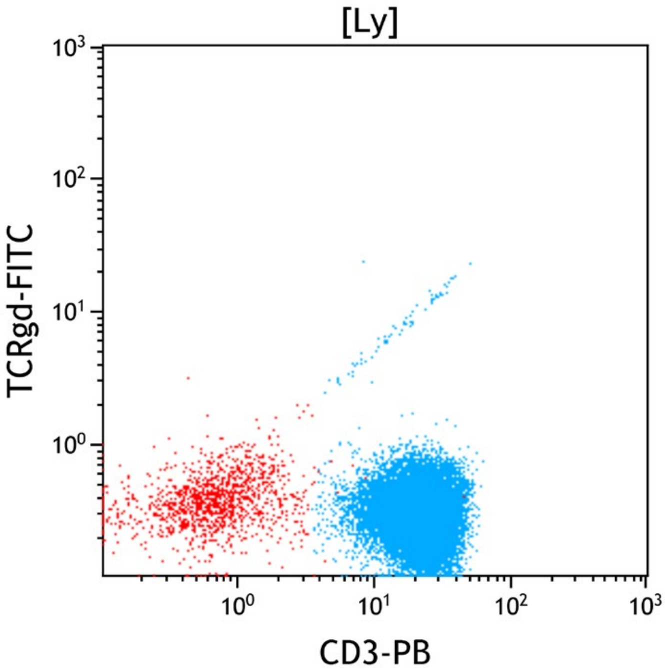

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other. Most T cells do not express TCRγδ, thus are not gamma/delta T cells.

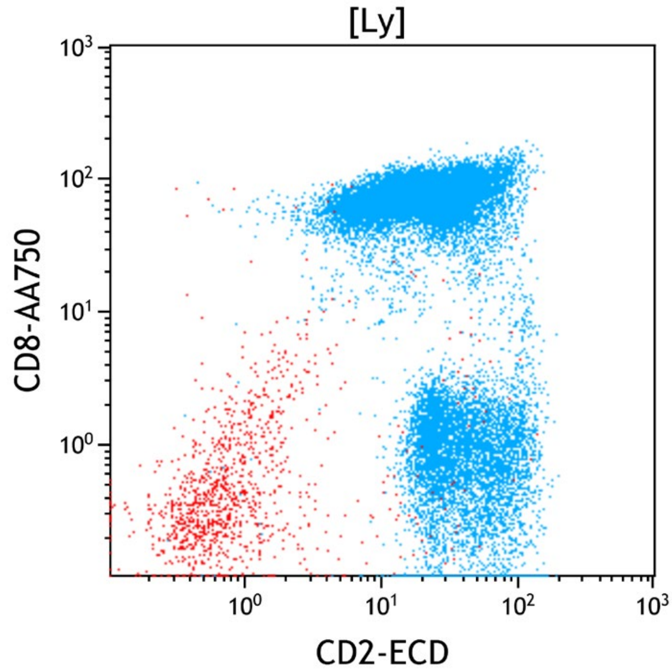

Figure 22. This CD2 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8-negative T cells (blue, lower right), a large subset of CD8 positive T cells (blue, upper right) show aberrantly decreased CD2 expression.

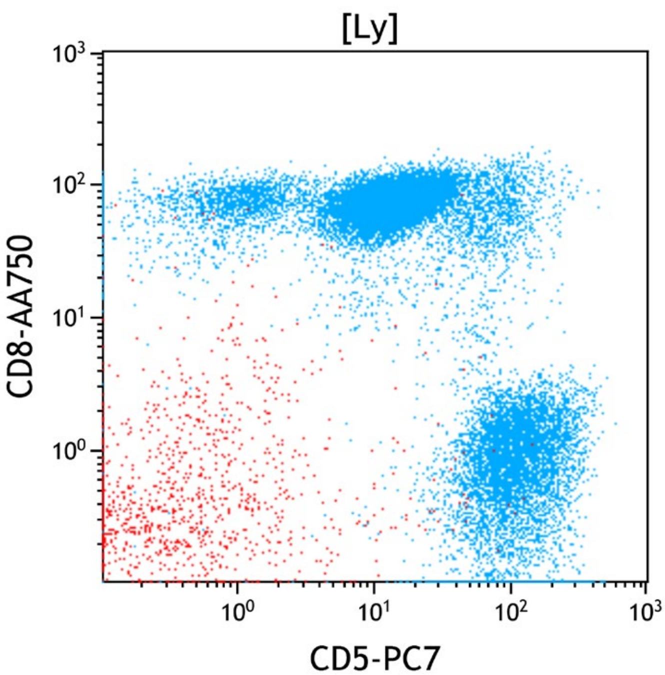

Figure 23. This CD5 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8-negative T cells (blue, lower right), a large subset of CD8 positive T cells (blue, upper) show aberrantly decreased CD5 expression.

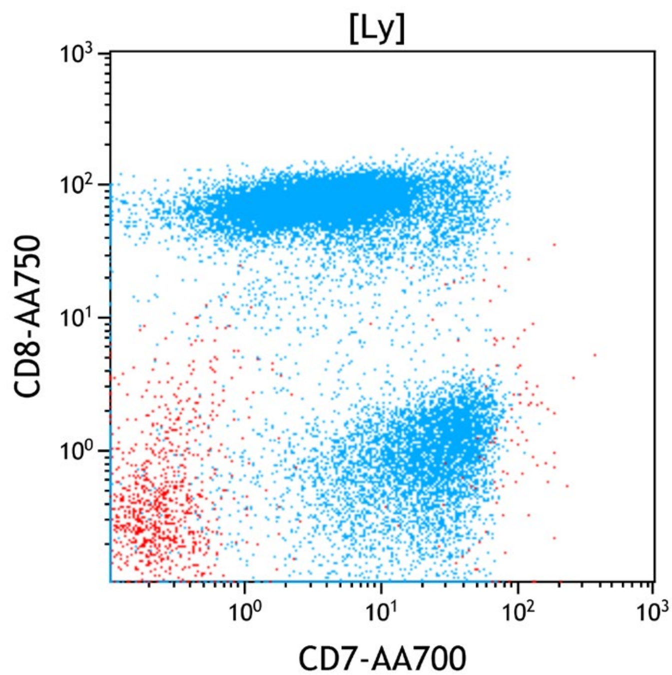

Figure 24. This CD7 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8-negative T cells (blue, lower right), a large subset of CD8 positive T cells (blue, upper) show aberrantly decreased CD7 expression.

M1 Cell Tube

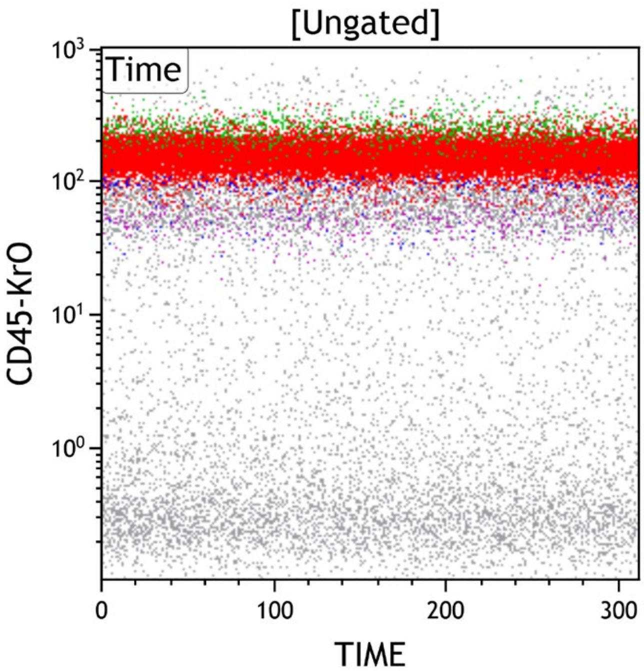

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

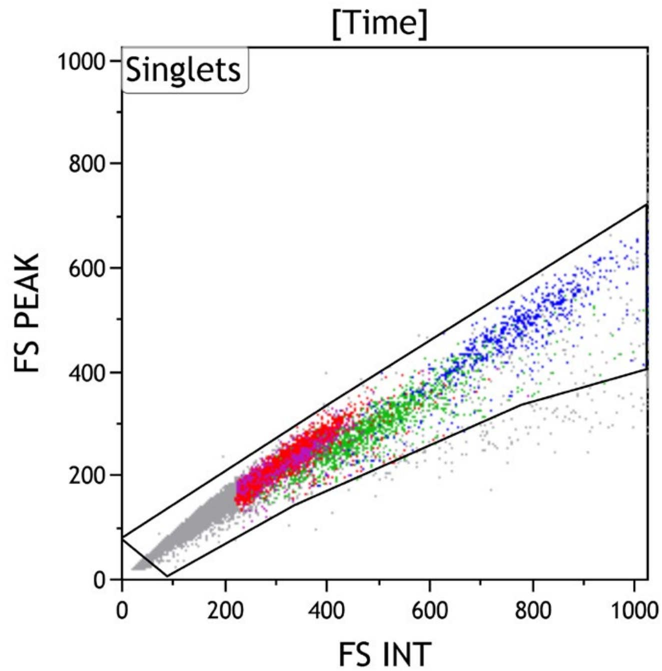

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

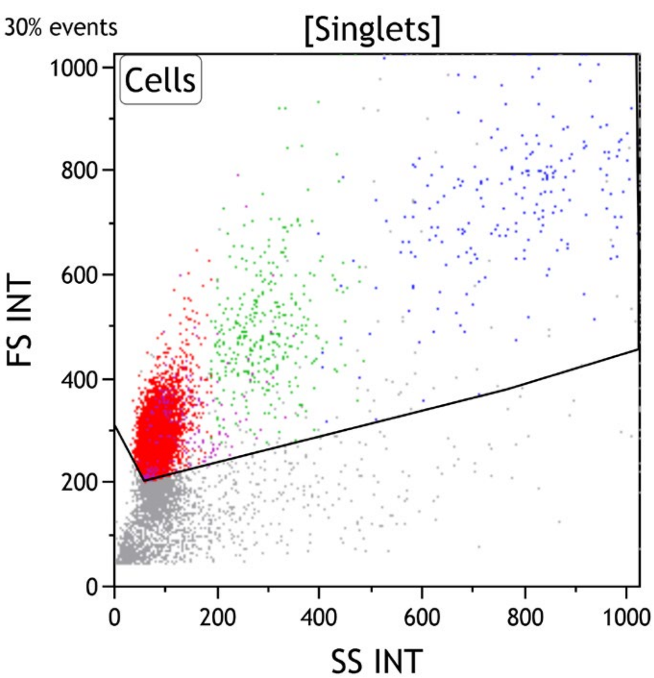

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

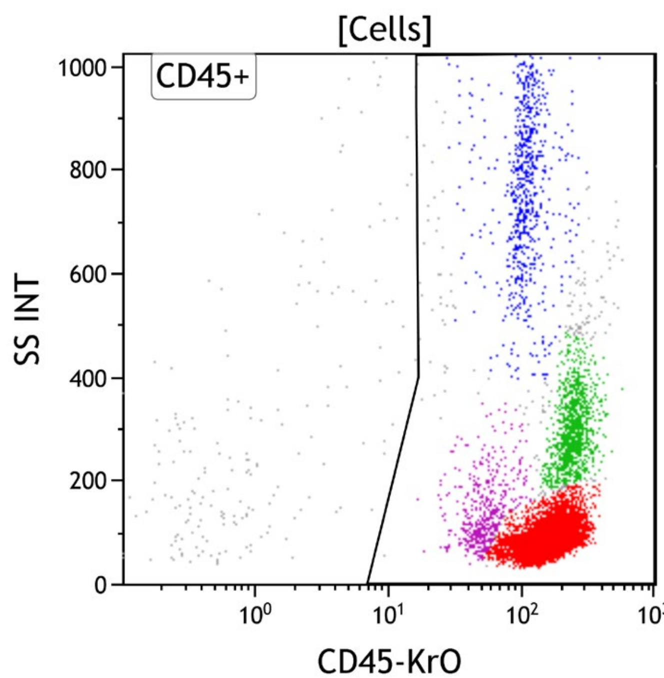

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

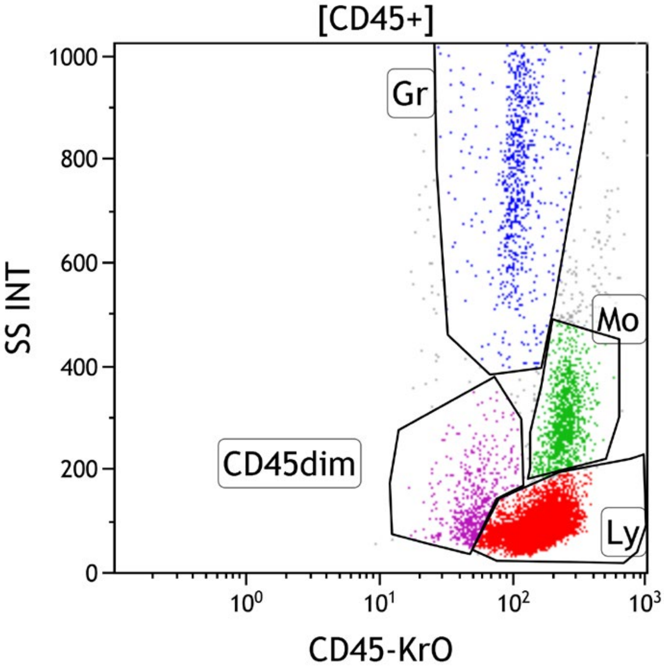

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased number of lymphocytes (red).

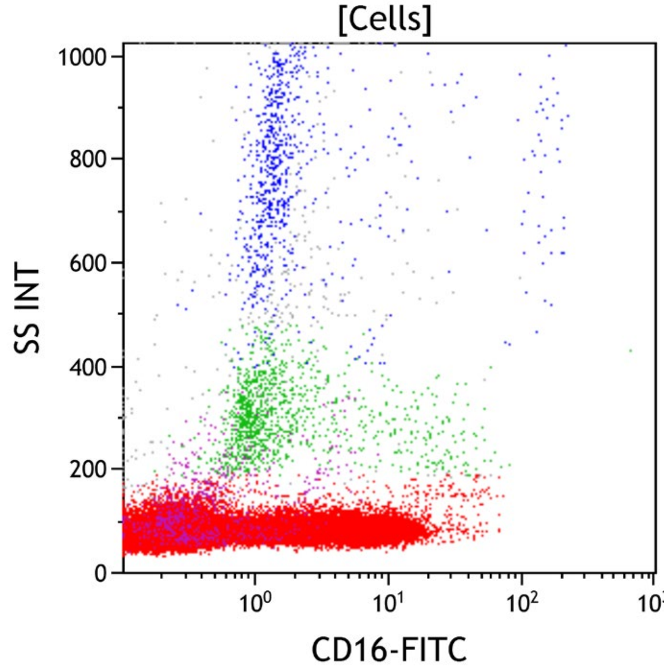

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). CD16 is expressed on a larger subset of lymphocytes (red) than is normal.

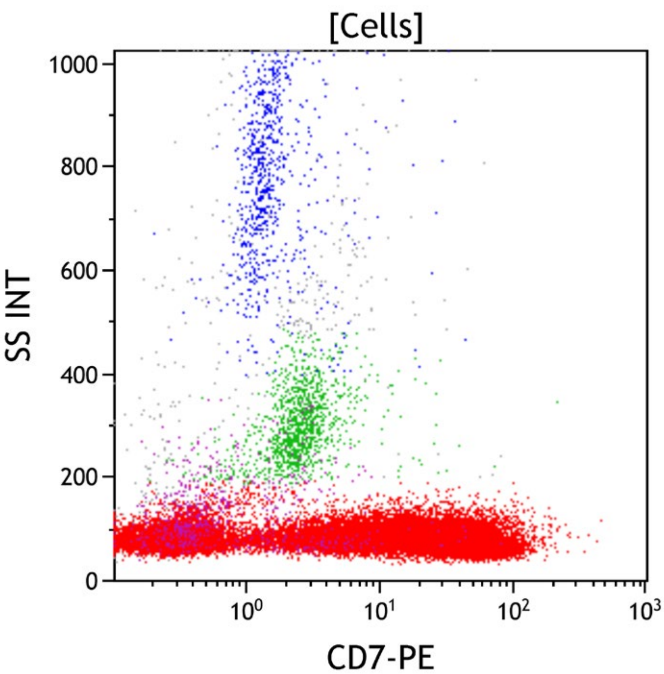

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. Variable CD7 expression is present on a large subset of lymphocytes (red).

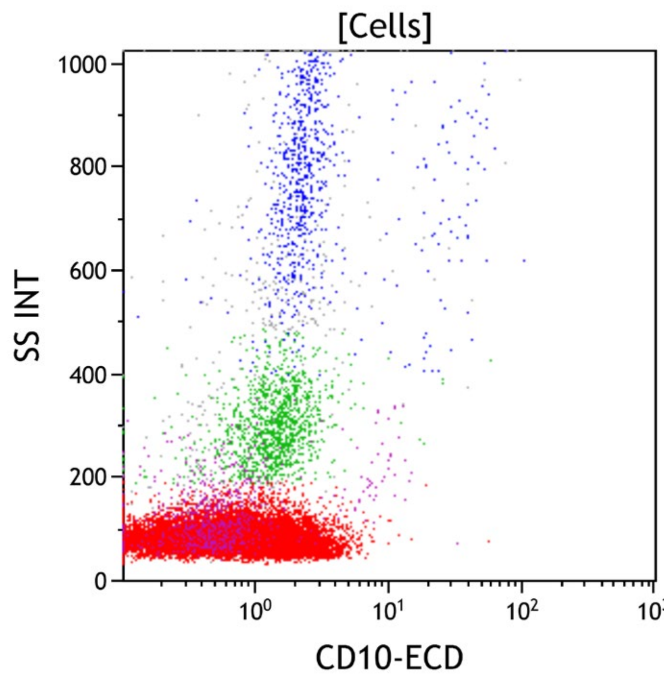

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The lymphocytes (red) do not express CD10.

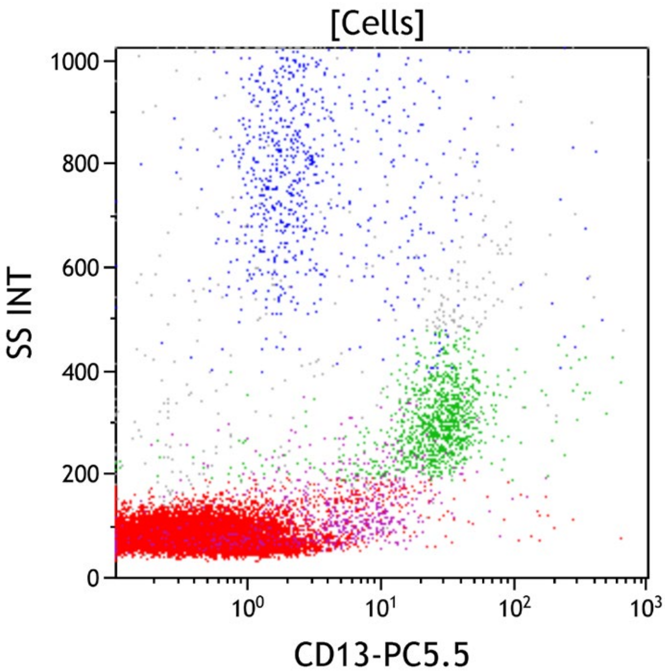

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The lymphocytes (red) do not express CD13.

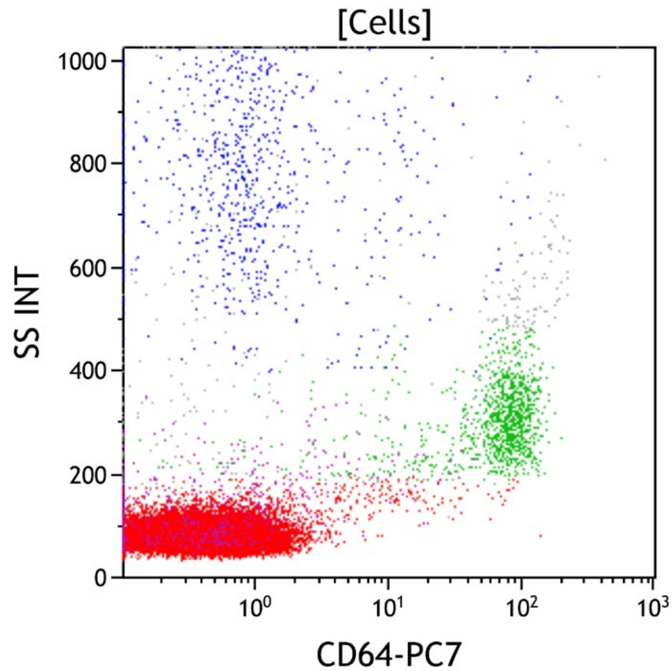

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The lymphocytes (red) do not express CD64.

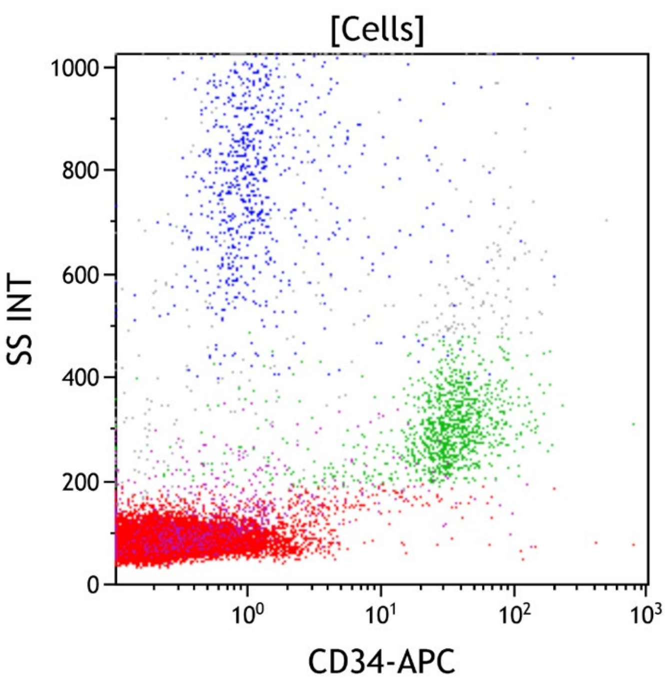

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34.

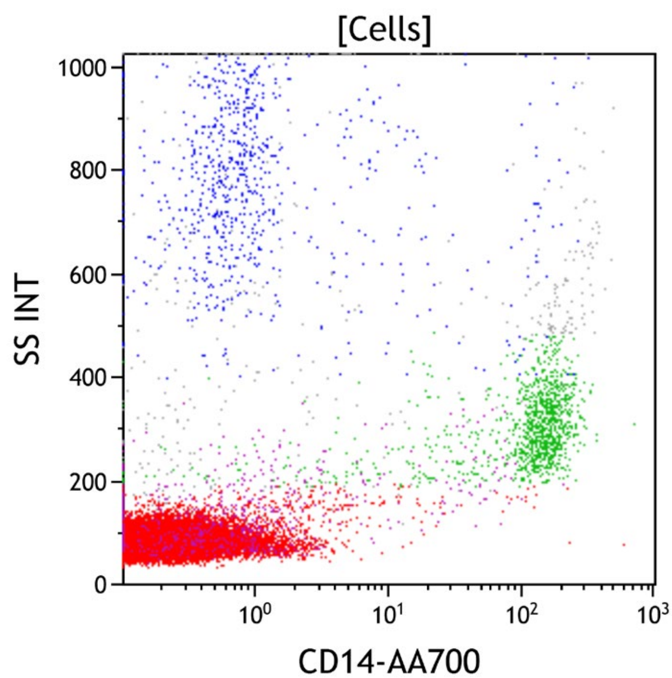

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The lymphocytes (red) do not express CD14.

M1 Cell Tube

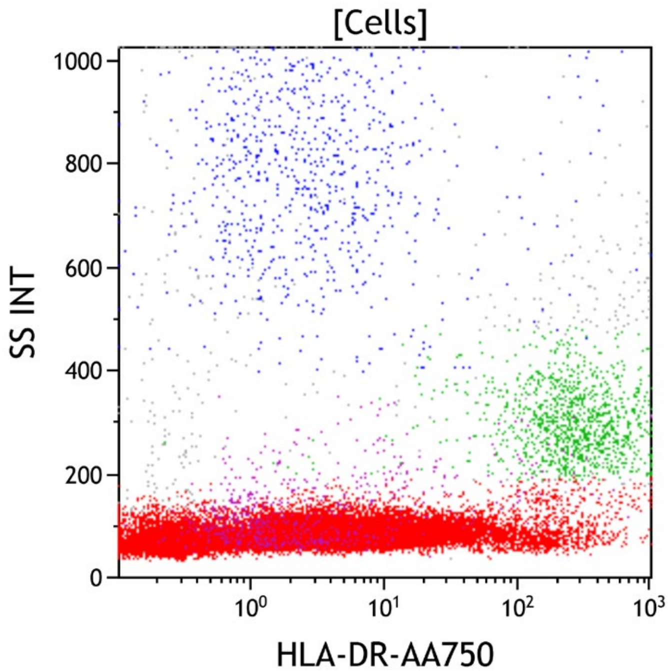

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. HLA-DR is variably expressed on the lymphocytes (red).

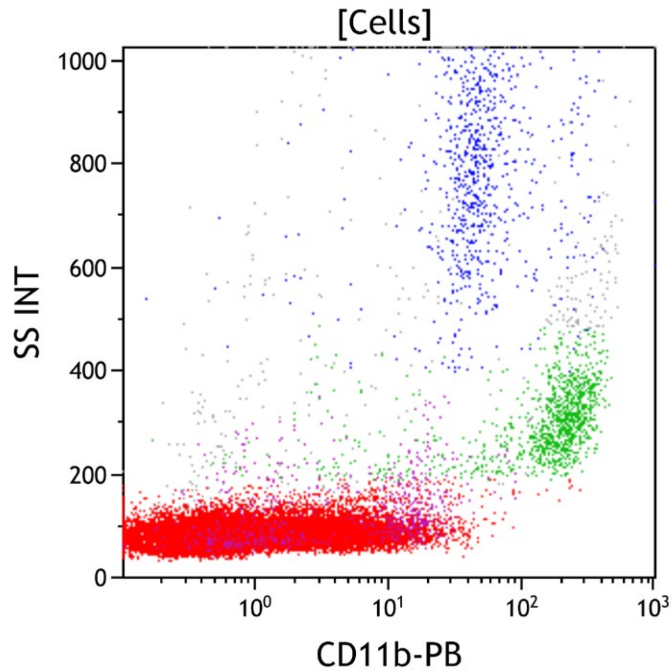

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. Dim CD11b expression is present on a subset of lymphocytes (red).

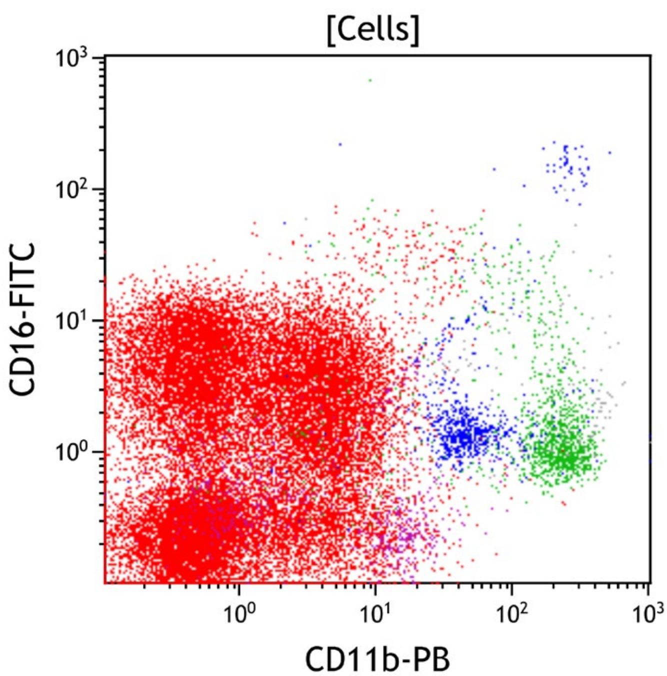

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. Dim CD11b and/or CD16 are expressed on a subset of lymphocytes.

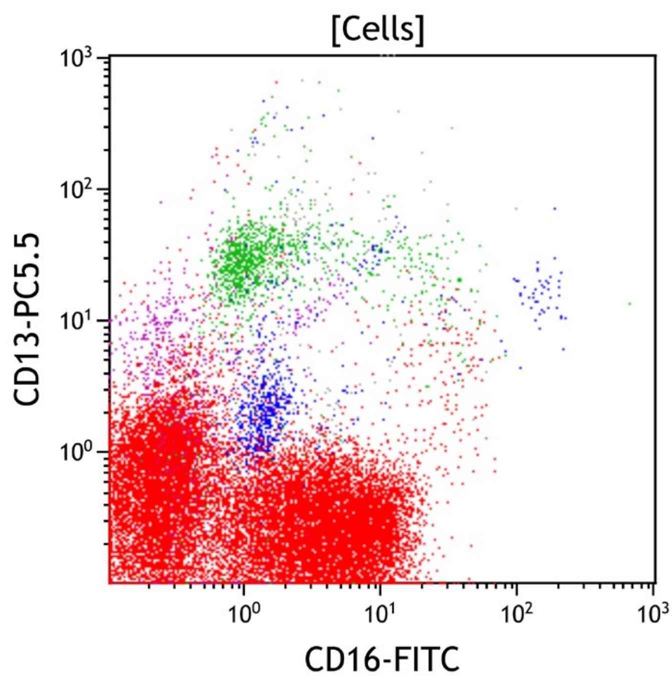

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils or CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. CD16 is expressed on a subset of lymphocytes.

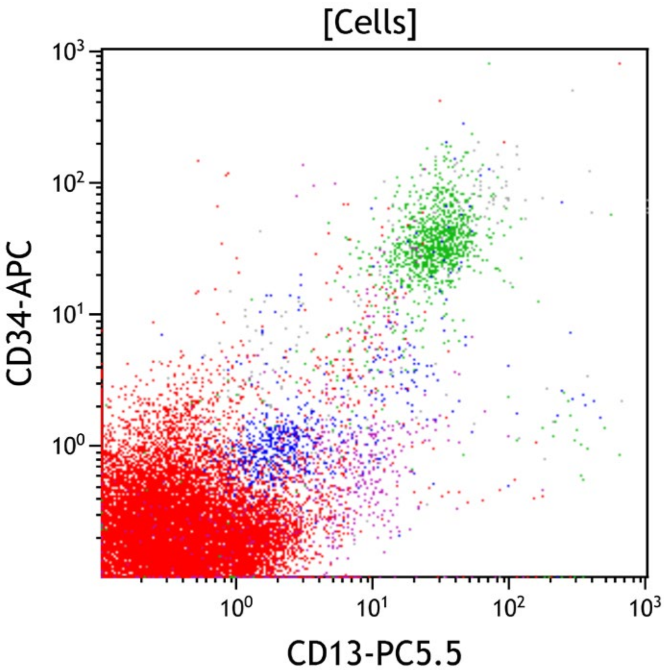

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive progenitors or mature lymphoid cells. The lymphocytes (red) do not express CD34.

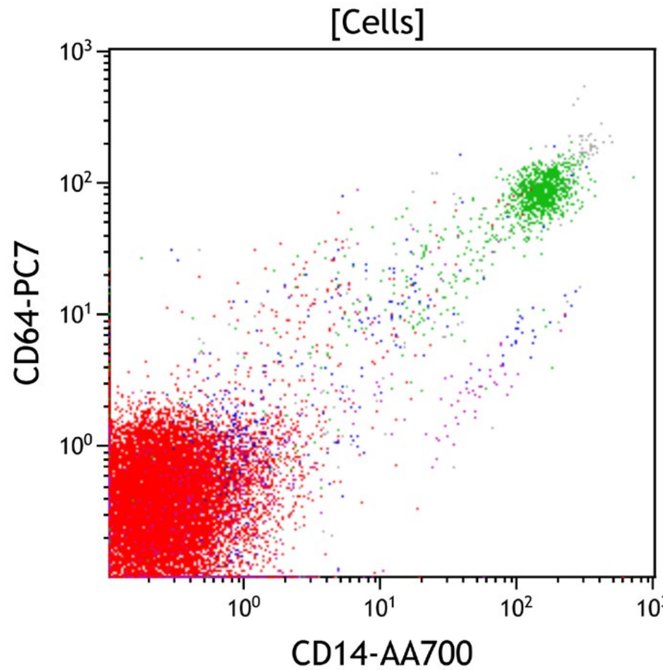

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. The lymphocytes (red) do not express CD14 and CD64.

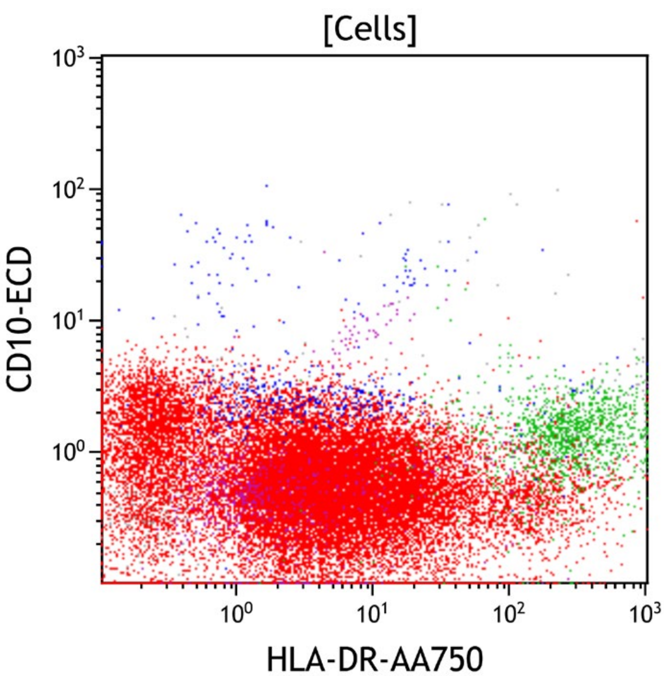

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue) and immature B cells. Immature B cells express both CD10 and HLA-DR. A large subset of lymphocytes (red) expresses HLA-DR.

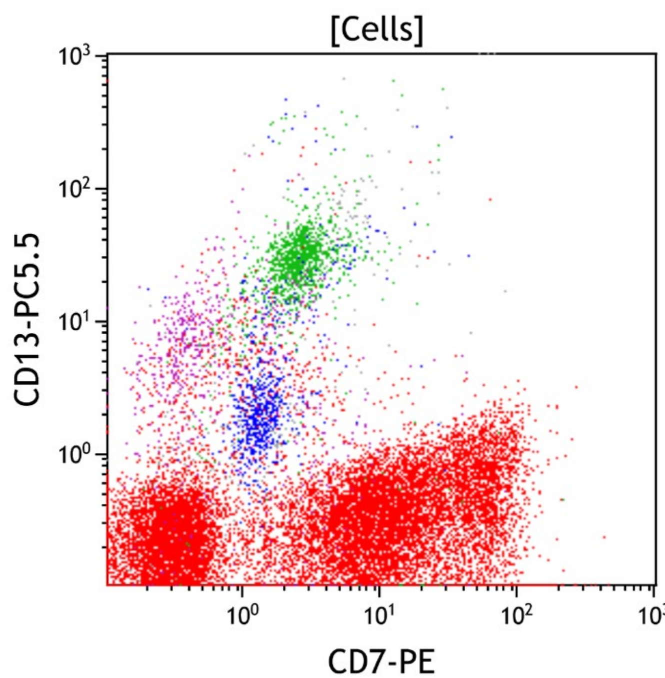

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. A large subset of lymphocytes (red) expresses CD7 but not CD13. The apparent dim CD13 expression on lymphocytes (lower right) is due to compensation artifact.

M2 Cell Tube

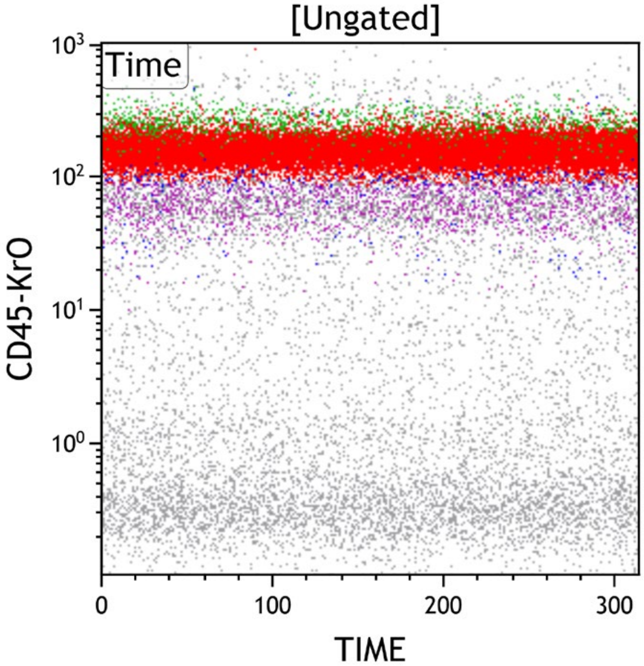

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

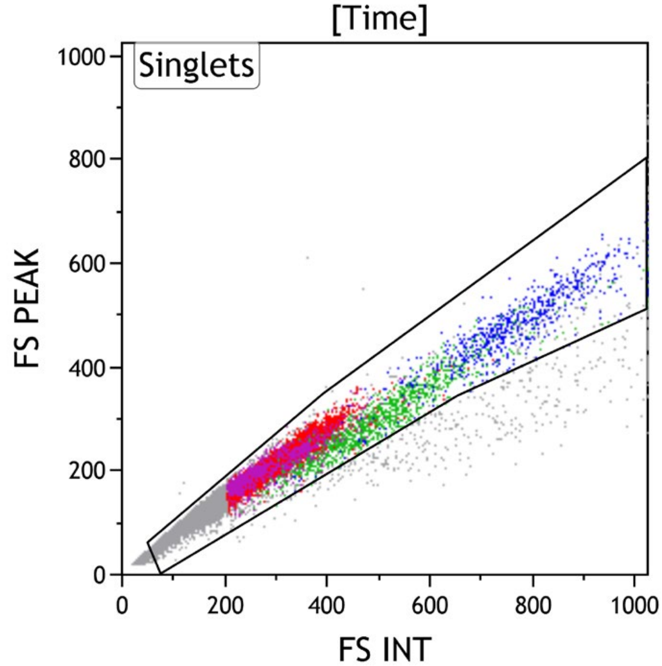

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

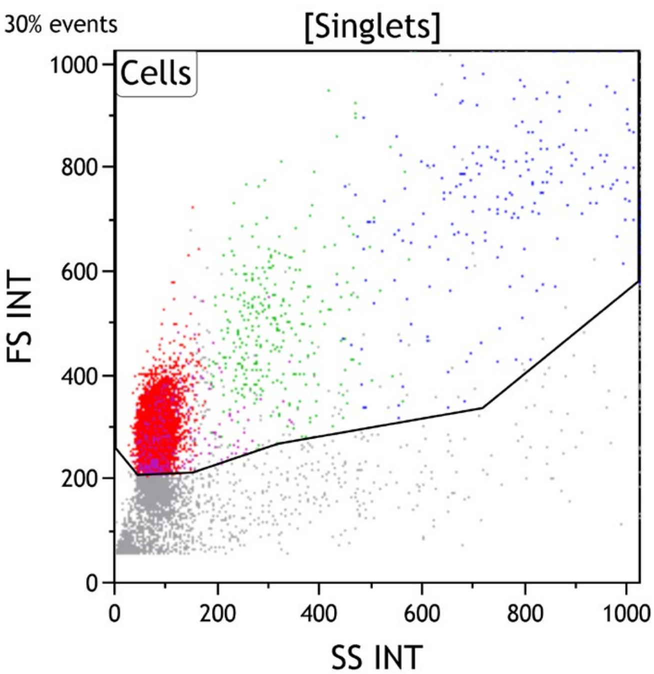

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

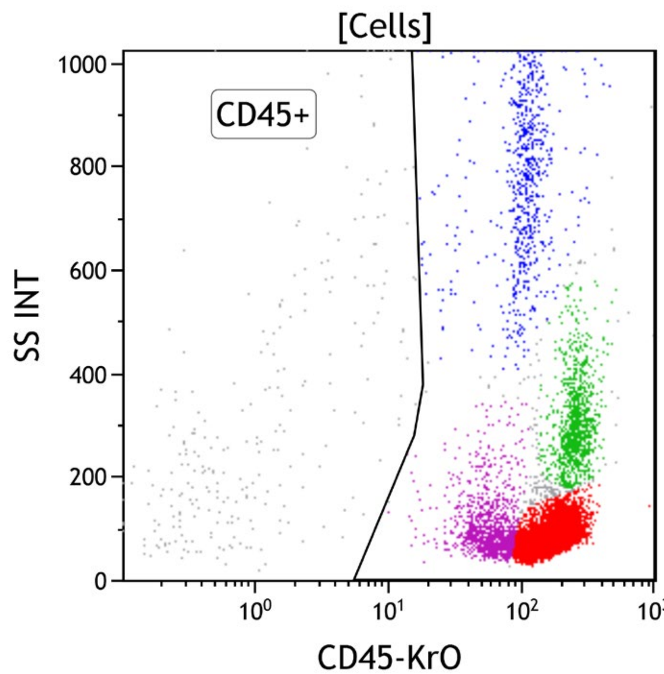

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

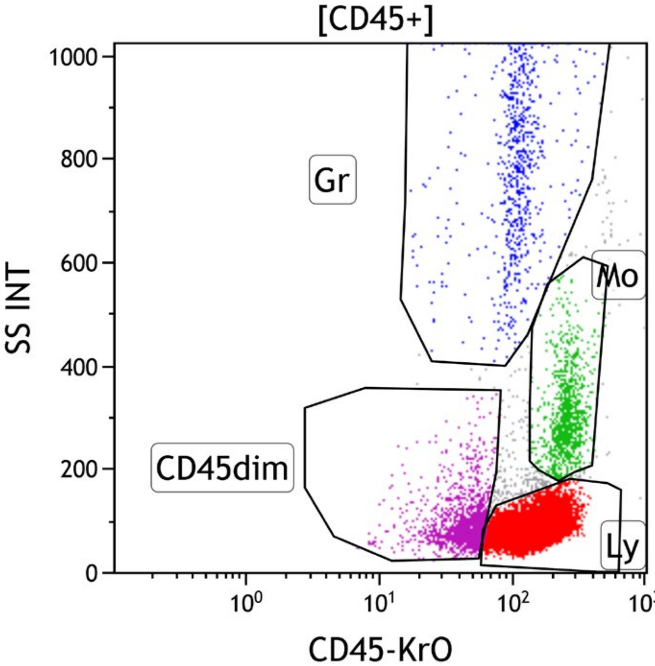

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased lymphocytes (red).

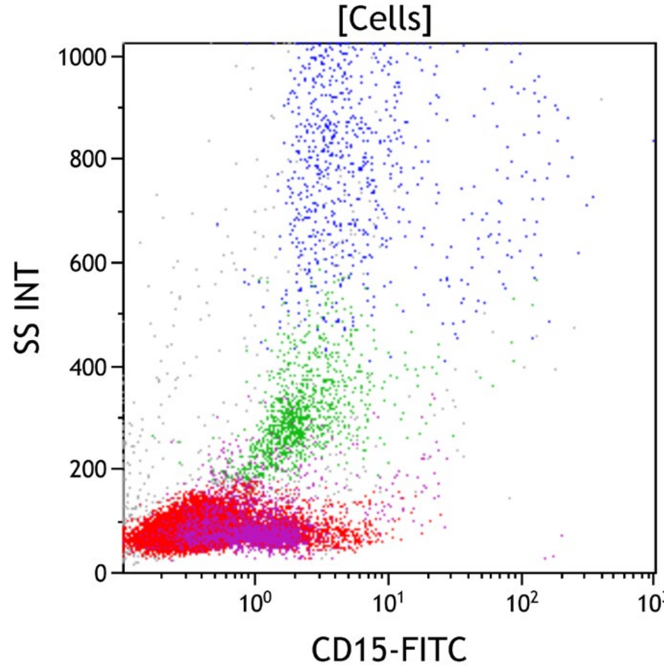

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes. The lymphocytes (red) do not express CD15.

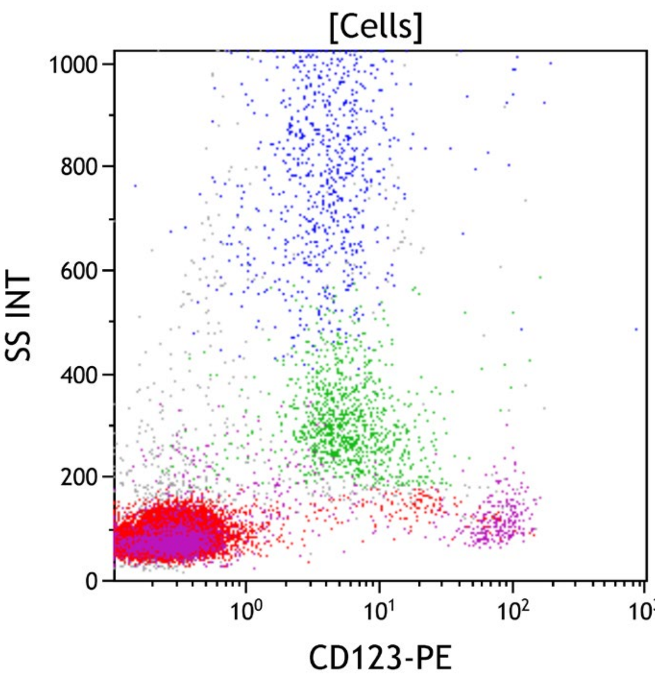

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The lymphocytes (red) do not express CD123.

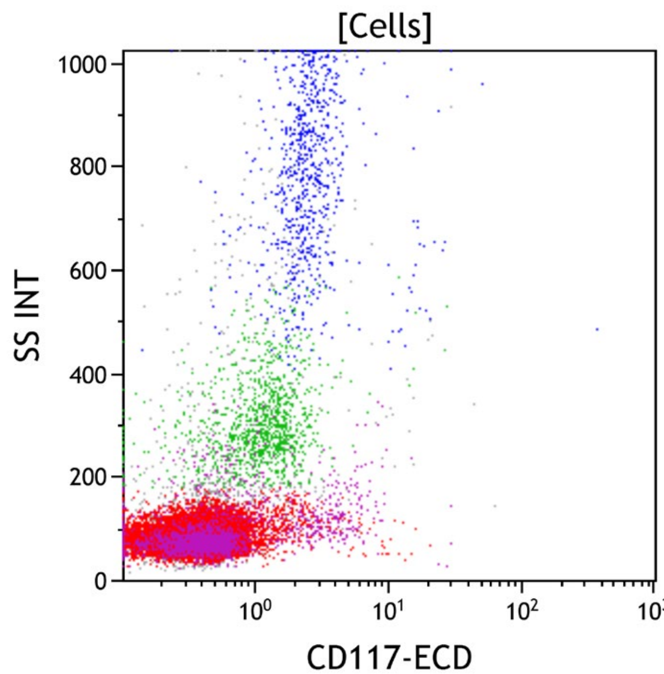

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The lymphocytes (red) do not express CD117.

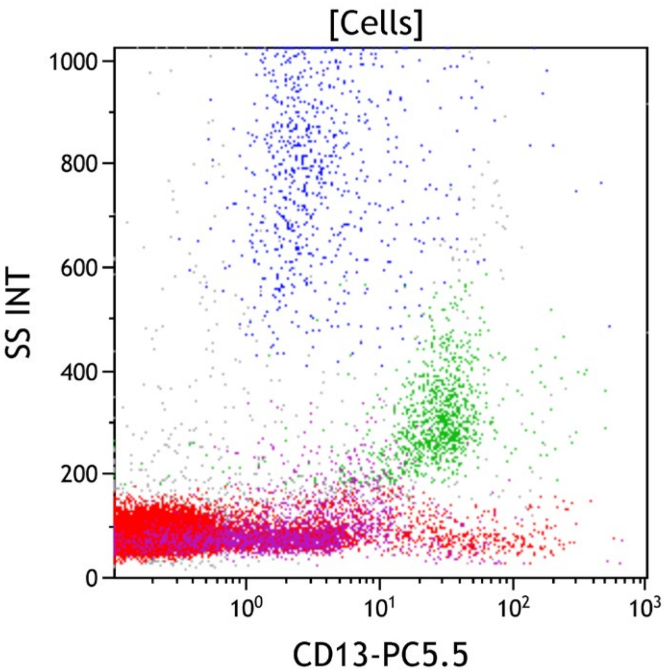

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The lymphocytes (red) do not express CD13.

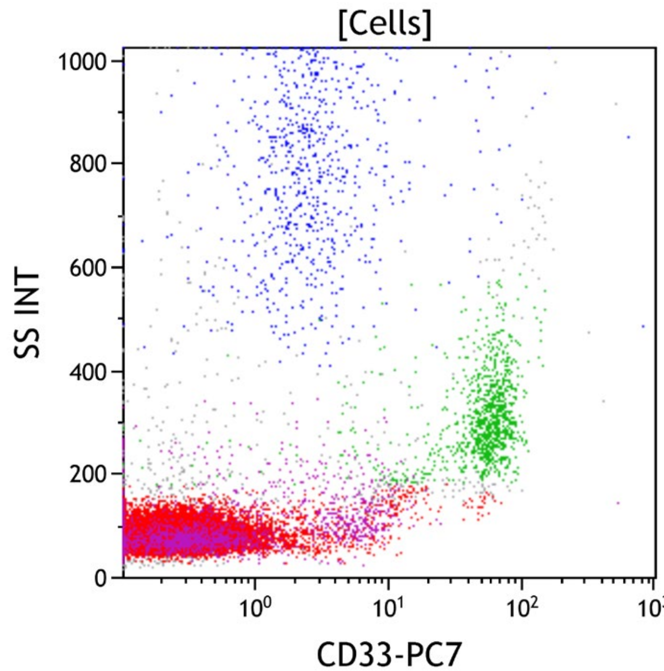

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors .The lymphocytes (red) do not express CD33.

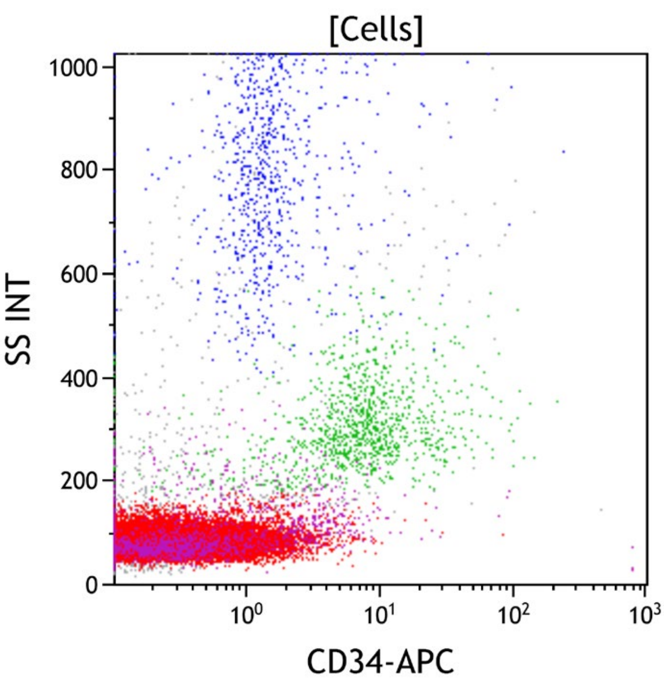

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate .The lymphocytes (red) do not express CD34.

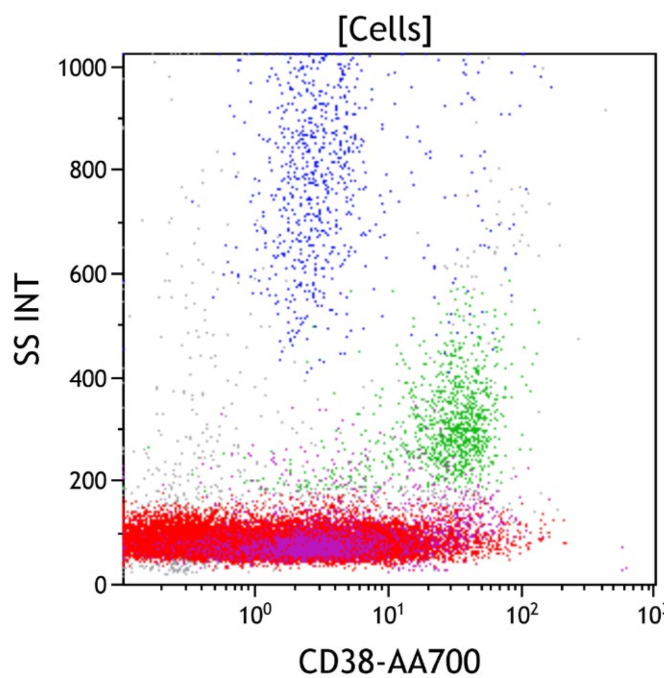

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. A subset of lymphocytes (red) expresses CD38.

M2 Cell Tube

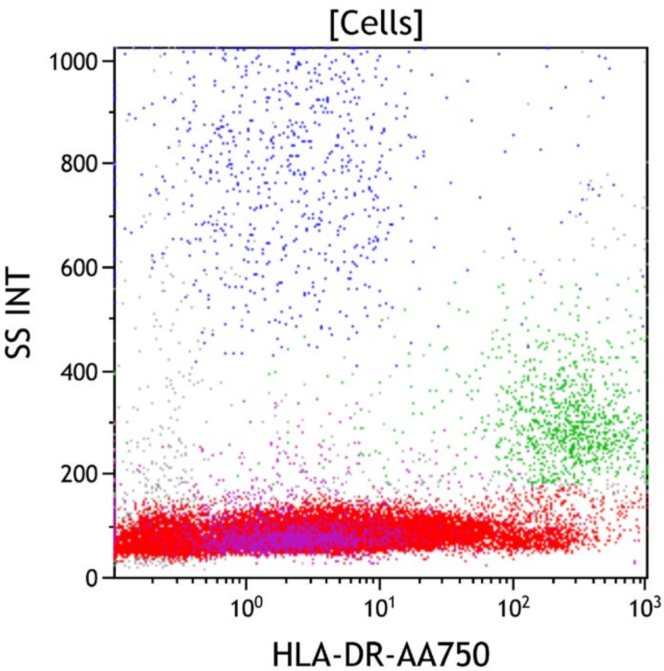

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. A subset of lymphocytes (red) variably expresses HLA-DR.

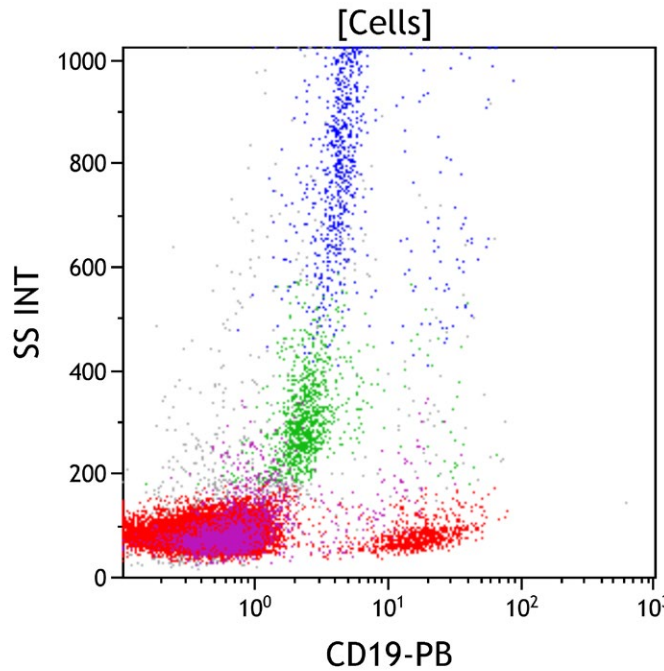

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Most of lymphocytes (red) do not express CD19.

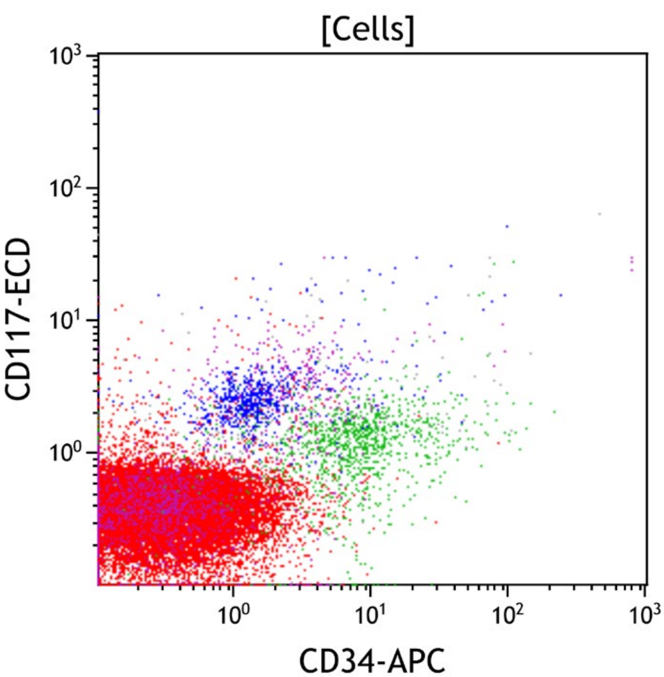

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors. The lymphocytes (red) do not express CD34.

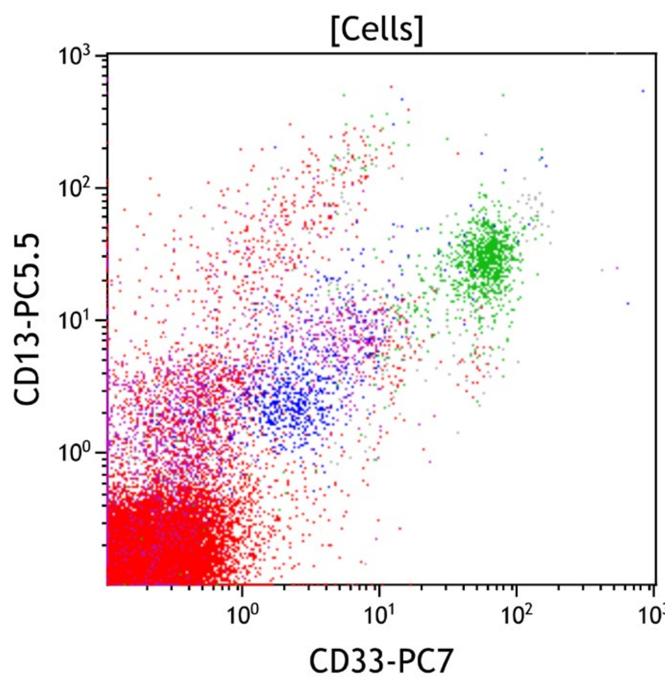

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red).

M2 Cell Tube

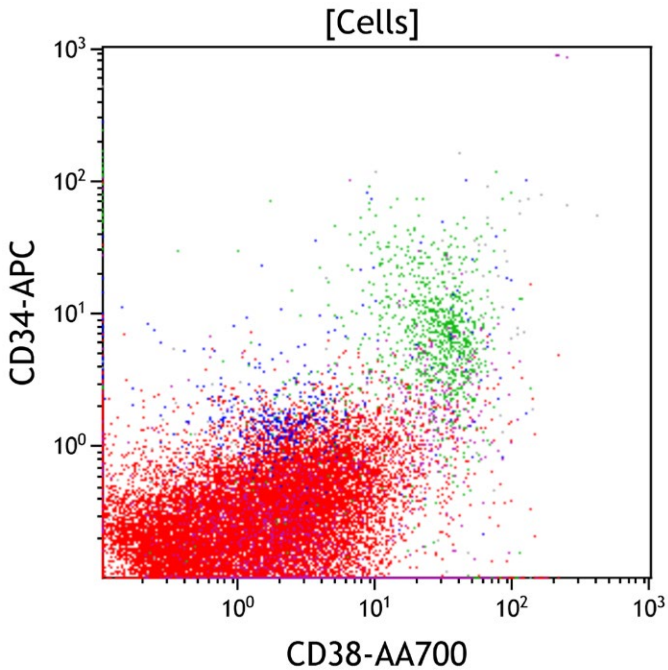

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Most of lymphocytes (red) express dim CD38 but do not express CD34.

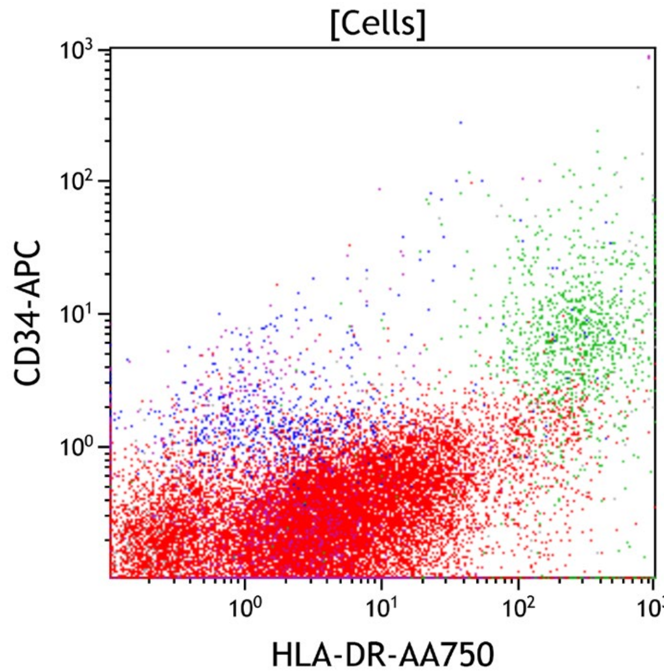

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. A major subset of lymphocytes (red) expresses dim HLA-DR.

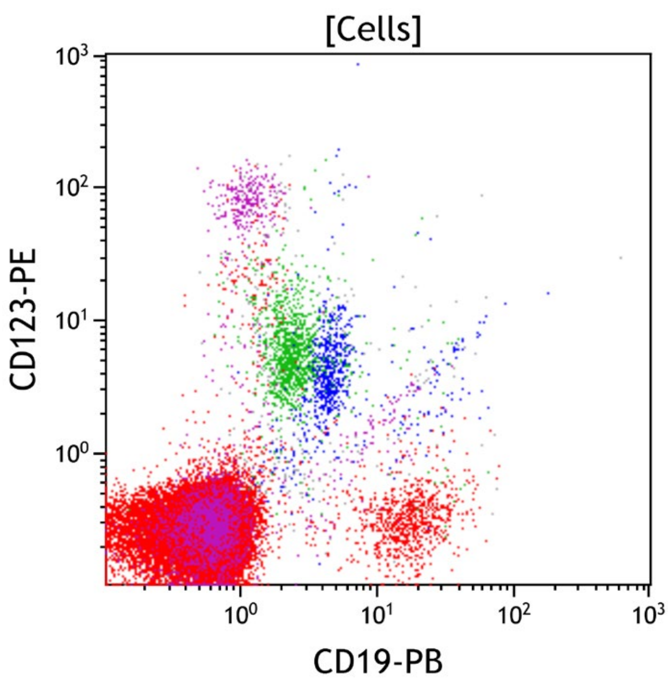

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. A small subset of lymphocytes (red) are CD19 positive B cells without expression of CD123.

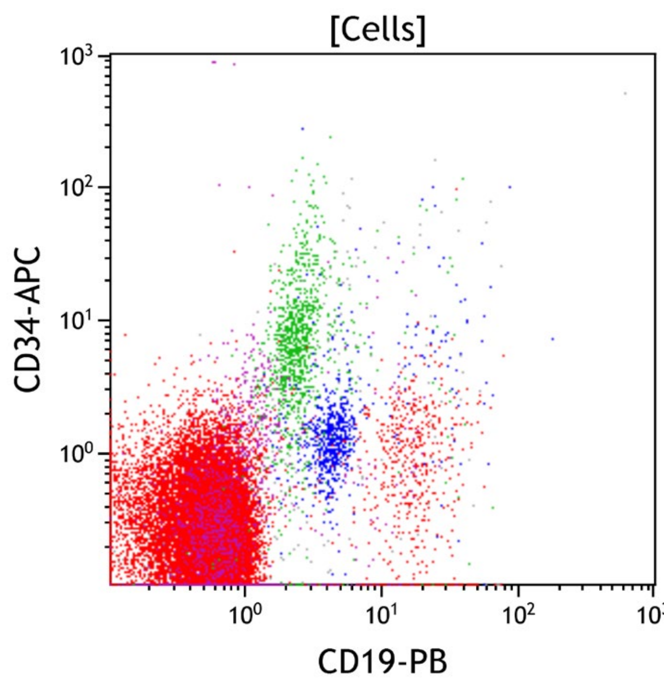

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. A small subset of lymphocytes (red) are CD19 positive B cells without expression of CD34.

M2 Cell Tube

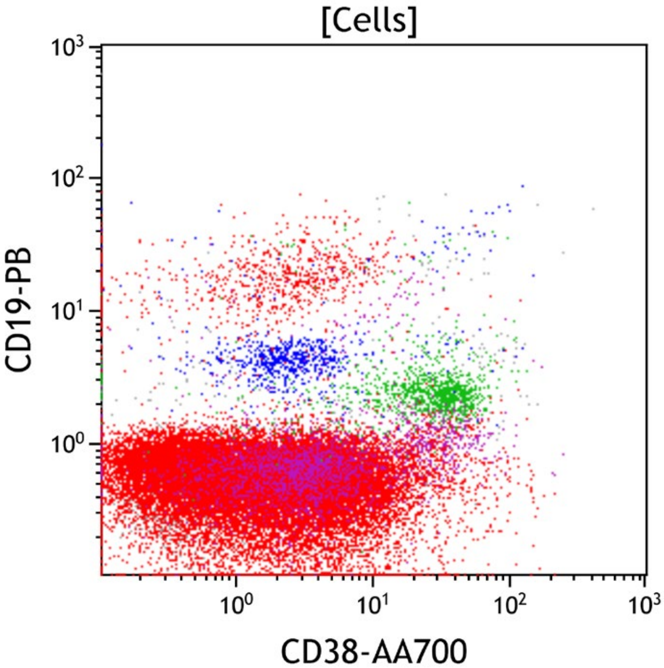

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38 (red left). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

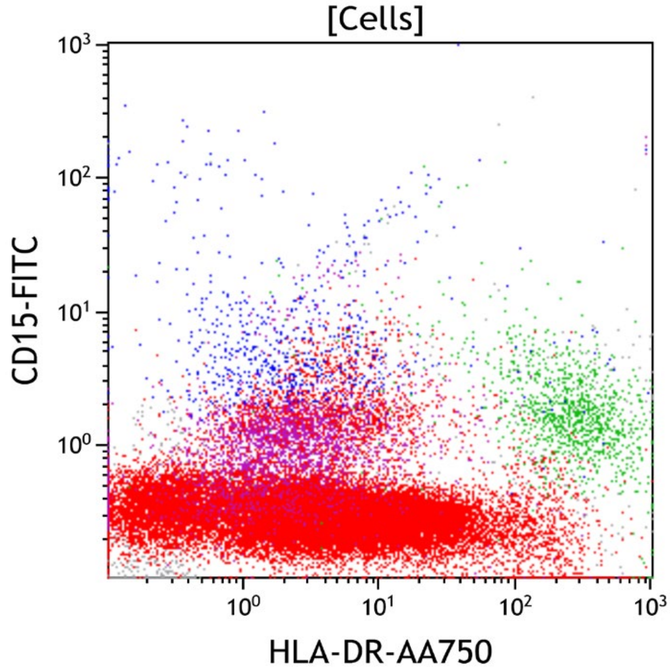

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR, except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. A subset of lymphocytes (red) variably expresses HLA-DR.

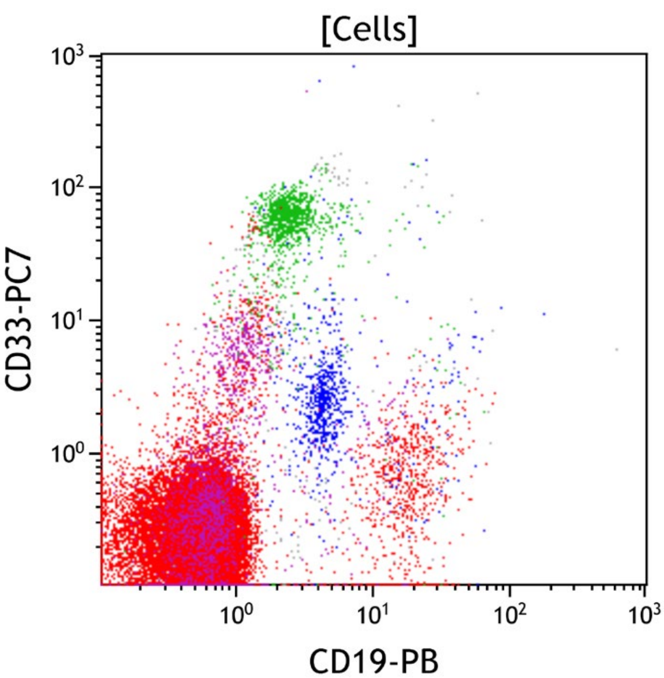

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. A small subset of lymphocytes (red) are CD19 positive B cells without expression of CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of variable CD2, intermediate CD3, variable CD5, variable CD7, bright CD8, bright CD45 and low CD56 on a subset. This population also likely expresses CD16 on a subset. Compared with normal mature T cells, the decreased expression of CD2, CD5 and CD7 with increased CD16 and CD56 is aberrant.

The immunophenotype of the abnormal population is consistent with expanded abnormal large granular lymphocytes of T cell type. In the appropriate clinical context of prolonged cytopenias without other explanation, this finding would be consistent with a large granular lymphoproliferative disorder.

Case #17: T–cell lymphoproliferative disorder

Clinical Vignette

This 62-year-old female presents with atypical cells on peripheral whole blood smear. A bone marrow aspirate sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

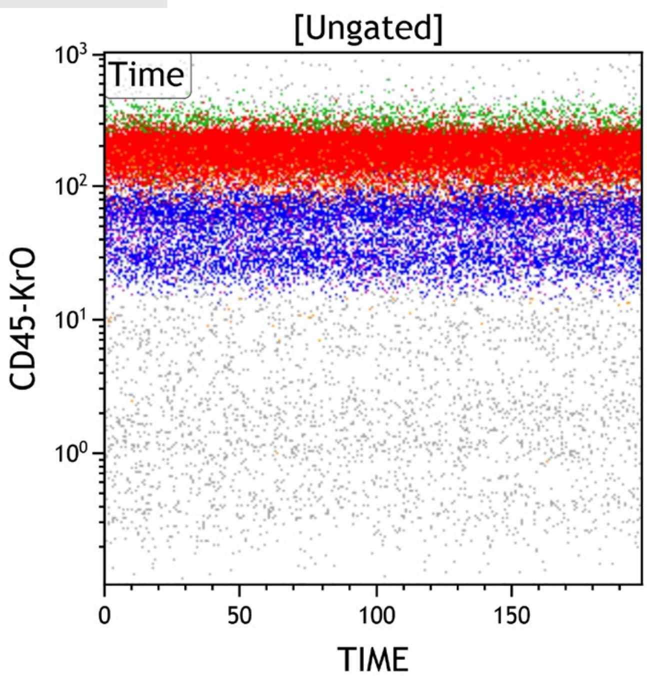

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

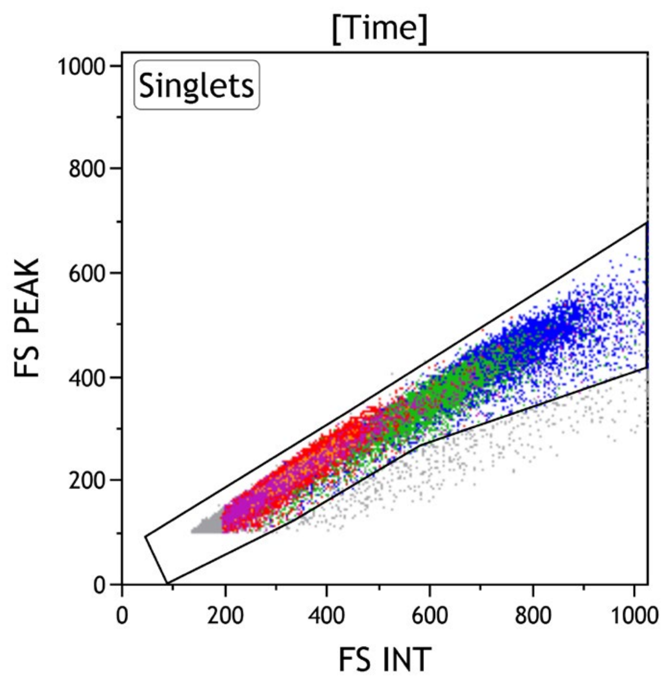

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

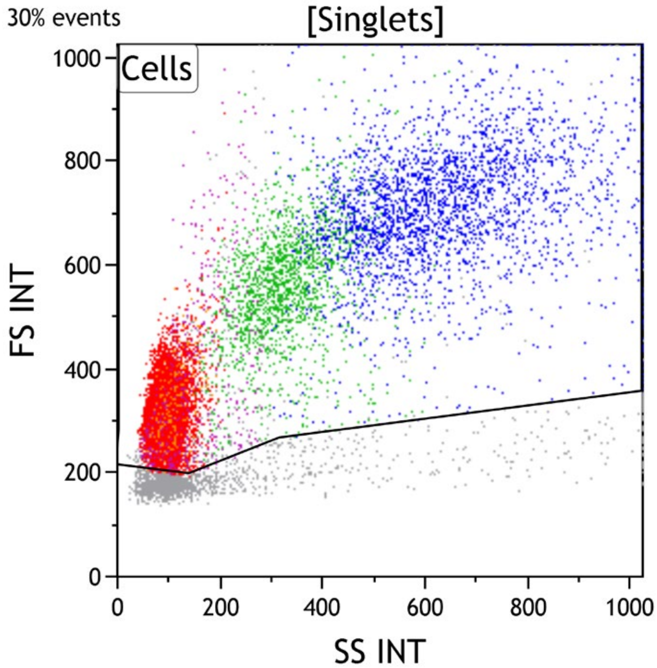

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

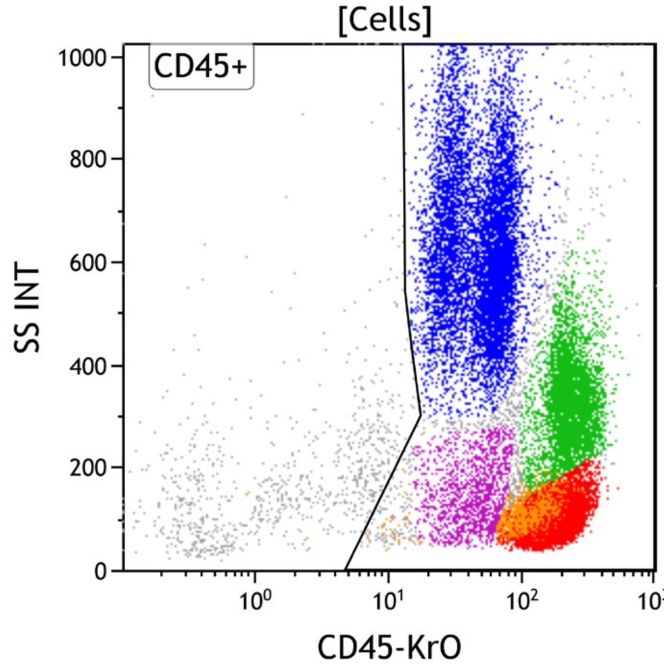

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

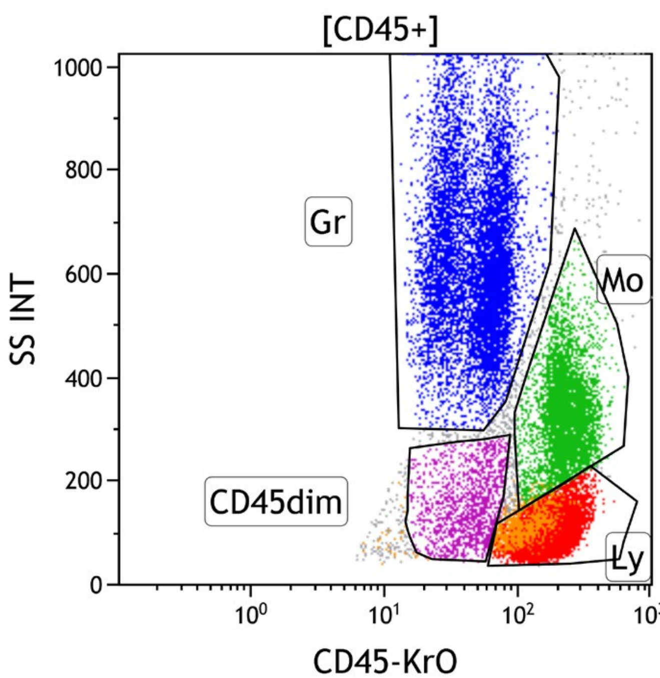

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note the relatively increased number of lymphocytes (red).

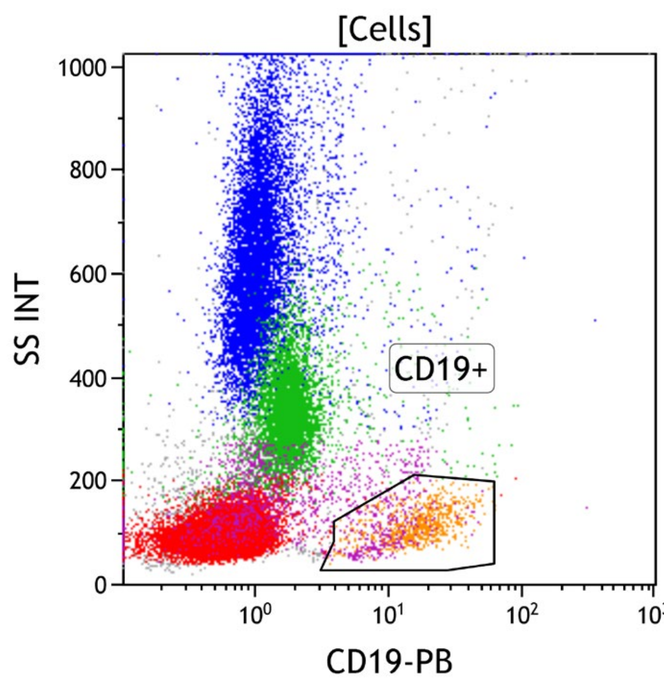

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. A minority of the lymphocytes (red) express CD19 (orange).

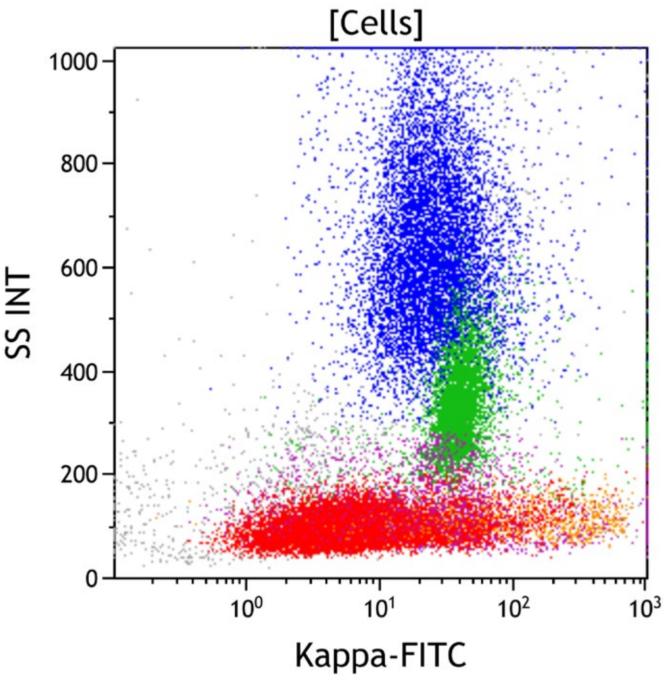

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The lymphocytes (red) show variably increased background for kappa light chains.

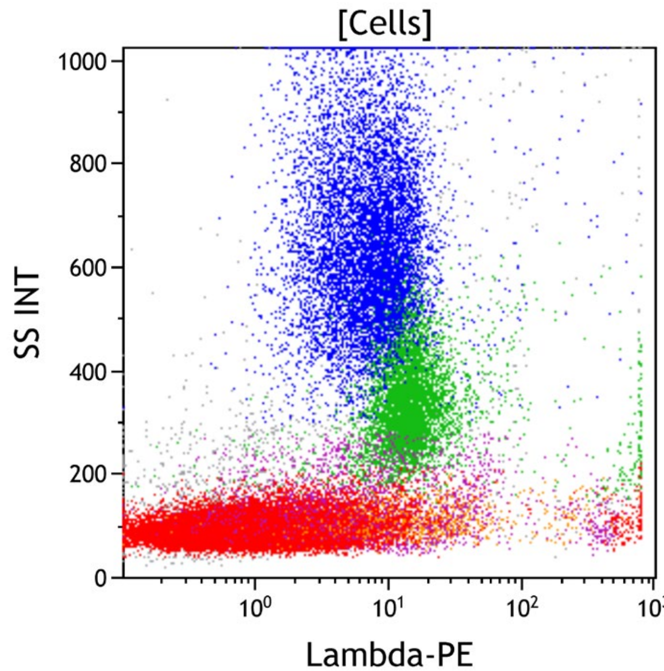

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The lymphocytes (red) show variably increased background for lambda light chains.

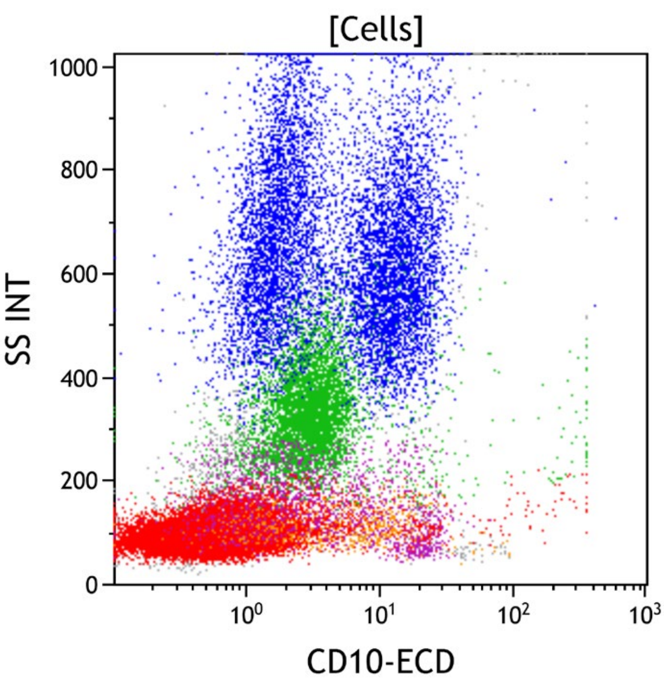

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) do not express CD10.

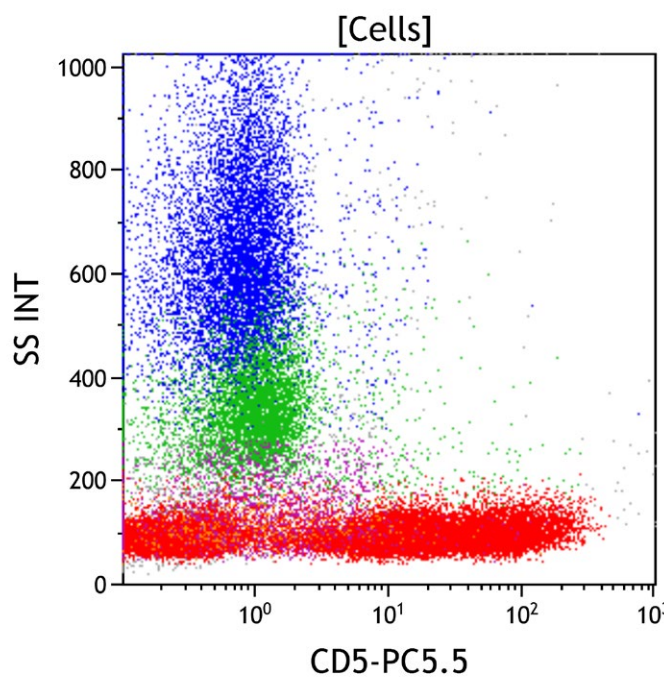

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. A subset of lymphocytes (red) express decreased to absent CD5.

B Cell Tube

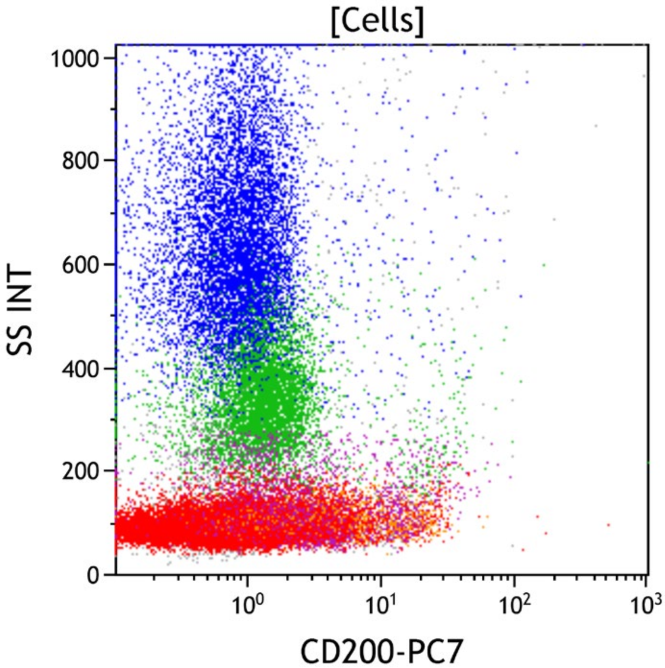

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The lymphocytes do not express CD200, the apparent low level expression is increased compensation background from CD5.

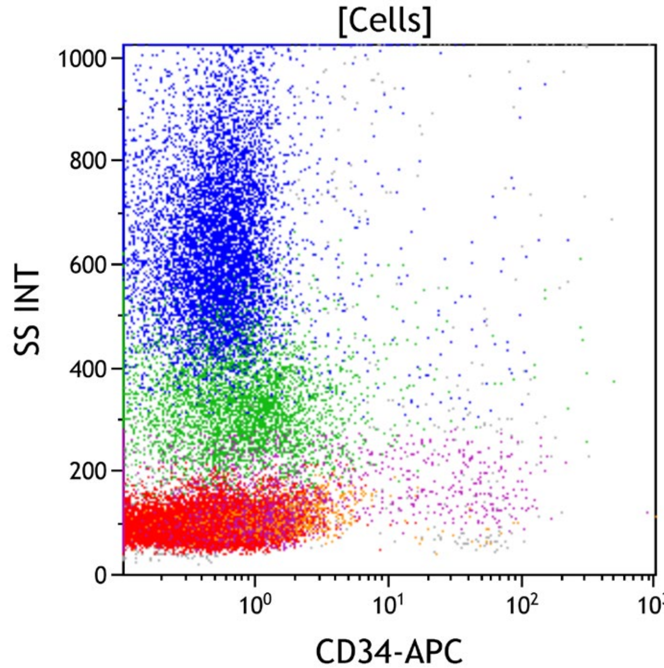

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The mature lymphocytes (red) do not express CD34.

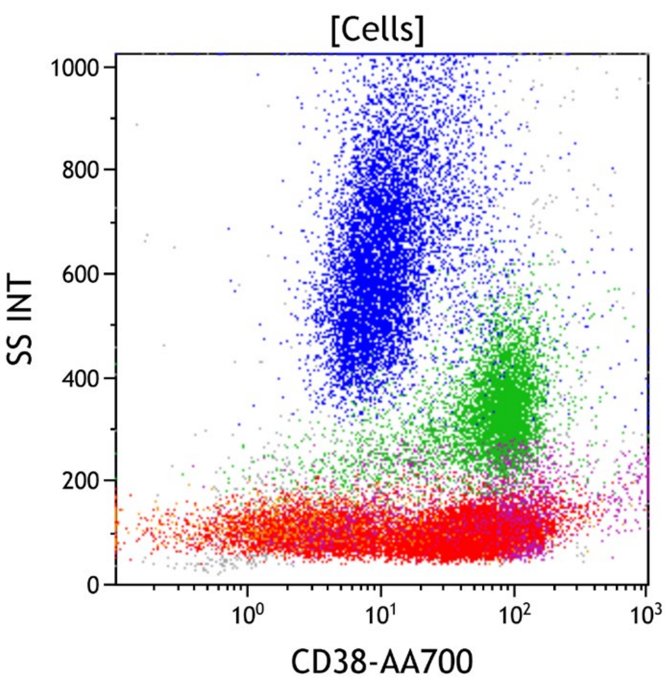

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Most of the lymphocytes (red) variably express CD38.

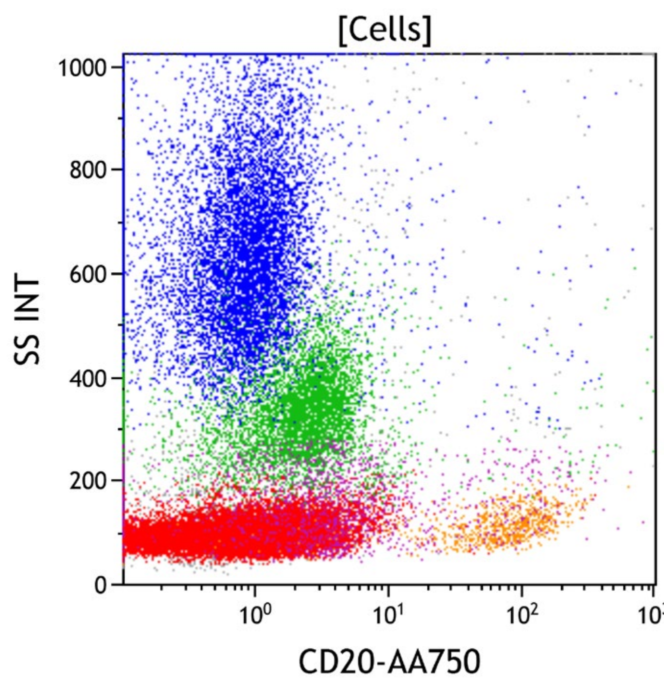

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The mature lymphocytes (red) do not express CD20, the apparent dim CD20 is due to increased compensation background from CD38.

B Cell Tube

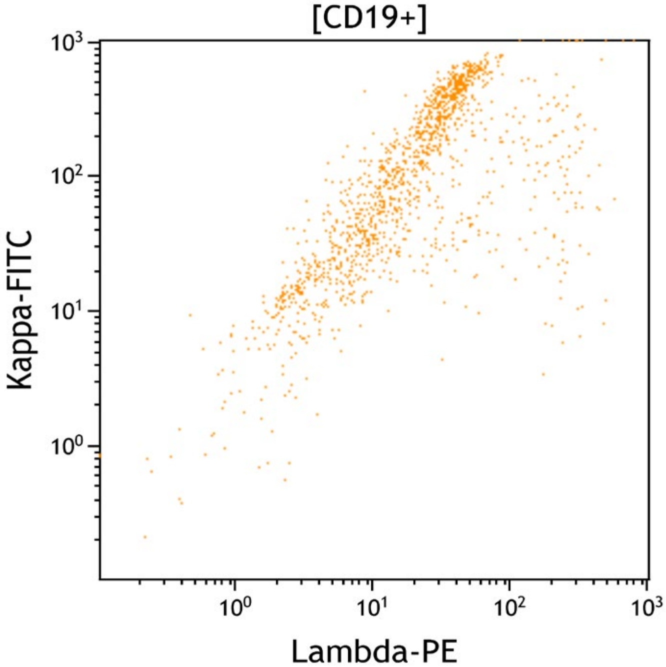

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

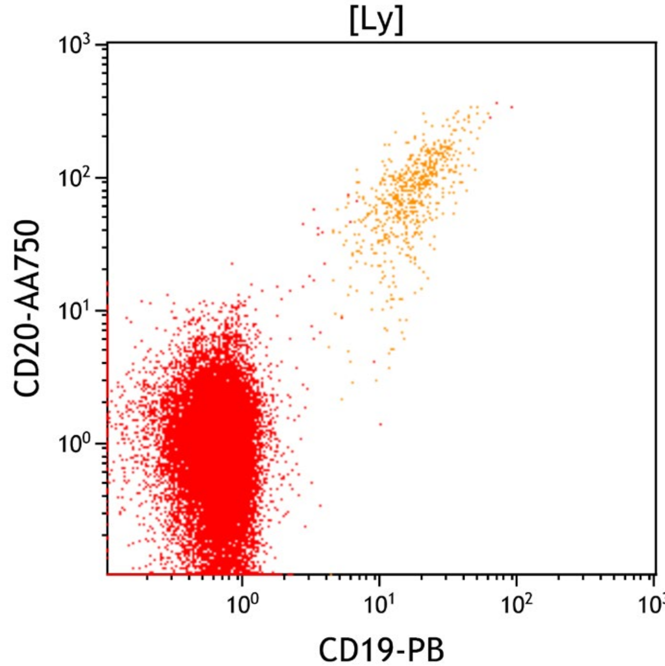

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate. Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

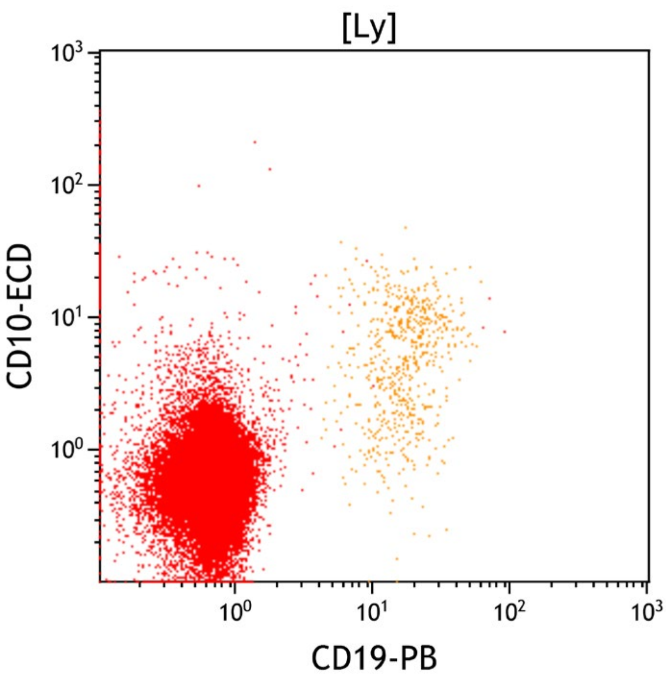

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

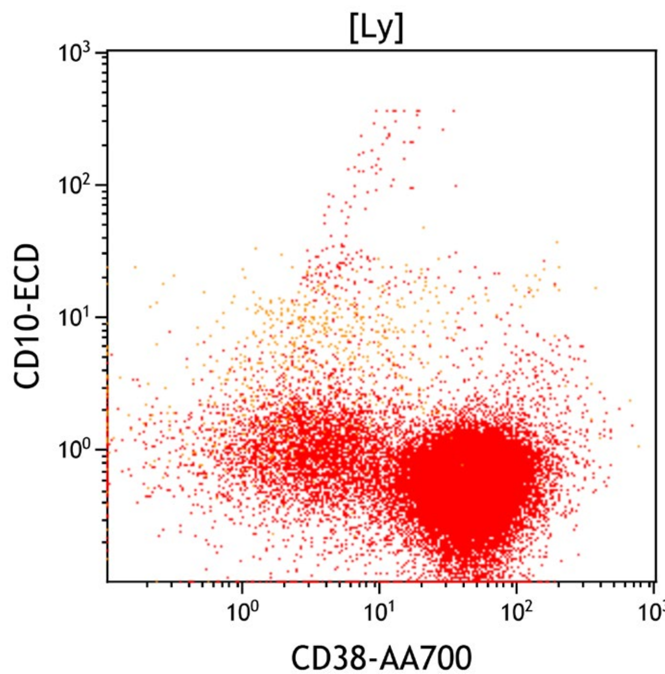

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate. CD38 is variably expressed on most of the lymphocytes (red).

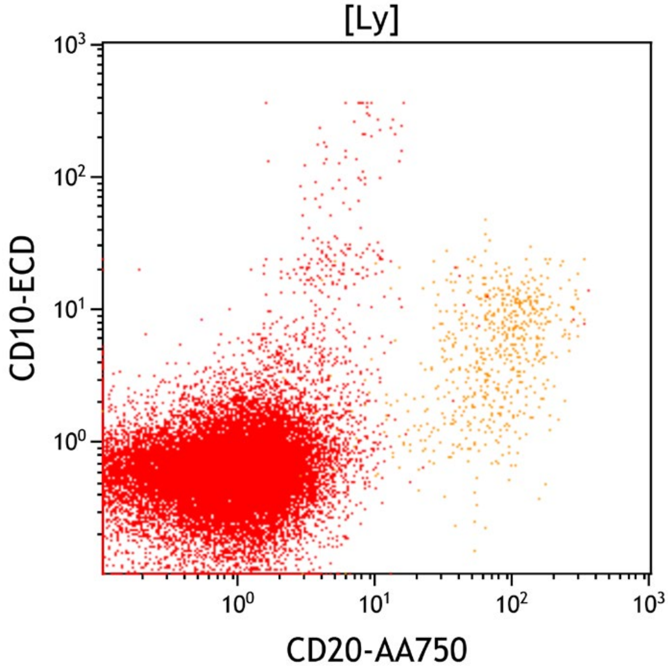

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20.

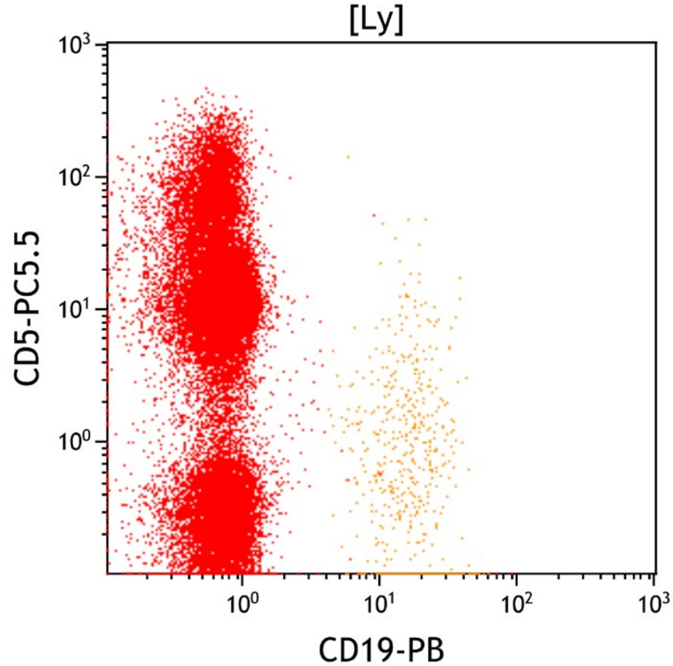

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells. CD5 is variably expressed on non-B cell lymphocytes.

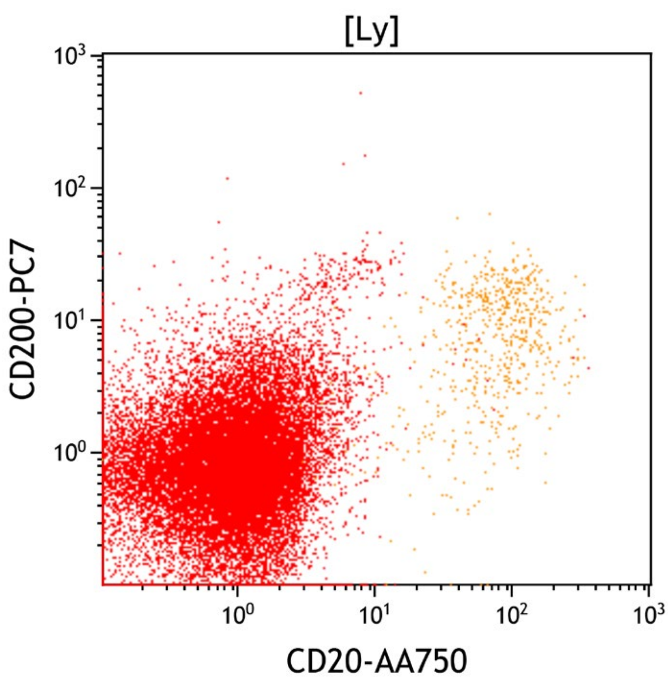

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange). The lymphocytes do not express CD200, the apparent low level expression is increased compensation background from CD5.

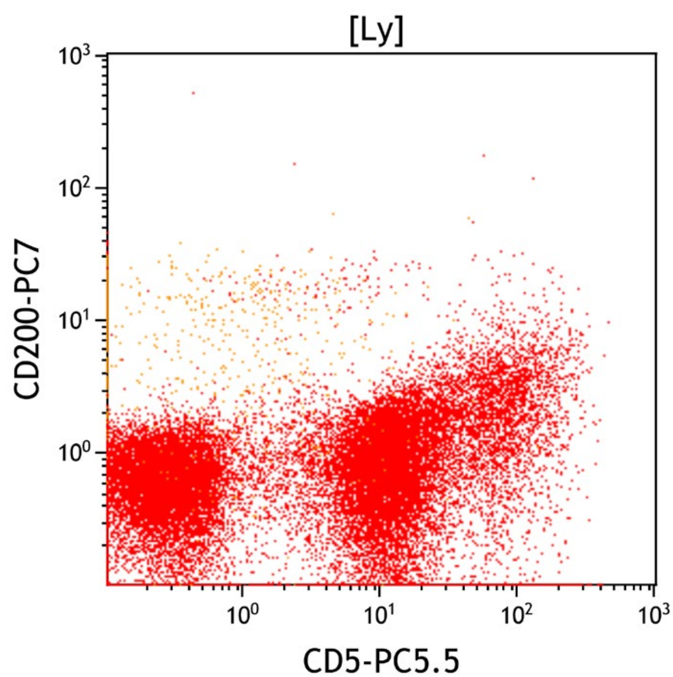

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. The lymphocytes do not express CD200, the apparent low level expression is increased compensation background from CD5.

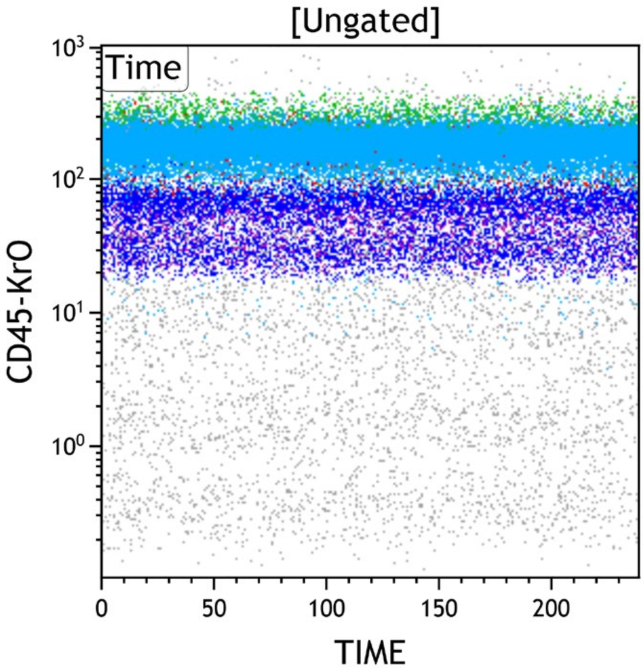

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

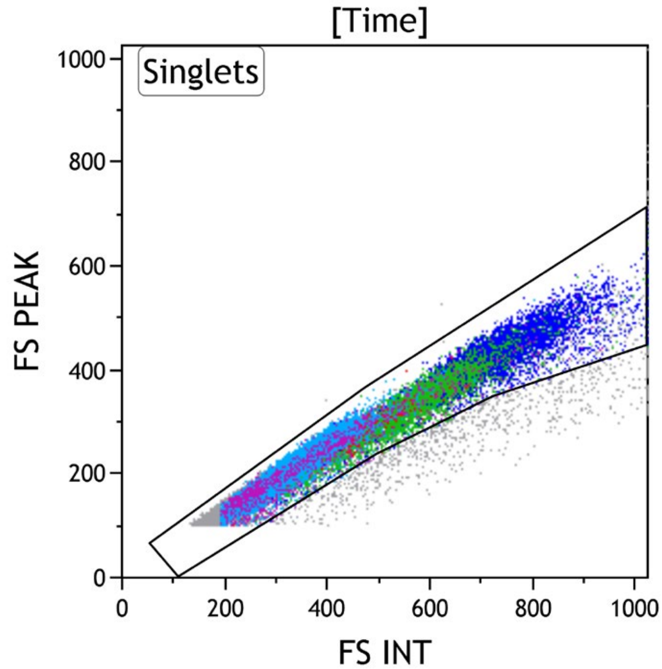

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

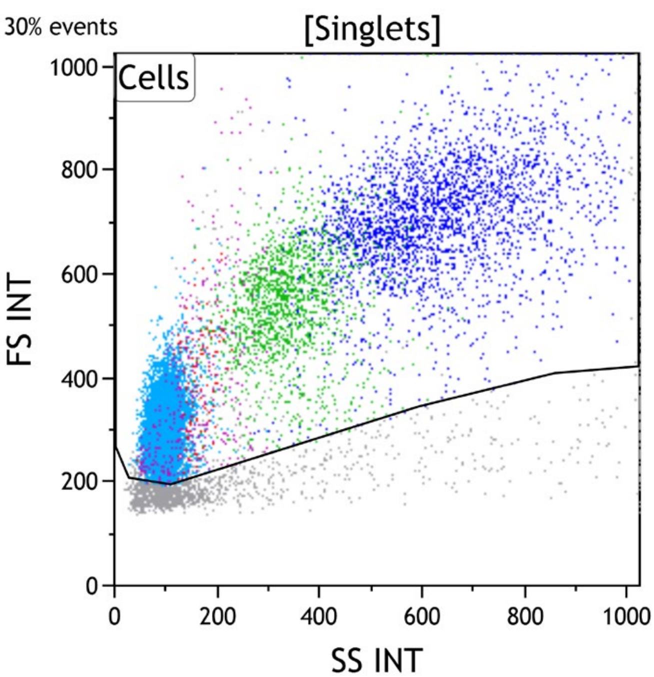

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

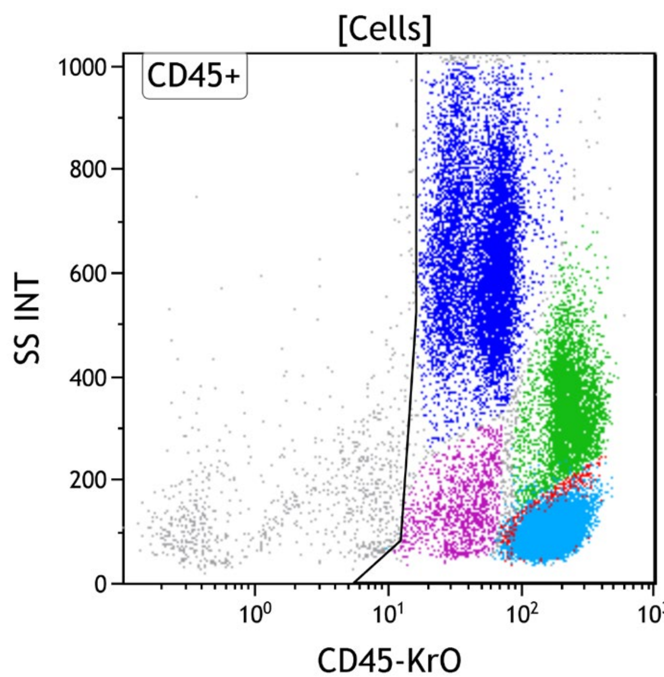

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

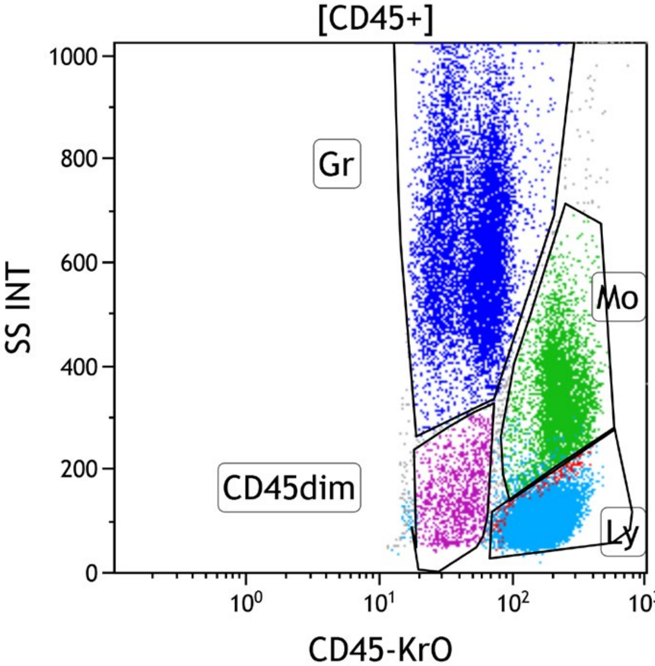

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased lymphocytes (red and aqua).

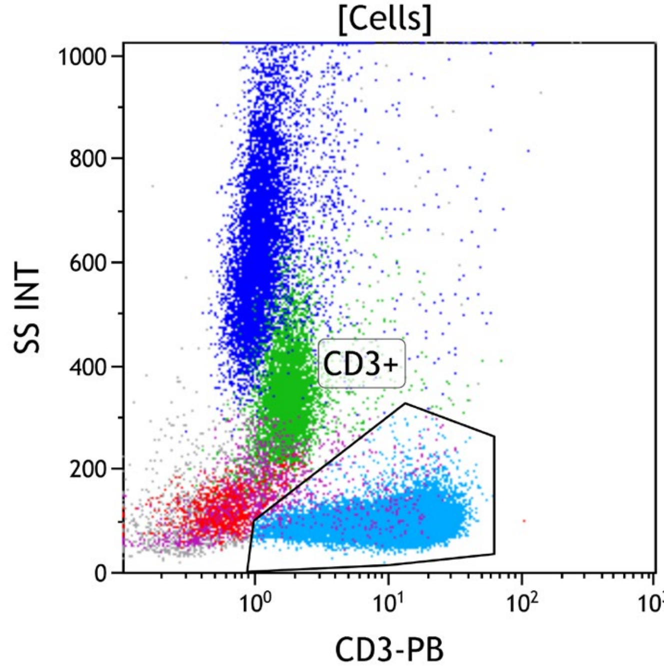

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. A subset of T cells (aqua) expresses decreased CD3.

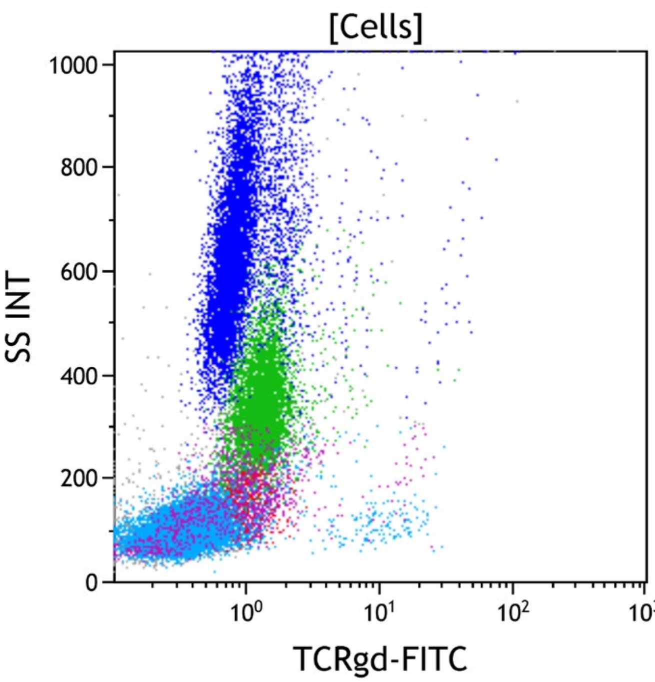

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ i s a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). T cells (aqua) express TCRγδ only rarely.

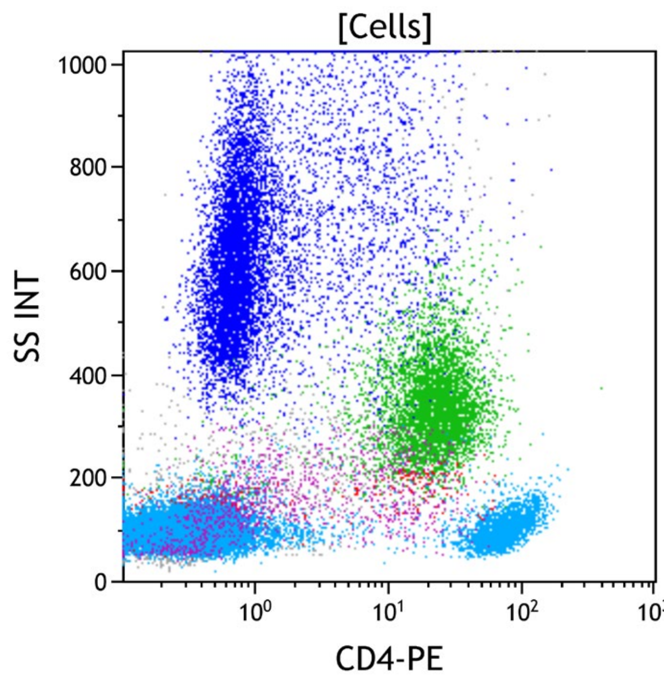

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. CD4-positive T cells represent the minority of T cells (aqua).

T Cell Tube

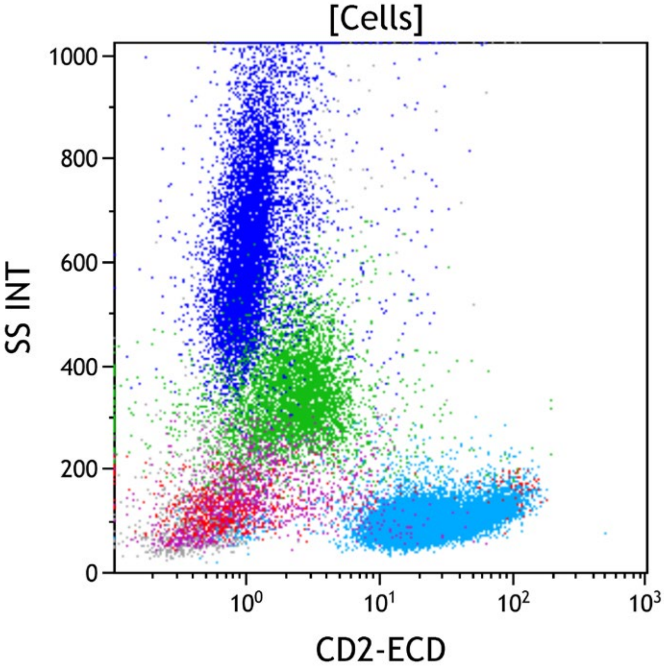

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The T cells (aqua) show variably decreased expression of CD2.

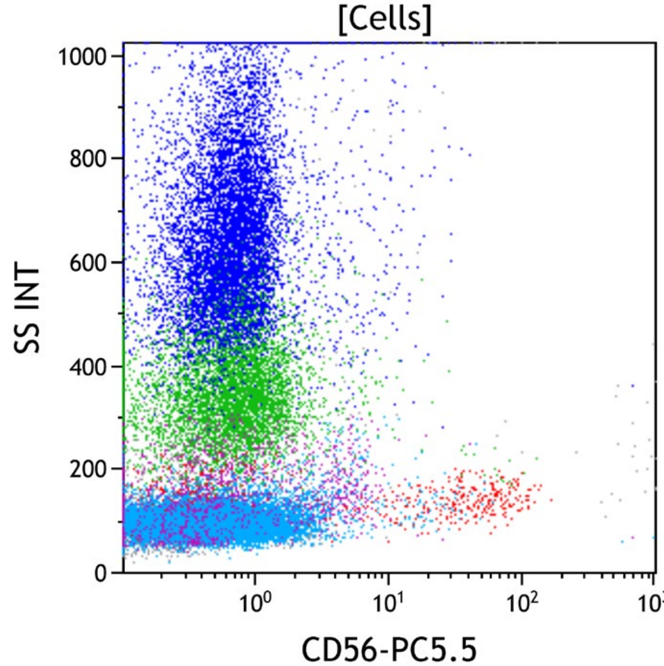

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. Most T cells (aqua) do not express CD56.

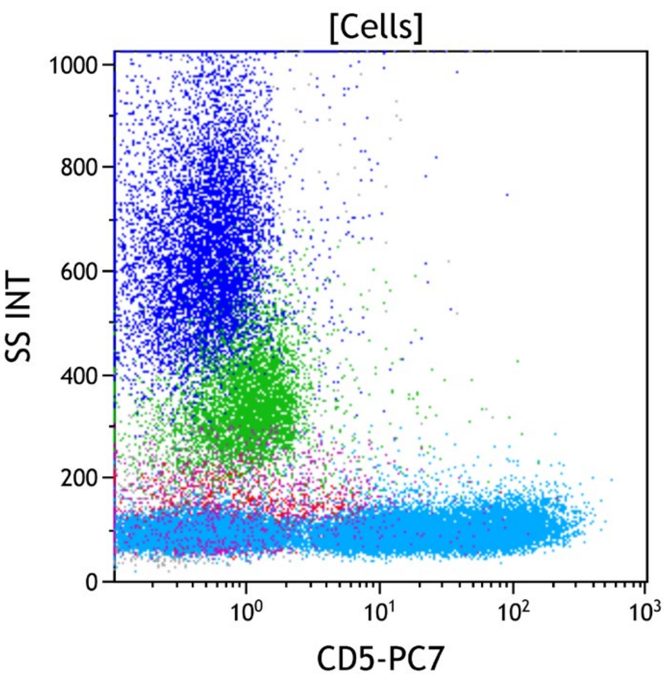

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. A major subset of T cells (aqua) have variably decreased CD5 expression.

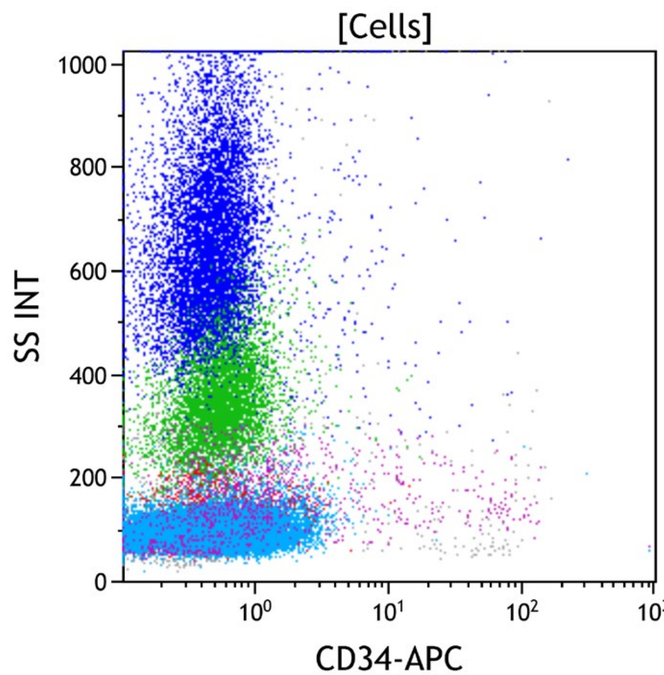

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. T cells (aqua) do not express CD34.

T Cell Tube

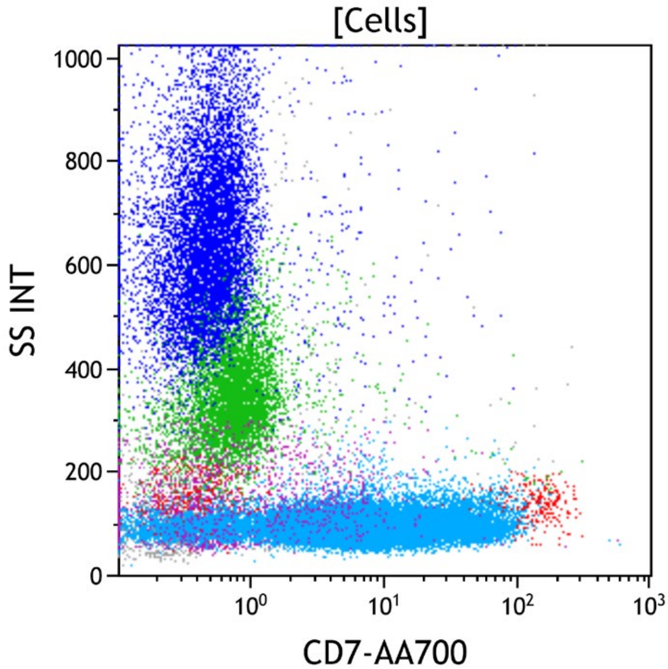

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. T cells (aqua) have variably decreased CD7 expression.

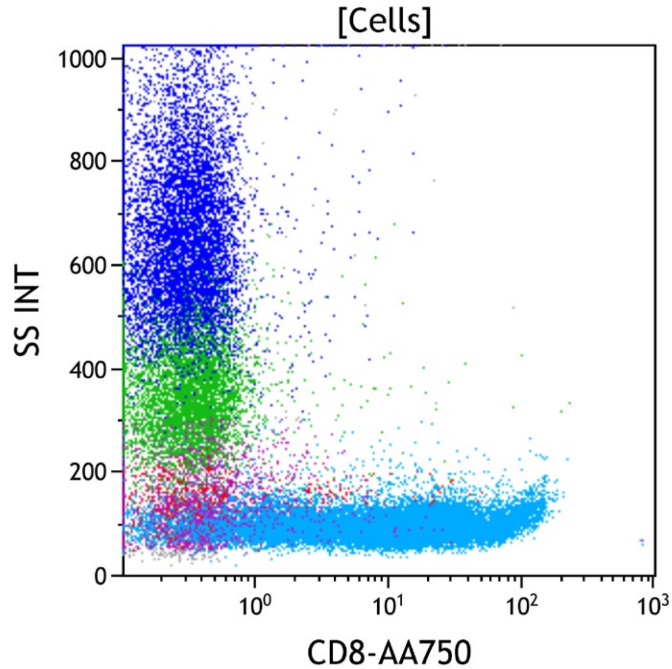

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. Most T cells (aqua) have variably decreased CD8 expression.

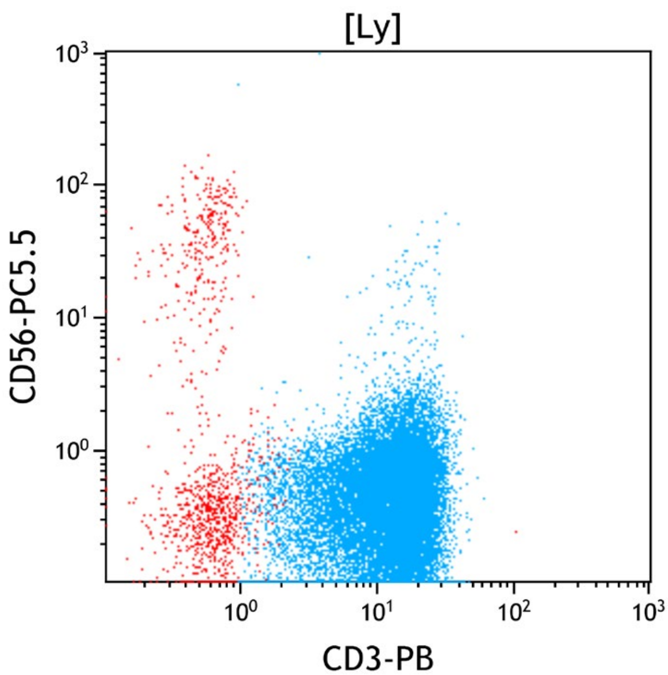

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells. Rare T cells (aqua) express CD56.

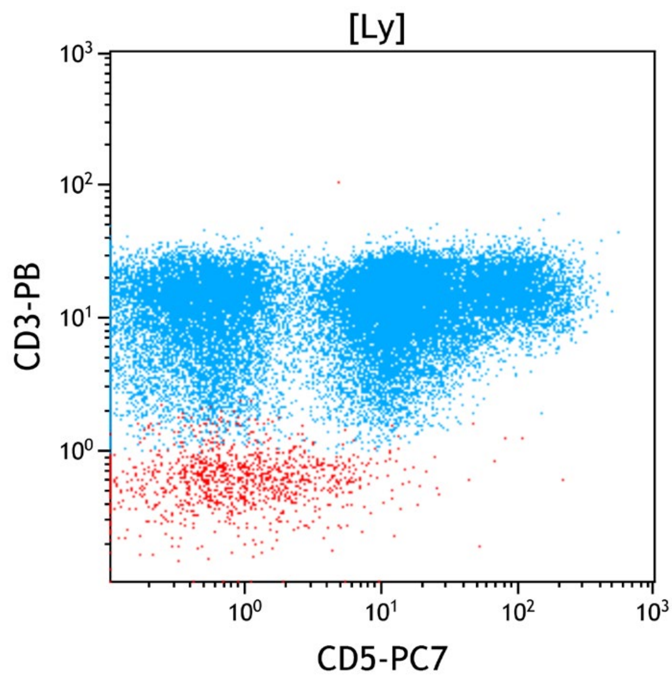

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red). A large subset of T cells (aqua) shows both decreased or absent CD5 expression and variably decreased CD3.

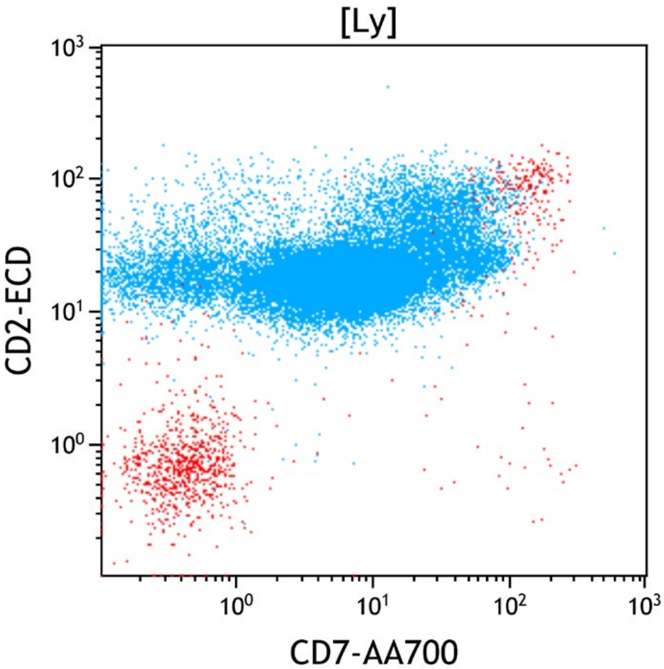

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right). A large subset of T cells (aqua) shows both decreased CD2 and variably decreased CD7 expression.

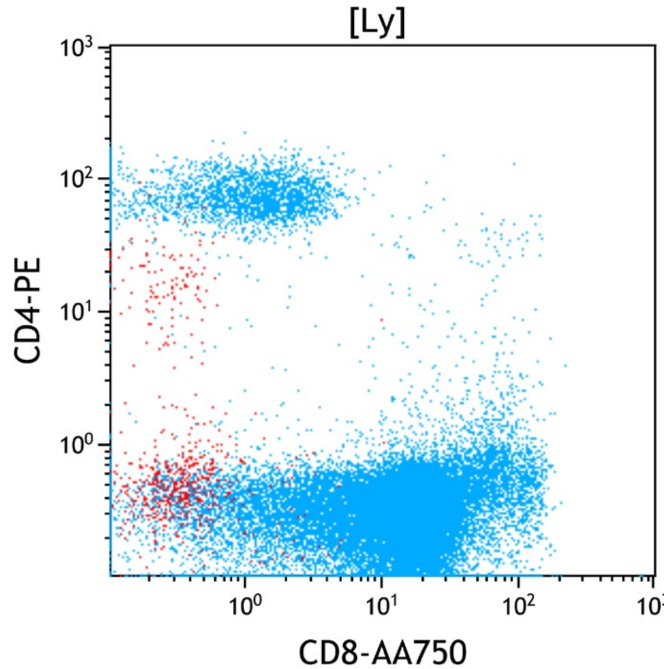

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells (red, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification. A large subset of T cells (aqua) expresses decreased CD8 without CD4.

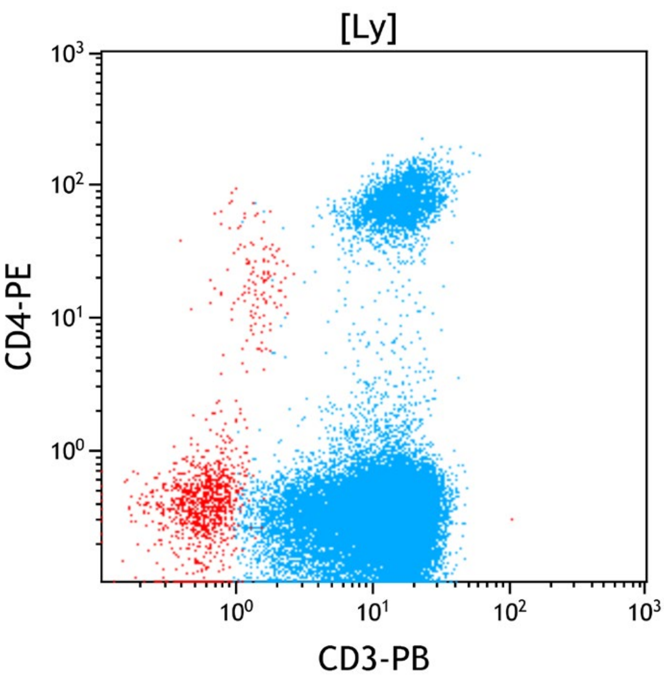

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. A smaller subset of T cells (aqua) express CD4 with normal expression of CD3.

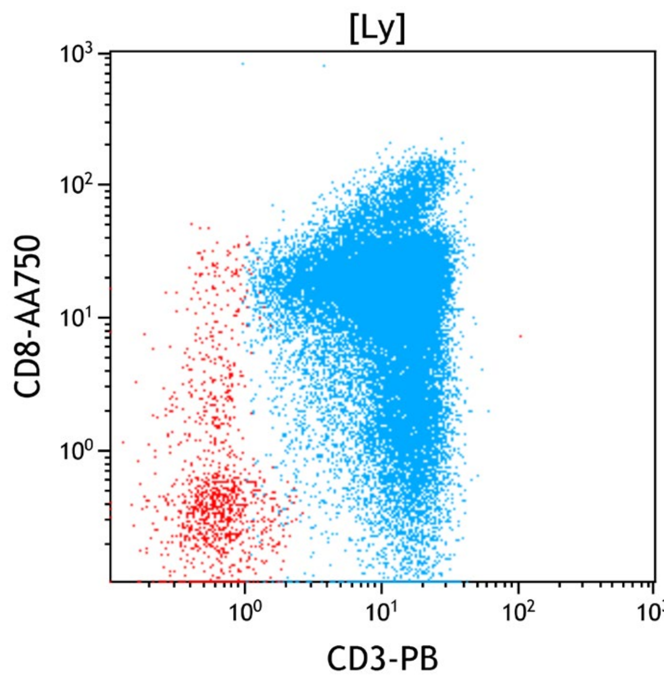

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3. Most T cells (aqua) express variably decreased CD8 with variably decreased CD3.

T Cell Tube

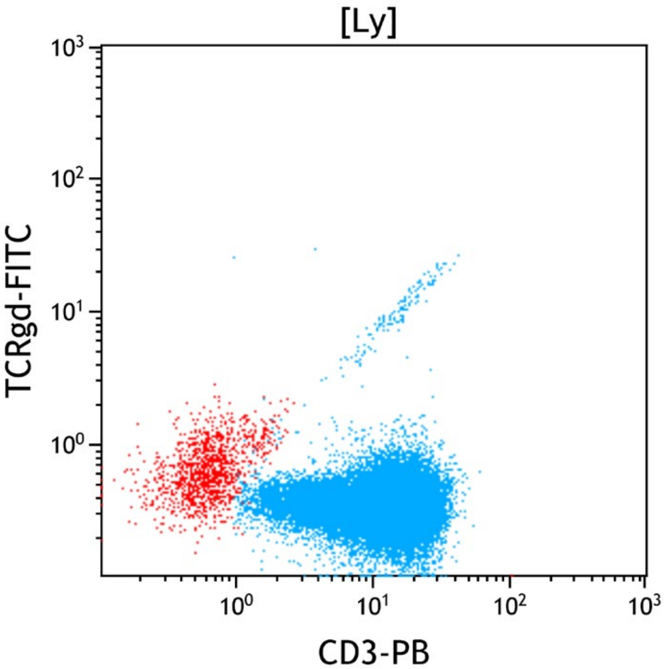

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other. Most T cells (aqua) express variably decreased CD3 without TCRγδ.

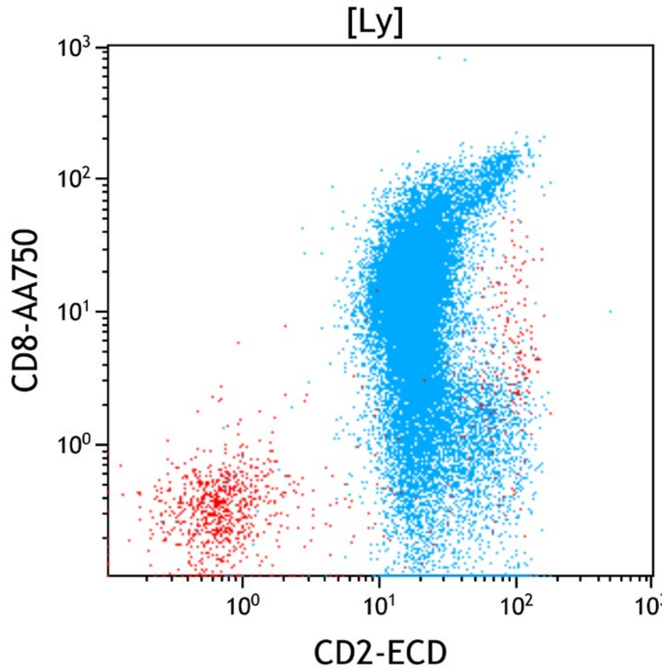

Figure 22. This CD2 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8-negative T cells (aqua, lower right), a large subset of CD8 positive T cells (aqua, upper right) show aberrantly decreased CD8 and CD2 expression.

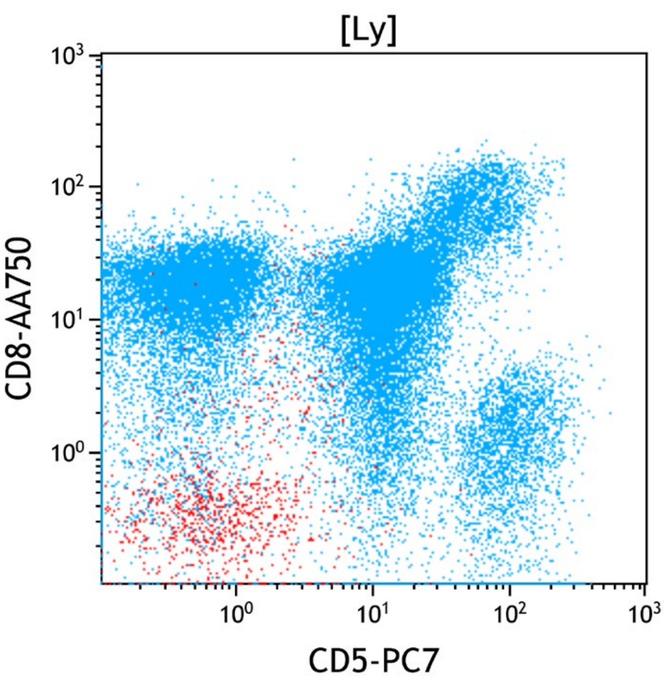

Figure 23. This CD5 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8-negative T cells (aqua, lower right), a large subset of CD8 positive T cells (aqua, upper) show aberrantly decreased CD8 expression and decreased to absent CD5 expression.

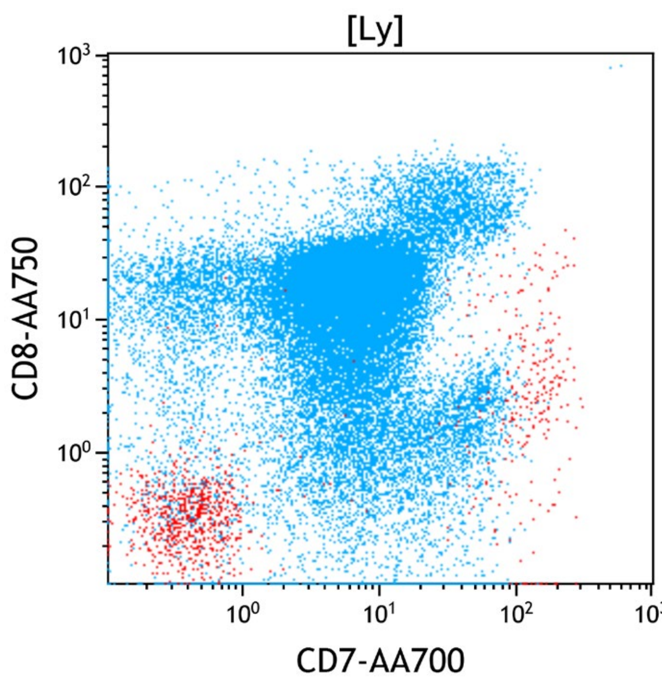

Figure 24. This CD7 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8-negative T cells (blue, lower right), a large subset of CD8 positive T cells (aqua, upper) show aberrantly decreased CD8 expression and decreased to absent CD7 expression.

M1 Cell Tube

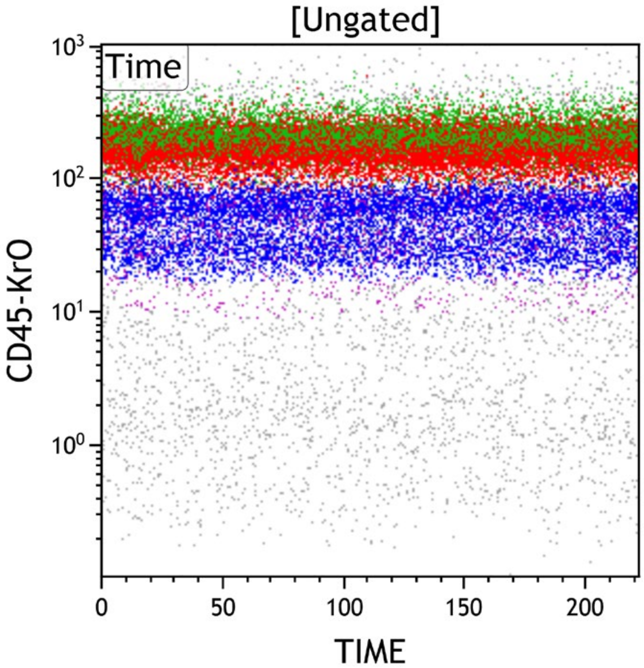

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

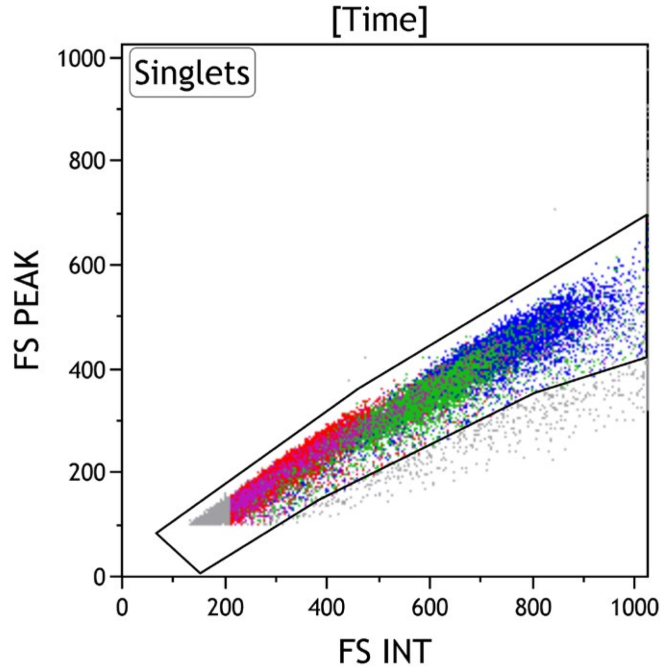

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

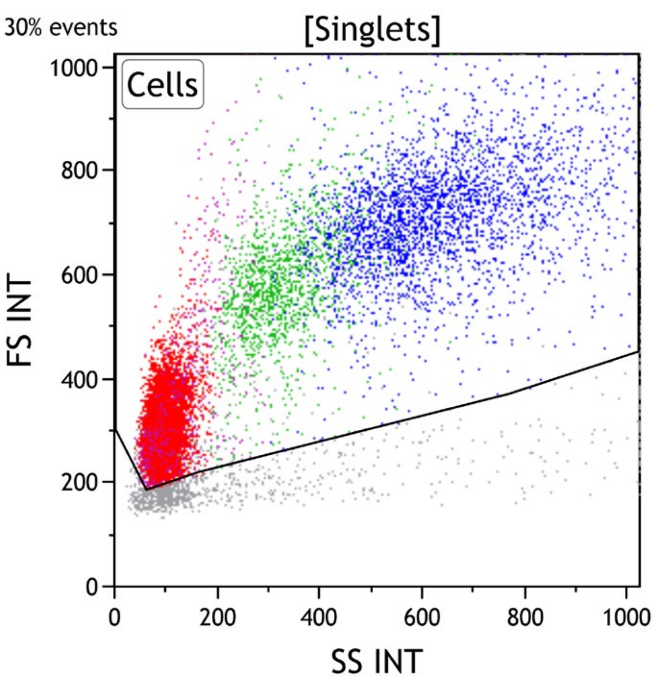

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

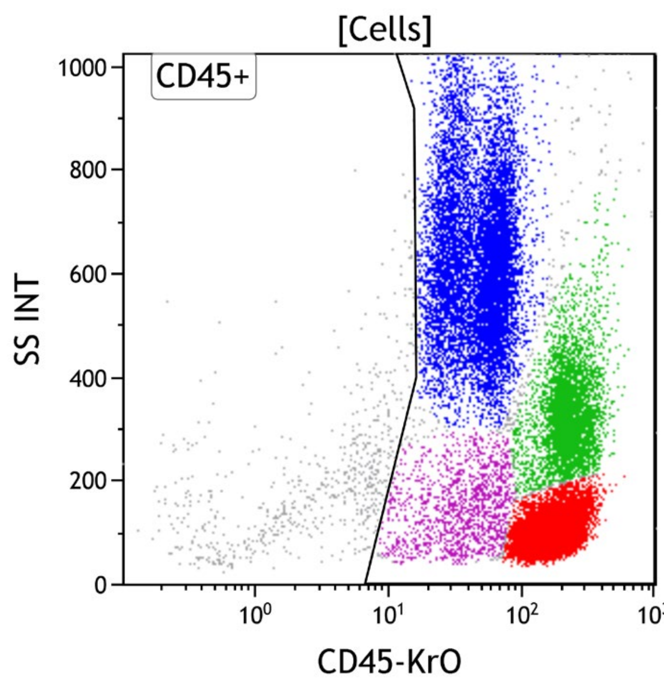

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

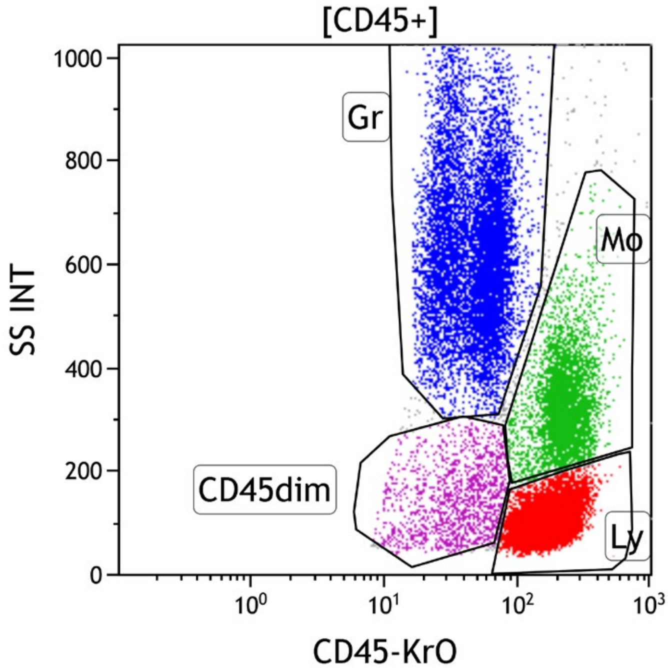

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased number of lymphocytes (red).

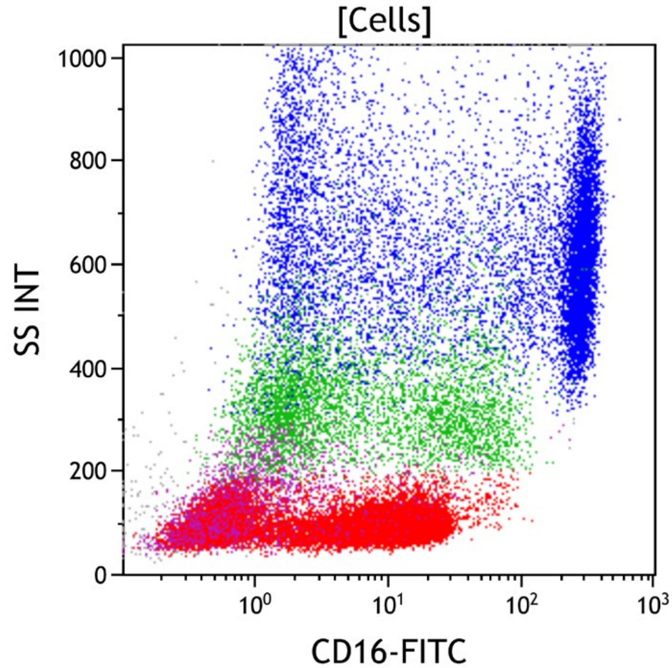

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). Dim to intermediate CD16 is expressed on most lymphocytes (red).

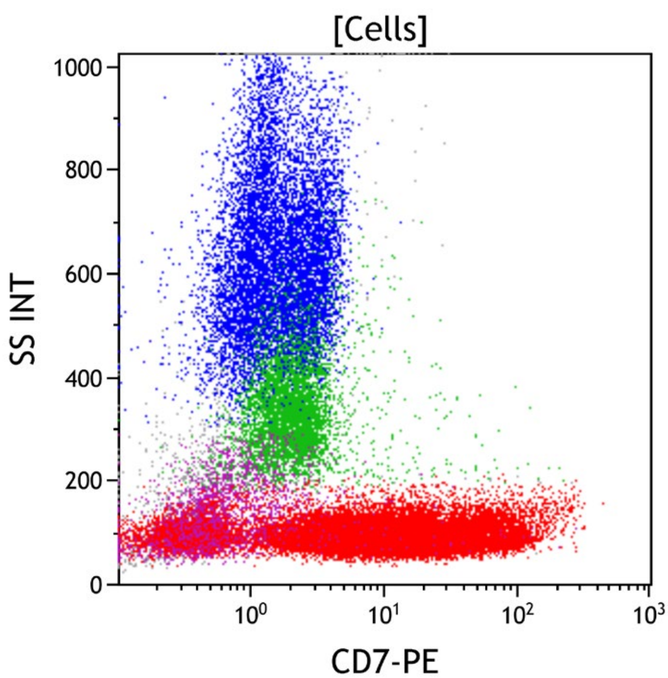

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors (purple). CD7 is variably expressed on most lymphocytes (red).

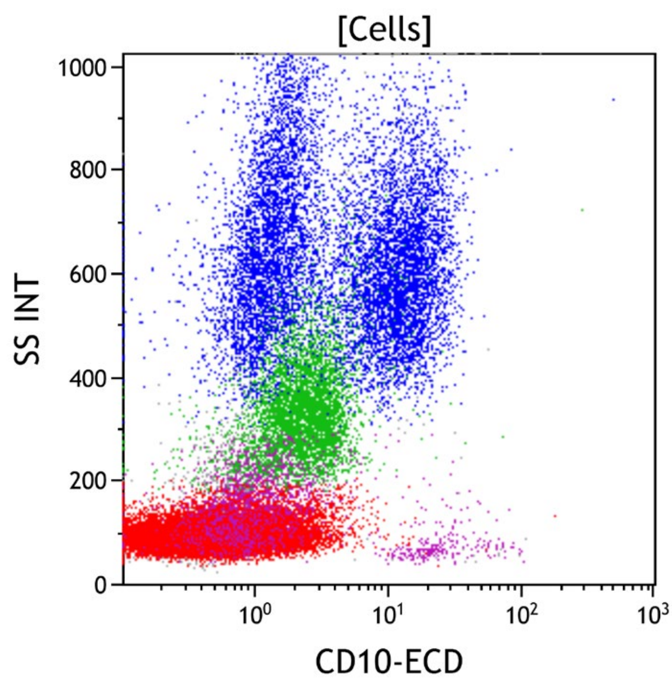

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Mature lymphocytes (red) do not express CD10.

M1 Cell Tube

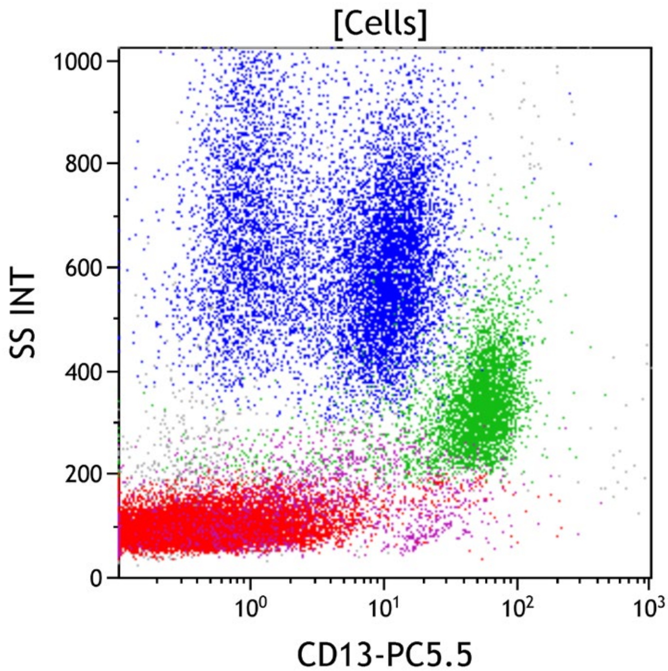

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple). Lymphocytes (red) do not express CD13.

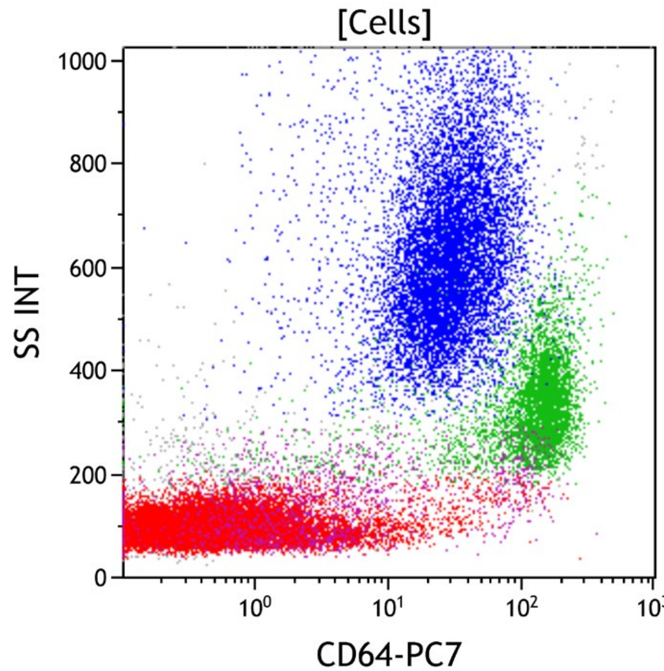

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. Lymphocytes (red) do not express CD64.

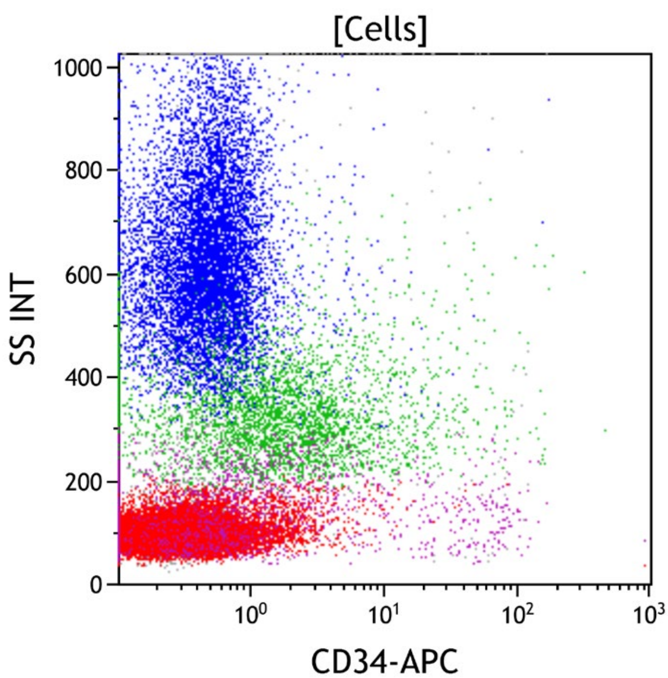

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. Lymphocytes (red) do not express CD34.

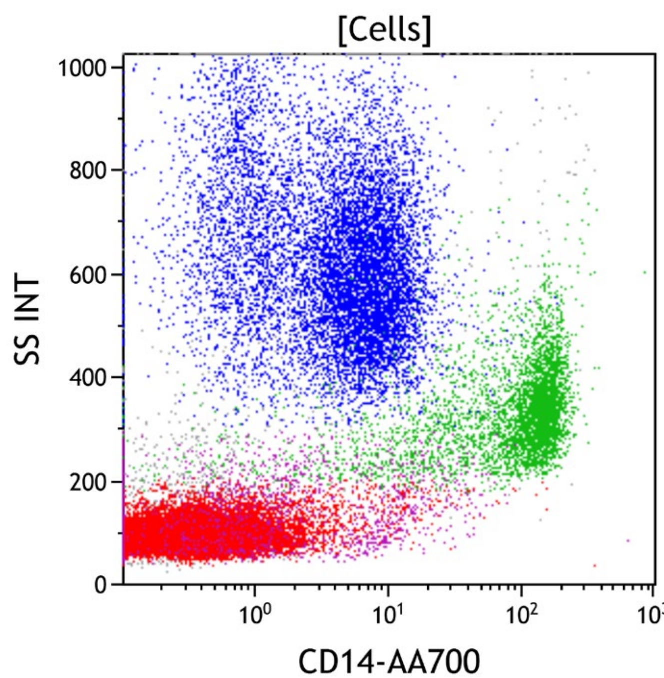

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Lymphocytes (red) do not express CD14.

M1 Cell Tube

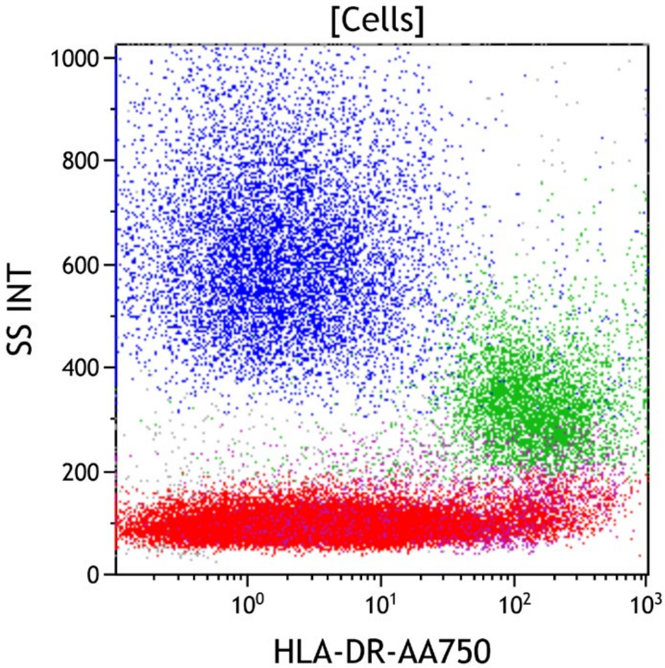

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red). Lymphocytes (red) variably express HLA-DR.

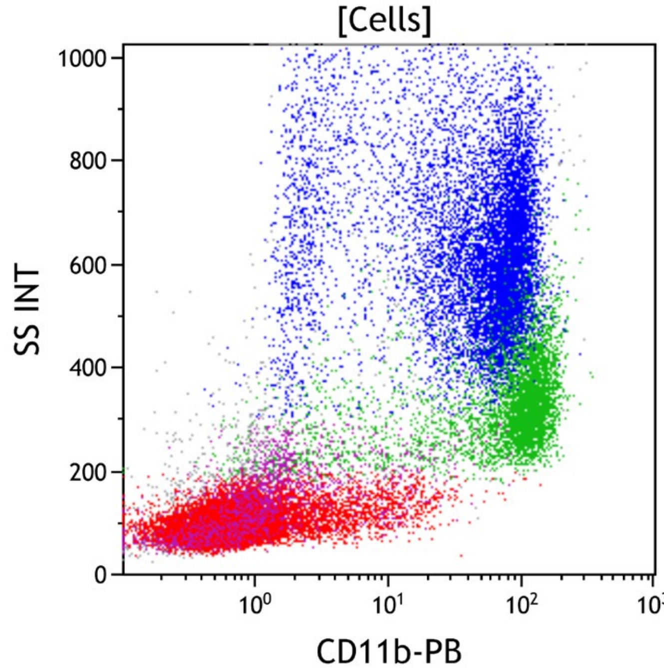

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple). Most lymphocytes (red) do not express CD11b.

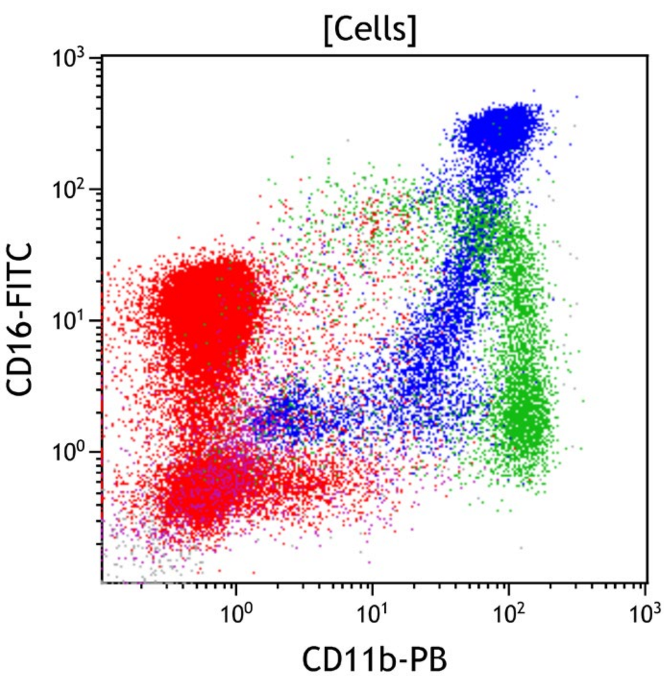

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. A major subset of lymphocytes (red) expresses CD16 without CD11b.

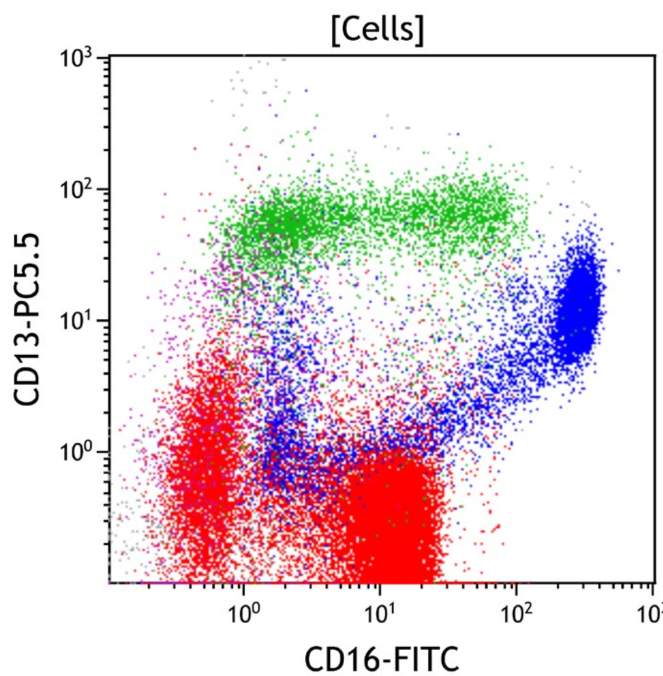

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. A major subset of lymphocytes (red) expresses CD16 without CD13.

M1 Cell Tube

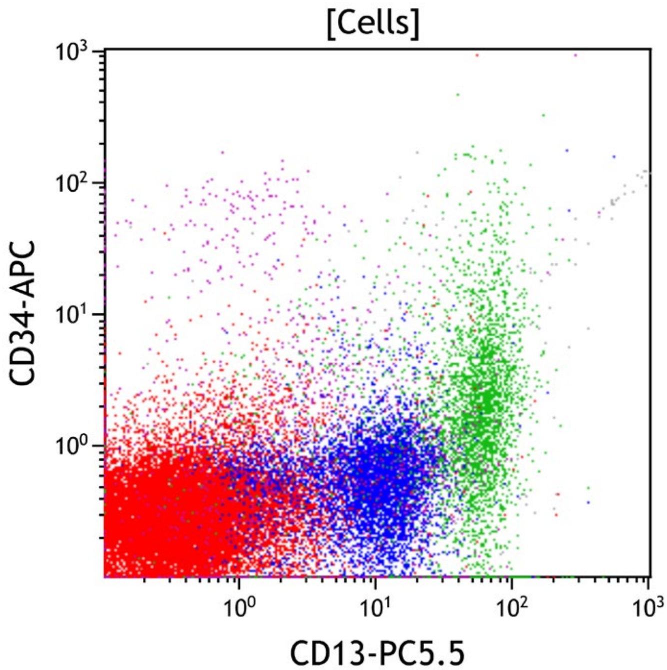

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphoid cells (red).

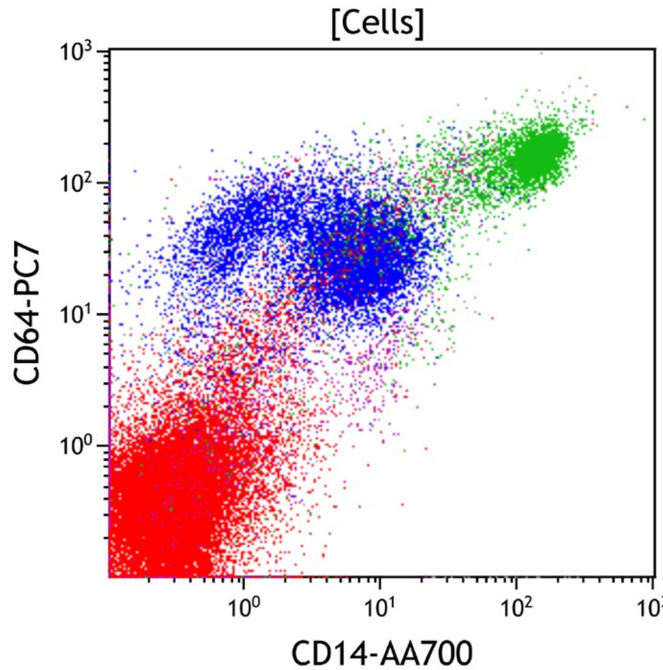

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64 (green). Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes.

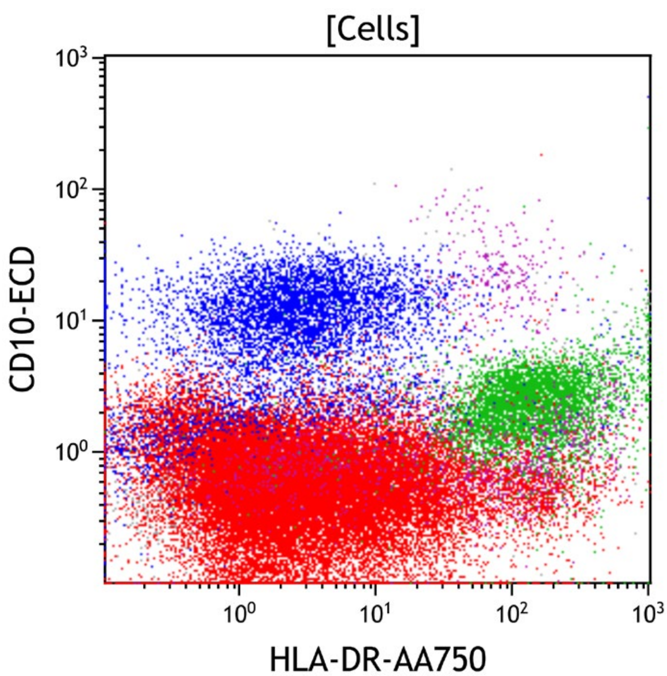

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple). Most lymphocytes (red) variably express HLA-DR.

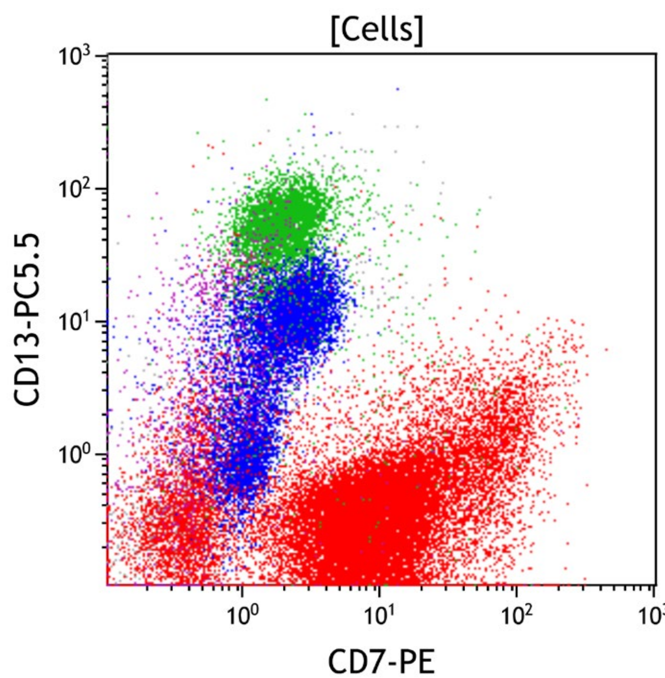

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen. Most lymphocytes (red) express variably decreased CD7 without CD13.

M2 Cell Tube

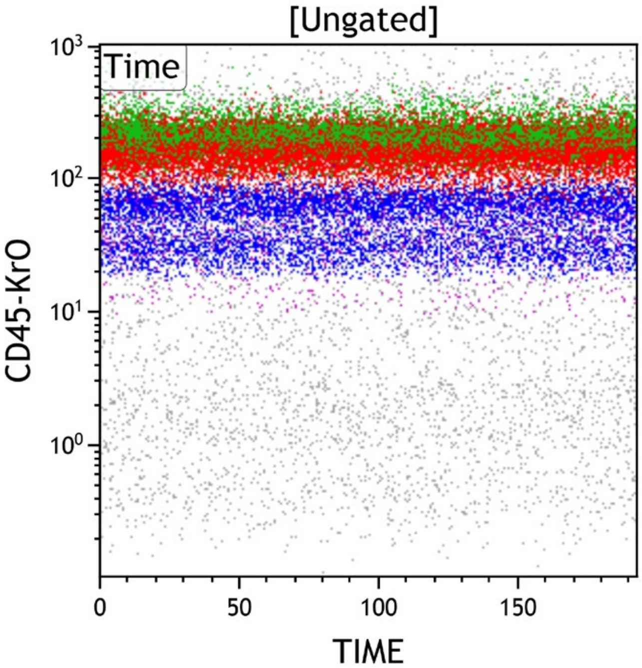

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

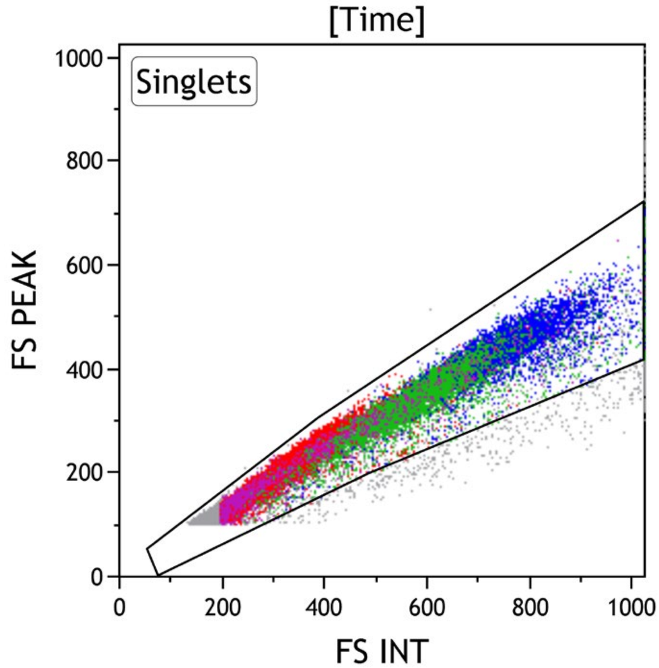

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

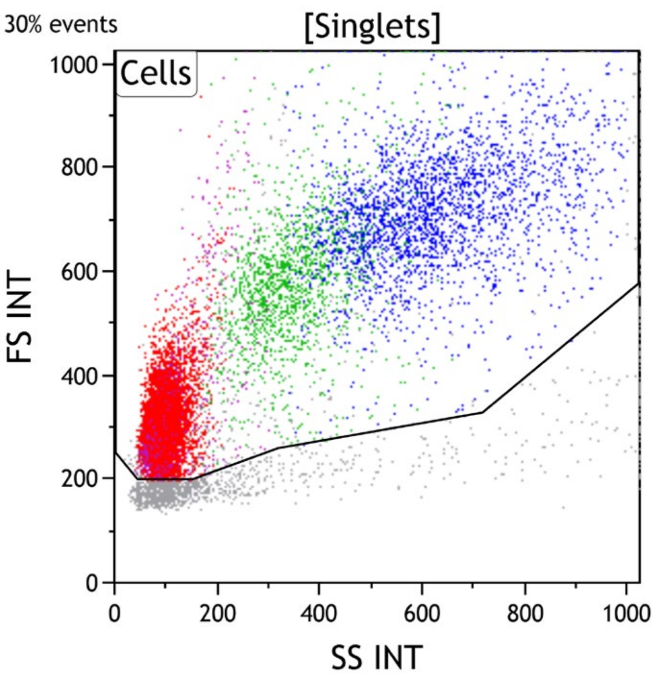

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

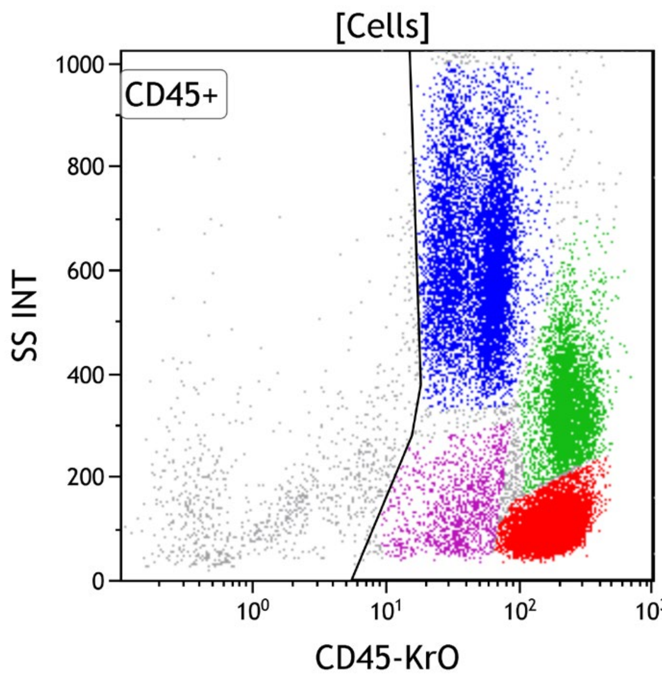

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

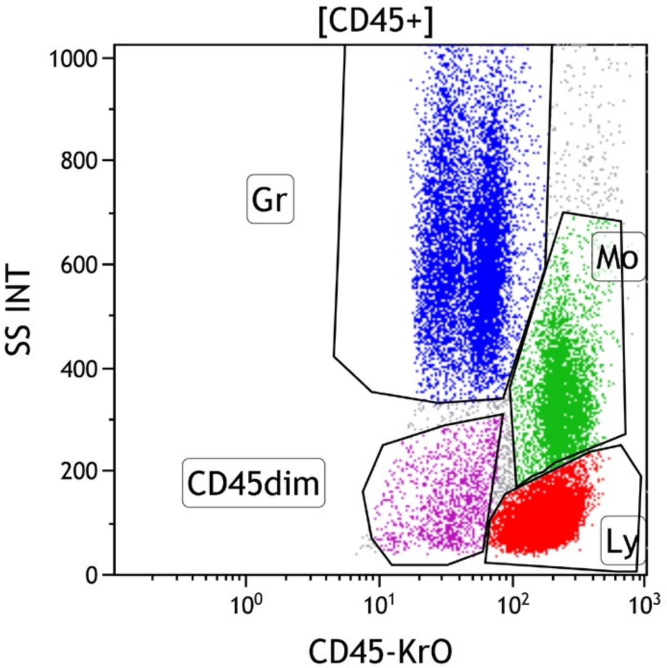

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased lymphocytes (red).

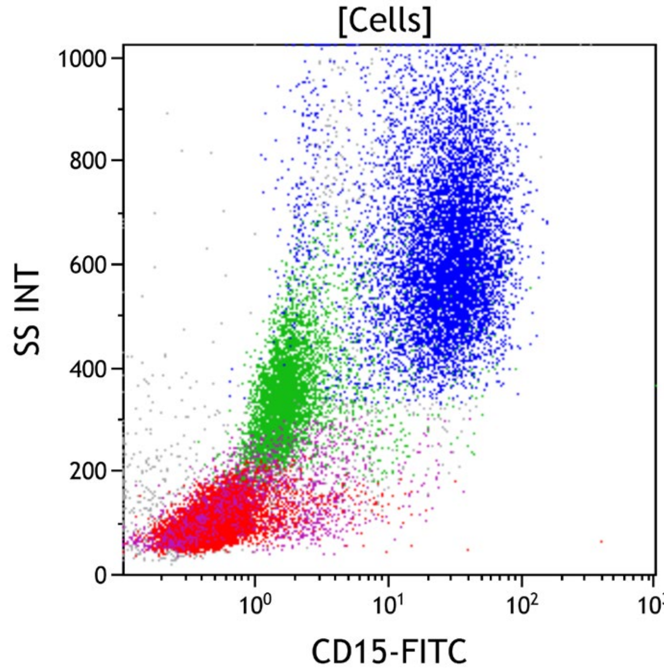

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). Lymphocytes (red) do not express CD15.

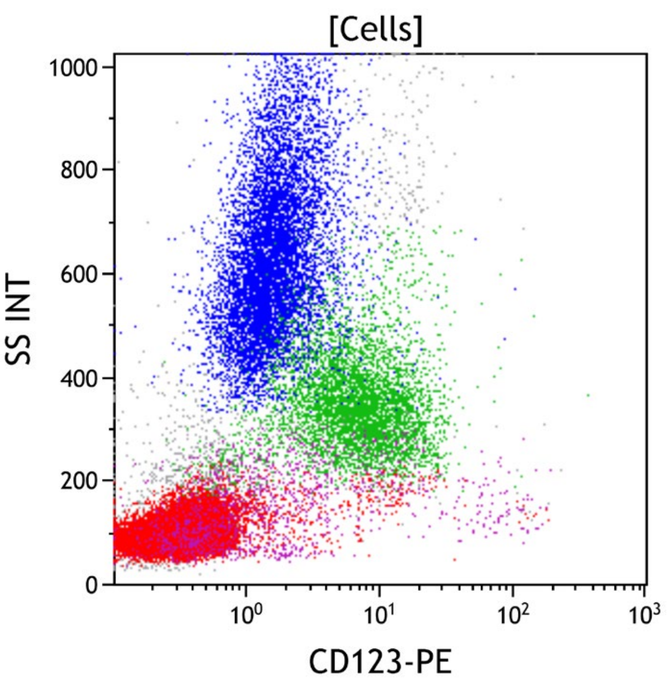

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green). Lymphocytes (red) do not express CD123.

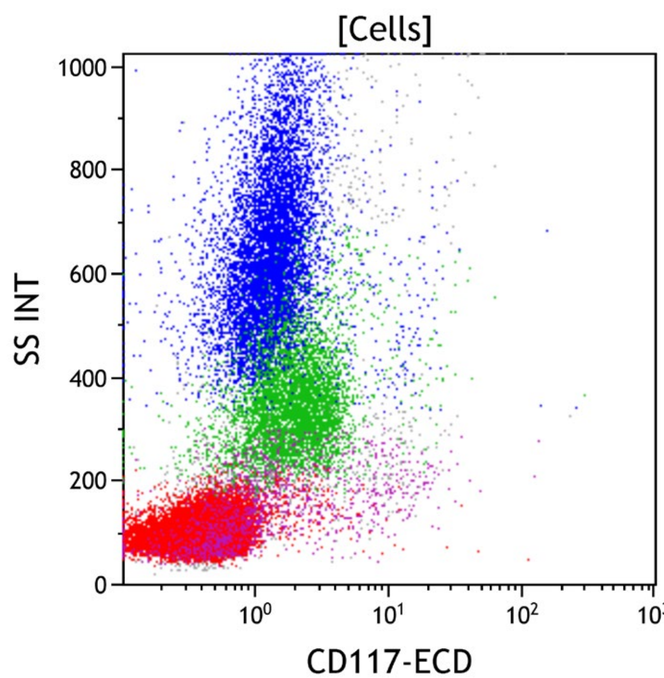

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Lymphocytes (red) do not express CD117.

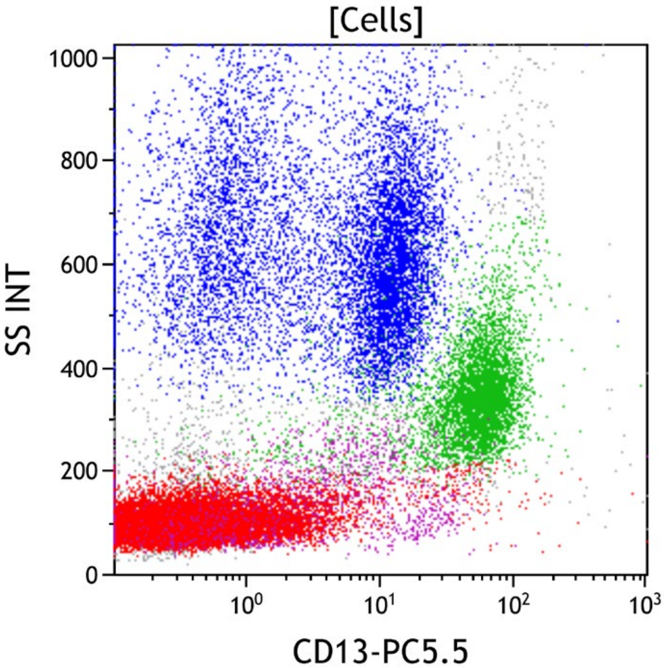

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors (purple). Lymphocytes (red) do not express CD13.

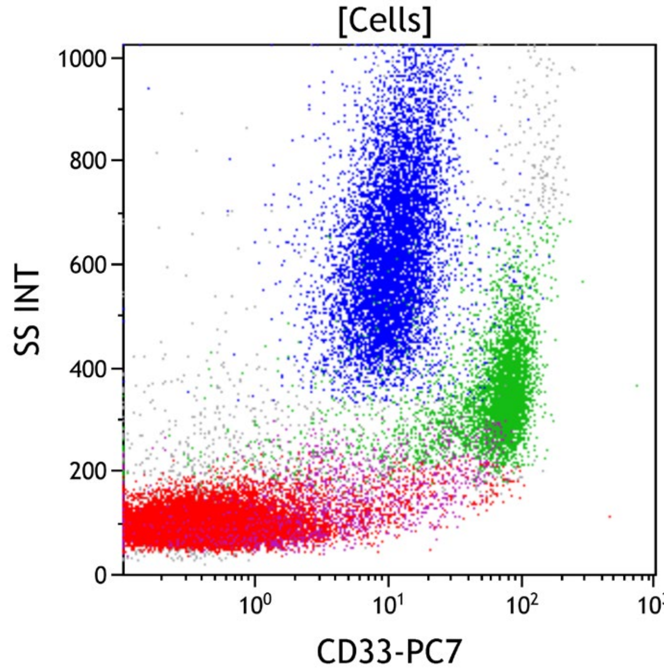

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors (purple). Lymphocytes (red) do not express CD33.

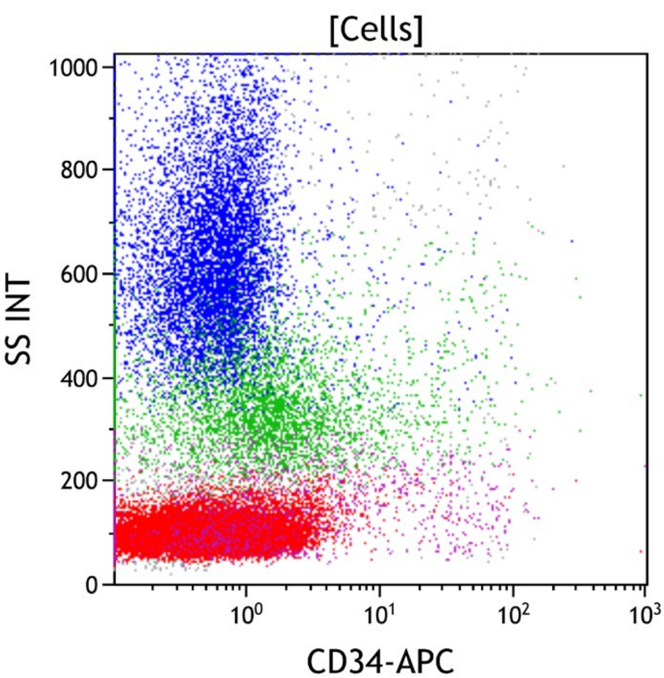

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate (purple). Lymphocytes (red) do not express CD34.

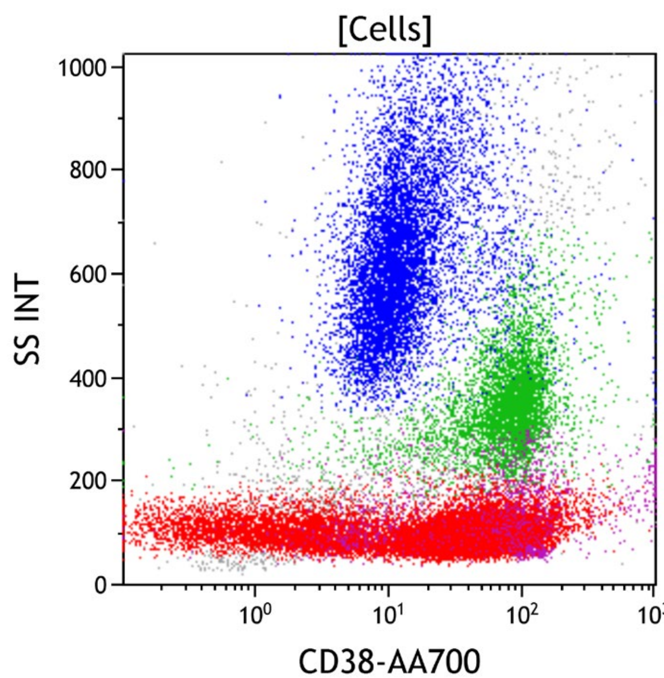

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Lymphocytes (red) variably express CD38.

M2 Cell Tube

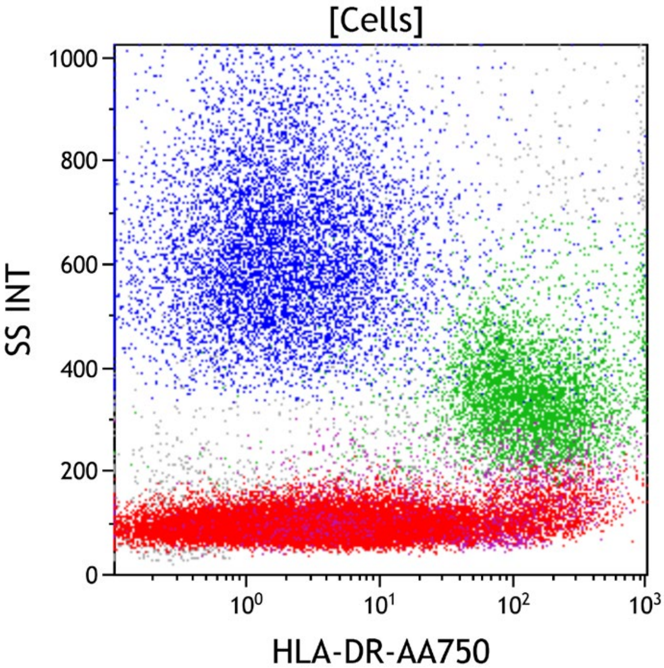

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red). Lymphocytes (red) variably express HLA-DR.

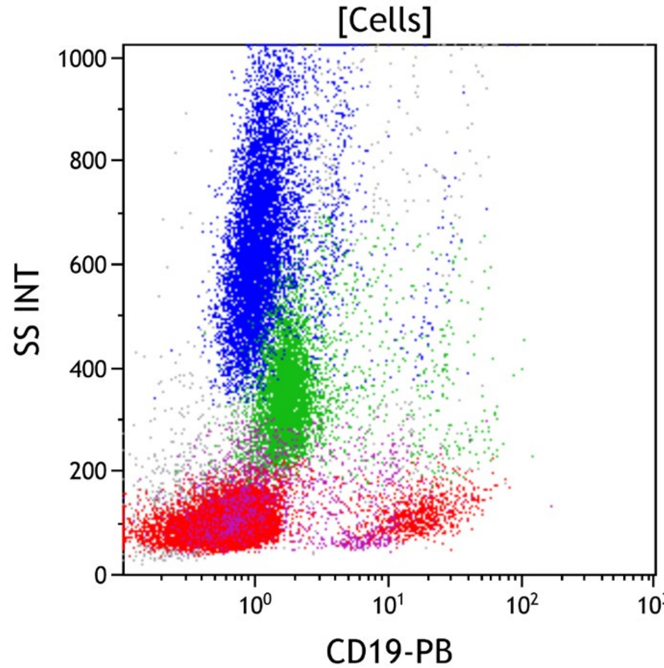

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Most lymphocytes (red, lower left) do not express CD19.

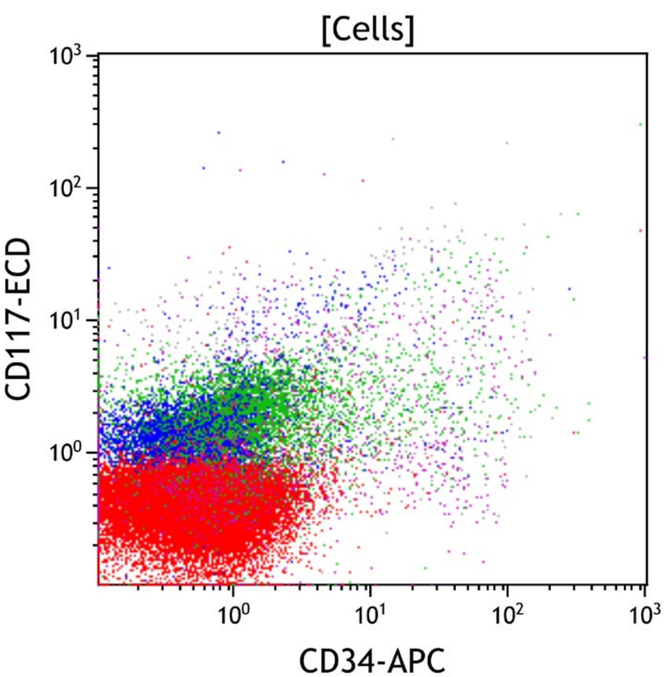

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors (purple). CD117 is expressed on myeloid blasts (upper right in purple), promyelocytes (blue), and early erythroid precursors, but negative on early B cell precursors (lower right in purple).

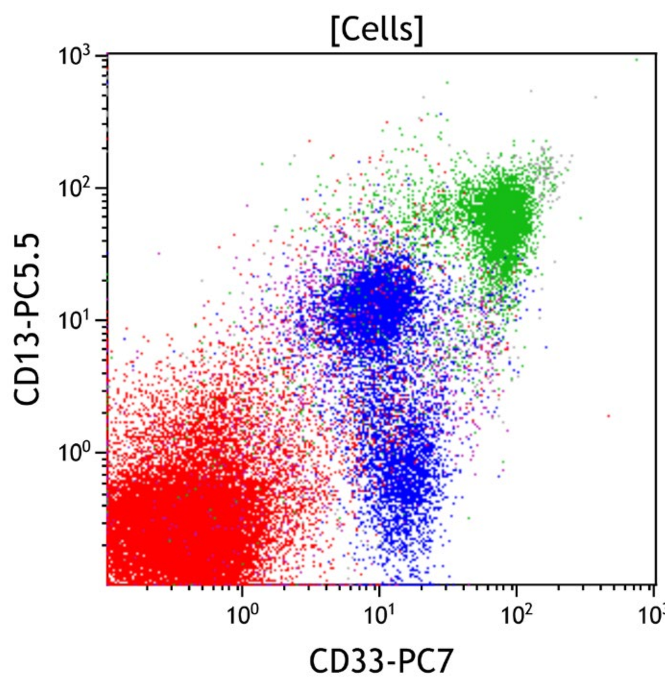

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red).

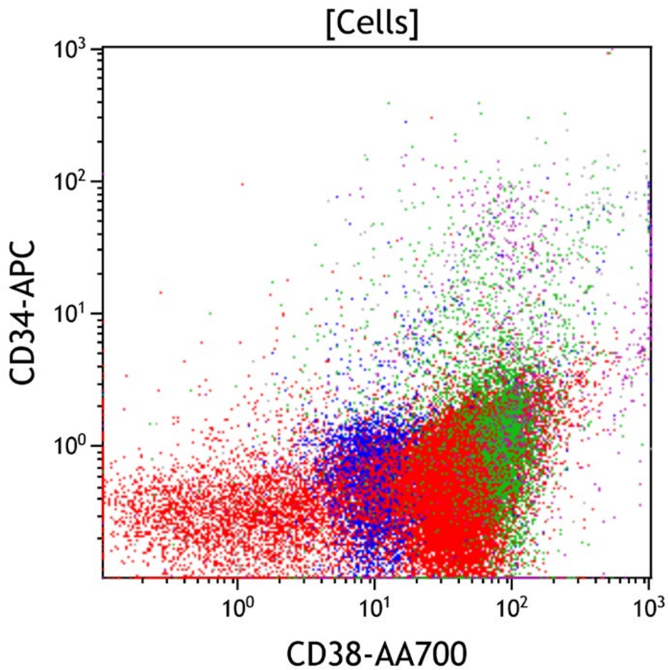

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable CD34 expression by plasma cells (purple extreme right) is a compensation artifact due to the extremely high level of CD38 that extends beyond the visible scale. Lymphocytes (red) express variable CD38 without CD34.

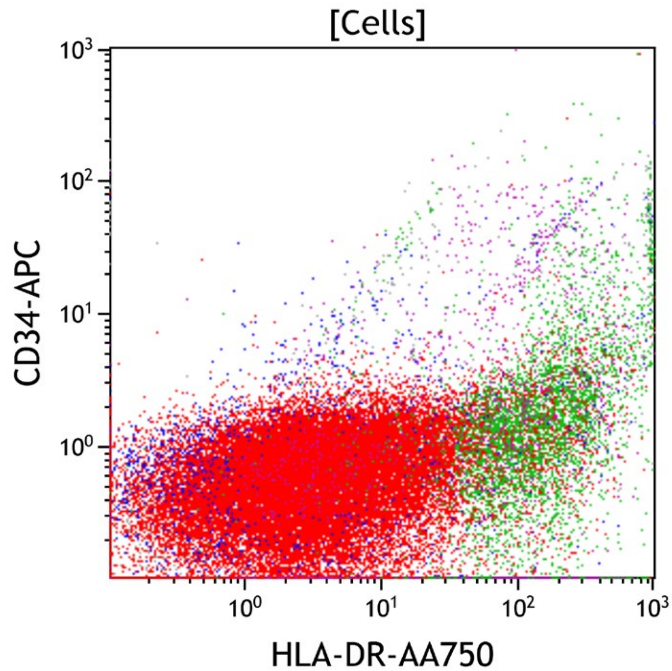

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes (purple right). Lymphocytes (red) express variable HLA-DR without CD34.

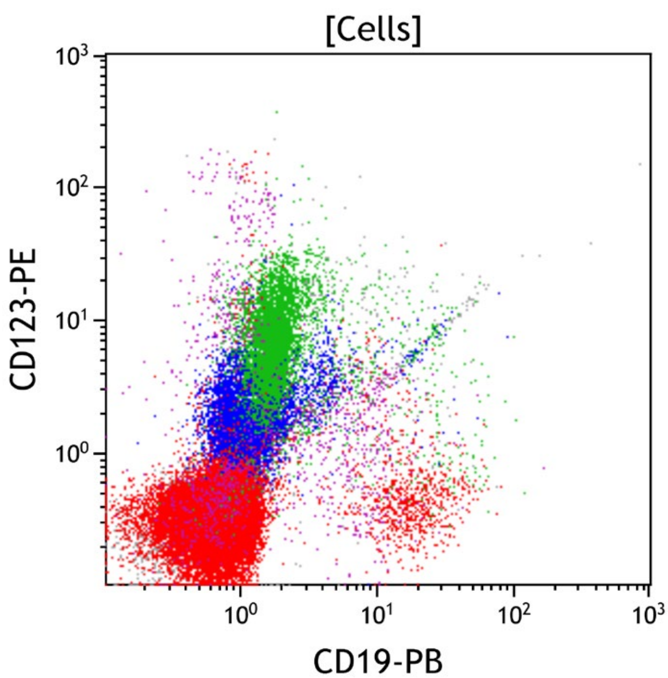

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

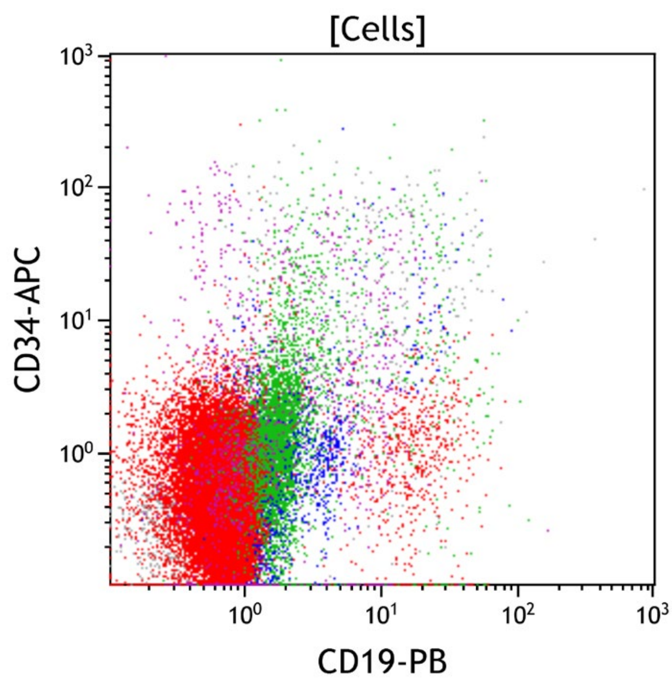

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19.

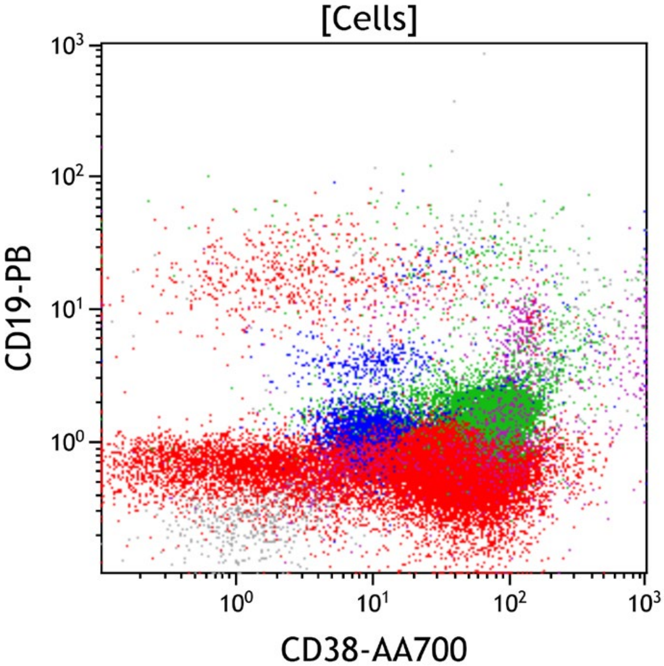

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19 positive B cells show lower expression of CD38 (red left). Plasma cells show extremely high CD38 expression that is largely off scale (purple extreme right), but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Lymphocytes (red) express variable CD38 without CD19.

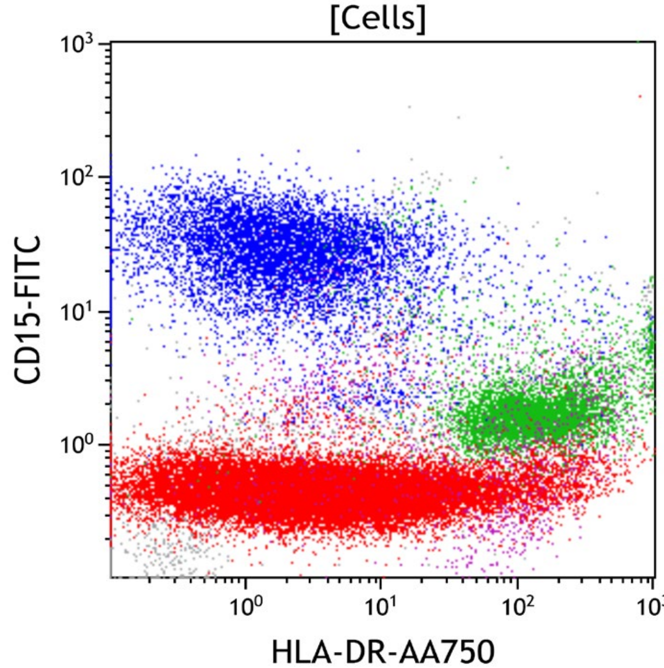

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors (purple) express HLA-DR but only transiently express CD15. Lymphocytes (red) express variable HLA-DR without CD15.

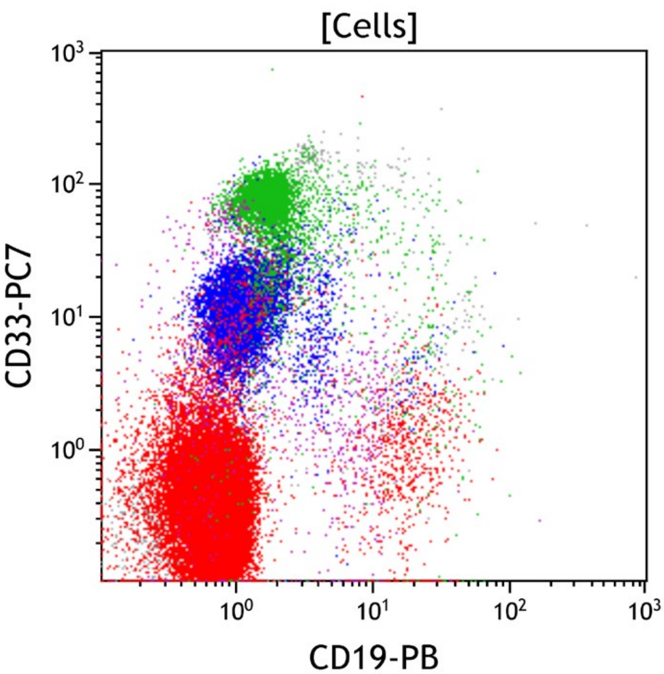

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate CD2, intermediate CD3, variable CD5, variable CD7, variable CD8, low CD16, variable CD38, bright CD45 and variable HLA-DR without CD56 or other B cell or myeloid antigens. Compared with normal mature T cells, the decreased expression of CD2, CD5, CD7 and CD8 with increased CD16, CD38 and HLA-DR is aberrant.

The immunophenotype of the abnormal population is consistent with expanded abnormal large granular lymphocytes of T cell type. In the appropriate clinical context of prolonged cytopenias without other explanation, this finding would be consistent with a large granular leukemia of T cell type.

NEOPLASTIC PROCESS OF MYELOID ORIGIN

Myeloid malignancies are clonal diseases of hematopoietic stem or progenitor cells. These malignancies can be present in the bone marrow and peripheral whole blood. Some myeloid disorders, such as the myeloid leukemias, have long been considered malignant while other myeloid disorders have been considered non-malignant or pre-leukemia blood disorders which may become malignant over time. Based on the morphology, cytochemistry, immunophenotype, genetics, and clinical features of myeloid disorders, the World Health Organization (WHO) categorizes myeloid malignancies into five primary types: (1) acute myeloid leukemia; (2) myelodysplastic syndromes (MDS); (3) myeloproliferative neoplasms (MPN); (4) myelodysplastic and myeloproliferative (MDS/MPN) neoplasms; and (5) myeloid neoplasms associated with eosinophilia and abnormalities of growth factor receptors derived from platelets or fibroblasts.

ACUTE MYELOID LEUKEMIA

Case #18: Acute Myeloid Leukemia

Clinical Vignette

This 55-year-old male presents with circulating blasts on peripheral whole blood smear. A bone marrow aspirate sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

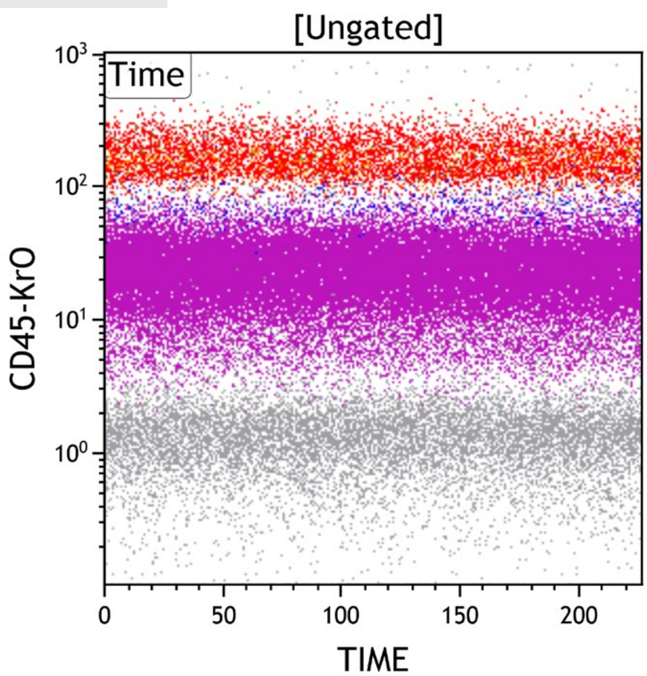

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

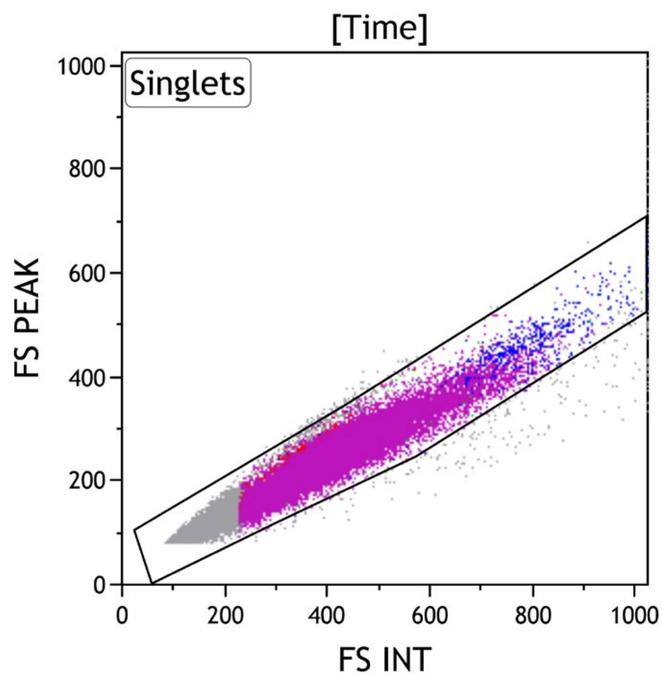

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

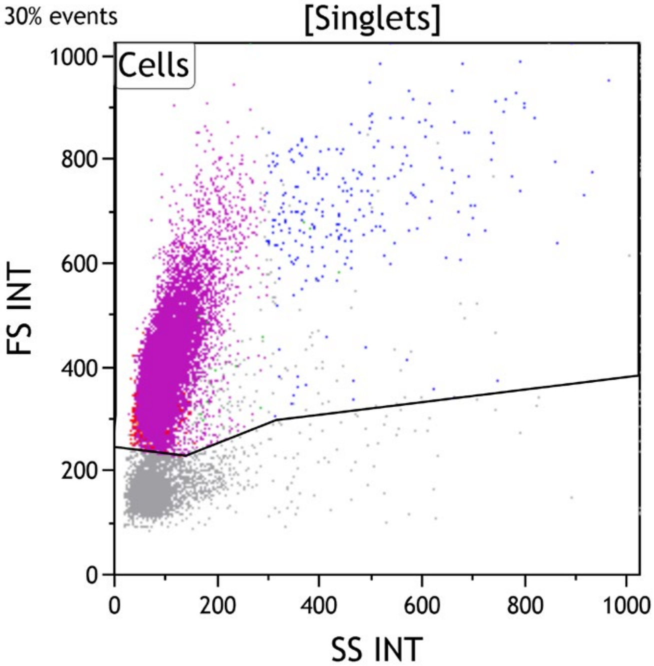

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

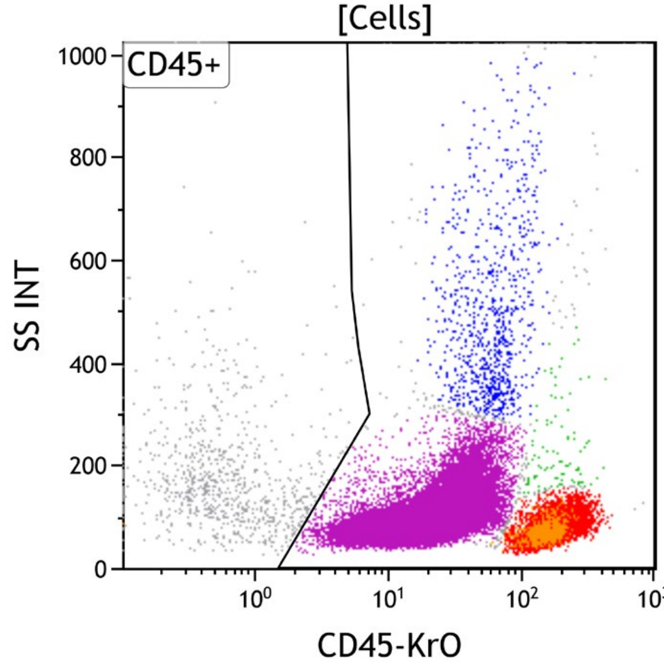

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

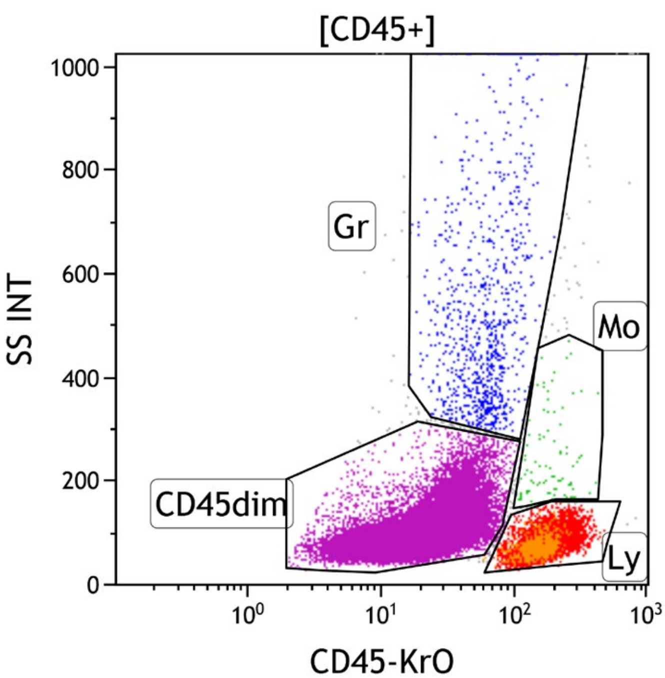

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note the expanded progenitors in the CD45 dim gate (purple).

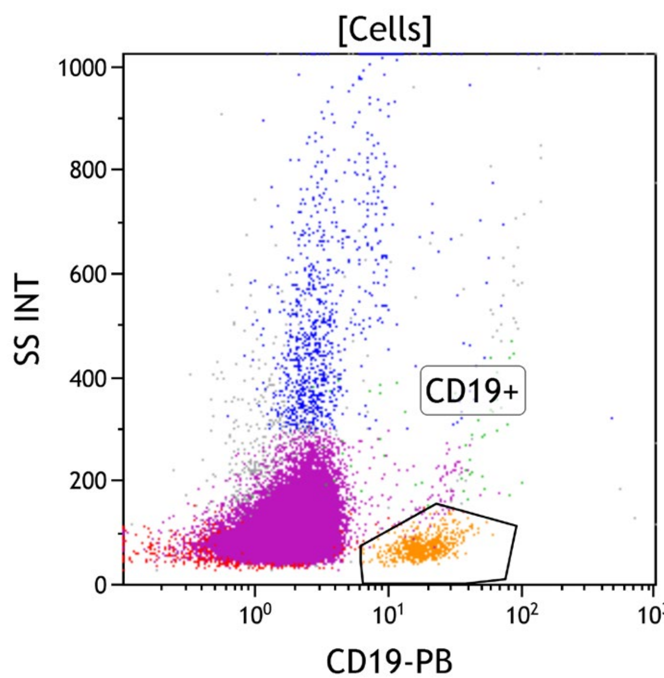

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The expanded progenitors (purple) do not express CD19.

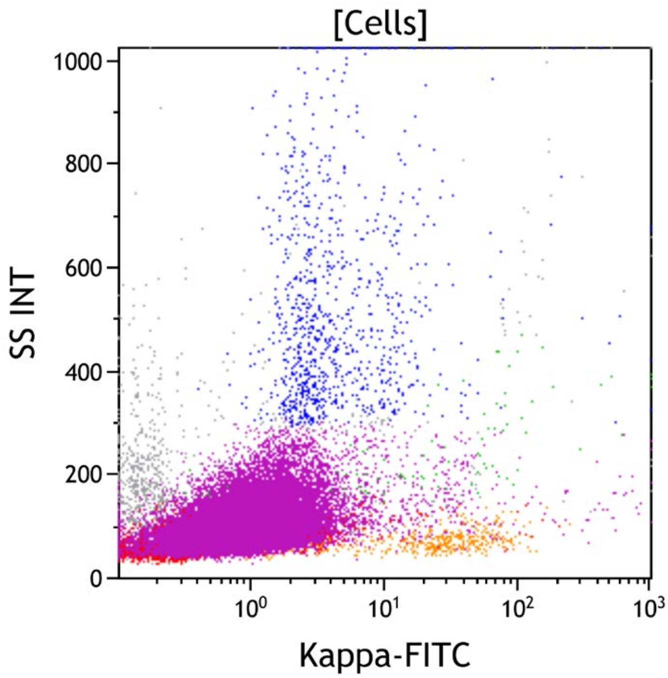

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The expanded progenitors (purple) do not express kappa light chain.

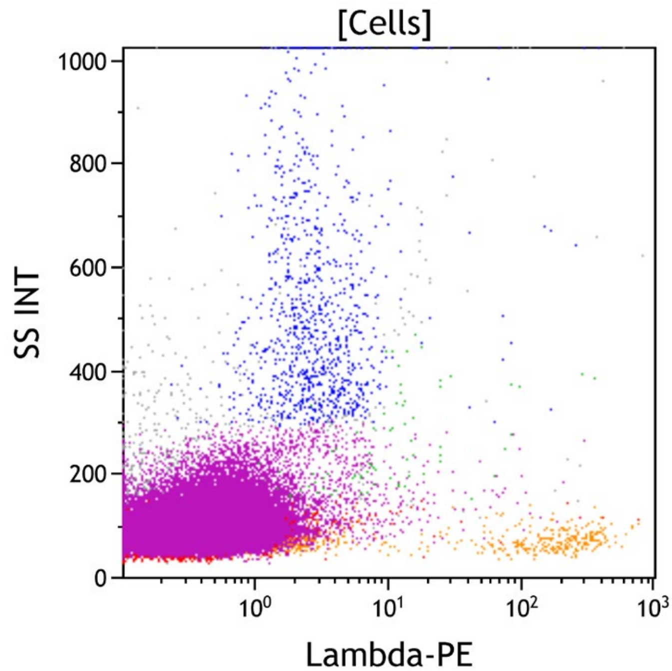

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The expanded progenitors (purple) do not express lambda light chain.

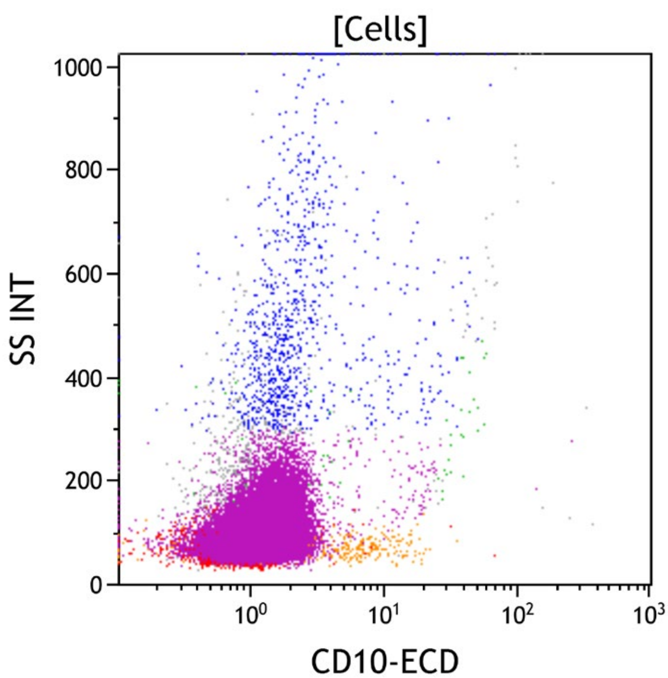

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The expanded progenitors (purple) do not express CD10.

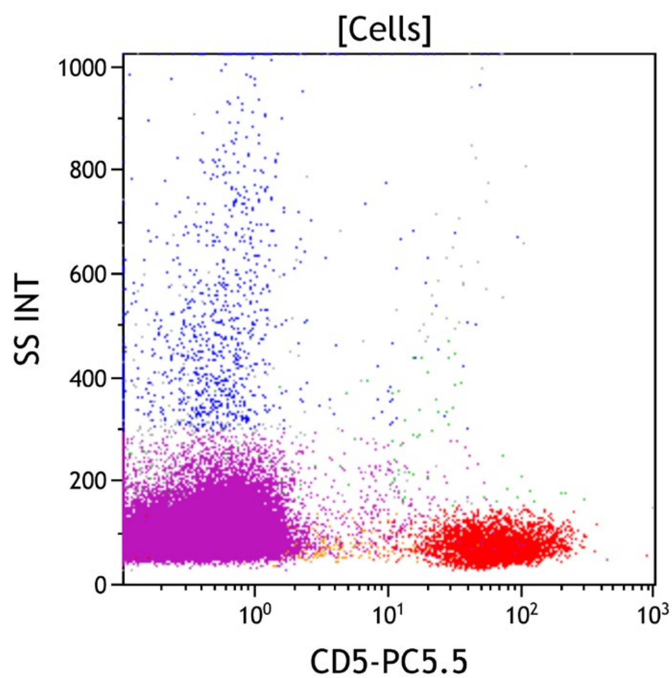

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The expanded progenitors (purple) do not express CD5.

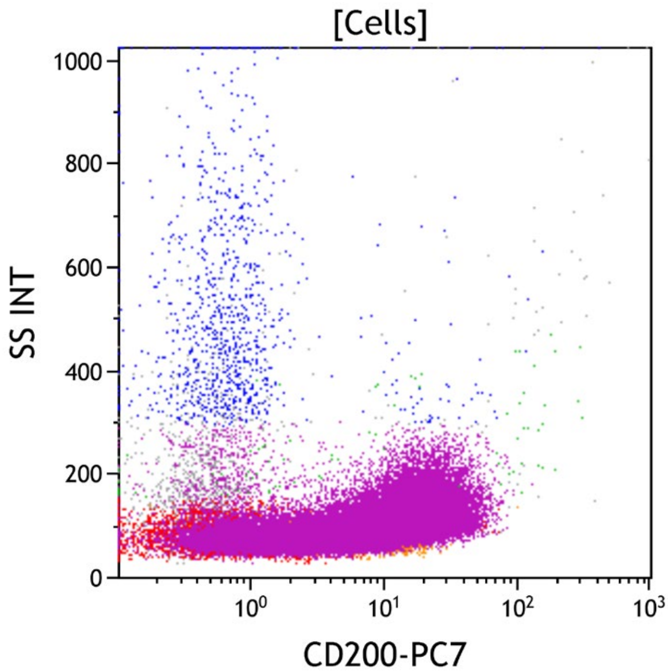

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The expanded progenitors (purple) express variable CD200.

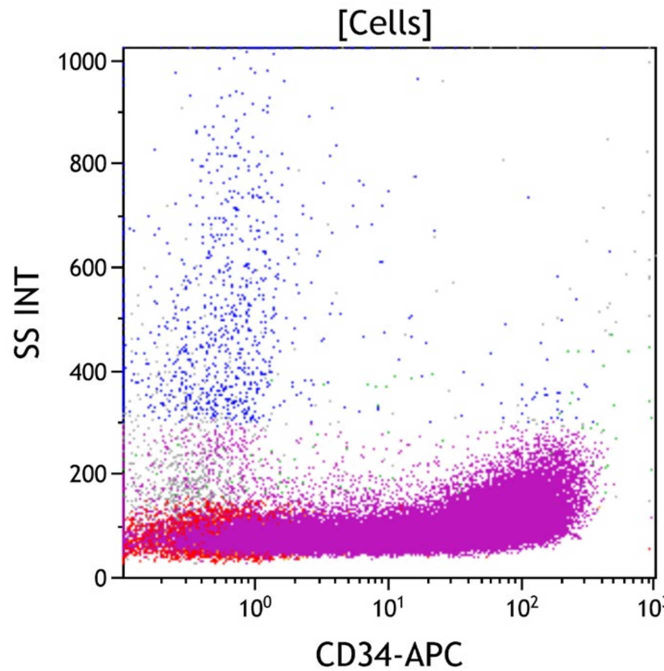

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The expanded progenitors (purple) variably express CD34.

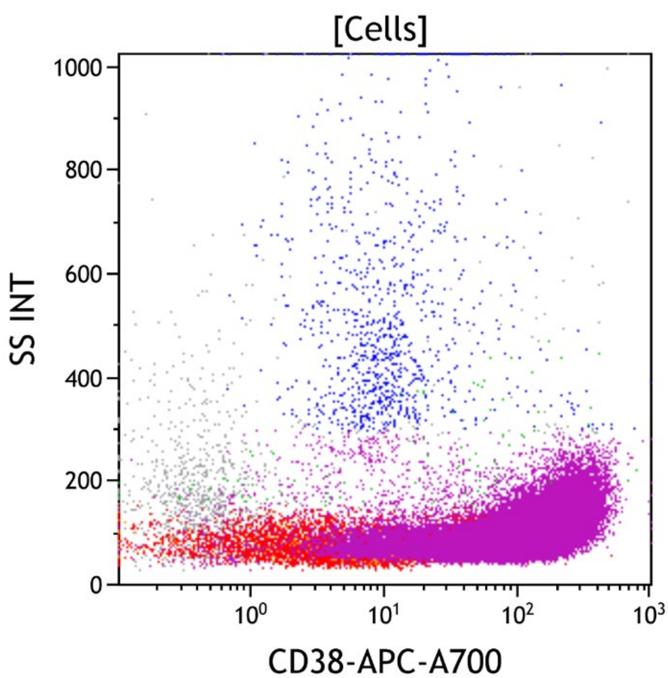

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The expanded progenitors (purple) variably express CD38.

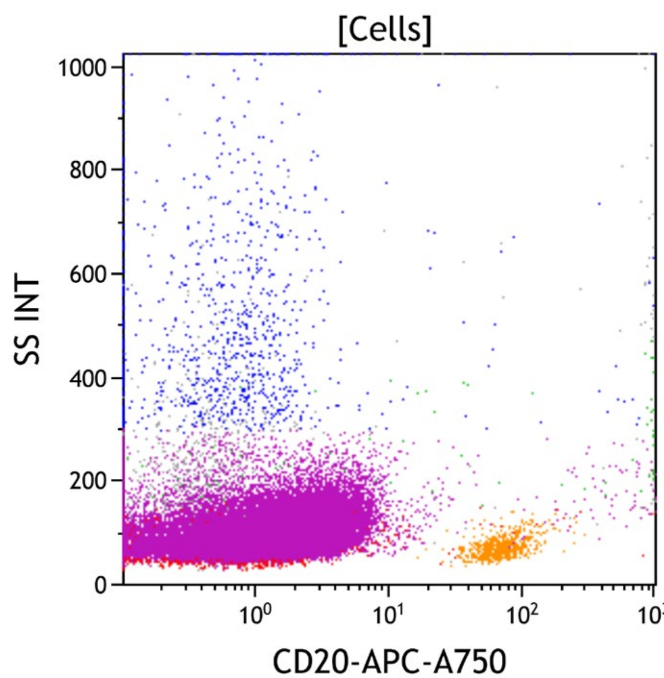

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The expanded progenitors (purple) do not express CD20.

B Cell Tube

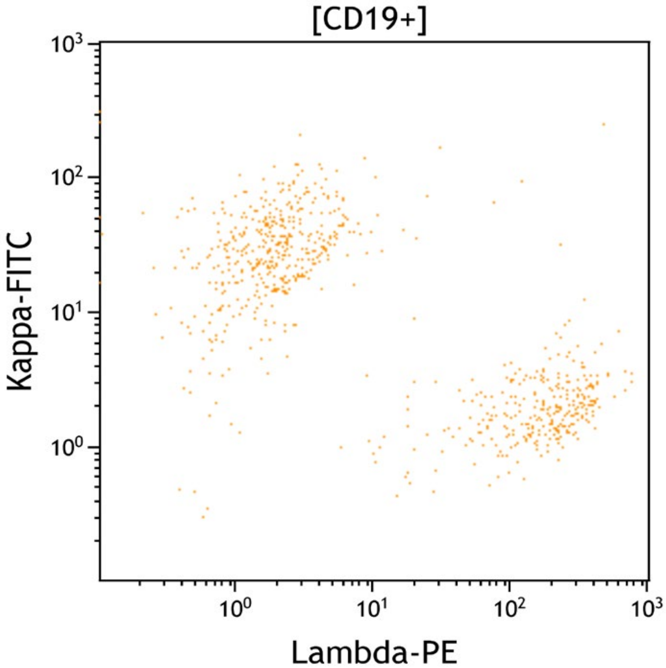

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

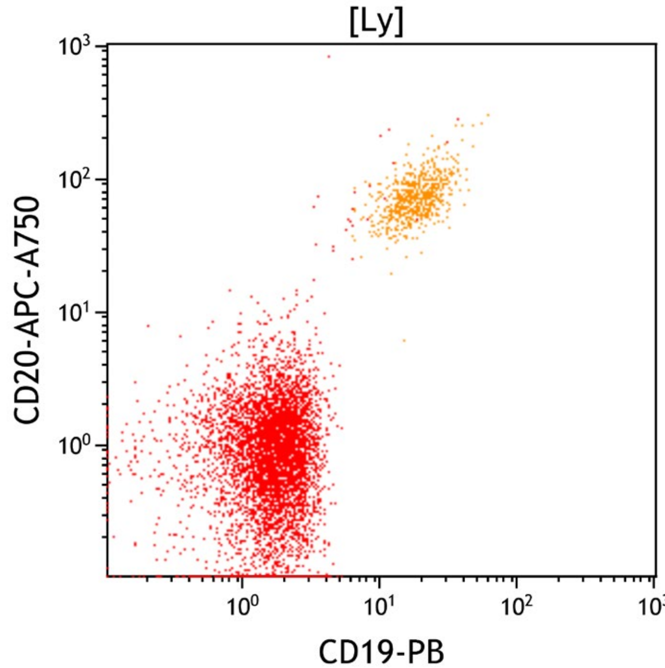

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

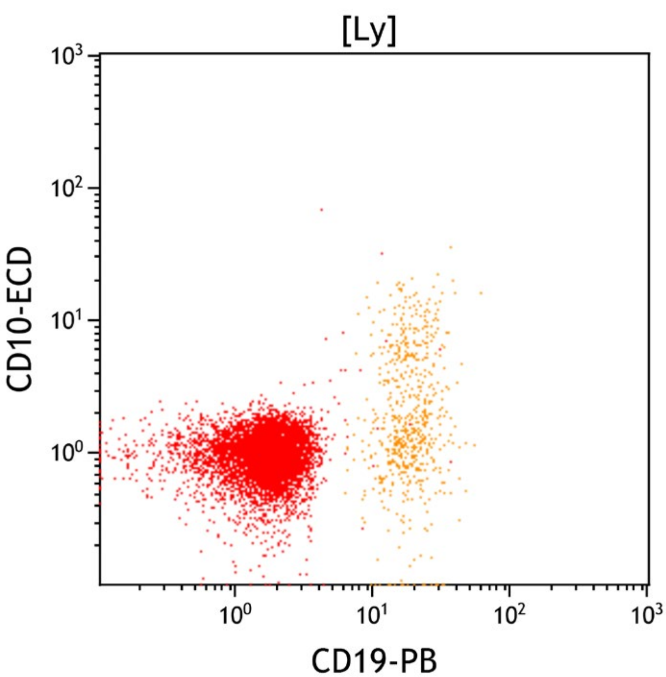

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

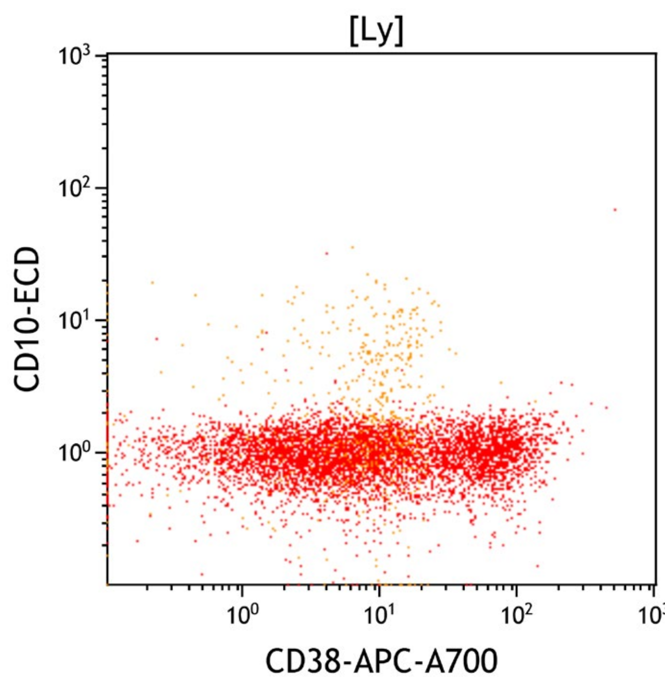

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate (Ly), though most immature B cells are in the CD45 dim gate.

B Cell Tube

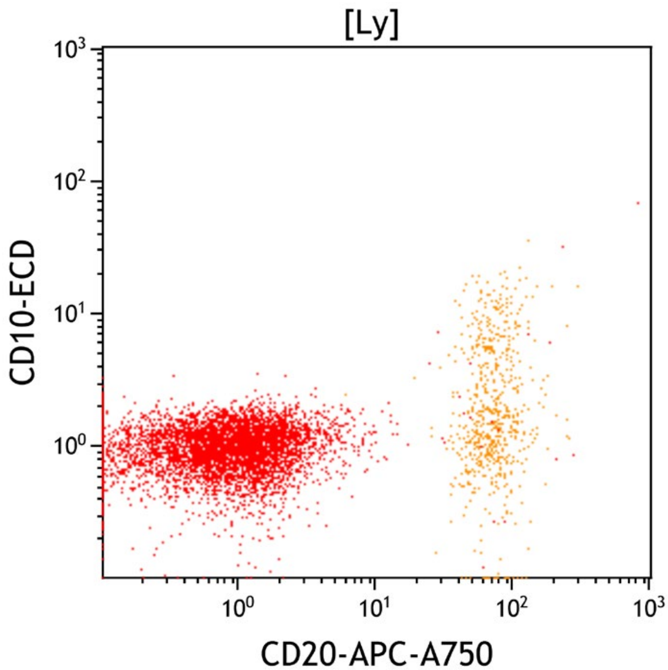

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

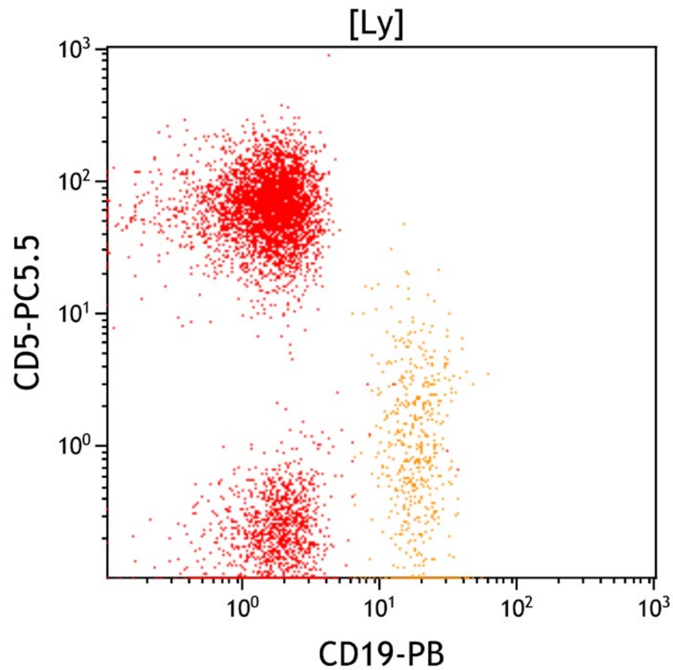

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

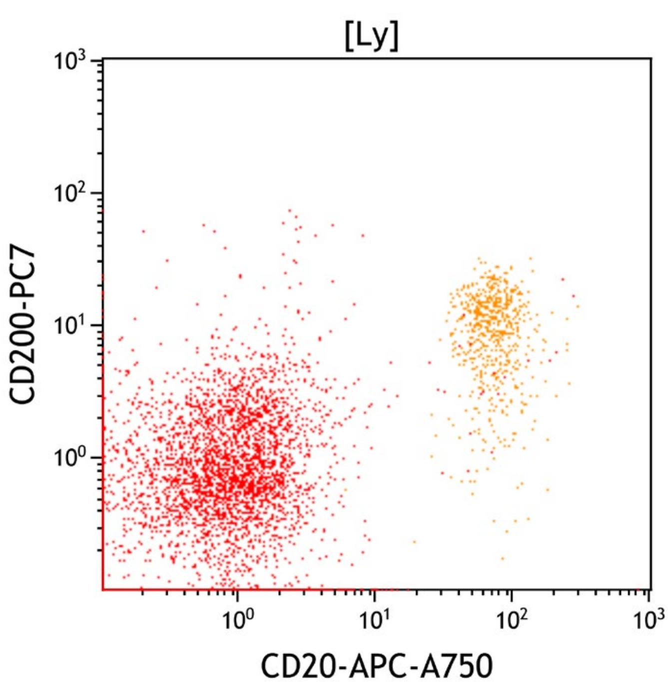

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

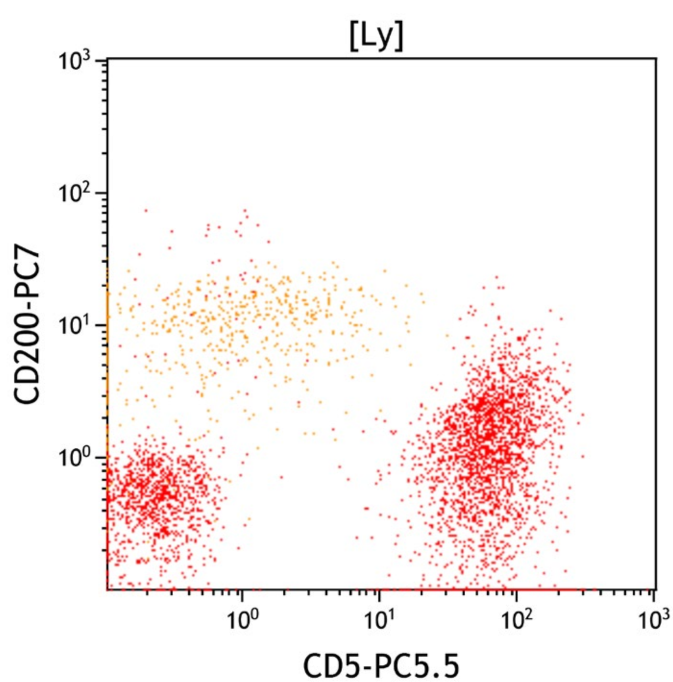

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

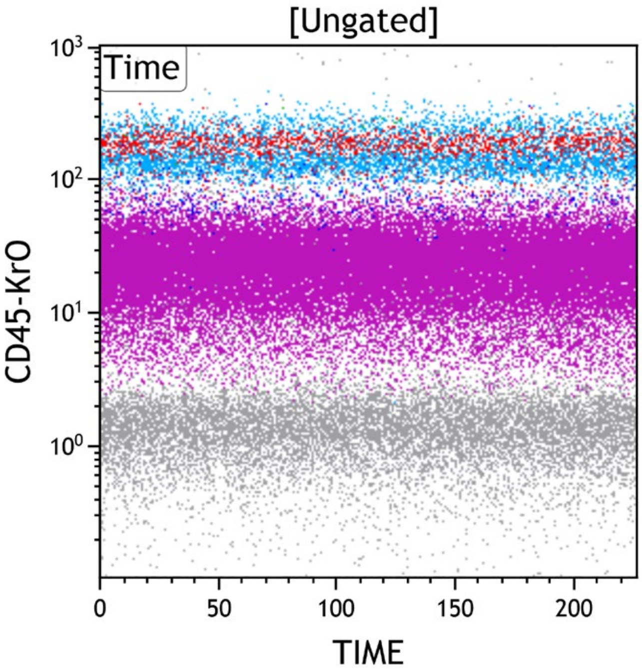

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

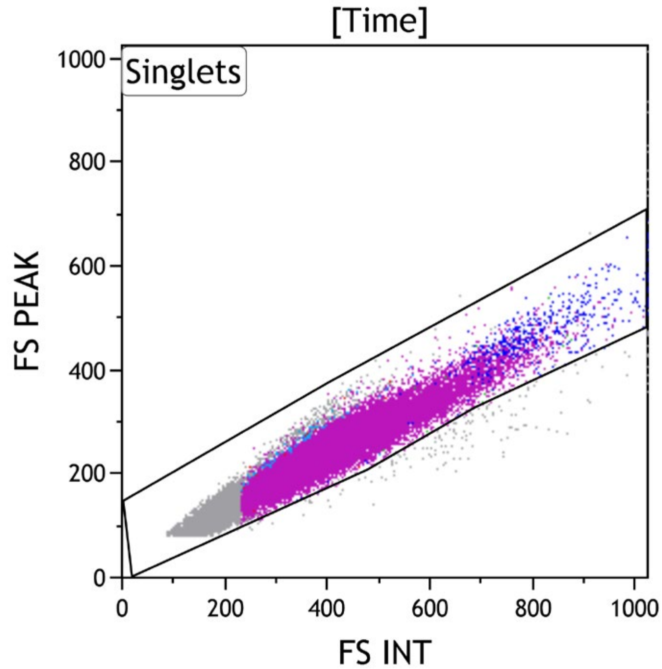

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship

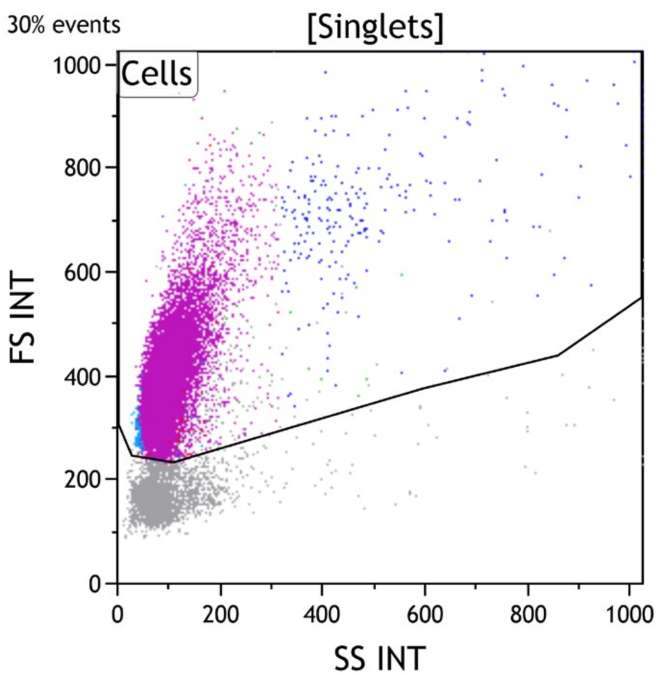

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

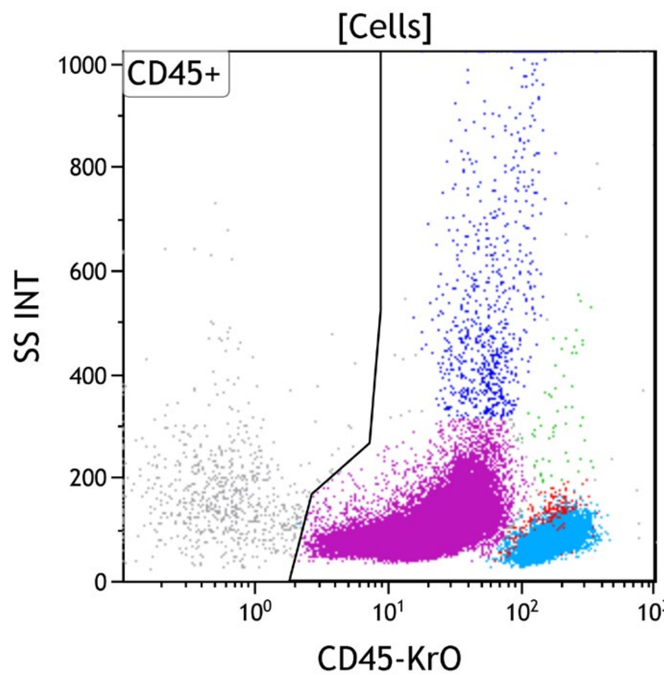

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

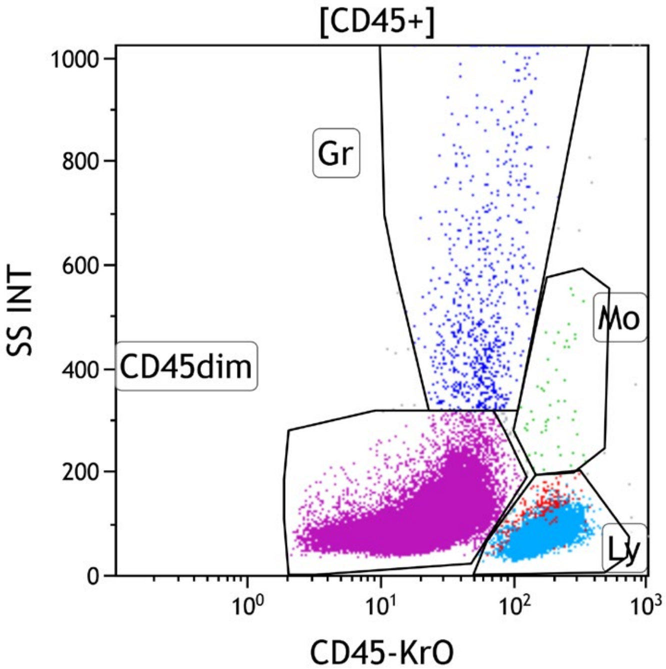

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the expanded progenitors in the CD45 dim gate (purple).

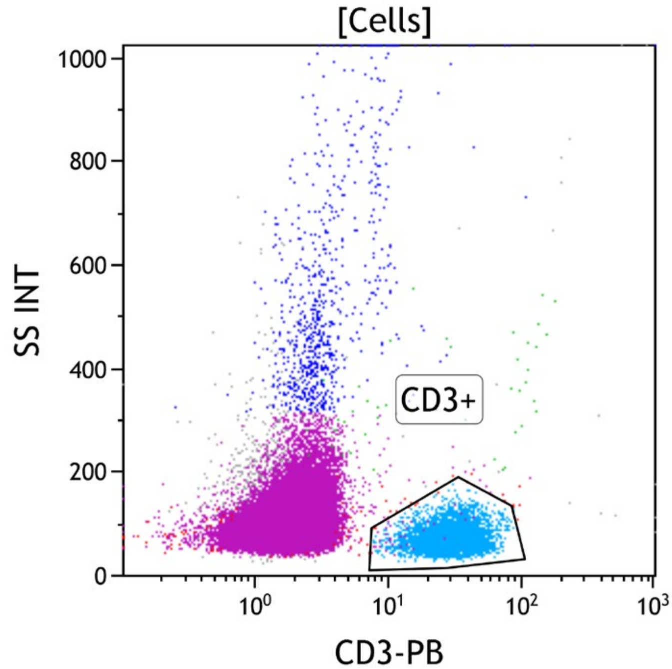

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The expanded progenitors (purple) do not express CD3.

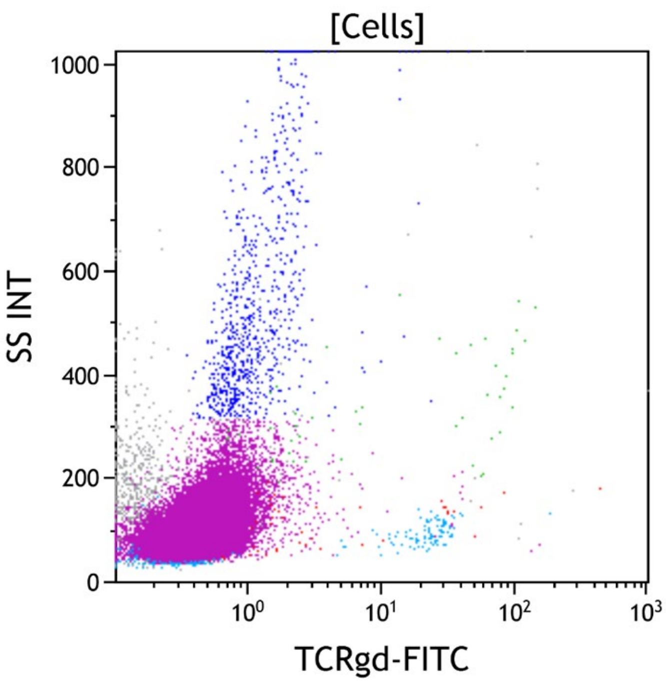

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The expanded progenitors (purple) do not express TCRγδ.

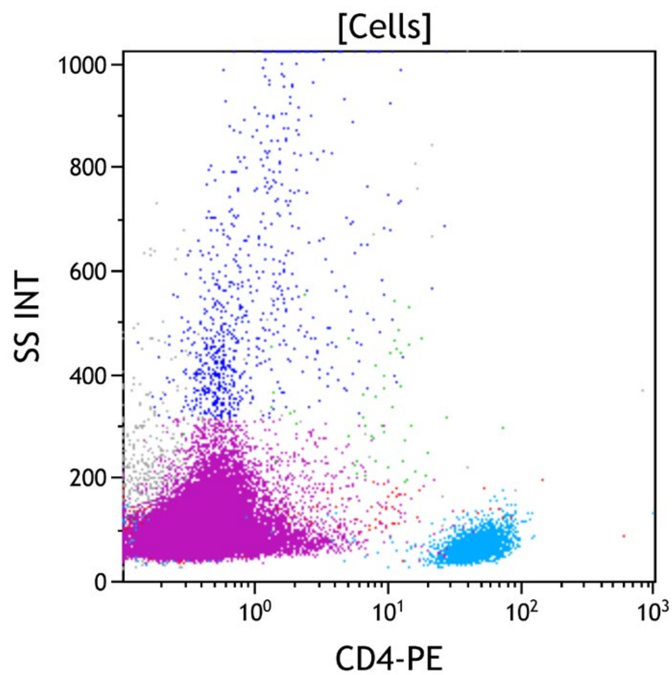

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The expanded progenitors (purple) do not express CD4.

T Cell Tube

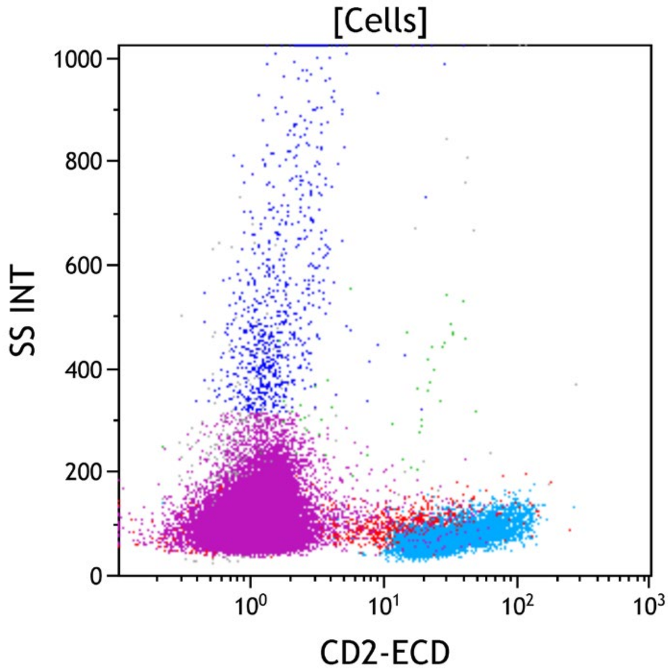

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The expanded progenitors (purple) do not express CD2.

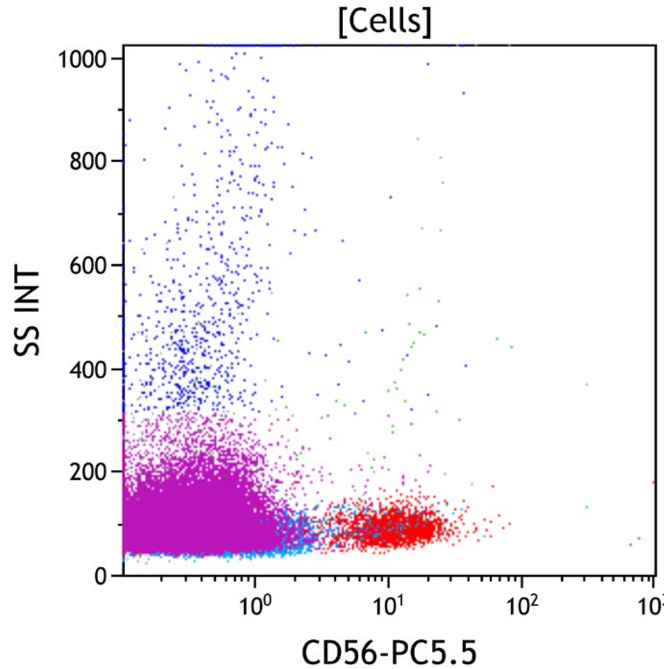

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The expanded progenitors (purple) do not express CD56.

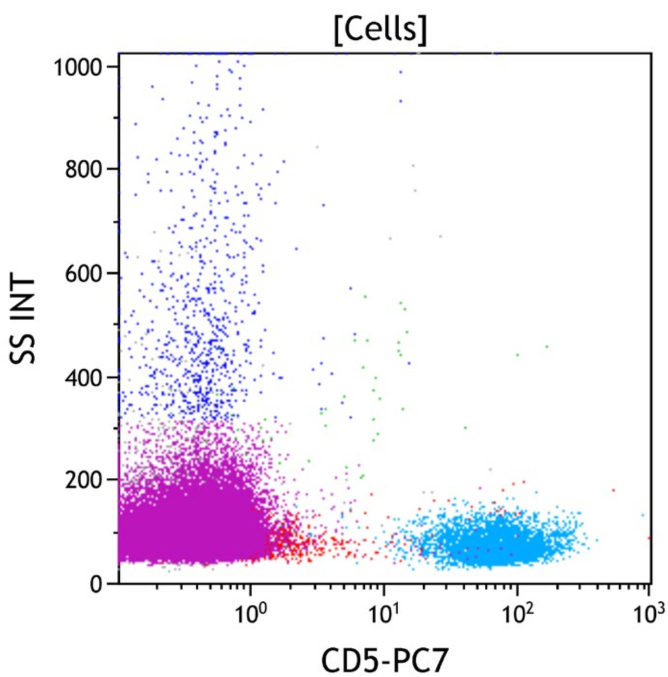

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The expanded progenitors (purple) do not express CD5.

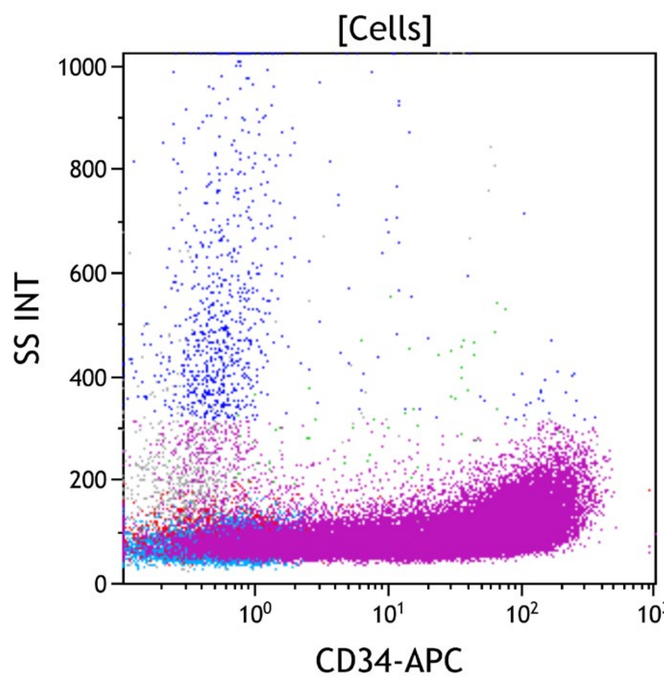

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The expanded progenitors (purple) variably express CD34.

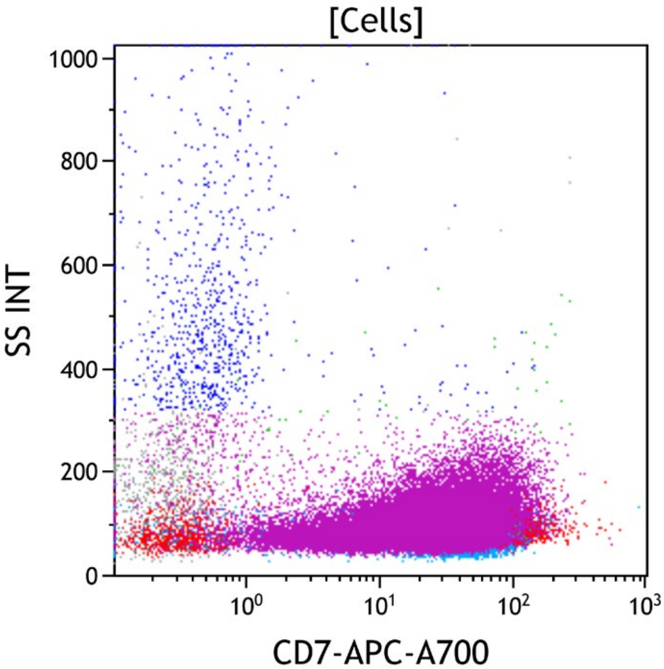

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The expanded progenitors (purple) express intermediate to bright CD7.

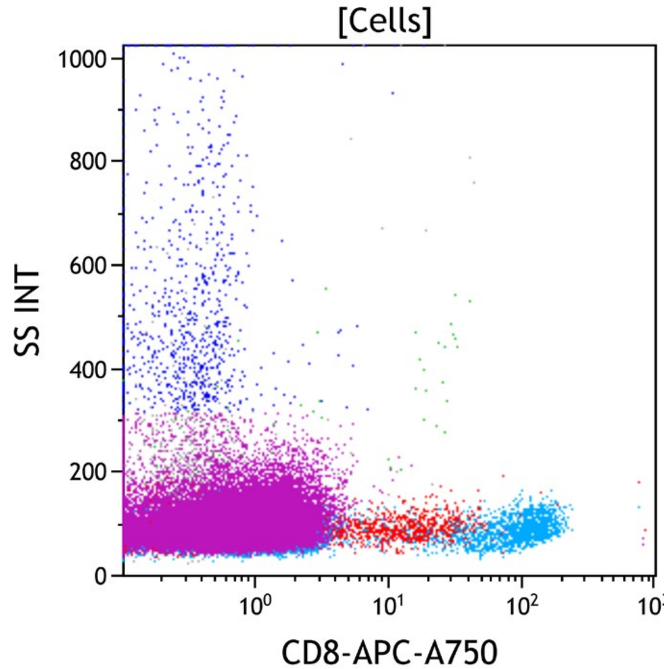

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The expanded progenitors (purple) do not express CD8.

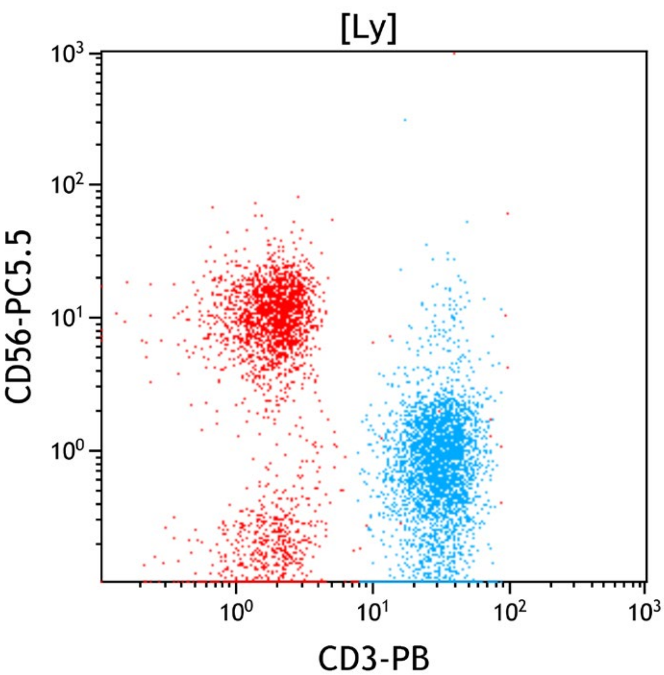

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

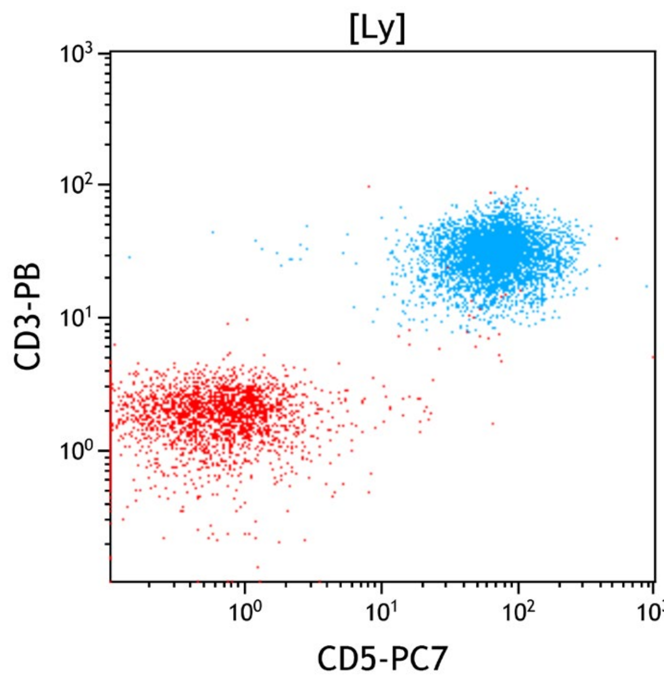

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

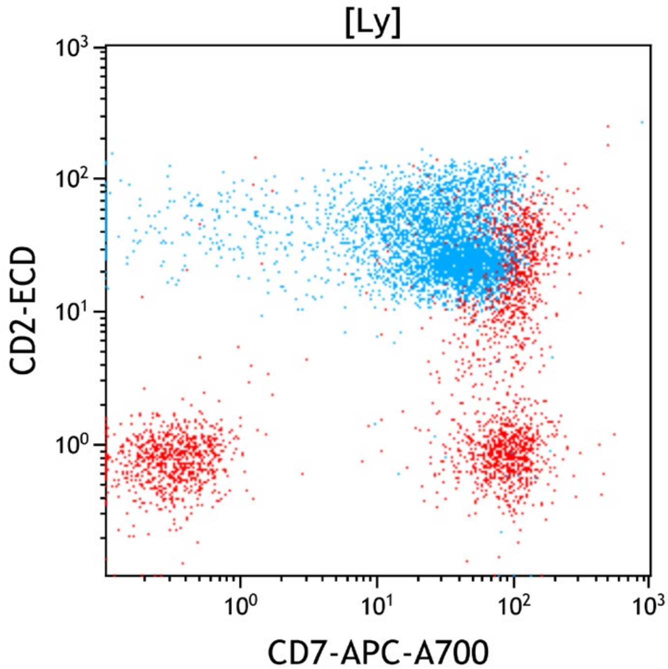

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

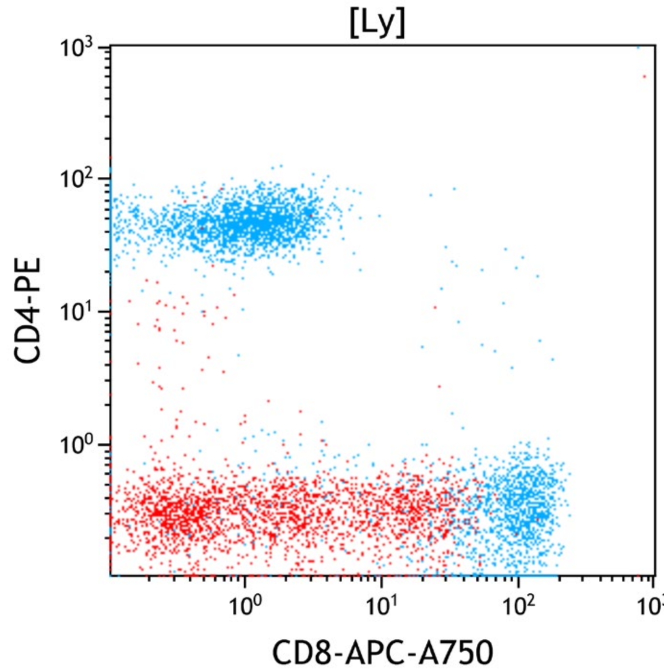

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

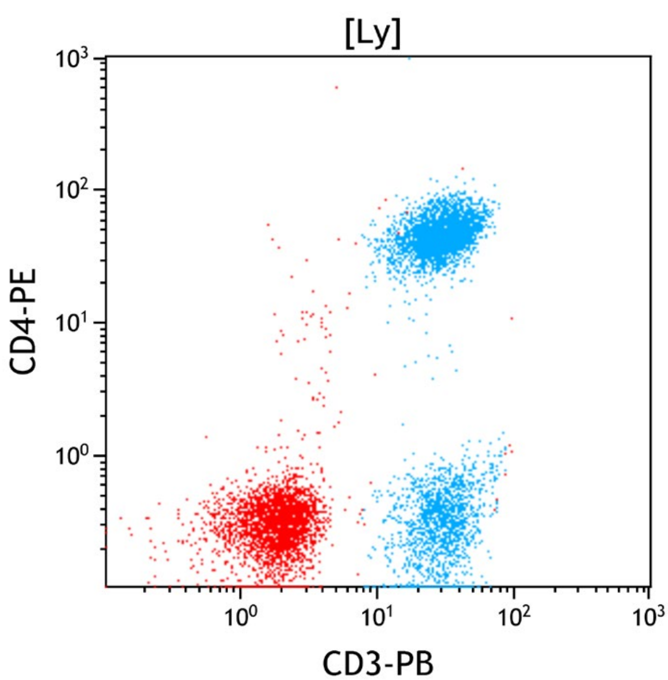

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

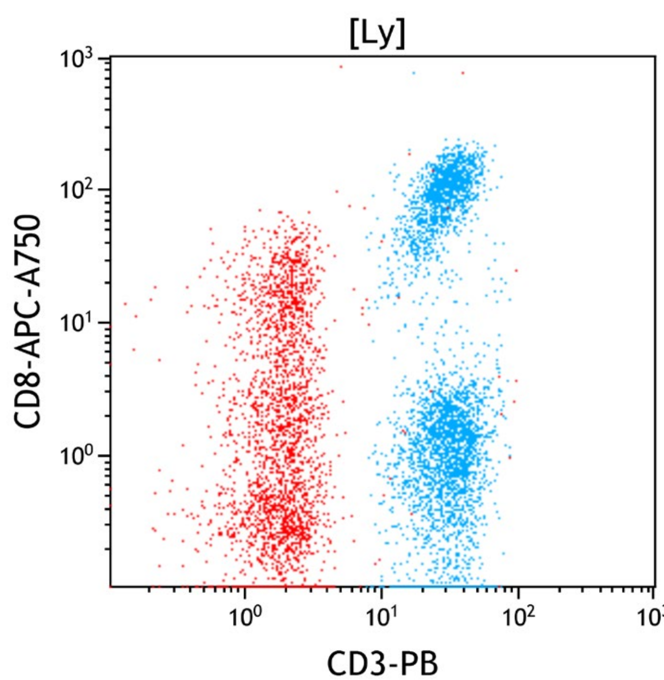

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

T Cell Tube

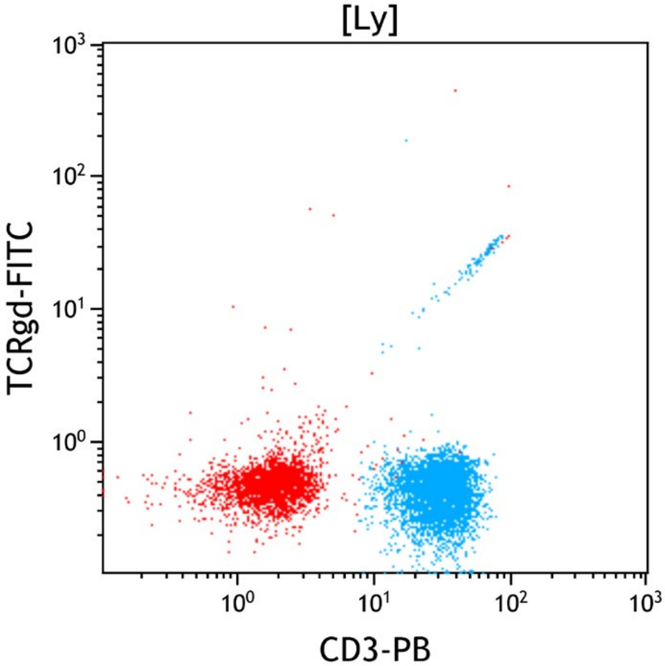

Figure 21. This CD3 vs TCRgd dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

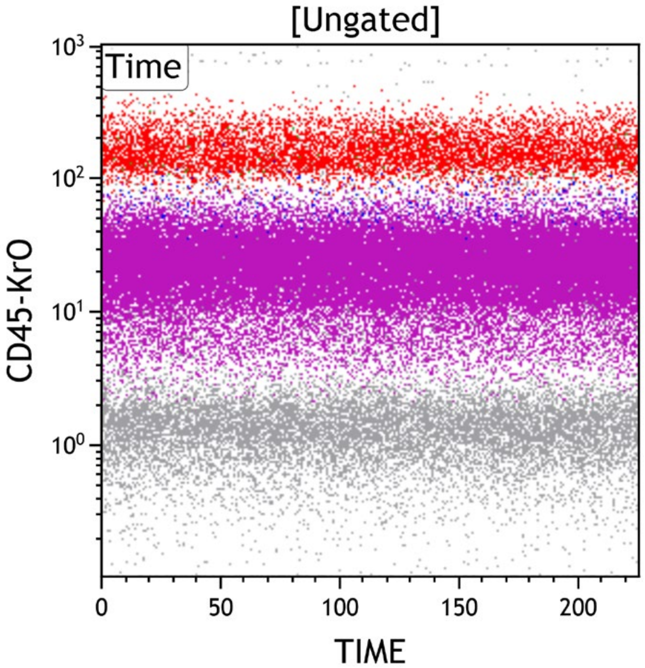

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

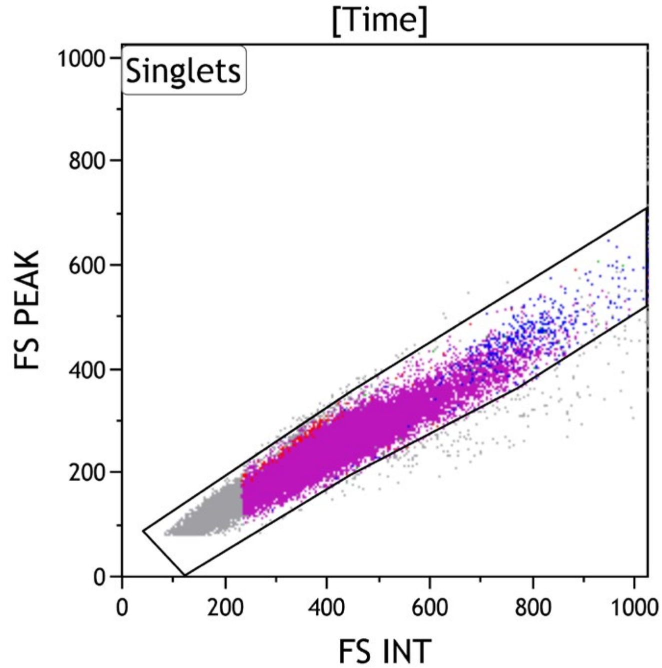

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

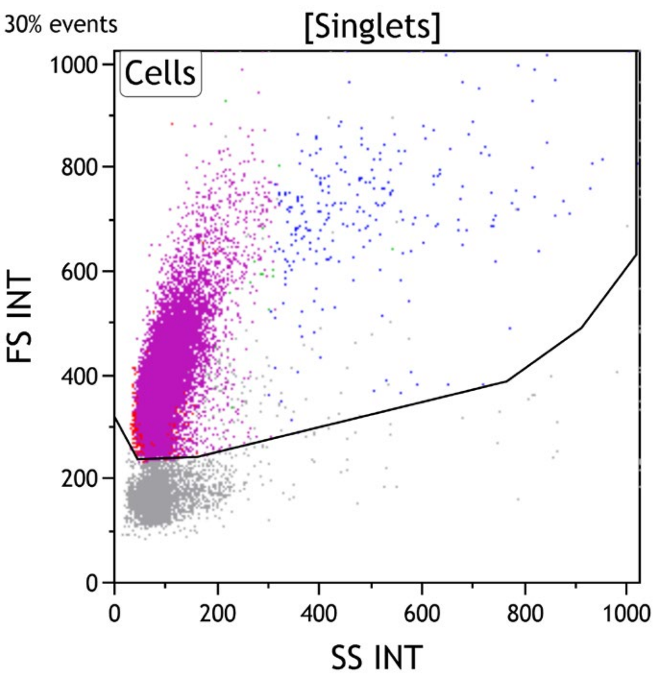

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

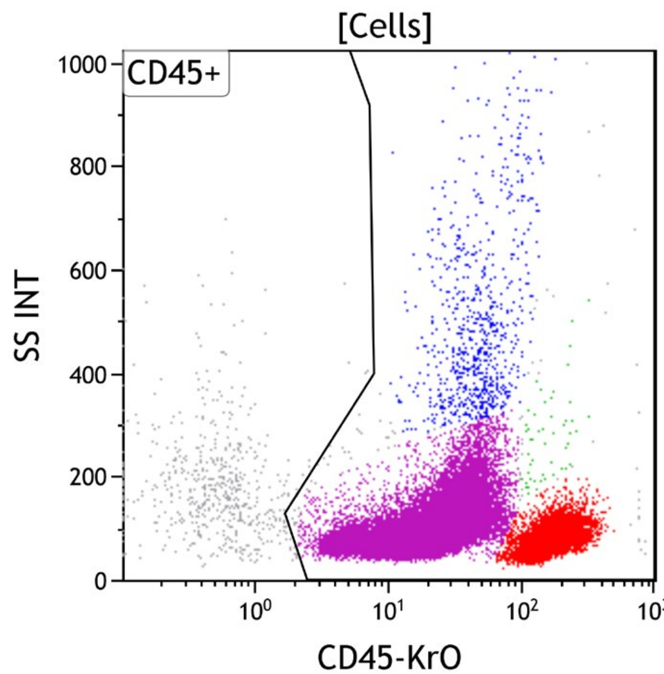

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

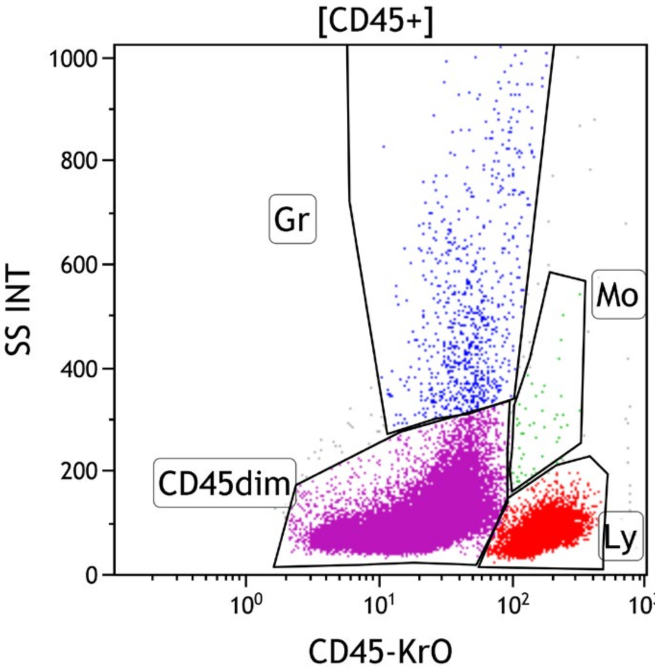

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the expanded progenitors in the CD45 dim gate (purple).

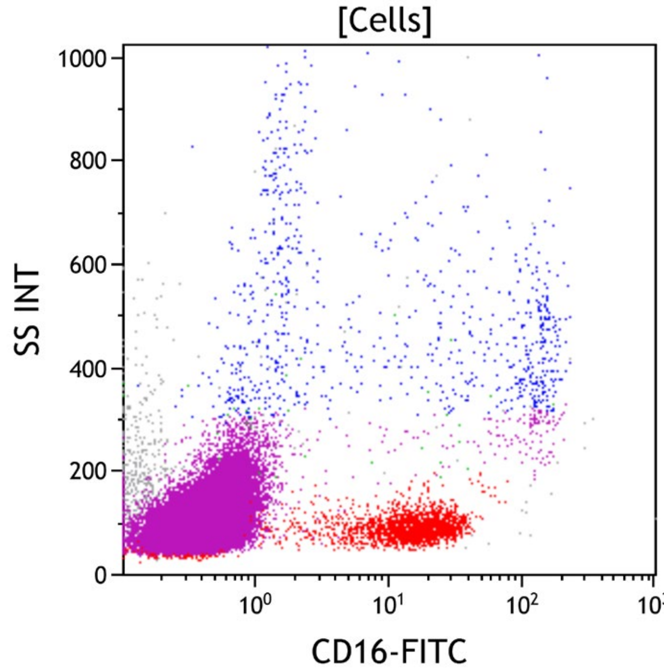

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The expanded progenitors (purple) do not express CD16.

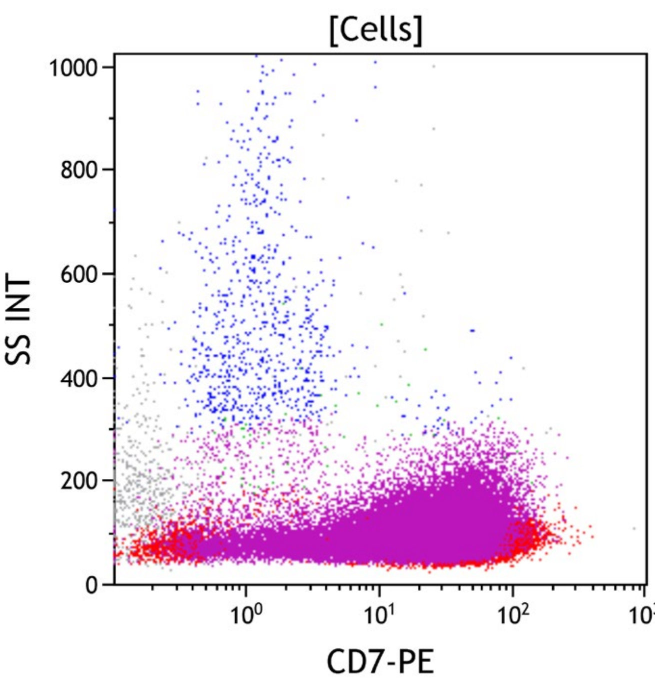

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The expanded progenitors (purple) express intermediate to bright CD7.

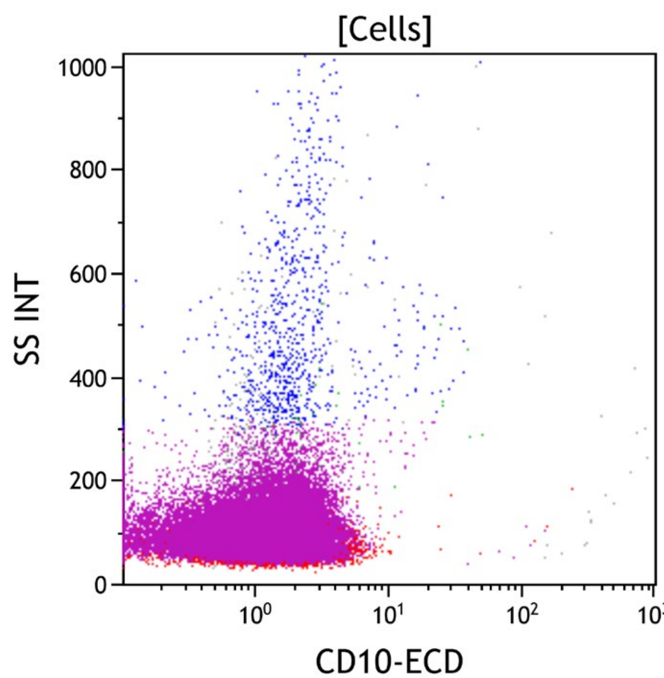

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells , mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The expanded progenitors (purple) do not express CD10.

M1 Cell Tube

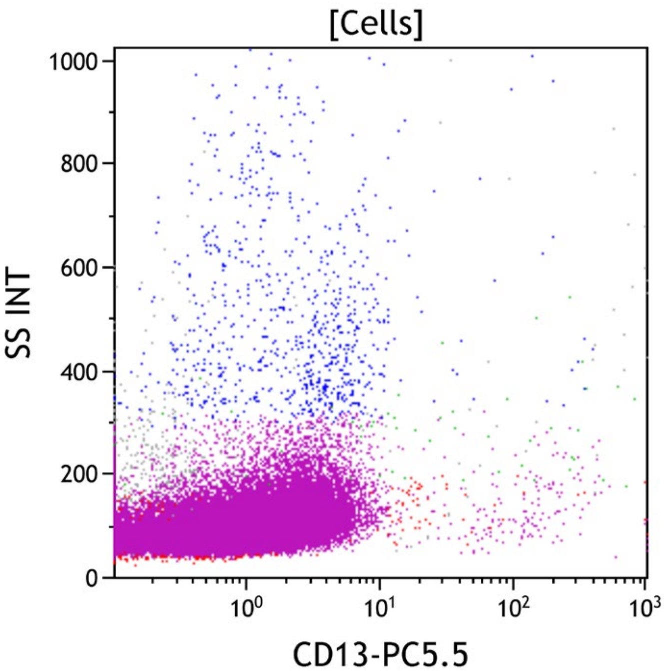

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The expanded progenitors (purple) express dim to absent CD13.

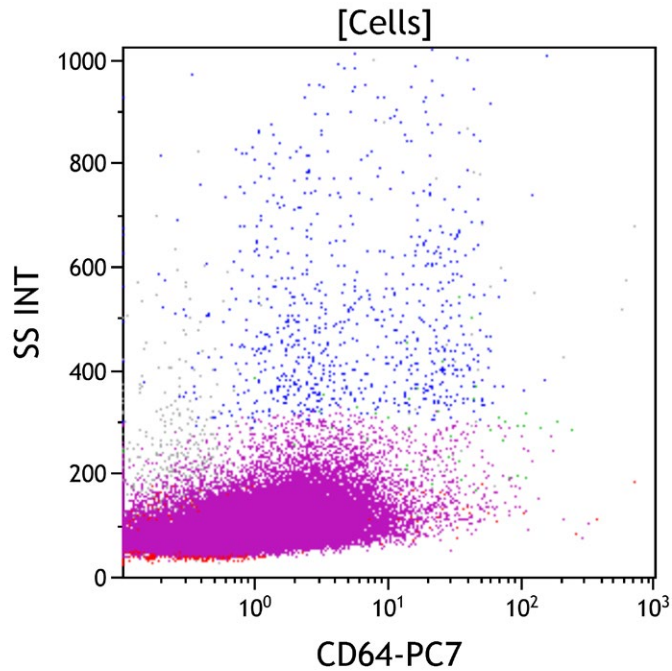

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The expanded progenitors (purple) do not express CD64.

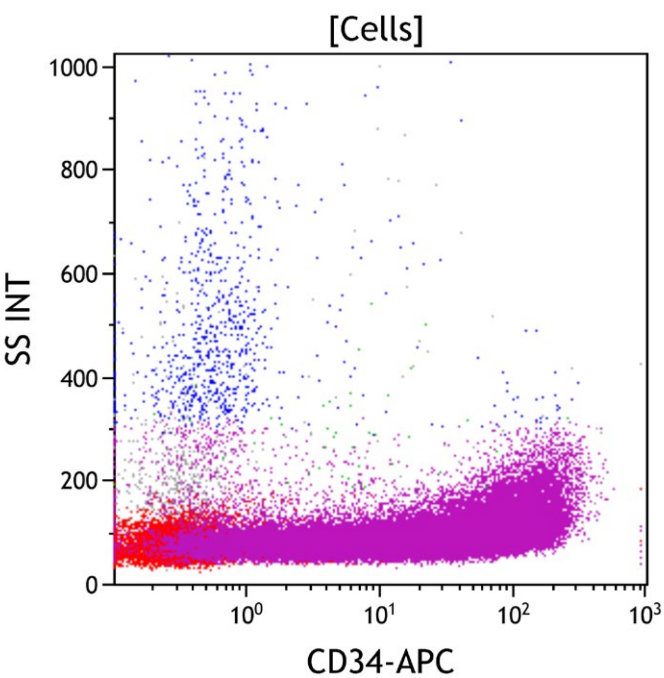

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The expanded progenitors (purple) variably express CD34.

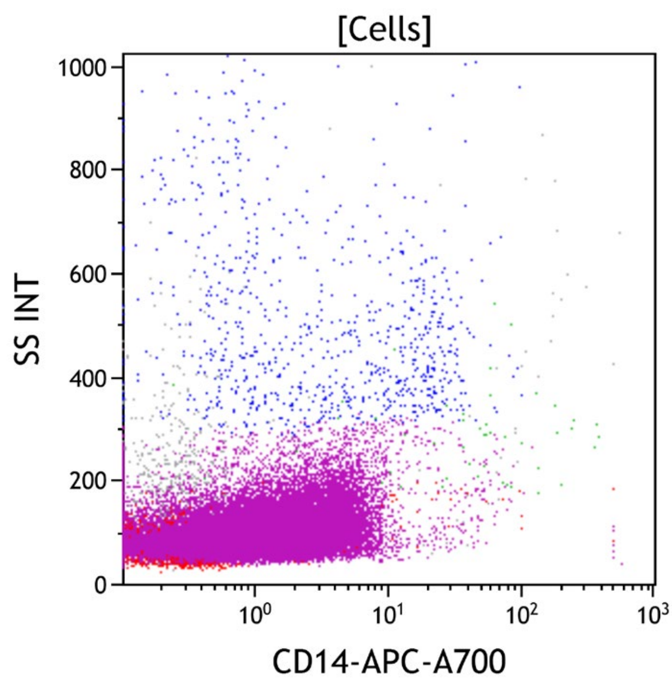

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The expanded progenitors (purple) do not express CD14. The apparent dim CD14 expression is due to increased compensation background from the bright CD34.

M1 Cell Tube

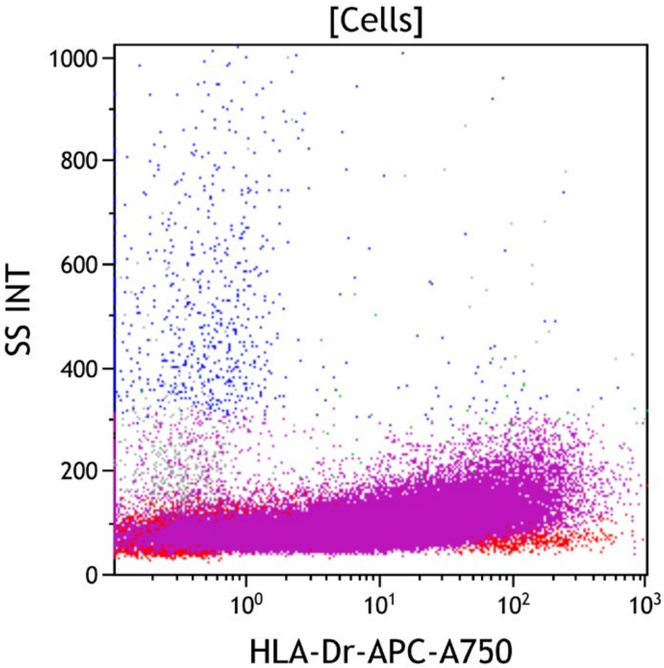

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). The expanded progenitors (purple) variably express HLA-DR.

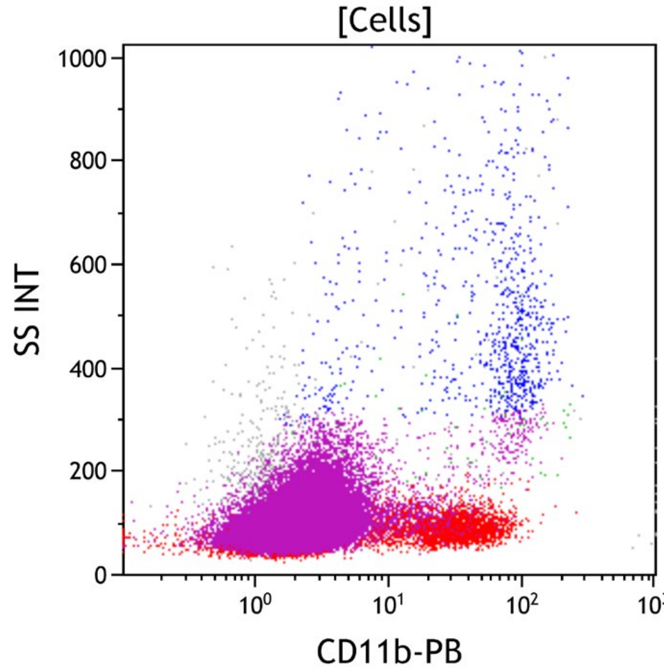

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The expanded progenitors (purple) do not express CD11b.

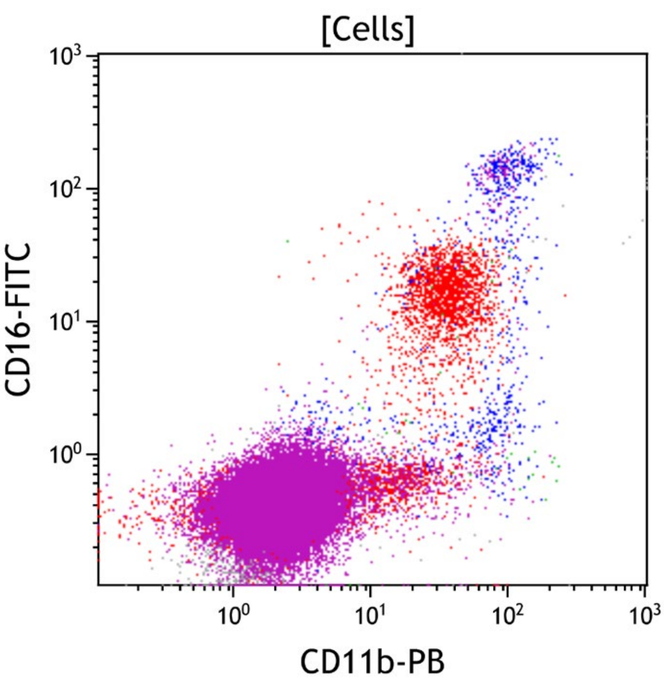

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (in green), immature and mature granulocytes (in blue) and NK cells (in red). CD16 is expressed on immature and mature granulocytes (in blue) and a subset of NK cells (in red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

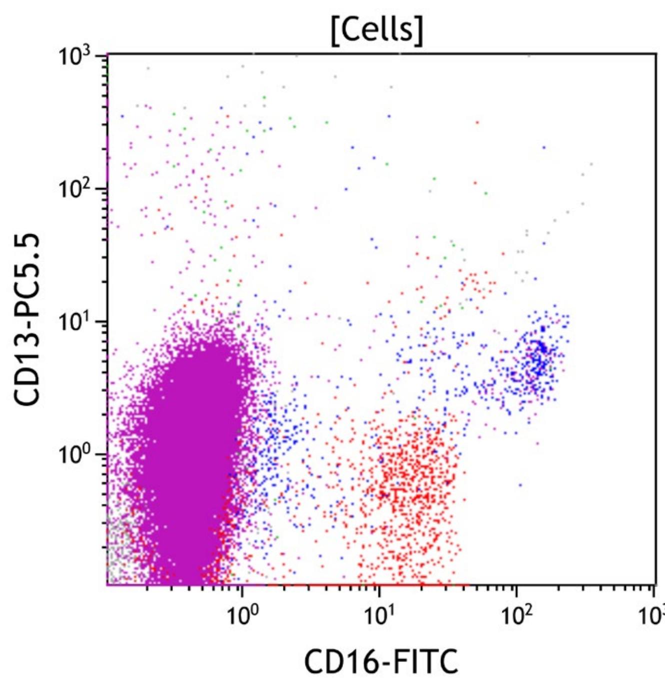

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16.

M1 Cell Tube

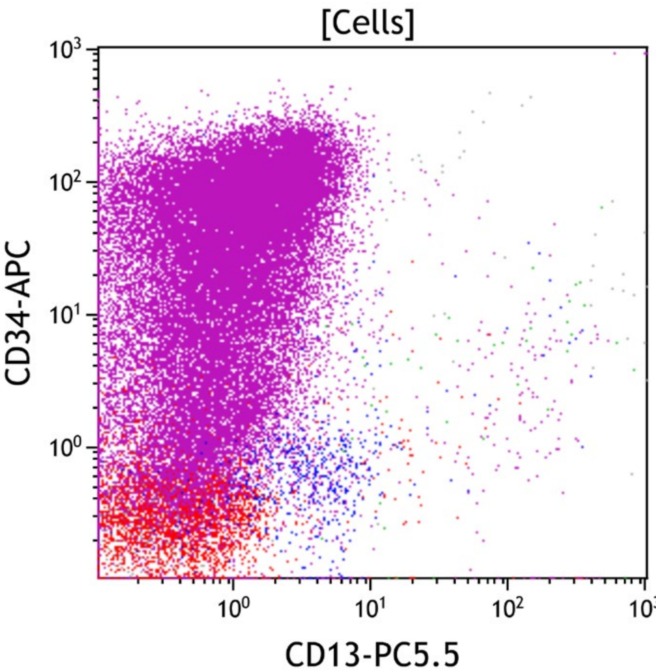

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells (red). The expanded progenitors express CD34 and dim to absent CD13.

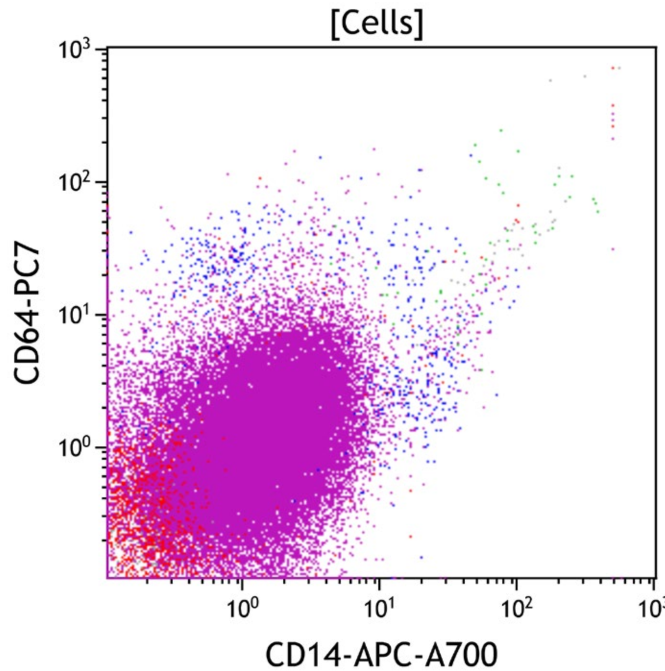

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. The expanded progenitors do not express CD14 or CD64.

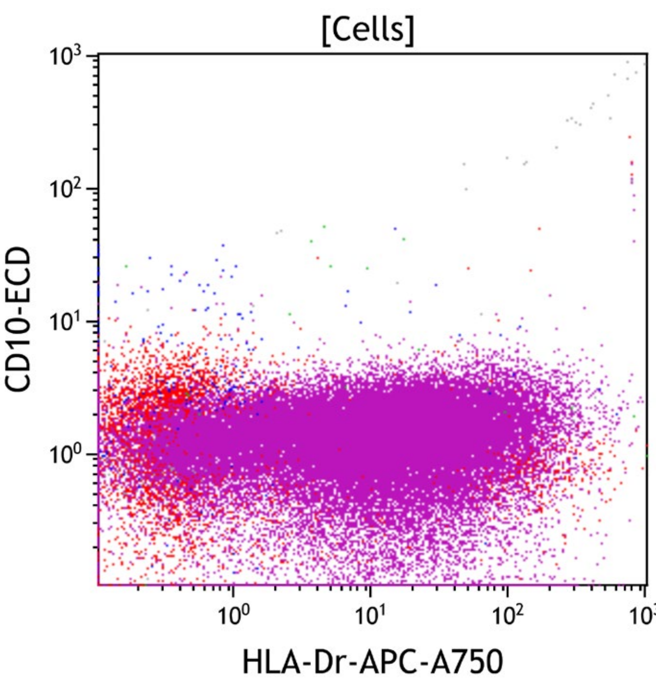

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors CD10 is expressed by mature granulocytes and immature B cells. Immature B cells express both CD10 and HLA-DR. The expanded progenitors variably express HLA-DR but do not express CD10.

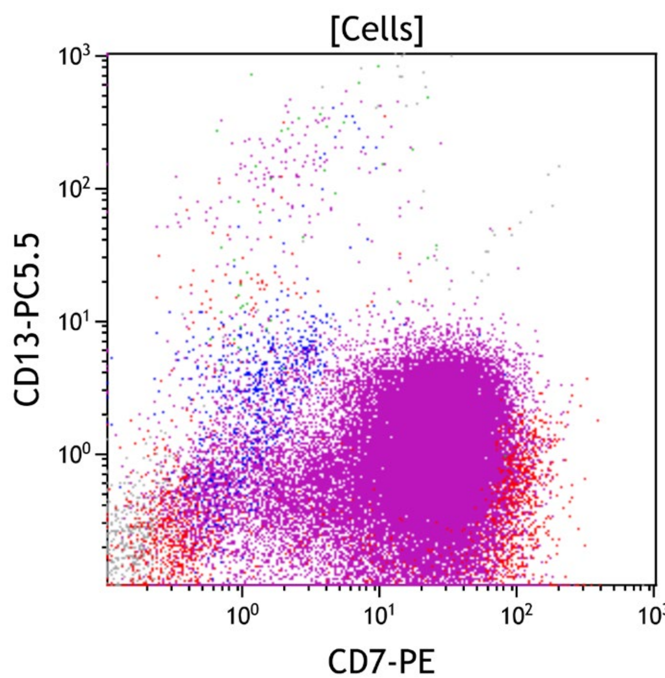

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The expanded progenitors express CD7 and dim to absent CD13.

M2 Cell Tube

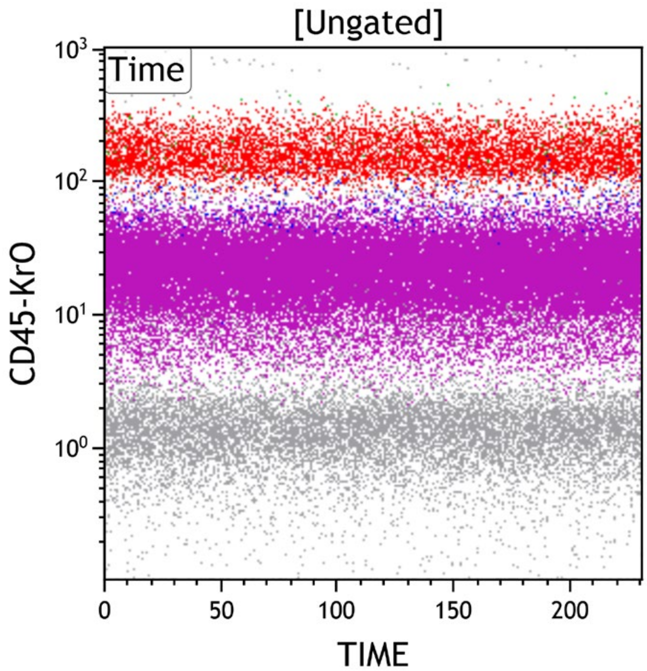

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

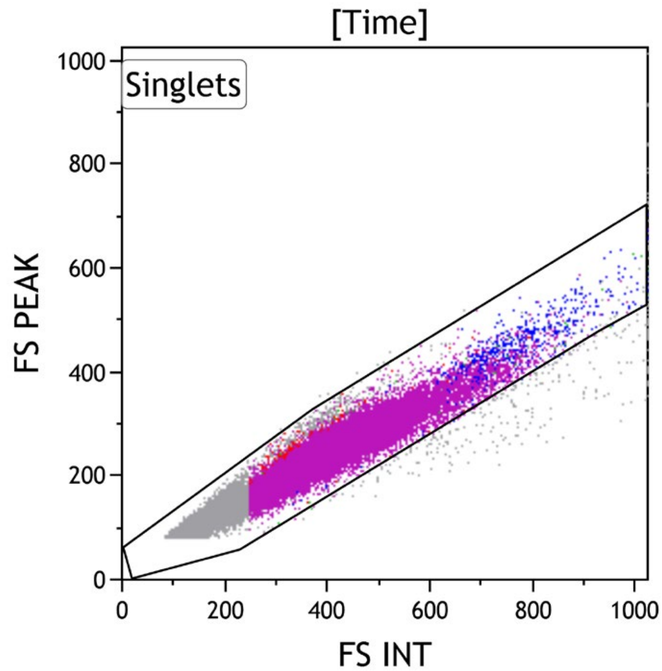

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

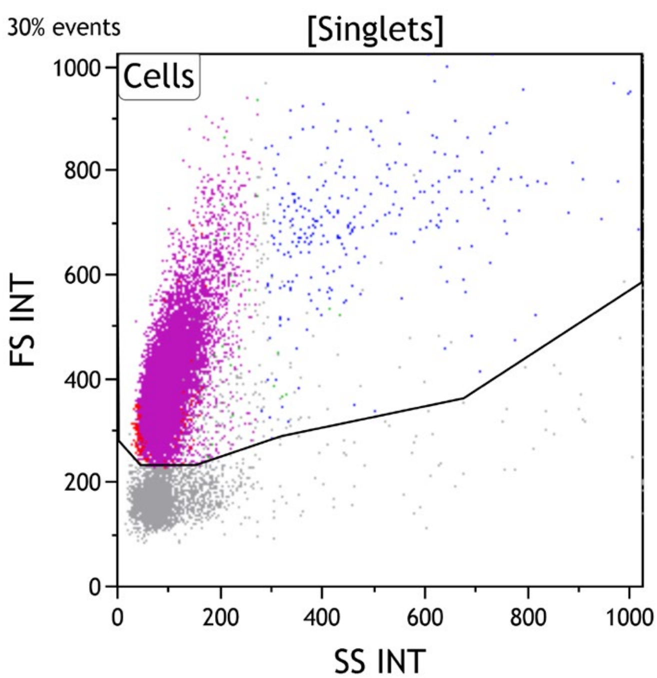

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

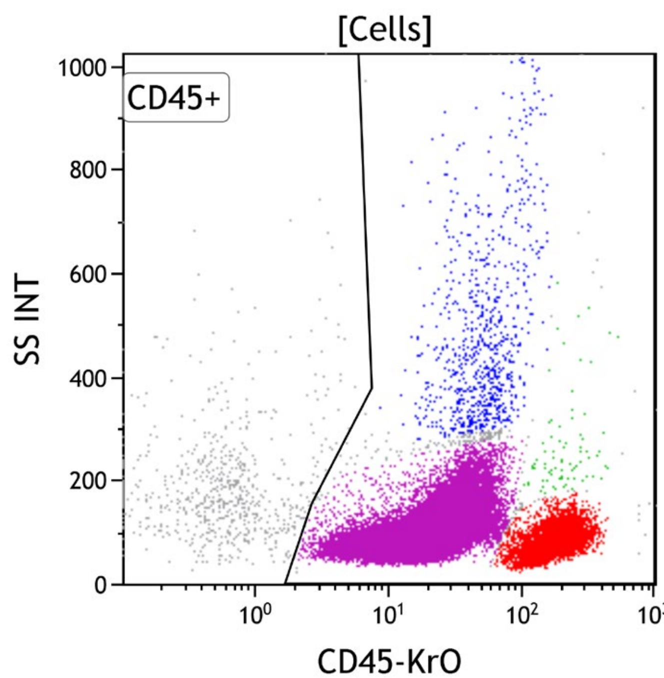

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

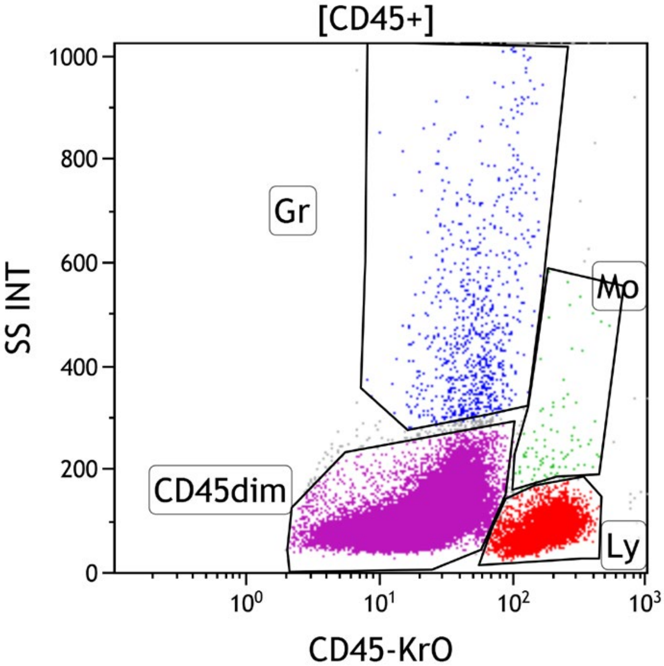

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the expanded progenitors in the CD45 dim gate (purple).

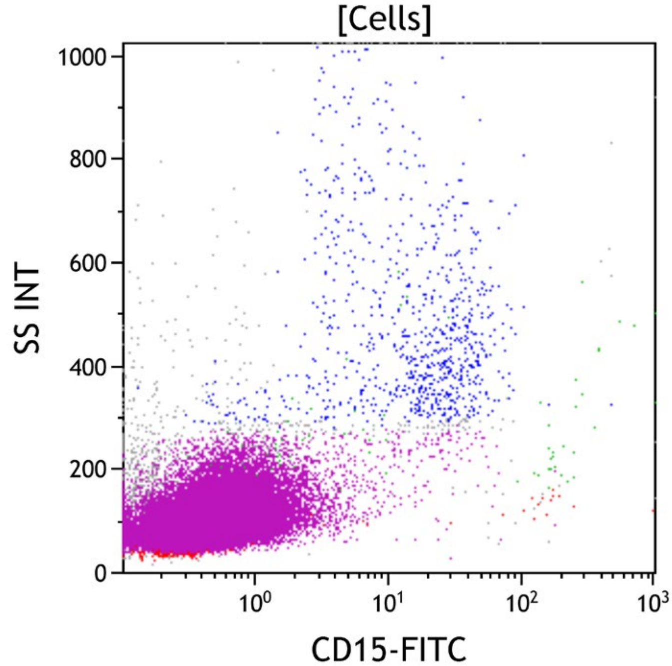

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The expanded progenitors (purple) do not express CD15.

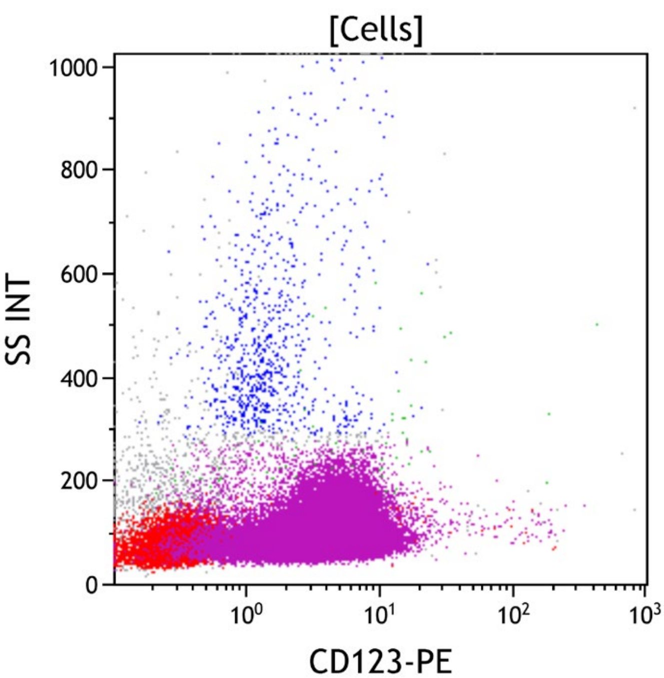

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The expanded progenitors (purple) express intermediate CD123.

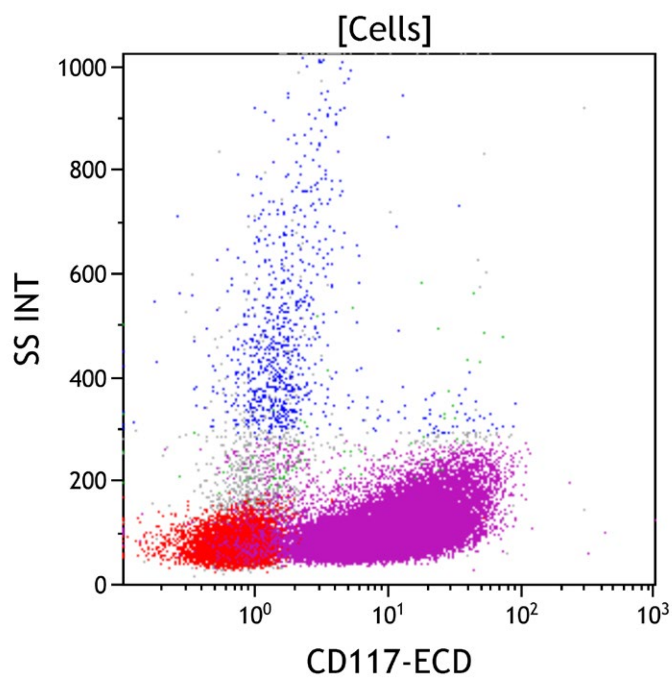

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The expanded progenitors (purple) express intermediate CD117.

M2 Cell Tube

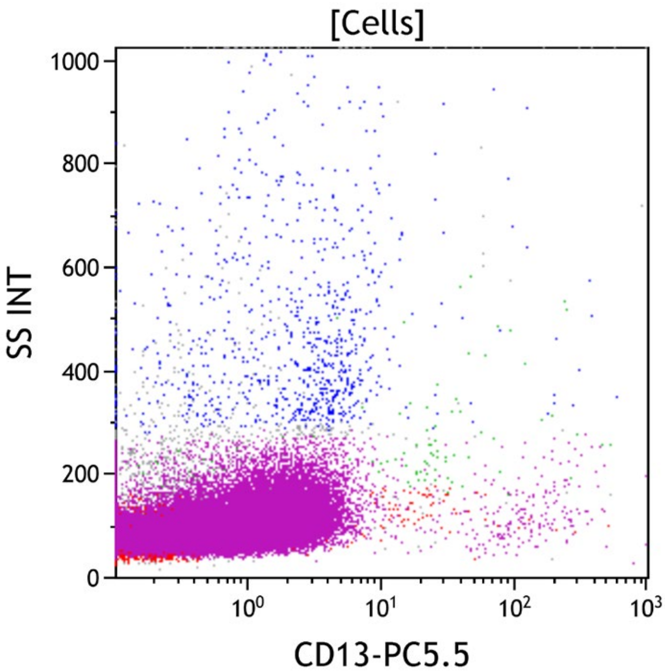

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The expanded progenitors (purple) express dim to absent CD13.

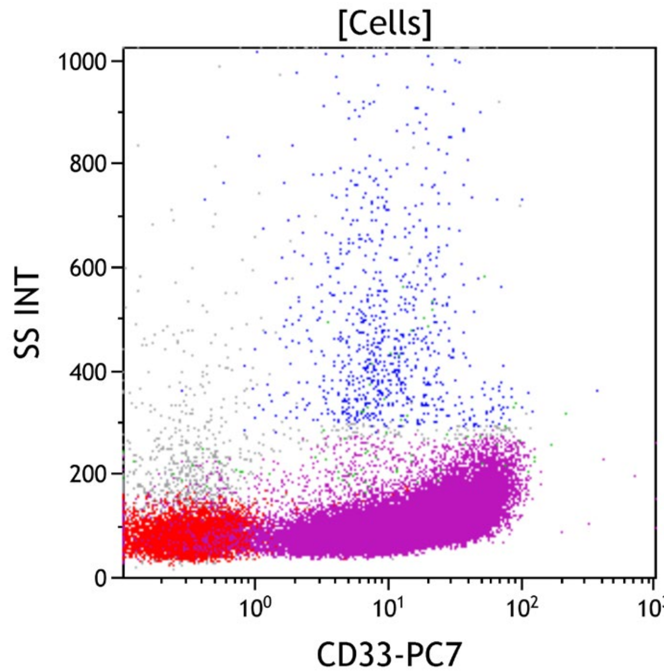

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. The expanded progenitors (purple) express intermediate to bright CD33.

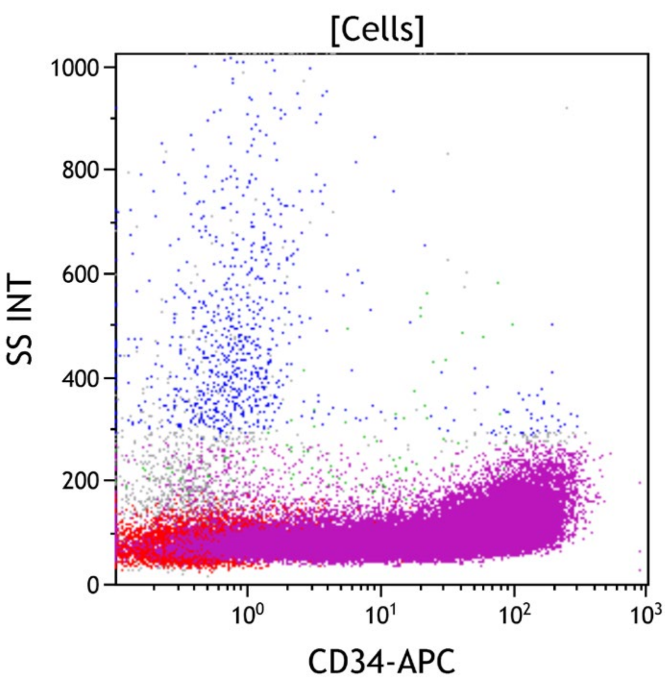

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The expanded progenitors (purple) variably express CD34.

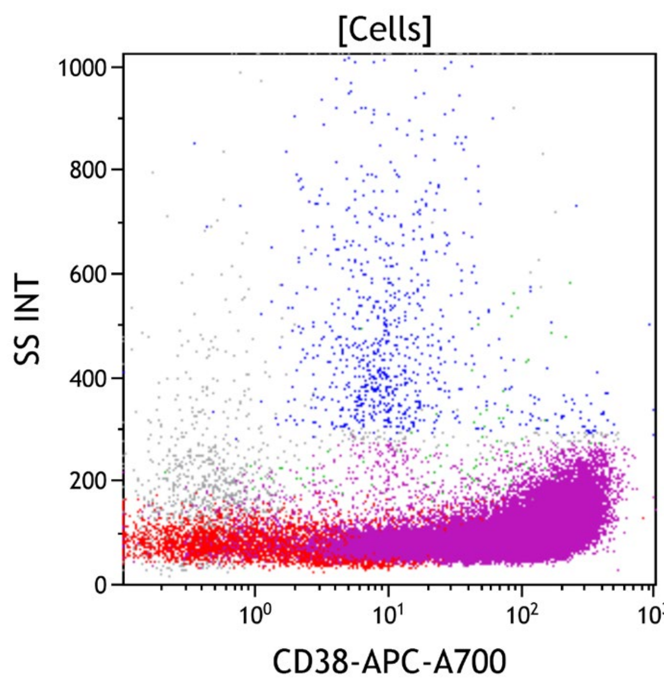

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The expanded progenitors (purple) express CD38.

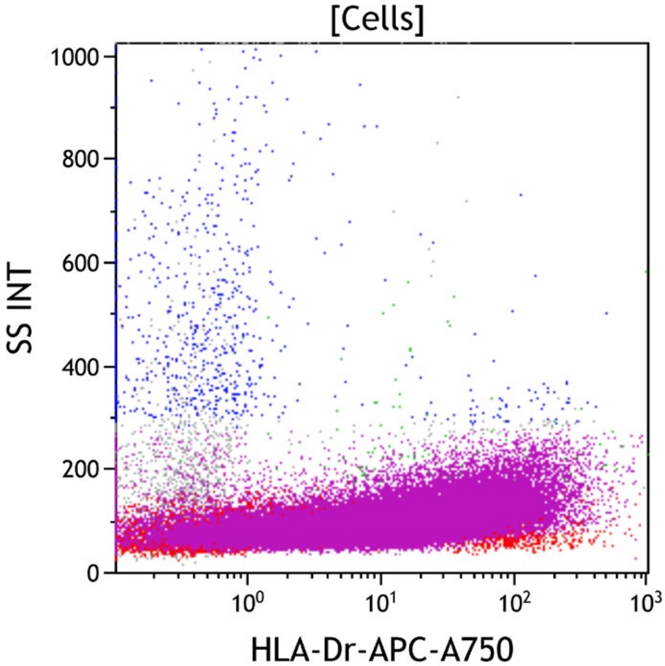

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). The expanded progenitors (purple) variably express HLA-DR.

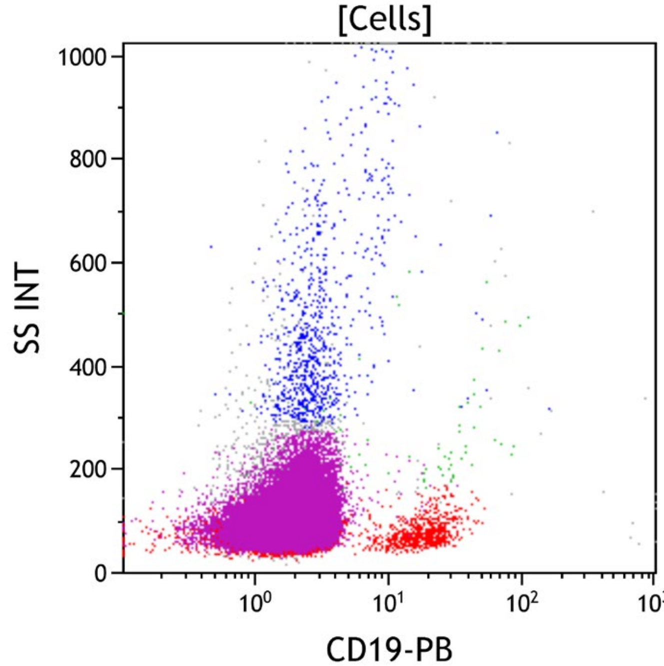

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The expanded progenitors (purple) do not express CD19.

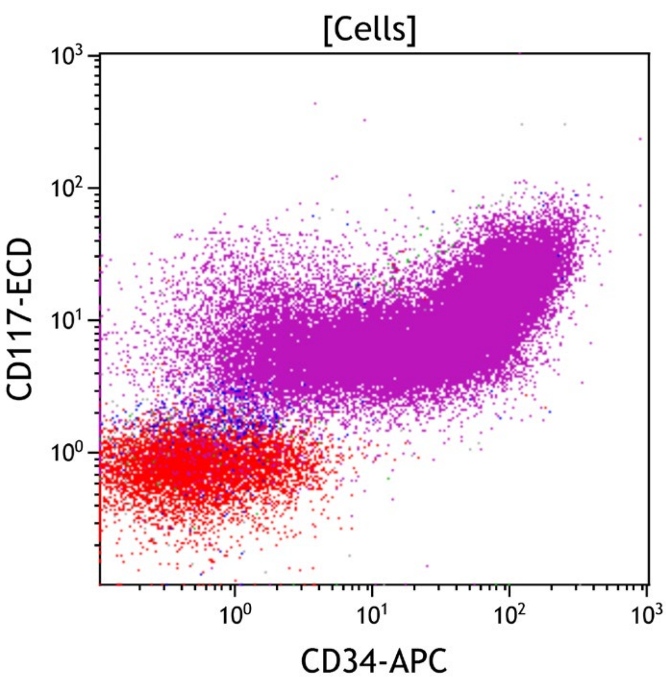

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors. The expanded progenitors (purple) express intermediate CD34 and CD117.

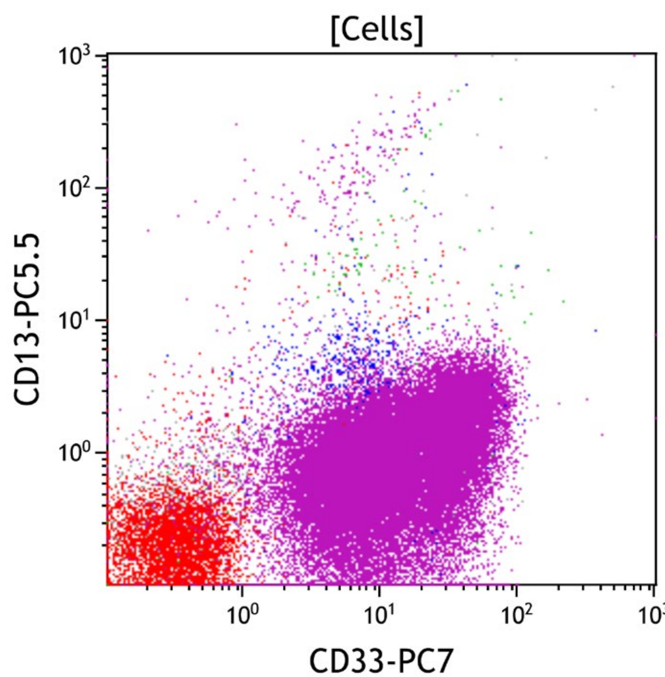

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red). The expanded progenitors (purple) express intermediate to bright CD33 and dim to absent CD13.

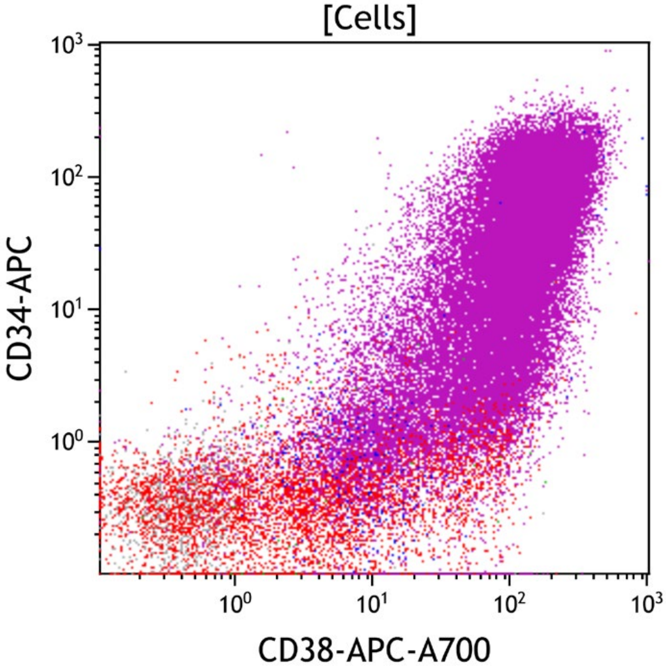

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. The expanded progenitors (purple) have coexpression of CD34 and CD38.

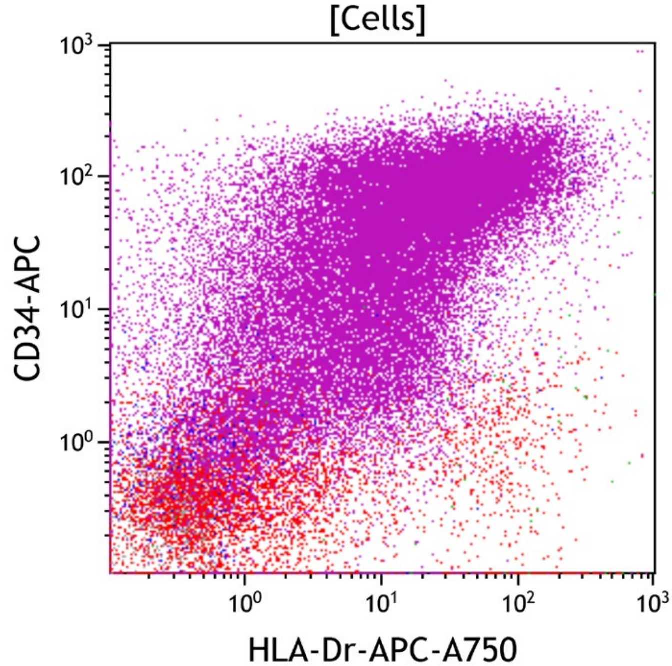

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The expanded progenitors (purple) have expression of intermediate CD34 and variable HLA-DR.

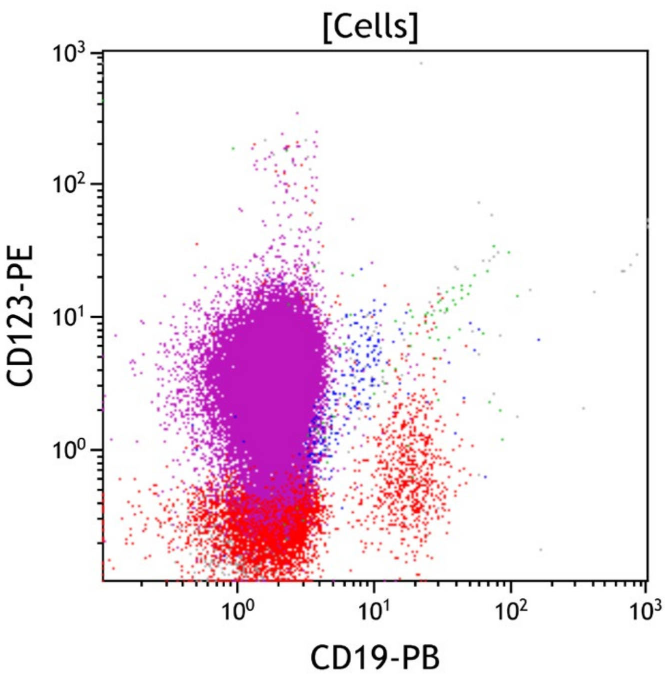

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The expanded progenitors (purple) express intermediate CD123 without CD19.

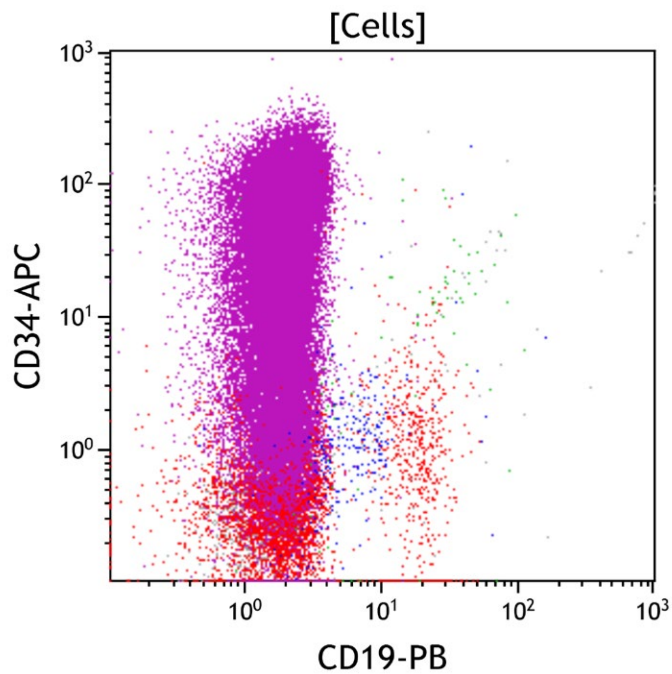

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors (purple) do not express CD19.

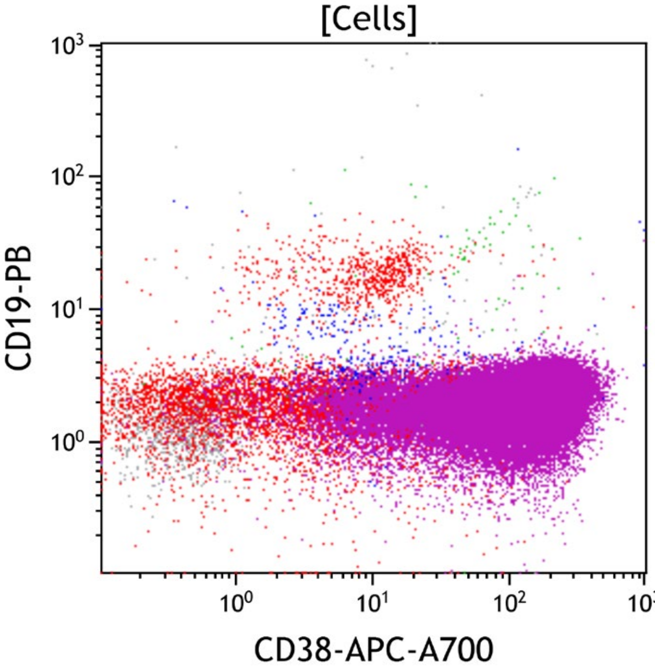

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors (purple) do not express CD19, but express intermediate to bright CD38.

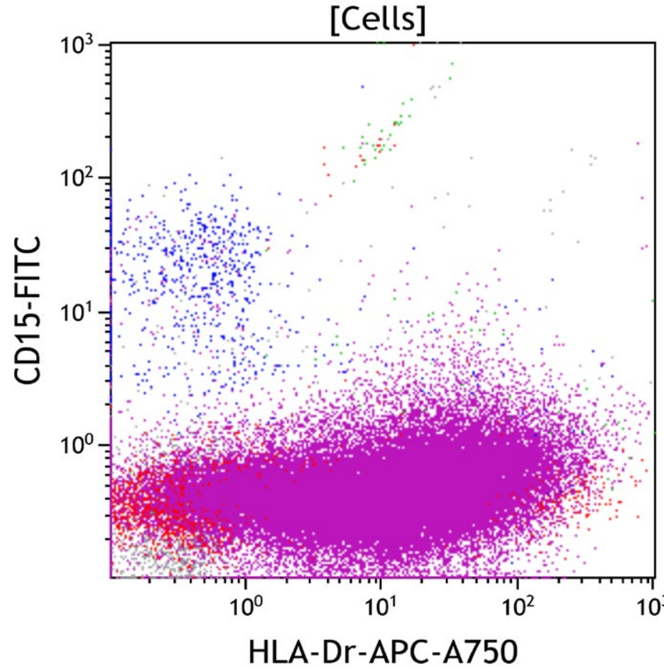

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and rare monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors (purple) express HLA-DR but only transiently express CD15.

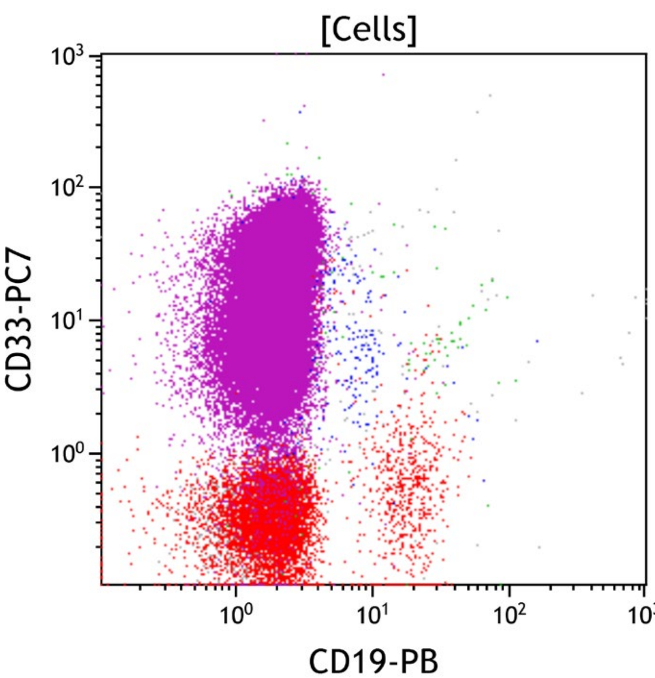

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red, lower right). CD33 is expressed by the expanded progenitors and rare monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The expanded progenitors (purple) express intermediate CD33.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of blasts with expression of intermediate CD7, dim to absent CD13, intermediate CD33, intermediate CD34, intermediate CD38, dim CD45, intermediate CD117, intermediate CD123 and variable HLA-DR without CD14, CD15, CD64 and other lymphoid or myeloid antigens. Compared with normal CD34-positive progenitors, the near-uniform expression of CD7 and decreased expression of CD13 is aberrant.

The immunophenotype of the abnormal population is that of expanded abnormal CD34-positive progenitors. This finding is consistent with an acute leukemia with myeloid differentiation (based on CD33 and CD117 expression). However, additional testing for cytoplasmic CD3 and cytoplasmic myeloperoxidase is indicated to exclude early precursor T-lymphoblastic leukemia and mixed phenotype acute leukemia. Correlation with clinical, morphologic and cytogenetic findings is required for definitive classification

Case #19: Acute Monocytic Leukemia

Clinical Vignette

This 70-year-old female presents with lymphocytosis, monocytosis and circulating blasts on peripheral whole blood smear. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

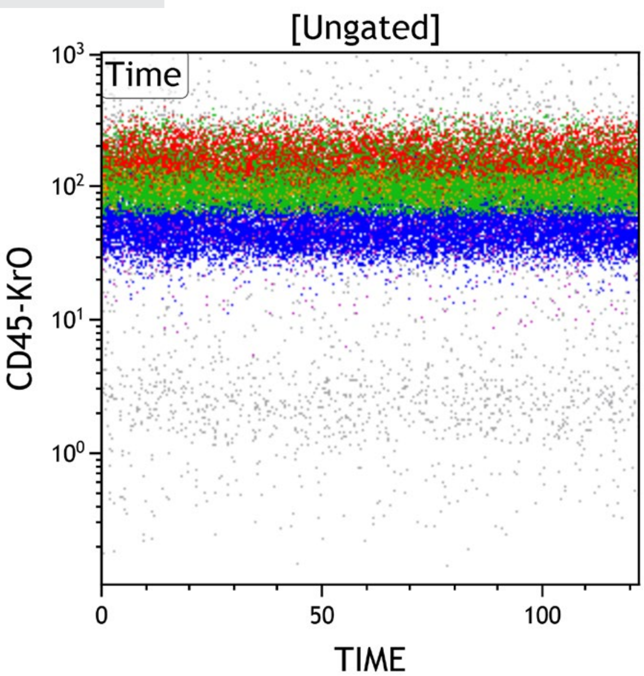

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from Time gate.

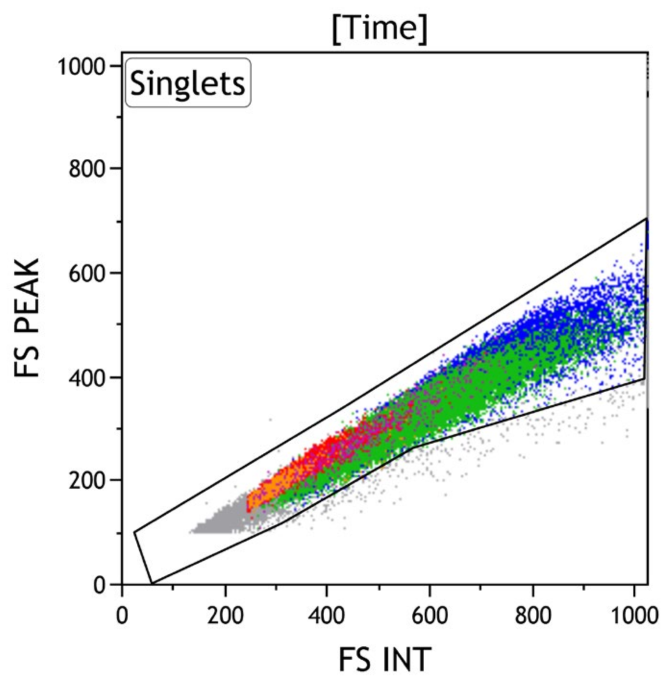

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

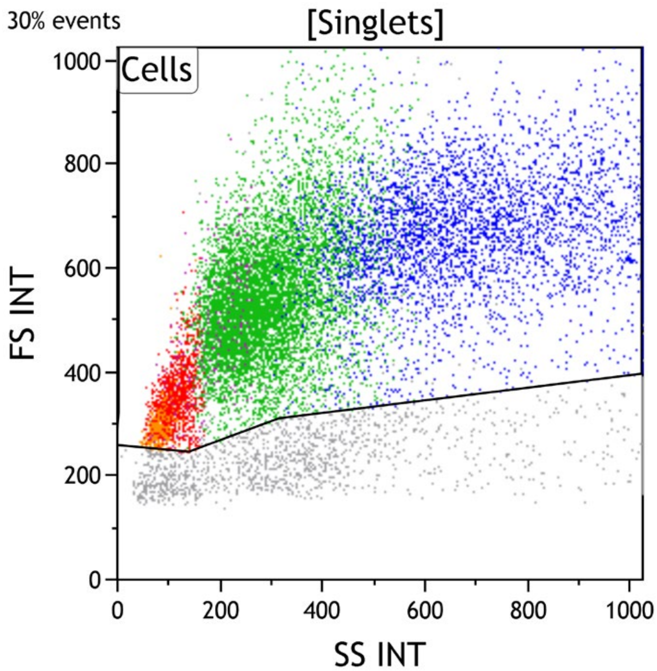

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

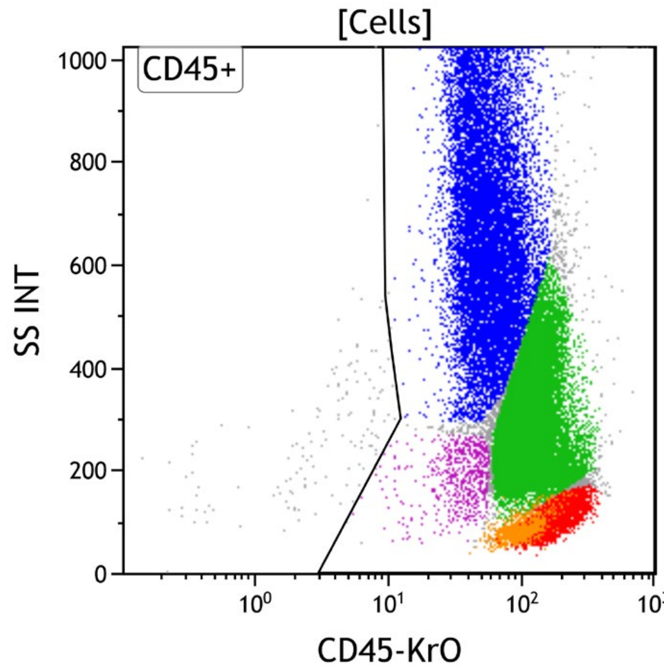

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

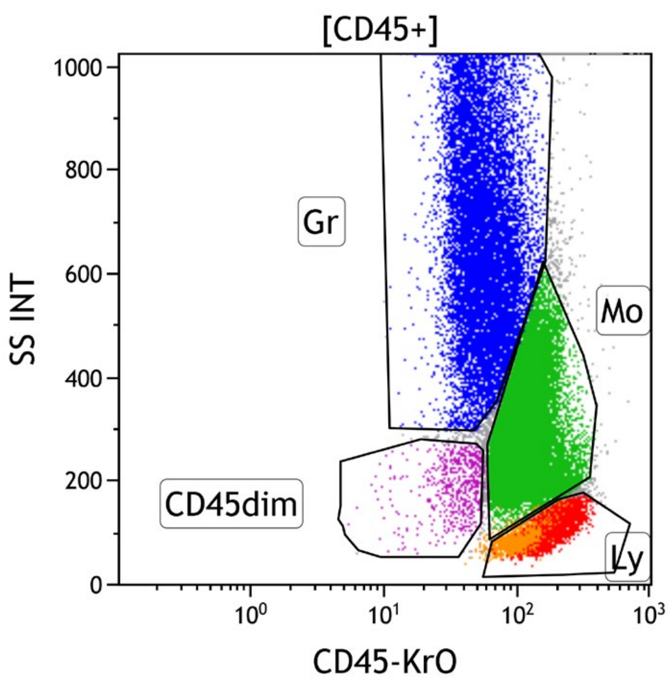

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note a relative increase in the monocytes (green).

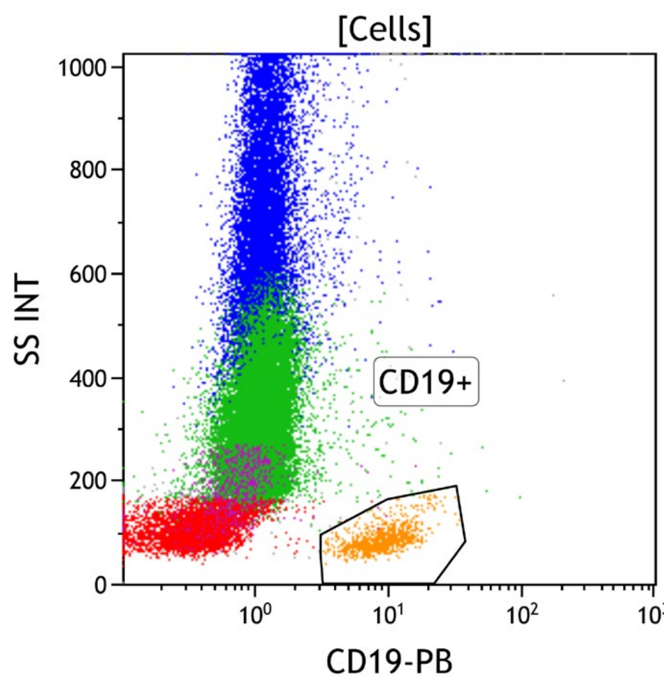

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The monocytes (green) do not express CD19.

B Cell Tube

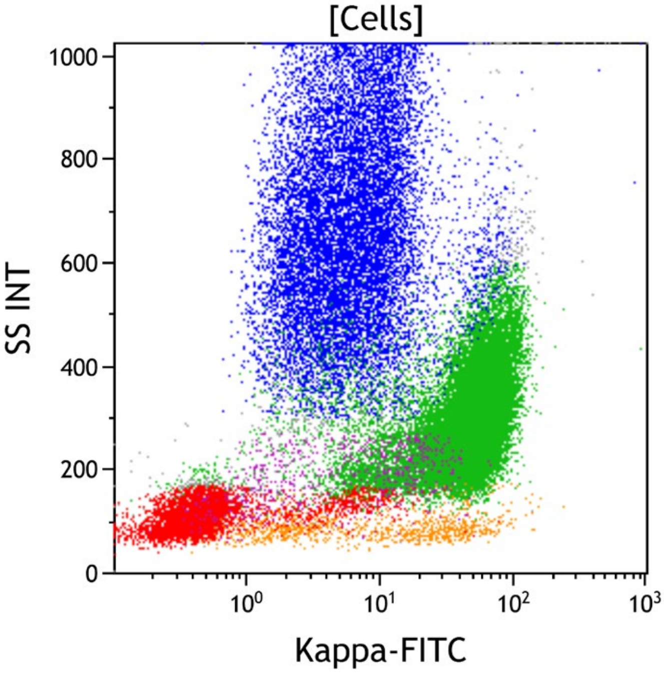

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The monocytes (green) appear to express kappa light chains likely due to Fc-receptor mediated binding of plasma immunoglobulin.

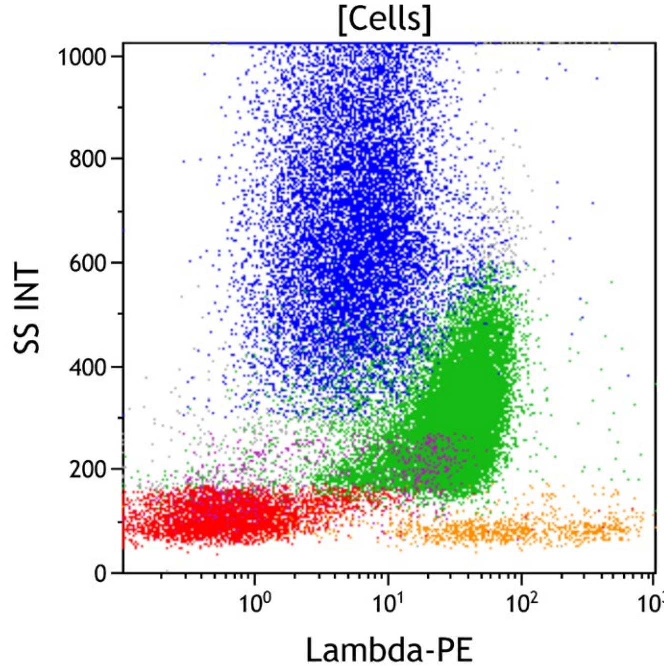

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The monocytes (green) appear to express lambda light chains likely due to Fc-receptor mediated binding of plasma immunoglobulin.

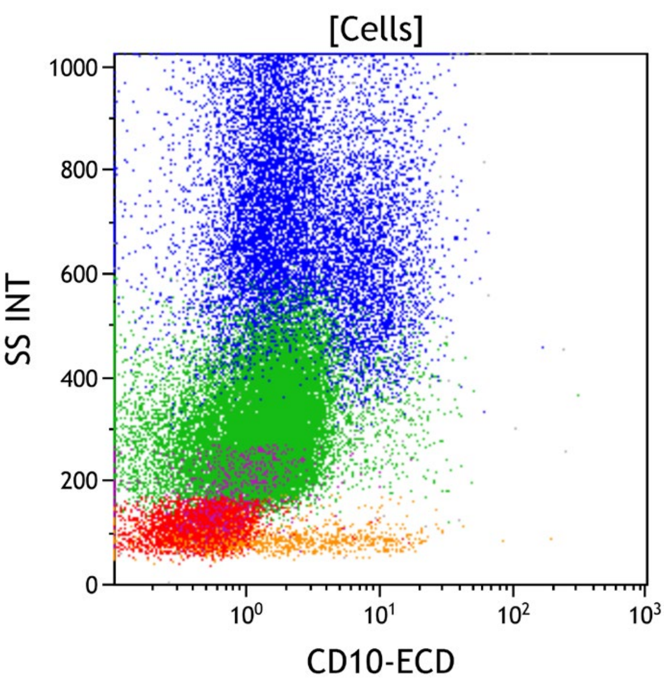

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The monocytes (green) do not express CD10.

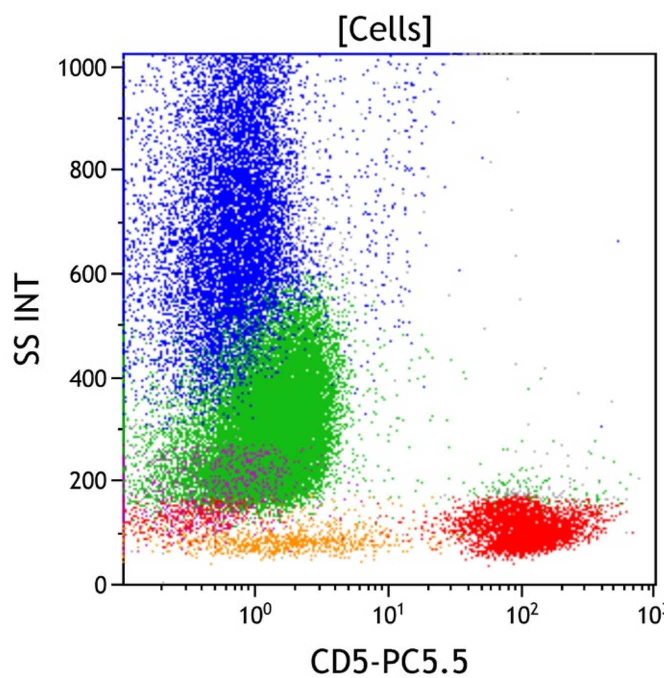

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The monocytes (green) do not express CD5.

B Cell Tube

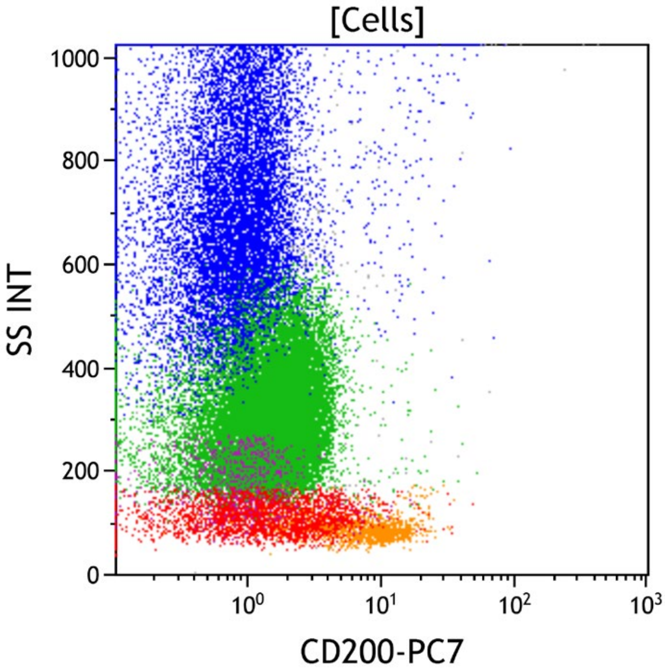

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The monocytes (green) do not express CD200.

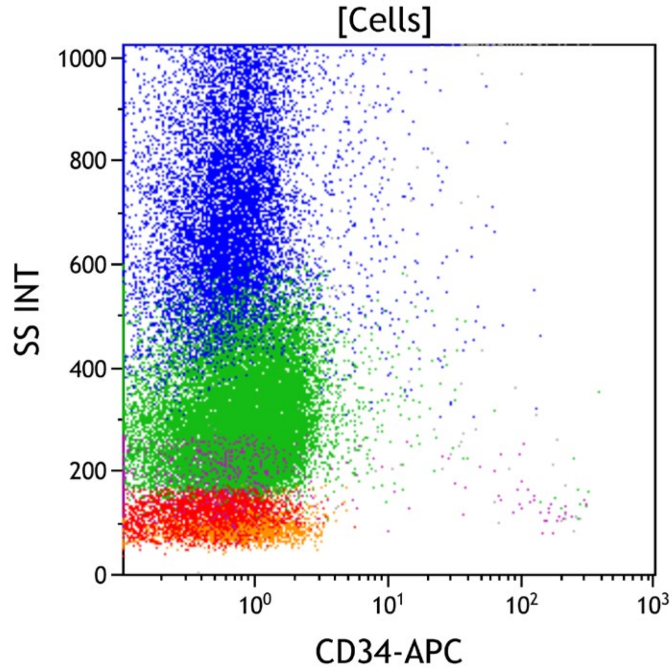

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The monocytes (green) do not express CD34.

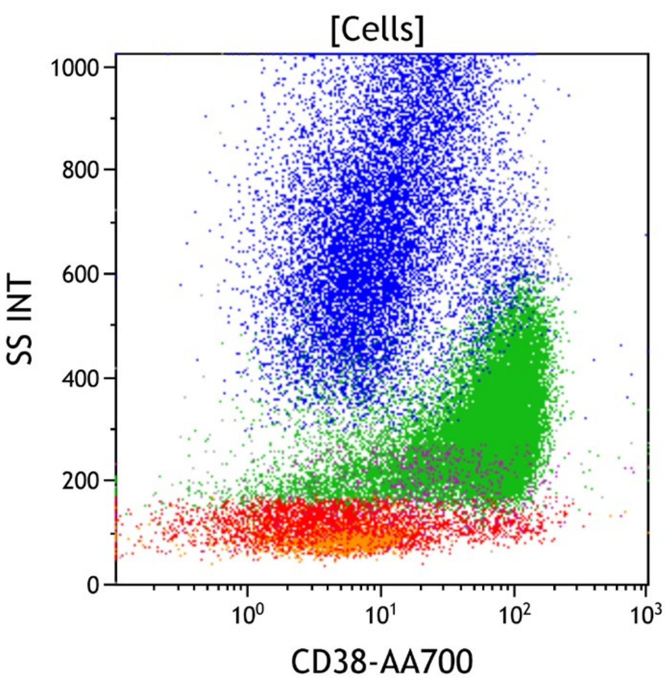

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The monocytes (green) have moderate CD38 expression.

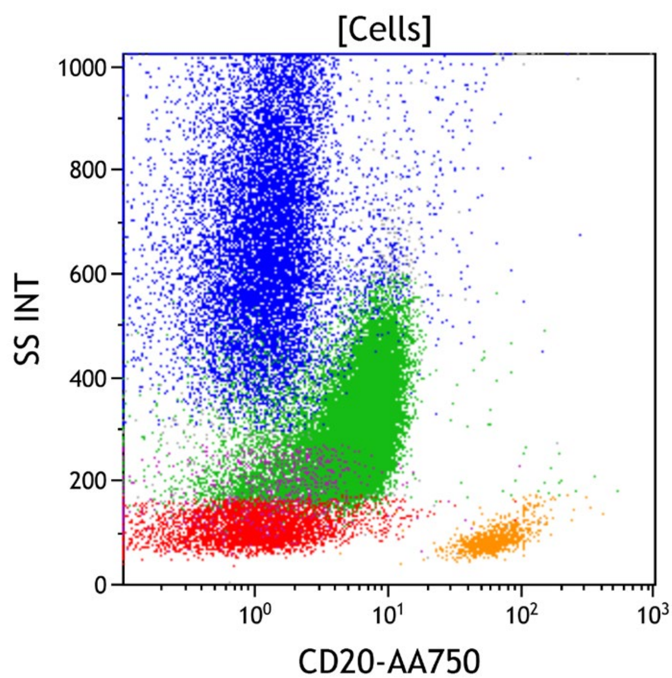

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The monocytes do not express CD20, the apparent low level expression is due to increased compensation background from CD38.

B Cell Tube

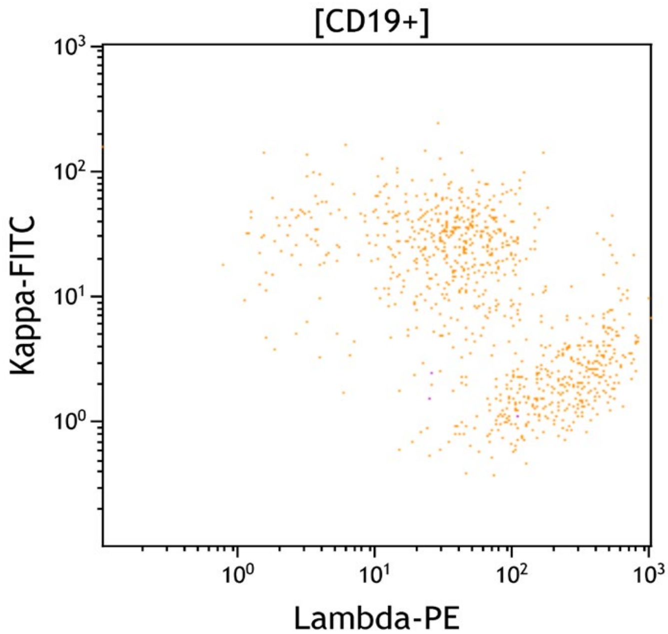

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

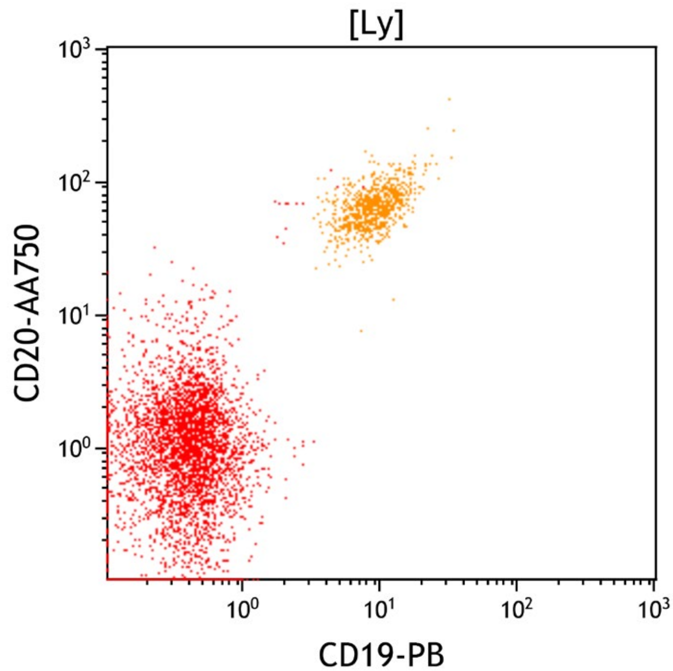

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate. Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

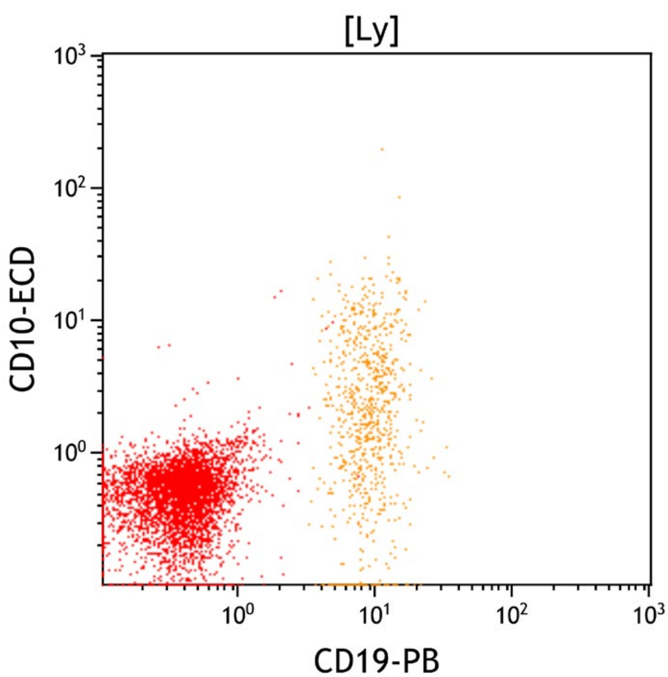

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate. B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

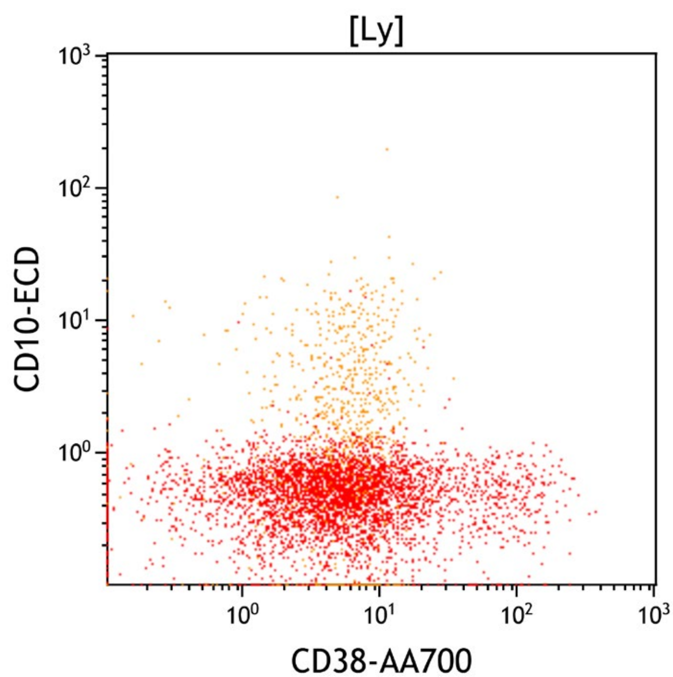

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

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B Cell Tube

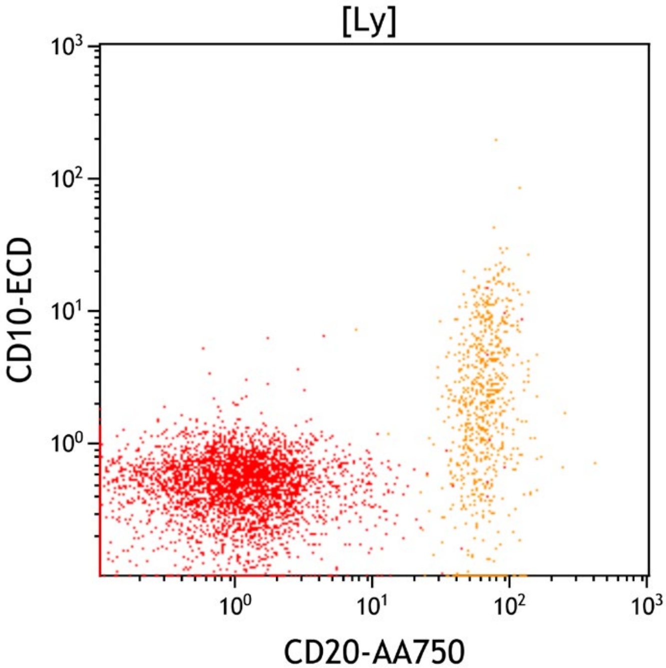


Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

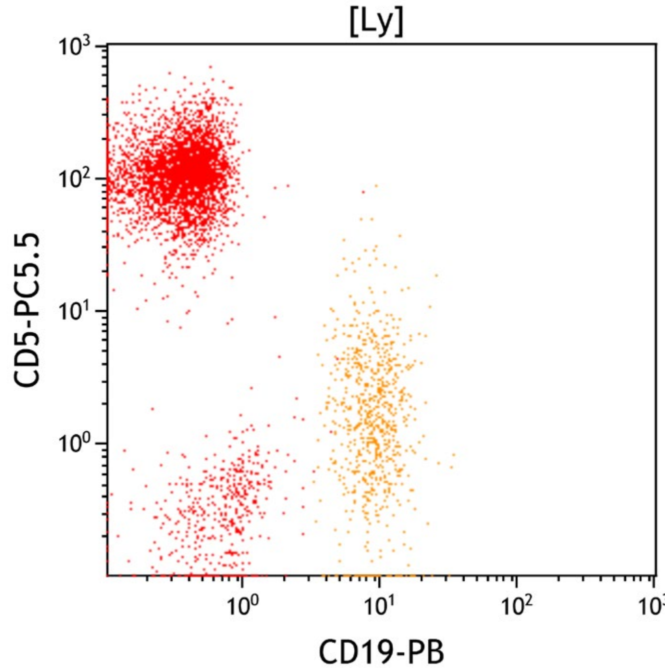


Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

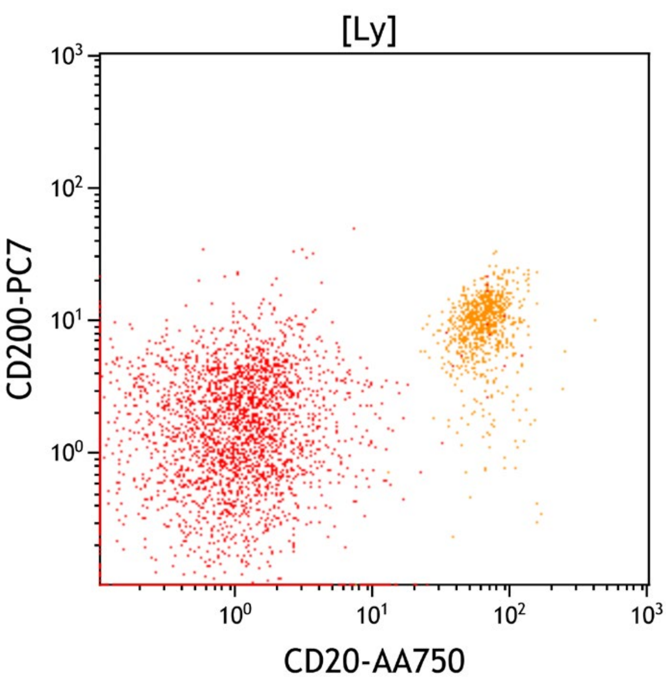


Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

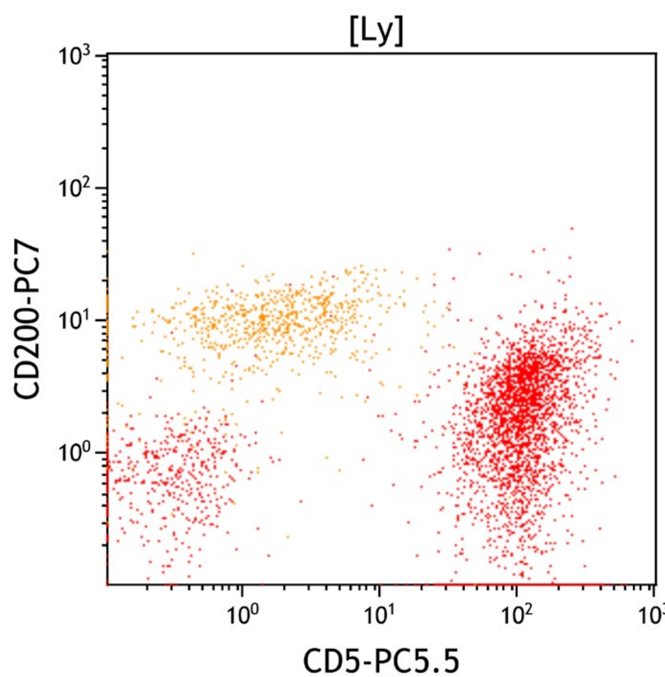


Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

Table of Contents > Neoplastic Process of Myeloid Origin > Case #19: Acute Monocytic Leukemia

T Cell Tube

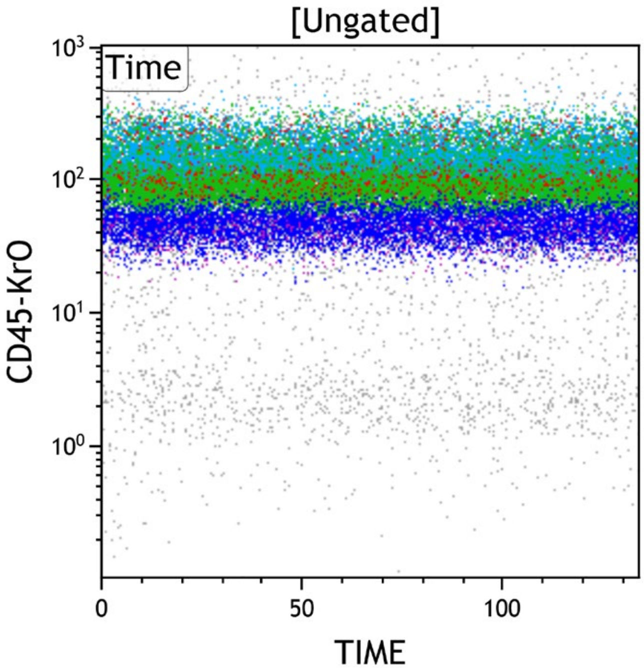


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

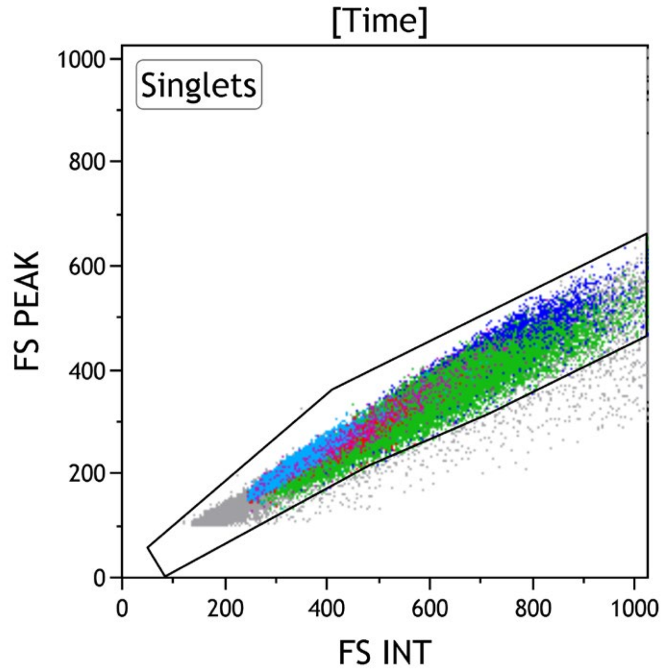


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

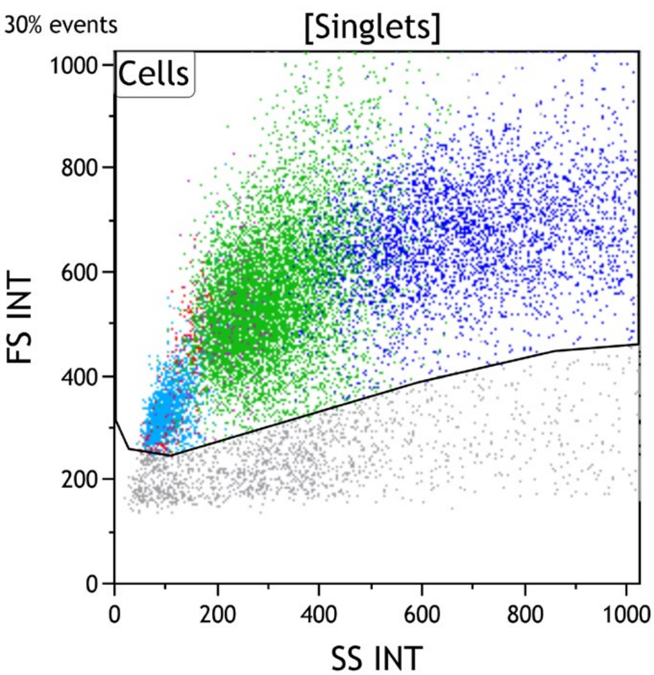


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

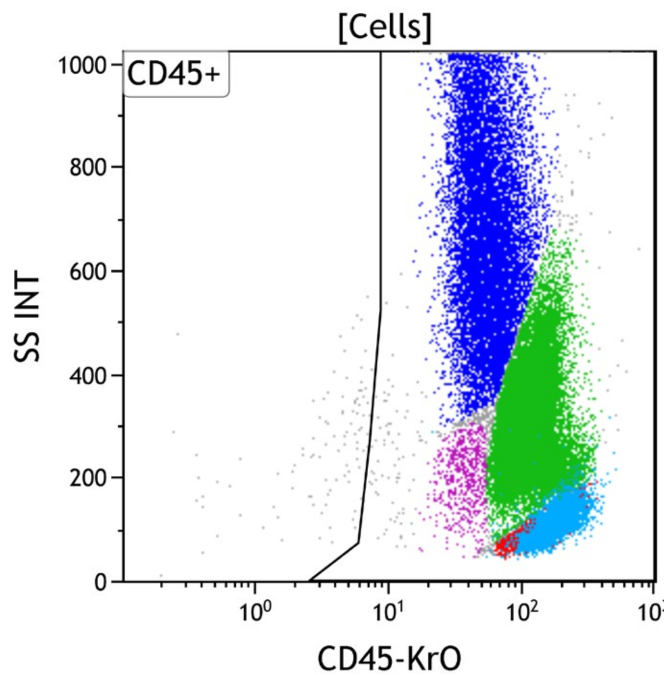


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

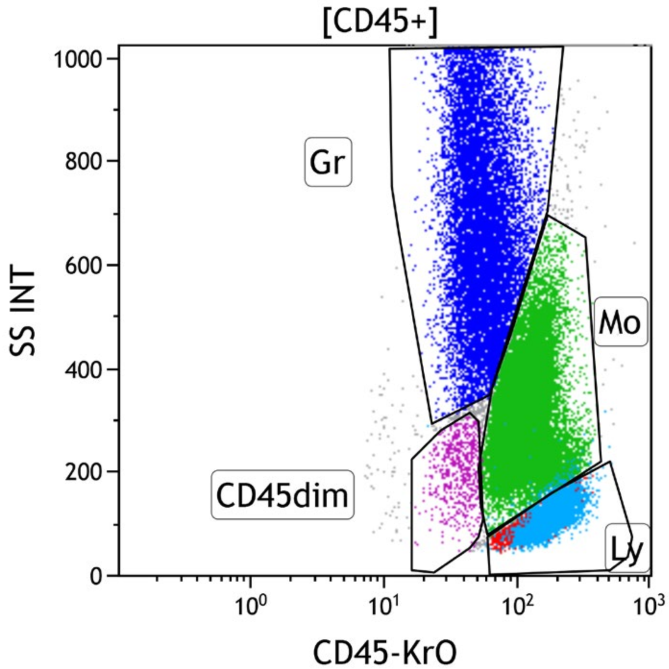


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note a relative increase in the monocytes (green).

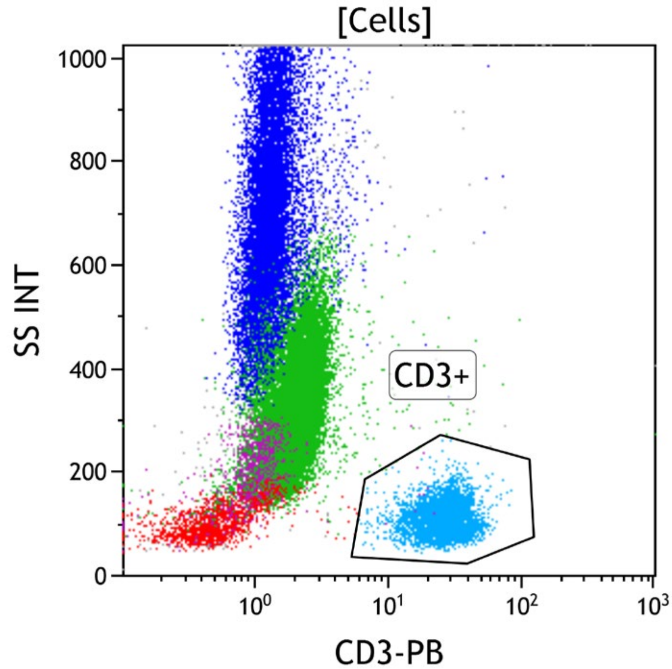


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The monocytes (green) do not express CD3.

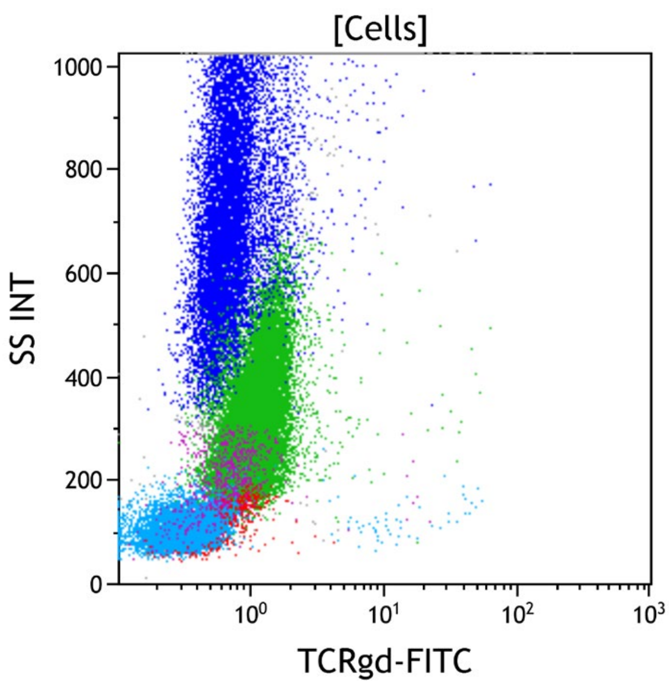


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The monocytes (green) do not express TCRγδ.

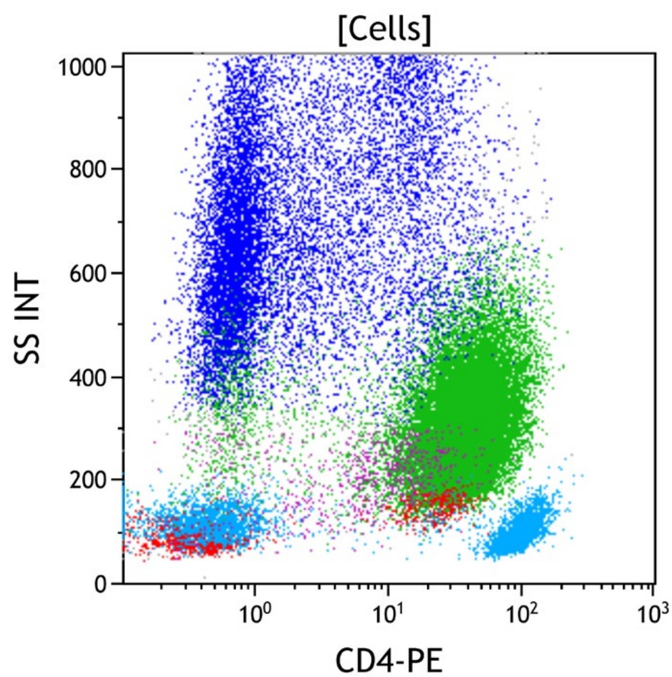


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow.

T Cell Tube

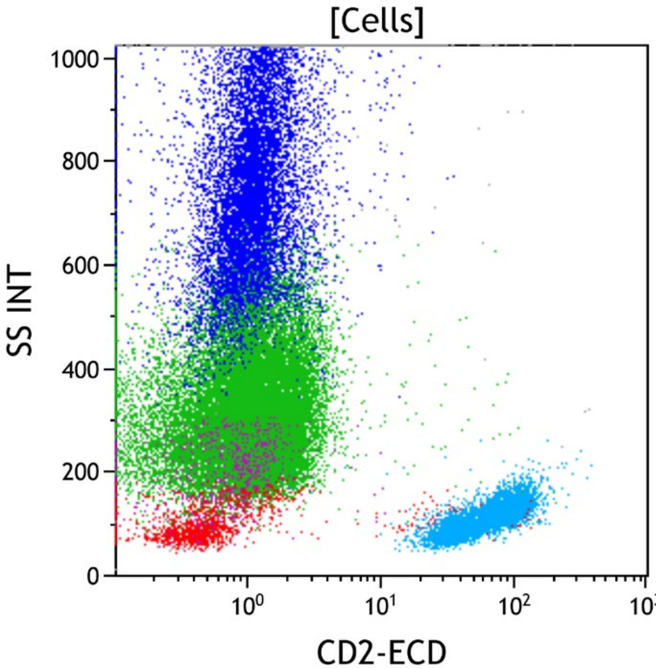


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The monocytes (green) do not express CD2.

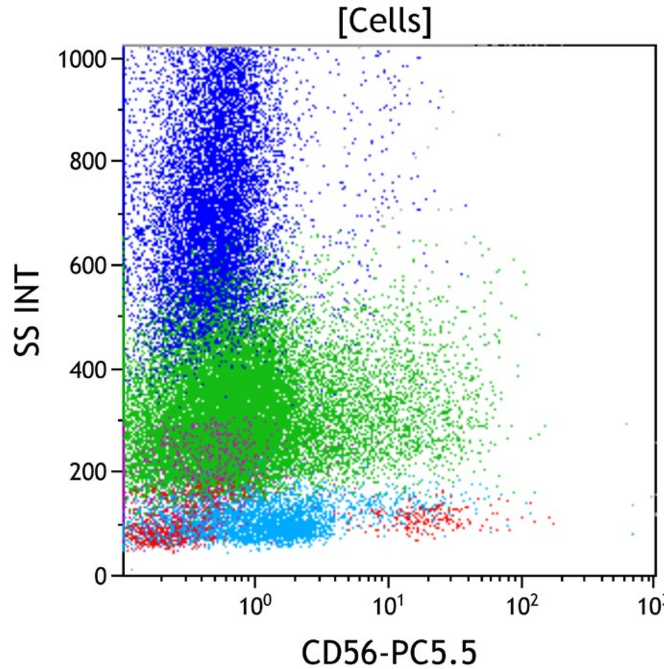


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The monocytes have dim, variable CD56 expression.

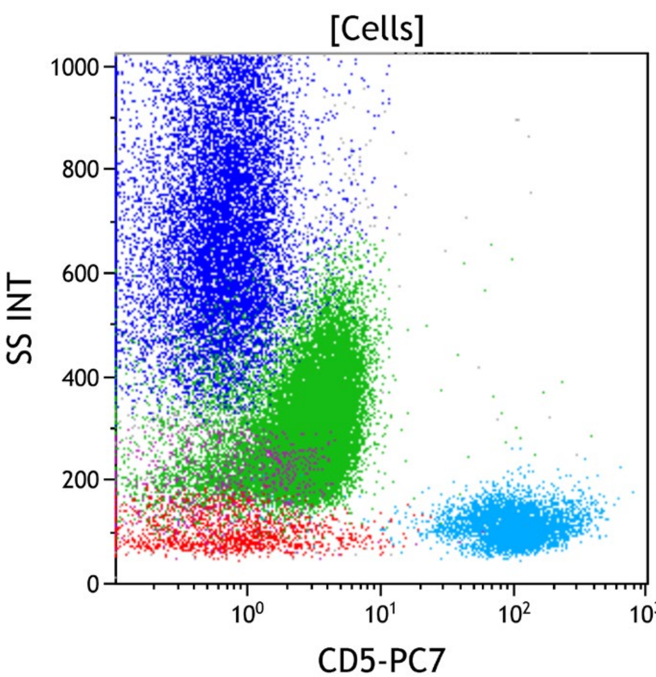


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The monocytes (green) do not express CD5.

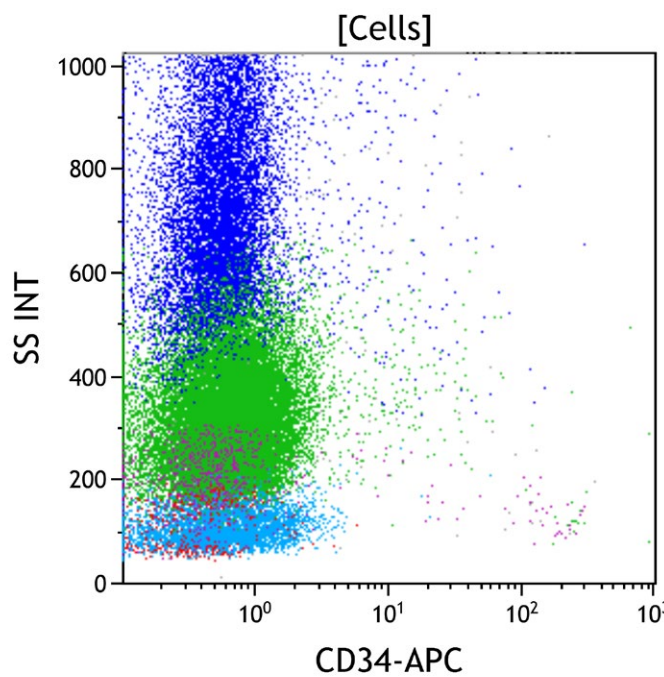


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The monocytes (green) do not express CD34.

T Cell Tube

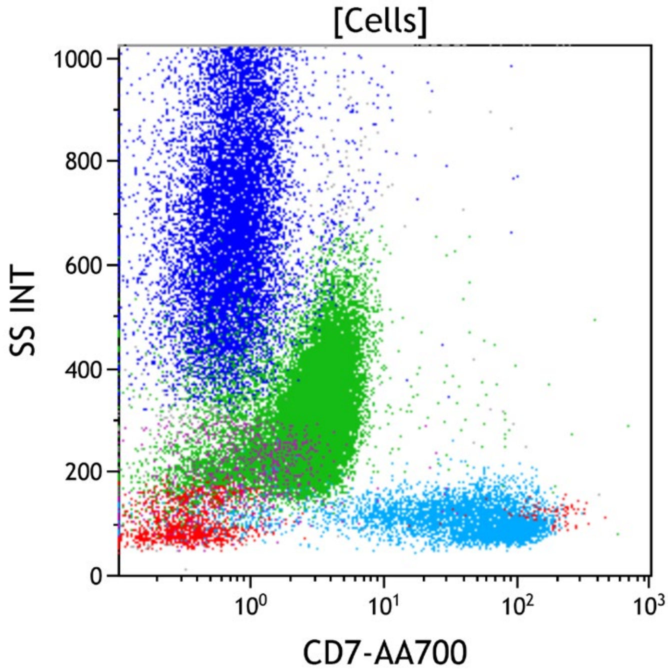


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The monocytes (green) do not express CD7.

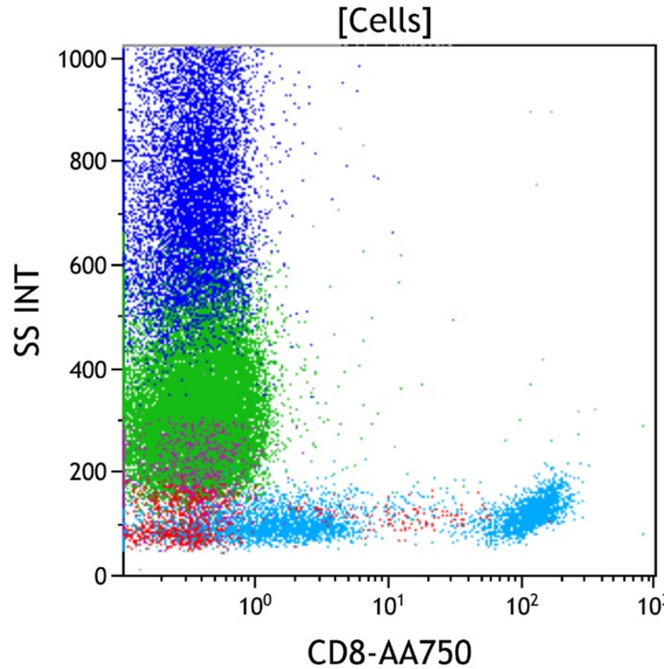


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The monocytes (green) do not express CD8.

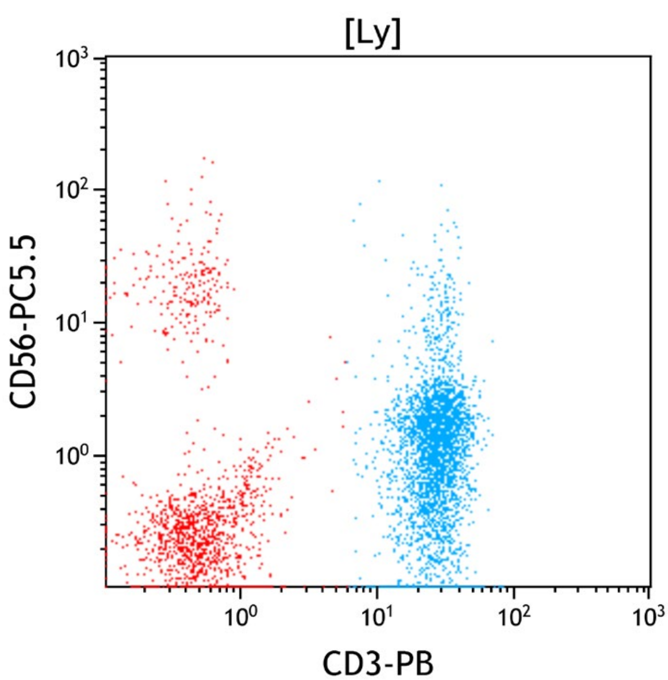


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

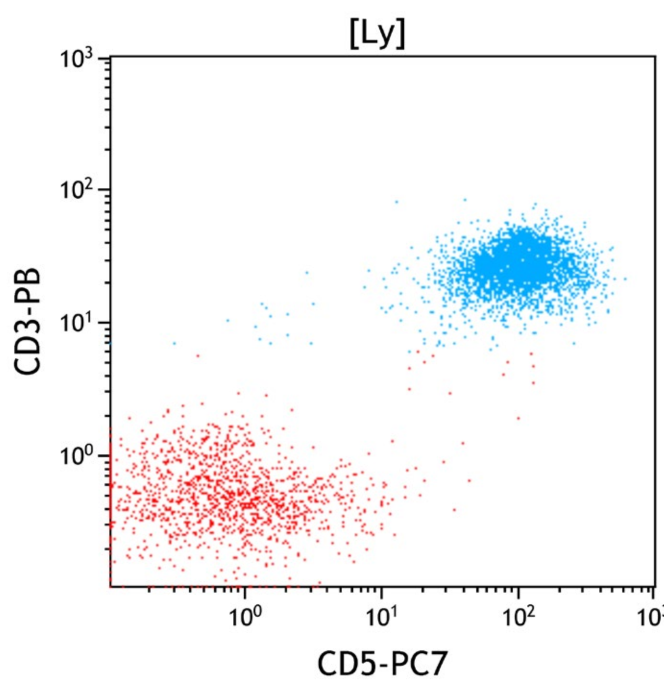


Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

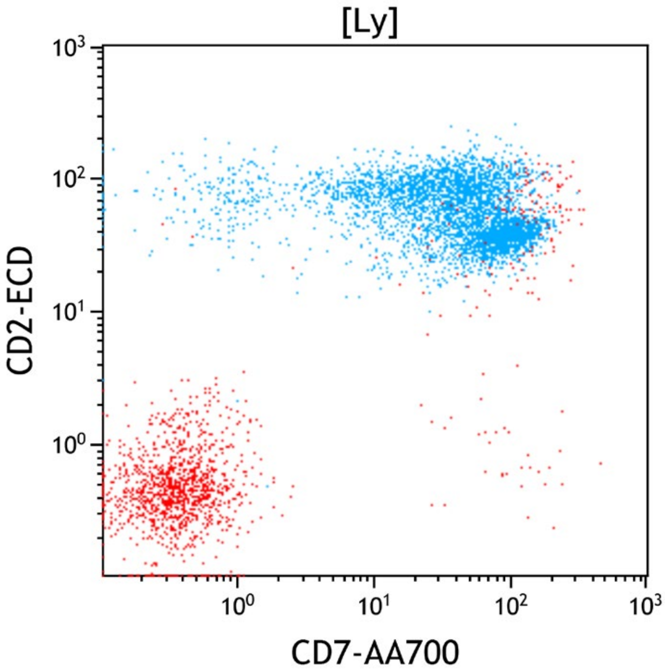


Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells.

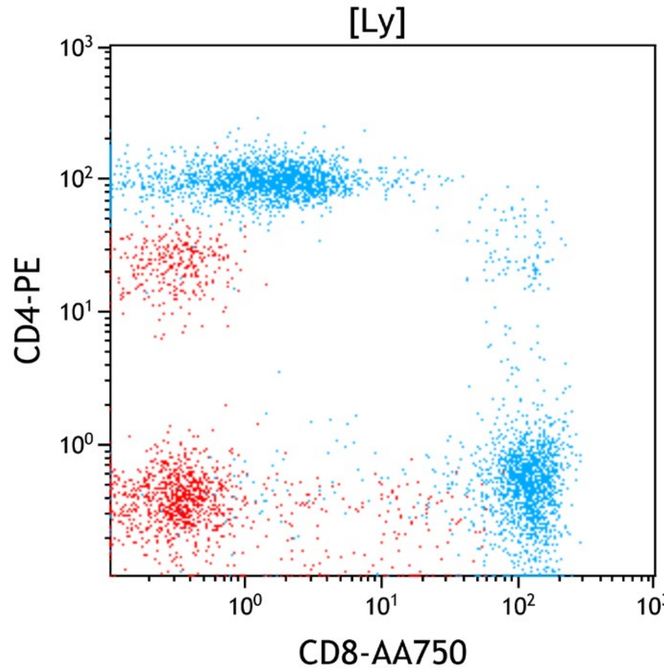


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

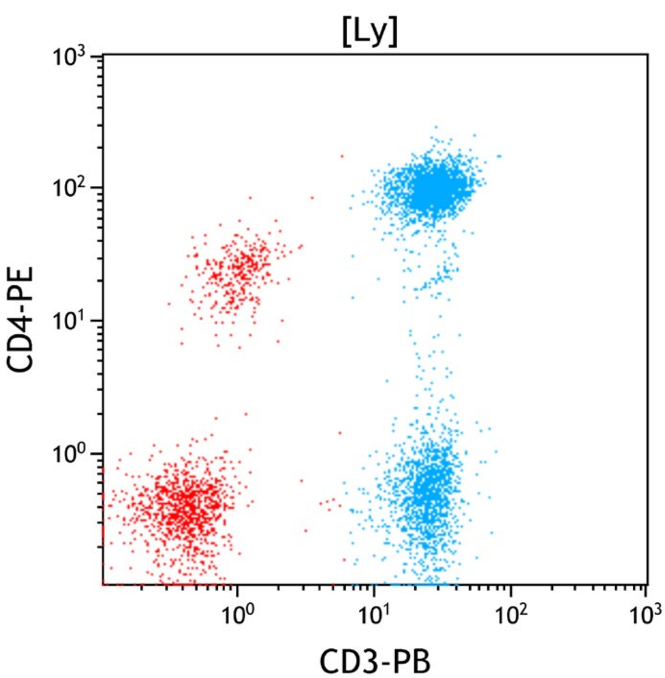


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

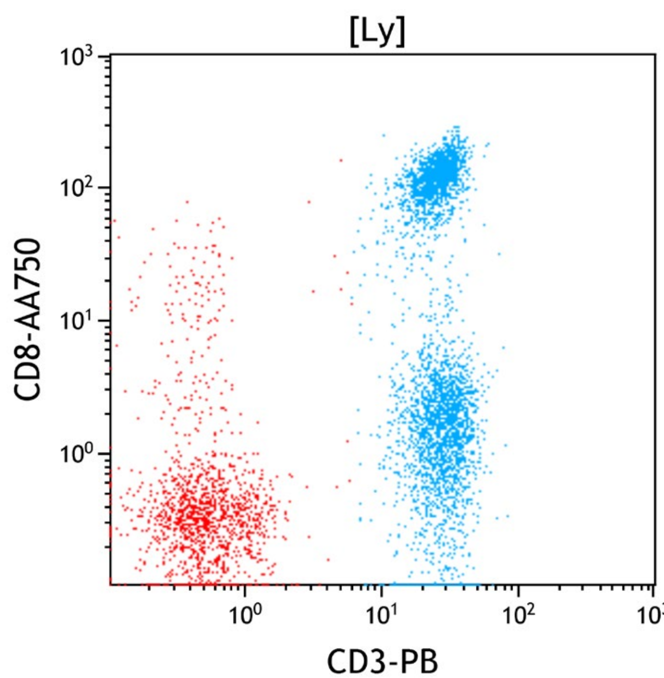


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

T Cell Tube

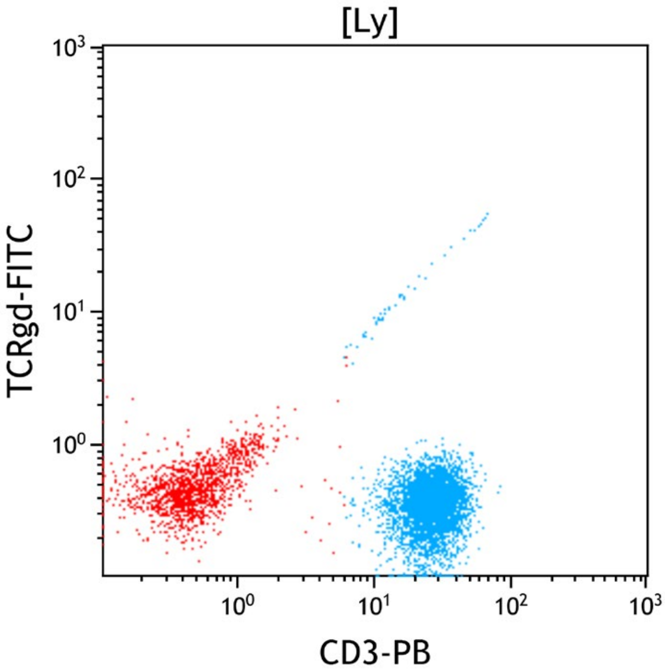
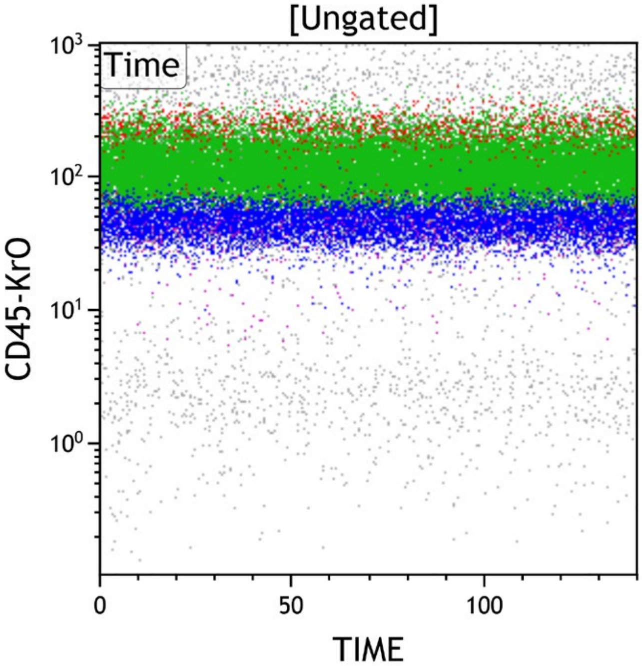


Figure 21. This CD3 vs TCRgd dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.



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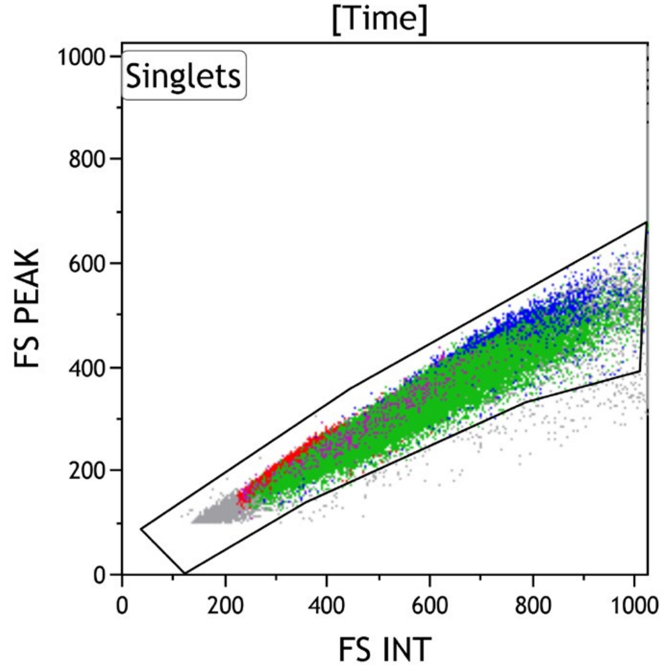

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

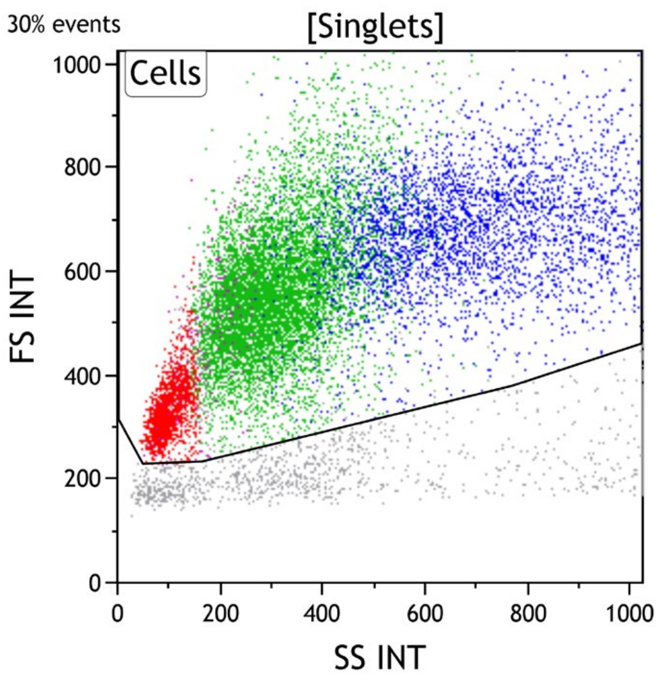

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

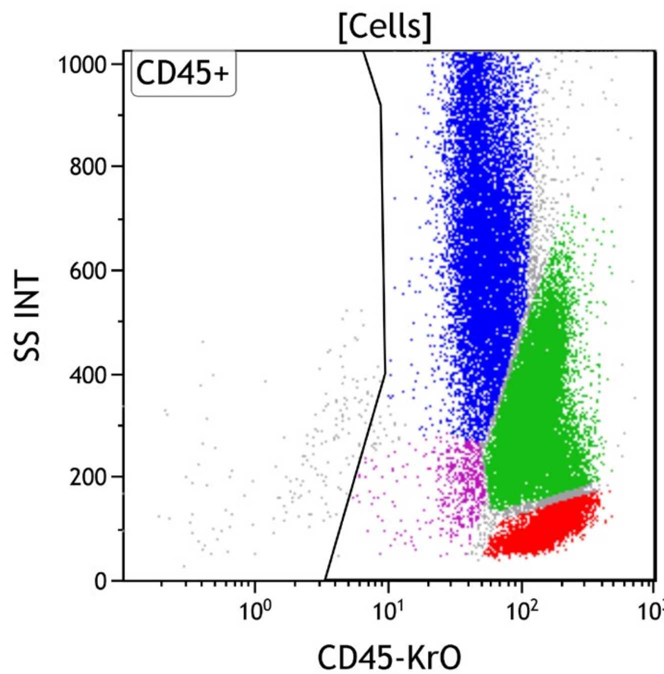

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

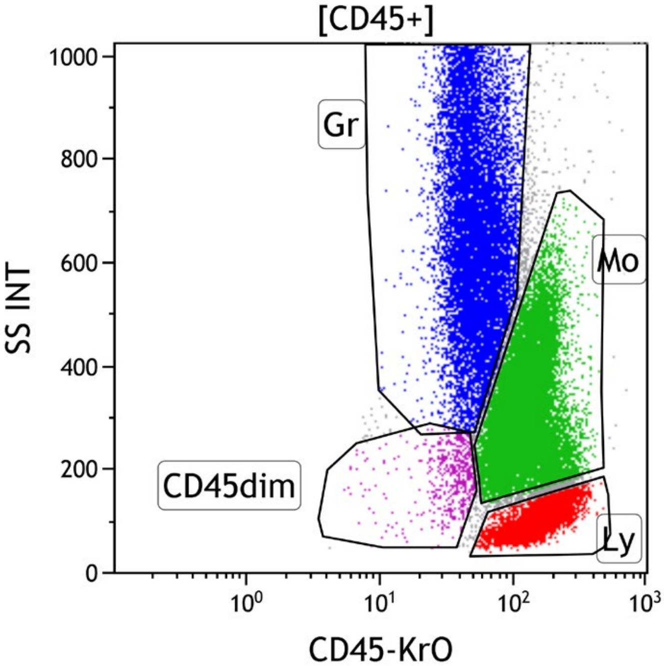

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note a relative increase in the monocytes (green).

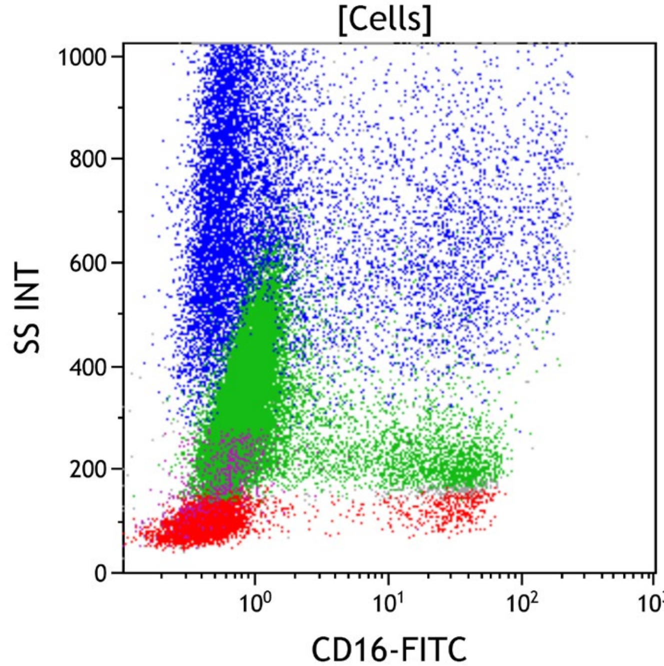

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). Most monocytes (green) do not express CD16.

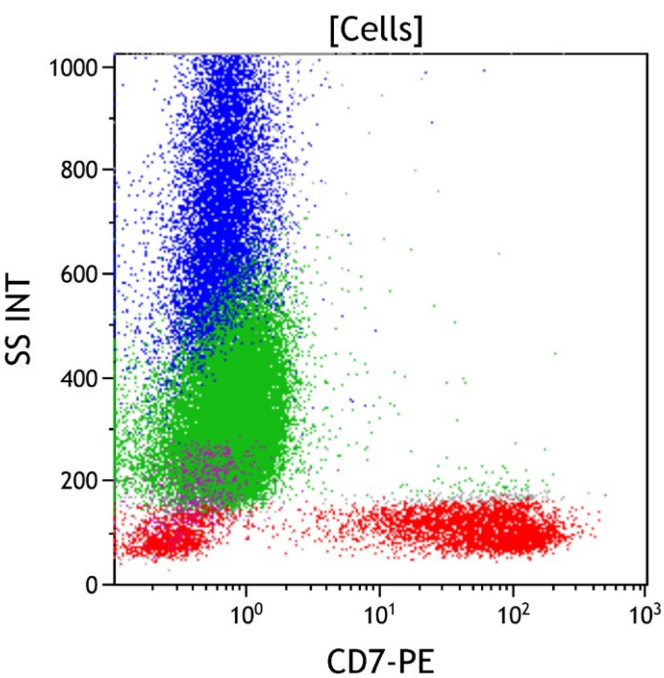

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The monocytes (green) do not express CD7.

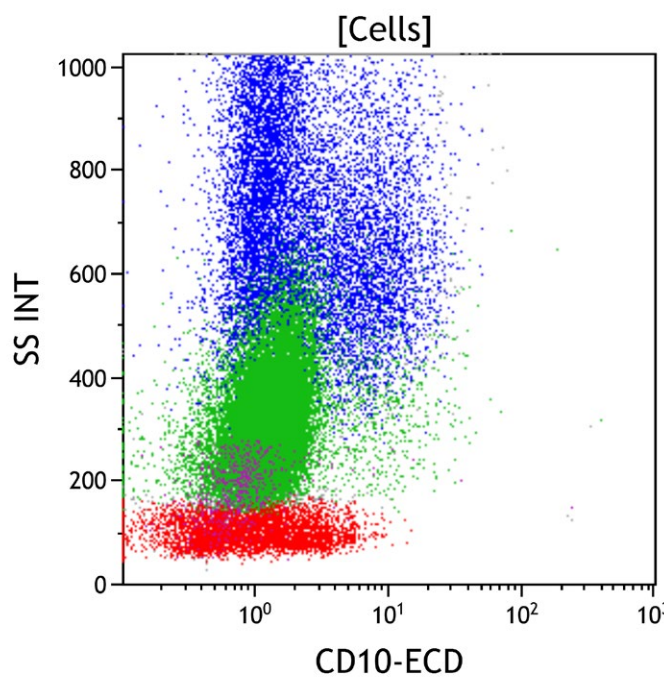

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The monocytes (green) do not express CD10.

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M1 Cell Tube

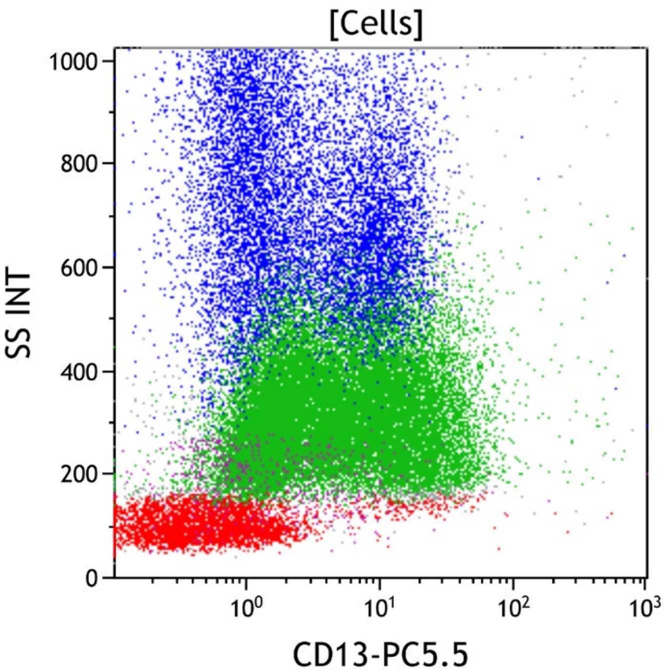

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. The monocytes (green) variably express CD13.

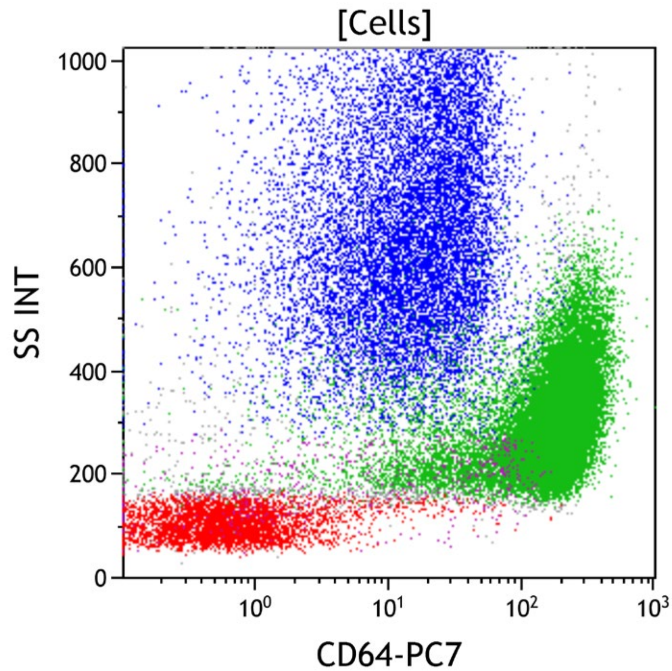

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The monocytes (green) express bright CD64.

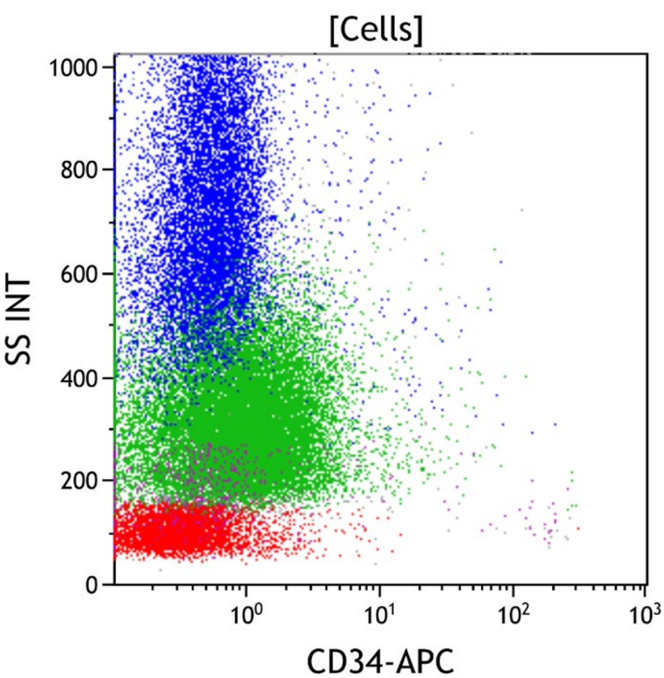

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The monocytes (green) do not express CD34.

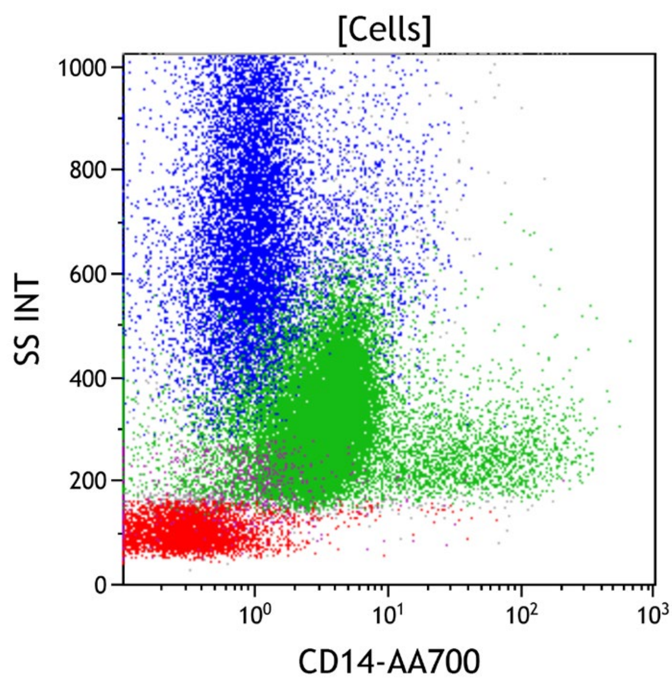

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Most of the monocytes (green) do not express CD14.

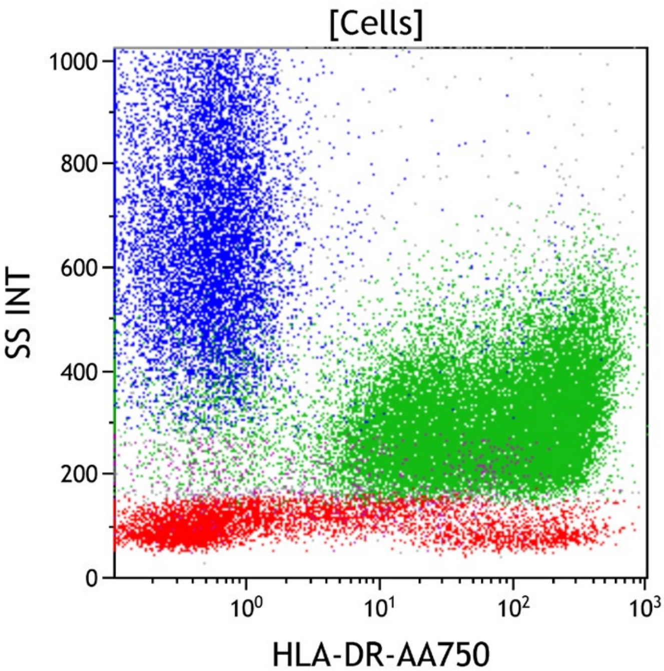

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). Monocytes (green) variably express HLA-DR.

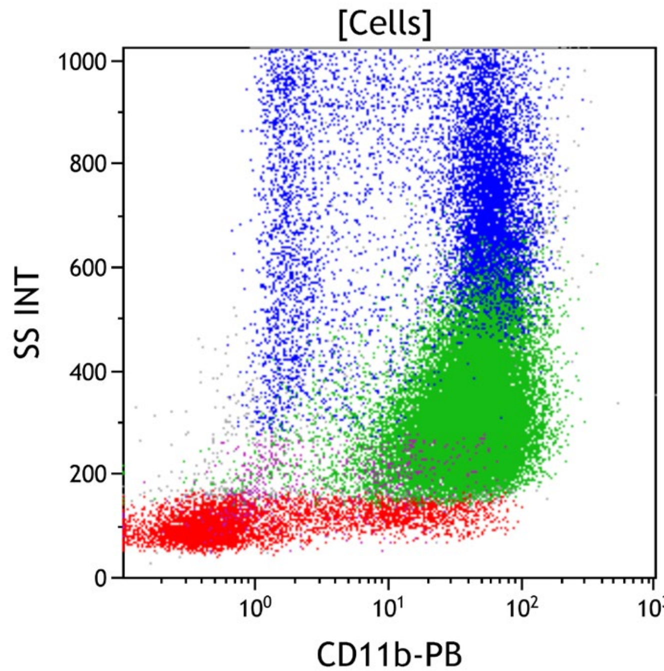

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The monocytes (green) express bright CD11b.

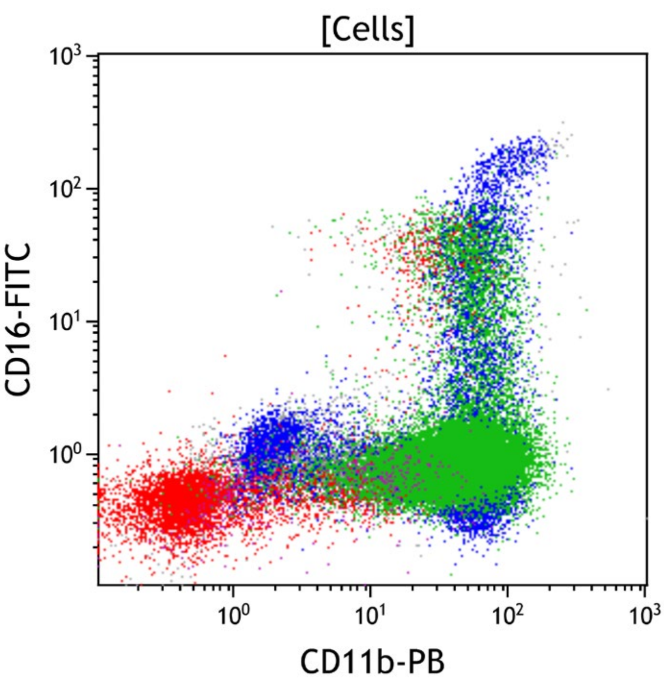

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (in green), immature and mature granulocytes (in blue) and NK cells (in red). CD16 is expressed on immature and mature granulocytes (in blue) and a subset of NK cells (in red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. Most monocytes (green) express CD11b without CD16.

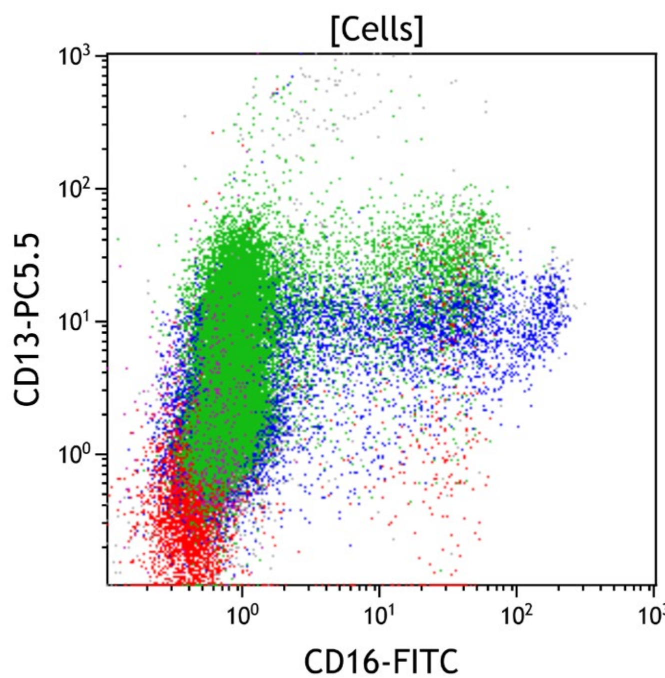

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. Monocytes (green) variably express CD13.

M1 Cell Tube

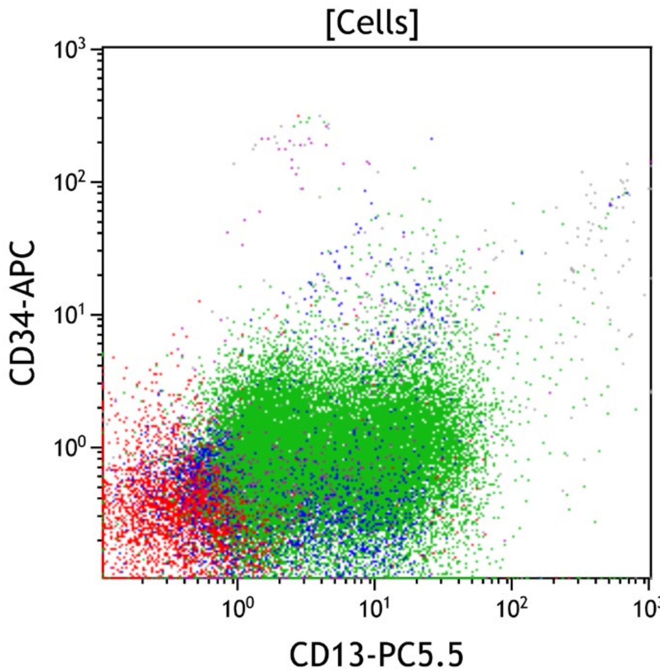

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells (red). The monocytes (green) variably express CD13.

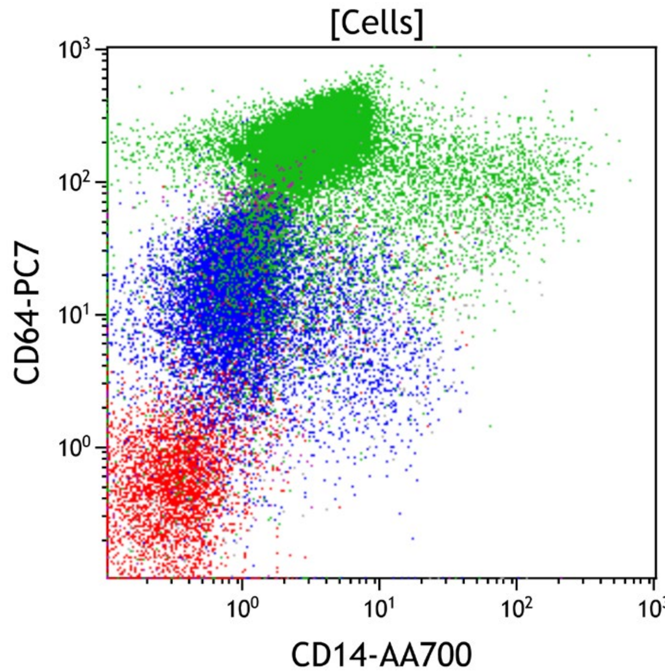

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (green top left) and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 (blue left) and acquire CD14 and lose CD64 at transition to mature granulocytes. Most monocytes (green) express bright CD64 without CD14, an immunophenotype consistent with immature monocytes

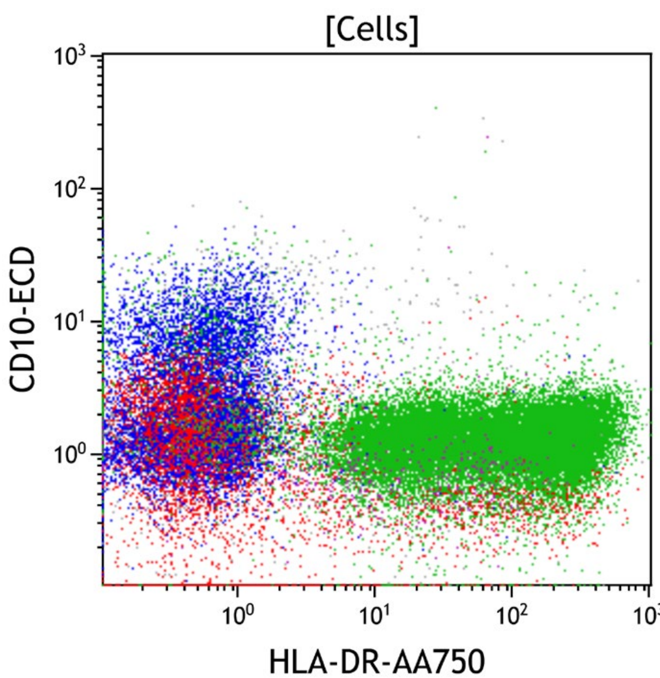

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue) and immature B cells. Immature B cells express both CD10 and HLA-DR. The monocytes (green) variably express HLA-DR without CD10.

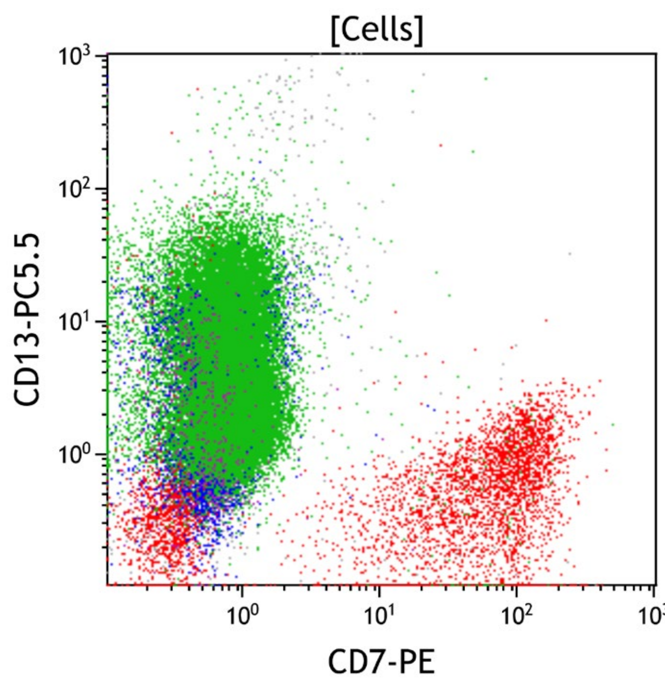

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The monocytes (green) variably express CD13 but not CD7.

M2 Cell Tube

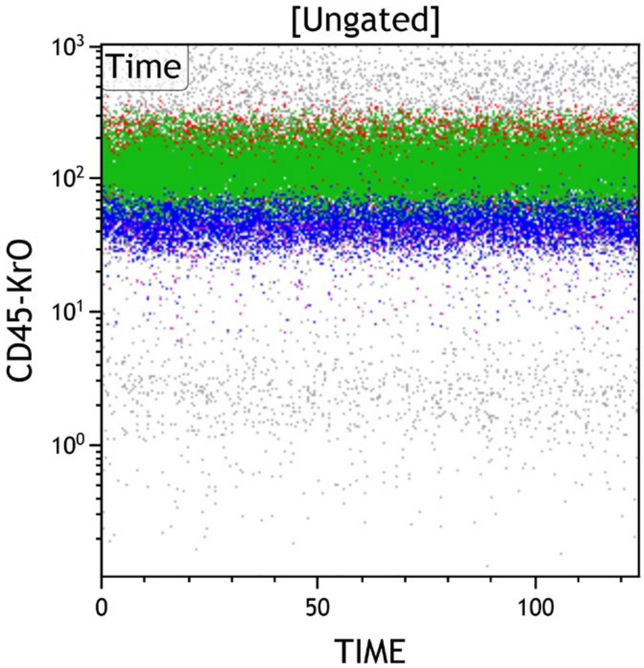

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

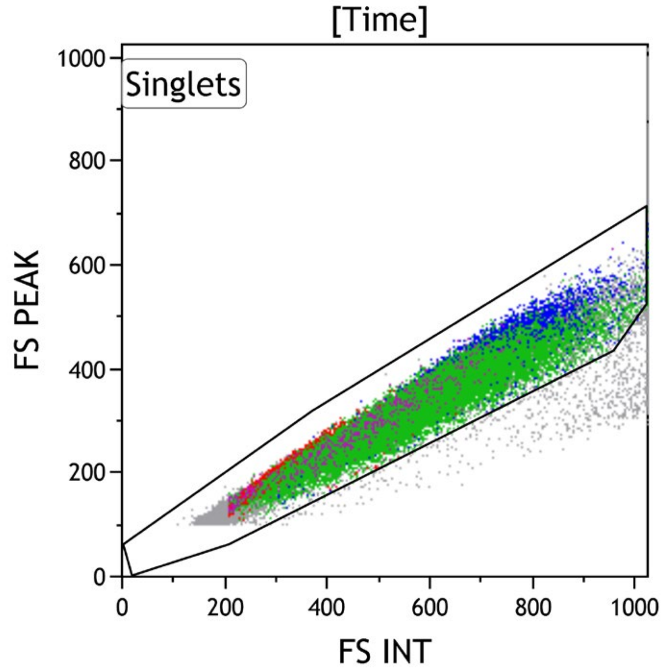

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

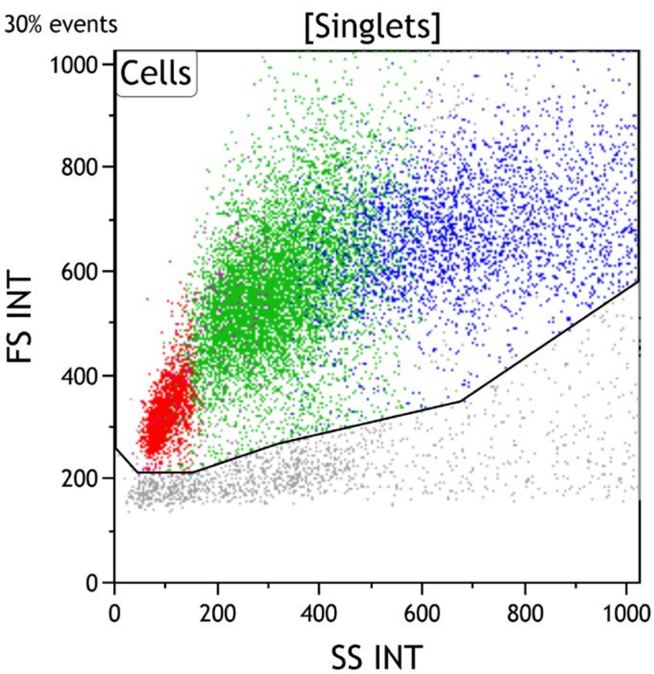

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

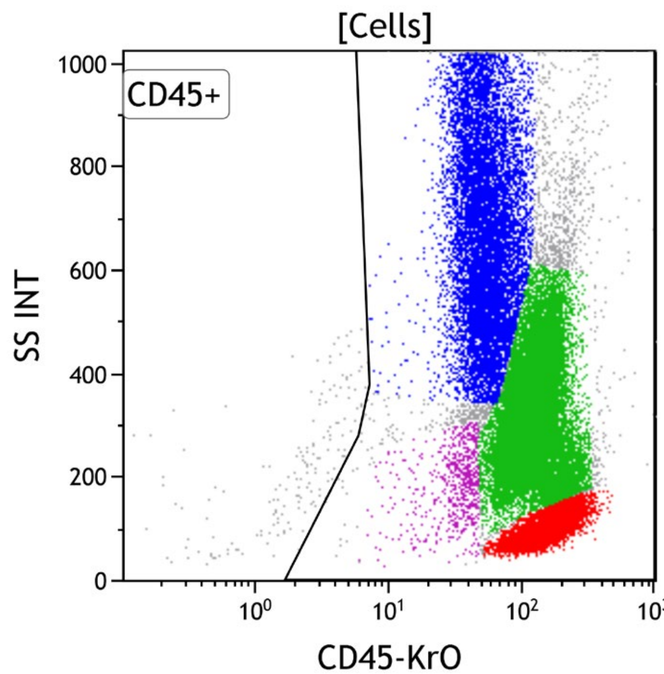

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

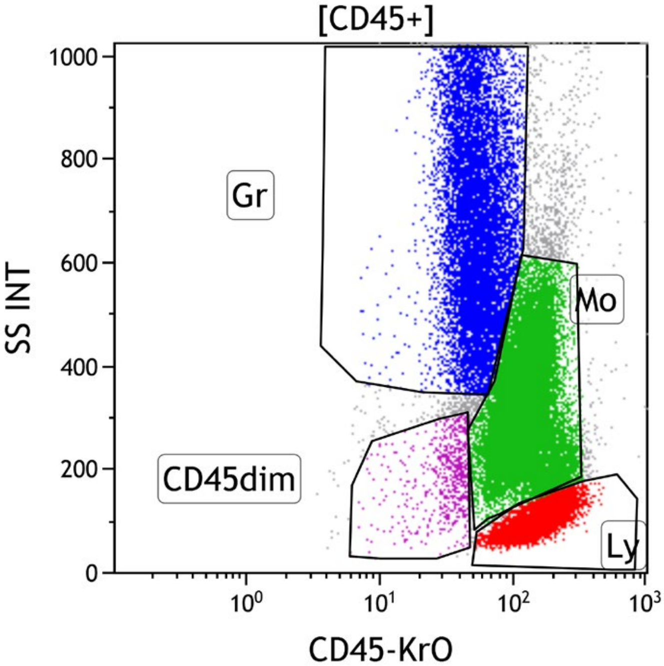

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note a relative increase in the monocytes (green).

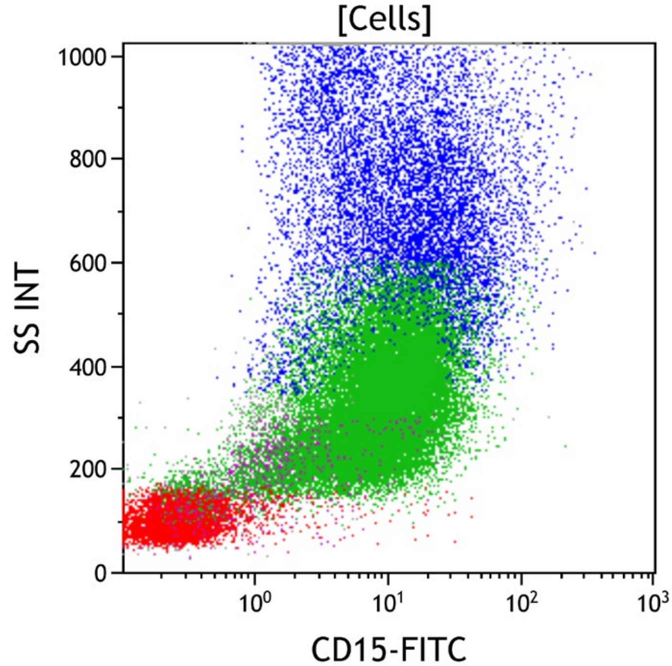

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes. The relatively expanded monocytes (green) express intermediate CD15.

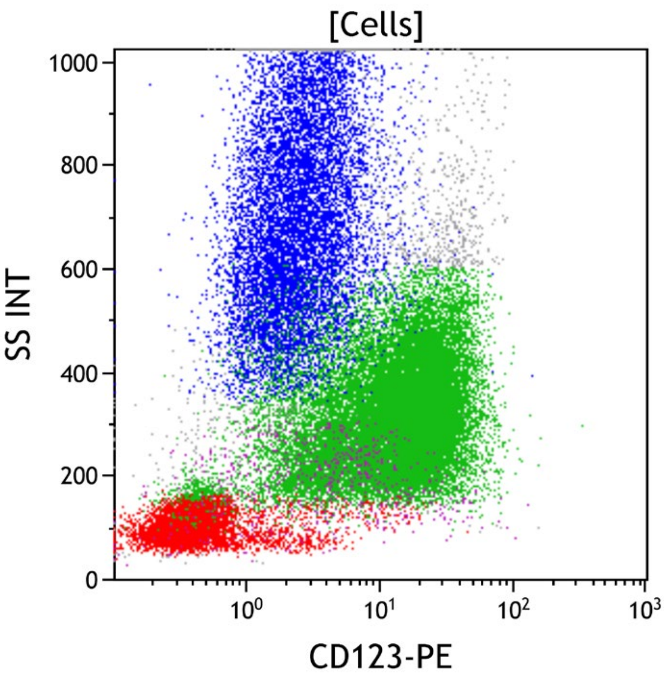

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. The relatively expanded monocytes (green) express intermediate CD123.

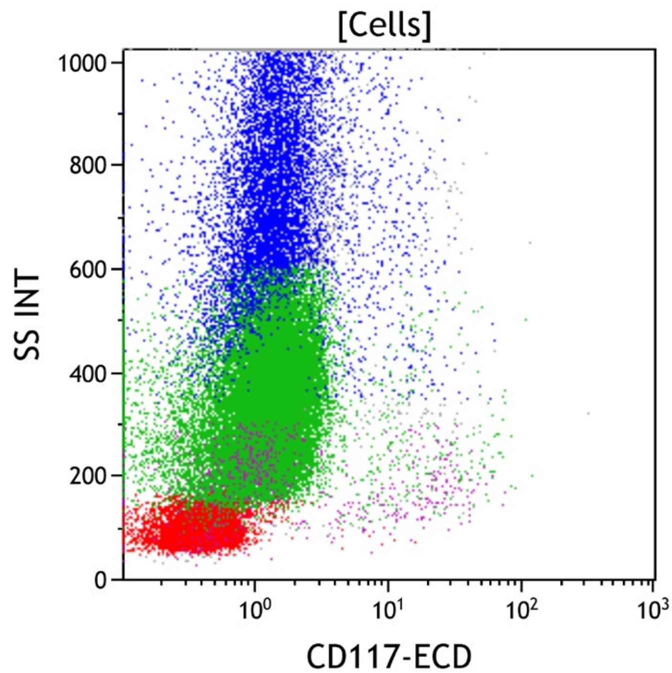

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The relatively expanded monocytes (green) do not express CD117.

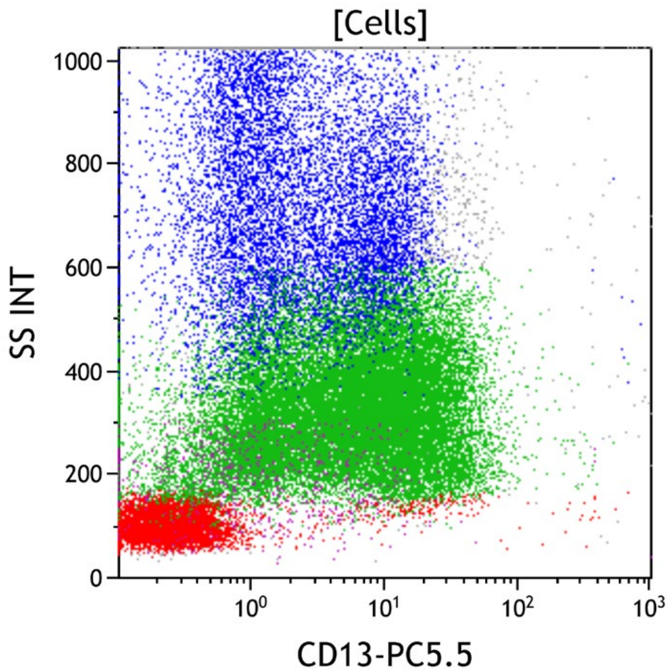

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. The monocytes (green) variably express CD13.

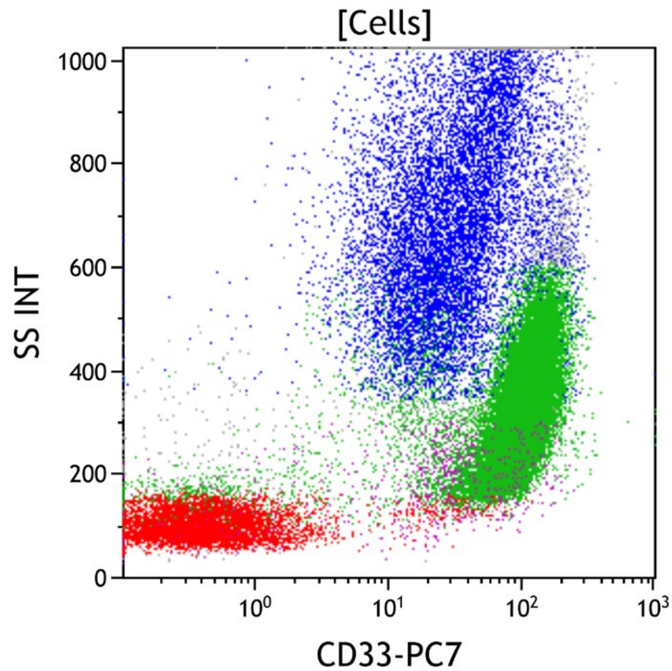

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The monocytes (green) express uniform CD33

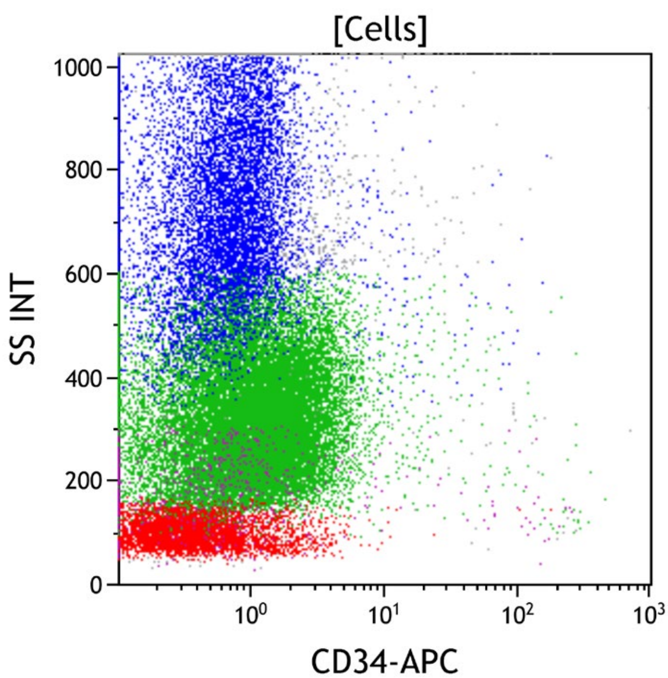

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The monocytes (green) do not express CD34.

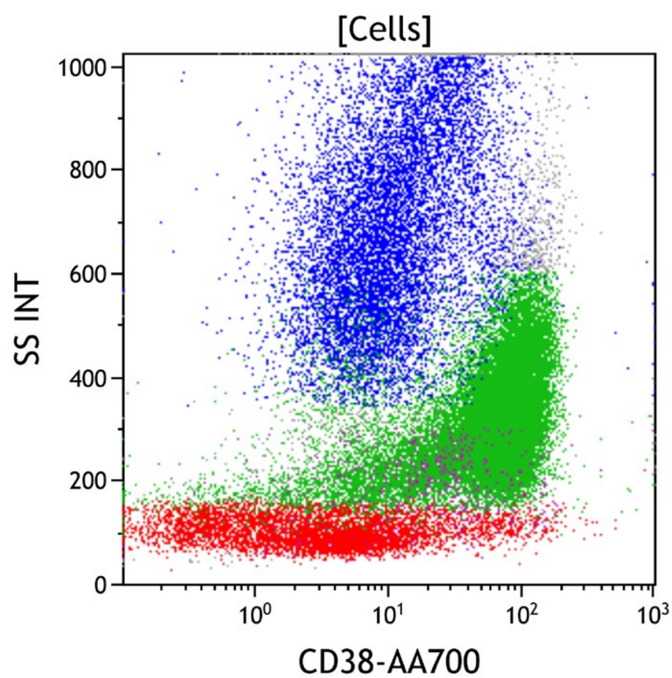

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The relatively expanded monocytes (green) express moderate CD38.

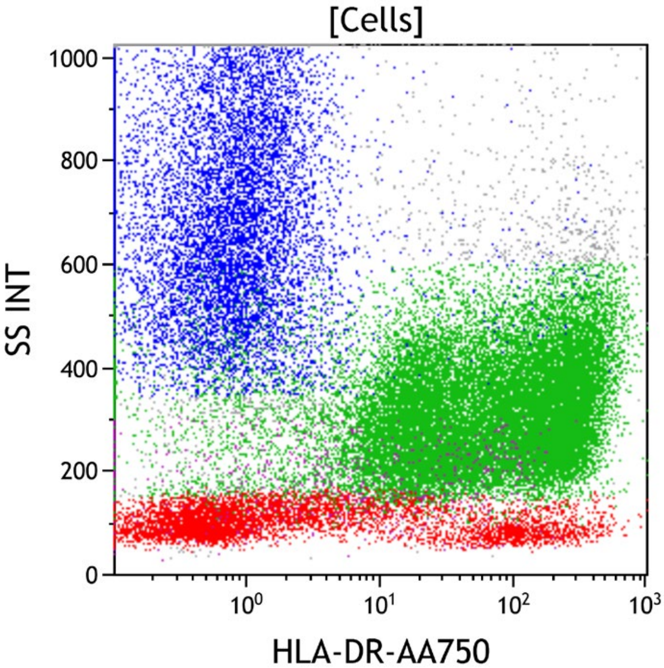

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). The relatively expanded monocytes (green) variably express HLA-DR.

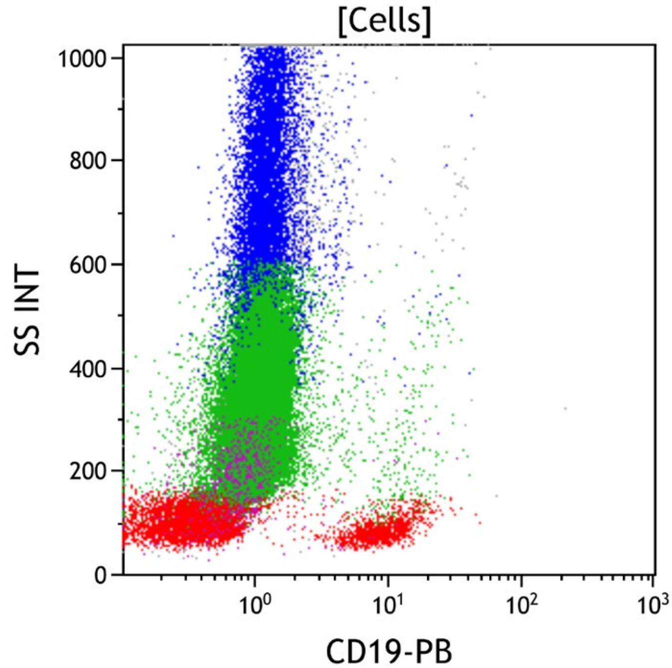

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The relatively expanded monocytes (green) do not express CD19.

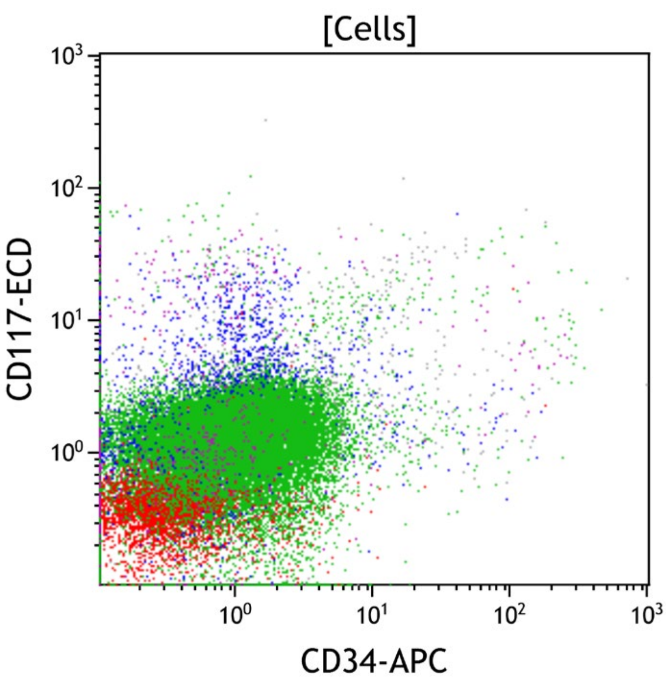

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors . CD117 is expressed on myeloid blasts (upper right in purple), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (lower right). Monocytes (green) are negative for CD34 and CD117.

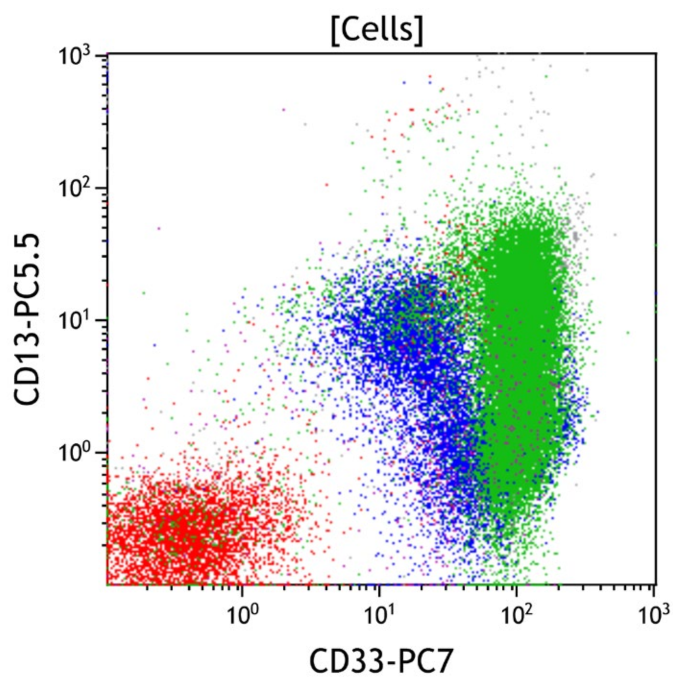

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red). The relatively expanded monocytes express bright CD33 and variable CD13.

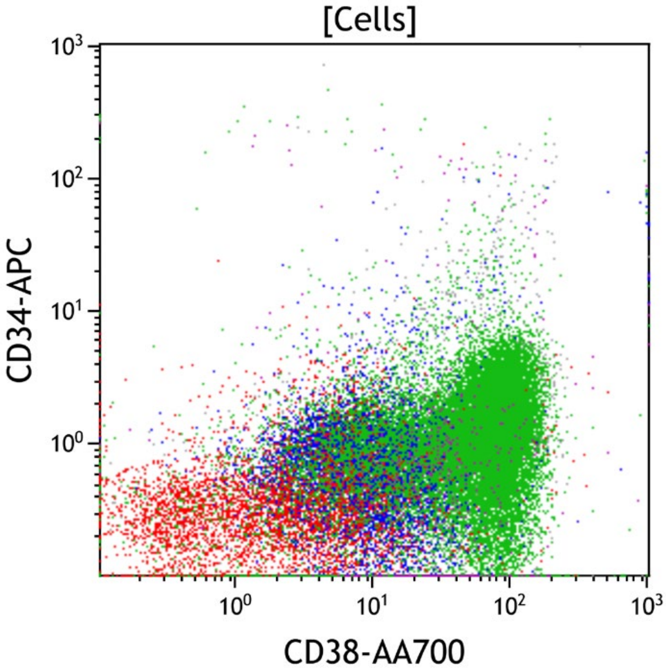

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent dim CD34 expression by monocytes (green) is a compensation artifact due to the high level of CD38.

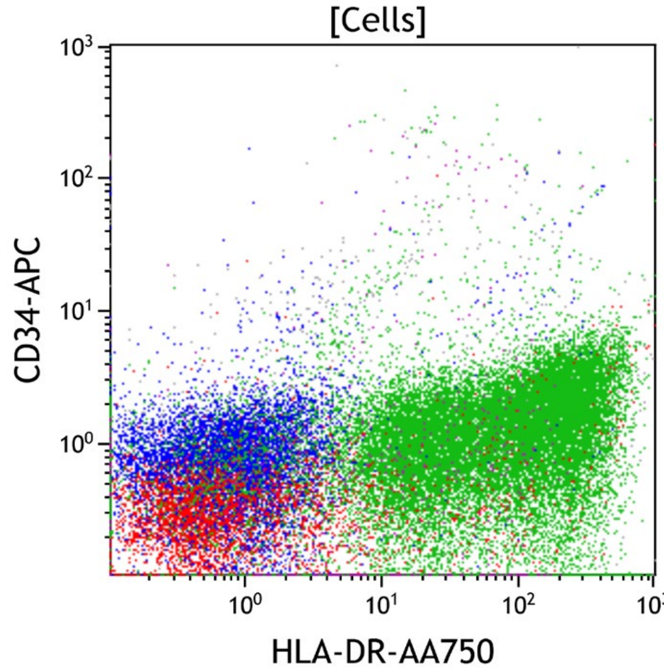

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The relatively expanded monocytes express variable HLA-DR and lack CD34 expression.

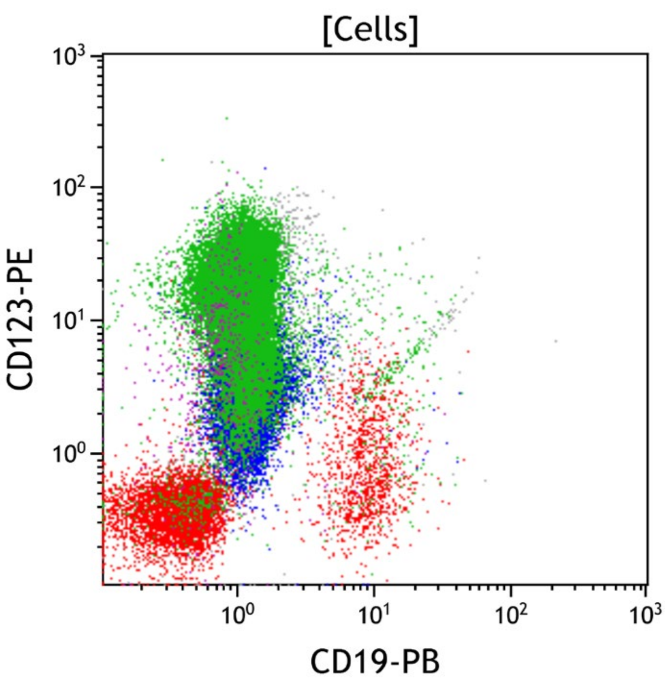

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The monocytes (green) express intermediate CD123 and do not express CD19.

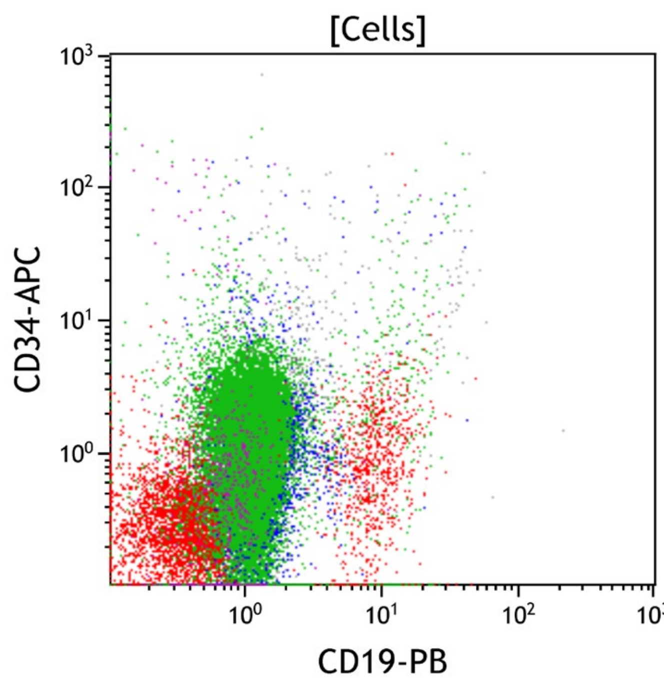

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. The monocytes (green) do not express CD34.

M2 Cell Tube

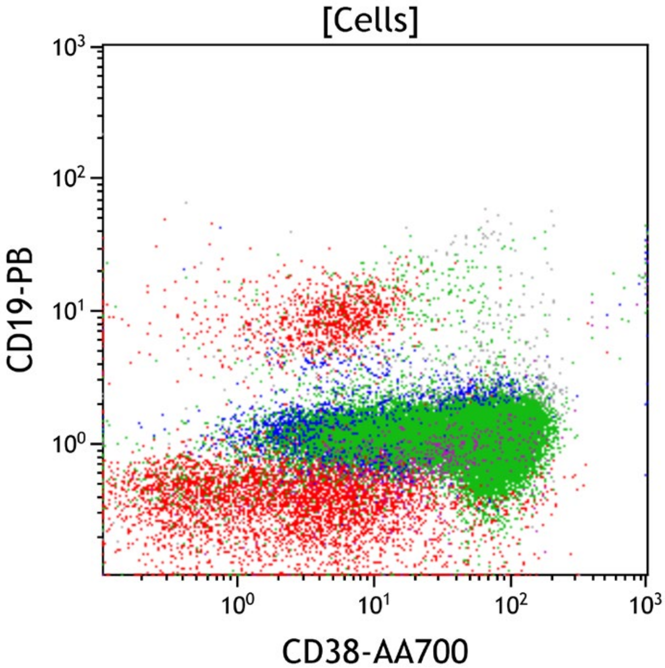

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38 (red left). Plasma cells show extremely high CD38 expression that is largely off scale, but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The relatively expanded monocytes express intermediate CD38 and lack CD19 expression.

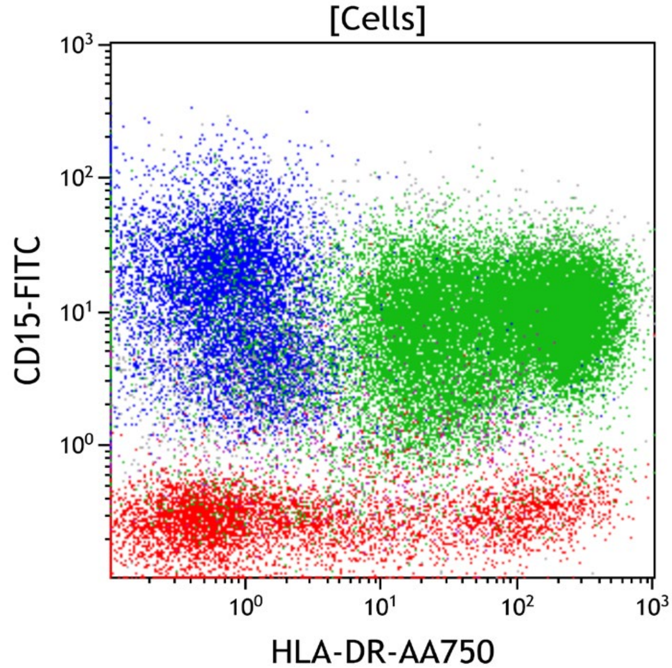

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. The relatively expanded monocytes express variable HLA-DR and intermediate CD15 expression.

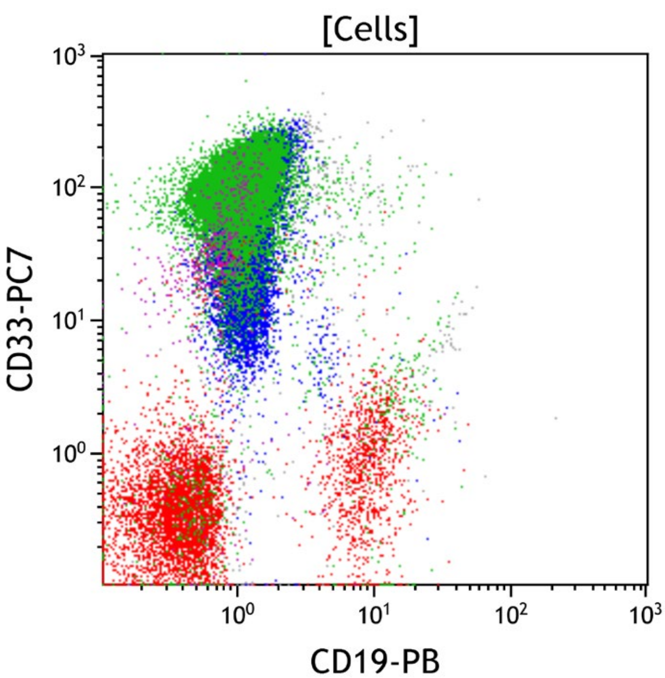

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate CD4, bright CD11b, variable CD13, intermediate CD15, bright CD33, intermediate CD38, bright CD45, dim CD56 on subset, bright CD64, intermediate CD123 and variable HLA-DR without CD14, CD34, CD117 and other B cell or myeloid antigens. Compared with normal mature monocytes, the decreased expression of CD14 and variable CD13 is aberrant.

The immunophenotype of the abnormal population is that of expanded abnormal immature monocytes and consistent with acute myeloid leukemia with monocytic differentiation. However, a diagnosis of acute myeloid leukemia requires identification of 20% blasts or blast equivalents by morphology.

The concurrent morphologic and immunohistochemical findings confirm monocytic differentiation with positivity for lysozyme. NGS identifies an NPM1 mutation without the FLT3 ITD. The findings are indicative of an acute myeloid leukemia with monocytic differentiation and NPM1 mutation.

Case #20: Acute Myeloid Leukemia-NOS

Clinical Vignette

This 75-year-old male presents with anemia and circulating blasts on peripheral whole blood smear. A peripheral blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

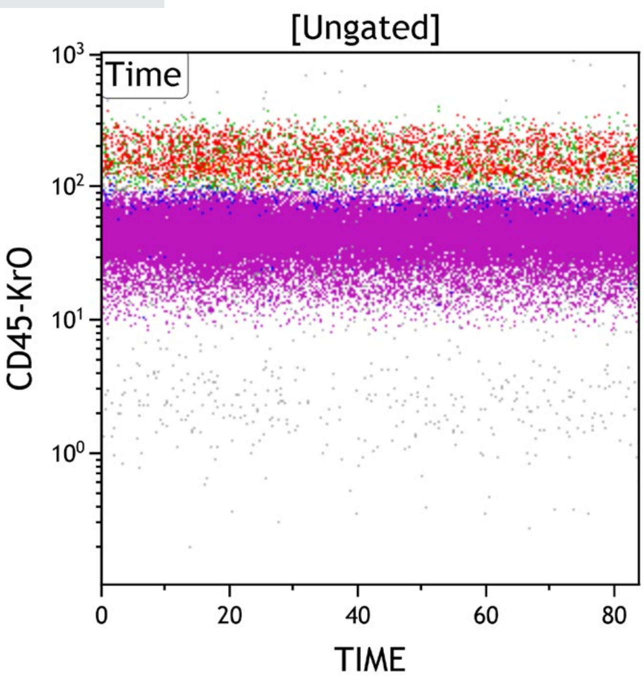

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

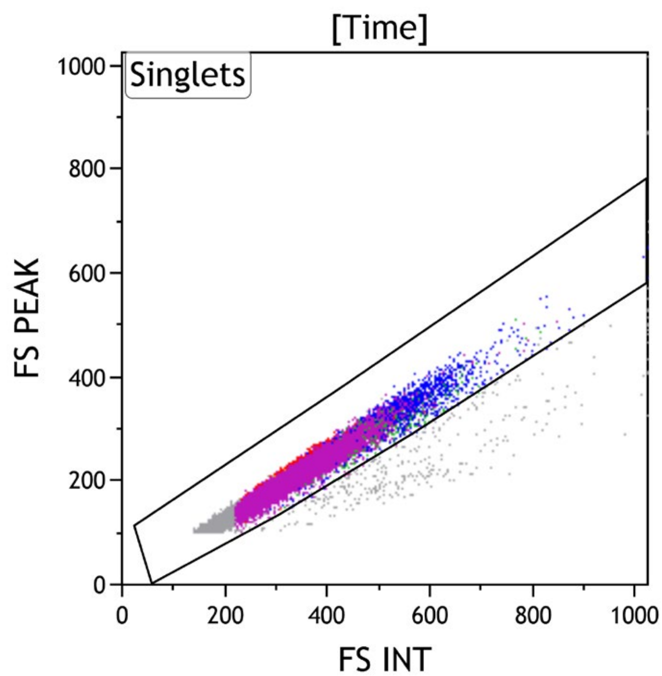

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

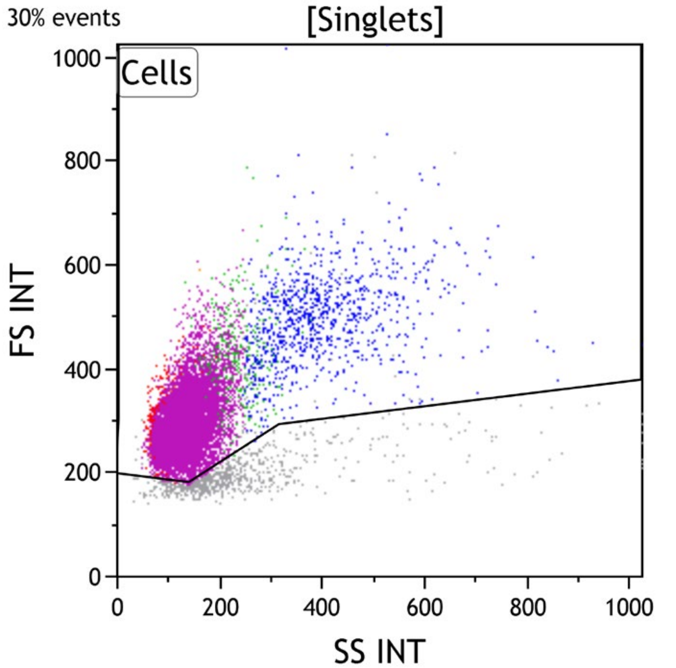

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

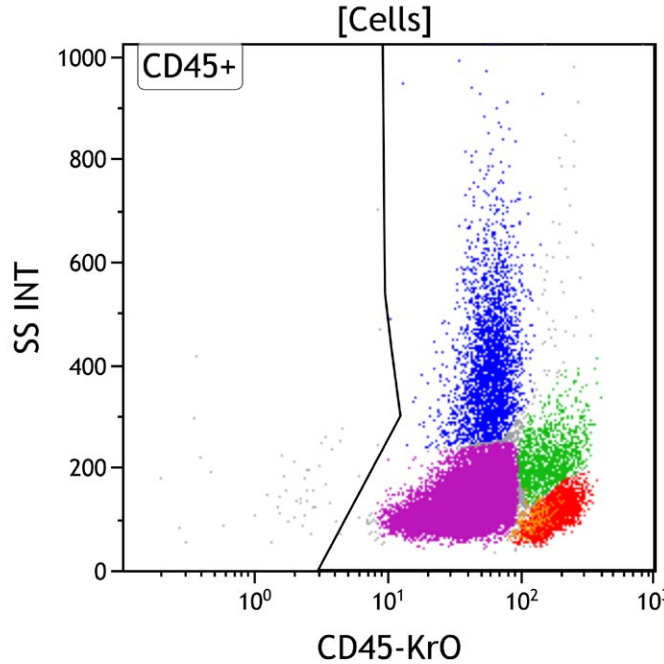

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

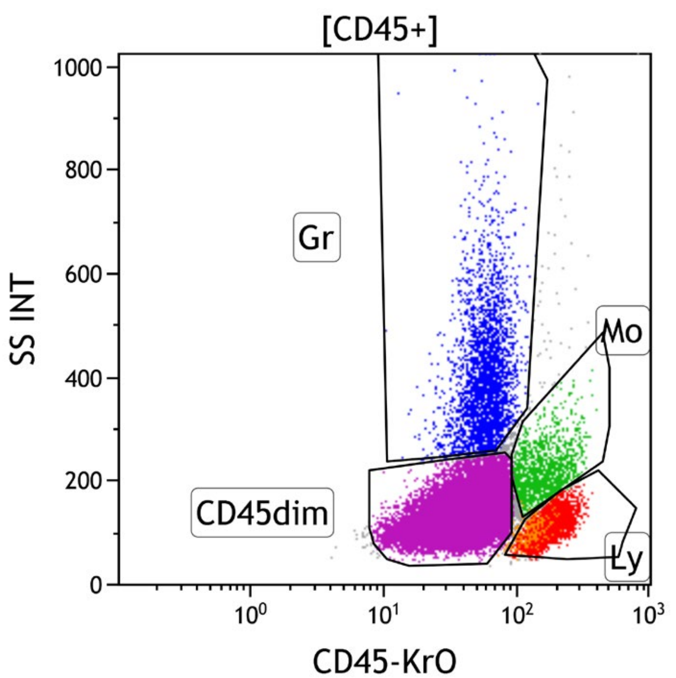

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note that the progenitor population (purple) is relatively increased.

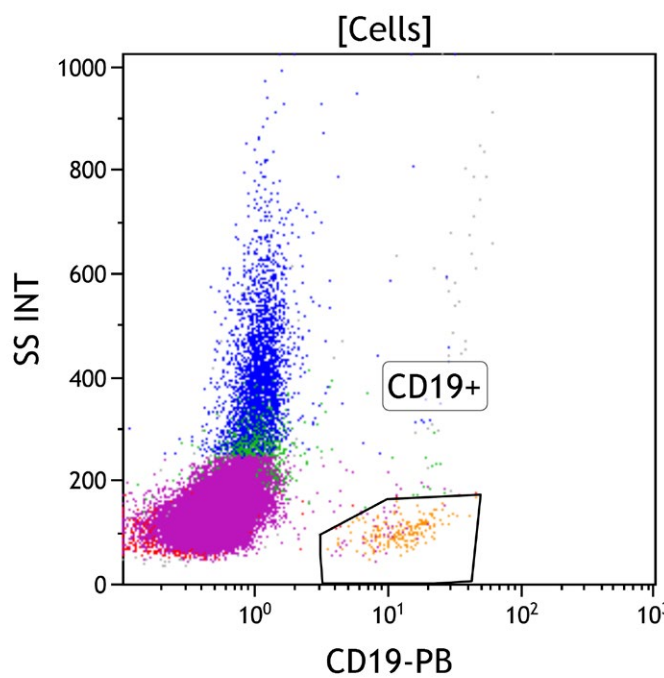

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The progenitors (purple) do not express CD19.

B Cell Tube

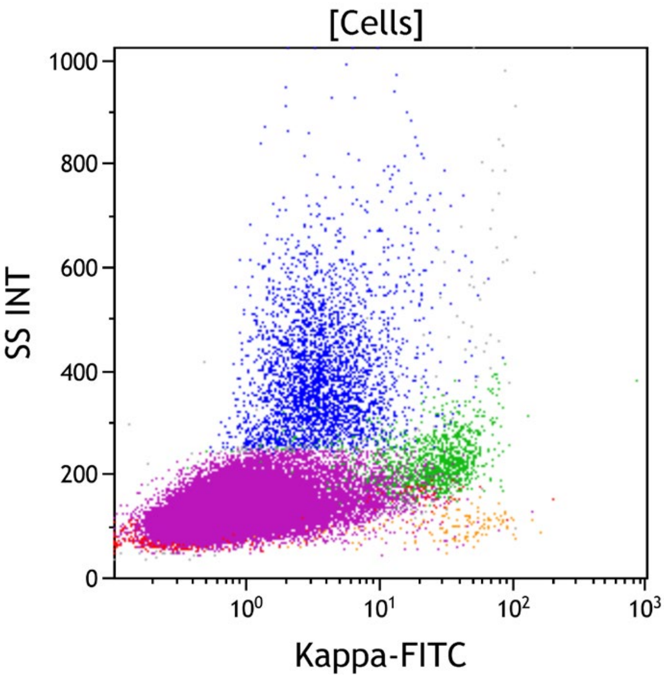

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The progenitors (purple) do not express kappa.

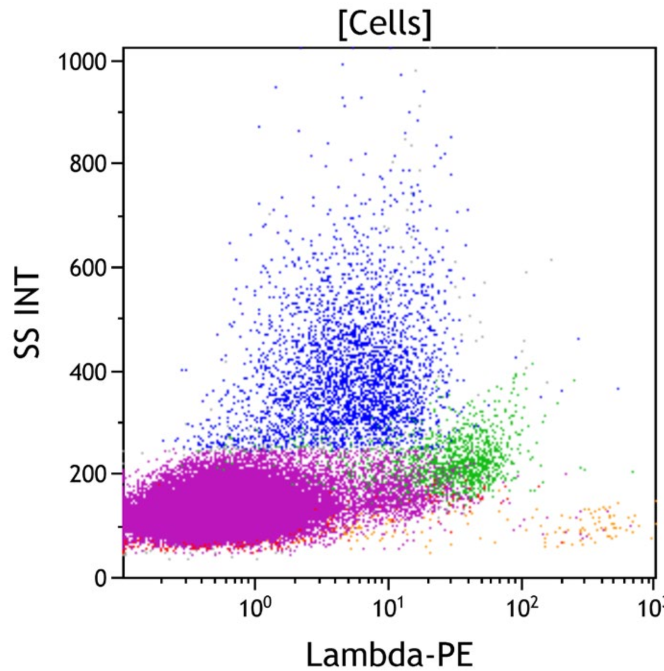

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The progenitors (purple) do not express lambda.

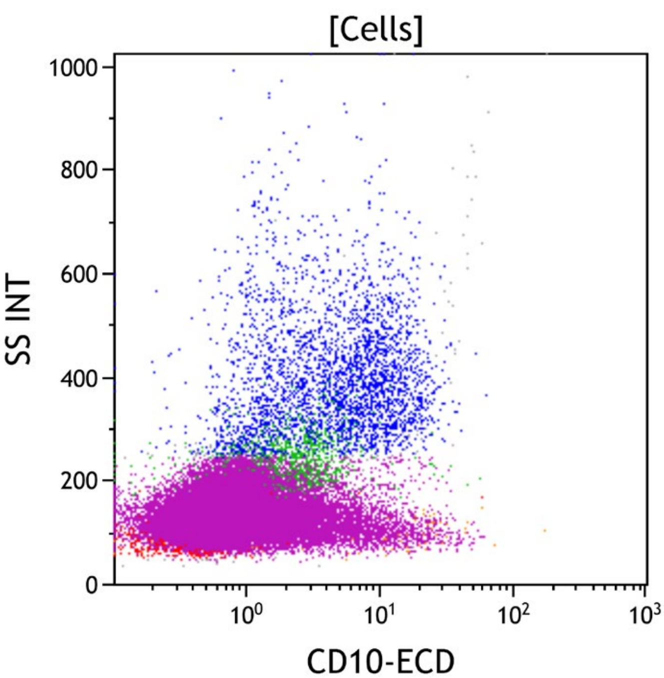

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The progenitors (purple) express dim variable CD10 on a subset.

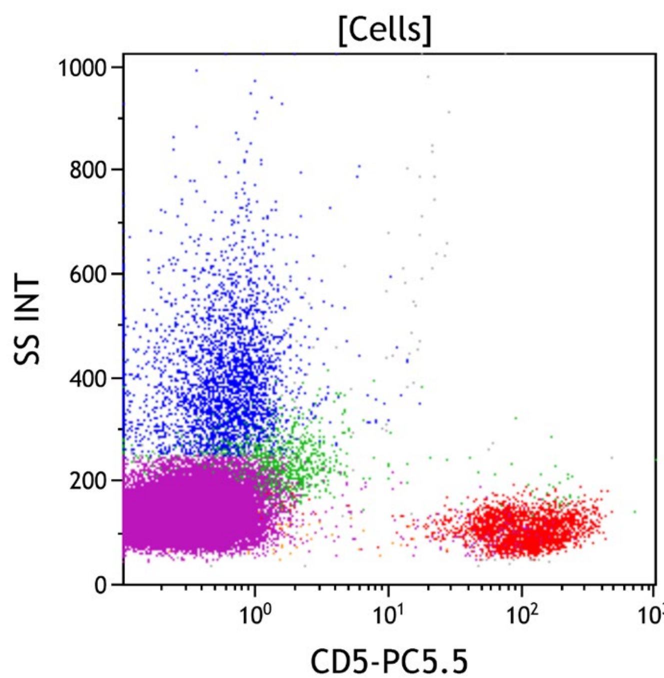

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells. These lymphoid cells typically have low side scatter. The progenitors (purple) do not express CD5.

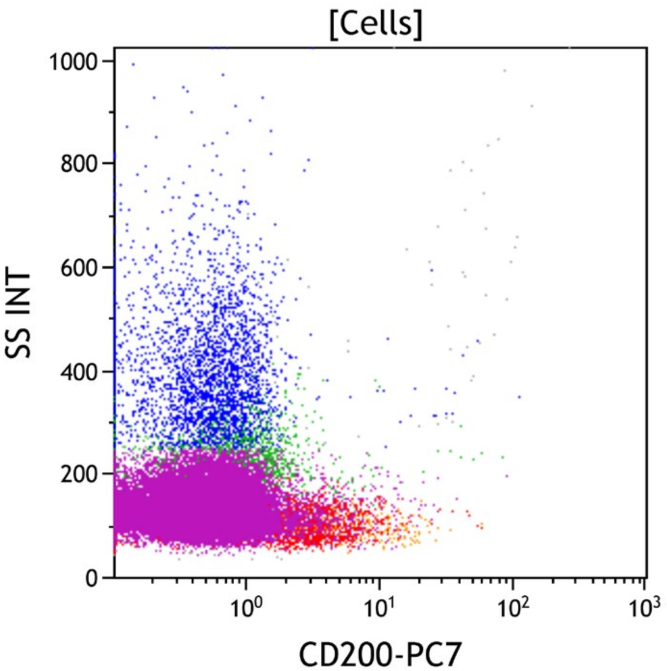

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The progenitors (purple) do not express CD200.

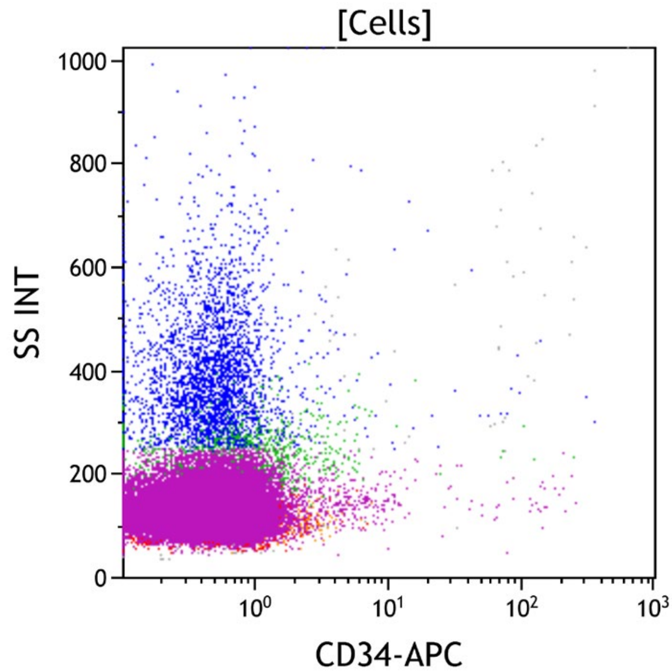

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The progenitors (purple) do not express CD34.

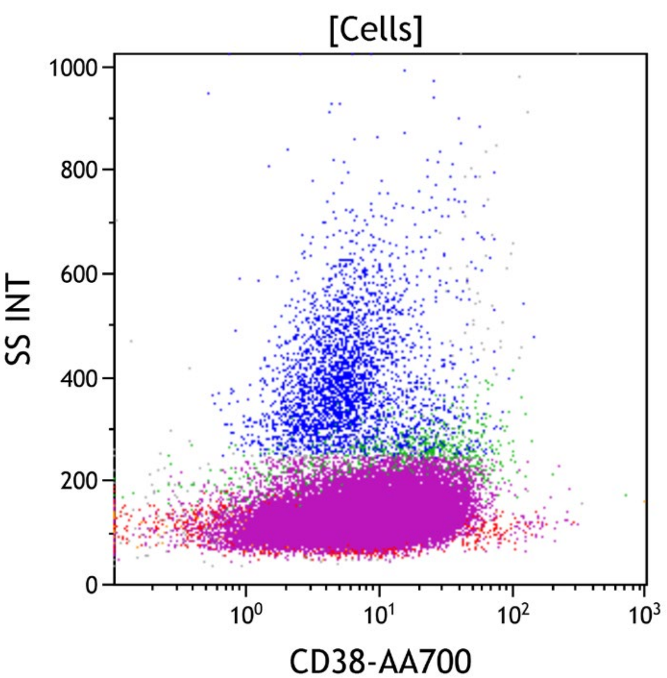

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The progenitors (purple) express dim to intermediate CD38.

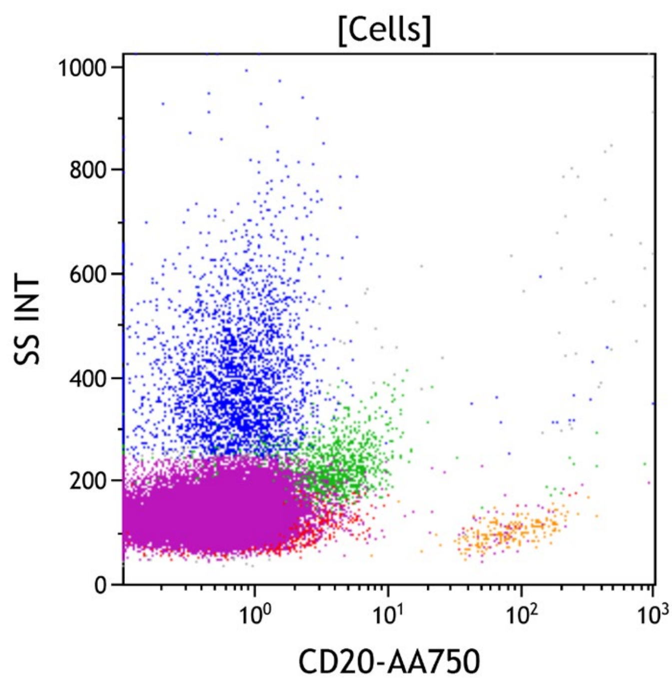

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The progenitors (purple) do not express CD20.

B Cell Tube

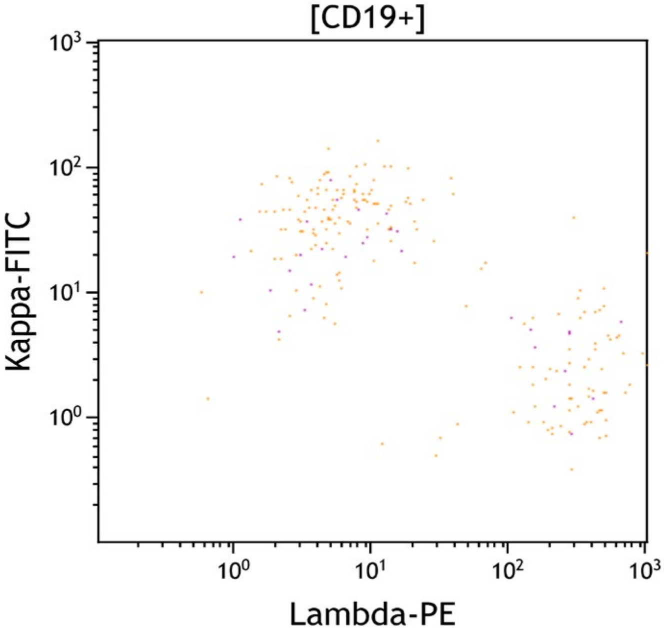

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

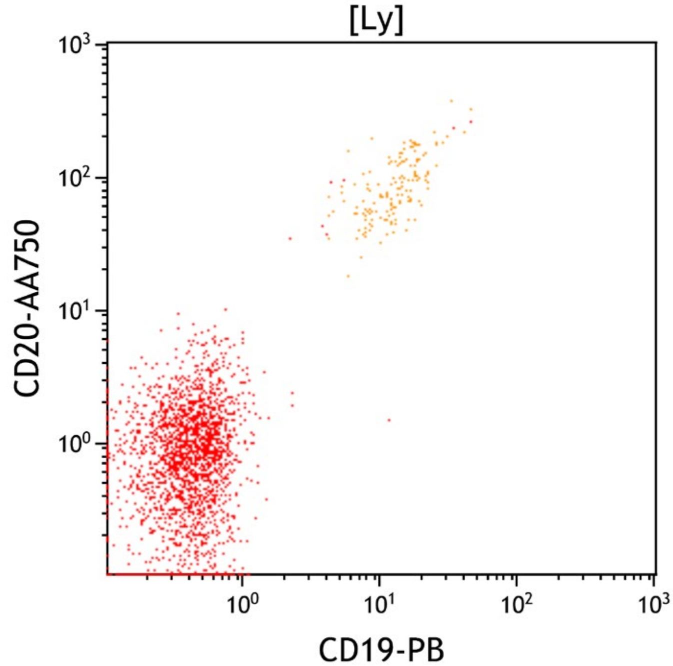

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (in orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

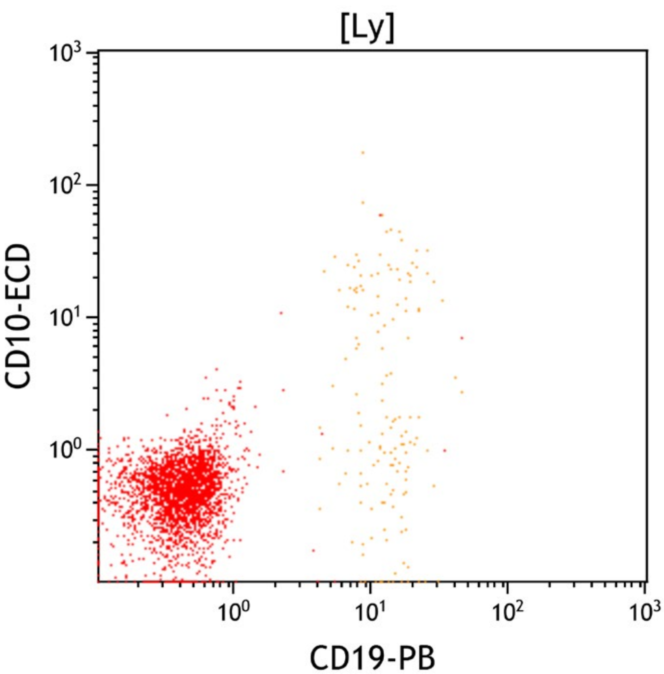

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (in orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

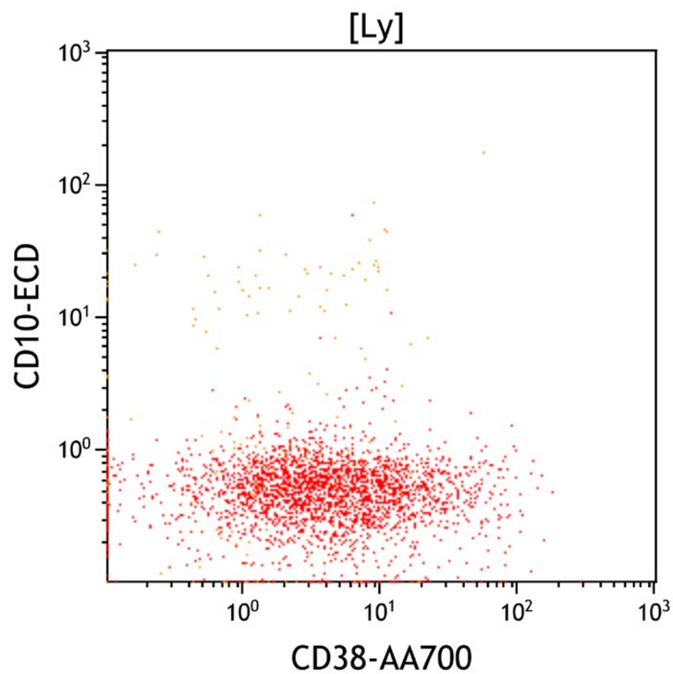

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

B Cell Tube

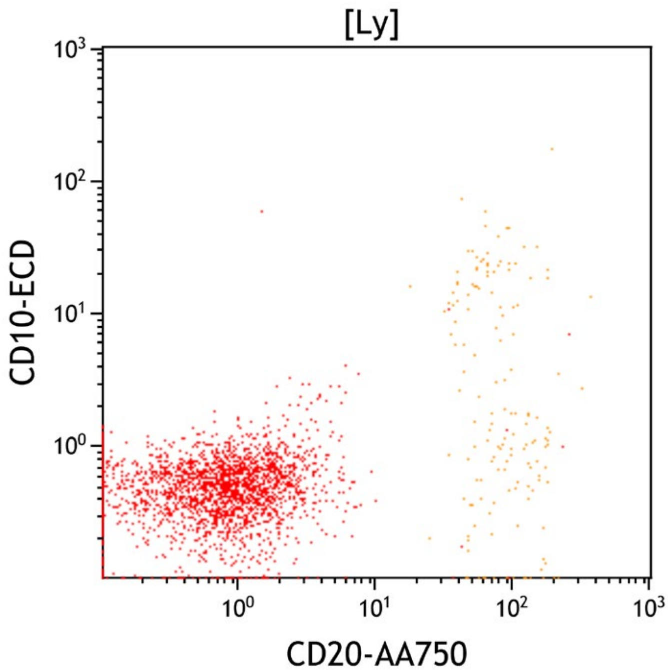

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

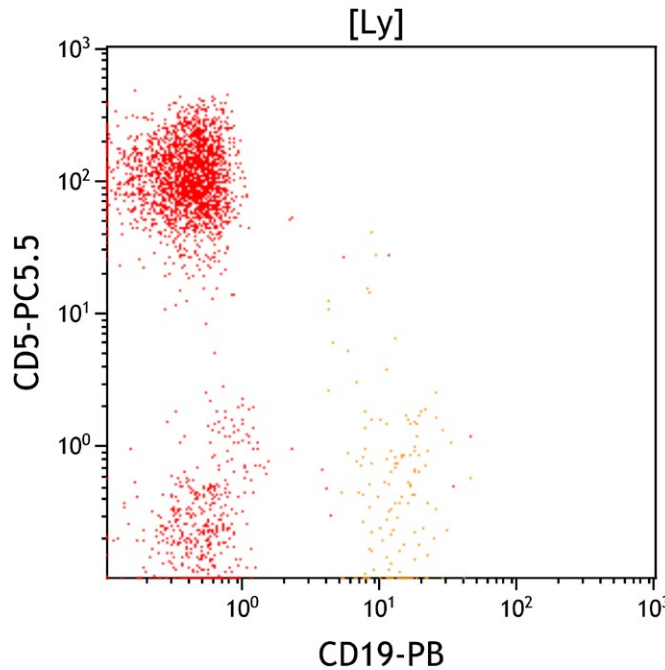

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

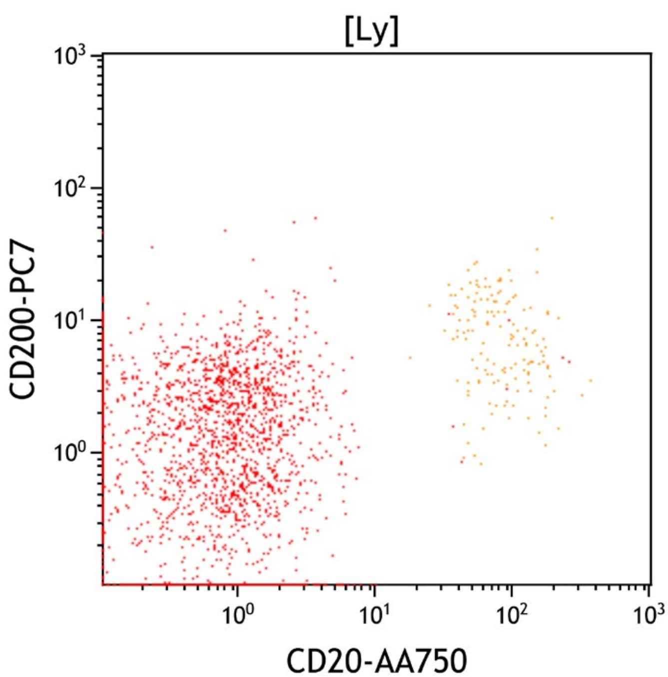

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (in orange).

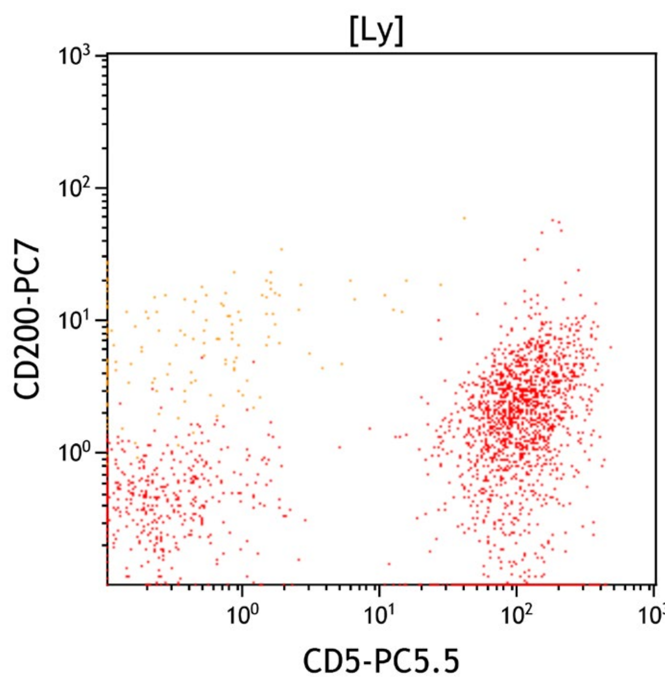

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

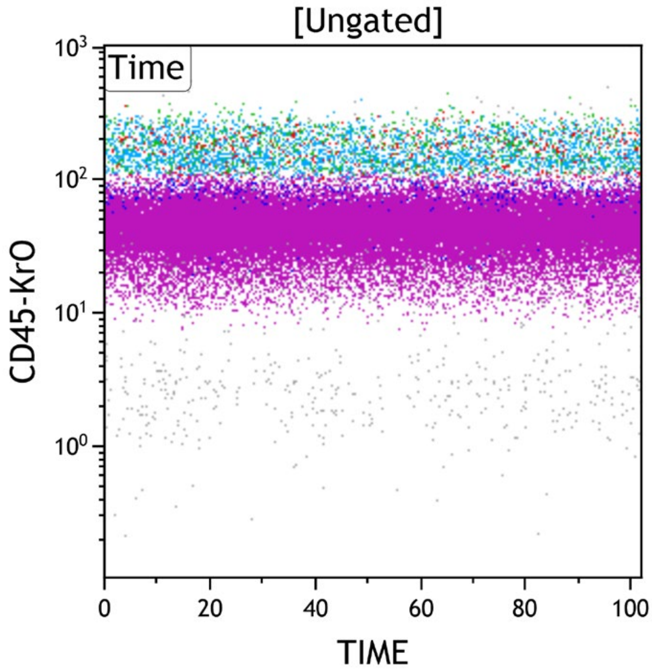

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

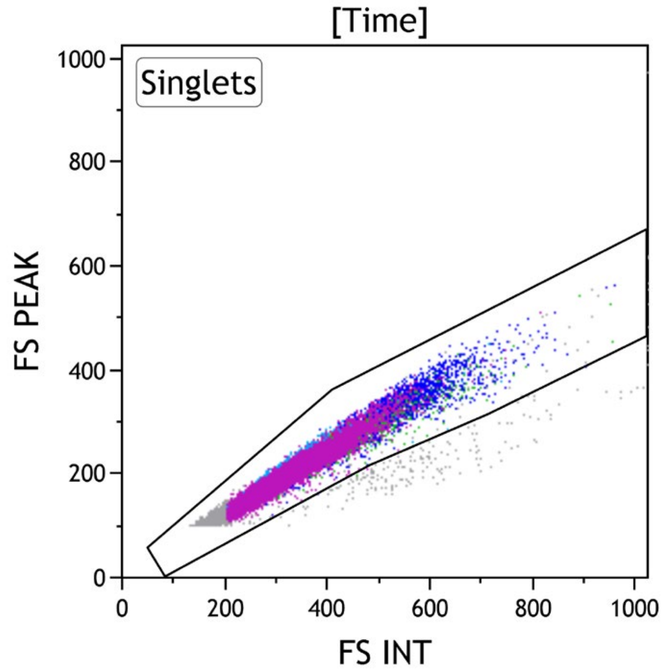

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

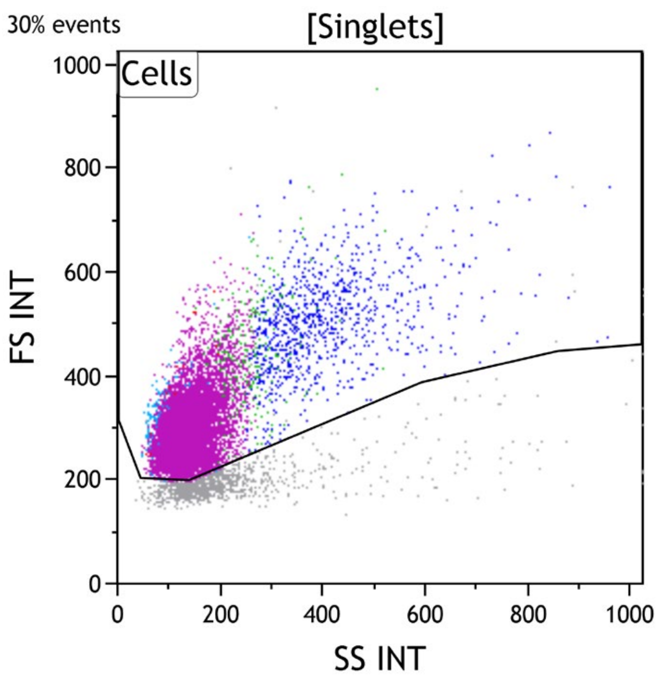

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

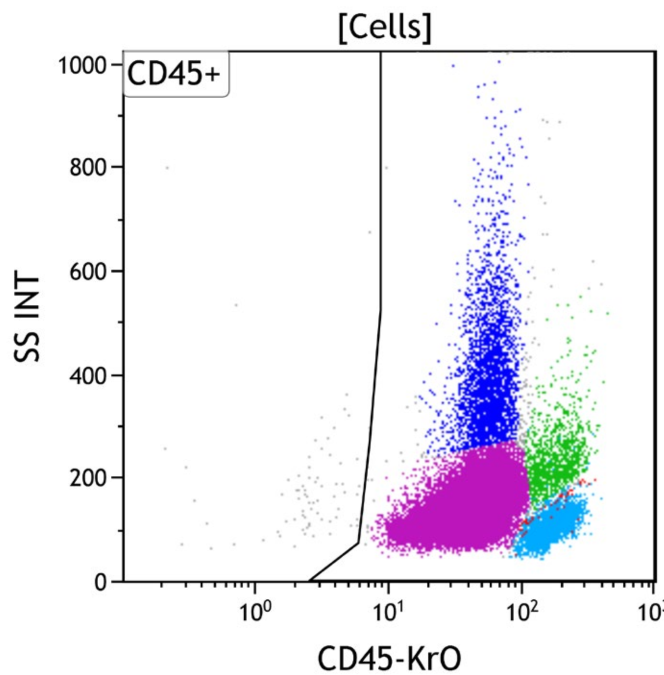

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

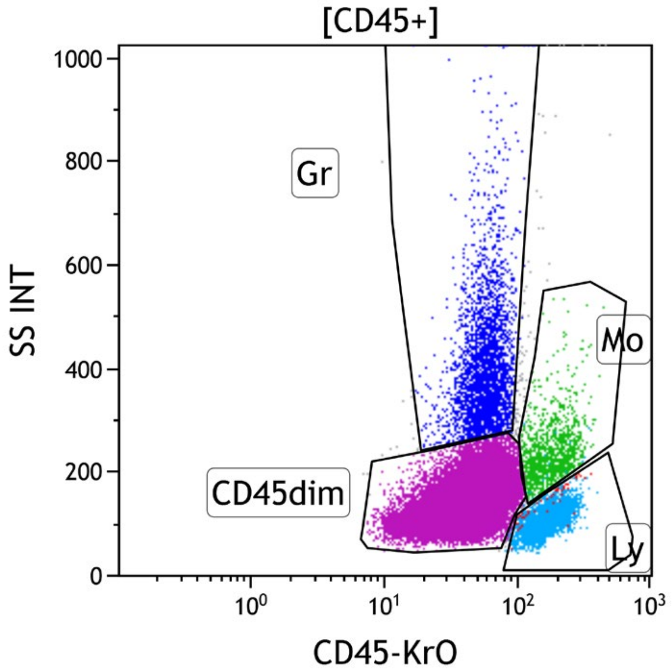

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that the progenitor population (purple) is relatively increased in number.

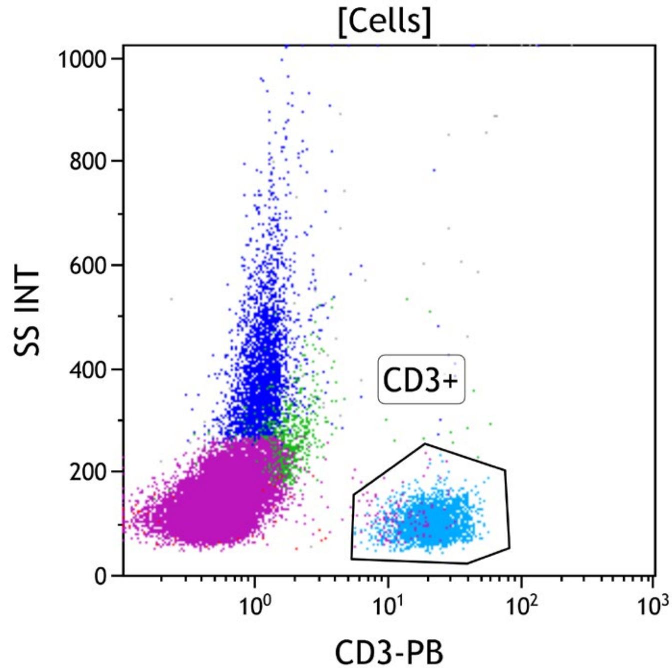

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The progenitors (purple) do not express CD3.

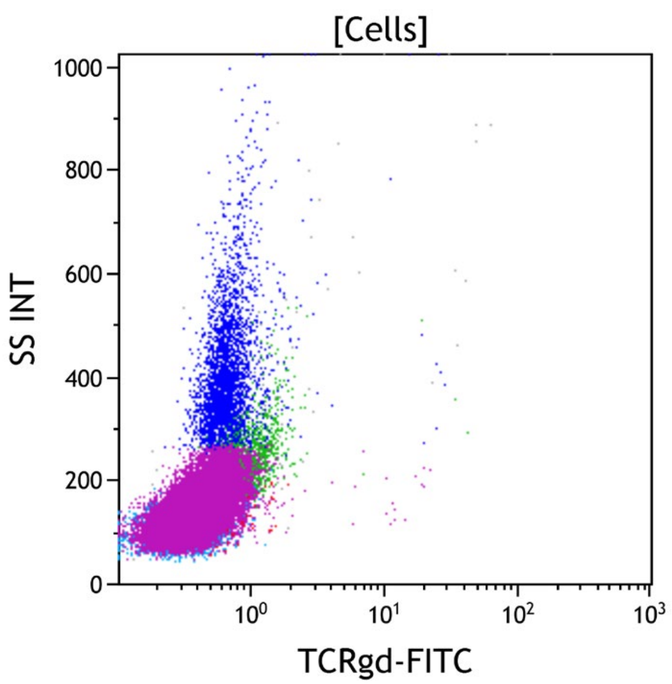

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The progenitors (purple) do not express TCRγδ.

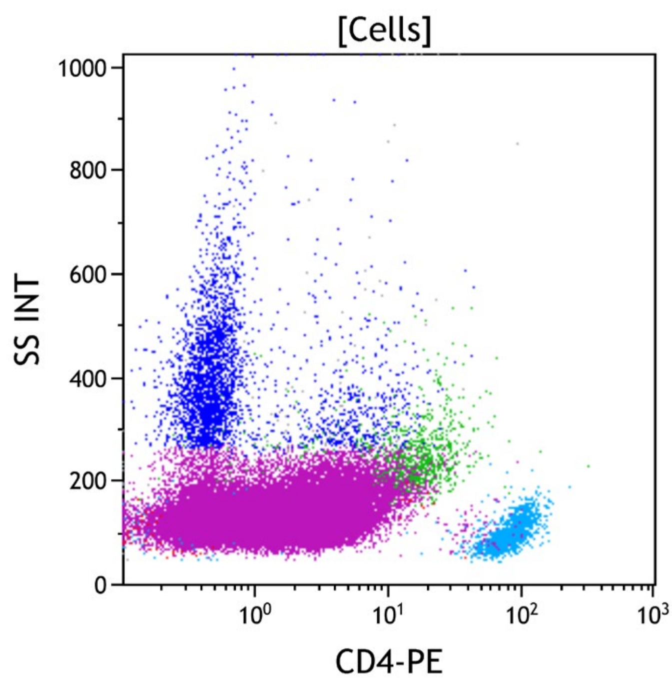

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The progenitors (purple) express dim CD4.

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T Cell Tube

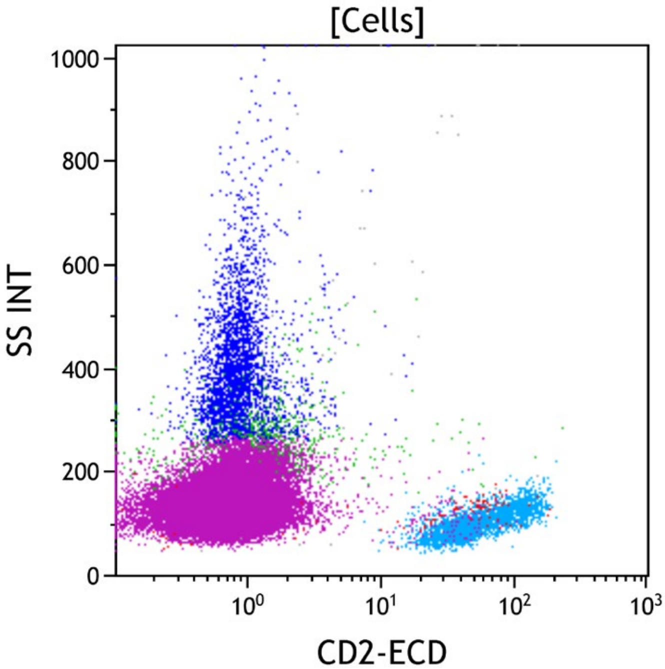

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The progenitors (purple) do not express CD2.

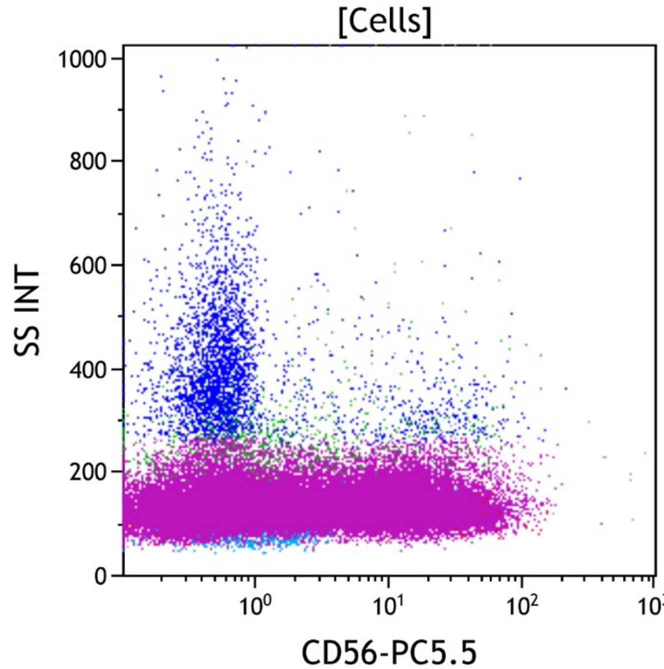

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The progenitors (purple) express variable CD56.

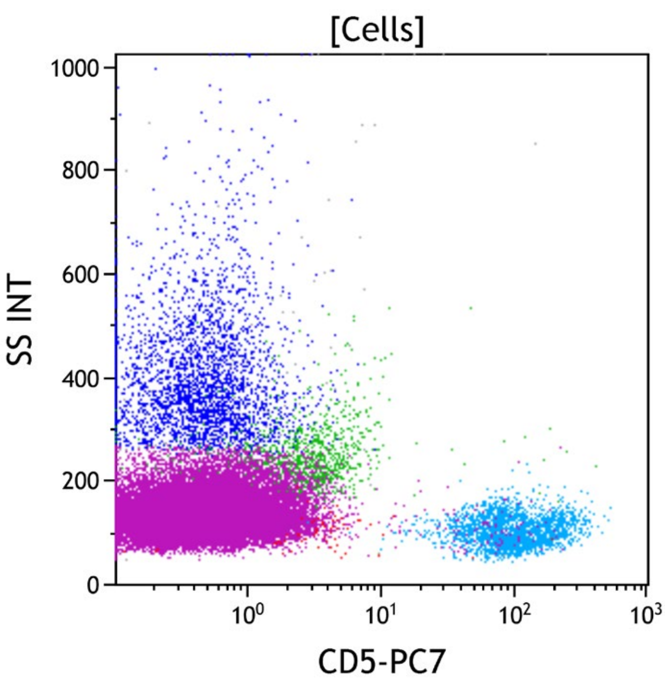

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The progenitors (purple) do not express CD5.

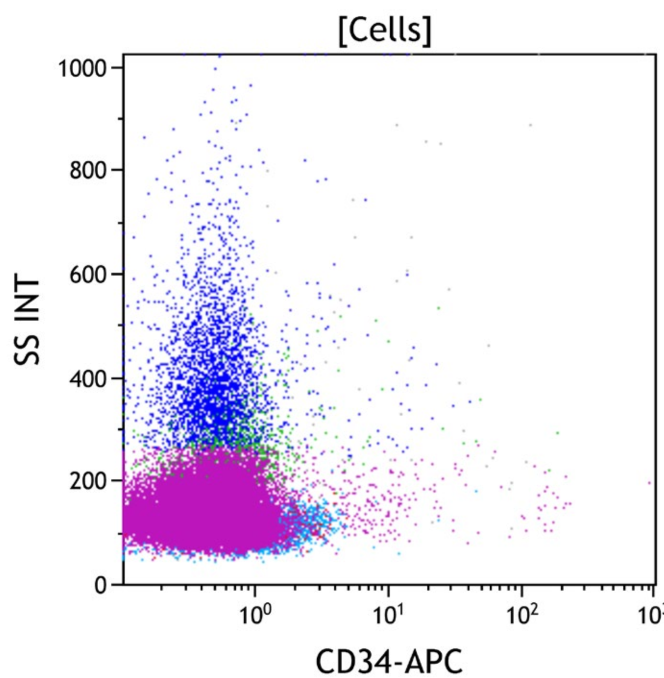

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The progenitors (purple) do not express CD34.

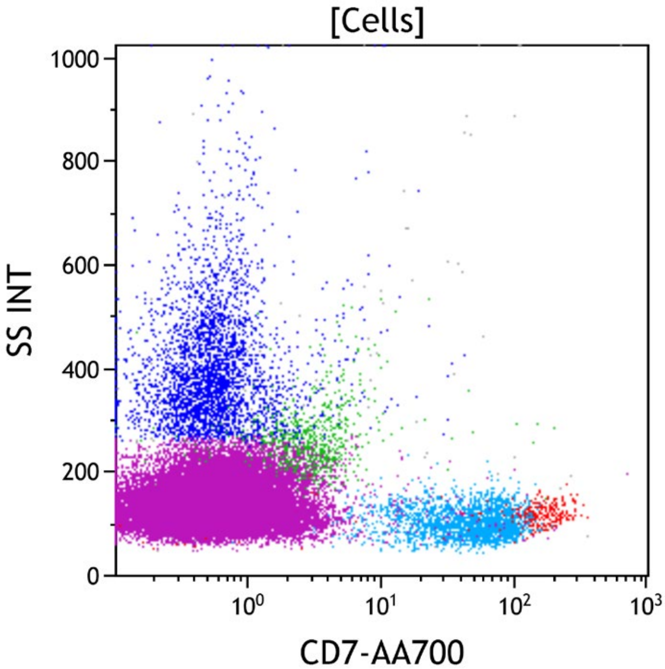

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The progenitors (purple) do not express CD7.

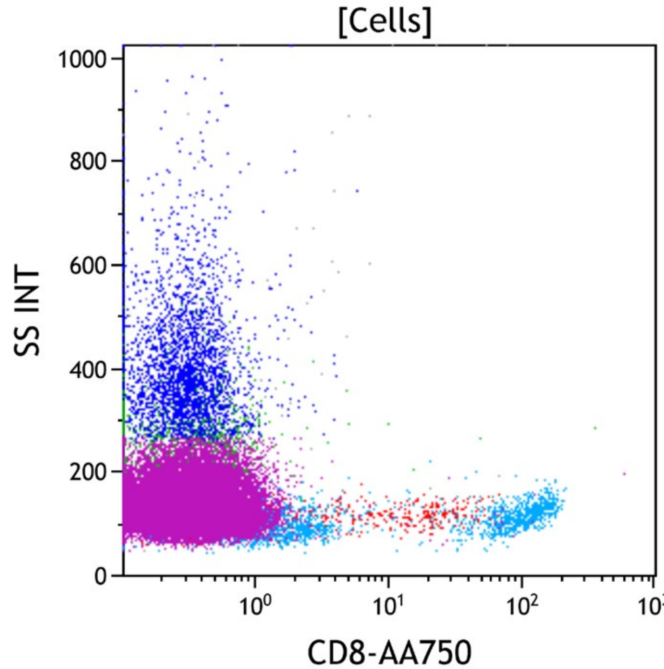

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The progenitors (purple) do not express CD8.

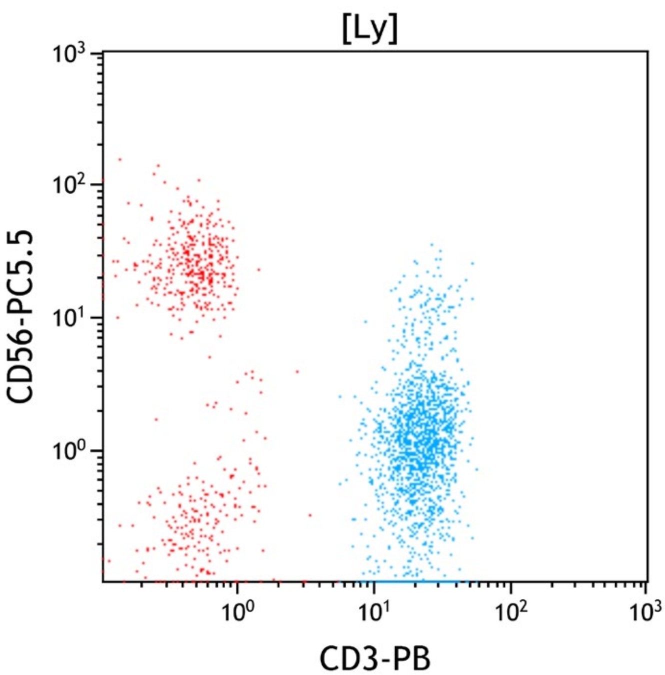

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

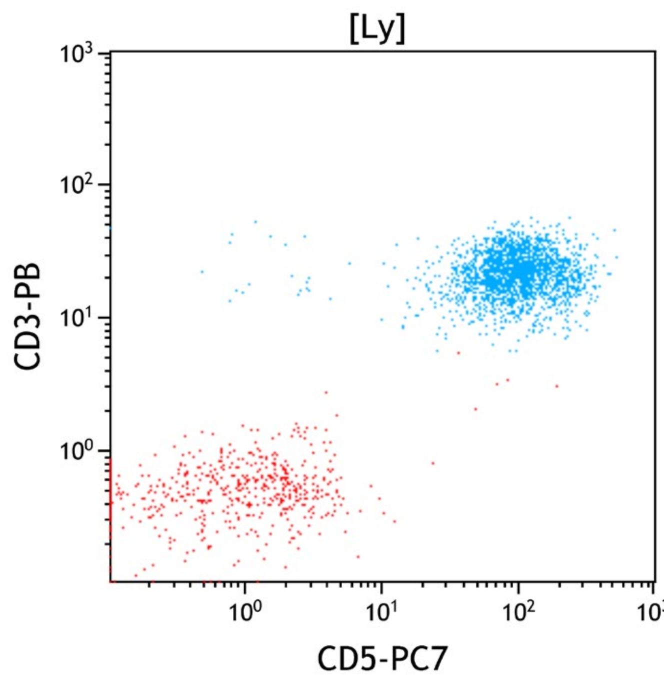

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

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T Cell Tube

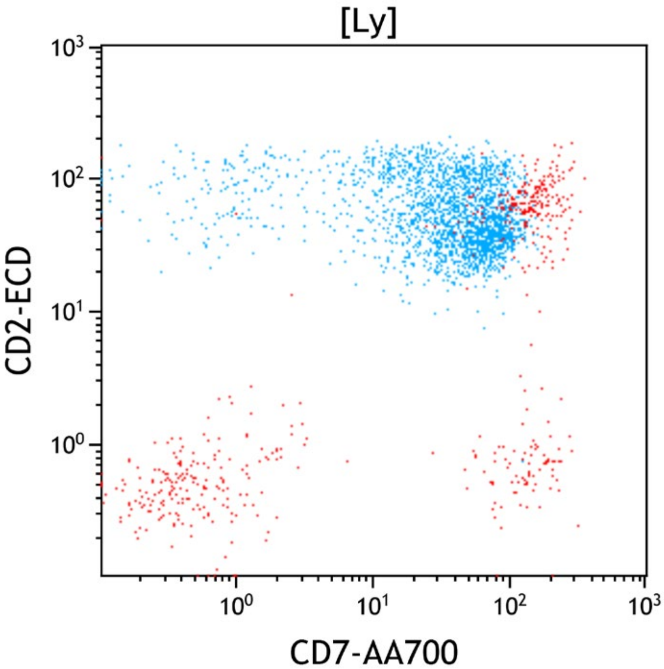

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

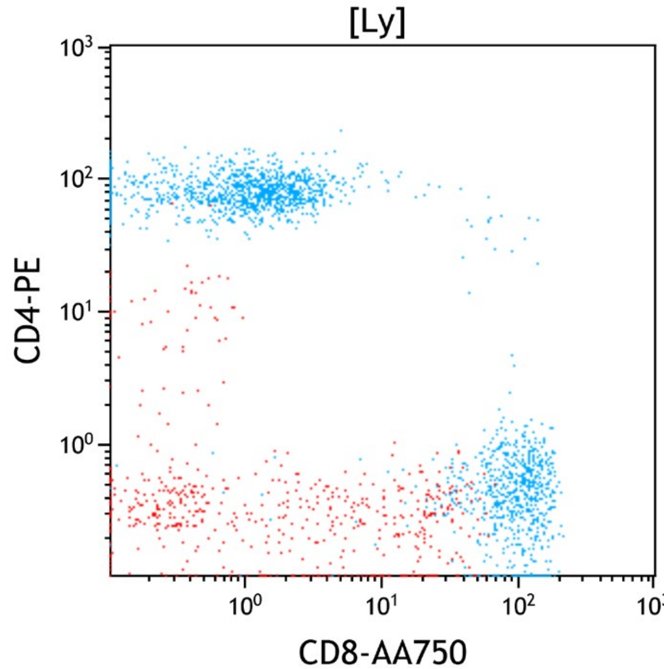

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

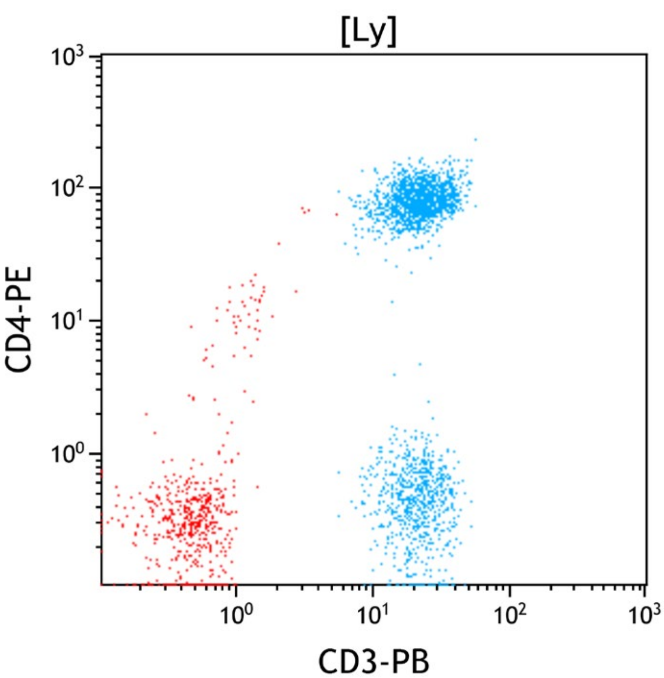

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

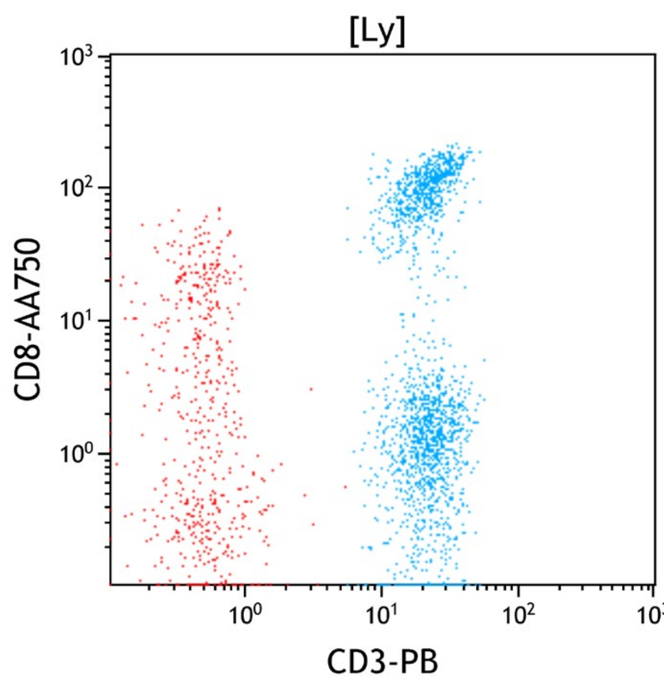

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 without CD3.

T Cell Tube

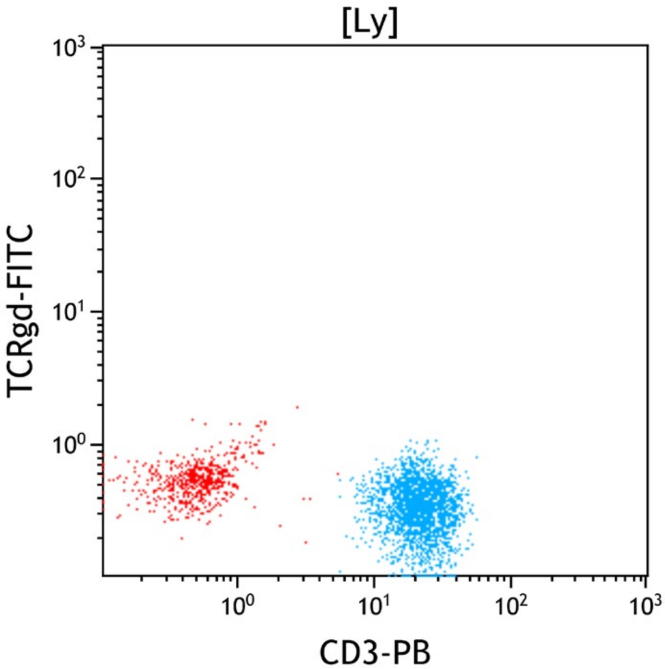

Figure 21. This CD3 vs TCRgd dot plot shows all cells in the lymphocyte gate (Ly). There are no gamma/delta T cells.

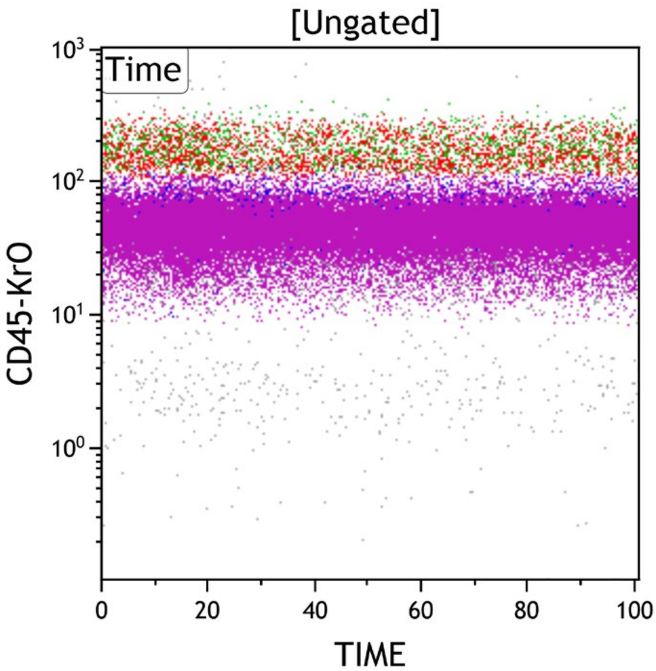

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

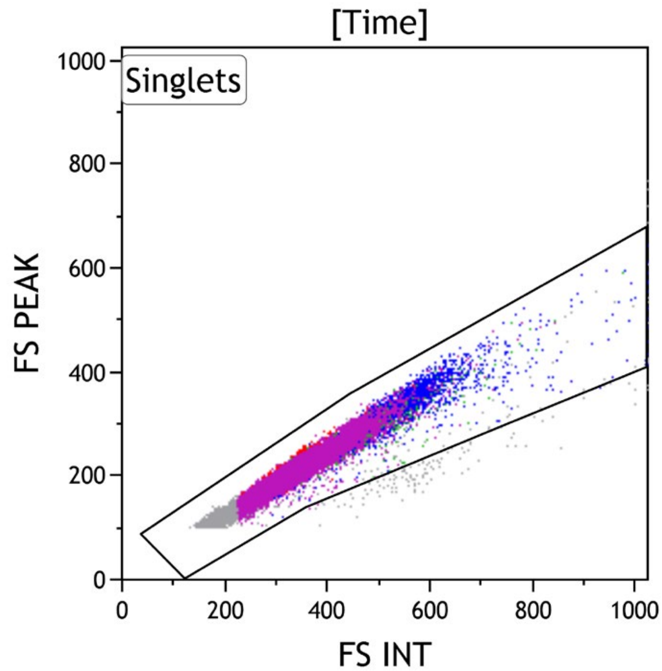

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

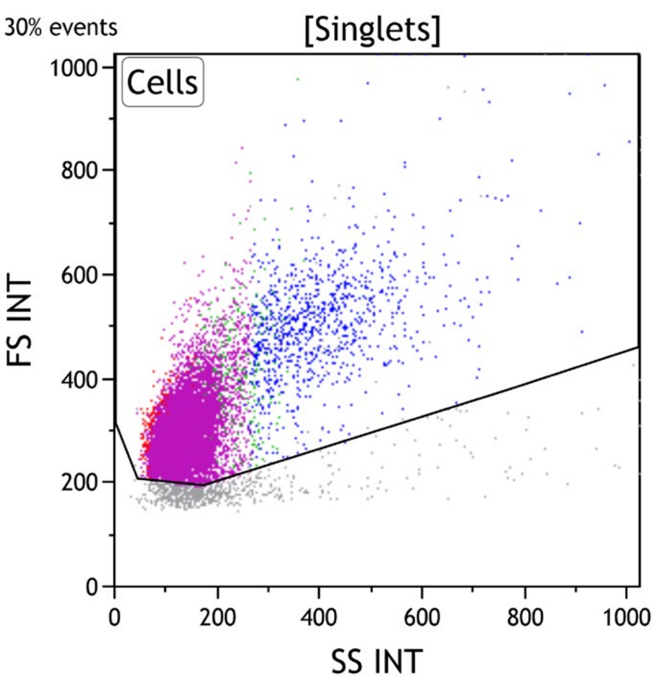

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

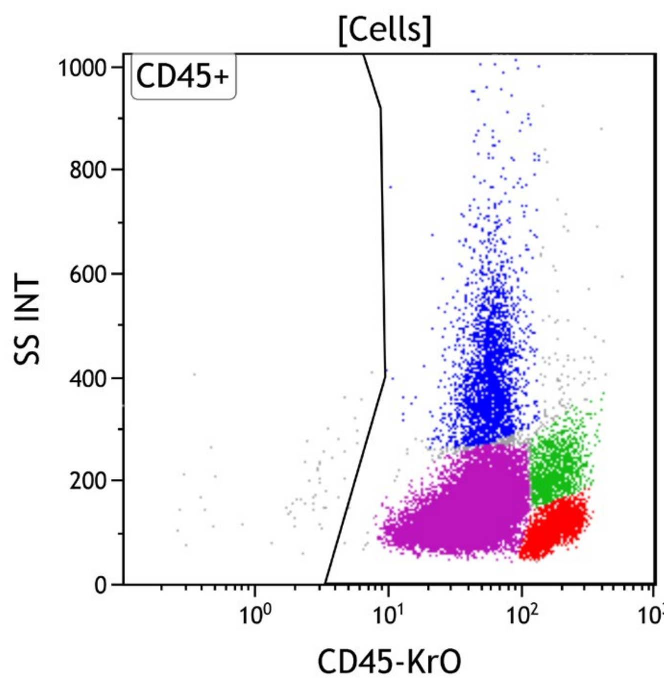

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

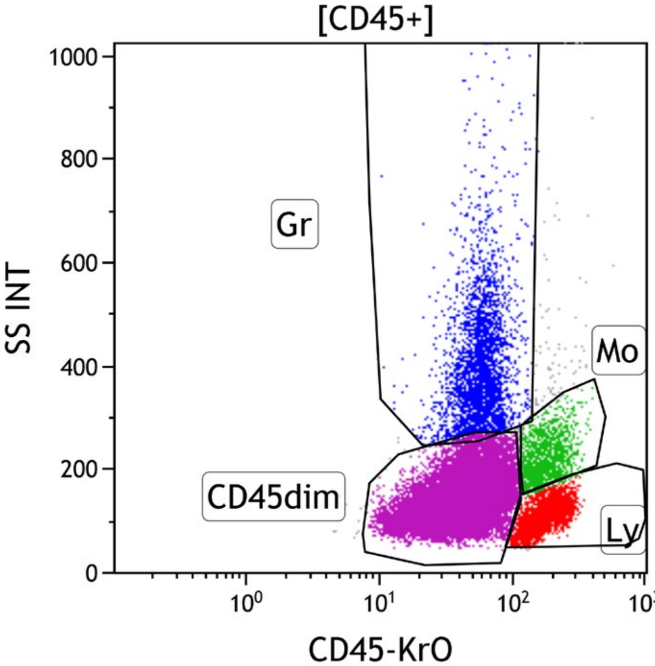

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that the progenitor population (purple) is relatively increased.

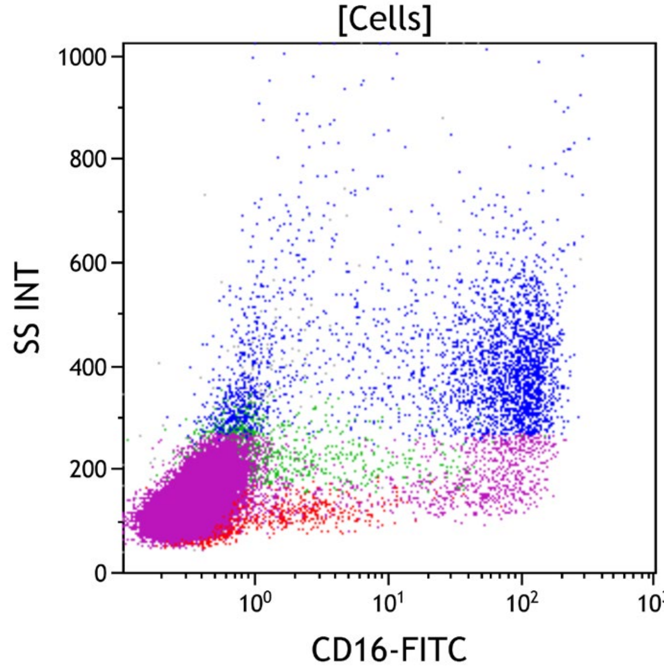

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The progenitors (purple) do not express CD16.

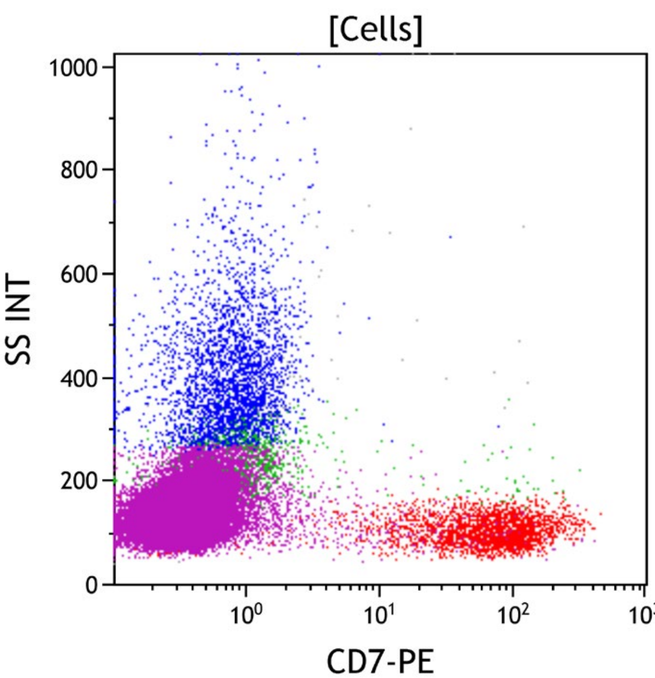

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The progenitors (purple) do not express CD7.

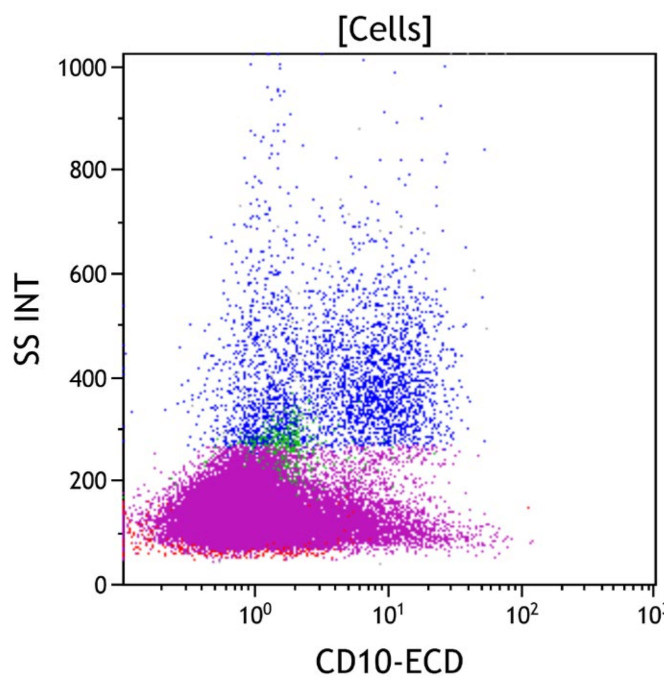

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The progenitors (purple) express dim variable CD10 on a small subset.

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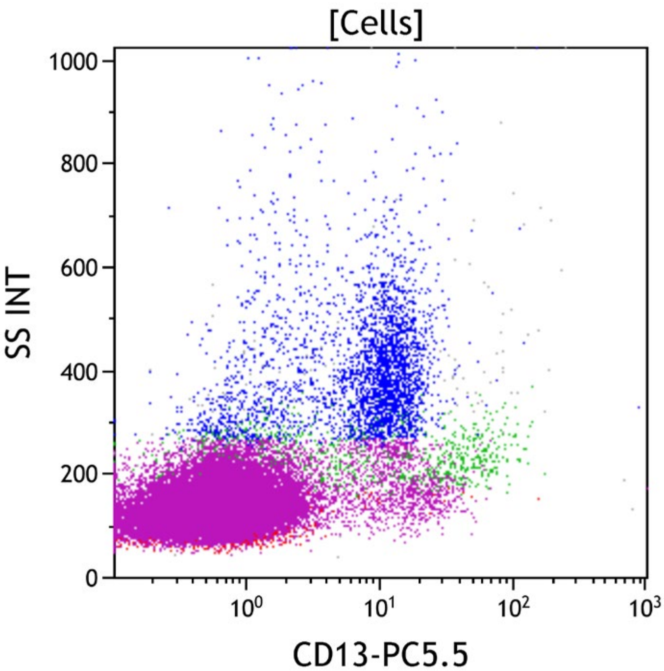

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The progenitors (purple) do not express CD13.

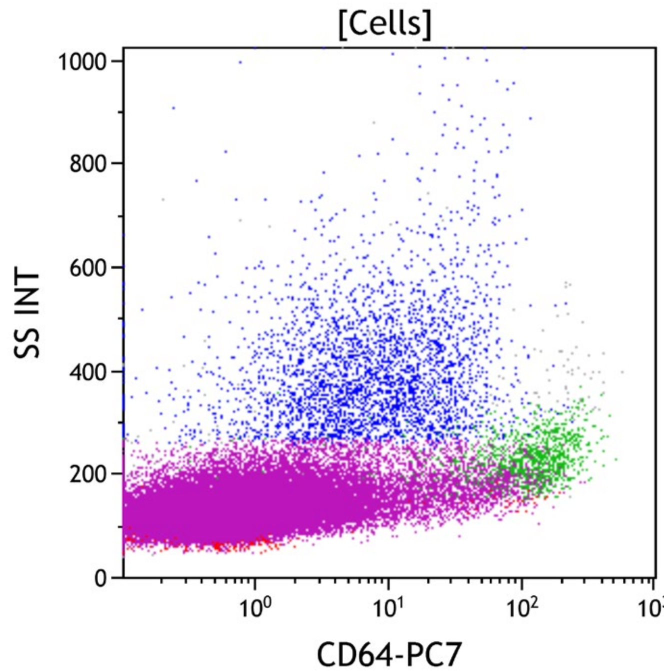

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The progenitors (purple) express dim variable CD64.

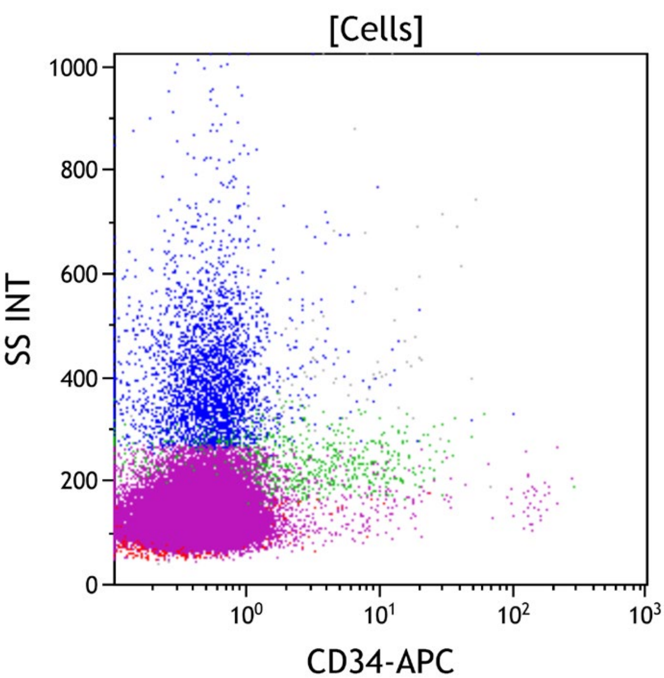

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The progenitors (purple) do not express CD34.

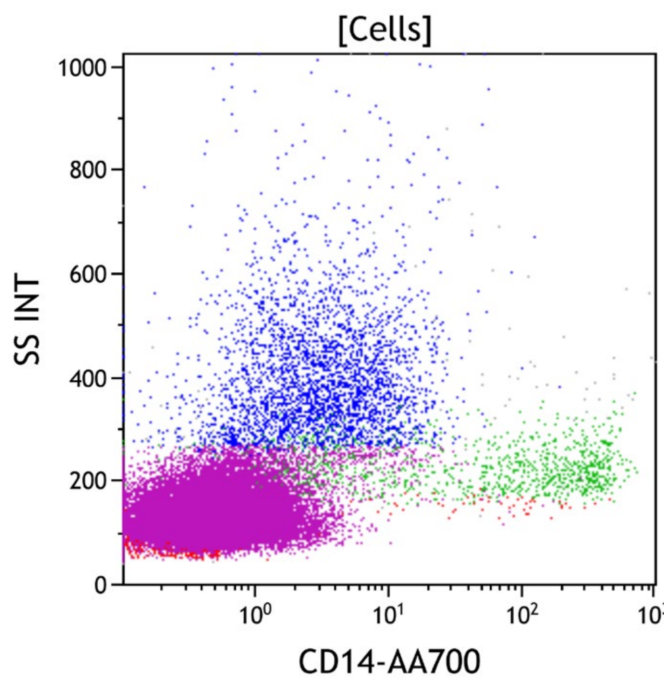

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The progenitors (purple) do not express CD14.

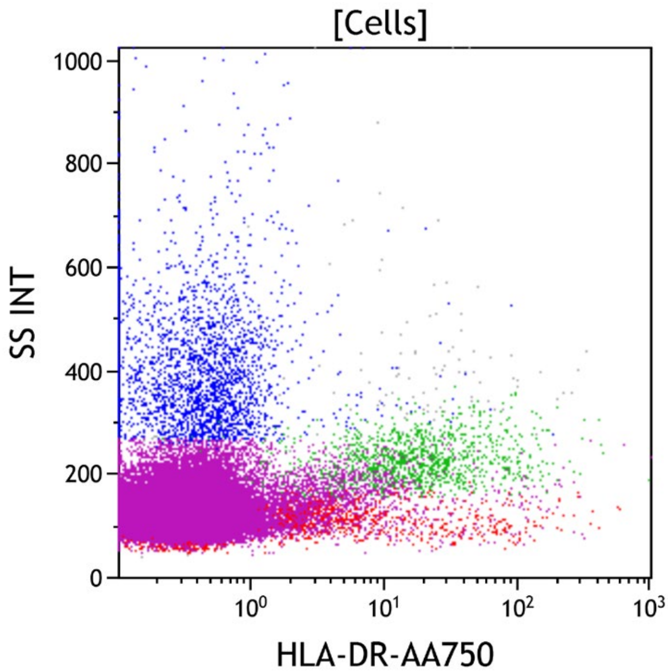

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). The progenitors (purple) do not express HLA-DR.

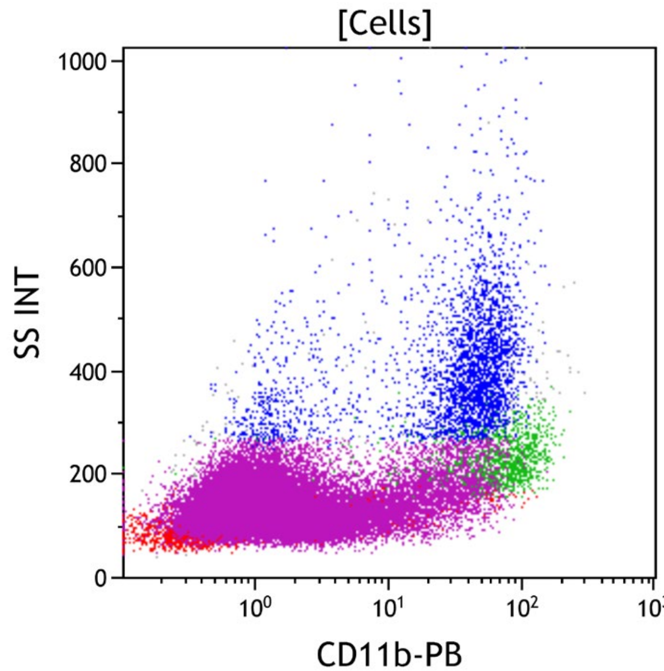

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The progenitors (purple) express dim variable CD11b on a subset.

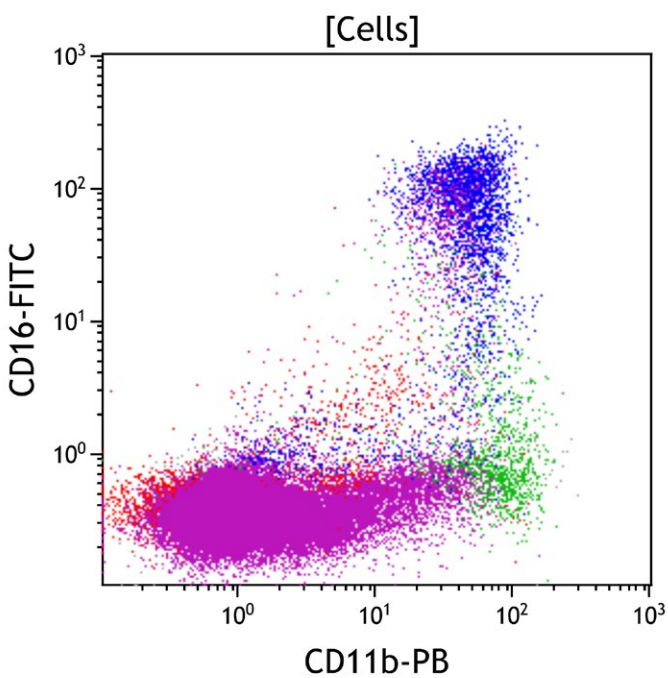

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (in green), immature and mature granulocytes (in blue) and NK cells (in red). CD16 is expressed on immature and mature granulocytes (in blue) and a subset of NK cells (in red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. The progenitors (purple) express dim variable CD11b on a subset without CD16.

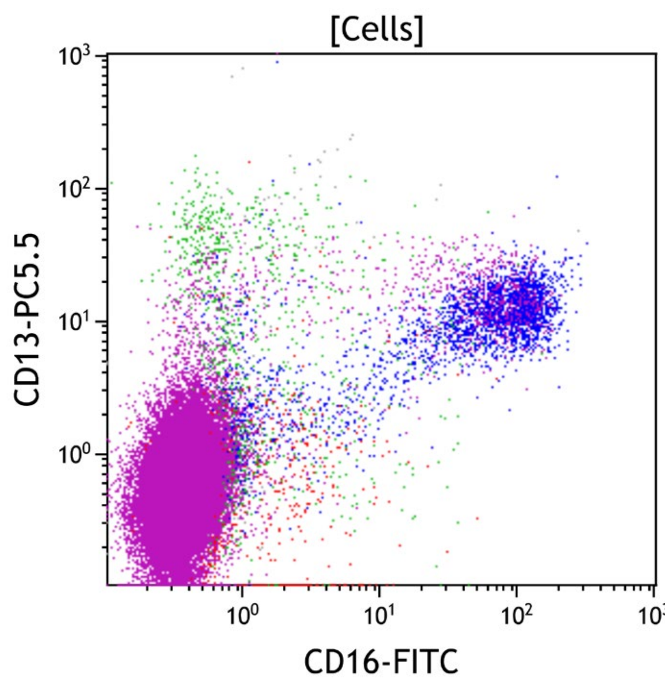

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. The progenitors (purple) do not express CD13 or CD16.

M1 Cell Tube

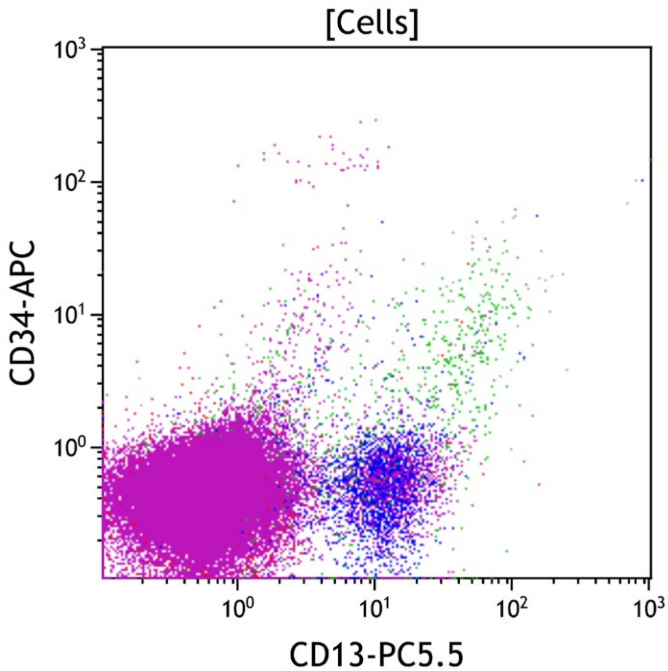

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells (red). The progenitors (purple) do not express CD13 or CD34.

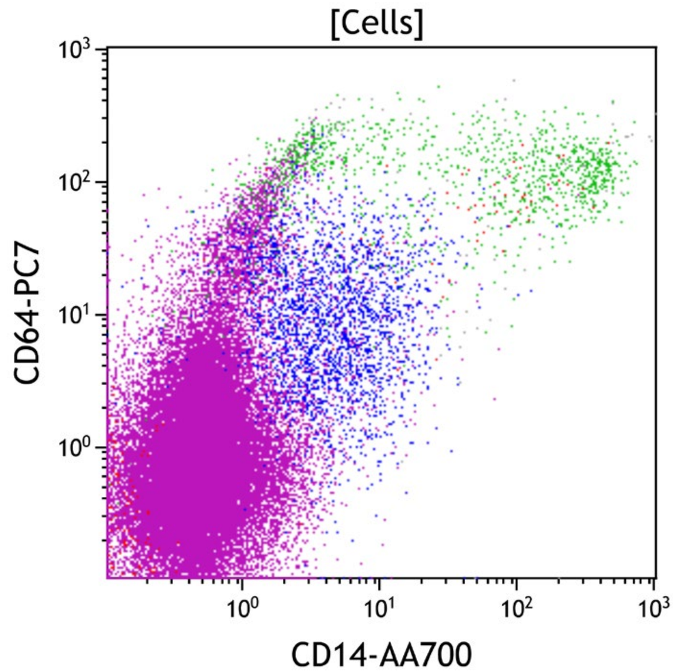

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (blue top left) and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64 (green). Immature granulocytes express moderate CD64 without CD14 (blue left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue bottom). The progenitors (purple) express dim variable CD64 on a small subset without CD14.

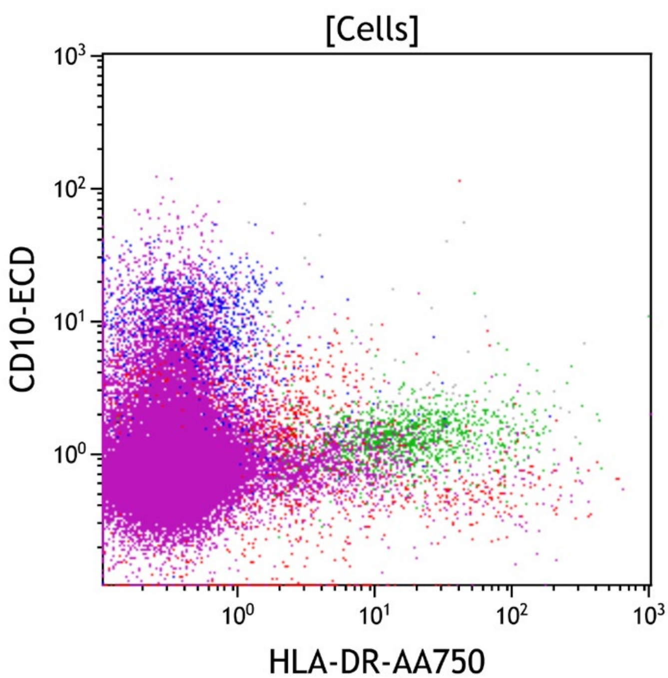

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue) and immature B cells. Immature B cells express both CD10 and HLA-DR. The progenitors (purple) express dim CD10 on a small subset with minimal HLA-DR.

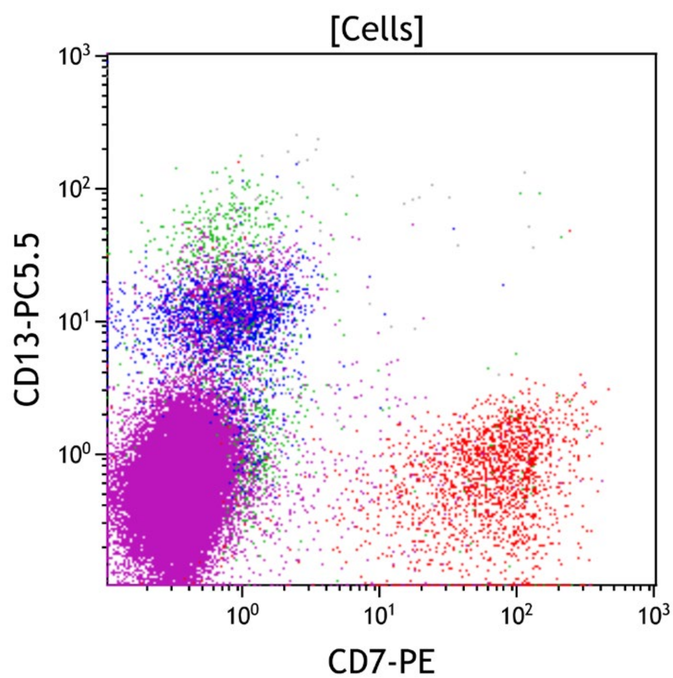

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The progenitors (purple) do not express CD7 and CD13.

M2 Cell Tube

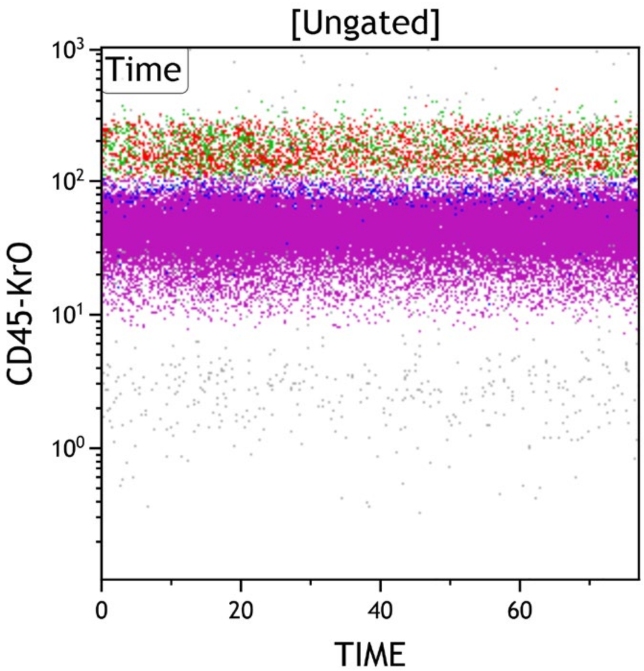

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

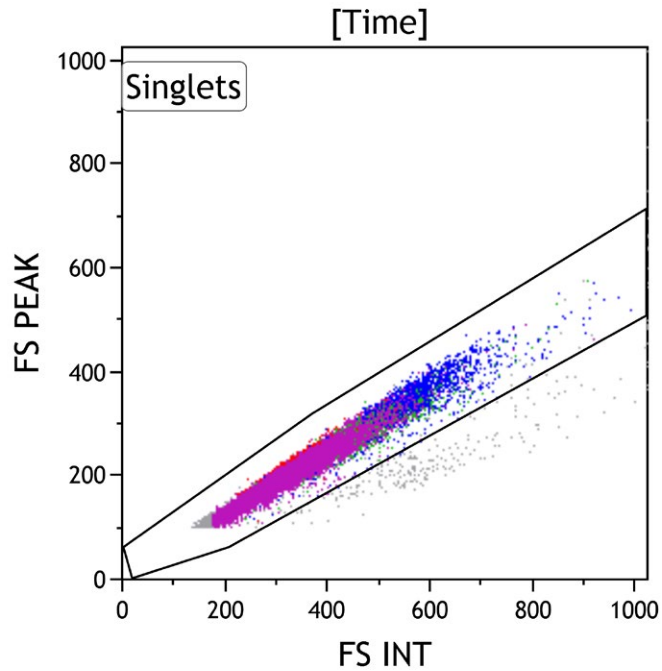

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

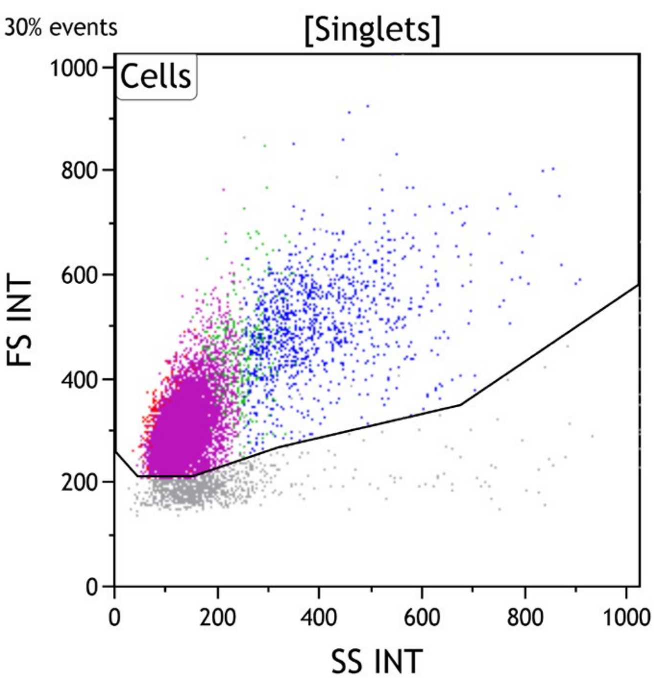

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

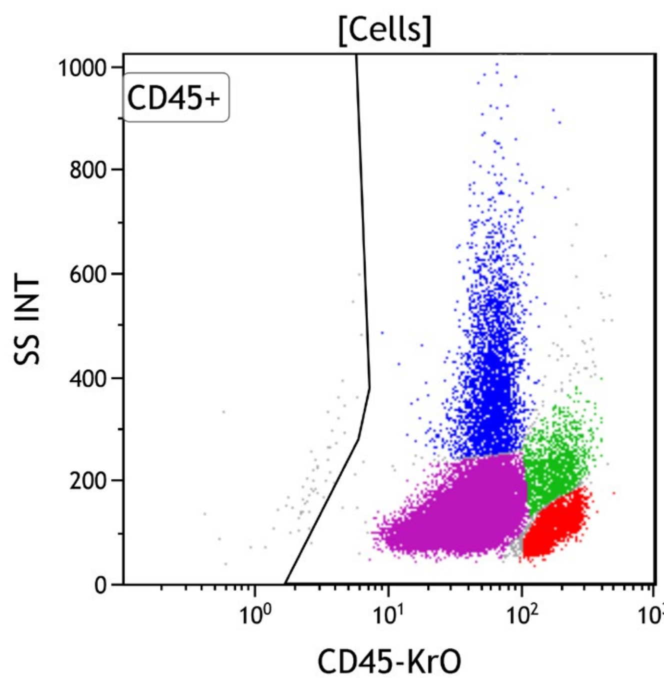

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

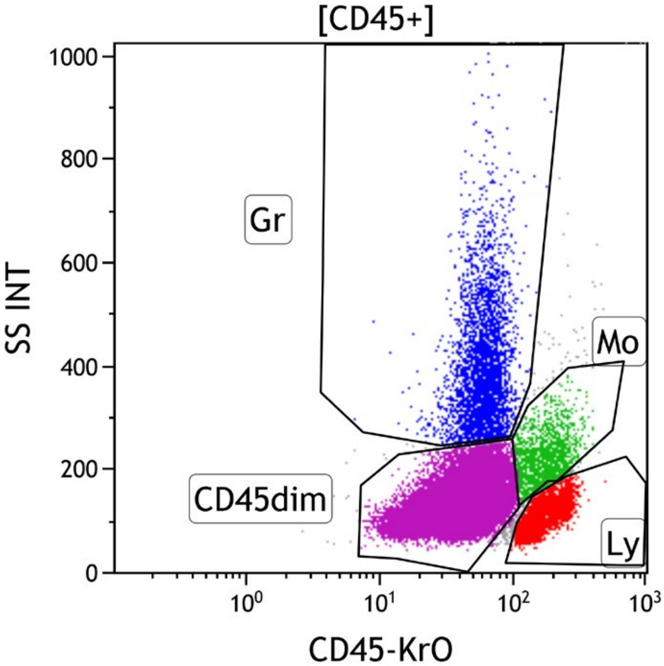

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that the progenitor population (purple) is relatively increased in number.

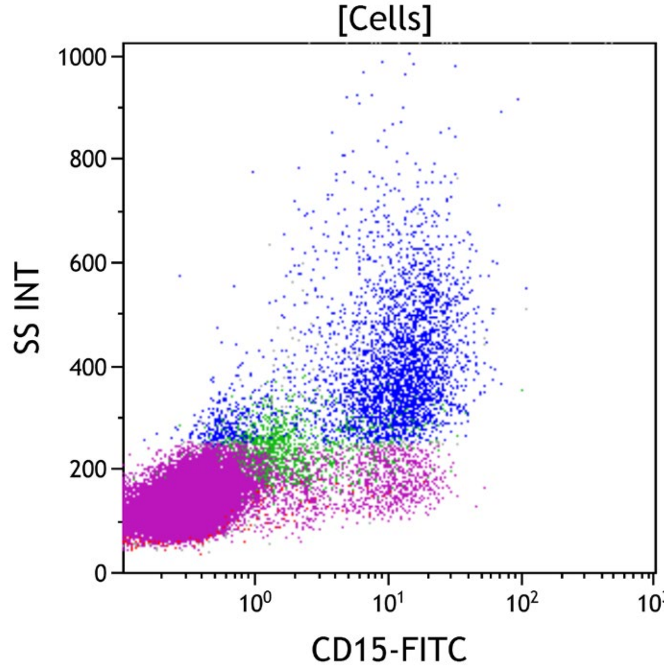

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The progenitors (purple) do not express CD15.

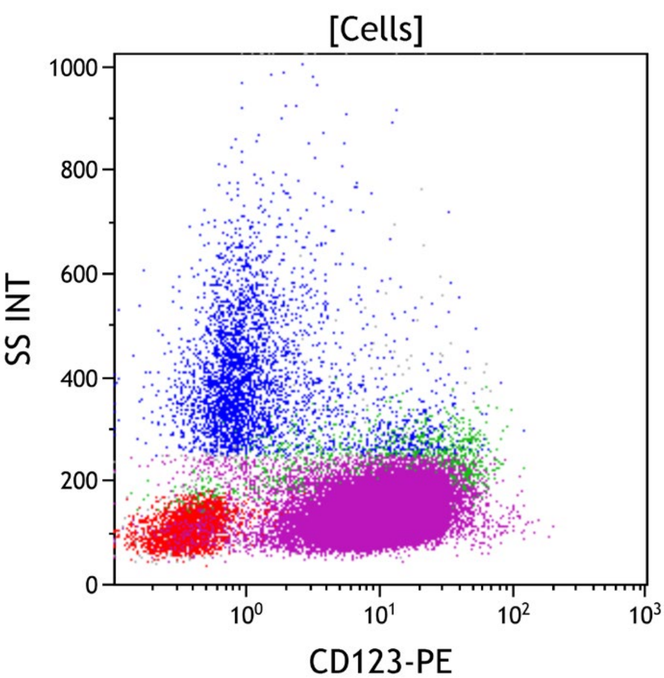

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The progenitors (purple) express intermediate CD123.

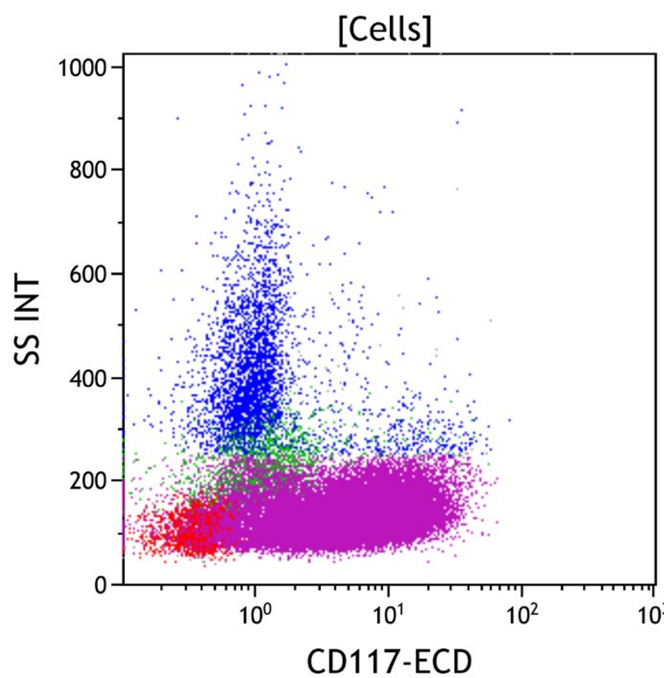

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The progenitors (purple) express dim to intermediate CD117.

M2 Cell Tube

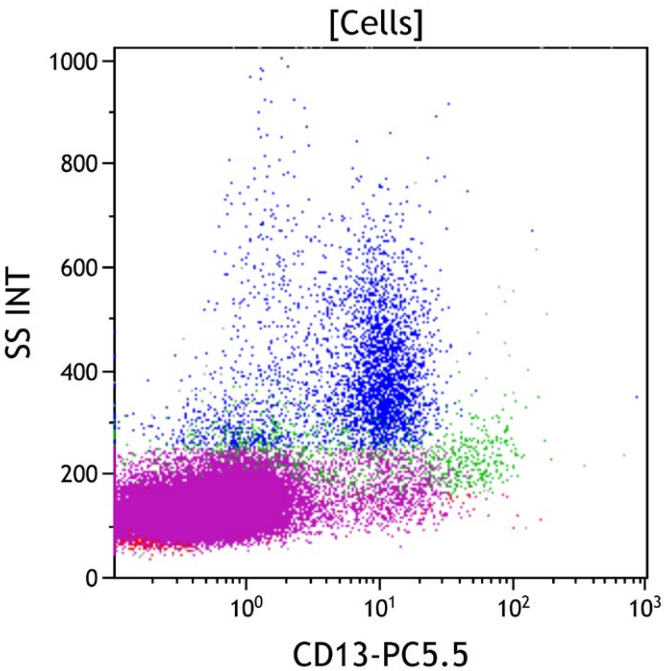

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The progenitors (purple) do not express CD13.

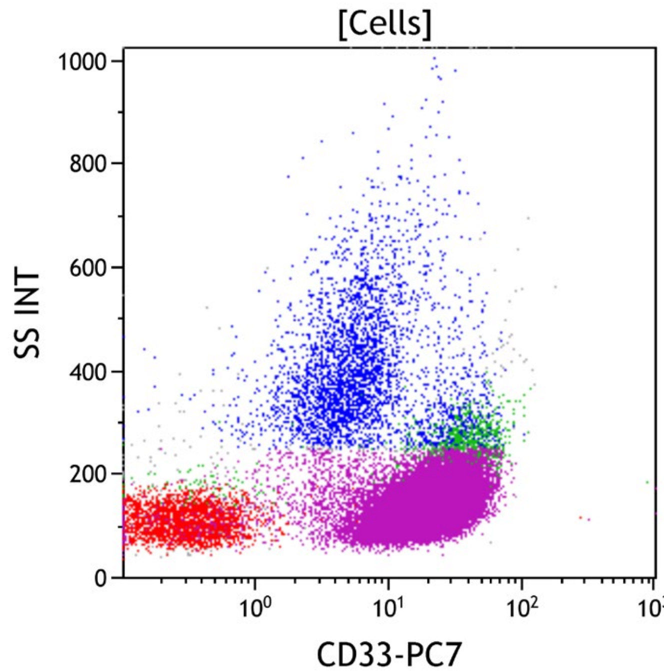

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The progenitors (purple) express bright CD33.

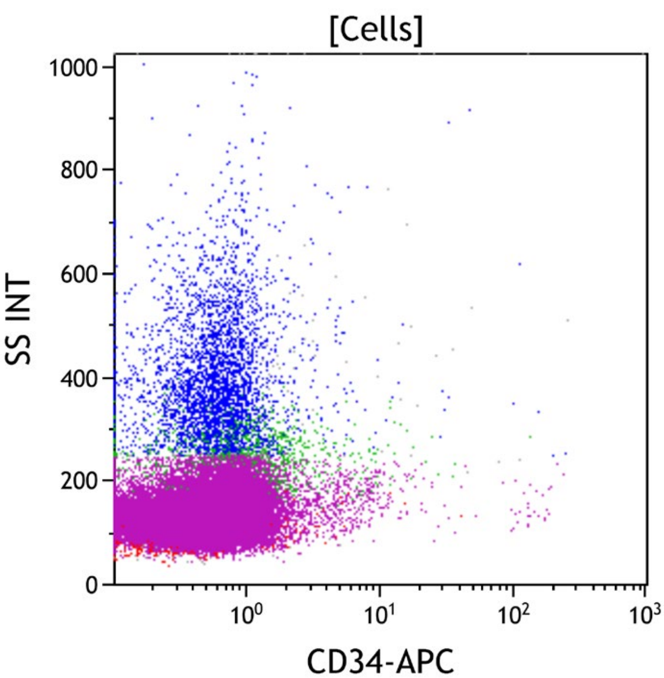

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The progenitors (purple) do not express CD34.

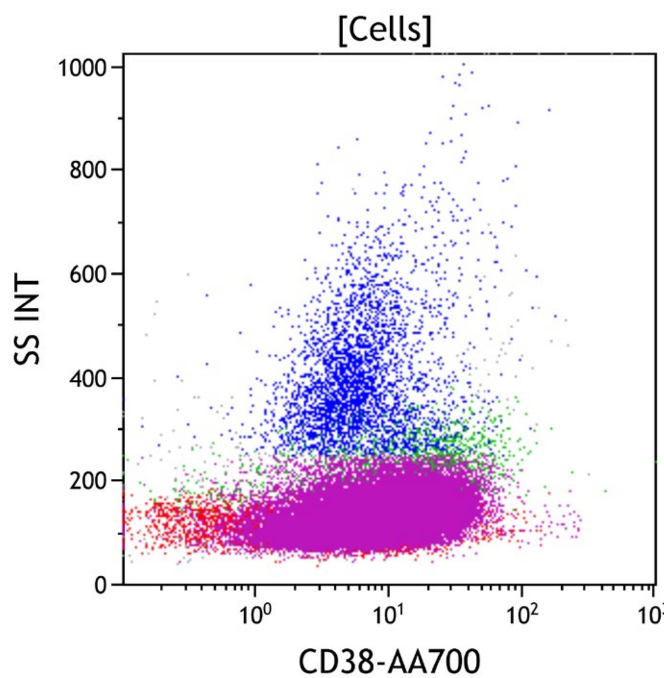

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The progenitors (purple) express dim to intermediate CD38.

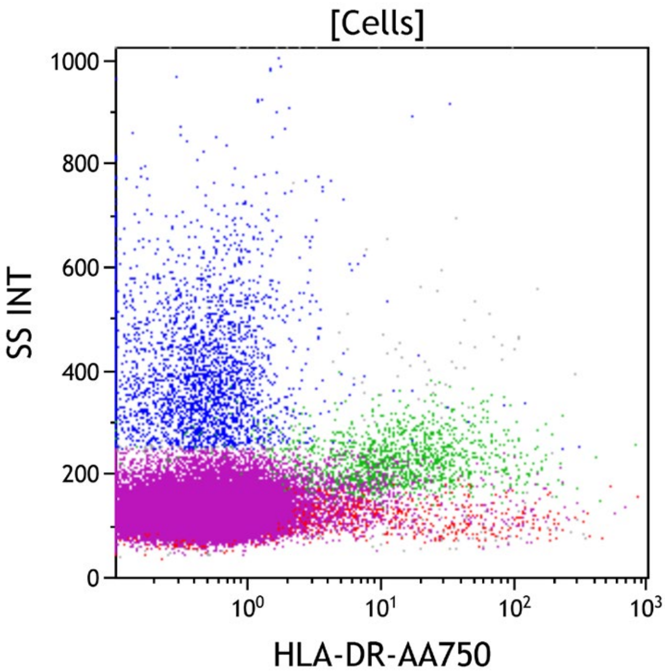

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). The progenitors (purple) do not express HLA-DR.

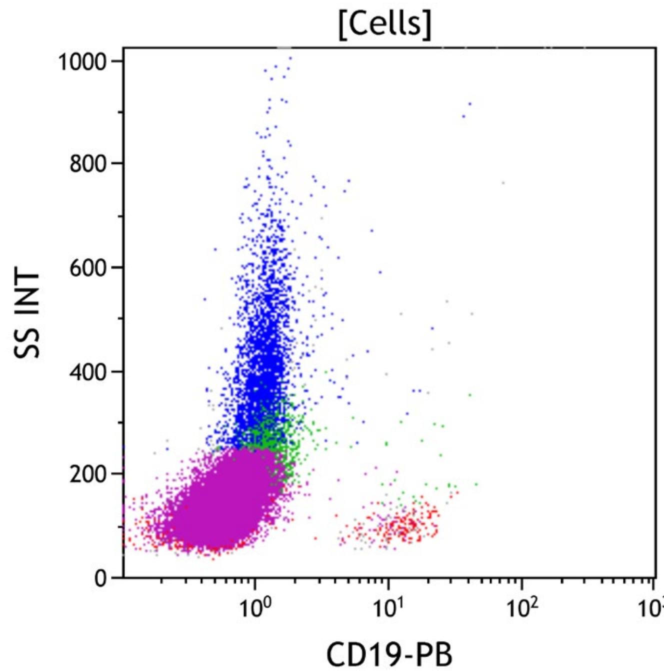

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The progenitors (purple) do not express CD19.

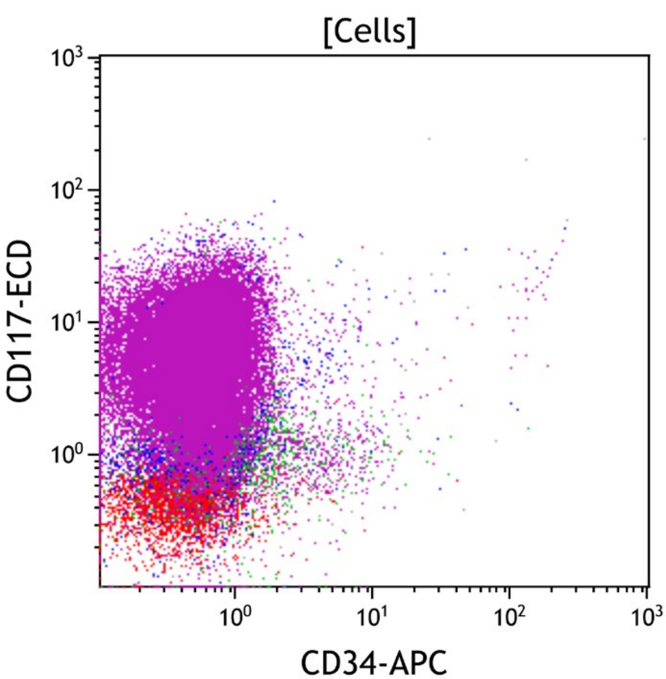

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts (upper right), promyelocytes (in blue), and early erythroid precursors, but negative on early B cell precursors (lower right). The progenitors (purple) express CD117 without CD34.

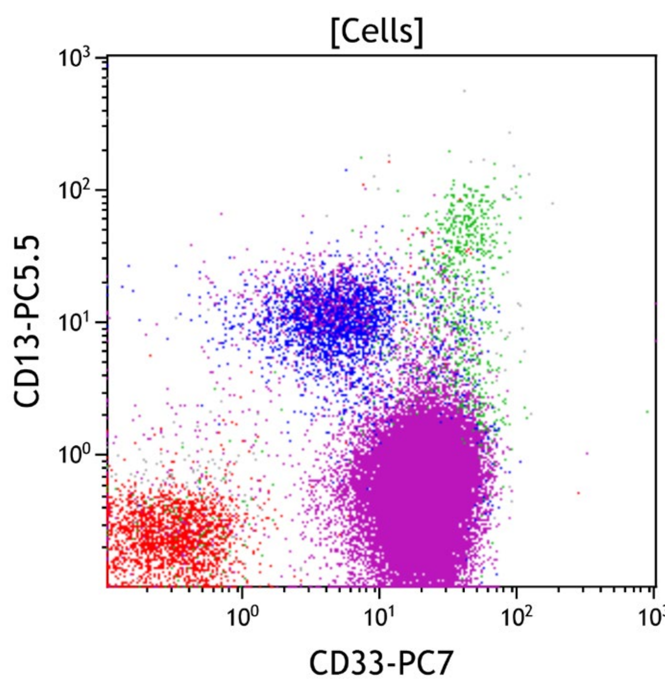

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue bottom) than more mature granulocytes (blue left). Lymphocytes largely do not express either CD13 or CD33 (red). The progenitors (purple) express bright CD33 without CD13.

M2 Cell Tube

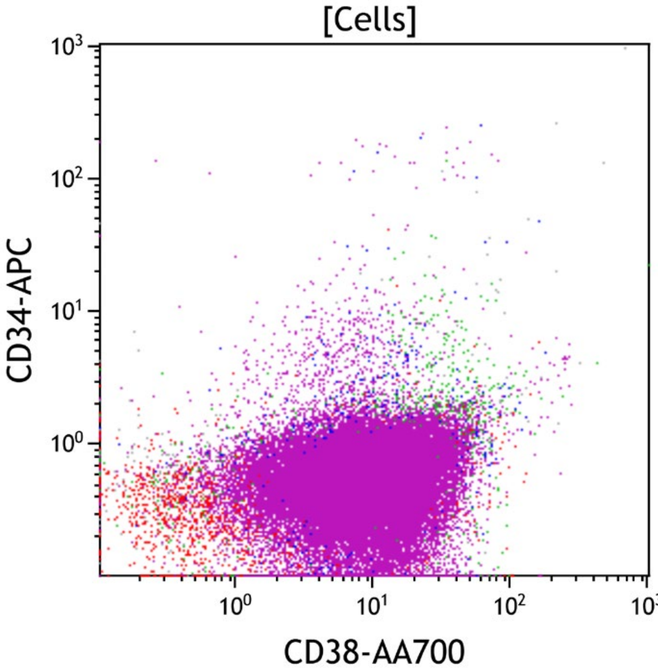

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (not many in this sample). The progenitors (purple) express dim to intermediate CD38 without CD34.

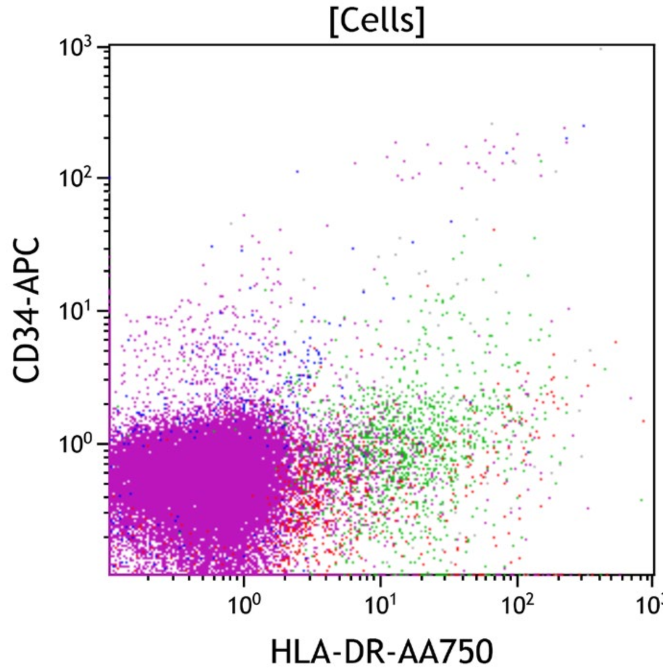

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes (right). The progenitors (purple) do not express CD34 or HLA-DR.

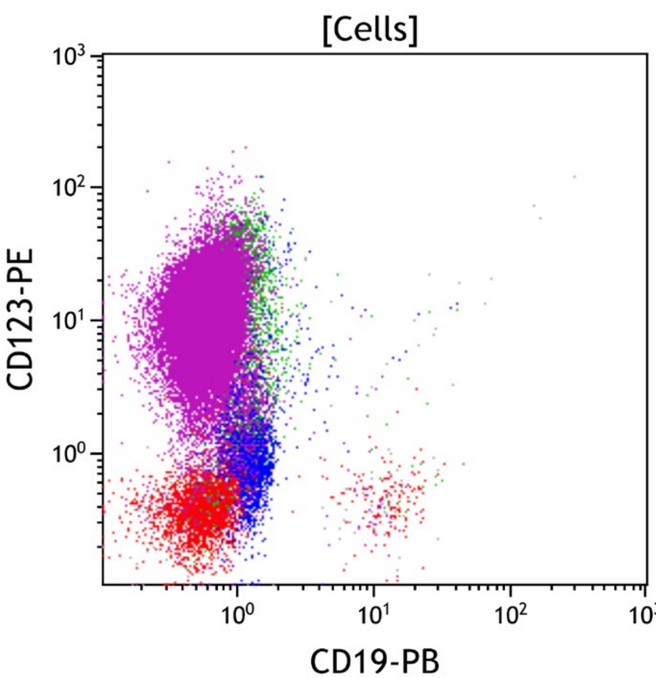

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The progenitors (purple) express intermediate CD123 without CD19.

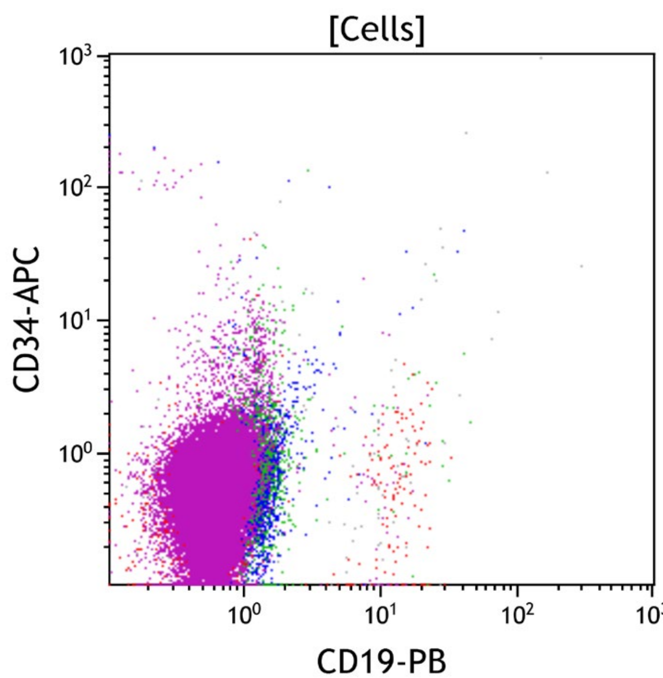

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. The progenitors (purple) do not express CD34 or CD19.

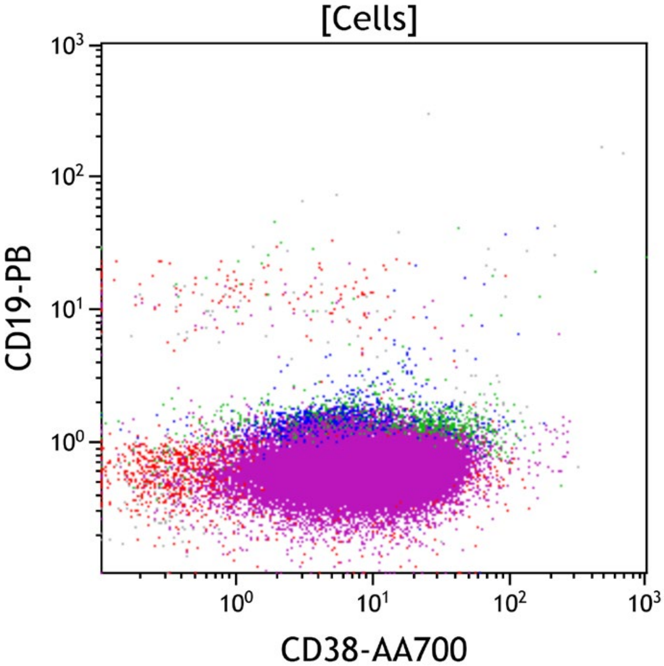

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38 (red left). Plasma cells show extremely high CD38 expression that is largely off scale (extreme right), but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The progenitors (purple) express dim to intermediate CD38 without CD19.

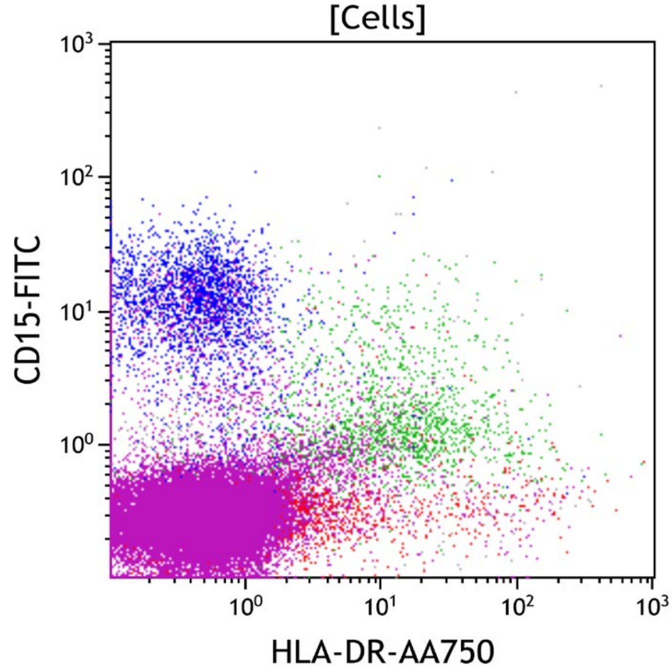

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. The progenitors (purple) do not express CD15 or HLA-DR.

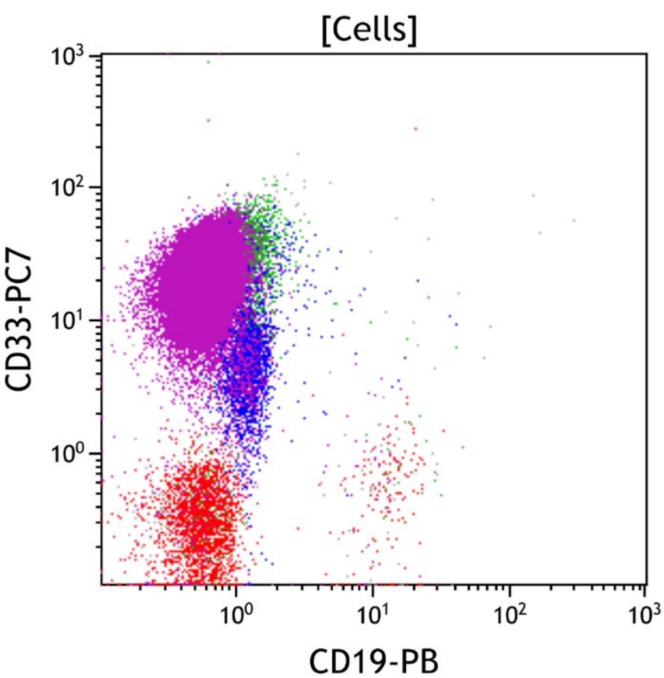

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The progenitors (purple) express bright CD33 without CD19.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate CD4, dim variable CD11b on a subset, bright CD33, dim to intermediate CD38, dim CD45, dim variable CD64 on a small subset, variable CD56, intermediate CD117, and intermediate CD123 without CD13, CD14, CD34, HLA-DR and other lymphoid or myeloid antigens. Compared with normal CD34-positive progenitors, the increased expression of CD33, absence of CD13, CD34 and HLA-DR, and variable CD56 is aberrant.

The immunophenotype of the abnormal population is that of expanded abnormal immature myelomonocytic progenitors and consistent with acute myeloid leukemia. However, a diagnosis of acute myeloid leukemia requires identification of 20% blasts or blast equivalents by morphology. In the context of acute myeloid leukemia, expression of bright CD33 without CD34 or HLA-DR on abnormal progenitors can be seen in NPM1 mutated AML, but is not entirely specific.

Case #21: Acute Promyelocytic Leukemia

Clinical Vignette

This 35-year-old female presents with pancytopenia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

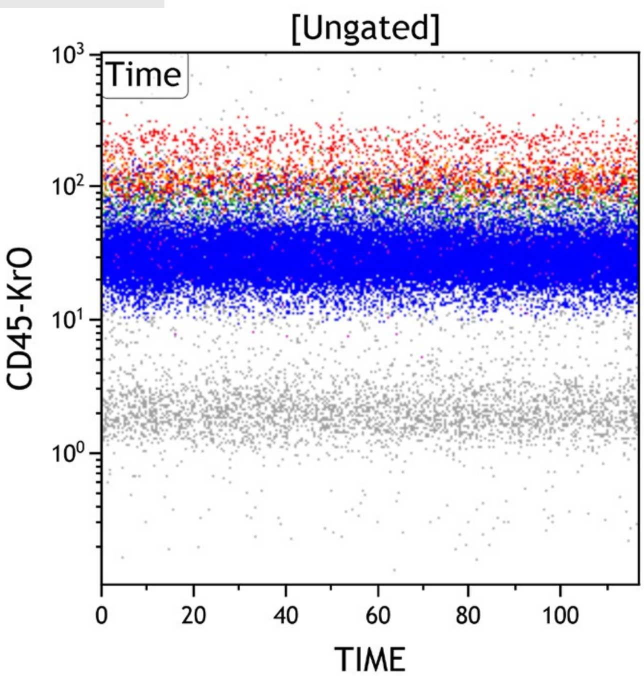

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

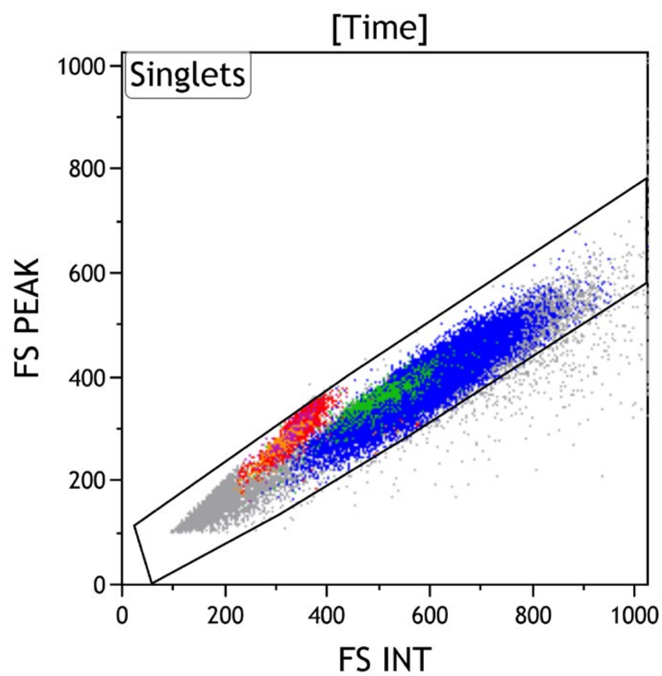

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

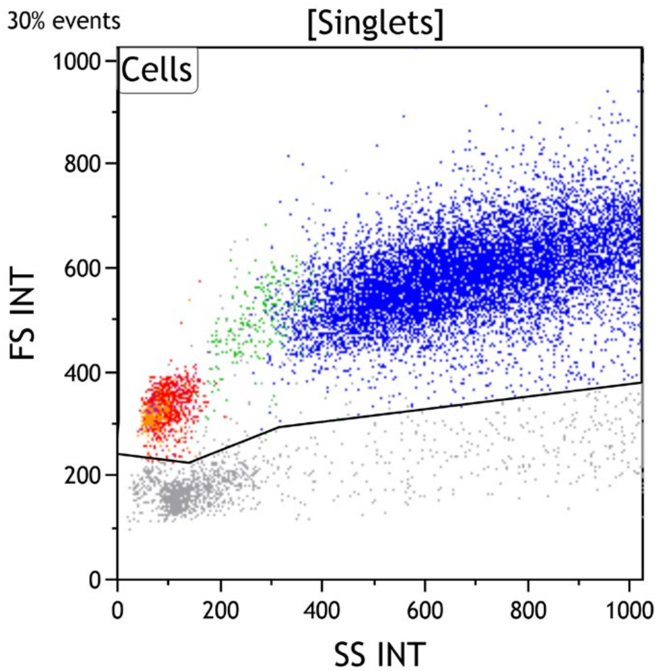

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

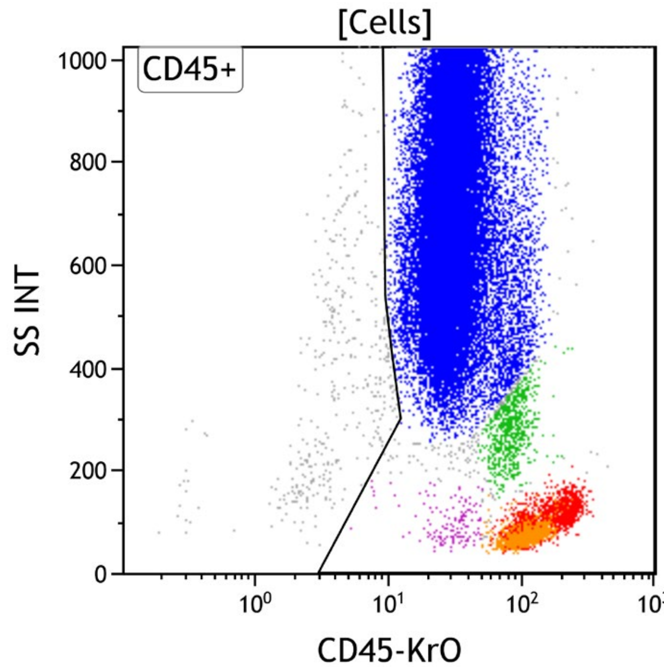

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

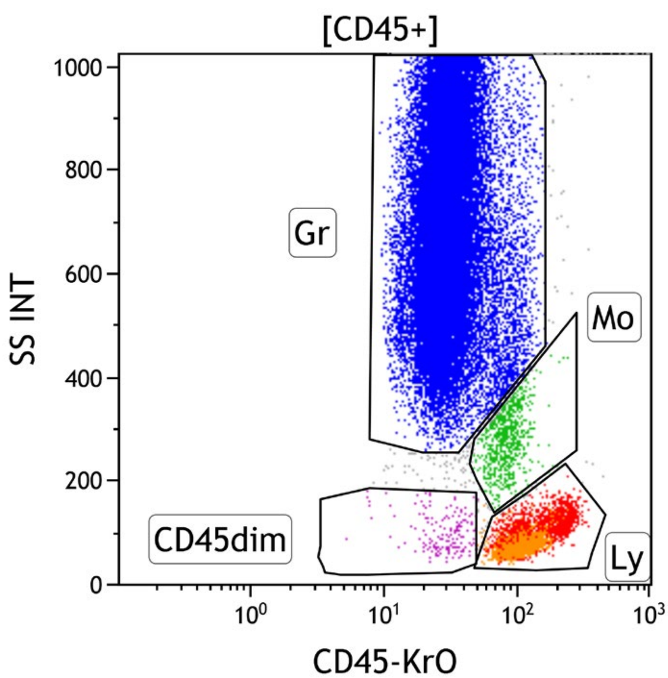

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note that the granulocytes (blue) are relatively expanded in number and have slightly decreased side scatter.

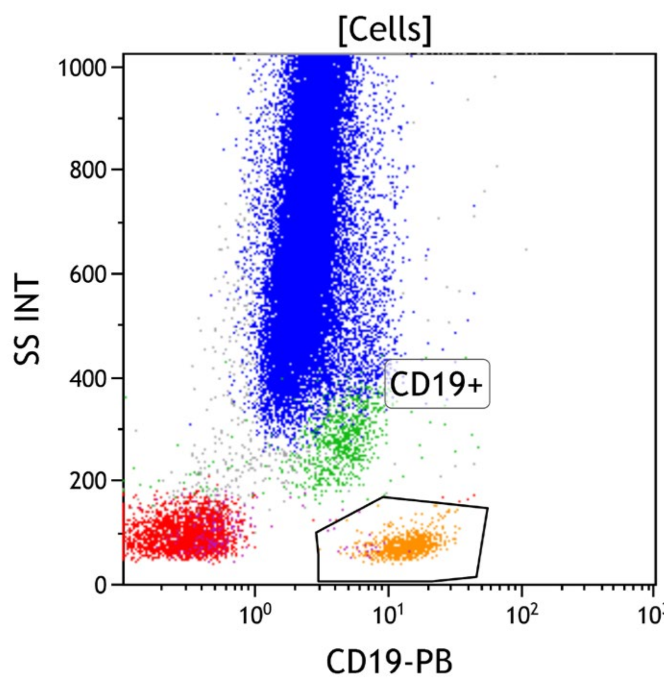

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The granulocytes (blue) do not express CD19.

B Cell Tube

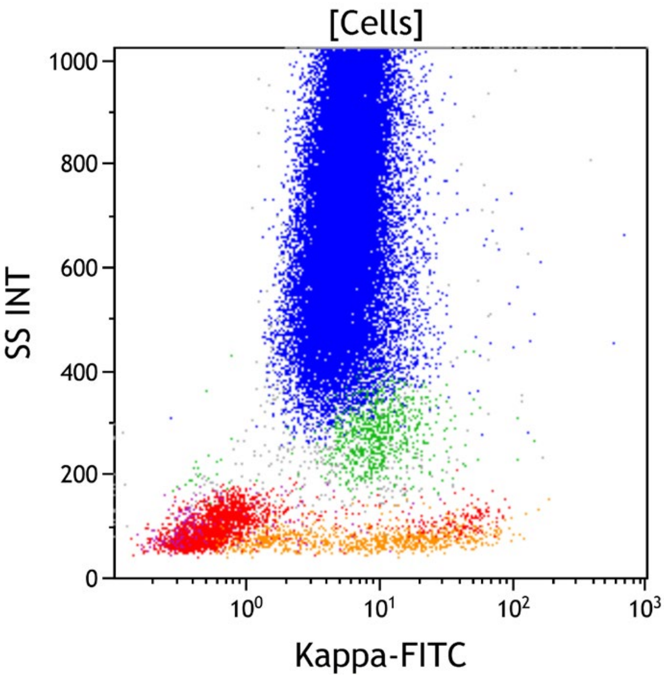

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The granulocytes (blue) do not express kappa light chains.

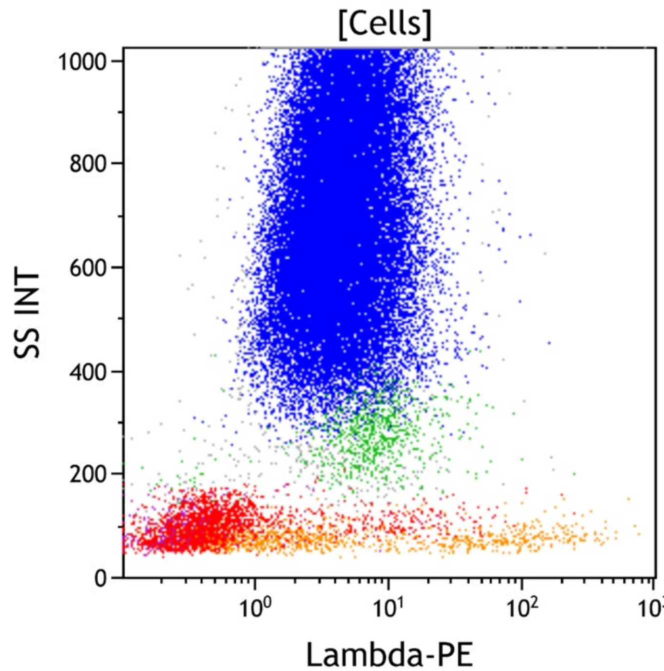

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The granulocytes (blue) do not express lambda light chains.

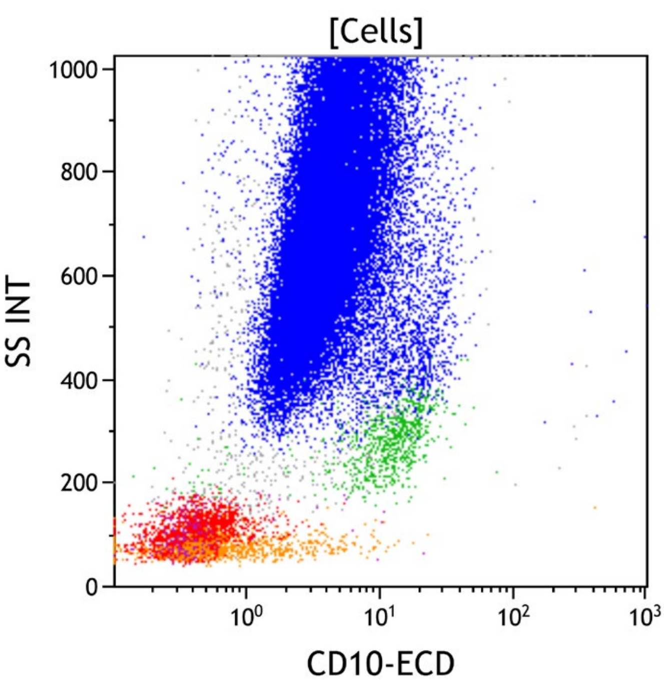

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes. The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The granulocytes (blue) do not express CD10, which is normally present on mature granulocytes.

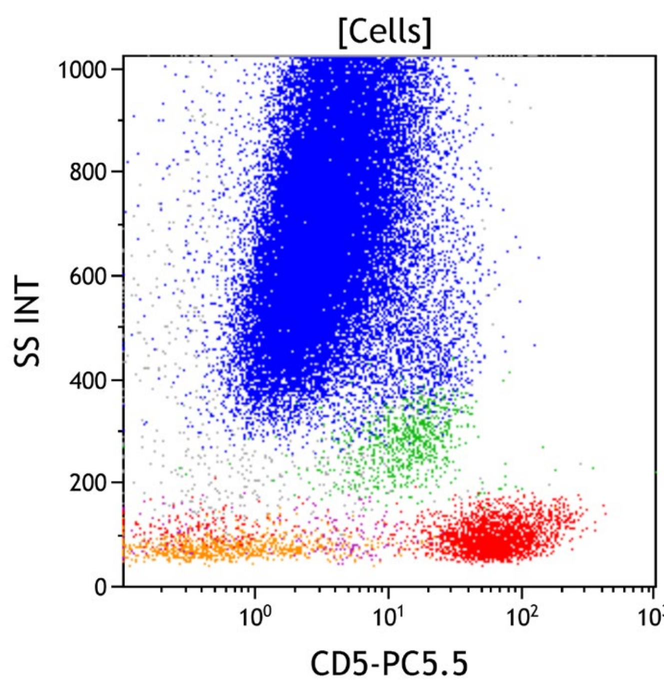

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The granulocytes (blue) do not express CD5.

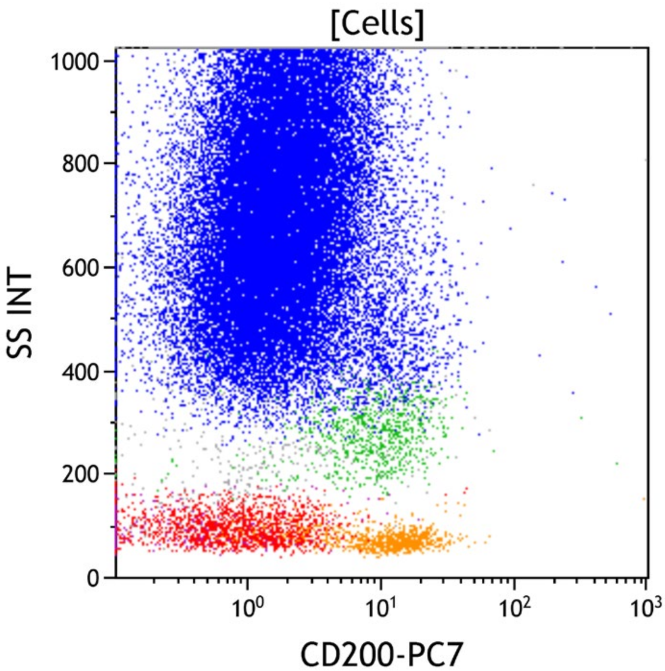

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The granulocytes (blue) do not express CD200.

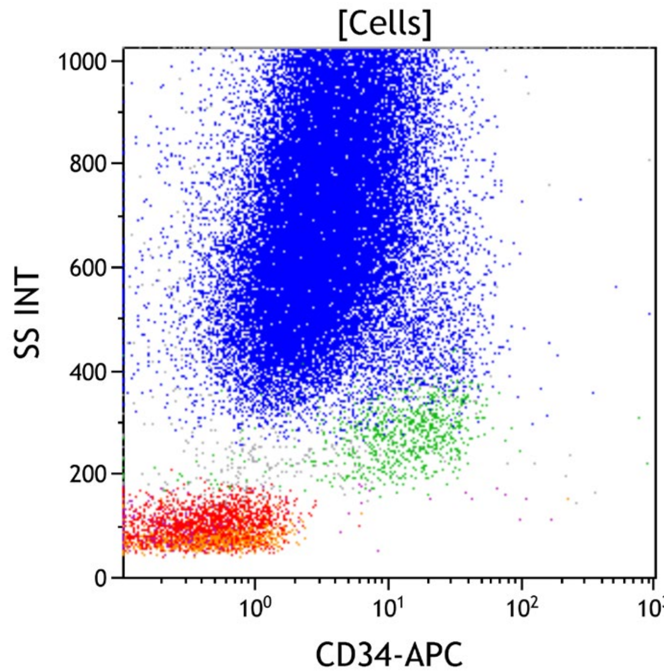

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The granulocytes (blue) do not express CD34.

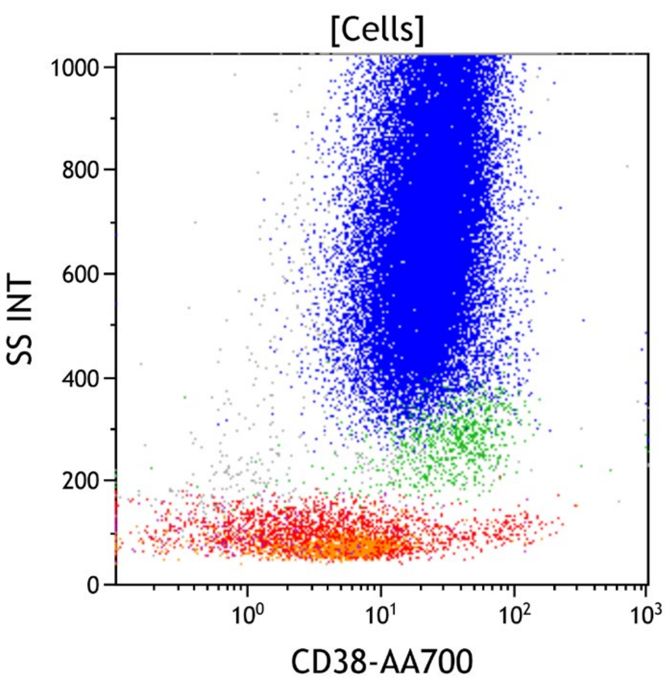

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The granulocytes (blue) express dim CD38.

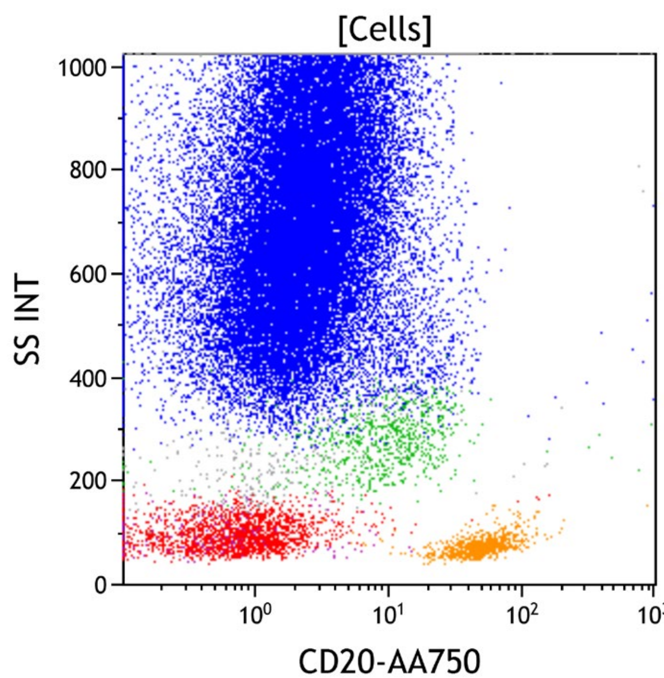

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The granulocytes (blue) do not express CD20.

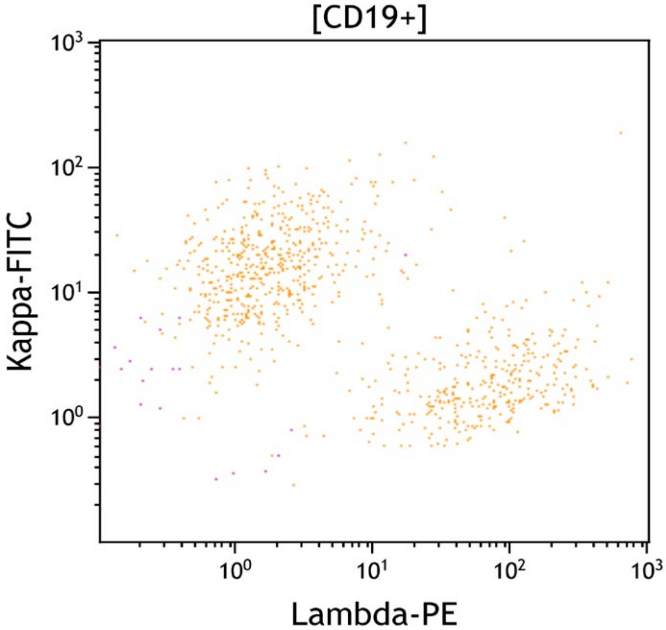

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

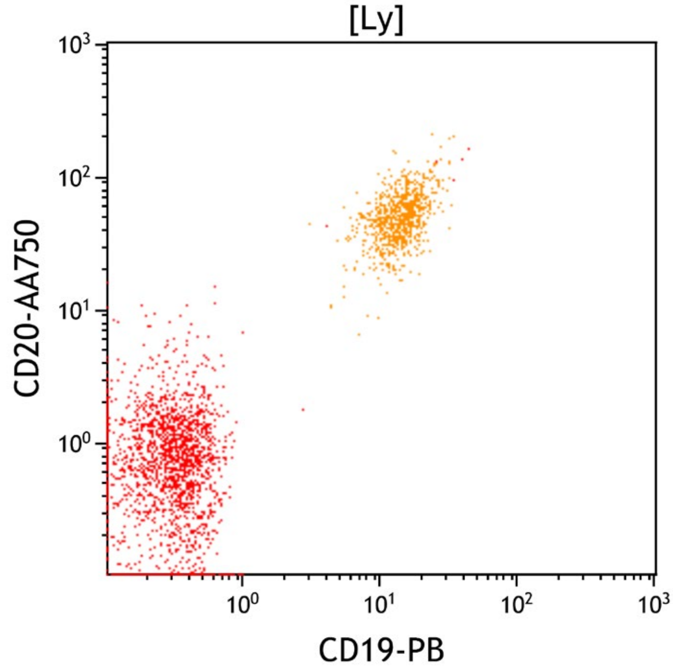

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

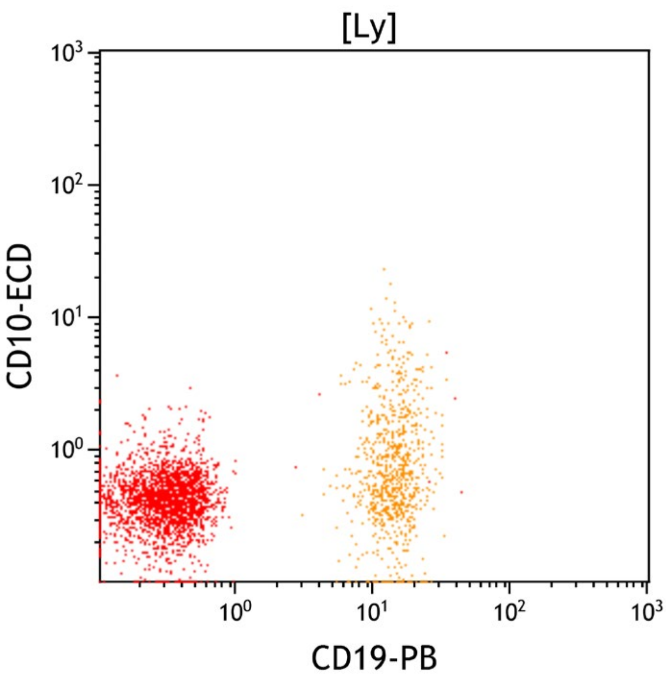

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

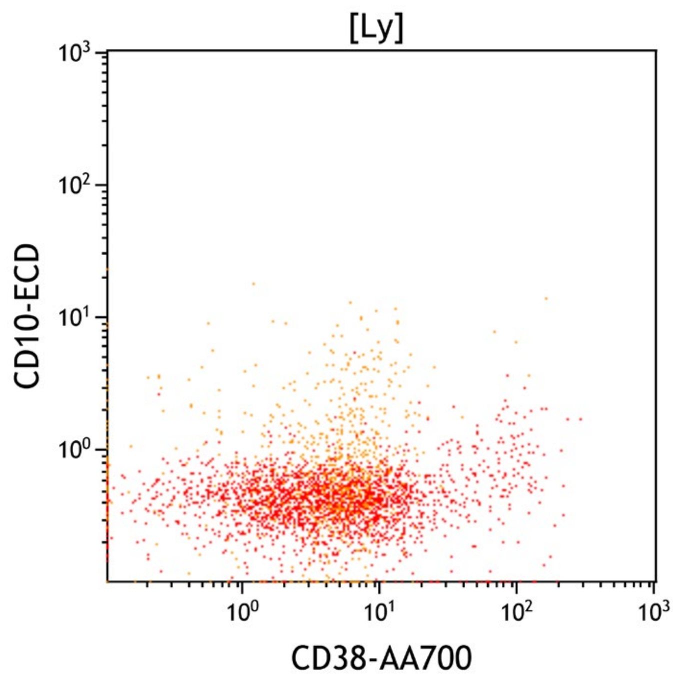

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

B Cell Tube

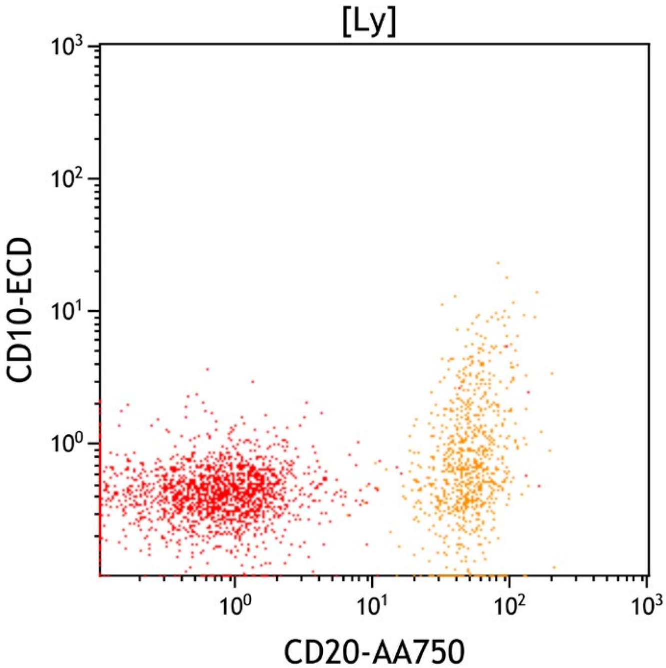

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

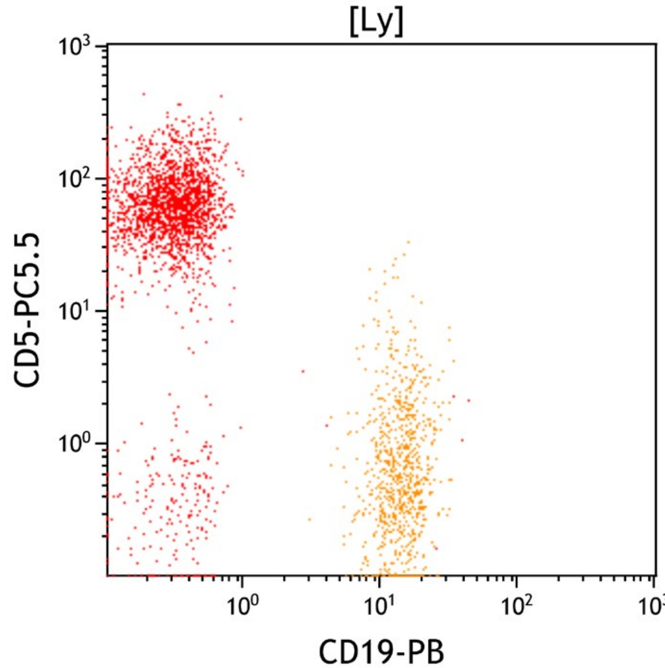

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper left), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

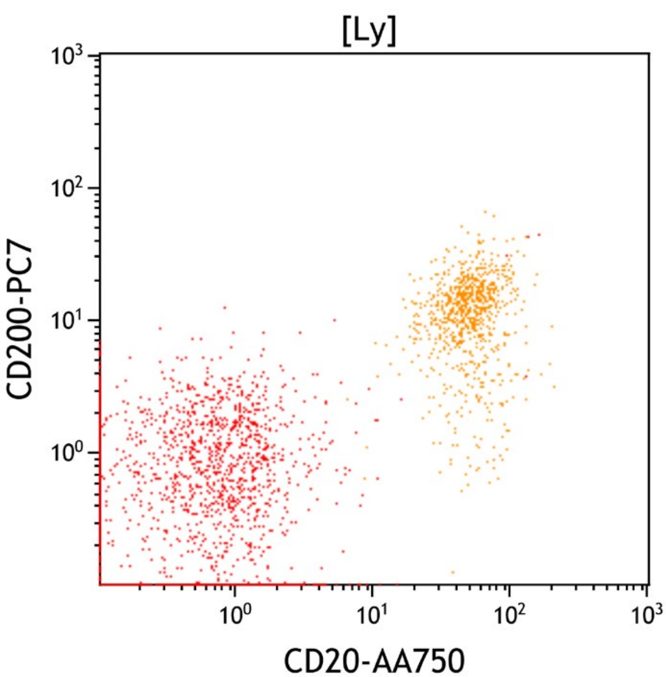

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

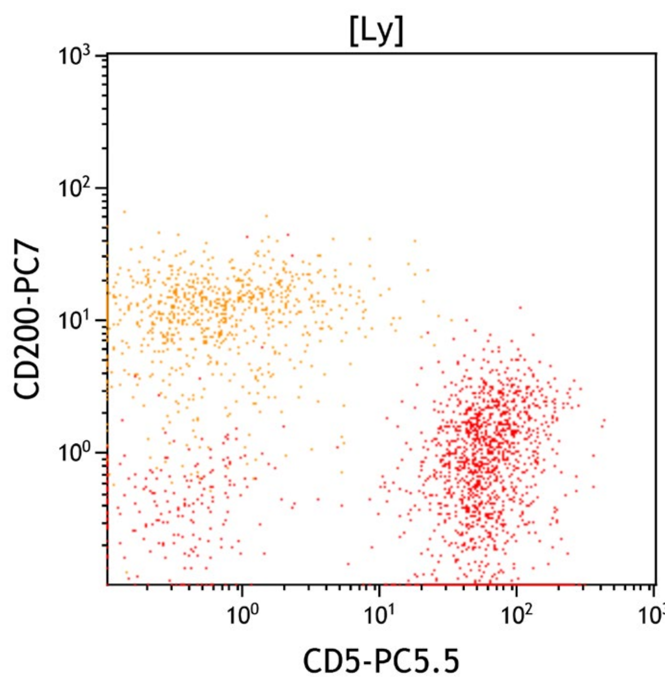

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

T Cell Tube

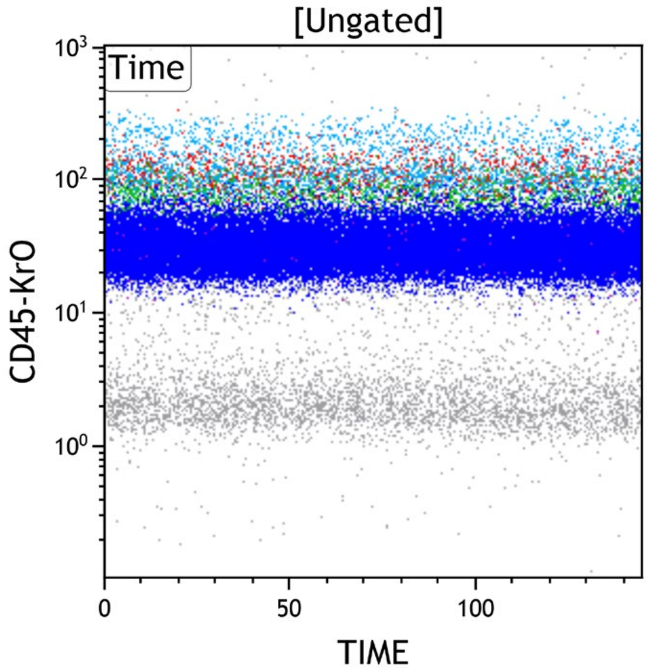

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

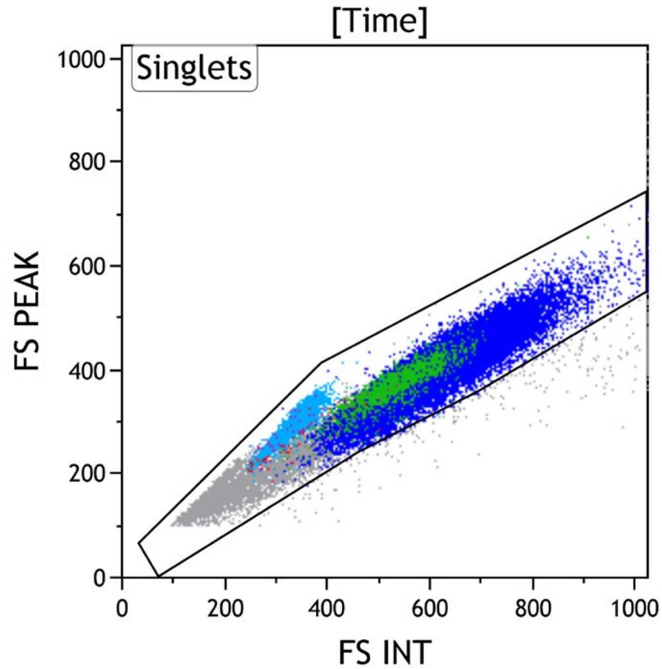

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

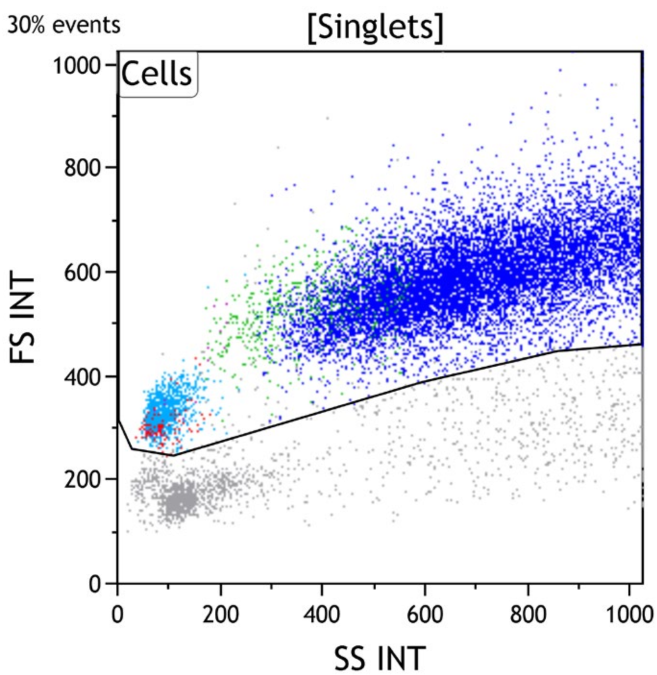

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

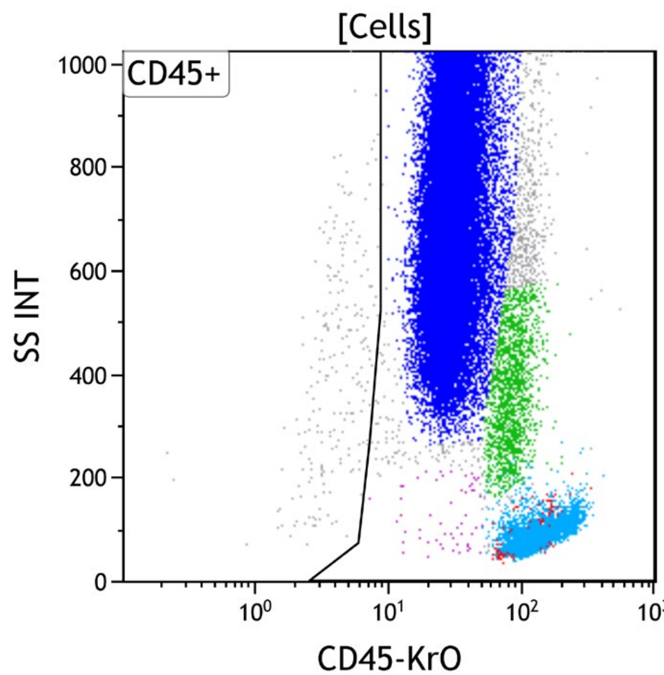

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

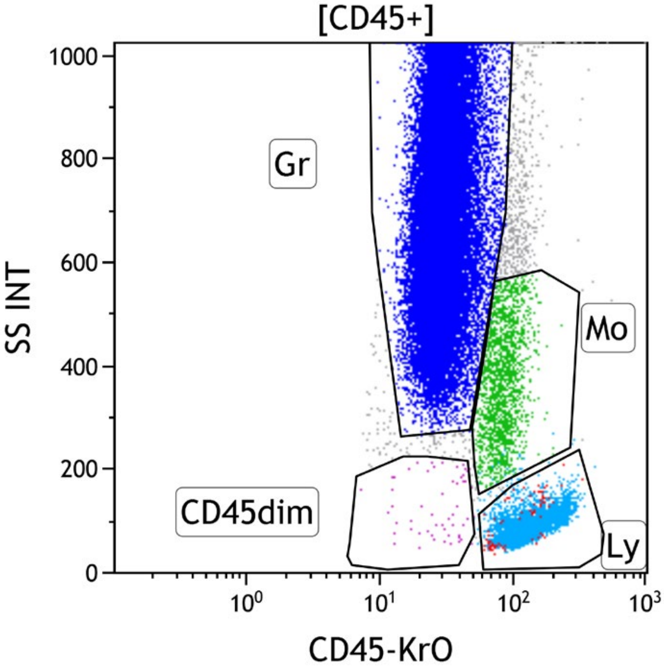

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that the granulocytes (blue) are relatively expanded in number and have slightly decreased side scatter.

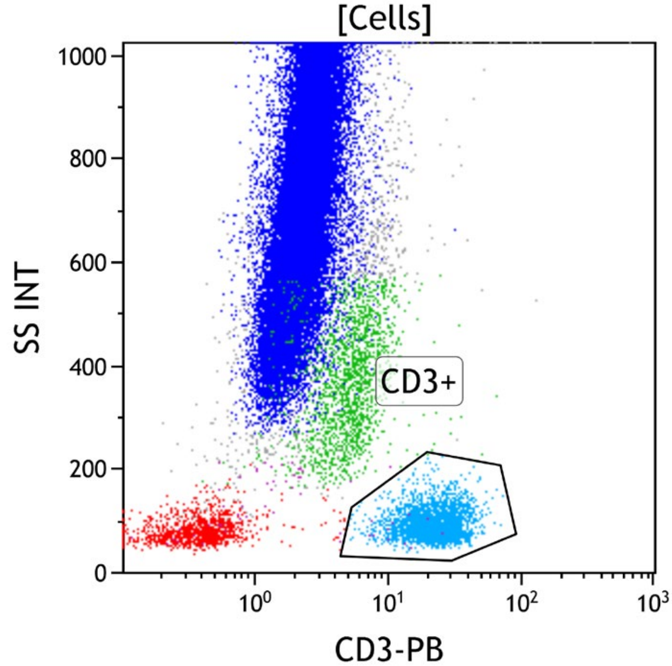

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The granulocytes (blue) do not express CD3.

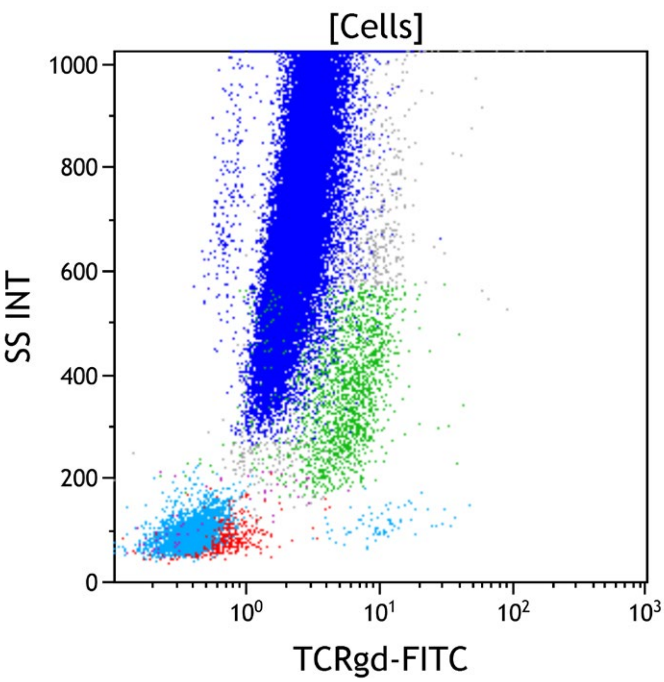

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The granulocytes (blue) do not express TCRγδ.

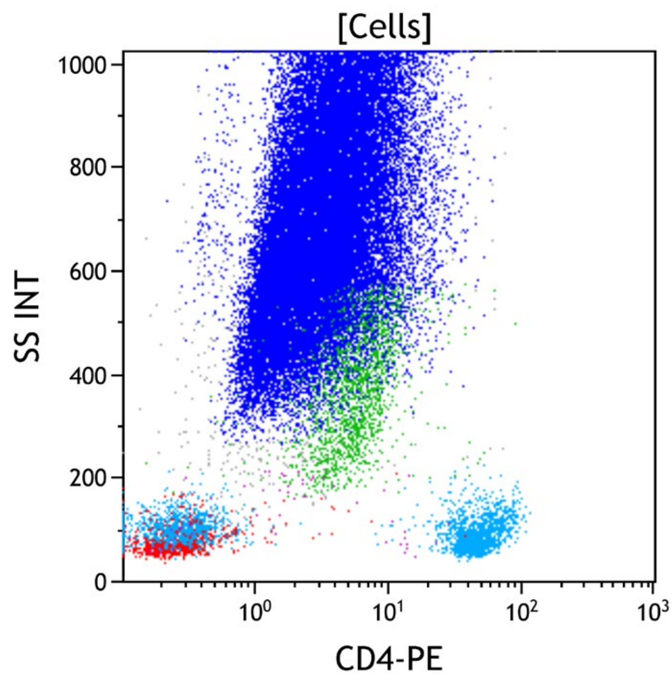

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The granulocytes (blue) do not express CD4.

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T Cell Tube

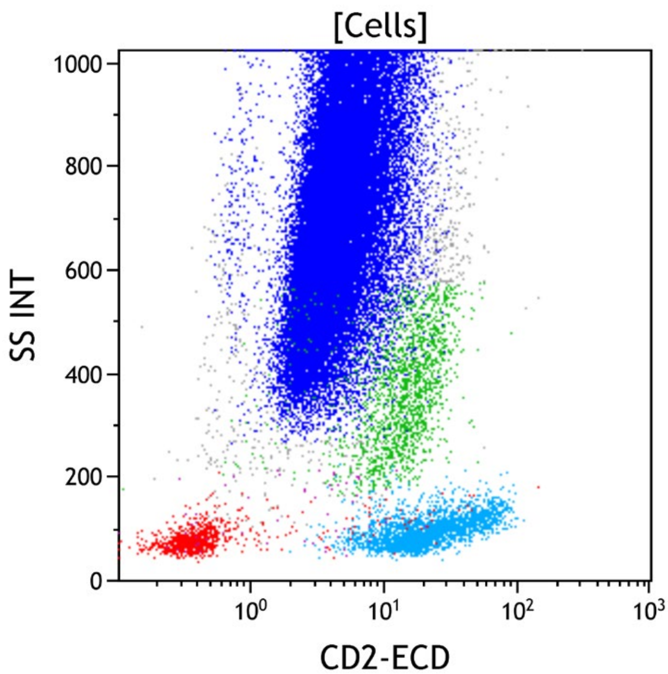

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The granulocytes (blue) do not express CD2.

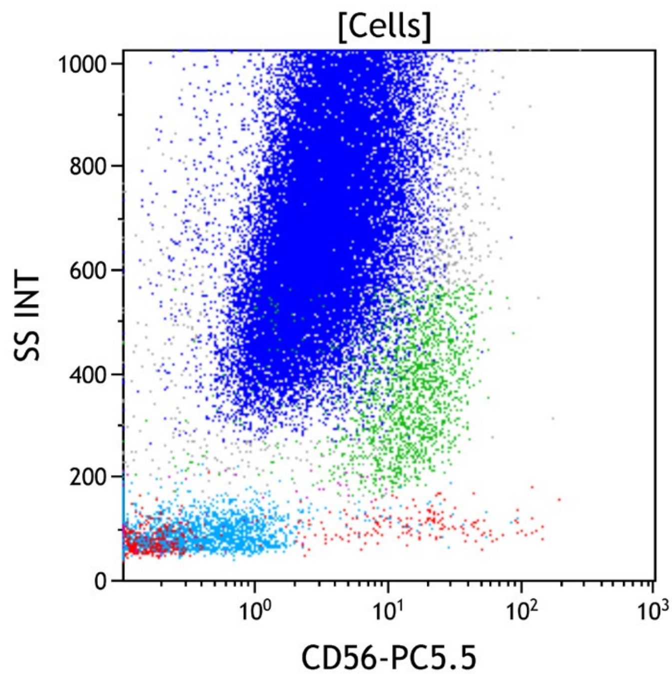

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red, lower right), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The granulocytes (blue) do not express CD56.

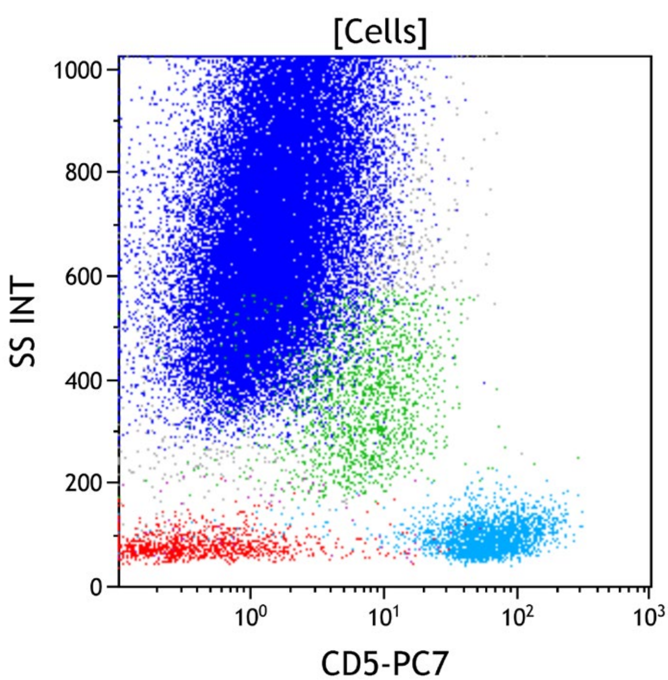

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The granulocytes (blue) do not express CD5.

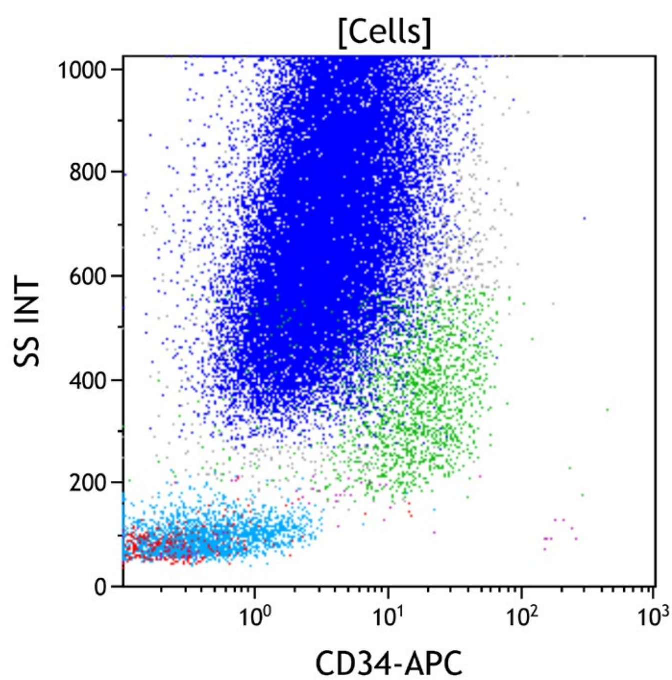

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The granulocytes (blue) do not express CD34.

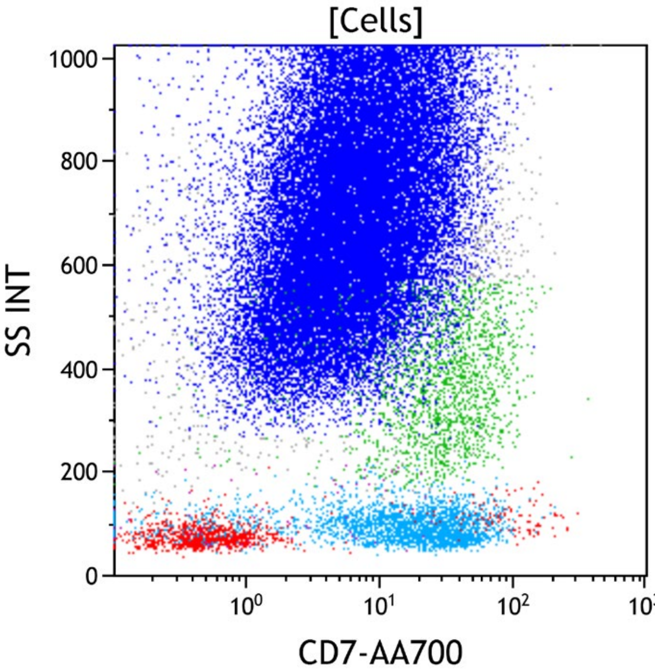

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The granulocytes (blue) do not express CD7.

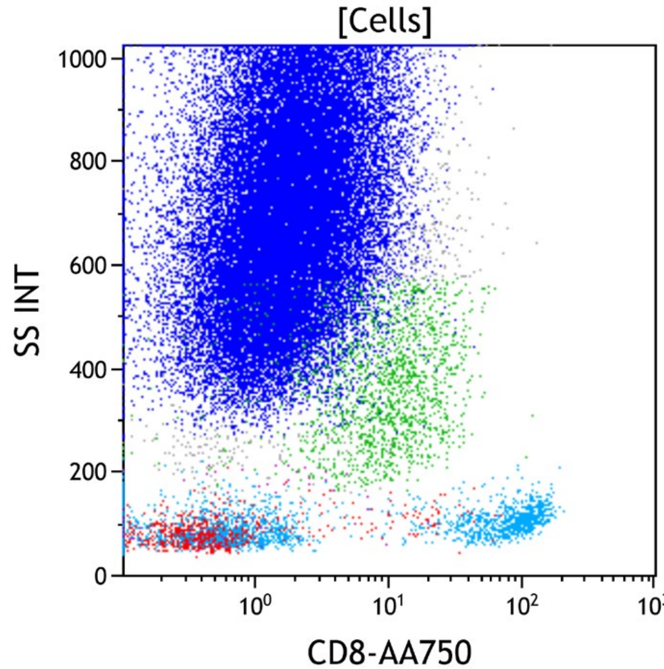

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The granulocytes (blue) do not express CD8.

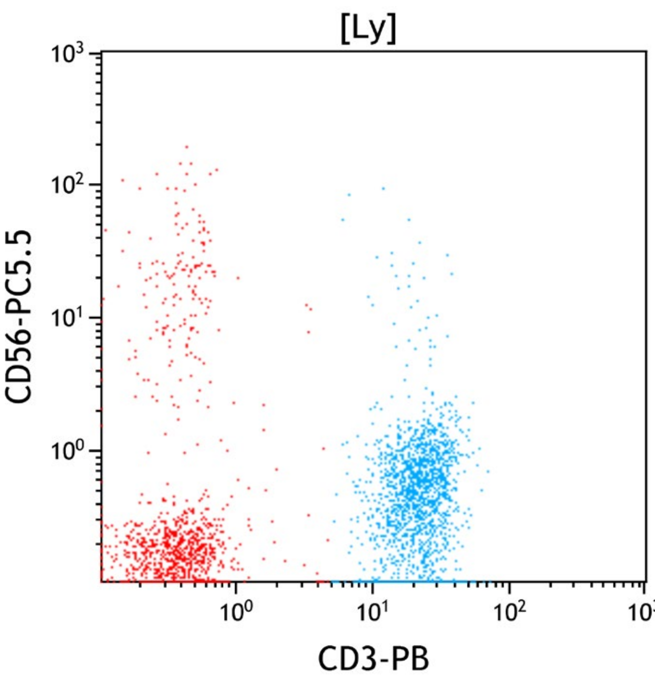

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

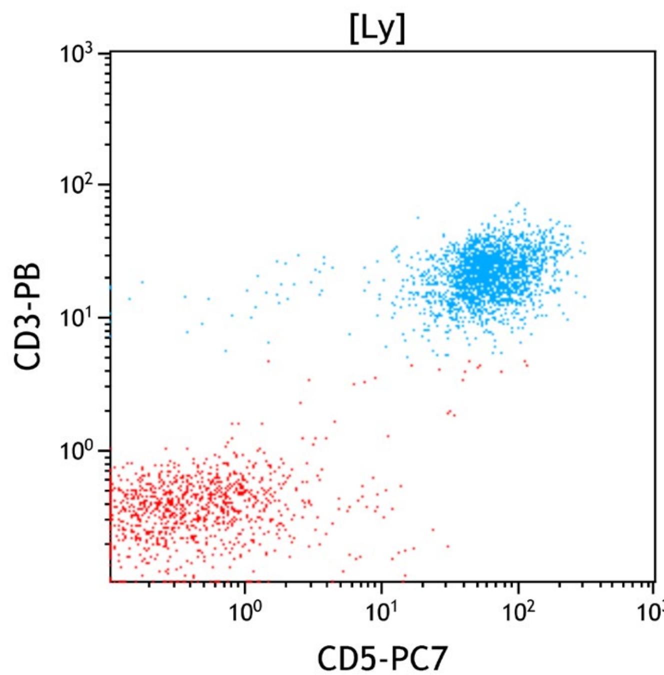

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

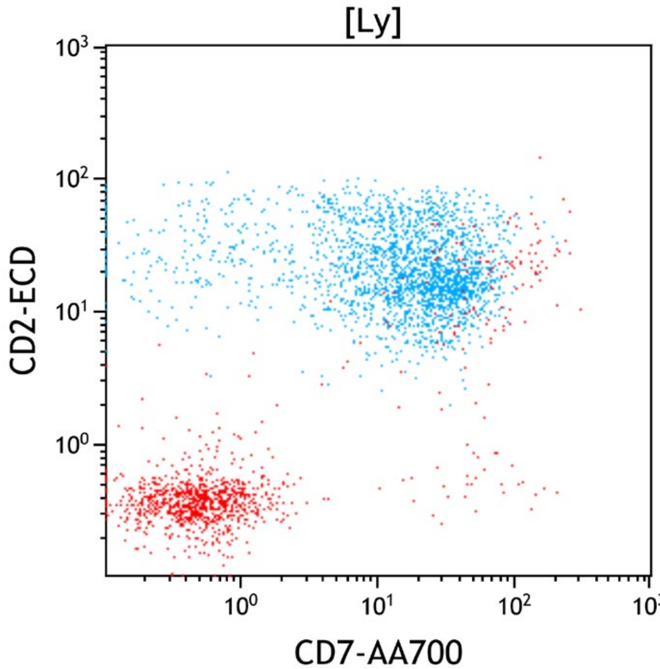

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

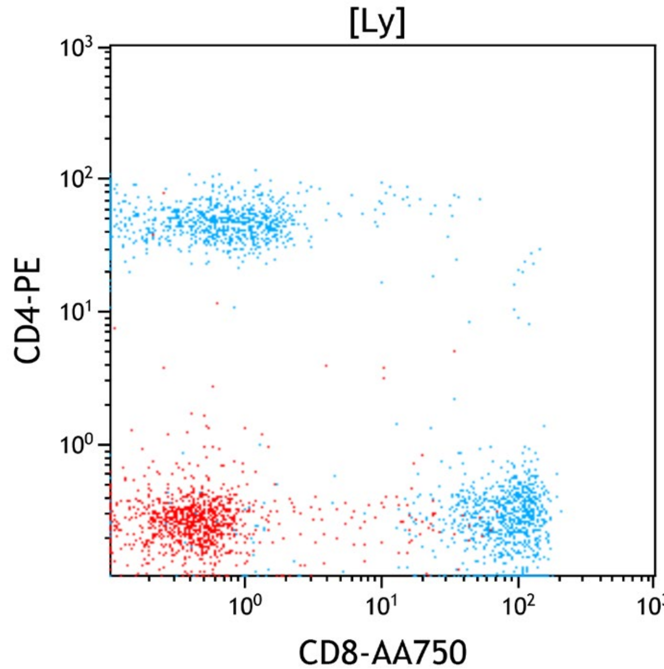

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells.

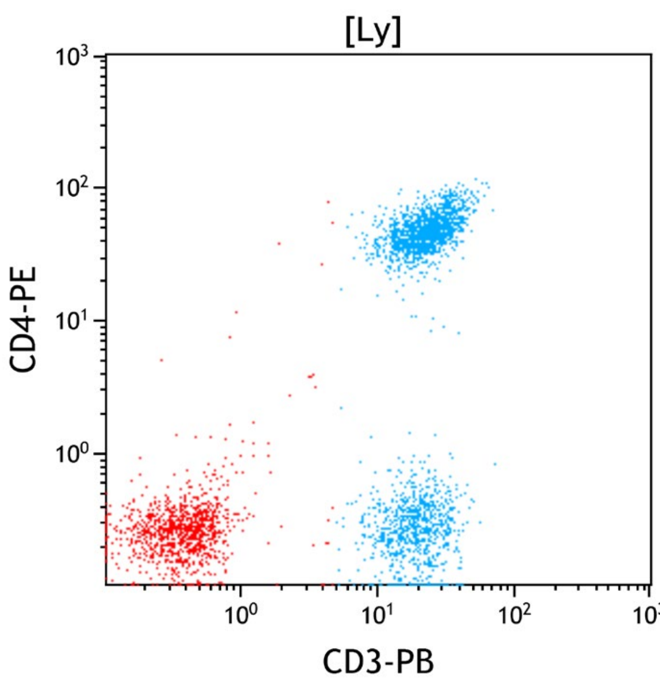

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

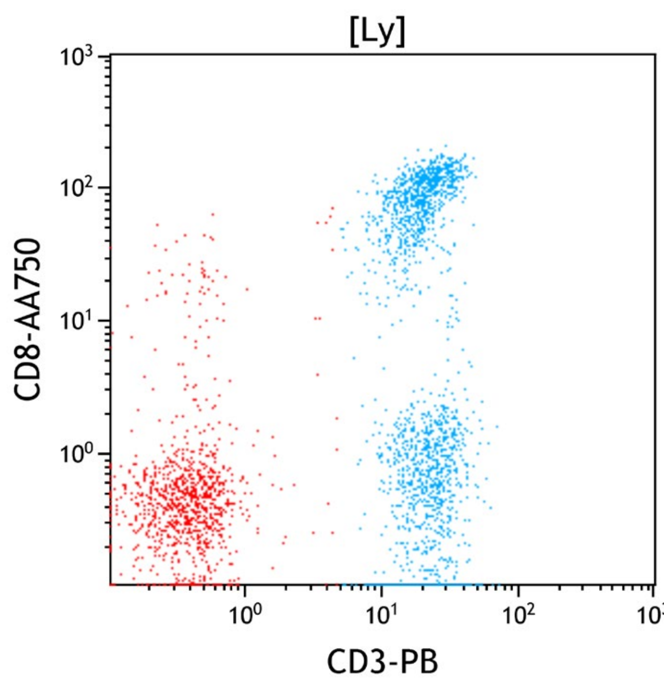

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

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T Cell Tube

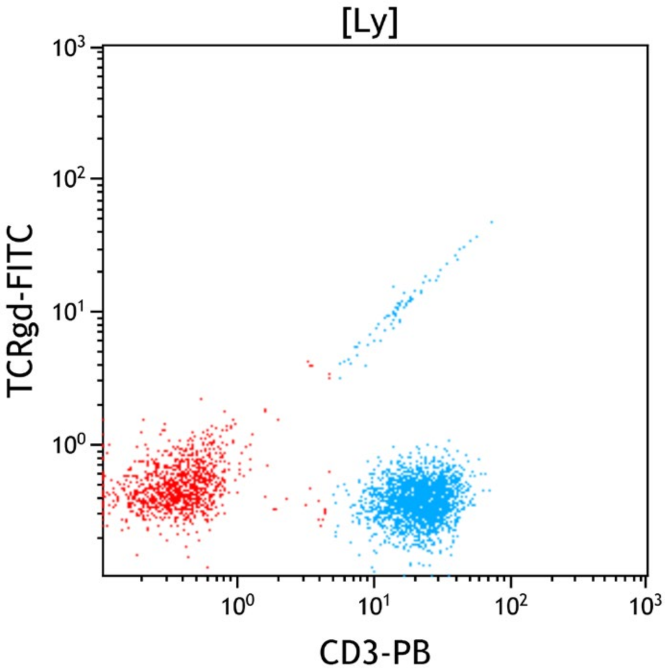

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

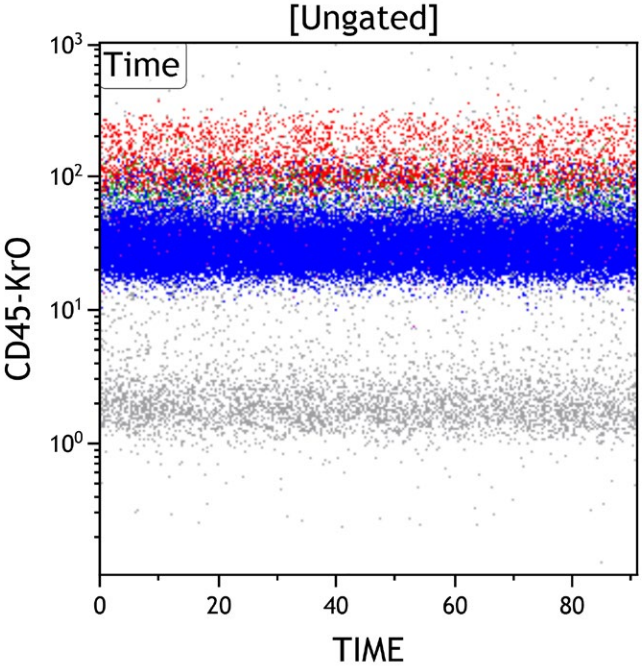

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

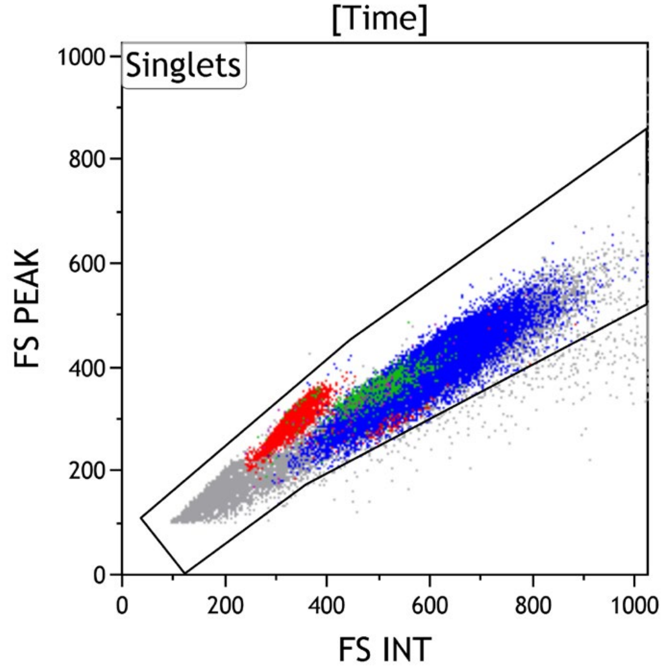

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

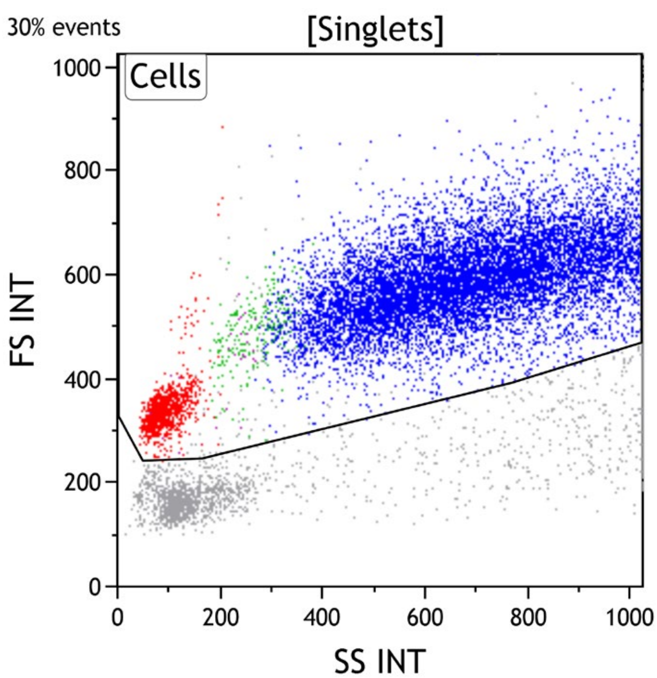

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

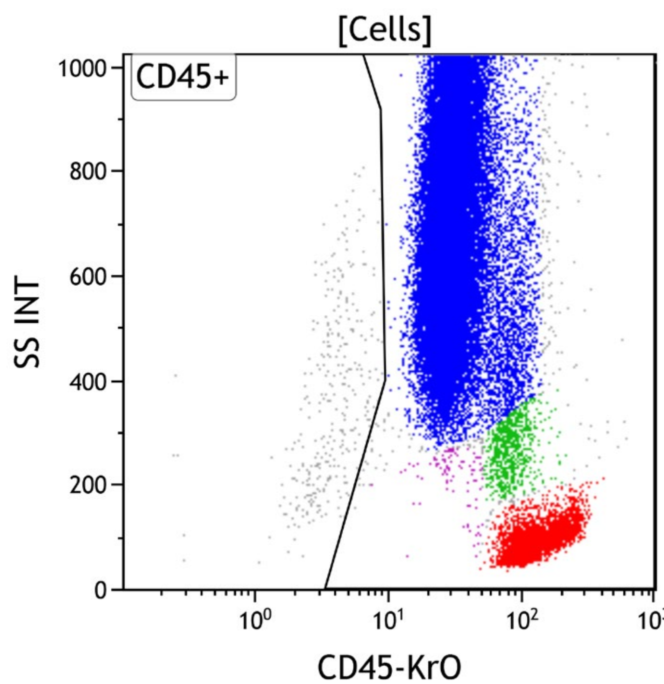

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

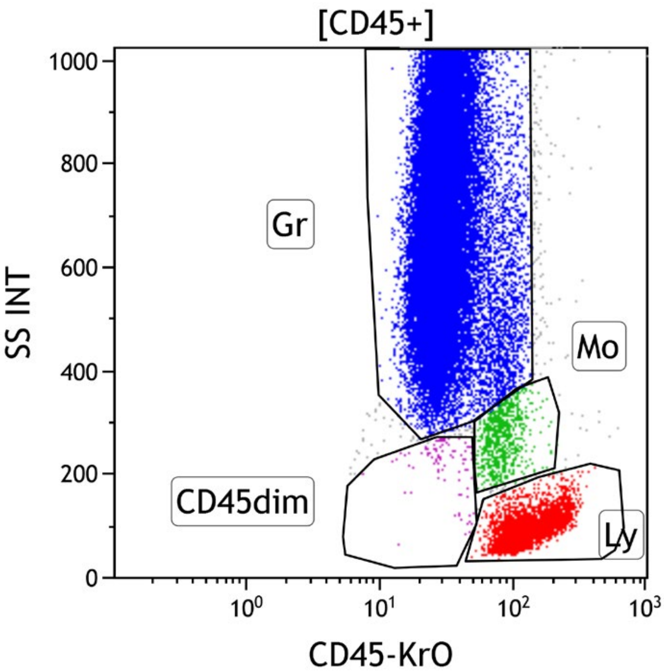

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that the granulocytes (blue) are relatively expanded and have slightly decreased side scatter.

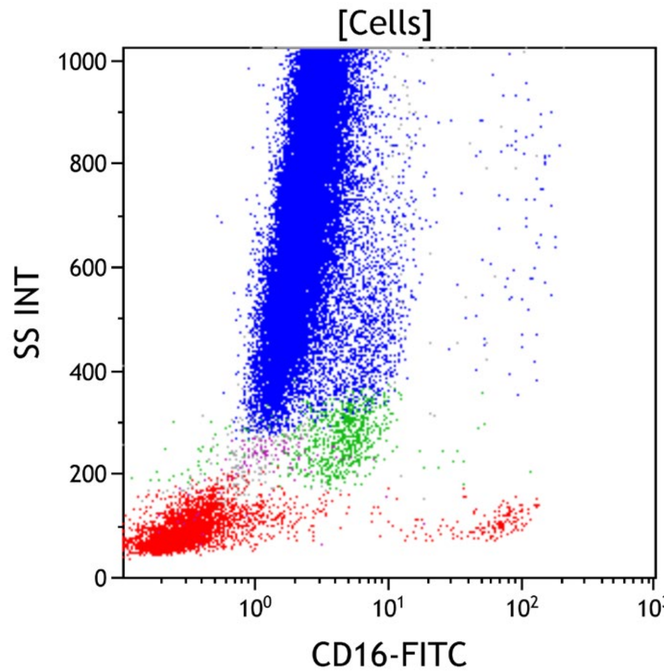

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands. Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The granulocytes (blue) do not express CD16.

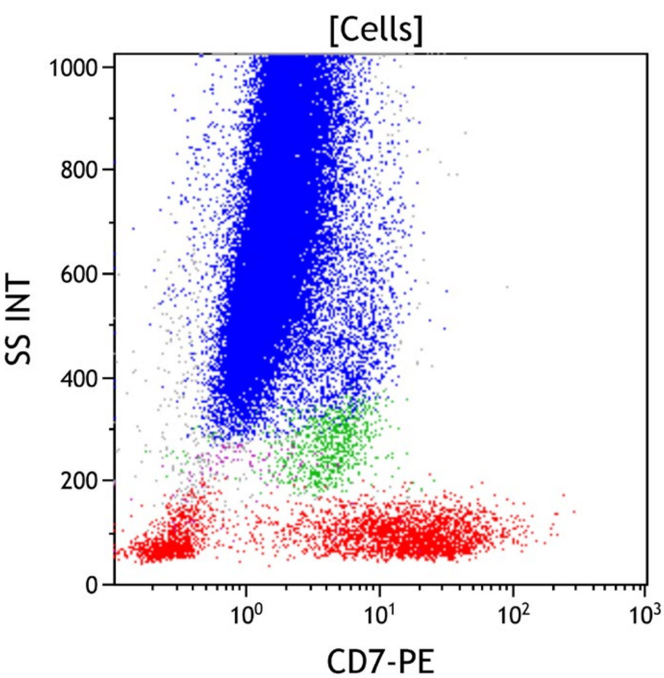

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The granulocytes (blue) do not express CD7.

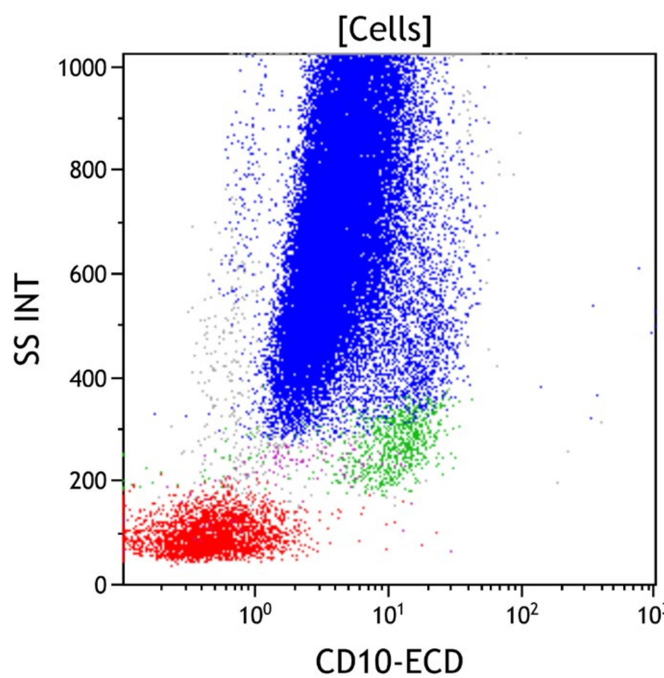

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes. The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The granulocytes (blue) do not express CD10.

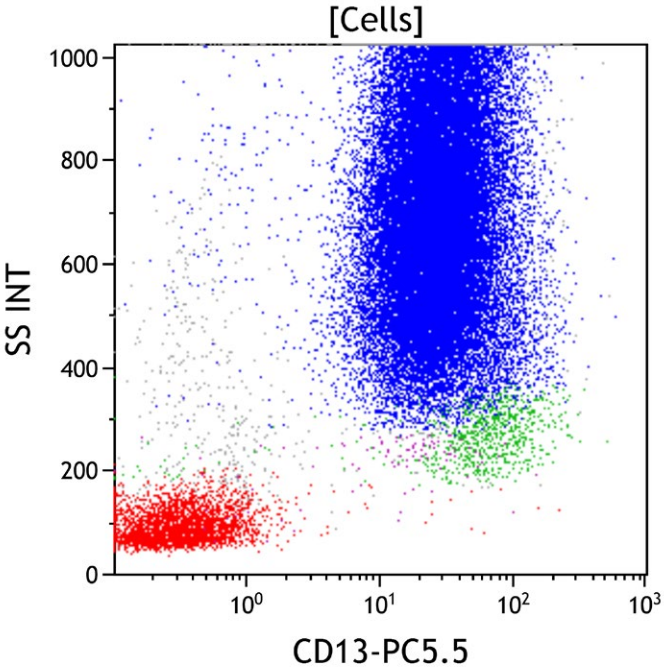

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The granulocytes (blue) express uniform intermediate CD13.

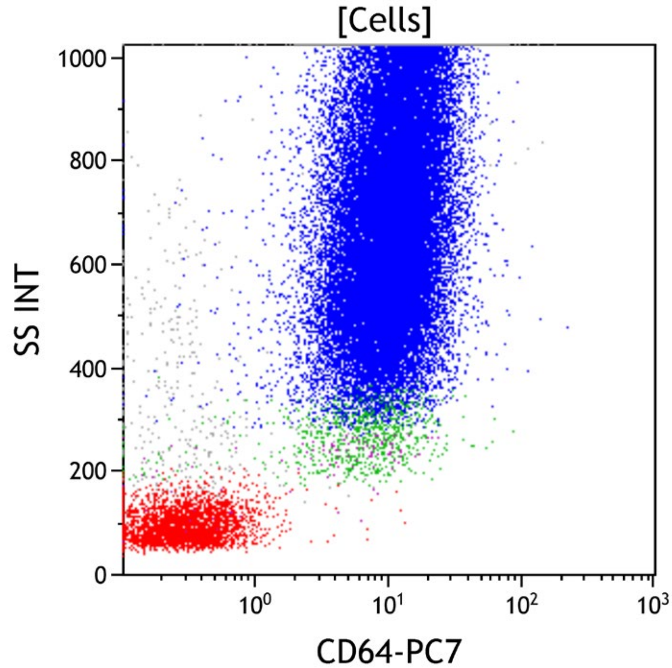

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes . CD64 is not well expressed on resting mature granulocytes, but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The granulocytes (blue)express dim CD64.

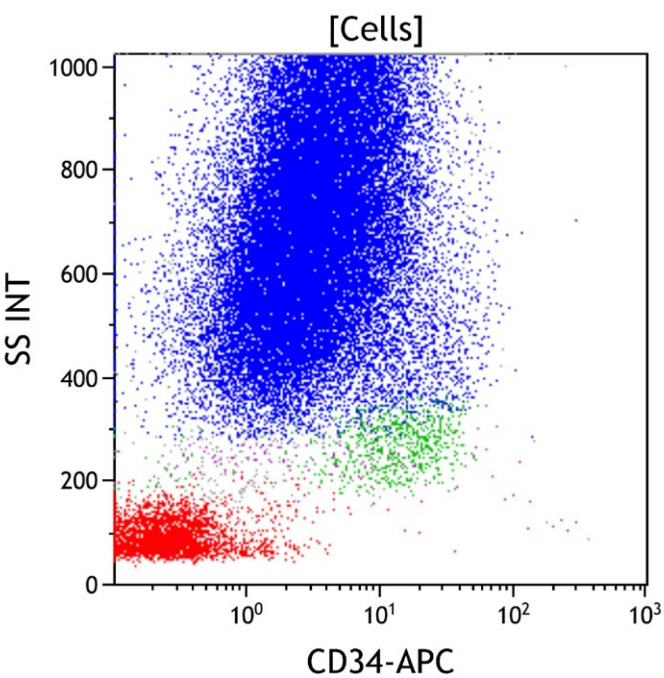

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The granulocytes (blue) do not express CD34.

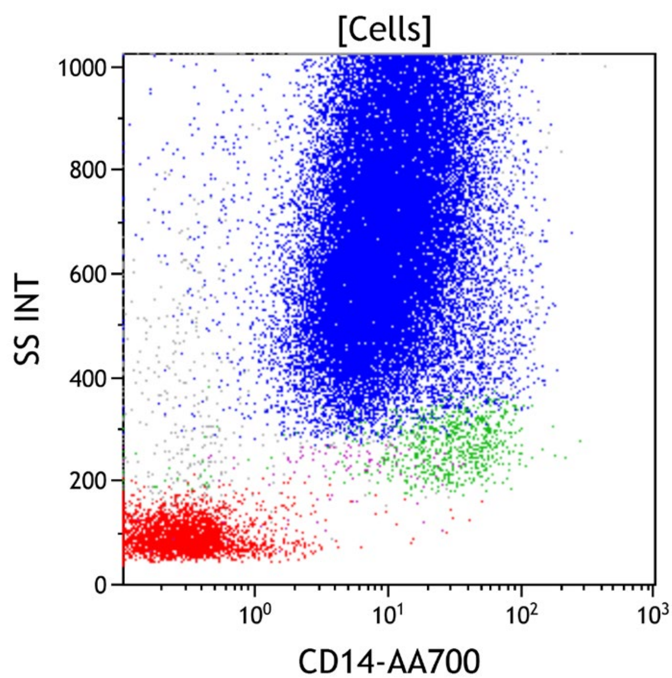

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The granulocytes do not express CD14, the apparent dim CD14 is due to increased compensation background from CD13.

M1 Cell Tube

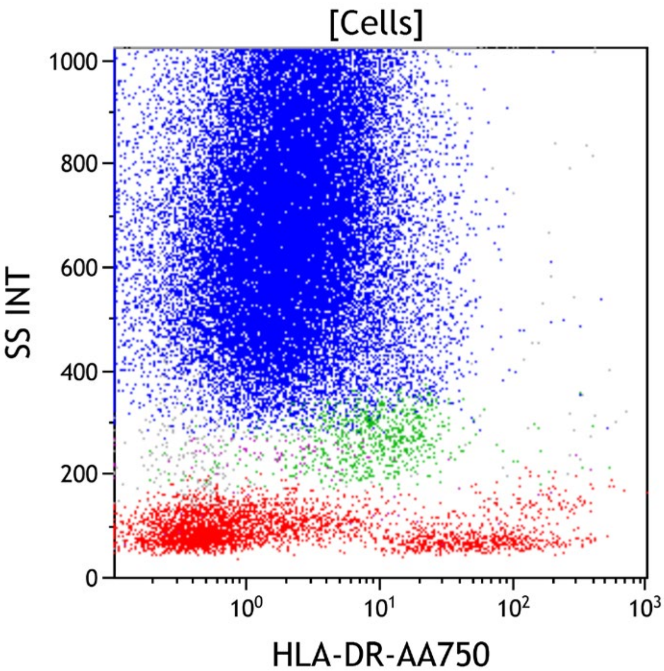

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The granulocytes (blue) do not express HLA-DR.

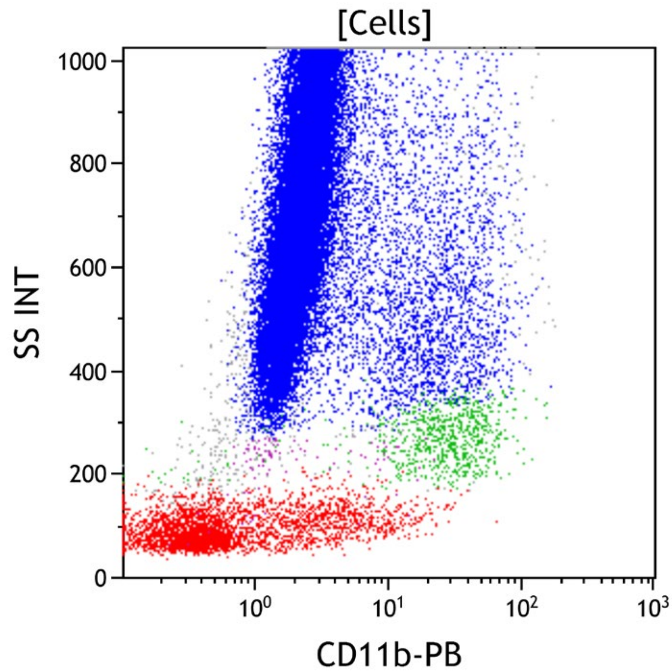

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage, and on monocytes (green). CD11b is also expressed on NK cells and basophils. The majority of the granulocytes (blue) do not express CD11b.

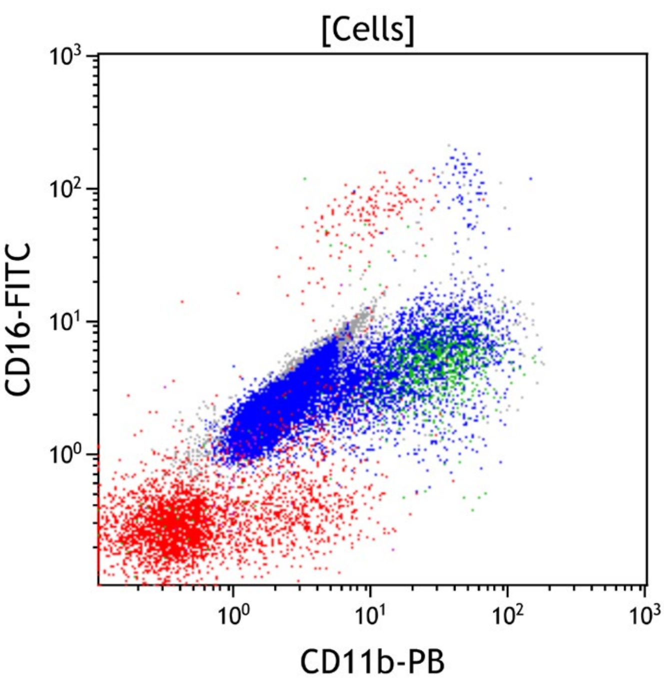

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (in green), immature and mature granulocytes and NK cells (in red). CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. Most of the granulocytes do not express CD11b or CD16.

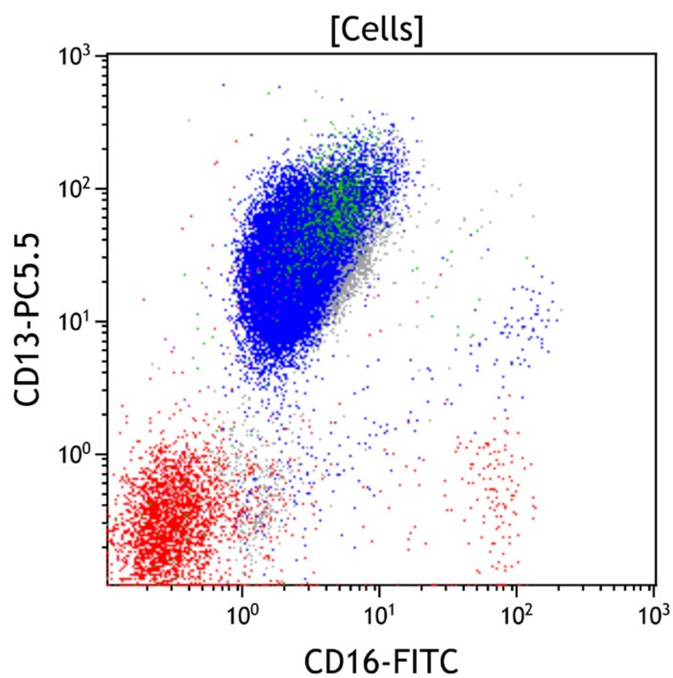

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. The granulocytes (blue) express CD13 without CD16.

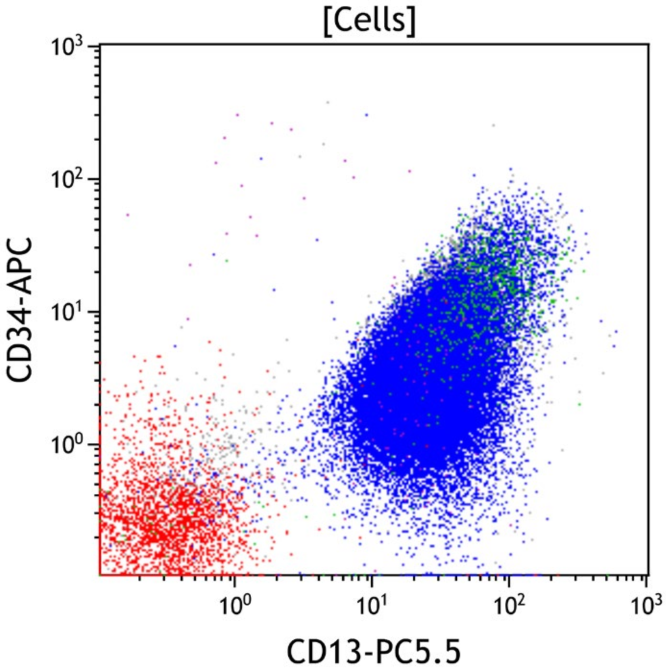

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells. The granulocytes (blue) express CD13 without CD34, the apparent dim CD34 expression is increased compensation background from CD13.

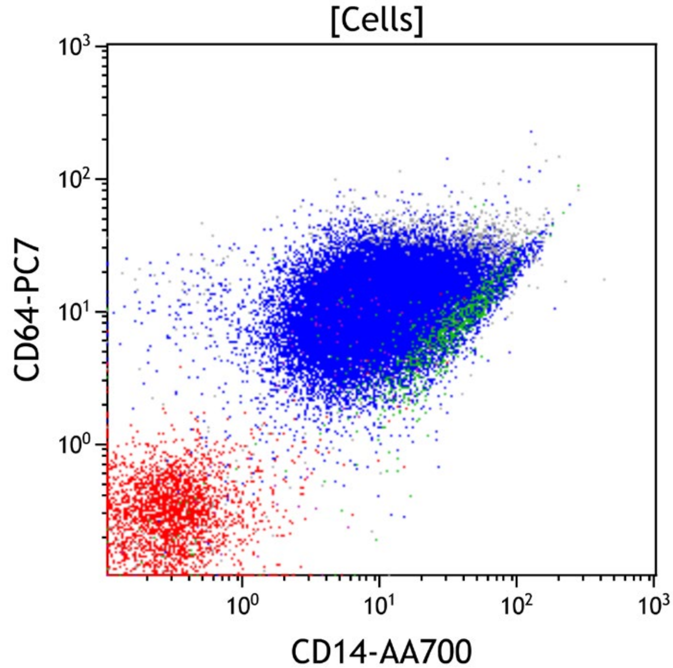

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. The granulocytes (blue) express CD64 without CD14, the apparent dim CD14 is due to increased compensation background from CD13.

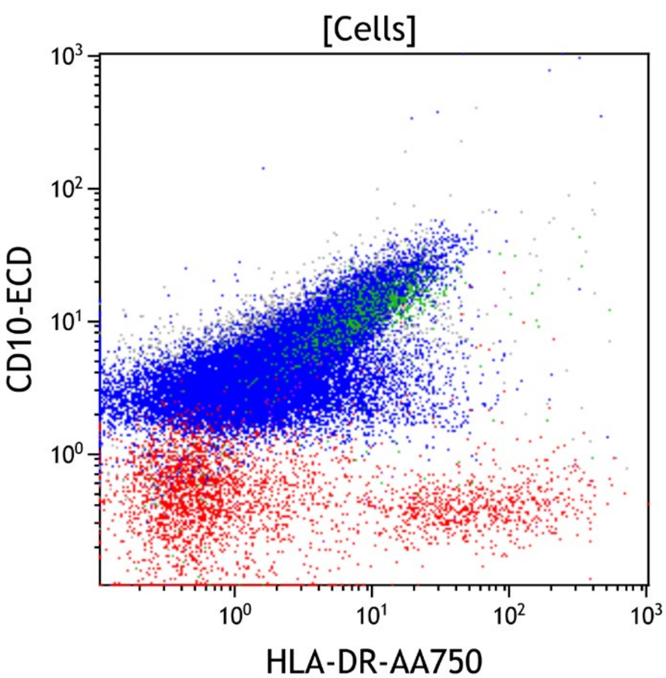

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes and immature B cells . Immature B cells express both CD10 and HLA-DR. The granulocytes (blue) do not express CD10 or HLA-DR. The strongly diagonal character of CD10 and HLA-DR expression suggests non-specific binding of reagents.

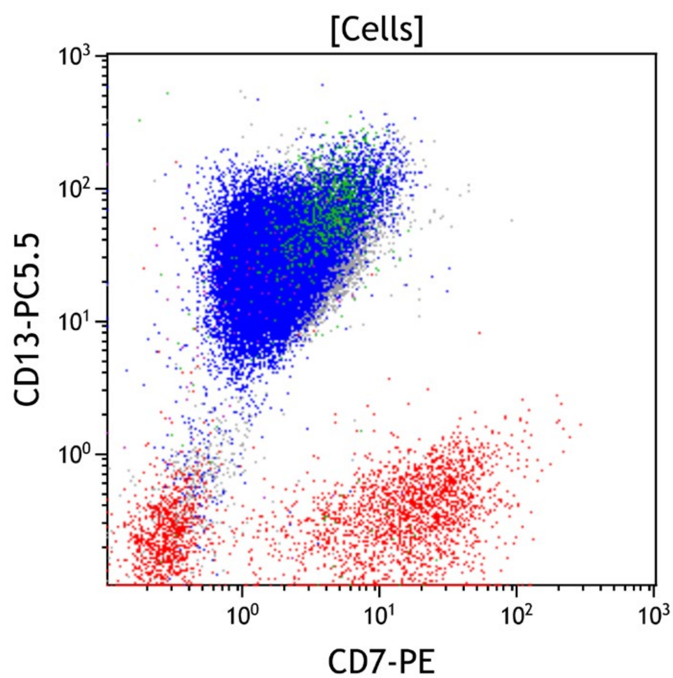

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on maturing granulocytes, monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The granulocytes (blue) express CD13 without CD7

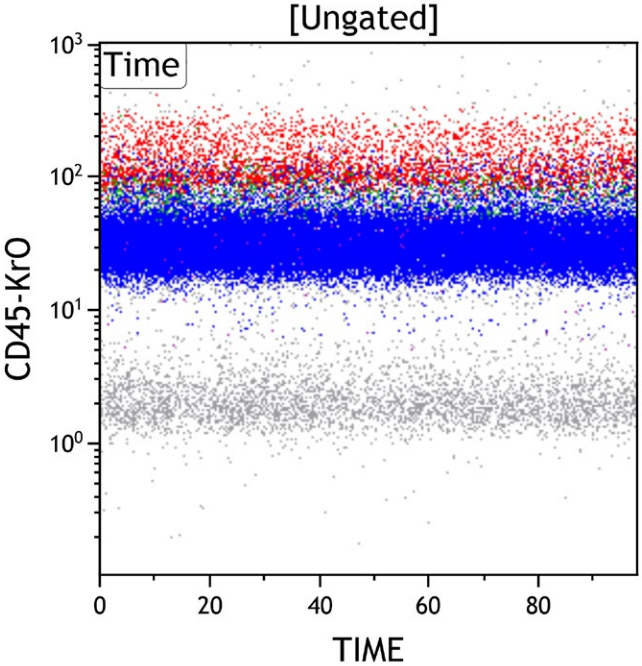

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

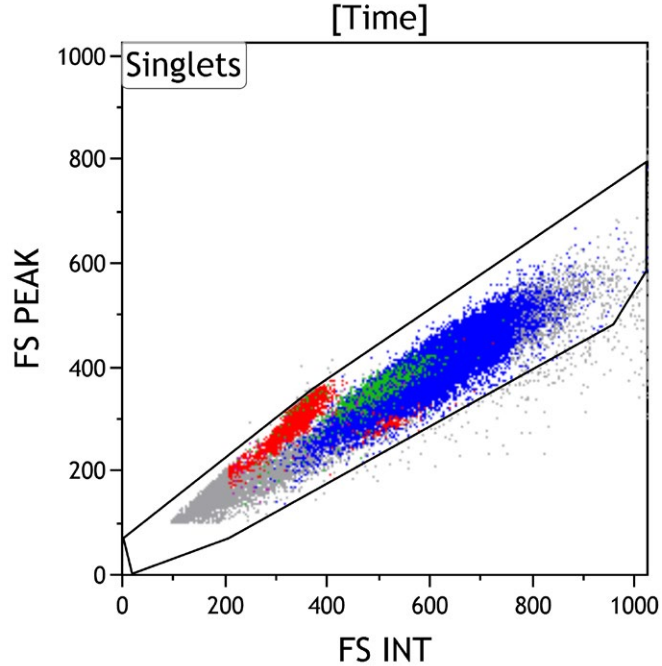

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

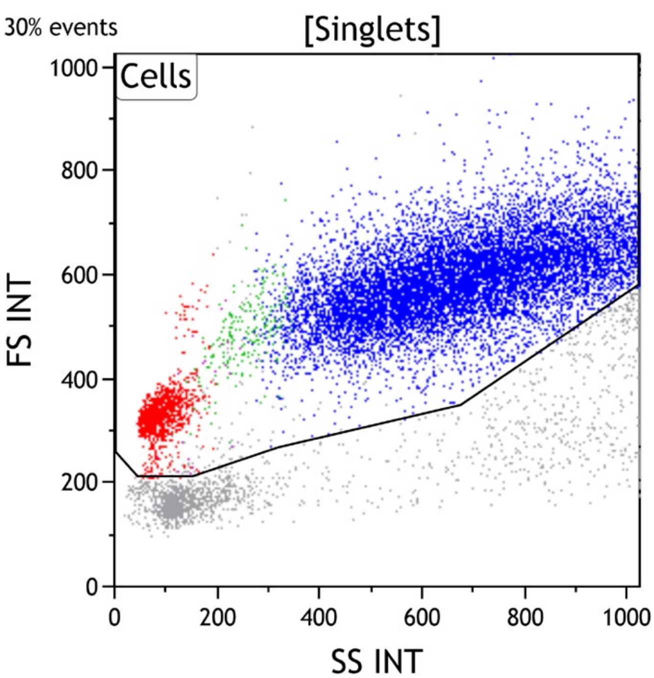

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

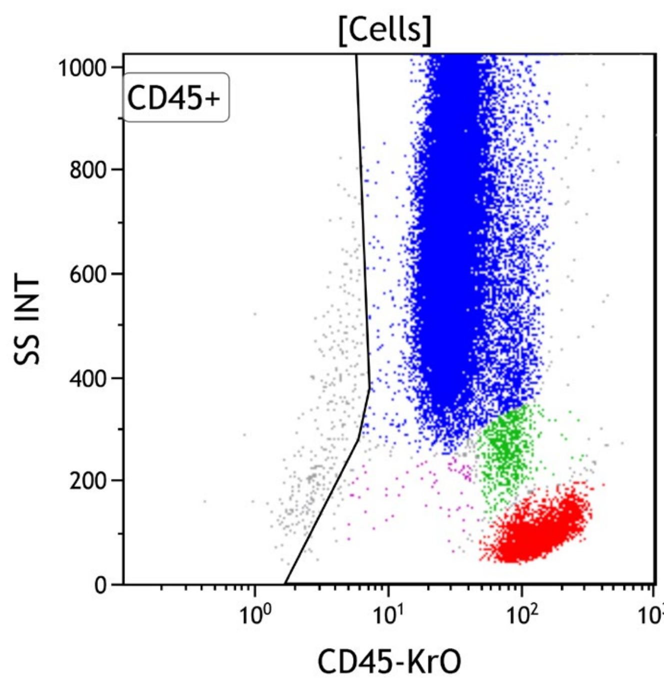

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

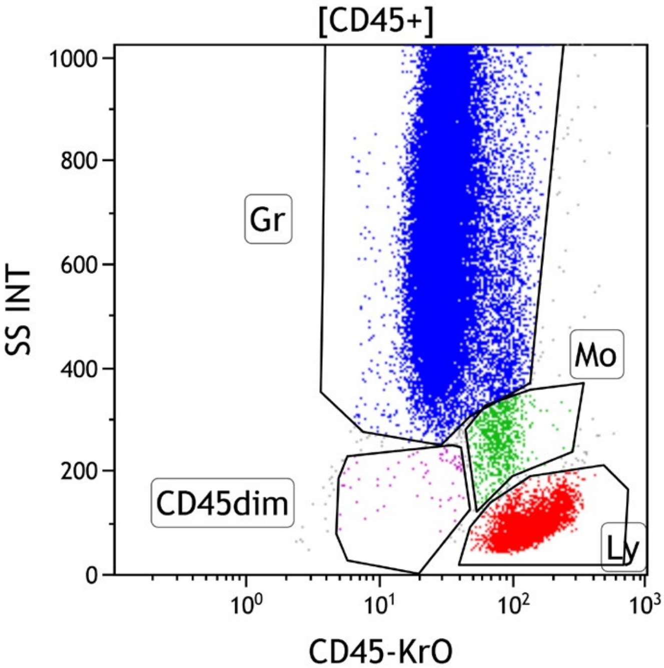

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that the granulocytes (blue) are relatively expanded in number and have slightly decreased side scatter.

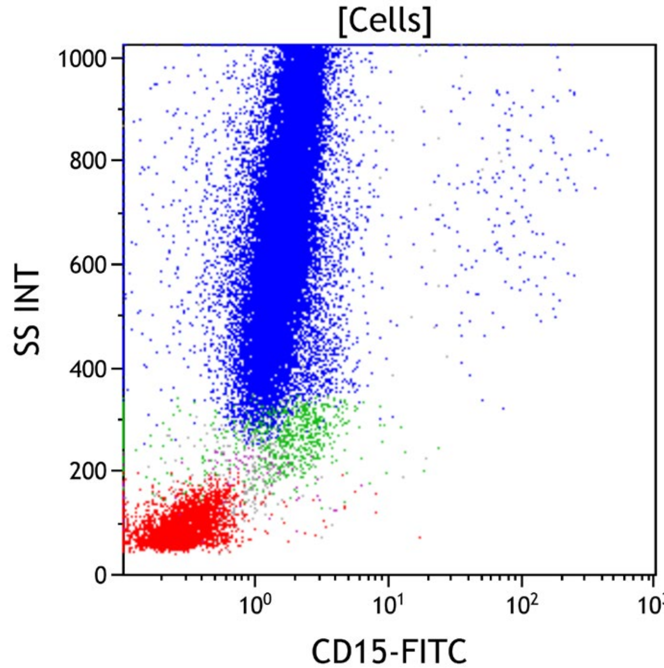

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The granulocytes (blue) do not express CD15.

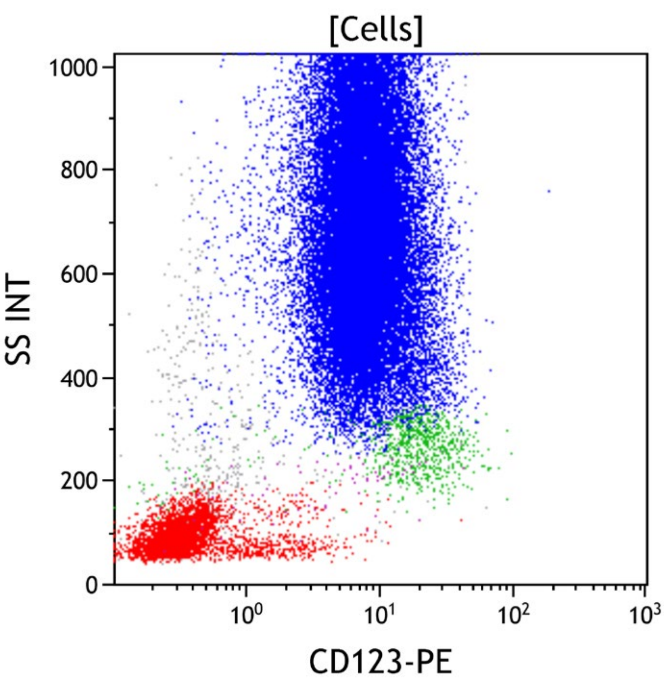

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The granulocytes (blue) express dim CD123.

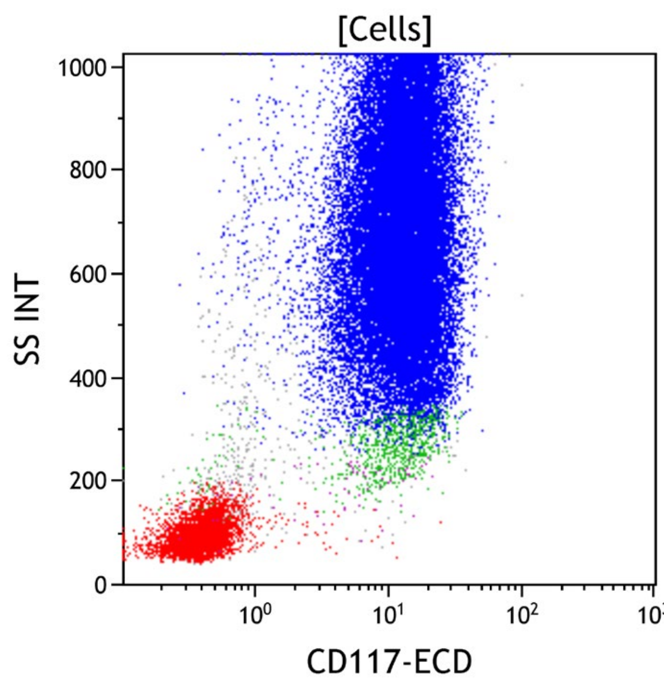

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The granulocytes (blue) express dim CD117.

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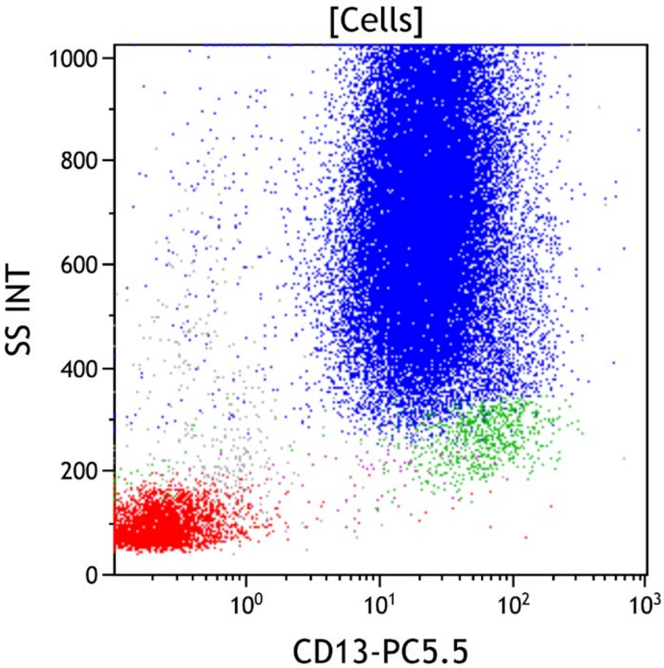

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The granulocytes (blue) express intermediate CD13.

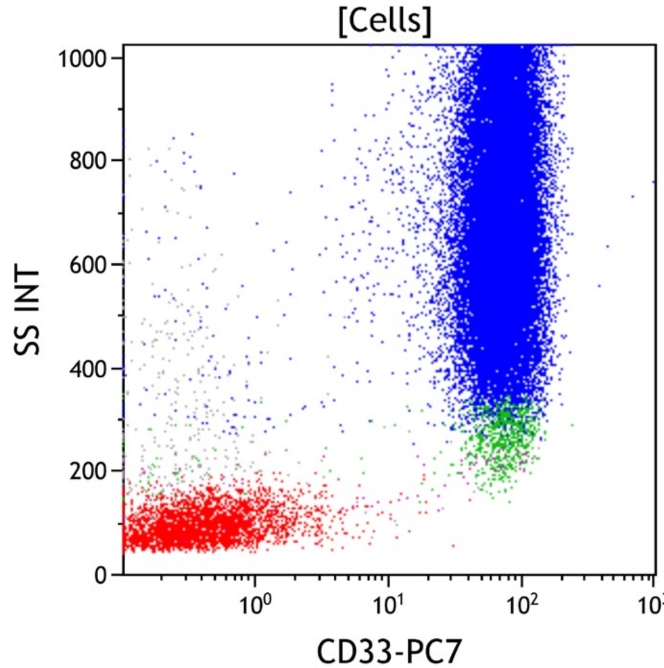

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes, and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The granulocytes (blue) express uniform bright CD33.

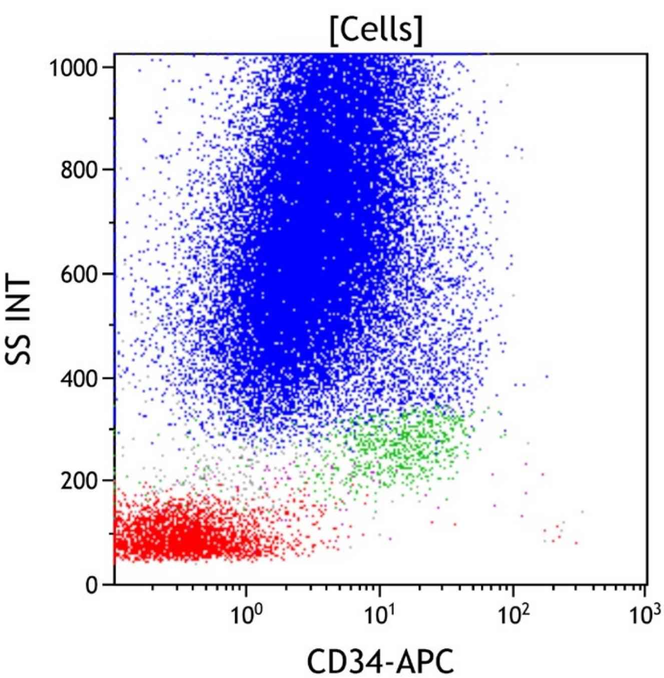

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The granulocytes do not express CD34.

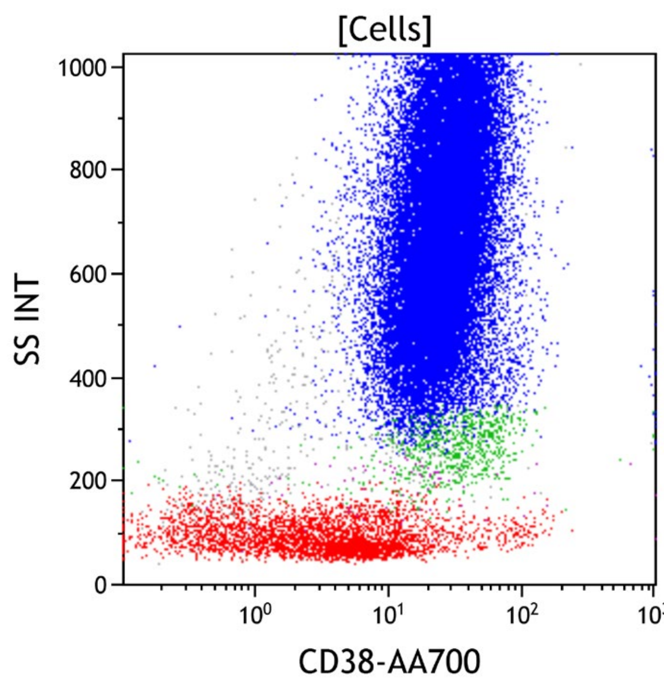

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The granulocytes (blue) express dim CD38.

M2 Cell Tube

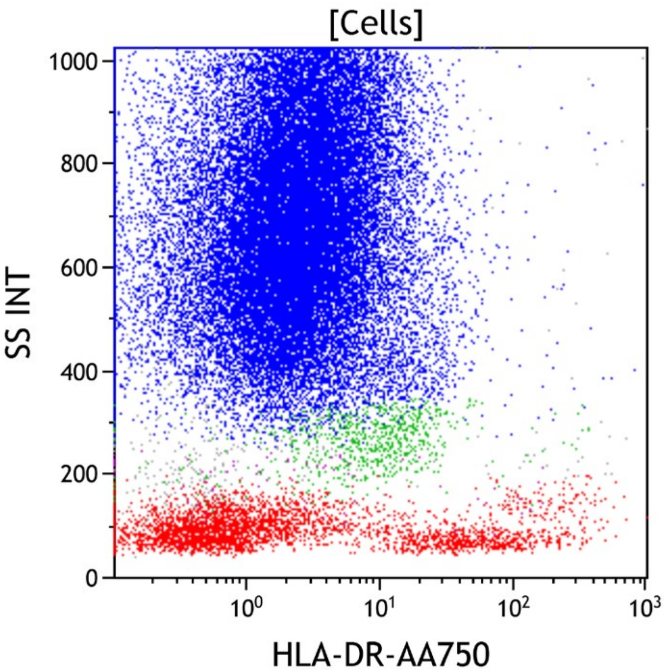

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The granulocytes (blue) do not express HLA-DR.

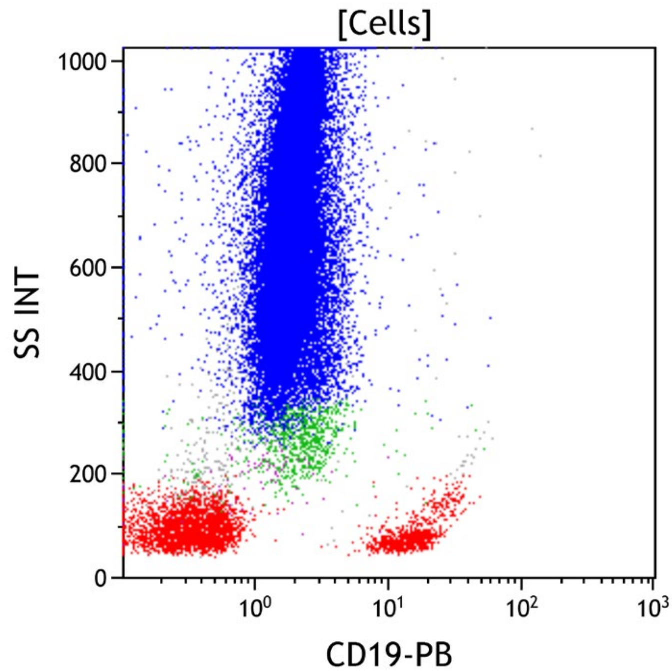

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The granulocytes (blue) do not express CD19.

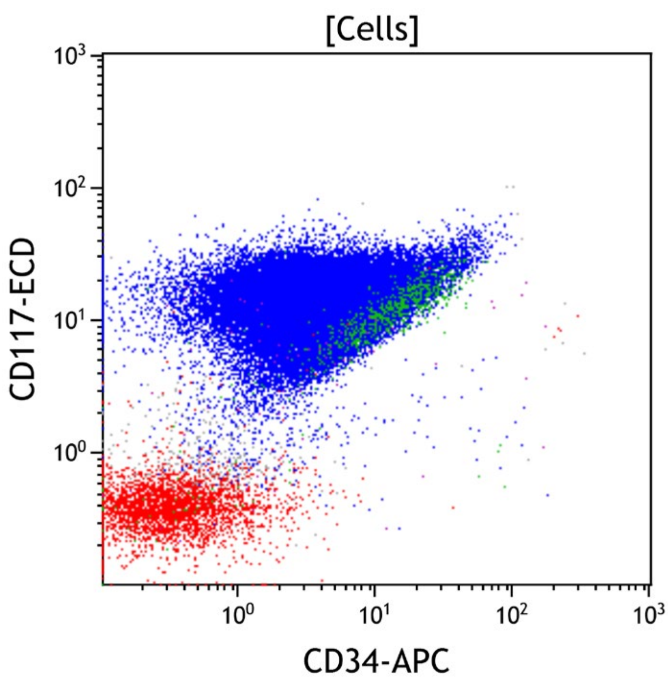

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The granulocytes (blue) express CD117 without CD34.

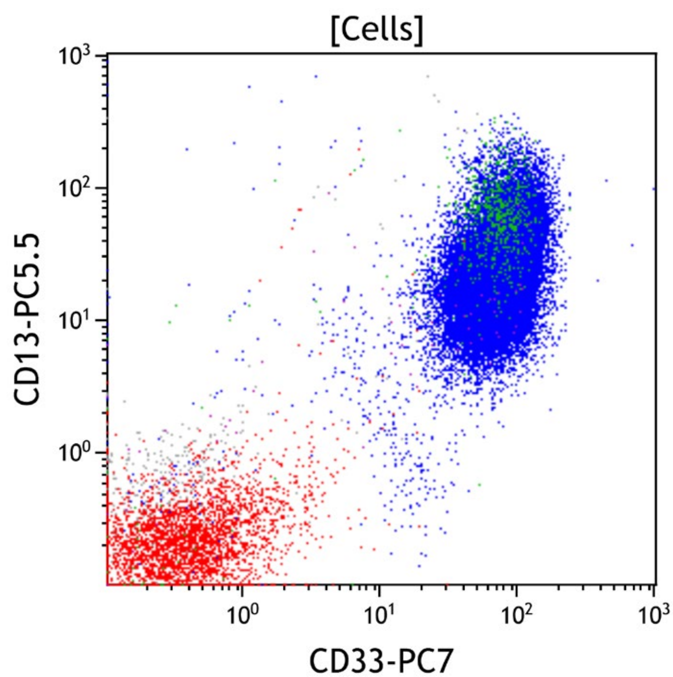

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red). The granulocytes (blue) express CD13 and bright CD33.

Table of Contents > Neoplastic Process of Myeloid Origin > Case #21: Acute Promyelocytic Leukemia

M2 Cell Tube

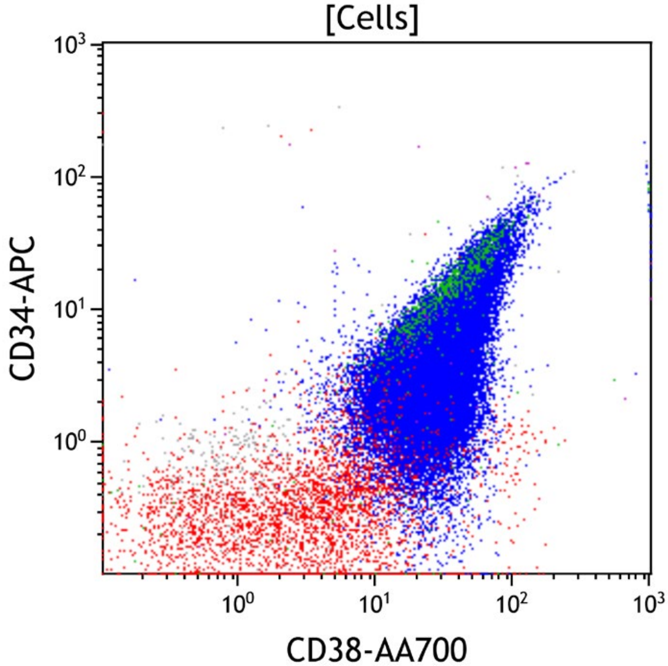

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38. The granulocytes (blue) express dim CD38 without CD34, the dim CD34 likely is due to non-specific antibody binding.

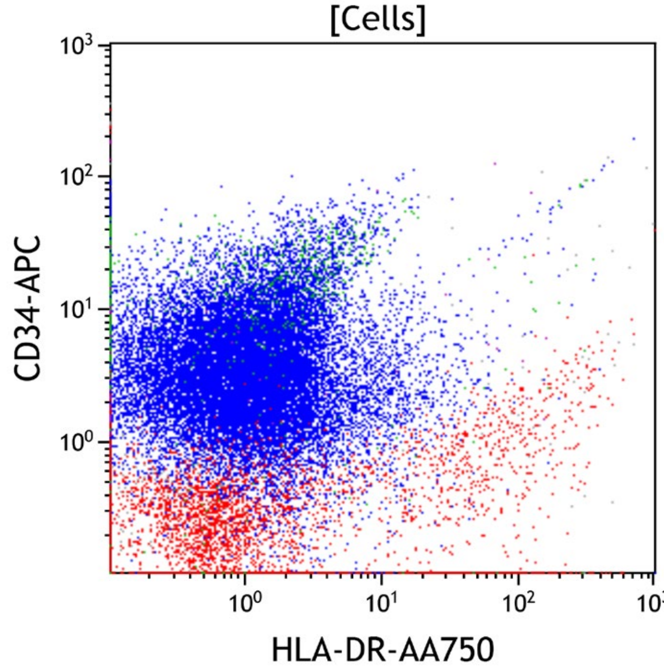

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The granulocytes (blue) do not express CD34 or HLA-DR, the apparent dim CD34 likely is due to non-specific antibody binding.

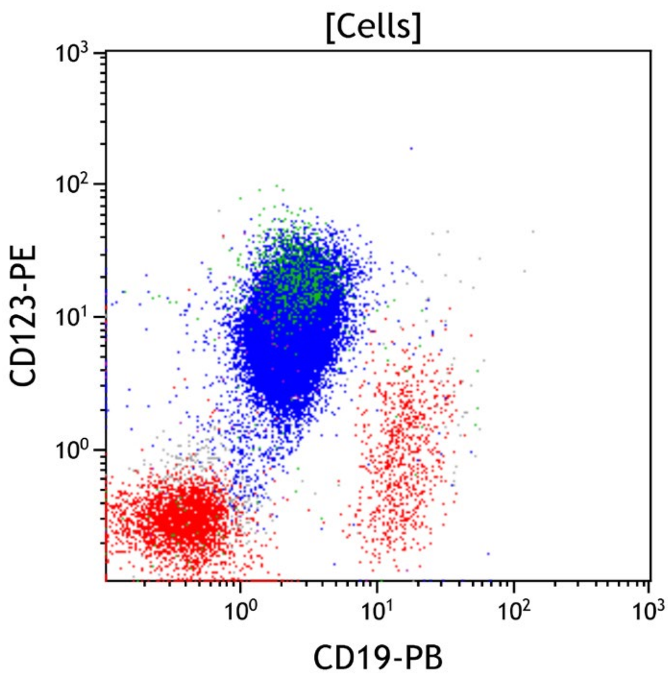

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes is due to autofluorescence, largely from eosinophils. The granulocytes (blue) express dim CD123 but not CD19. The apparent dim CD19 is due to increased autofluorescence.

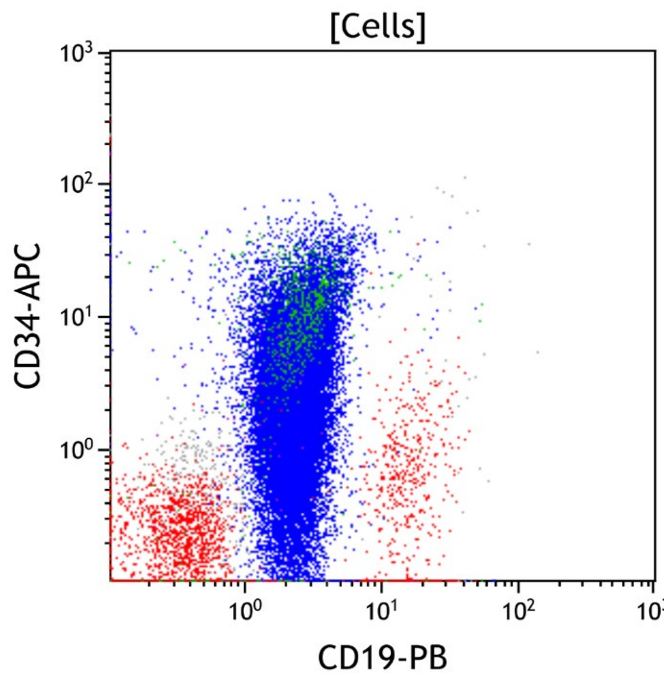

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. The granulocytes (blue) do not express CD19 or CD34. The apparent dim CD19 and CD34 is due to increased autofluorescence.

Table of Contents > Neoplastic Process of Myeloid Origin > Case #21: Acute Promyelocytic Leukemia

M2 Cell Tube

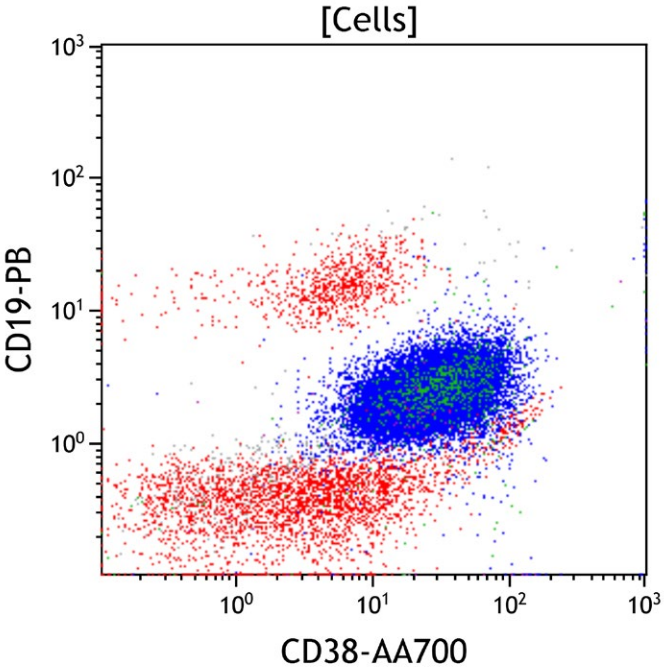

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells show lower expression of CD38. The apparent CD19 positivity on maturing granulocytes is due to autofluorescence, largely from eosinophils. The granulocytes express CD38 without CD19. The apparent dim CD19 is due to increased autofluorescence.

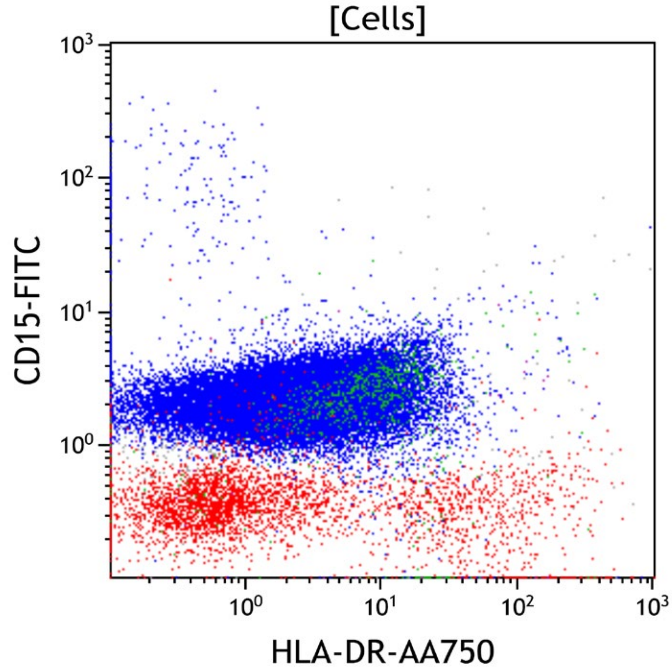

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR, except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. The granulocytes (blue) do not express CD15 or HLA-DR. The apparent dim CD15 is due to increased autofluorescence.

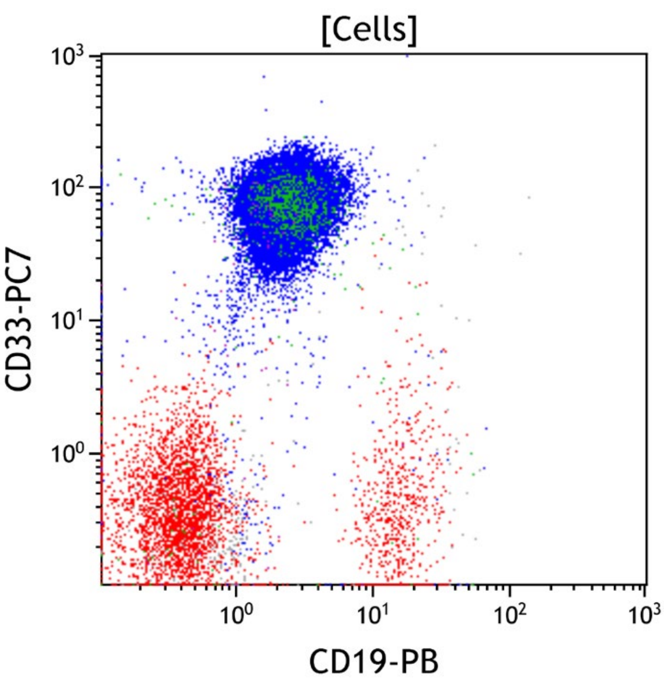

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes and maturing granulocytes. CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes is due to autofluorescence, largely from eosinophils. The granulocytes (blue) express CD33 but not CD19. The apparent dim CD19 is due to increased autofluorescence.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of high side scatter, intermediate CD13, bright CD33, dim CD38, intermediate CD45, dim CD64, intermediate CD117, and intermediate CD123 without CD11b, CD14, CD15, CD34, HLA-DR and other lymphoid or myeloid antigens. Compared with normal promyelocytes, the increased expression of CD33, and absence of CD15 are aberrant.

The immunophenotype of the abnormal population is that of expanded abnormal promyelocytes and consistent with acute promyelocytic leukemia. Additional testing identified a t(15;17) indicative of an acute promyelocytic leukemia.

Case #22: Acute Monocytic Leukemia

Clinical Vignette

This 51-year-old male presents with circulating blasts. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

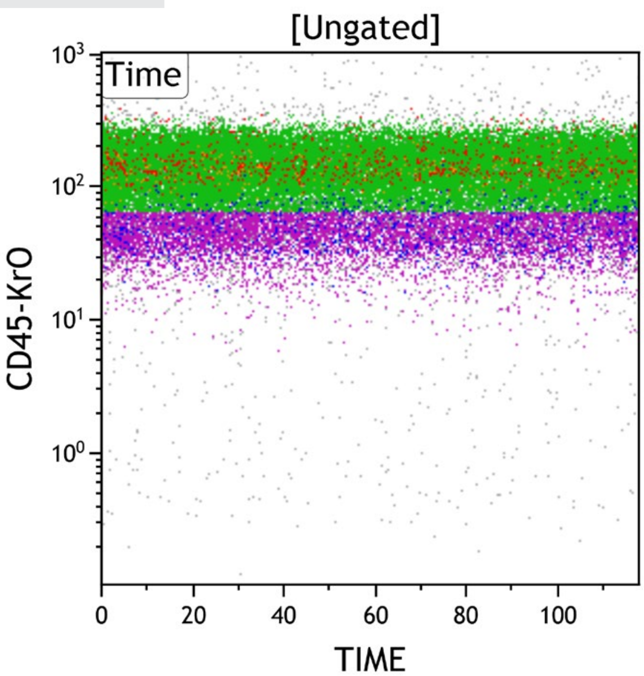

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

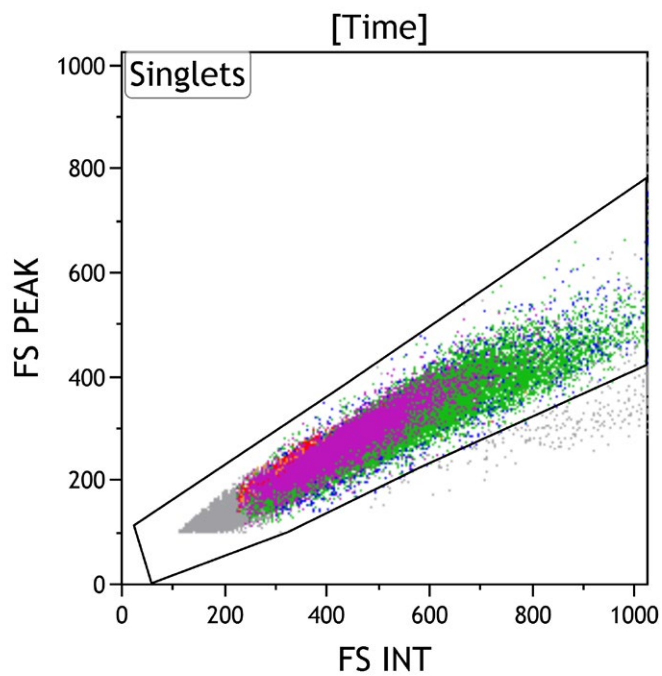

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

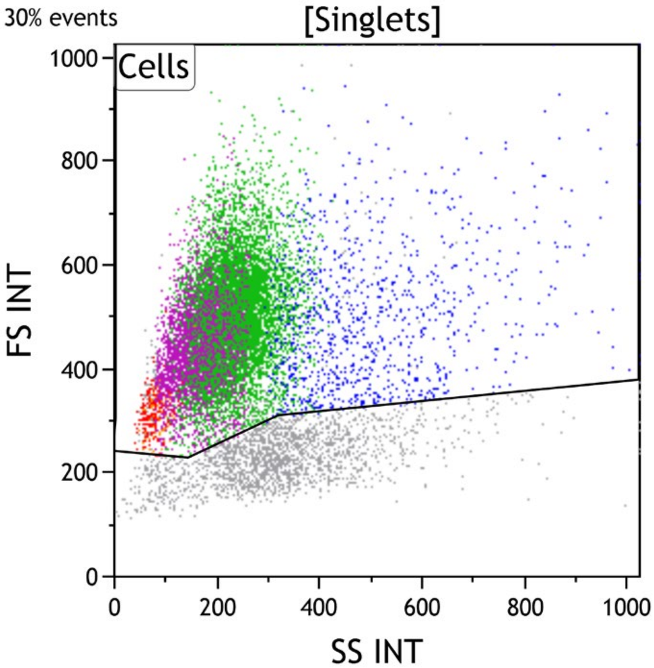

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

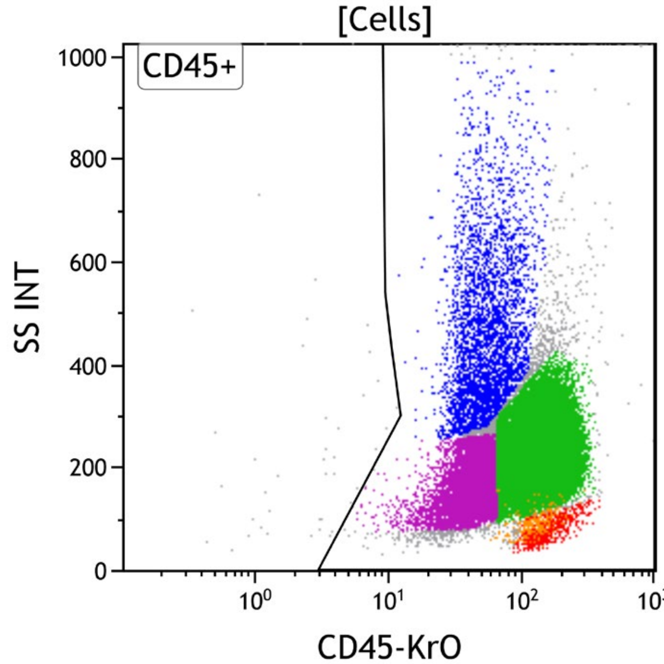

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

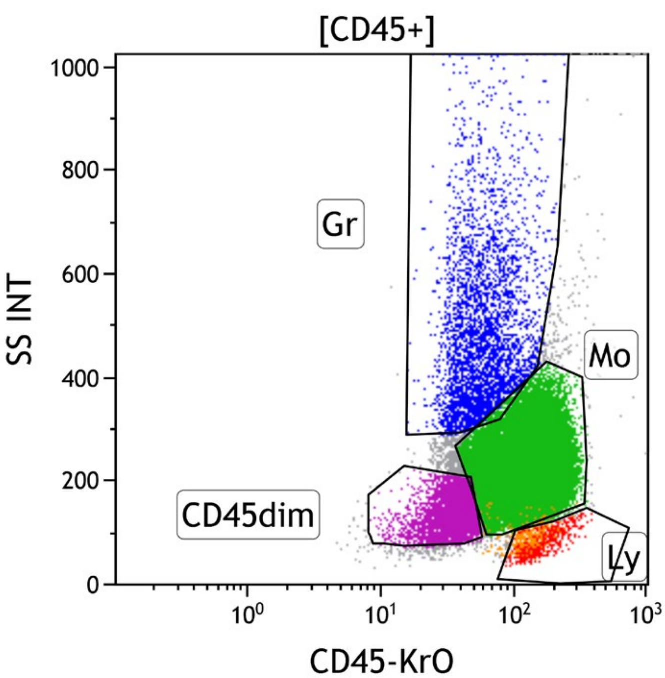

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note that progenitors (purple) and monocytes (green) are relatively expanded in number.

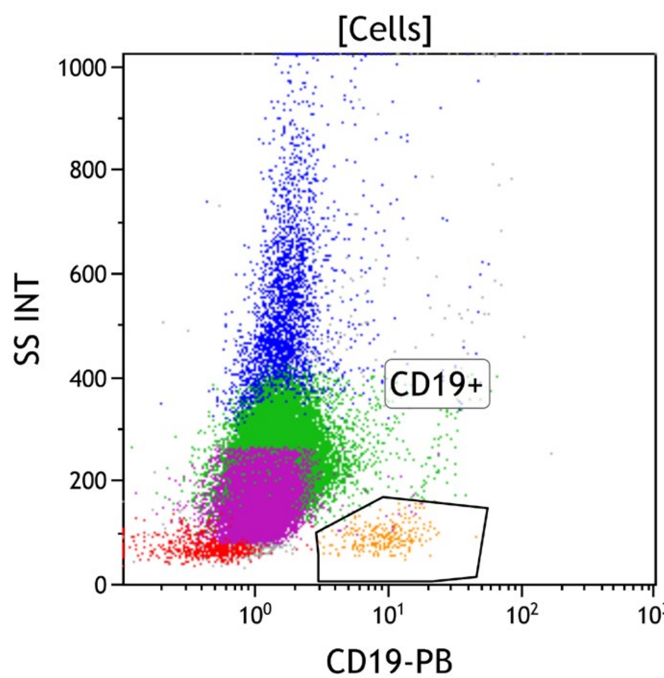

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The progenitors (purple) and monocytes (green) do not express CD19.

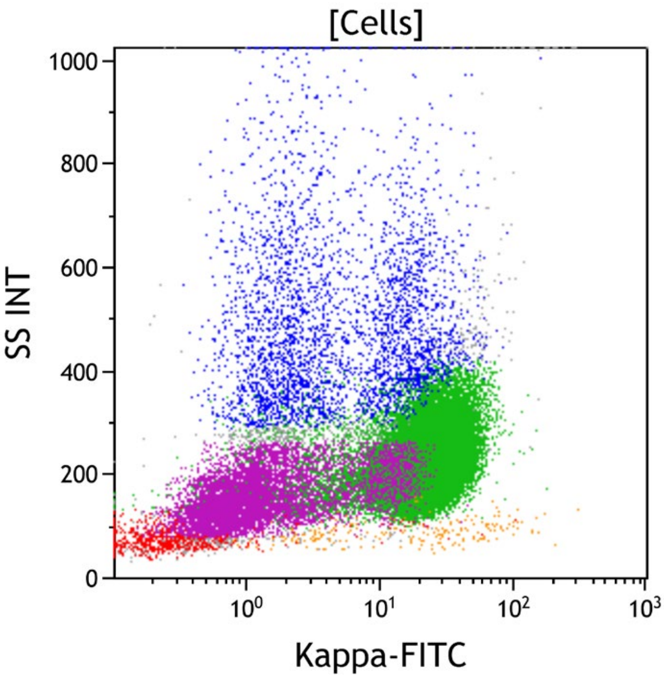

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The progenitors (purple) do not express kappa. The monocytes (green) show increased background for kappa light chains, likely a result of Fc-receptor mediated binding of plasma immunoglobulin.

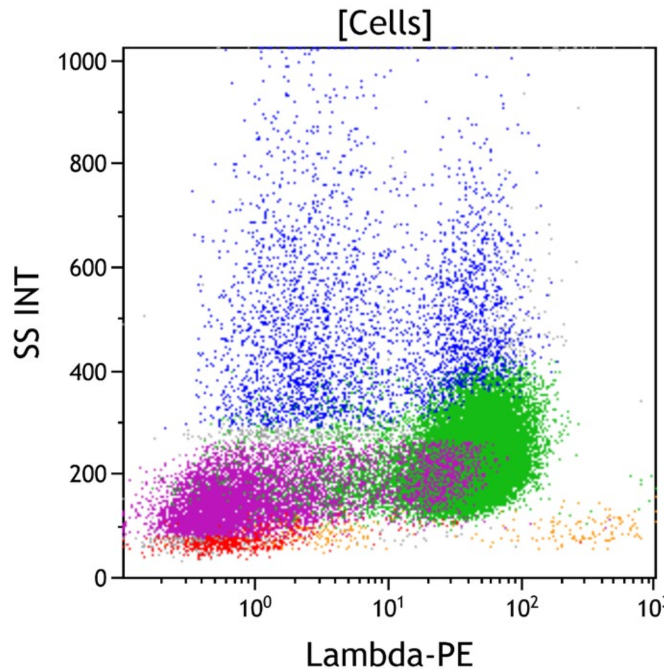

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The progenitors (purple) do not express lambda. The monocytes (green) show increased background for lambda light chains, likely a result of Fc-receptor mediated binding of plasma immunoglobulin.

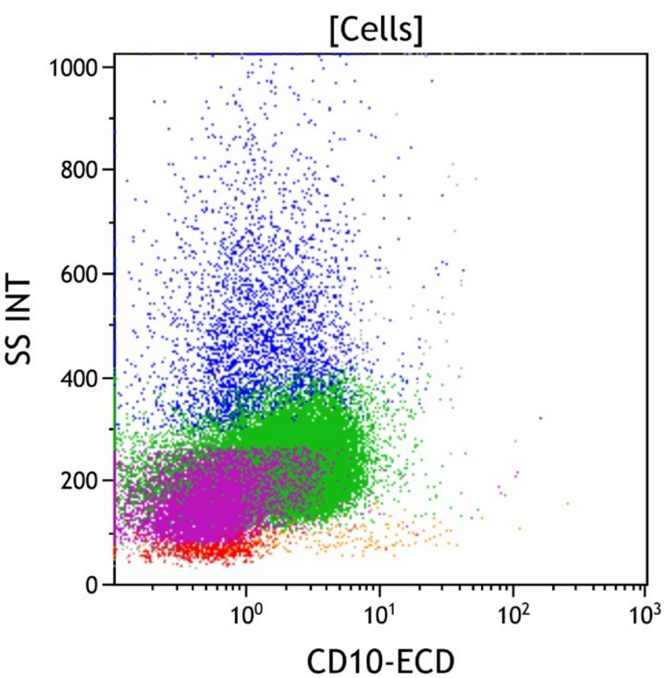

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The progenitors (purple) and monocytes (green) do not express CD10. The apparent dim CD10 on monocytes is due to increased compensation background from the bright lambda expression.

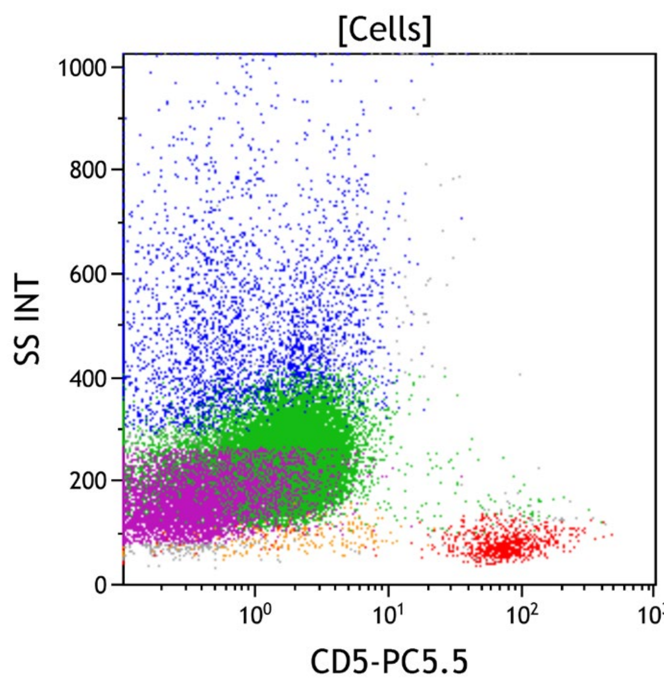

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The progenitors (purple) and monocytes (green) do not express CD5.

B Cell Tube

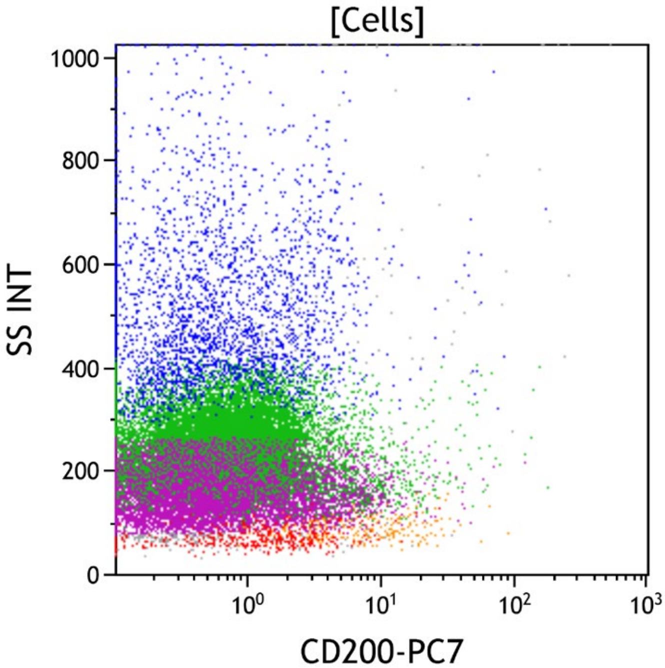

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The progenitors (purple) and monocytes (green) do not express CD200.

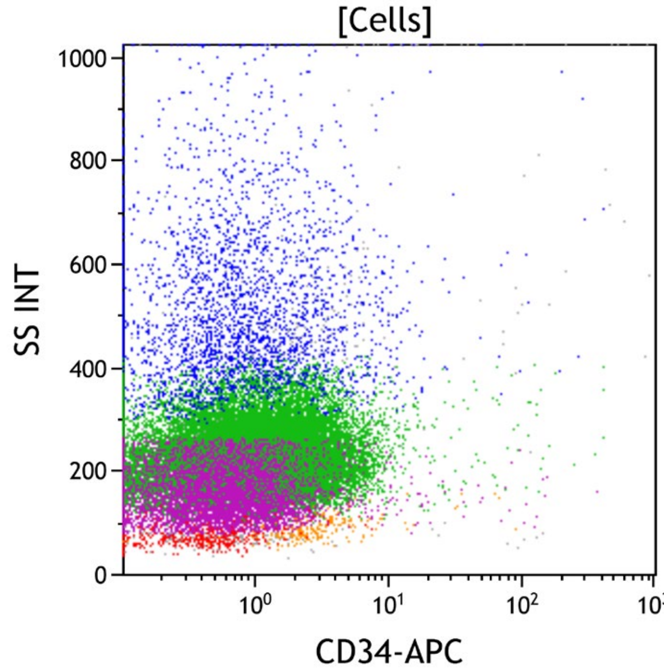

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The progenitors (purple) and monocytes (green) do not express CD34.

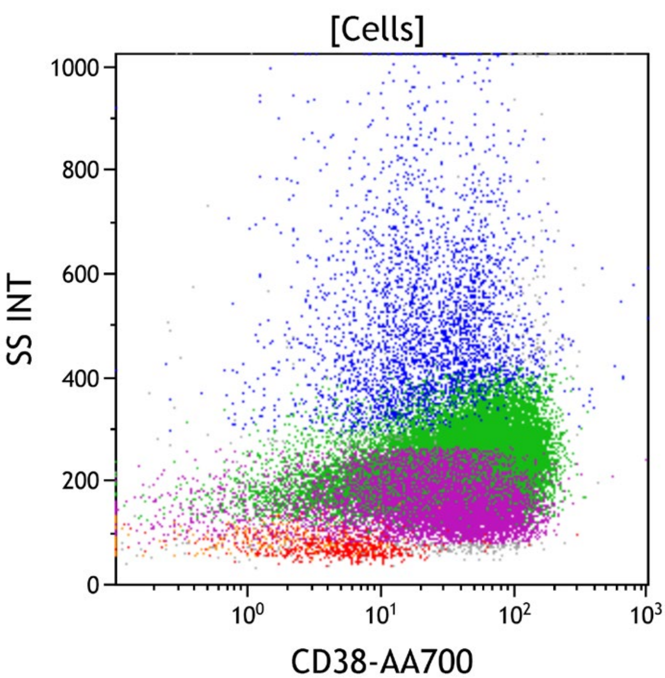

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on mature monocytes, and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The progenitors (purple) and monocytes (green) express intermediate CD38.

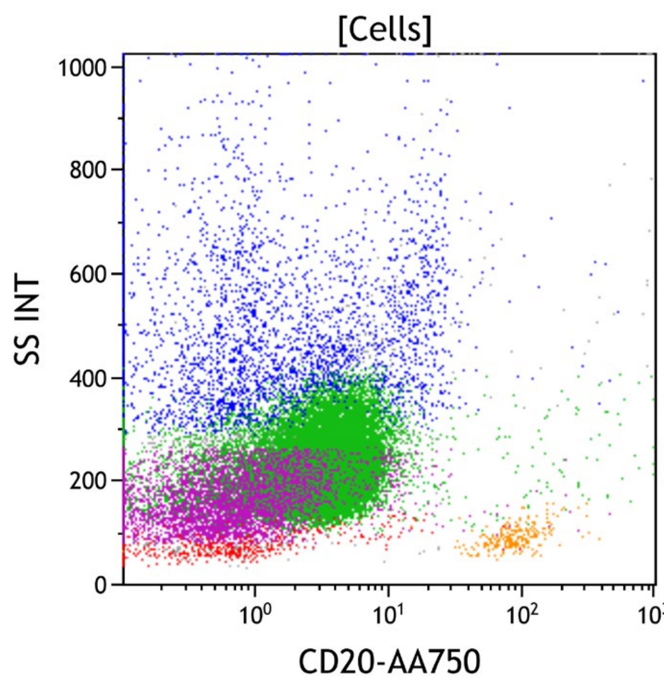

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The progenitors (purple) and monocytes (green) do not express CD20. The apparent low CD20 expression of monocytes is likely due to a combination of increased compensation background from the relatively bright CD38 and some non-specific antibody binding.

B Cell Tube

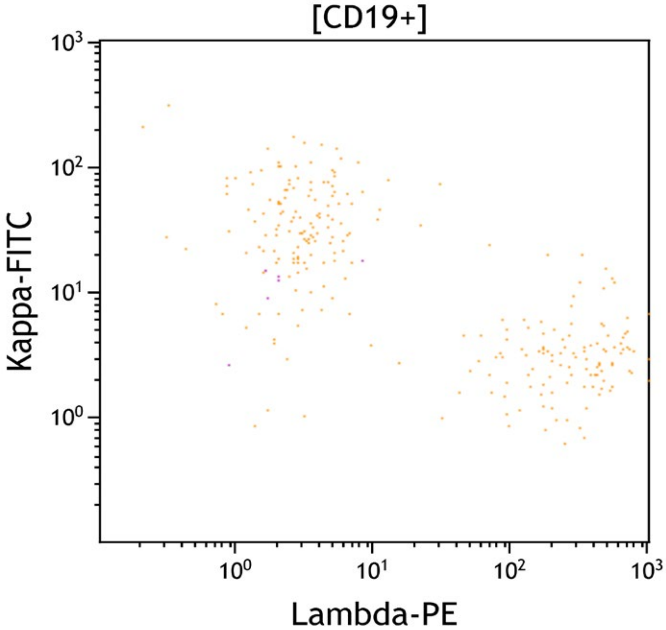

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

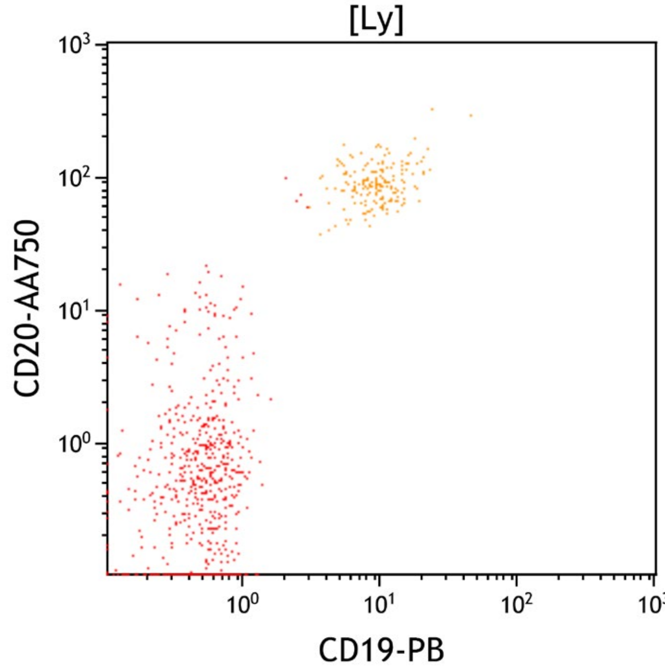

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

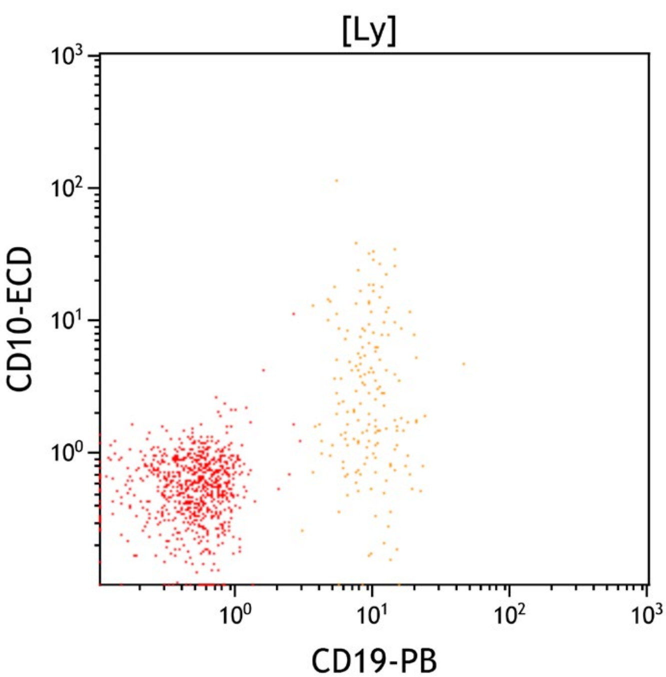

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

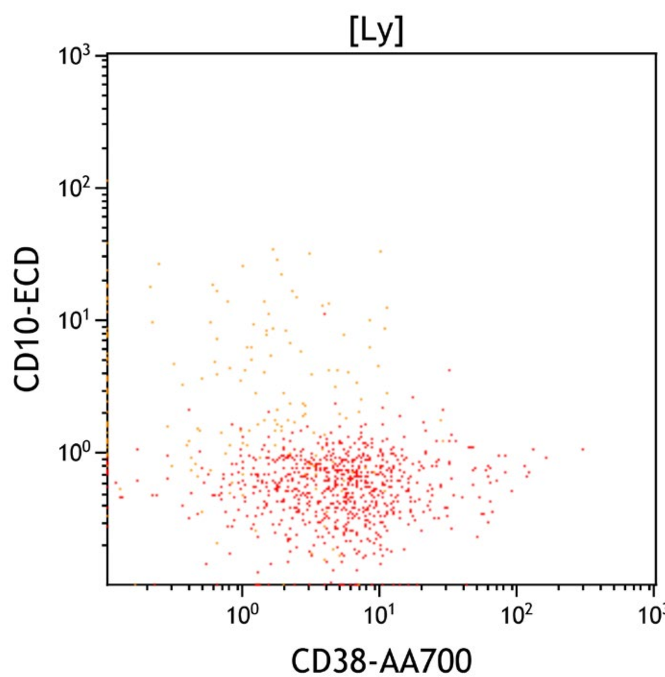

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells display low to absent expression of CD38. T cells show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

B Cell Tube

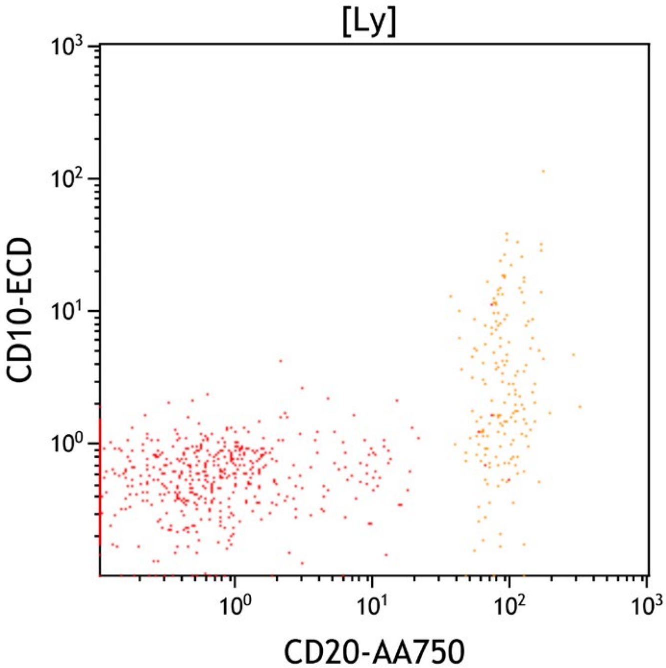

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

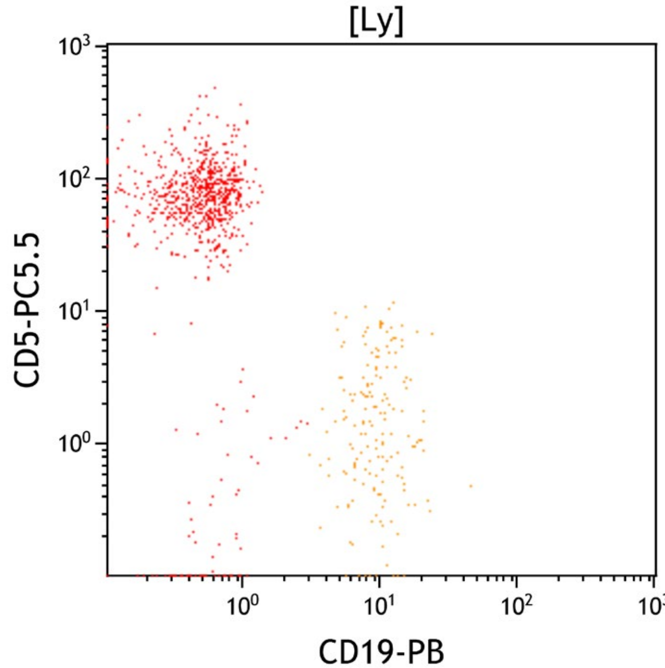

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

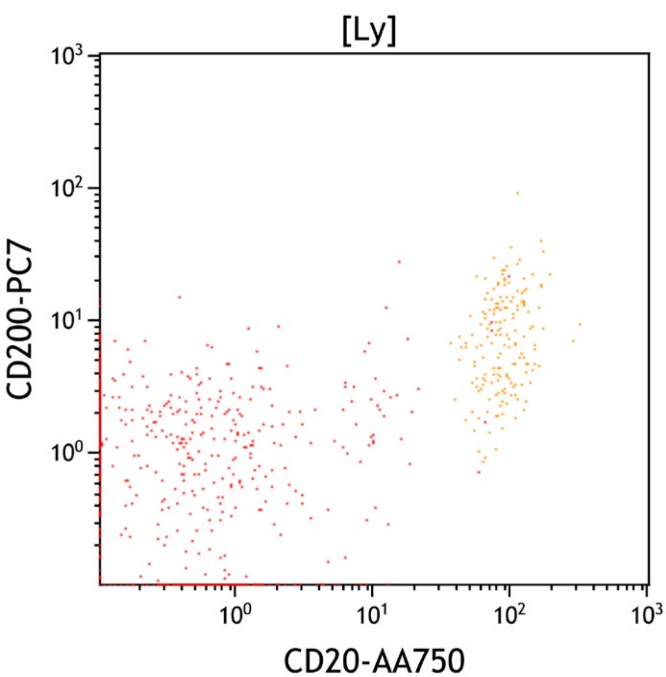

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

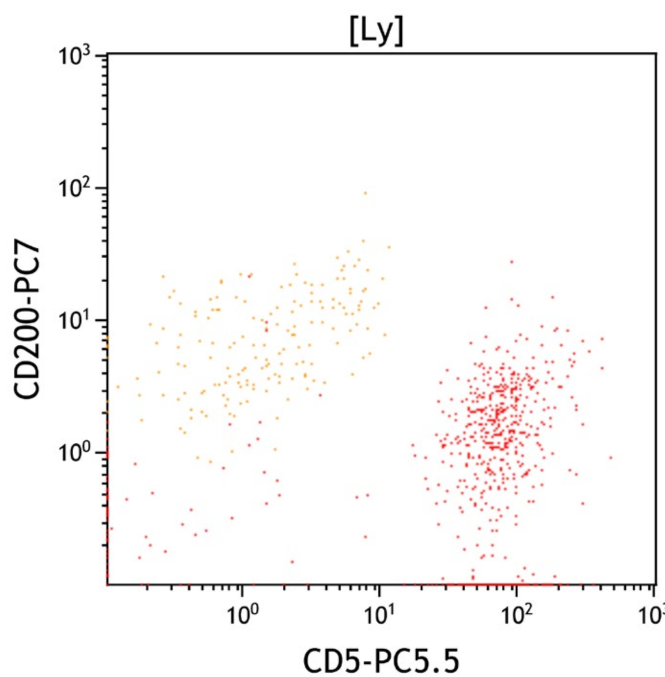

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

T Cell Tube

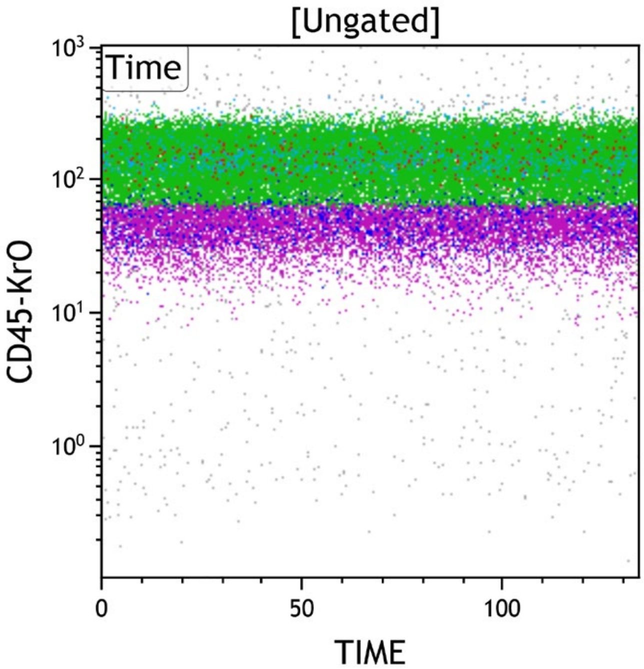

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

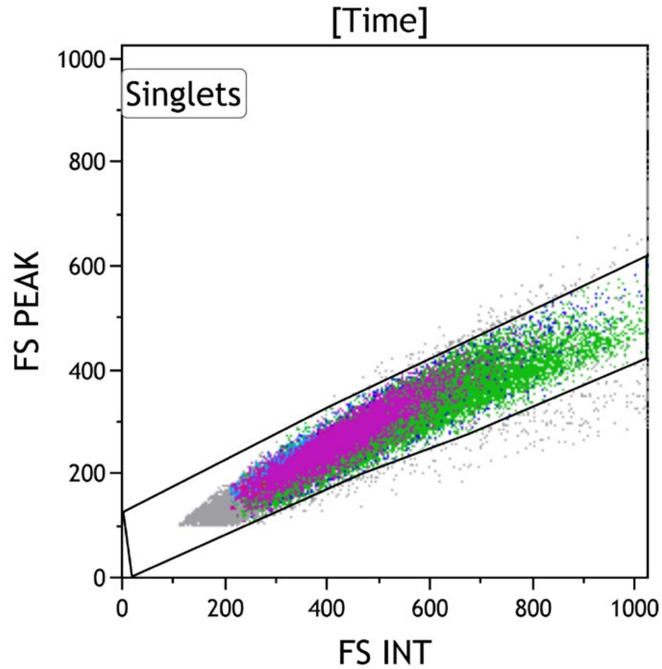

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

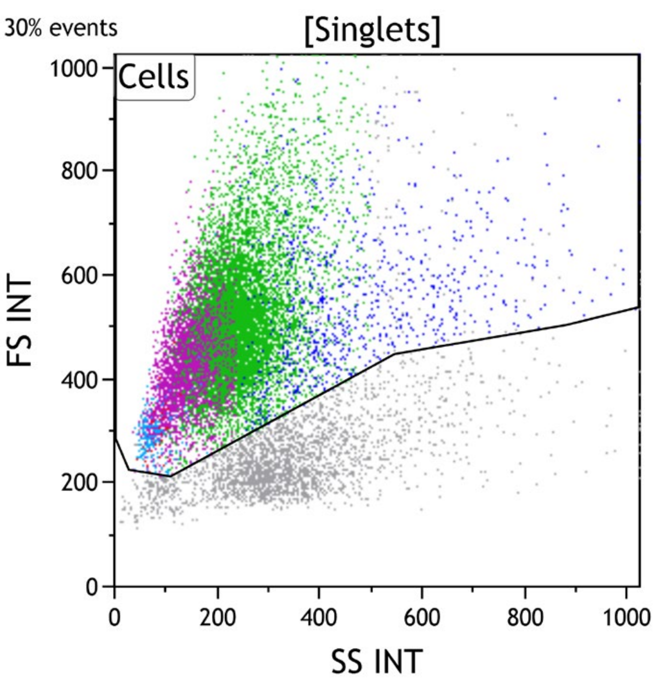

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

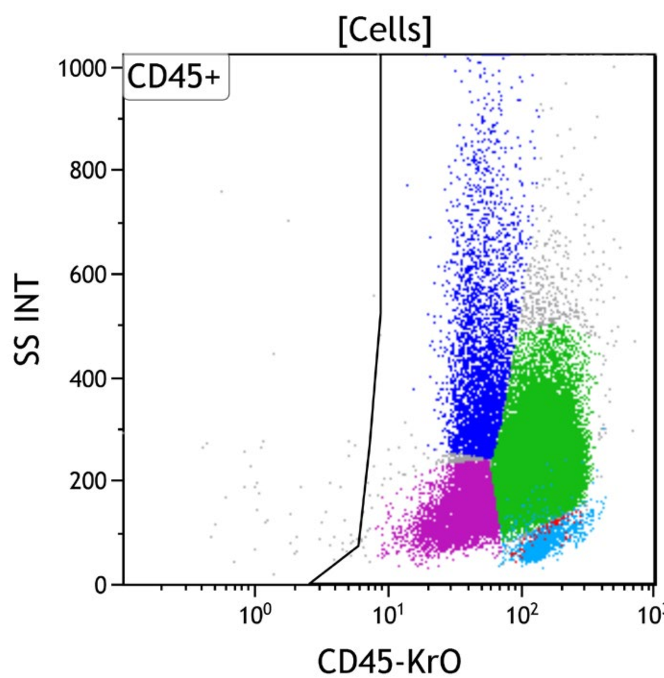

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

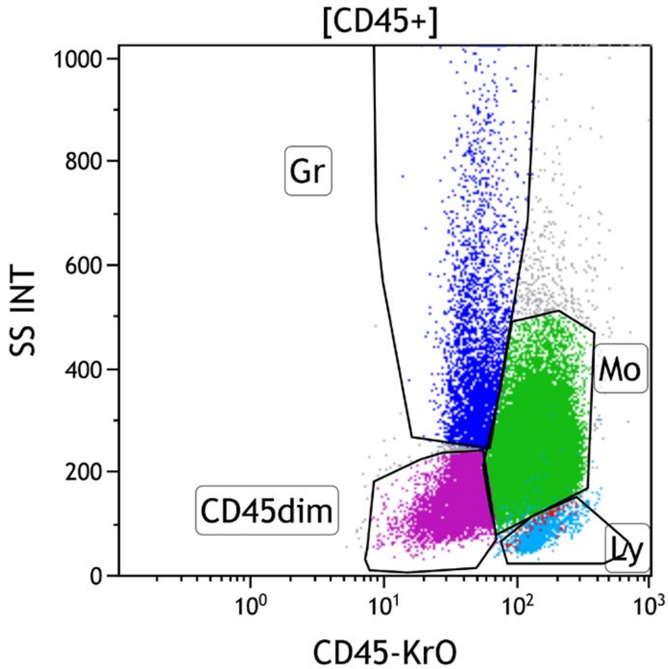

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that progenitors (purple) and monocytes (green) are relatively expanded in number.

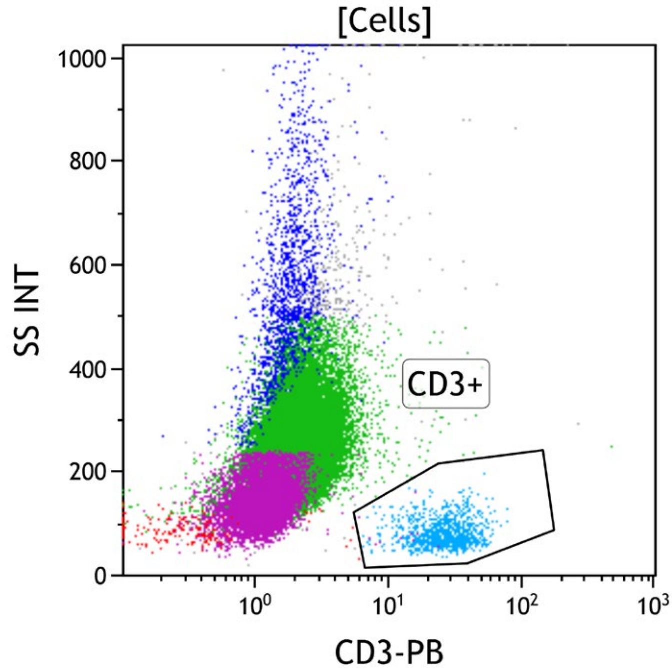

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The progenitors (purple) and monocytes (green) do not express CD3.

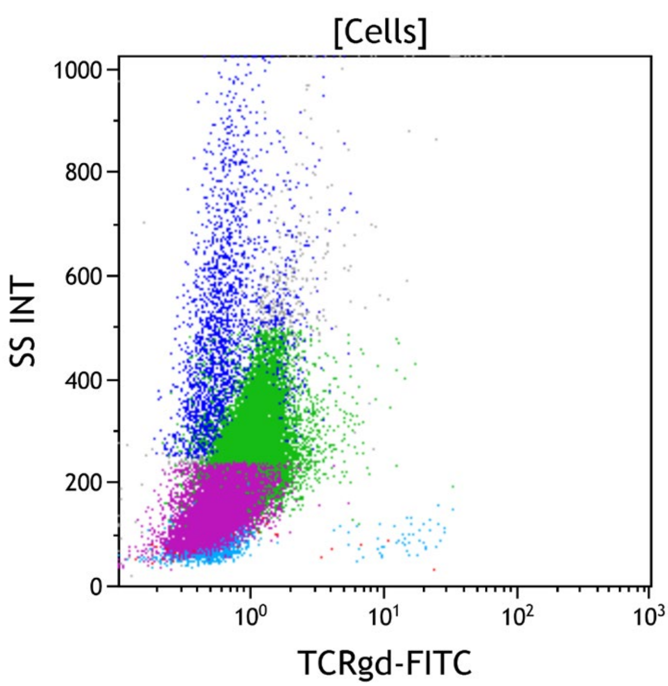

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The progenitors (purple) and monocytes (green) do not express TCRγδ.

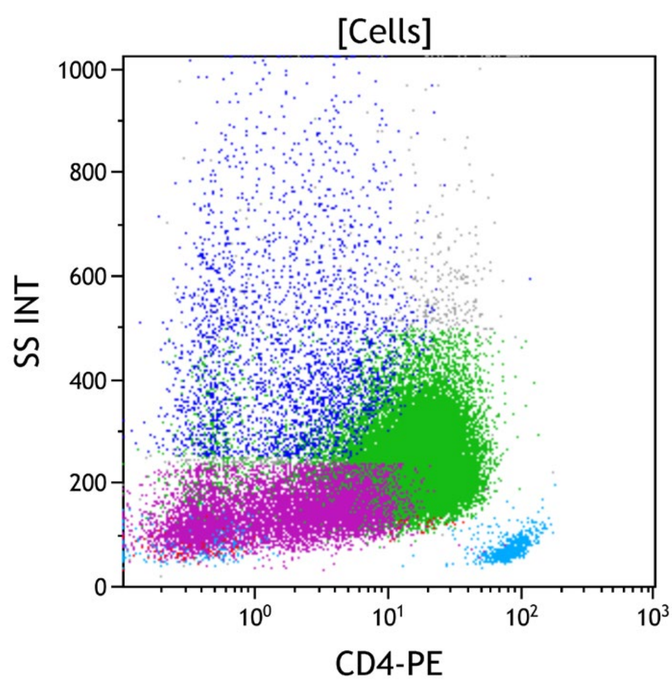

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The progenitors (purple) express variable dim CD4 and the monocytes (green) express intermediate CD4.

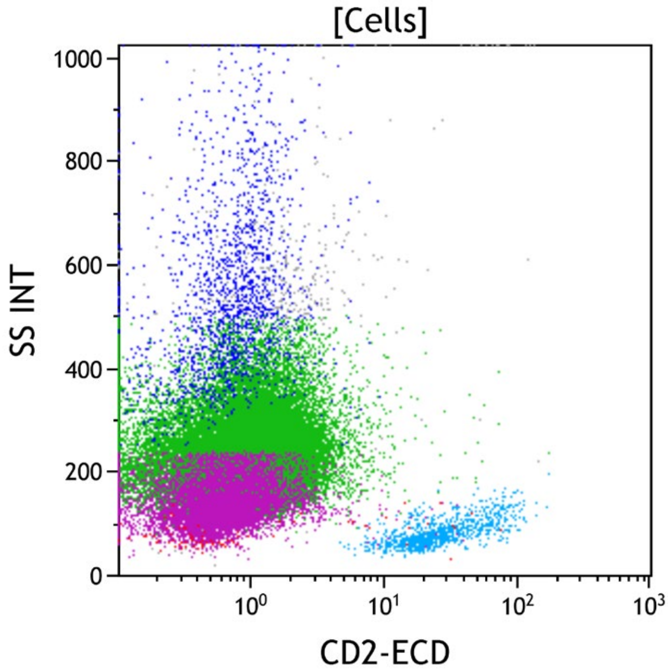

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red). The progenitors (purple) and monocytes (green) do not express CD2.

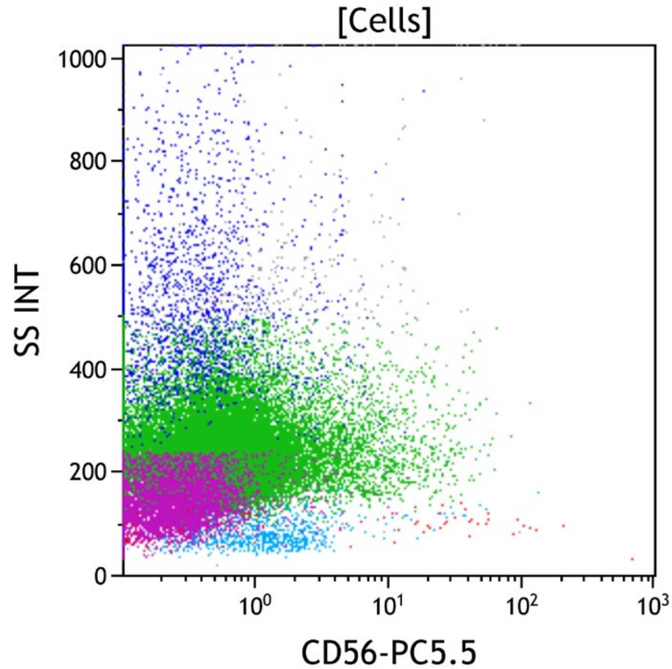

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The progenitors (purple) and monocytes (green) do not express significant CD56.

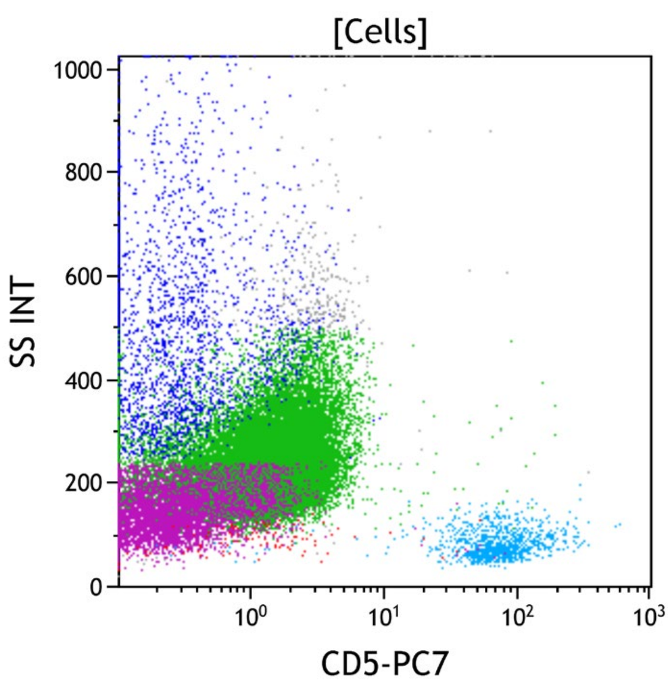

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The progenitors (purple) and monocytes (green) do not express CD5.

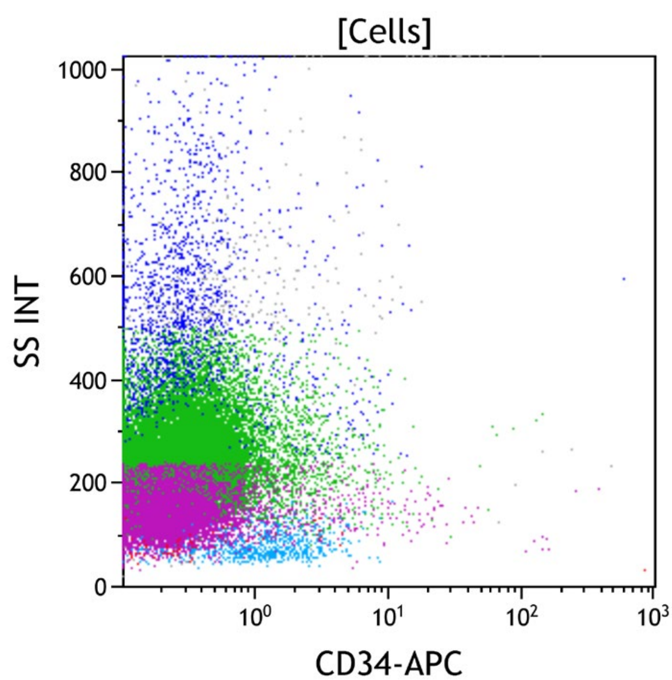

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The progenitors (purple) and monocytes (green) do not express CD34.

T Cell Tube

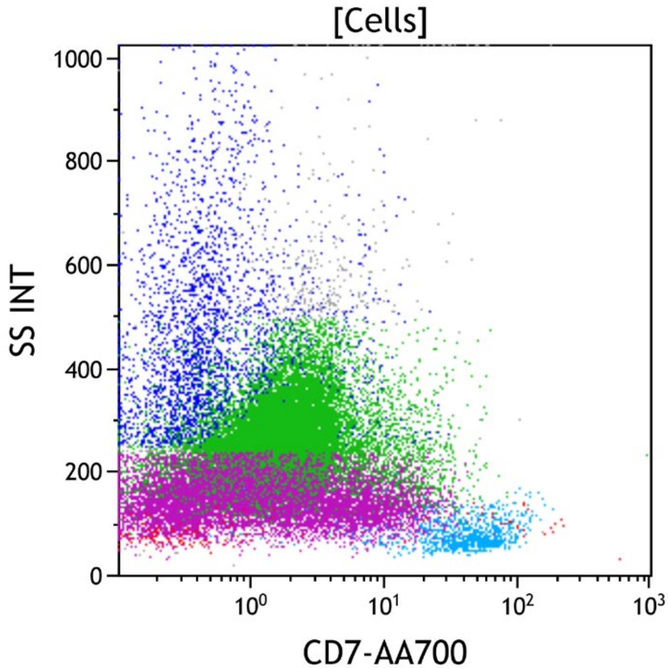

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The progenitors (purple) express variable dim CD7 and monocytes (green) do not express CD7.

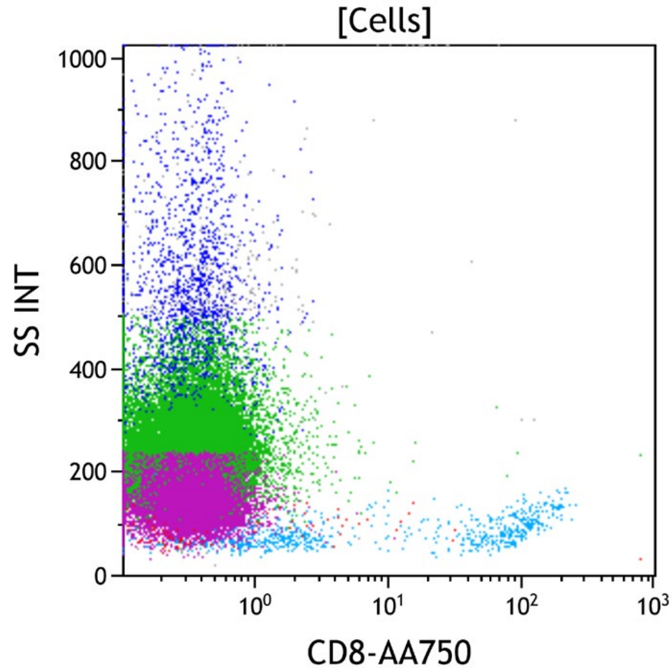

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The progenitors (purple) and monocytes (green) do not express CD8.

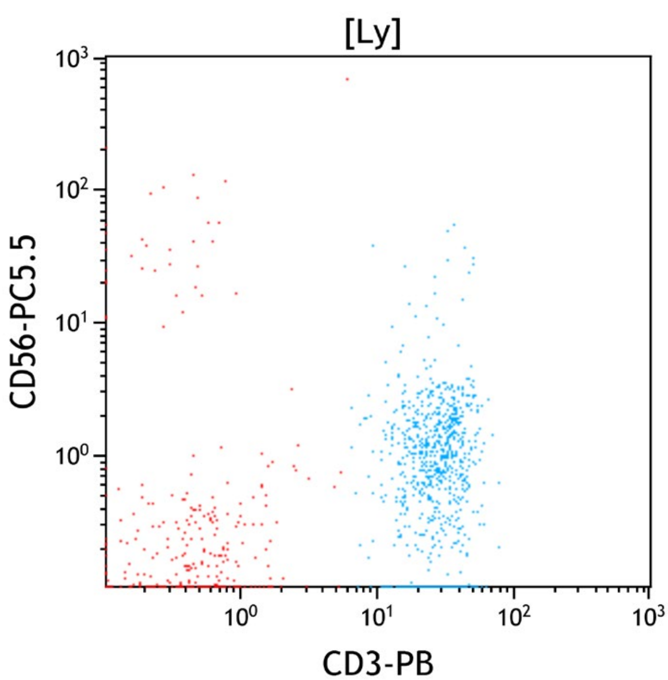

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

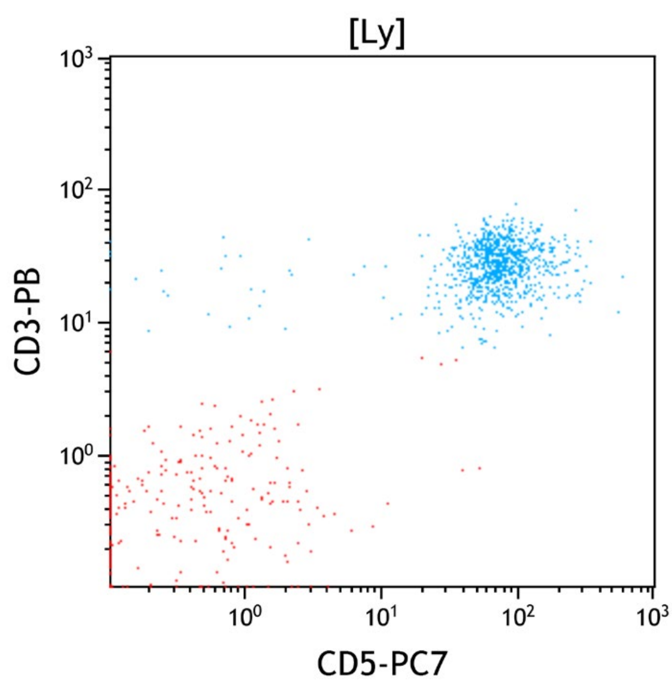

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

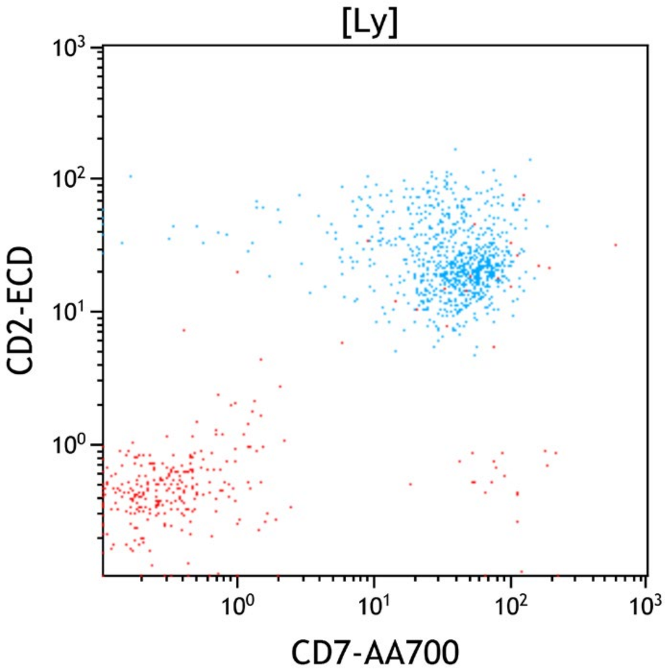

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

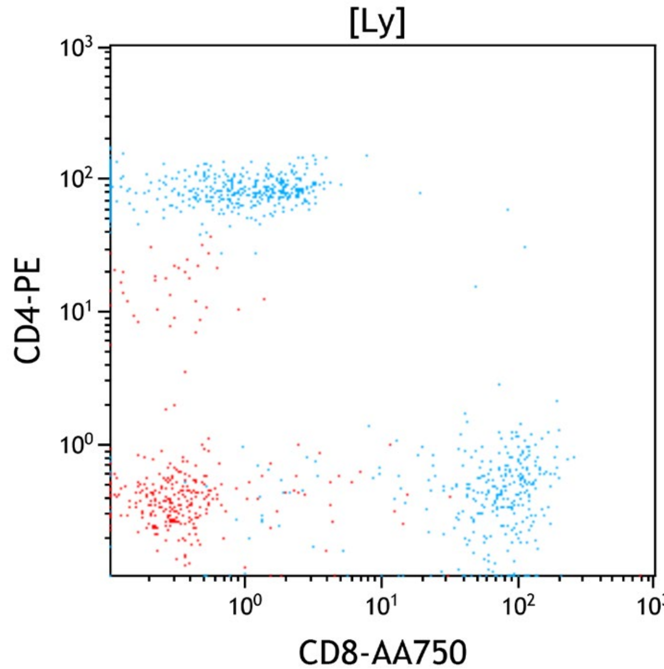

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

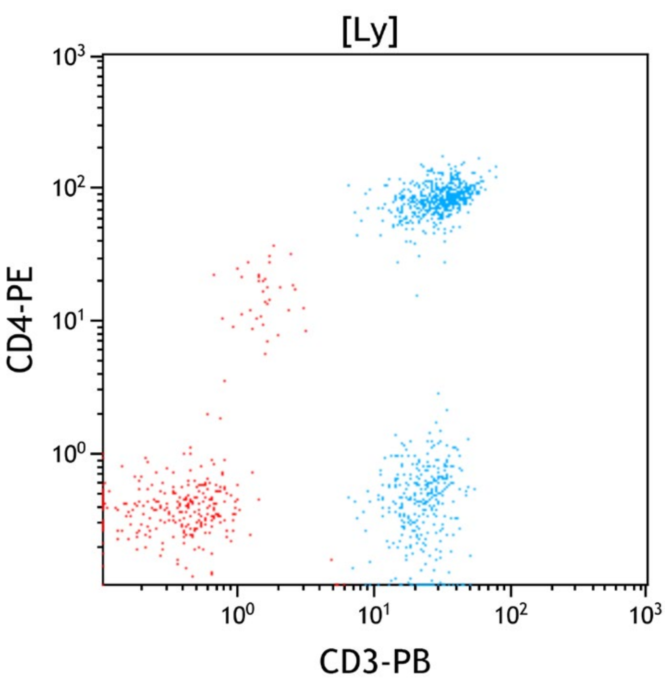

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

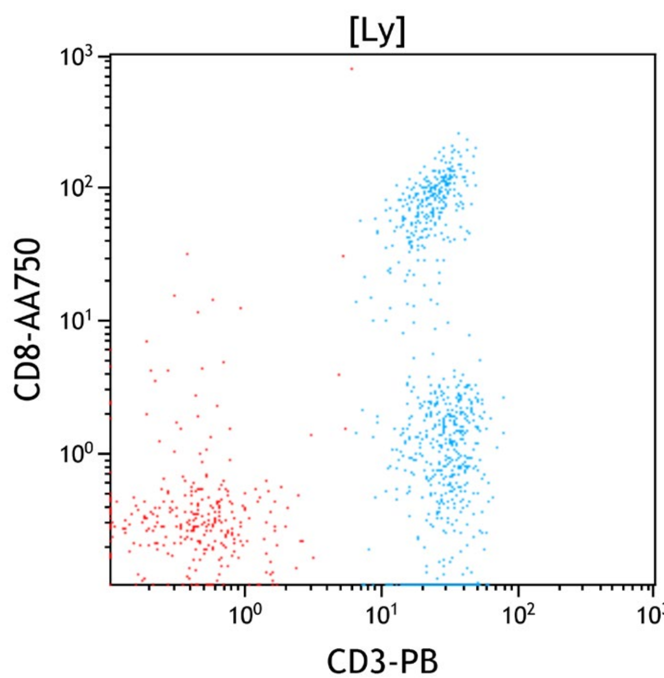

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 without CD3.

T Cell Tube

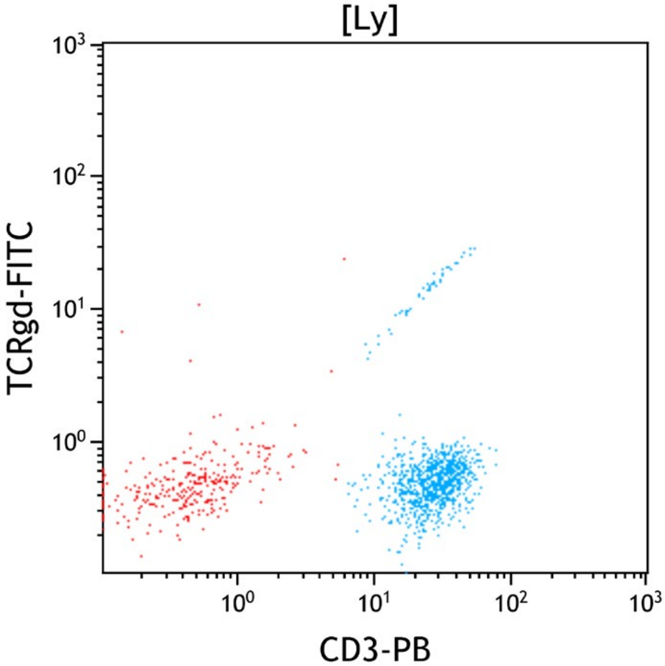

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

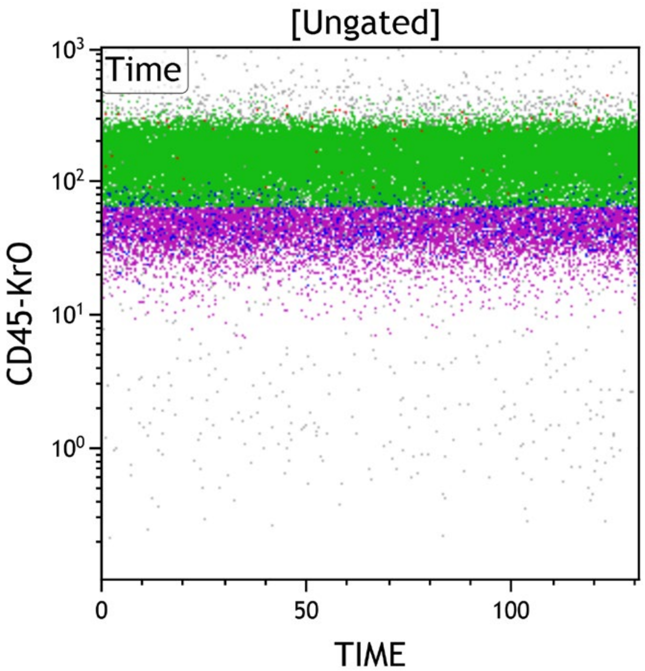

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

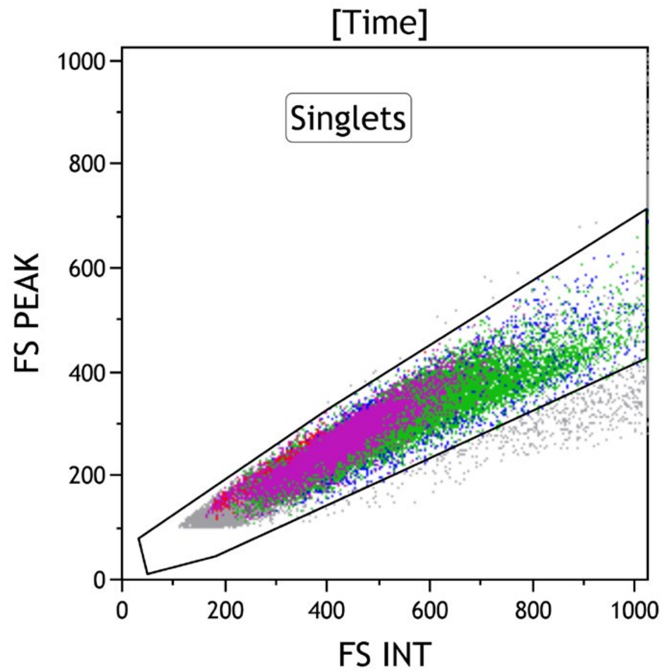

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

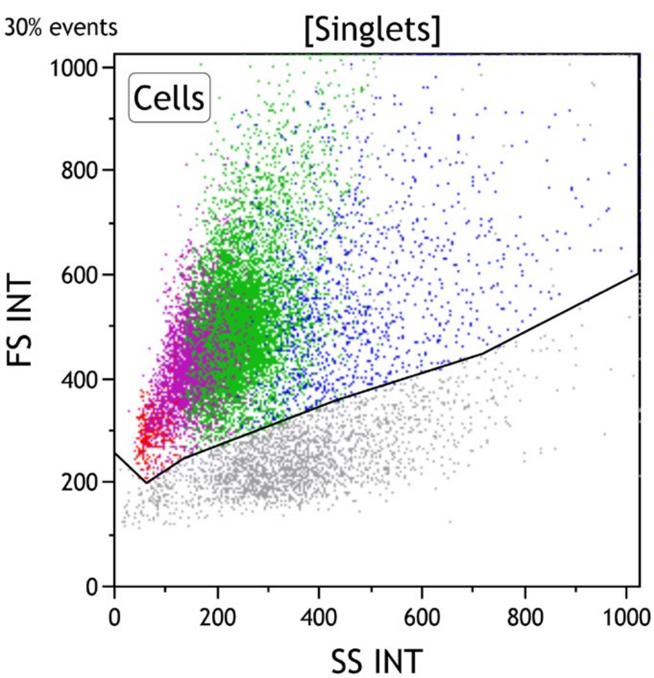

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

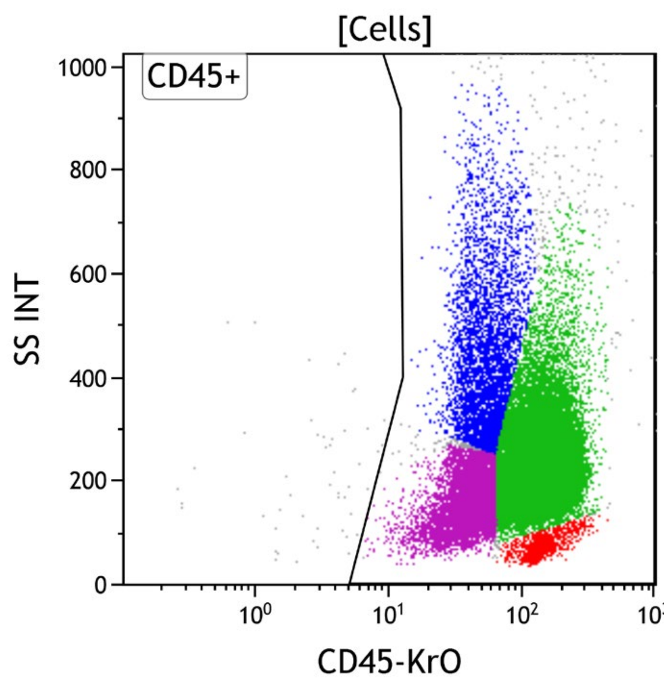

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

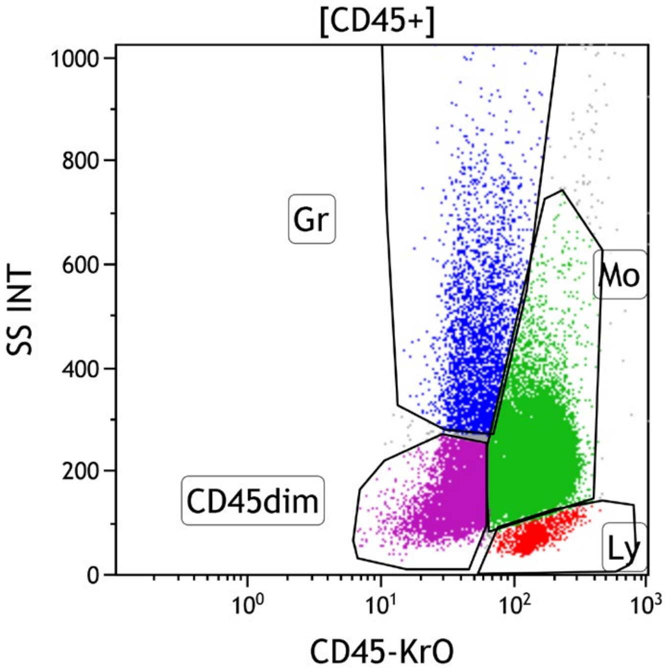

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that progenitors (purple) and monocytes (green) are relatively expanded in number.

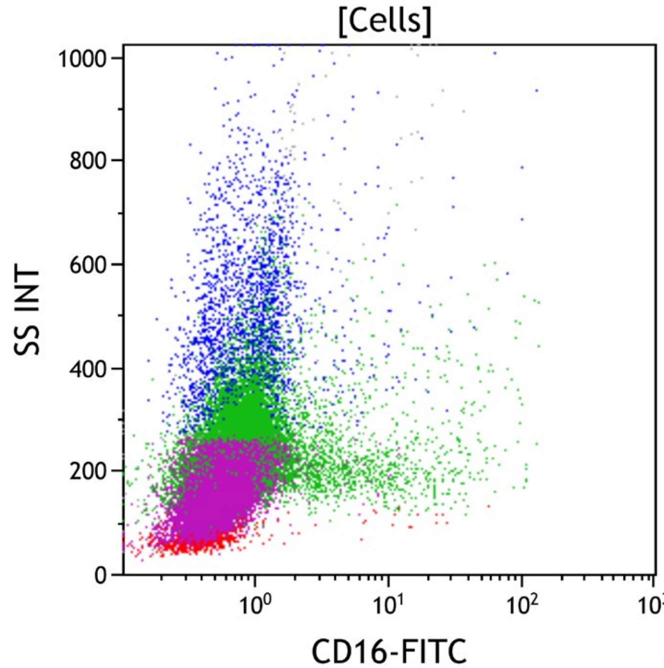

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands. Most NK cells express CD16, as do a subset of activated monocytes. The progenitors (purple) and monocytes (green) do not express CD16.

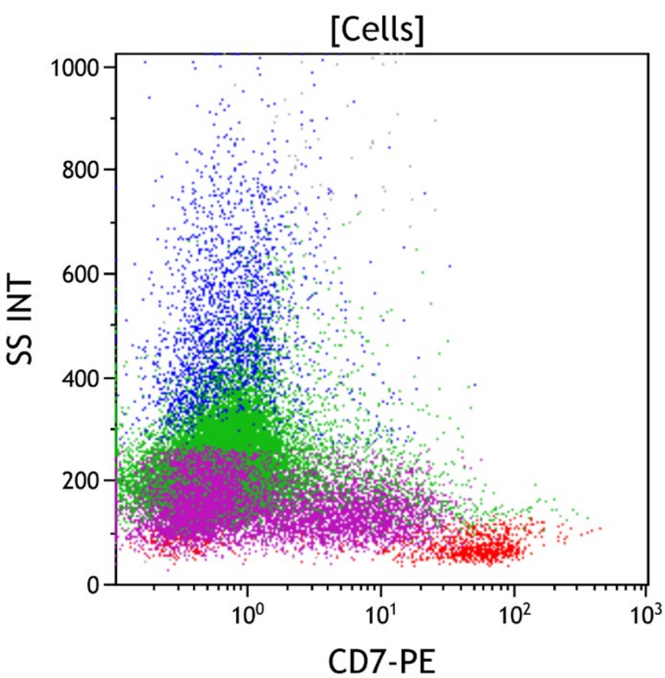

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The progenitors (purple) express variable dim CD7 and monocytes (green) do not express CD7.

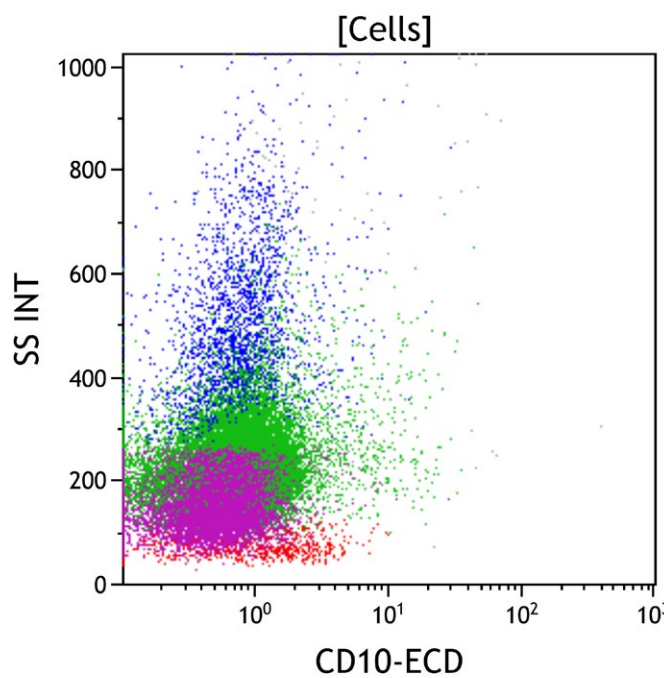

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes. The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The progenitors (purple) and monocytes (green) do not express CD10.

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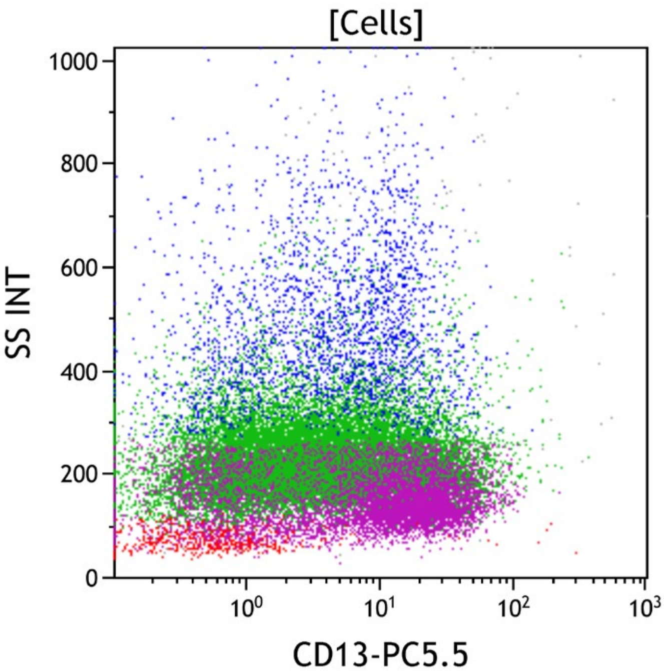

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. The progenitors (purple) express intermediate CD13 and monocytes (green) express variable dim CD13.

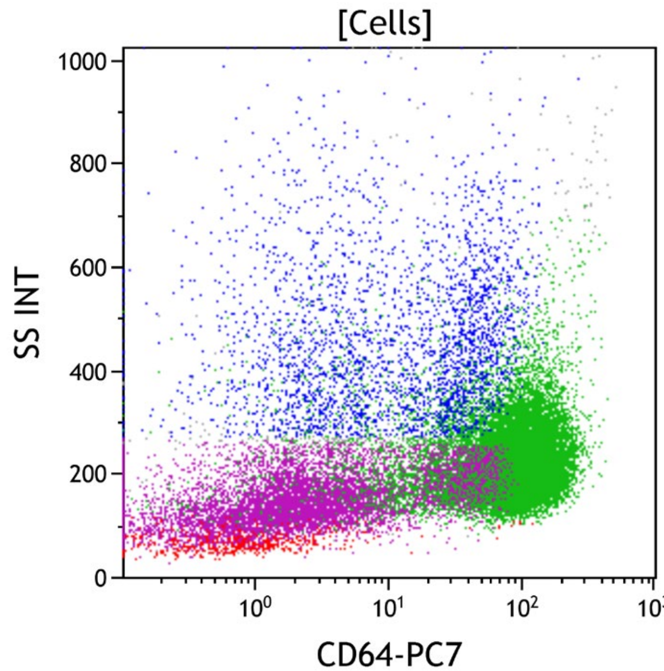

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes and at a slightly lower level on promyelocytes and myelocytes. CD64 is not well expressed on resting mature granulocytes, but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The majority of progenitors (purple) do not express CD64 and monocytes (green) express bright CD64.

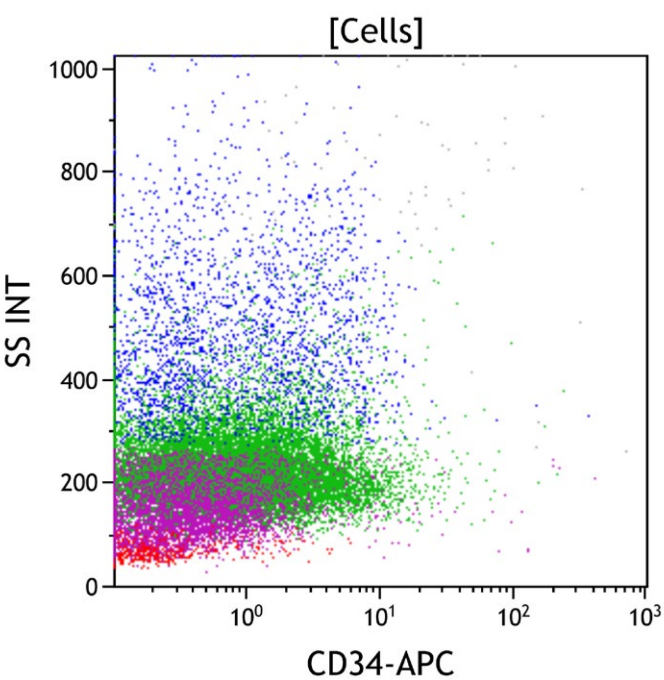

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The progenitors (purple) and monocytes (green) do not express CD34.

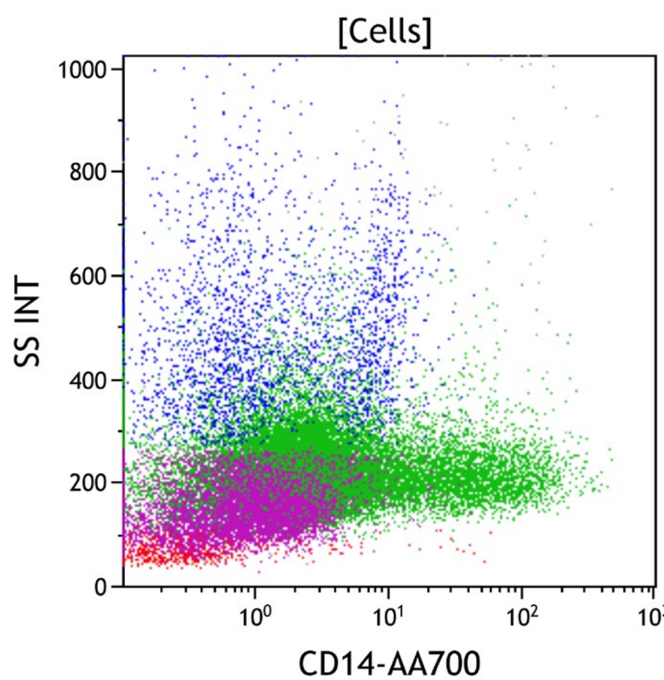

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The progenitors (purple) do not express CD14 and monocytes (green) express variable dim to intermediate CD14 on a small subset.

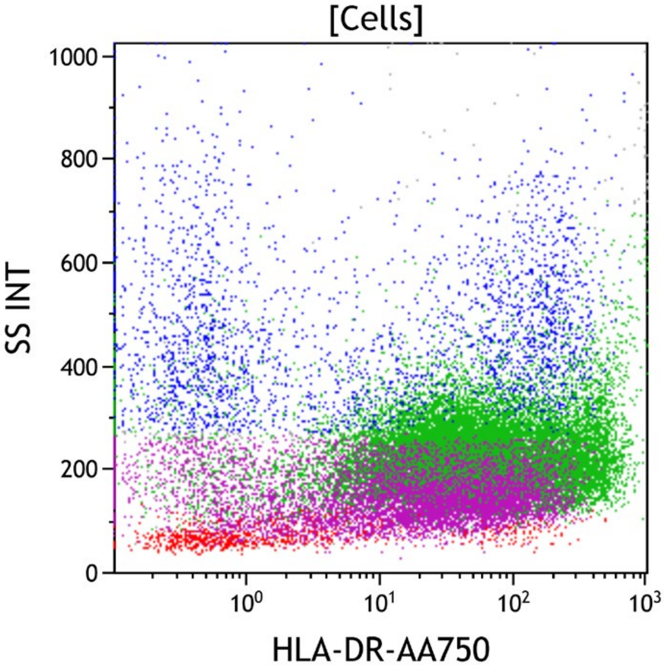

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The progenitors (purple) and monocytes (green) express intermediate to bright HLA-DR.

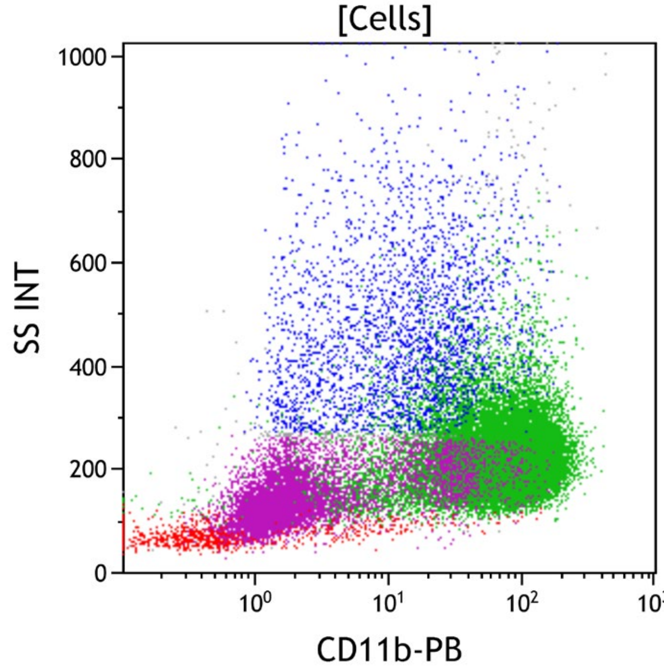

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage, and on monocytes. CD11b is also expressed on NK cells and basophils. The majority of progenitors (purple) do not expression CD11b and monocytes (green) express bright CD11b.

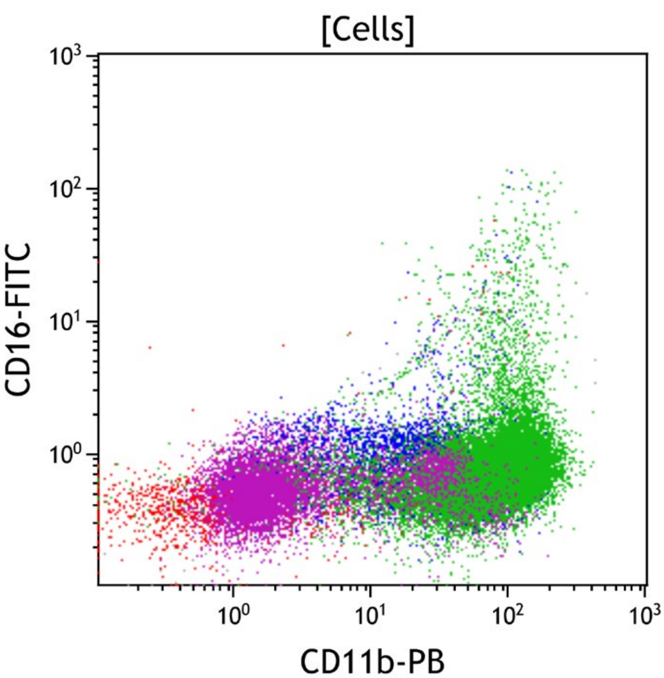

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. The majority of progenitors (purple) do not express CD11b or CD16 and monocytes (green) express CD11b without CD16.

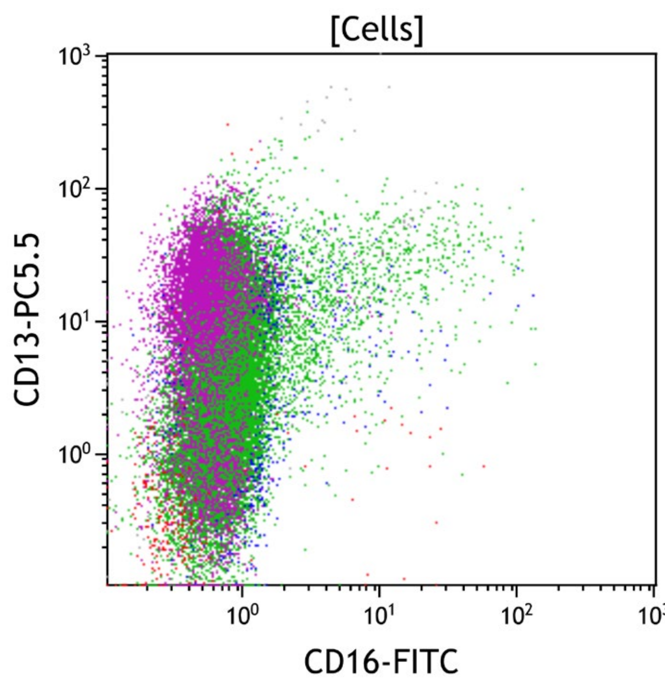

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. The progenitors (purple) and monocytes (green) express CD13 without CD16.

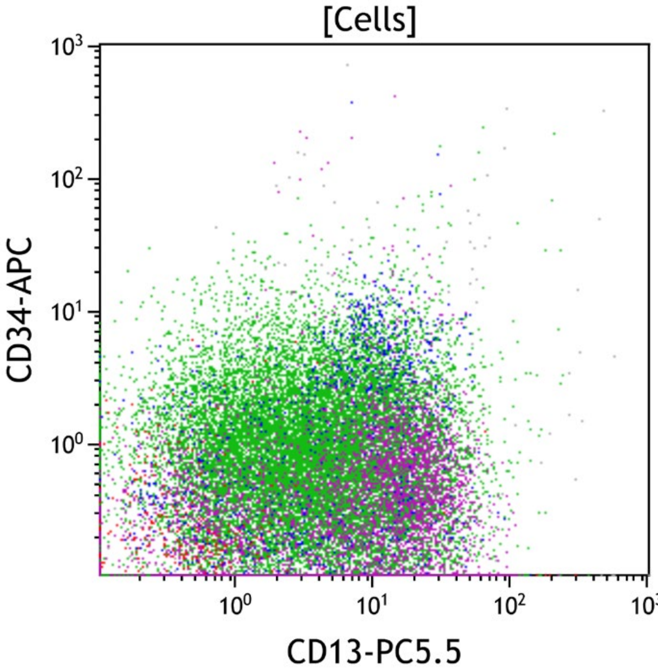

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells. The progenitors (purple) and monocytes (green) express CD13 without CD34.

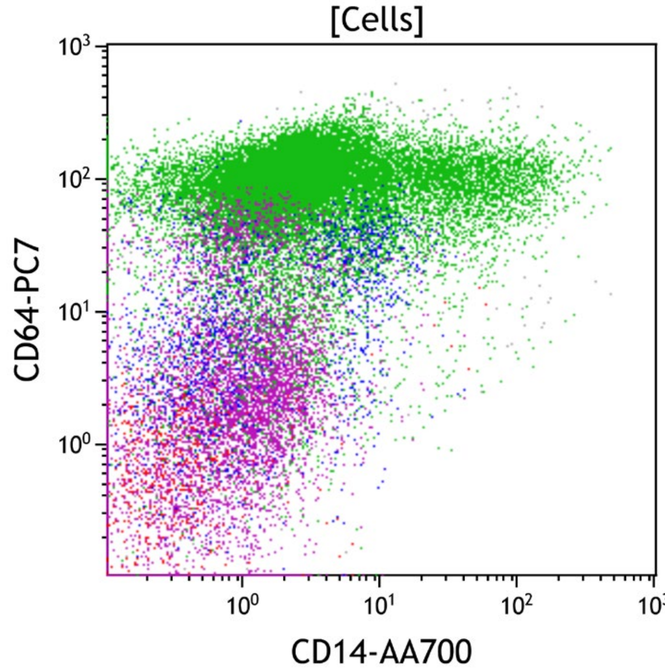

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. The progenitors (purple) do not expression CD14 or CD64 but monocytes (green) express bright CD64 with variable CD14 on a small subset.

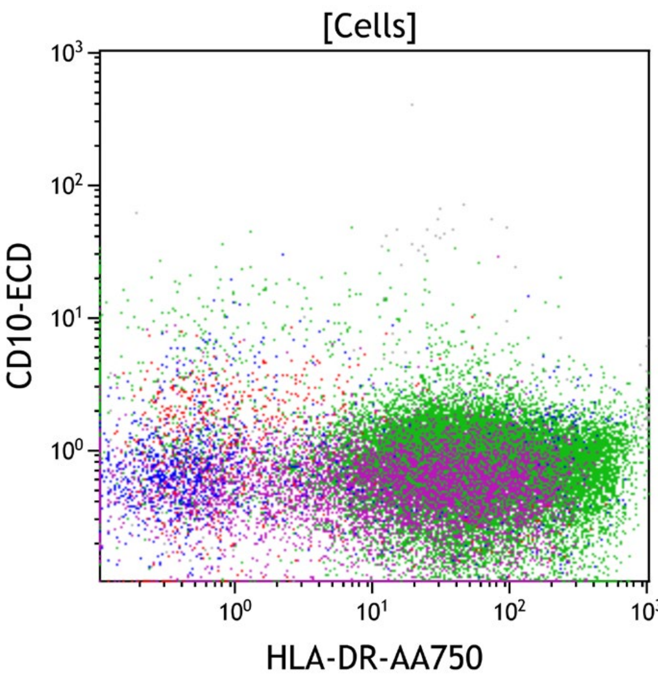

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes and immature B cells. Immature B cells express both CD10 and HLA-DR. The progenitors (purple) and monocytes (green) express variable HLA-DR without CD10.

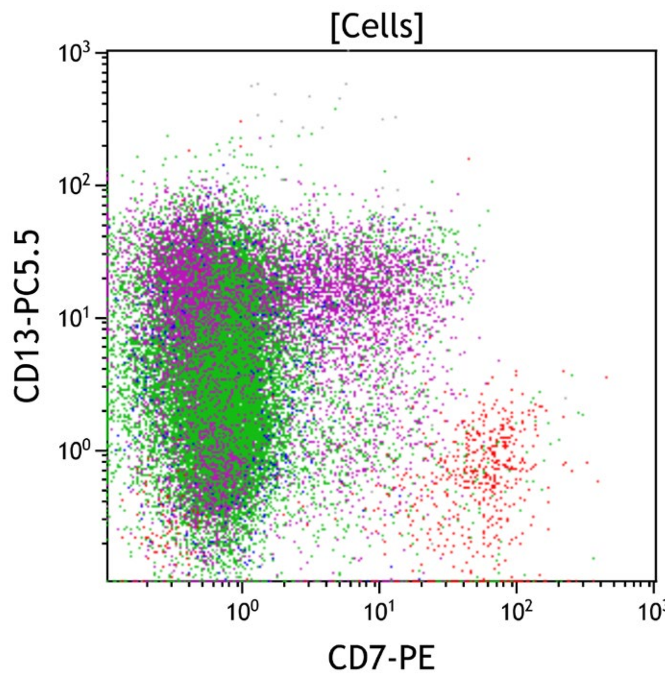

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The progenitors (purple) express CD7 on a subset and intermediate CD13 but the monocytes (green) express variable CD13 with minimal CD7.

M2 Cell Tube

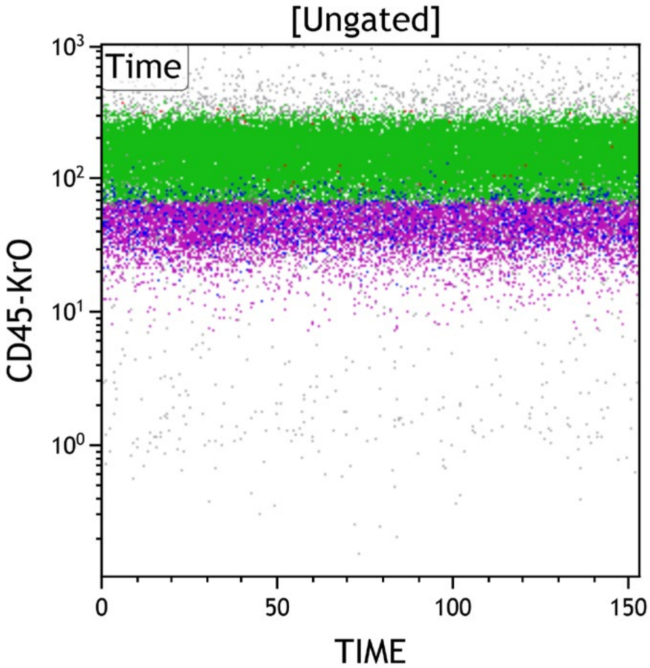

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

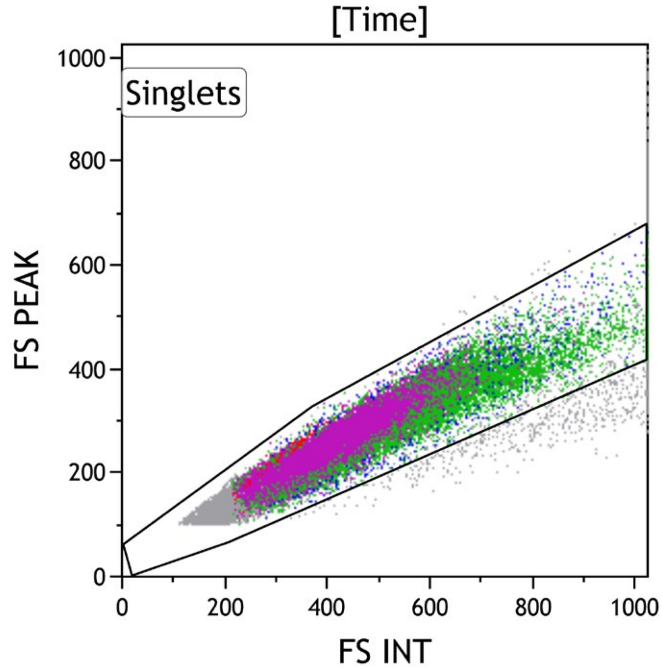

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

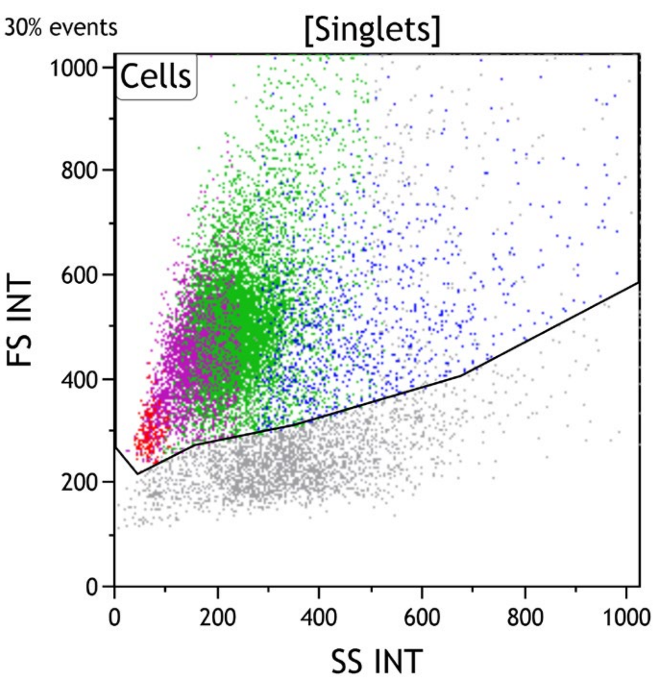

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

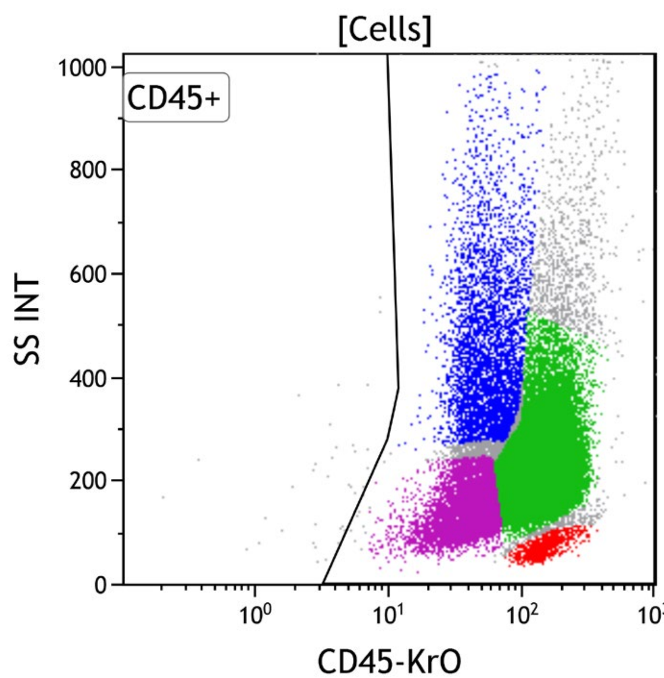

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

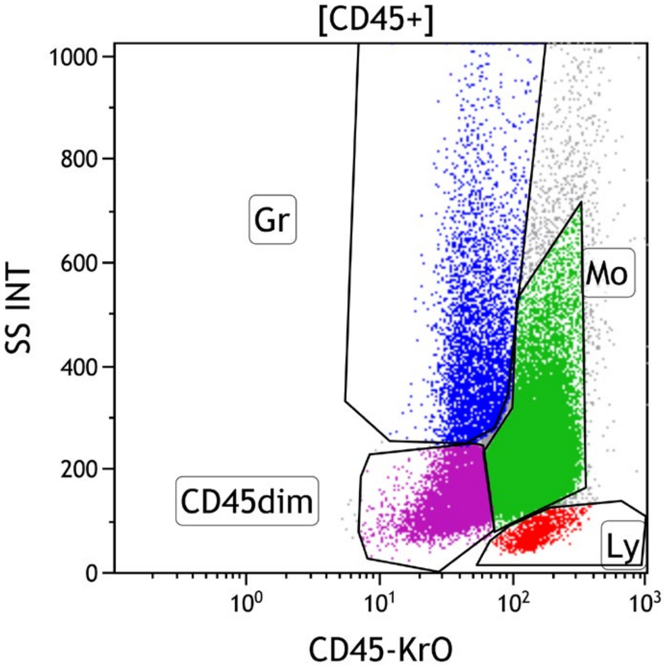

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note that progenitors (purple) and monocytes (green) are relatively expanded in number.

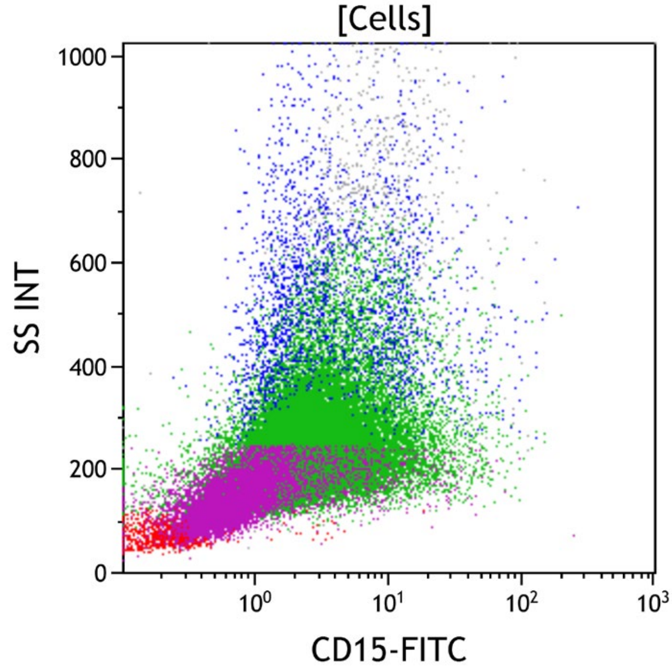

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes. The progenitors (purple) do not express CD15 and monocytes (green) express dim CD15.

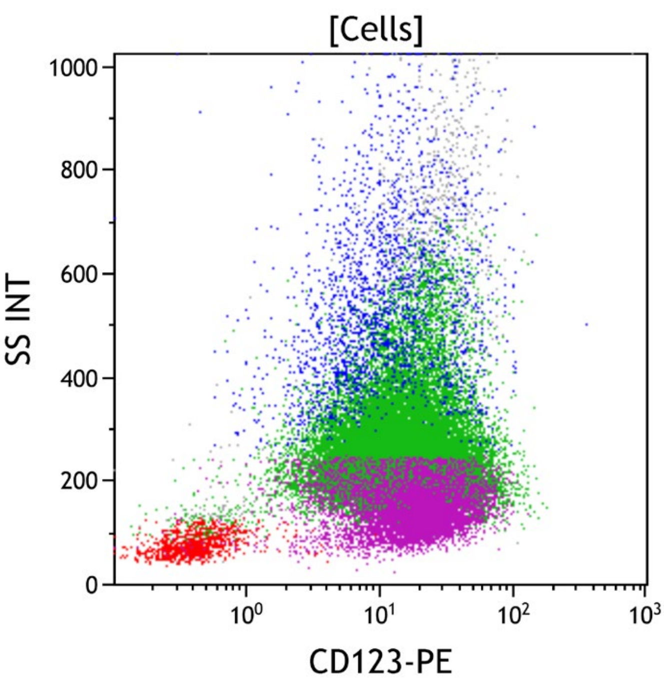

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. The progenitors (purple) and monocytes (green) express intermediate CD123.

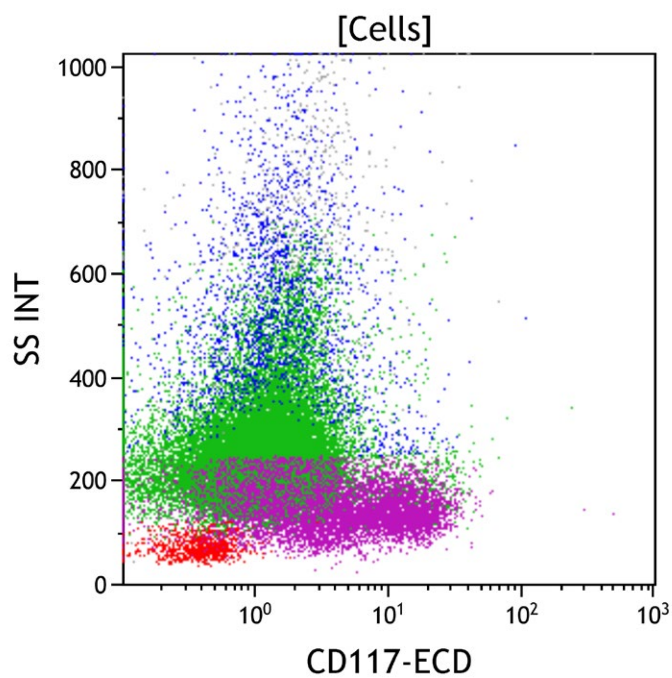

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The progenitors (purple) express dim to intermediate CD117 and monocytes (green) do not express CD117.

M2 Cell Tube

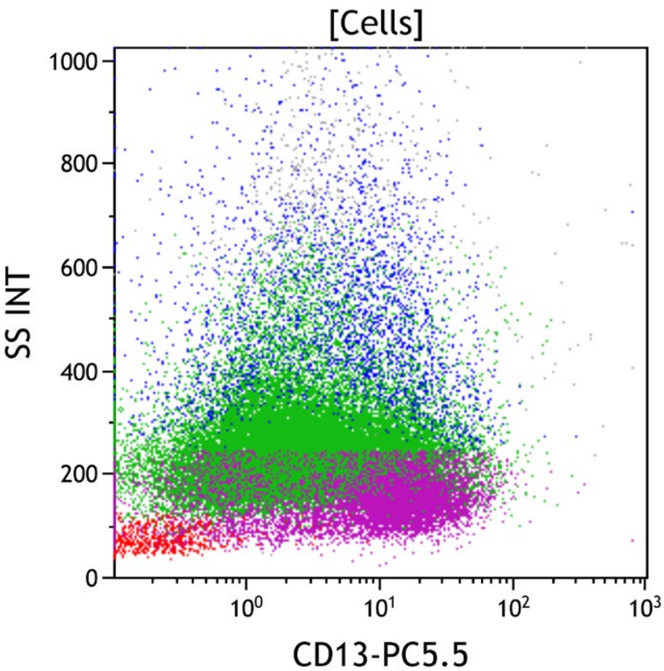

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. The progenitors (purple) express intermediate CD13 and monocytes (green) express dim to intermediate CD13.

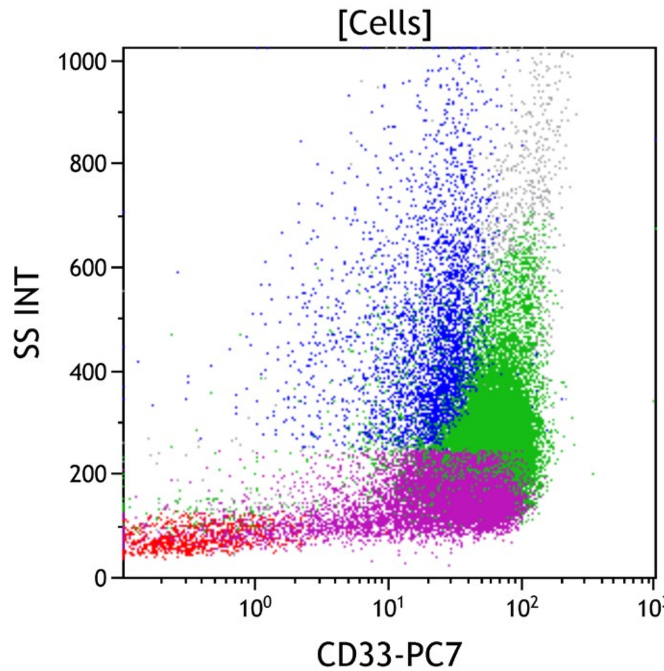

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes, and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. The progenitors (purple) and monocytes (green) express bright CD33.

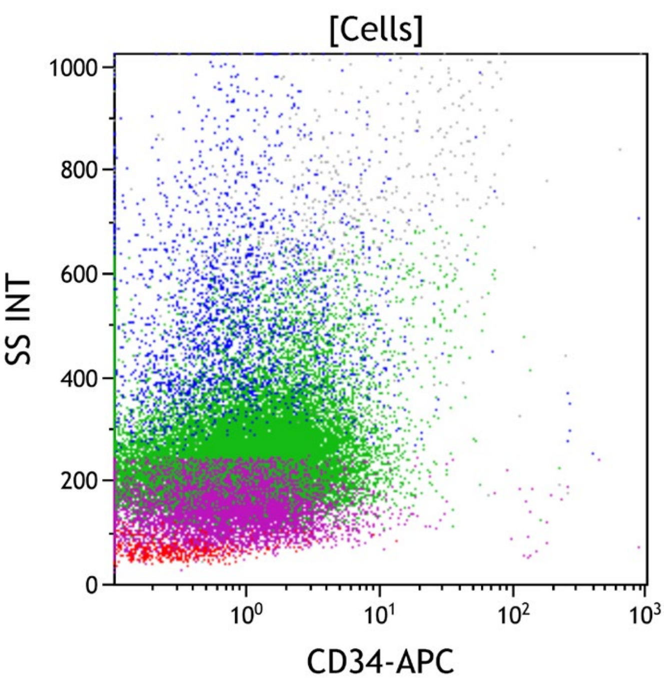

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The progenitors (purple) and monocytes (green) do not express CD34.

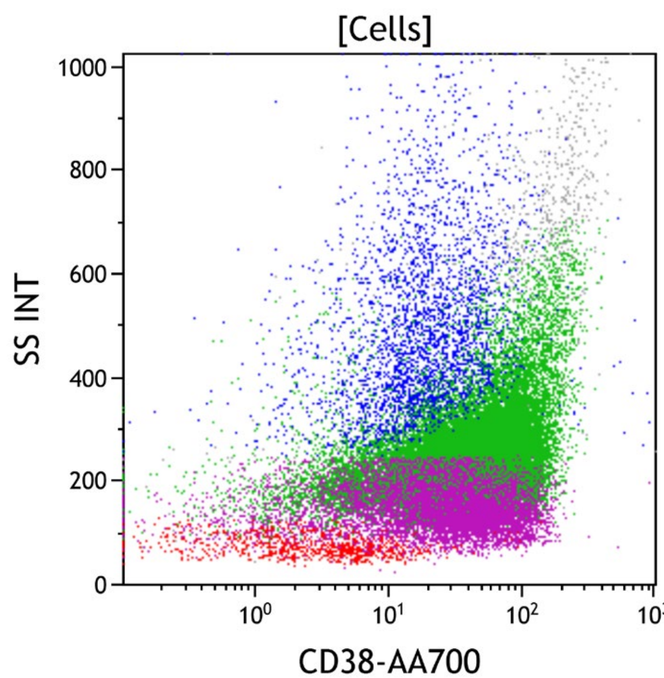

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The progenitors (purple) and monocytes (green) express intermediate CD38.

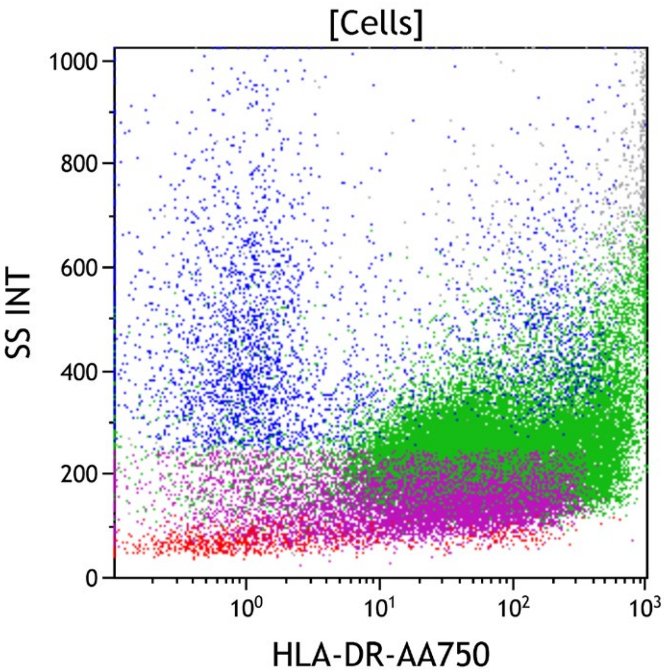

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The progenitors (purple) and monocytes (green) express intermediate to bright HLA-DR.

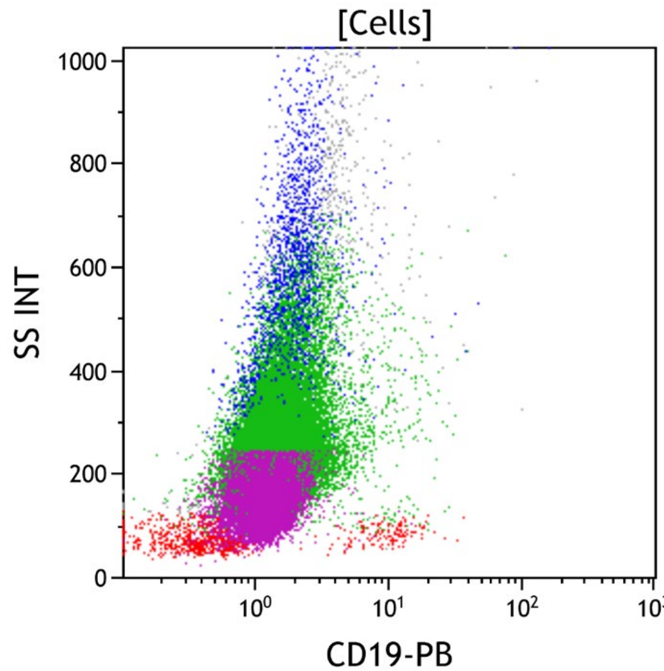

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The progenitors (purple) and monocytes (green) do not express CD19.

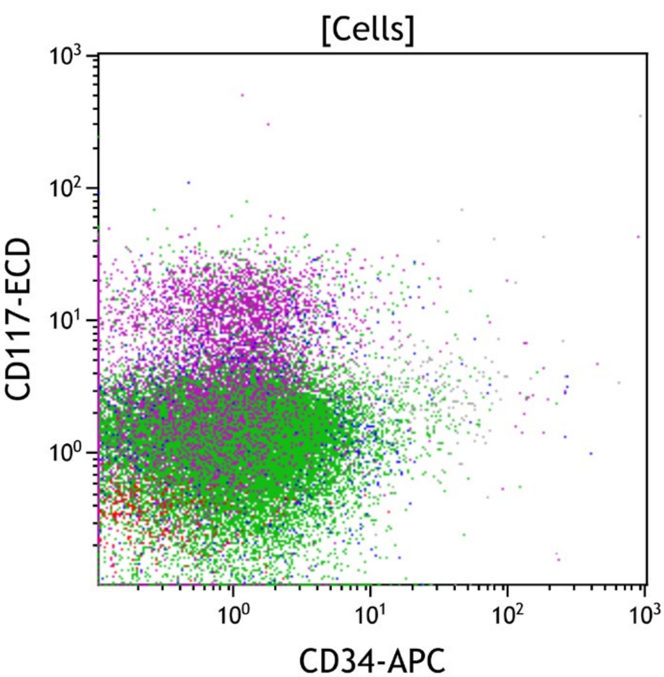

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The progenitors (purple) express CD117 without CD34 and monocytes (green) do not express CD34 or CD117.

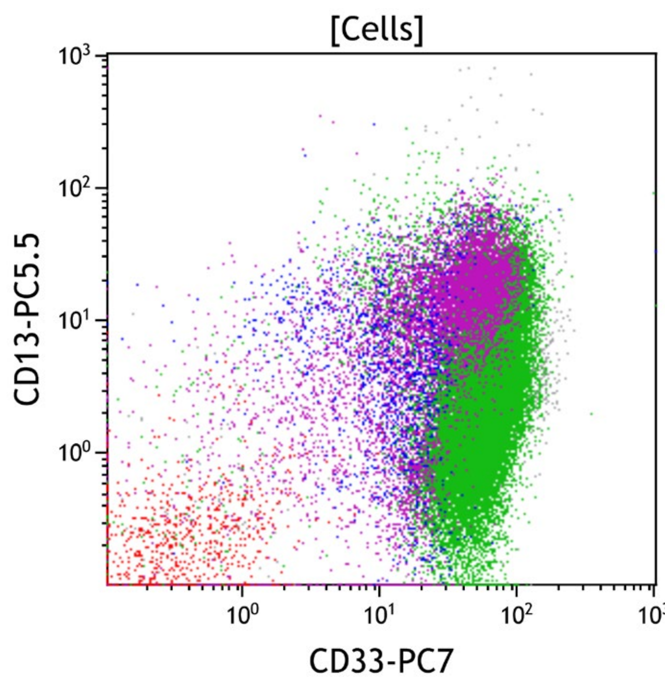

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33 (red). The progenitors (purple) express bright CD33 with intermediate CD13 but monocytes (green) express bright CD33 with dim to intermediate CD13.

M2 Cell Tube

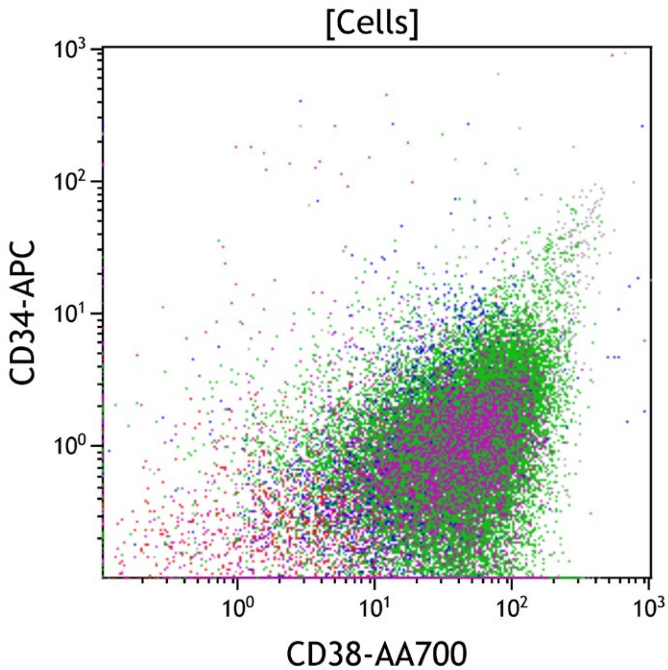

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38. The progenitors (purple) and monocytes (green) express intermediate CD38 without CD34.

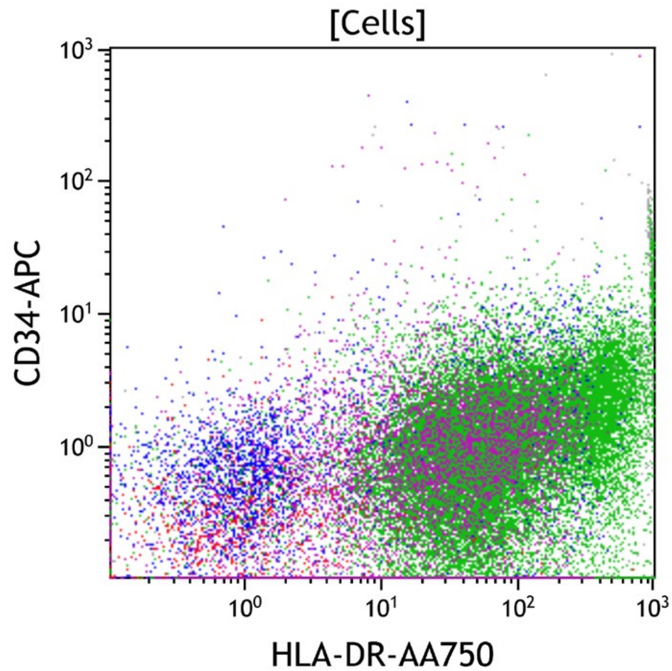

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The progenitors (purple) and monocytes (green) express intermediate to bright HLA-DR without CD34.

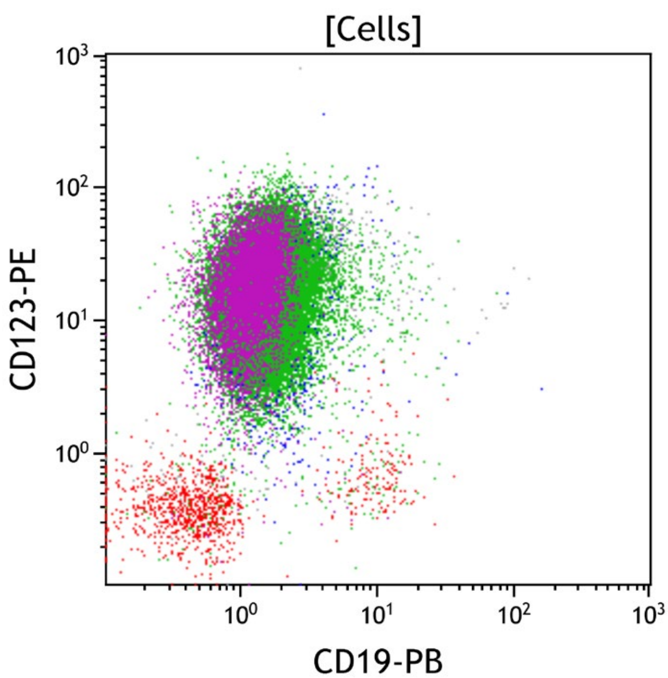

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The progenitors (purple) and monocytes (green) express intermediate CD123 without CD19.

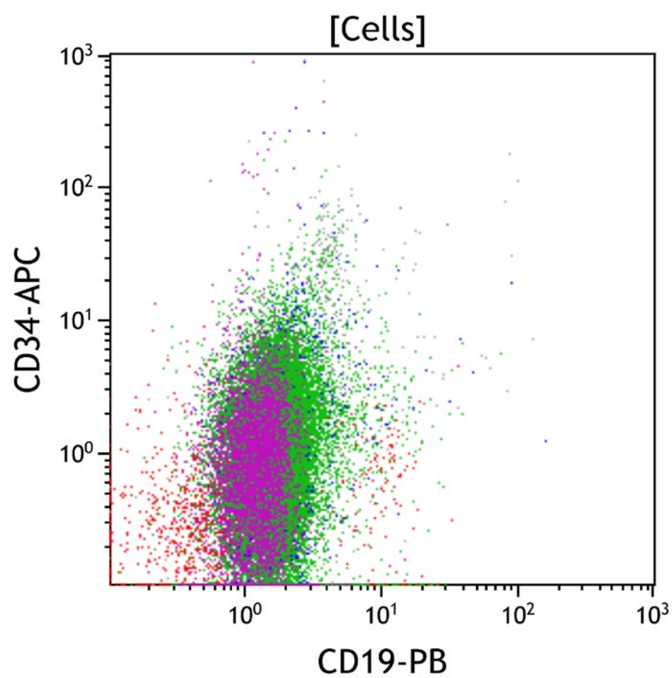

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. The progenitors (purple) and monocytes (green) do not express CD19 or CD34.

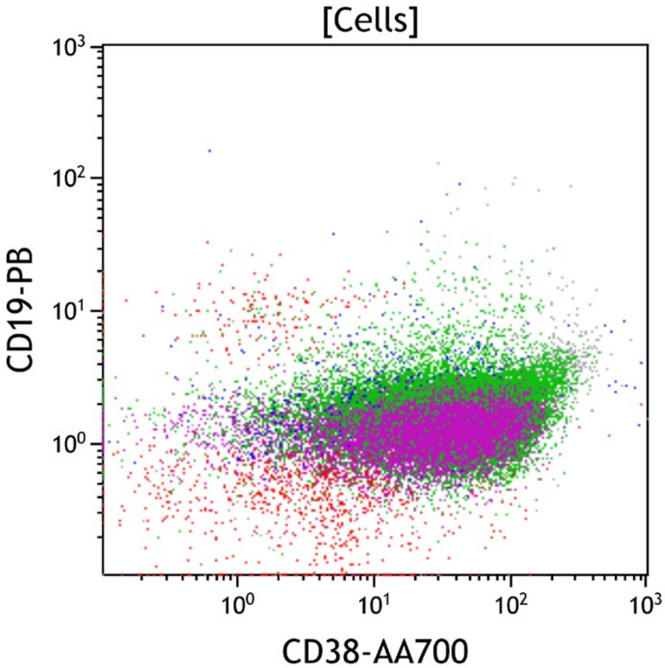

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38. The apparent CD19 positivity on maturing granulocytes is due to autofluorescence, largely from eosinophils. The progenitors (purple) and monocytes (green) express express intermediate CD38 without CD19.

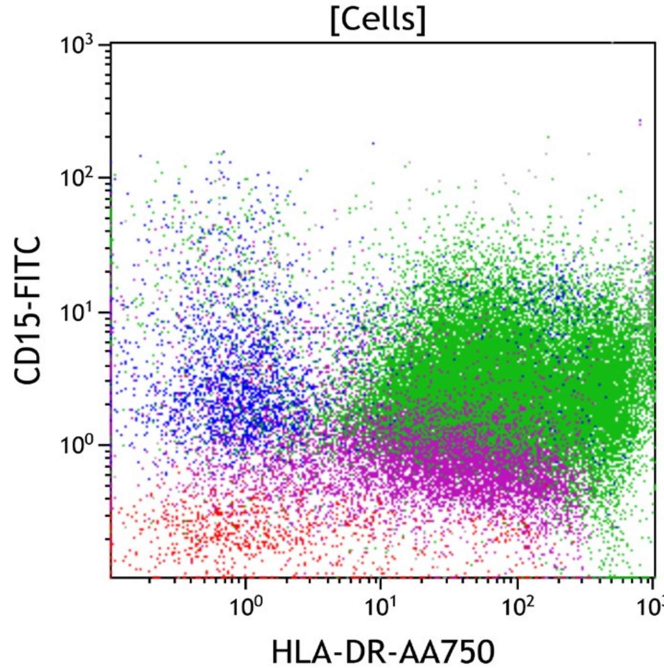

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR (blue), except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. The progenitors (purple) express intermediate to bright HLA-DR without CD15 and monocytes (green) express intermediate to bright HLA-DR with dim CD15.

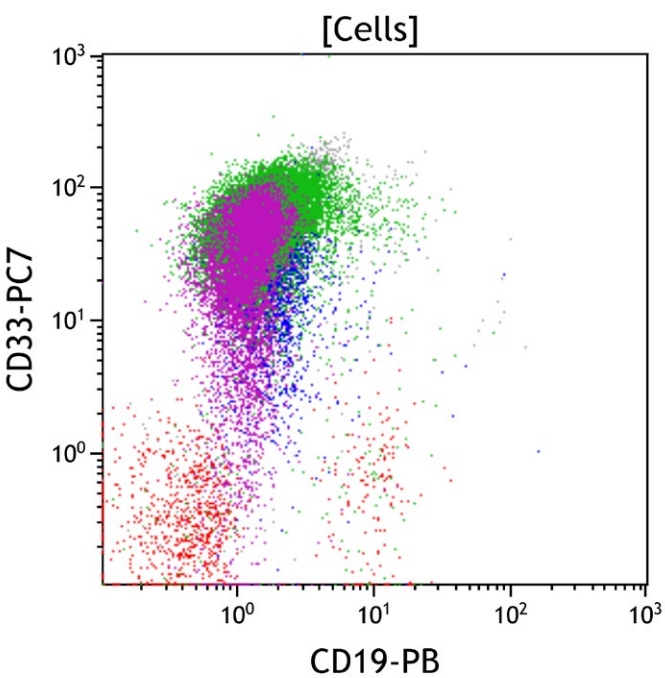

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes and maturing granulocytes. CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes is due to autofluorescence, largely from eosinophils. The progenitors (purple) and monocytes (green) express bright CD33 without CD19.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies two phenotypically distinct populations of cells. The first are progenitors with expression of intermediate CD4, dim variable CD7 on a subset, intermediate CD13, bright CD33, intermediate CD38, dim CD45, low to intermediate CD117, intermediate CD123 and intermediate to bright HLA-DR without CD14, CD15, CD34, CD64 and other lymphoid or myeloid antigens. Compared with normal CD34-positive progenitors, the expression of CD7, increased expression of CD33, and absence of CD34 is aberrant. The second population is monocytes with expression of intermediate CD4, bright CD11b, dim to intermediate CD13, dim to intermediate CD14 on a small subset, dim CD15, bright CD33, intermediate CD38, bright CD64, intermediate CD123 and intermediate to bright HLA-DR without CD34, CD117 and other lymphoid or myeloid antigens. Compared with normal mature monocytes, the decreased expression of CD13 and CD14 is aberrant.

The immunophenotype of the abnormal populations are those of expanded abnormal CD34-positive progenitors and immature monocytic progenitors, respectively, and consistent with acute myeloid leukemia having monocytic differentiation. Additional testing from the subject shows the presence of 96% blasts by morphology 3-4% positivity by myeloperoxidase, these findings in combination with the immunophenotypic data, is indicative of an acute monocytic leukemia.

Case #23: Myelodysplastic Syndrome

Clinical Vignette

This 72-year-old male presents with anemia and thrombocytopenia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

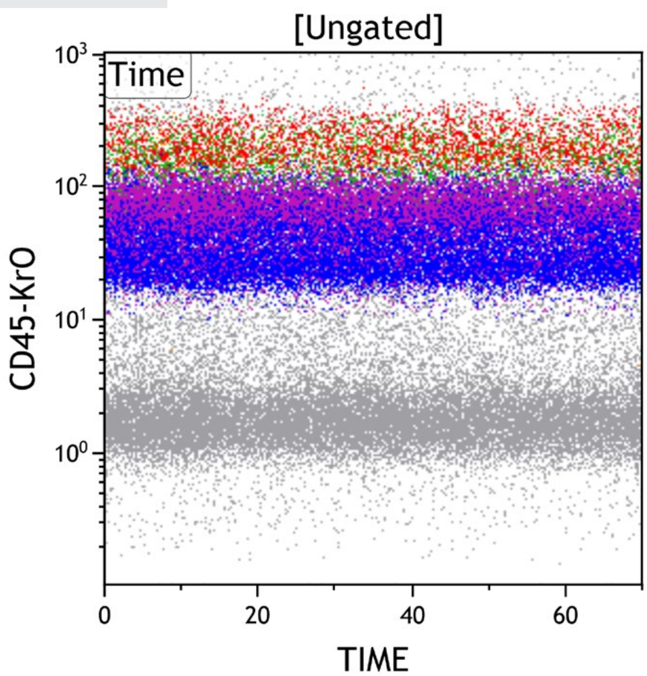

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

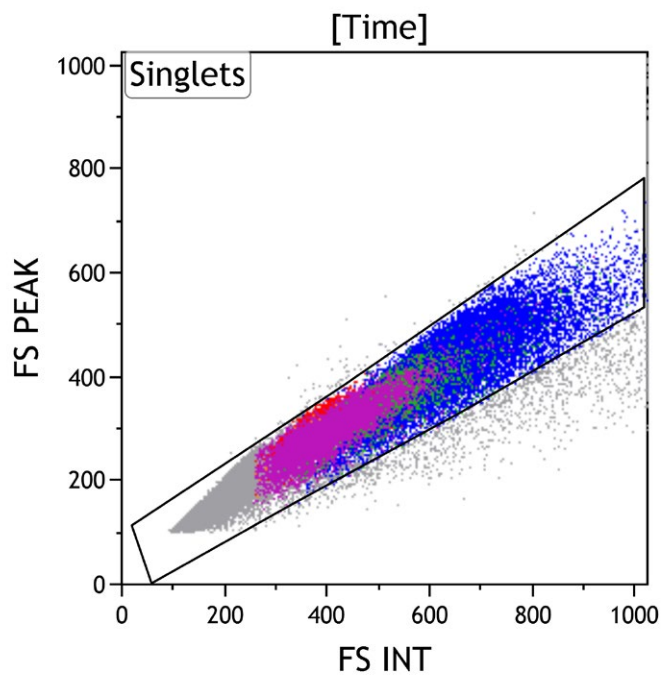

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

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B Cell Tube

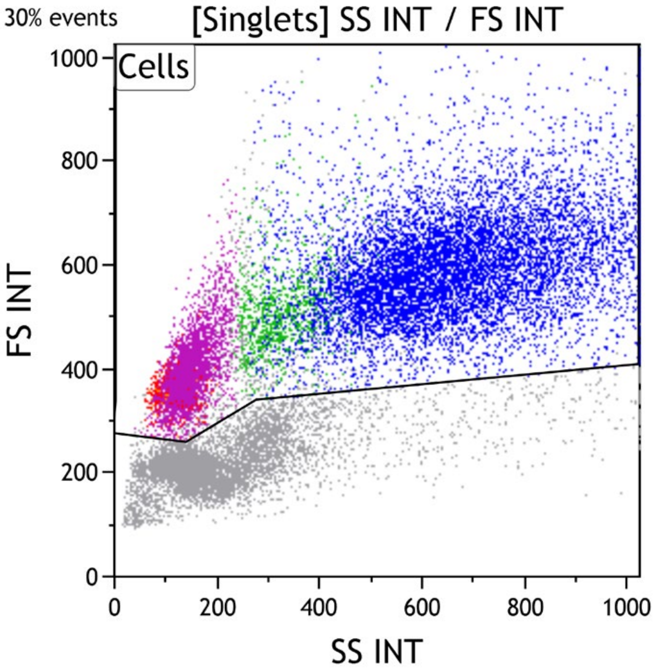


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

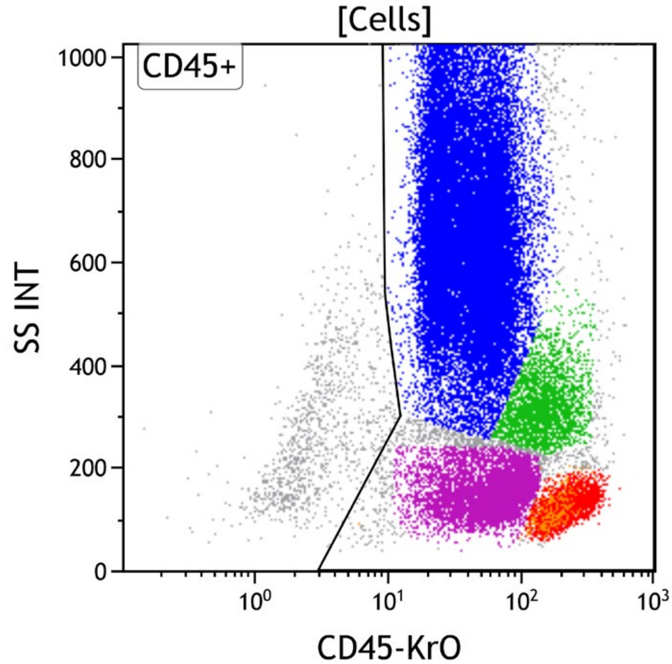


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells. Note the relatively increased number of progenitors (purple).

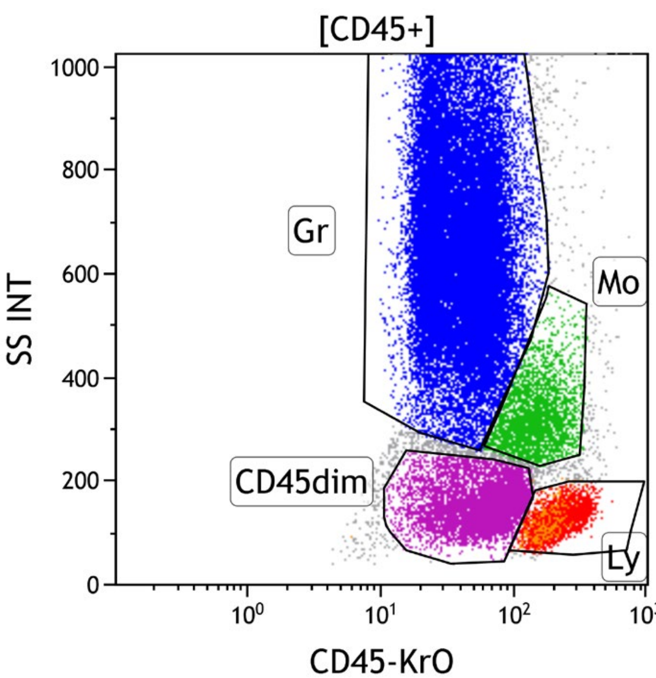


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. The CD45 dim population is mildly increased.

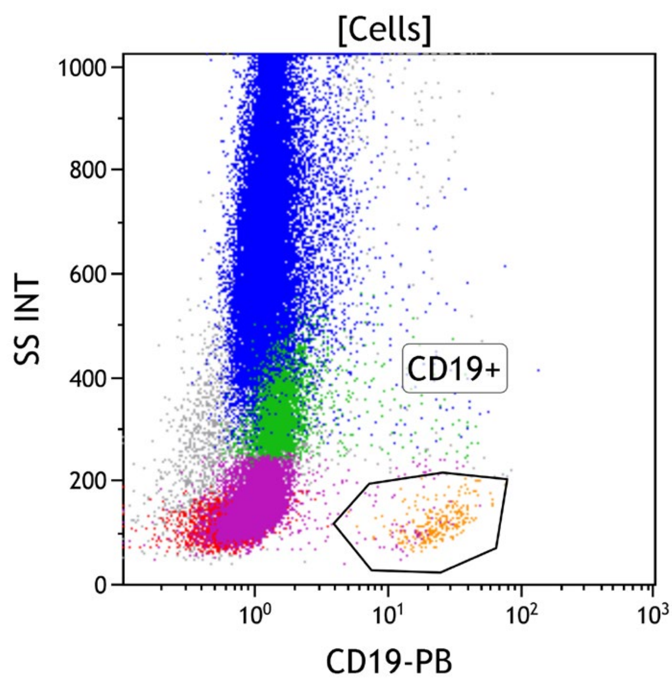


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The expanded progenitors (purple) do not express CD19.

B Cell Tube

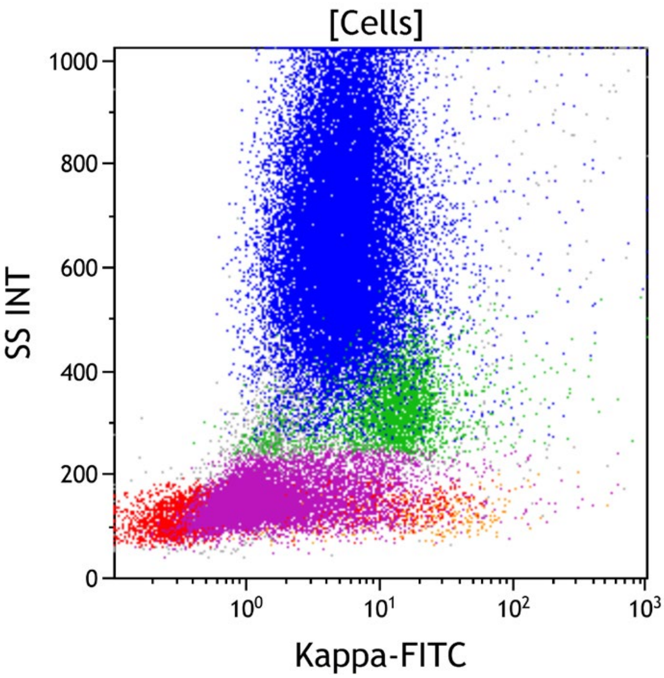


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The expanded progenitors (purple) do not express kappa.

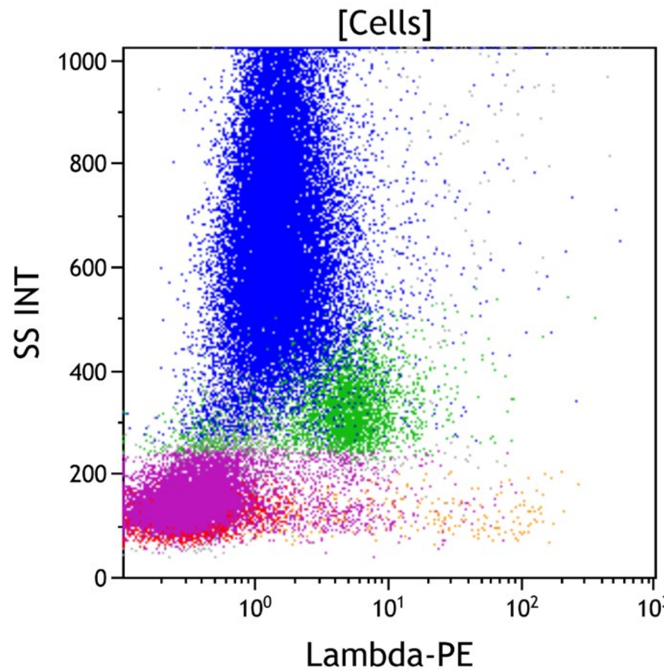


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The expanded progenitors (purple) do not express lambda.

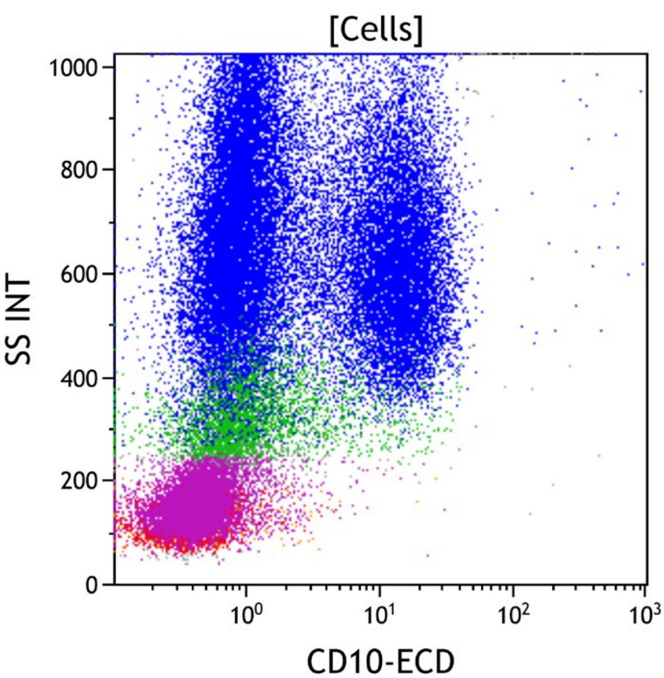


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells , mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The expanded progenitors (purple) do not express CD10.

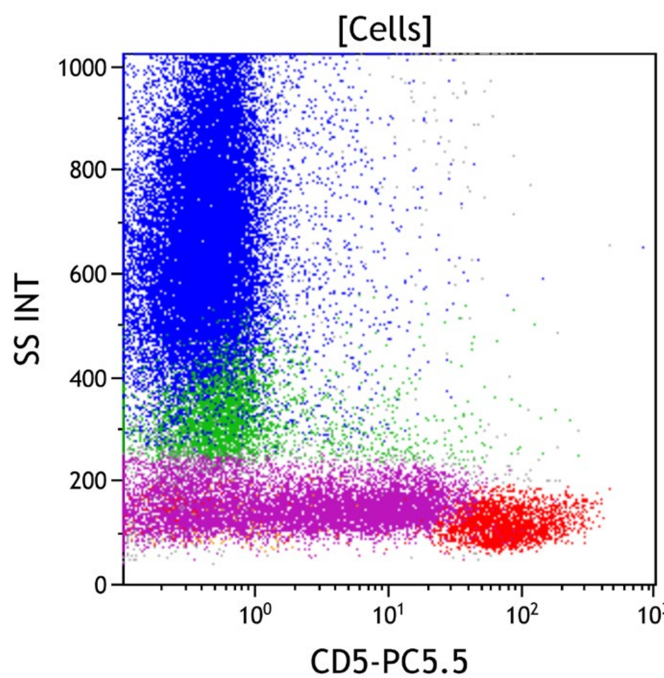


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. Most of the progenitors (purple) express dim CD5.

B Cell Tube

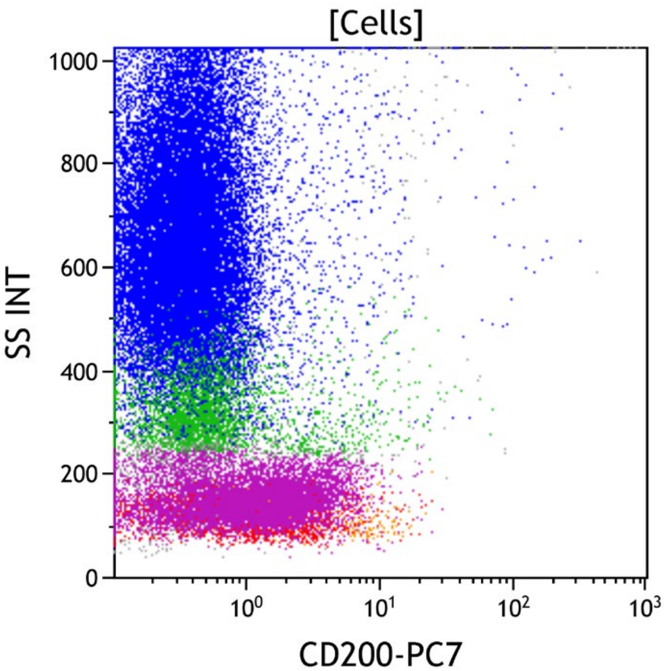


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The expanded progenitors (purple) do not express CD200.

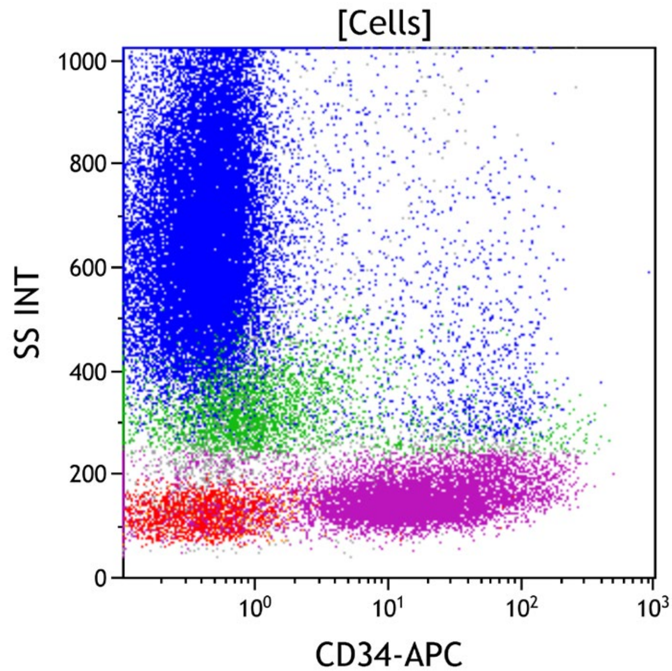


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The expanded progenitors (purple) express CD34.

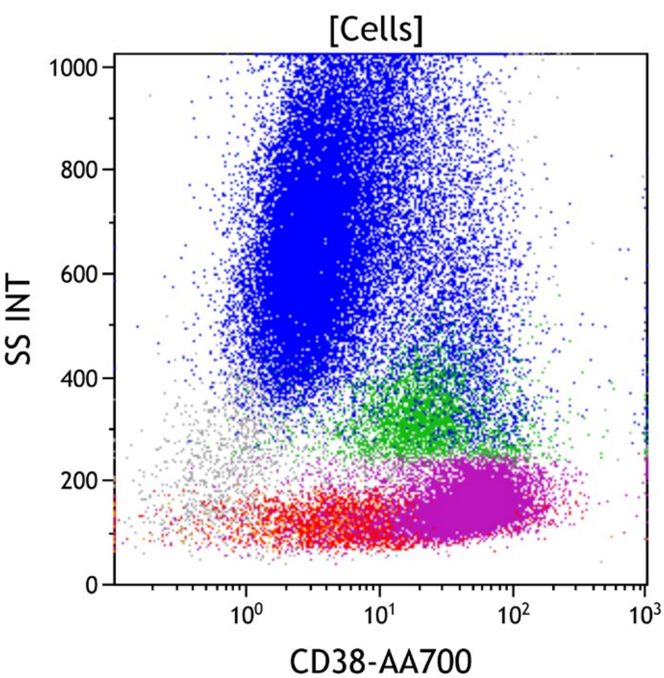


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The expanded progenitors (purple) express intermediate CD38.

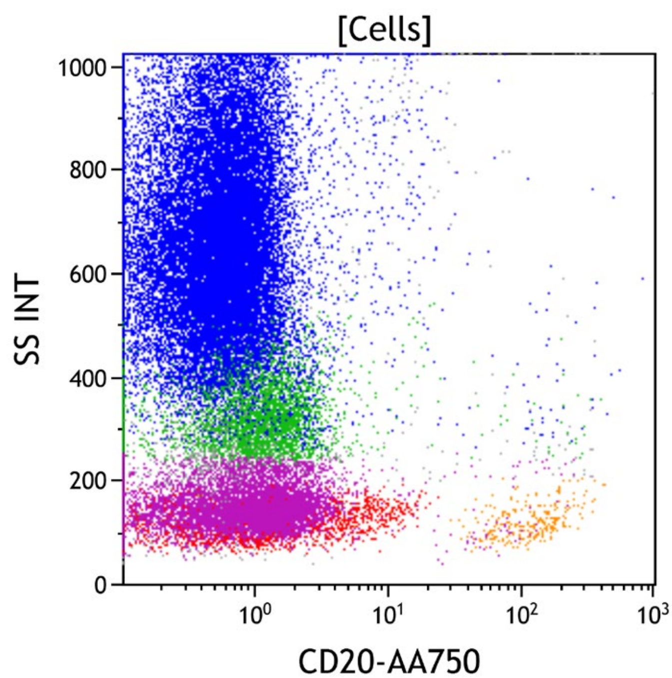


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The expanded progenitors (purple) do not express CD20.

B Cell Tube

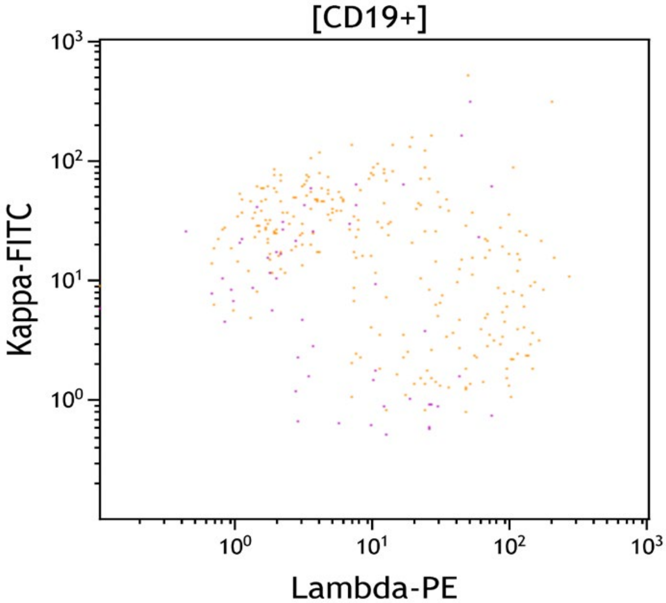


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

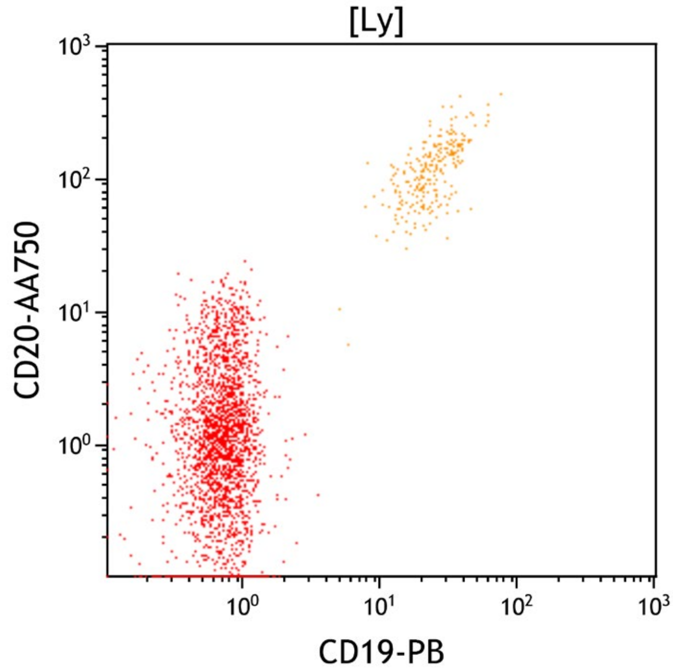


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

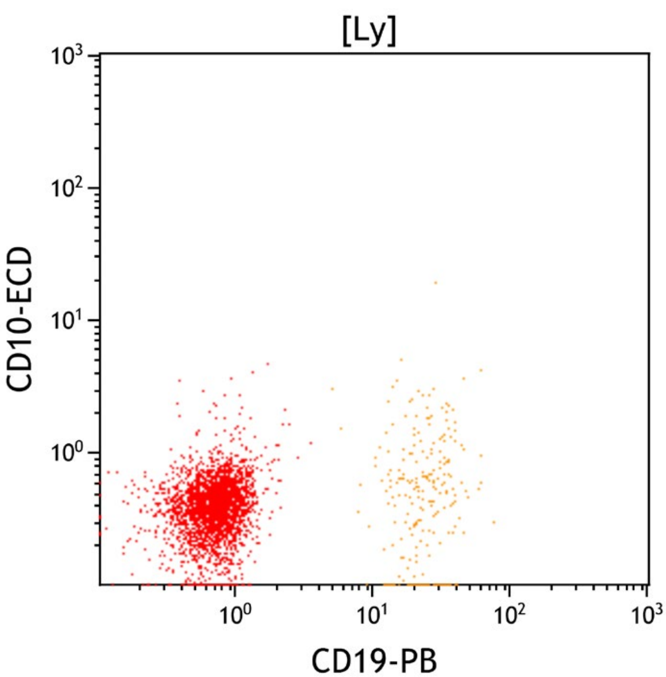


Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

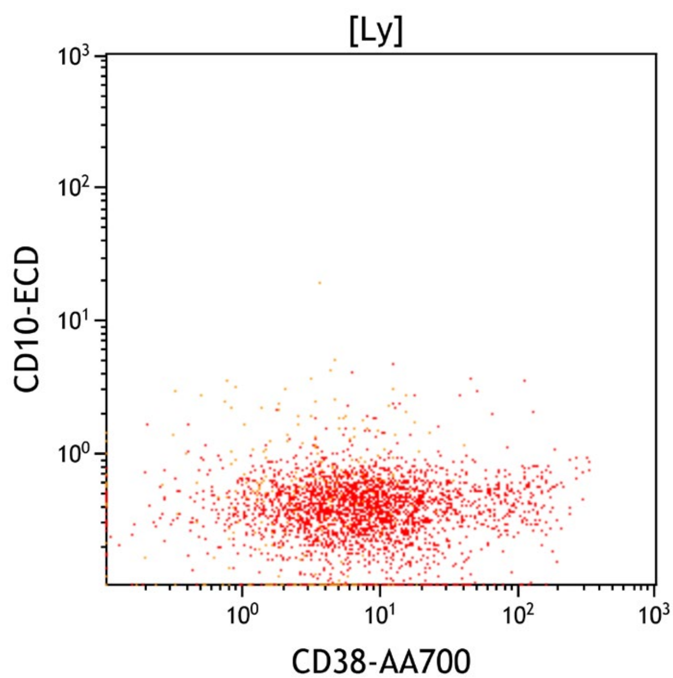
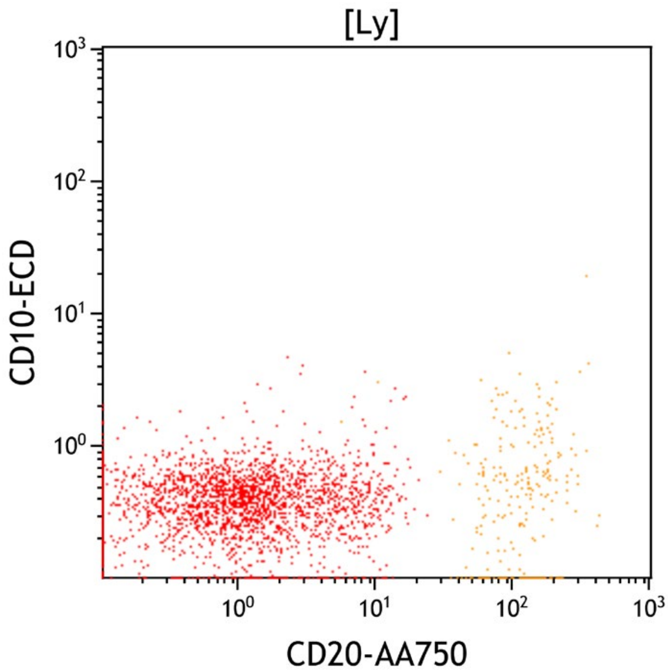


Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

B Cell Tube



igure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

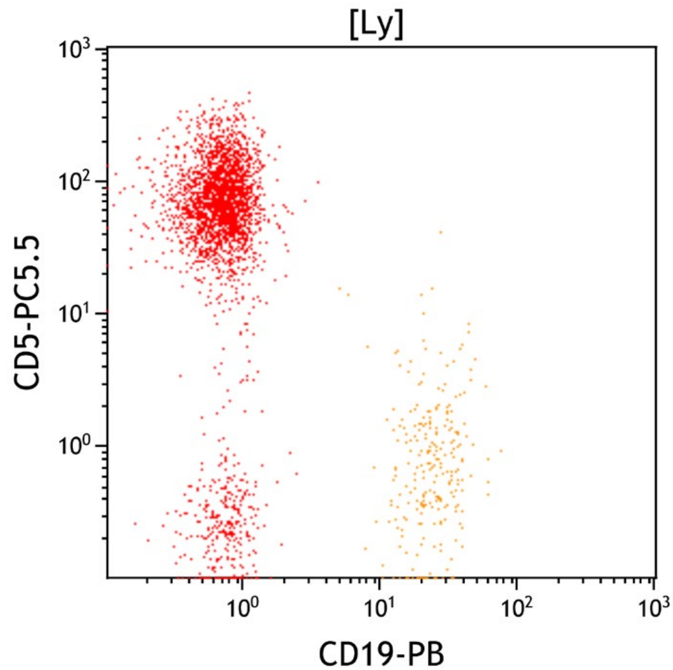


Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

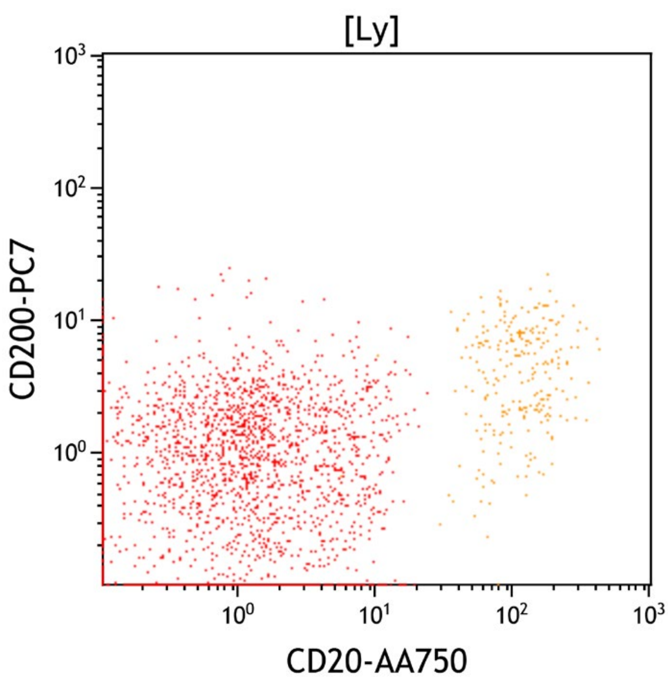


Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

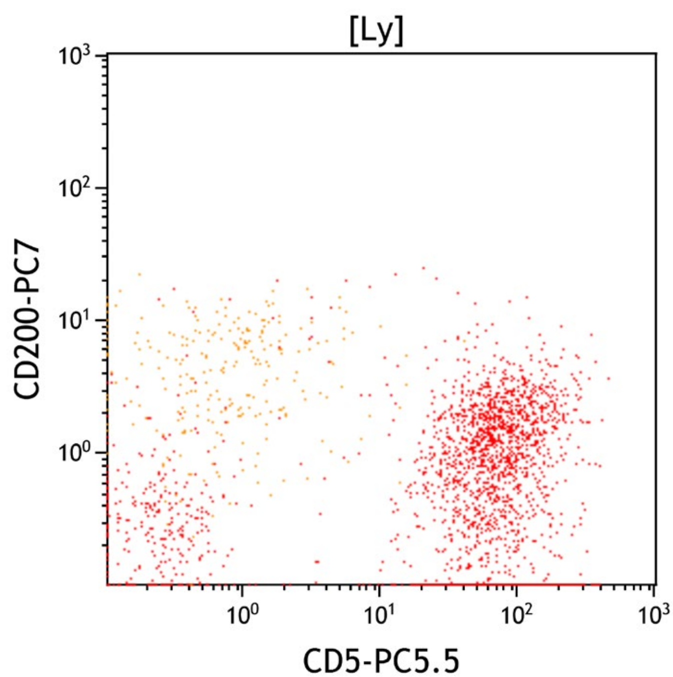


Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

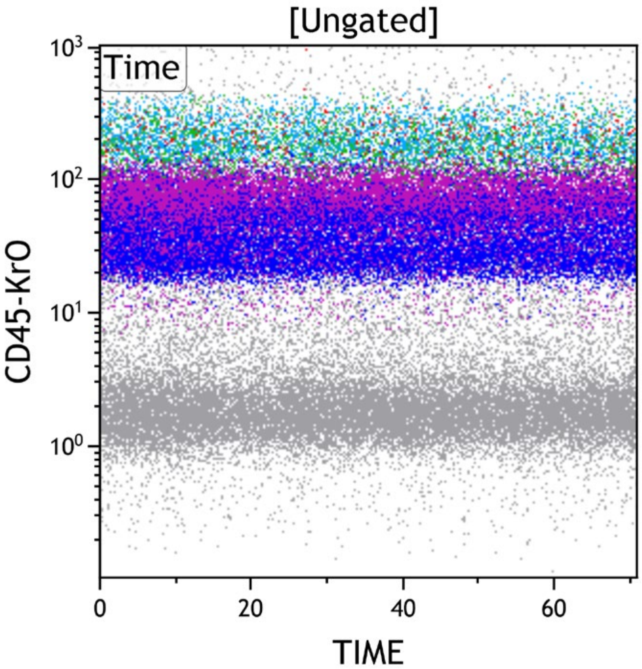


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

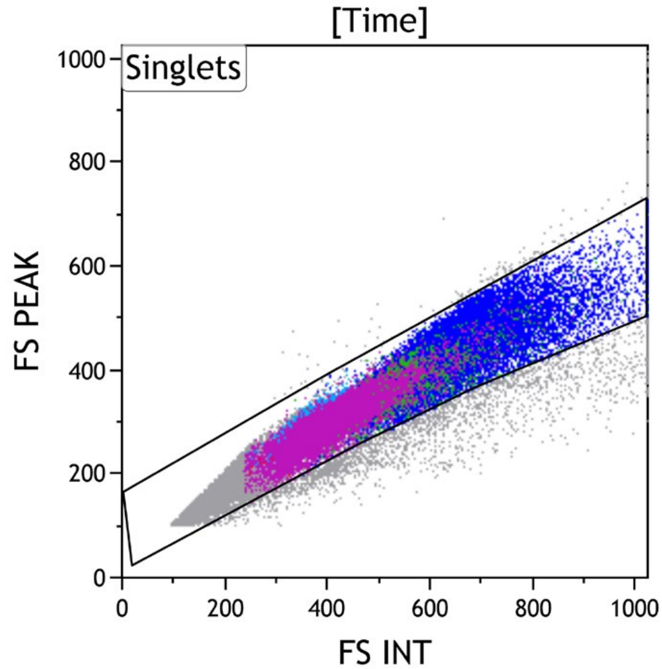


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

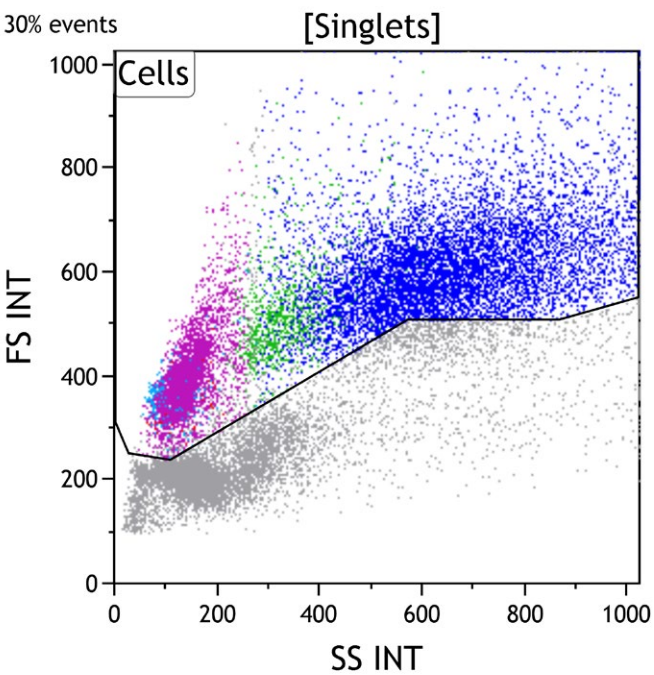


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

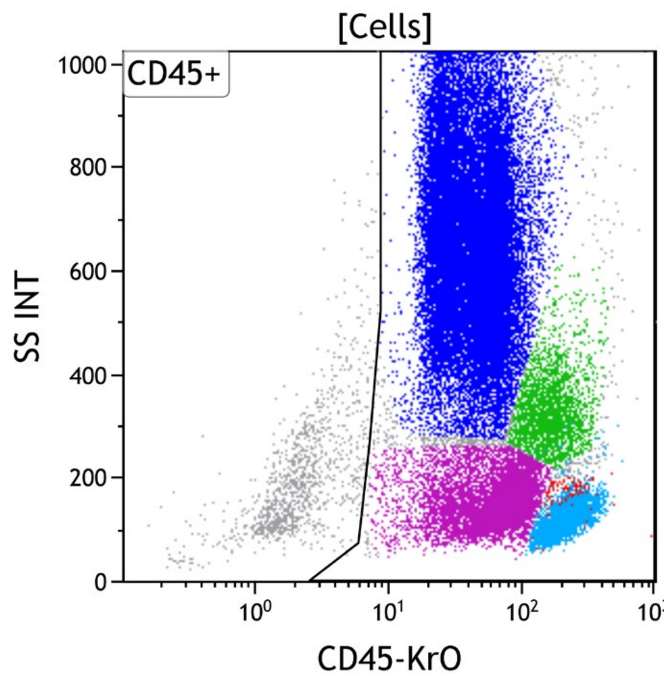


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

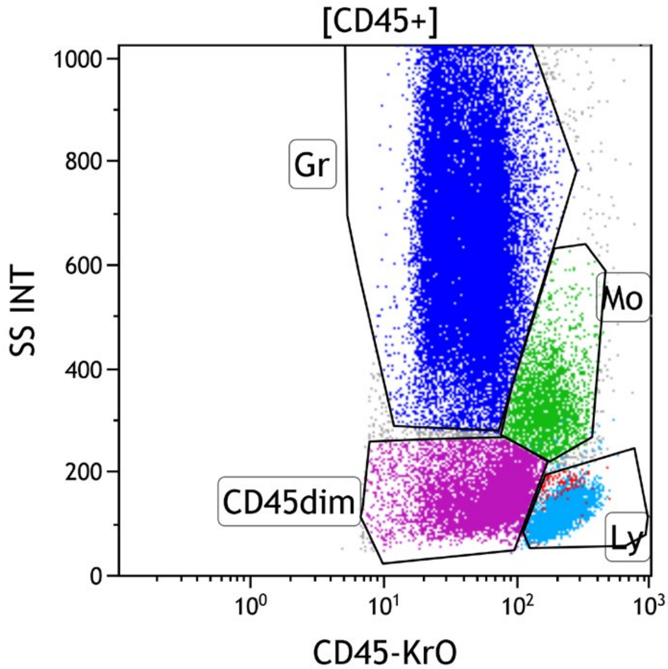


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased number of progenitors (purple)

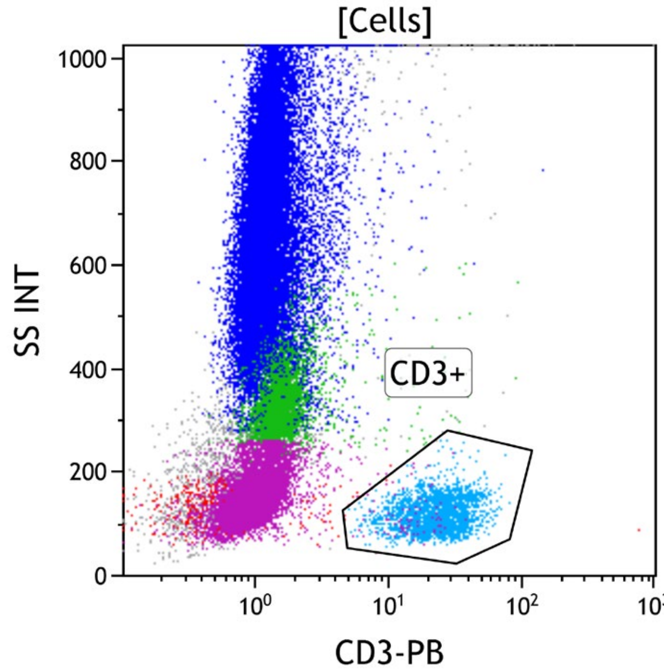


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The expanded progenitors (purple) do not express CD3.

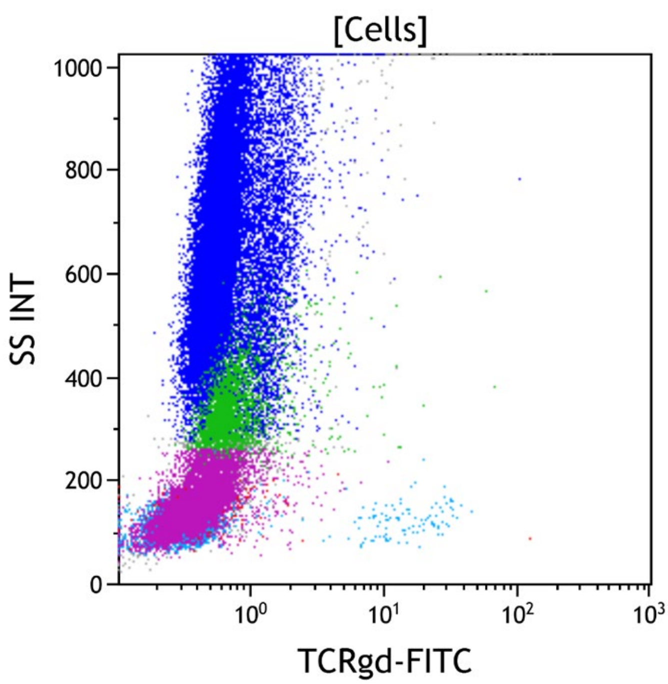


Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The expanded progenitors (purple) do not express TCRγδ.

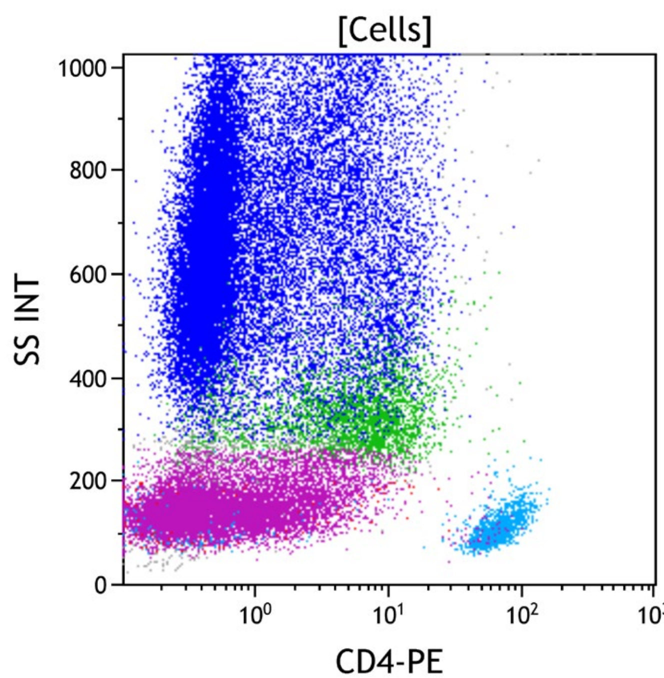


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The expanded progenitors (purple) express dim CD4 on a subset.

T Cell Tube

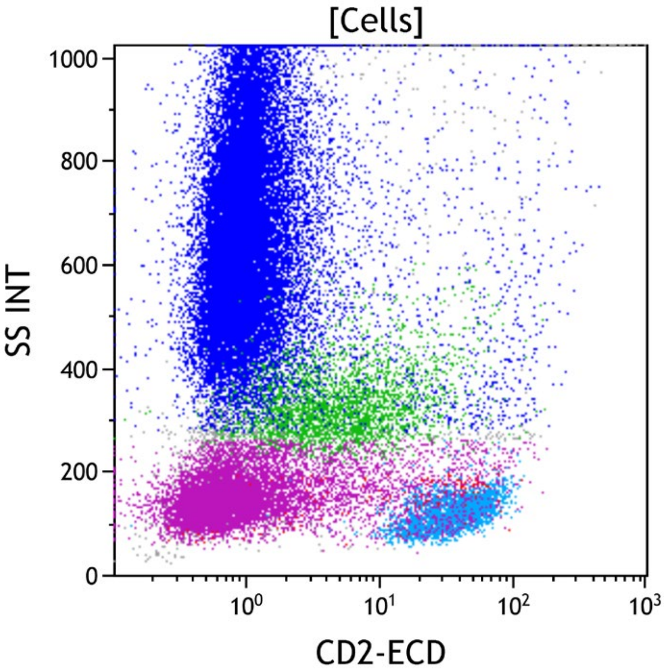


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on rare NK cells (red) and at a low level on monocytes (green). The expanded progenitors (purple) do not express CD2.

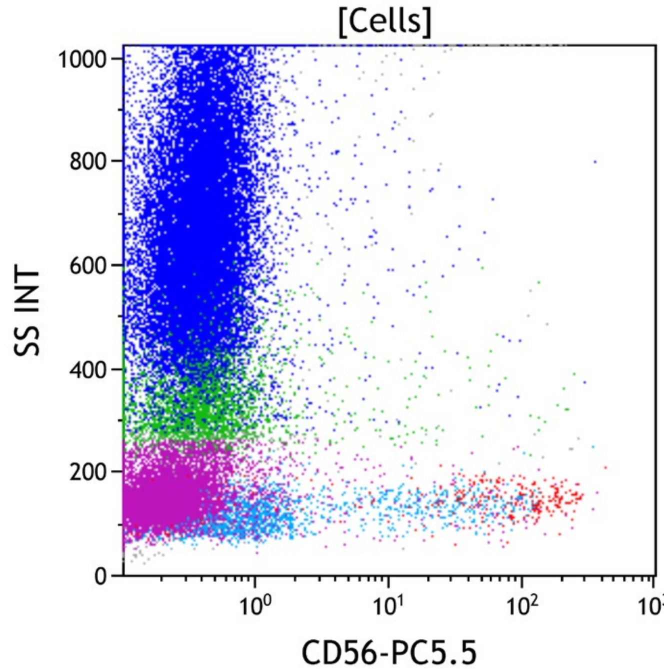


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The expanded progenitors do not express CD56.

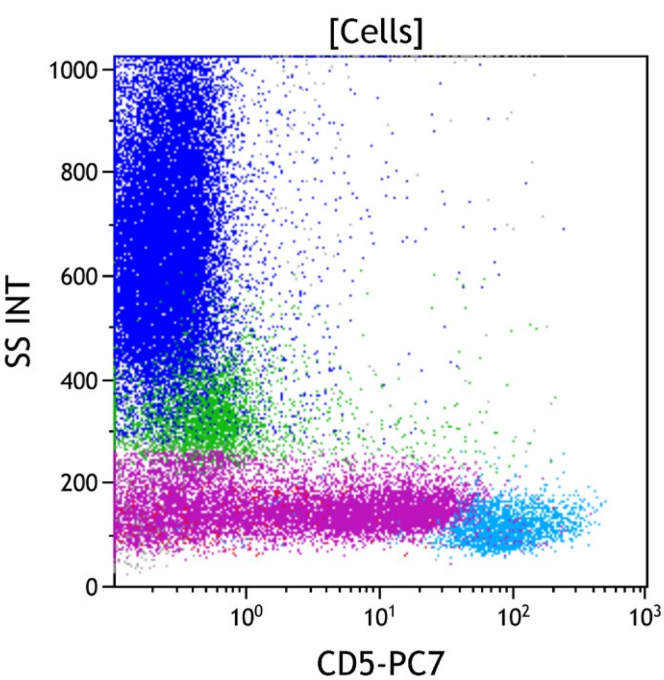


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Most of the expanded progenitors (purple) express variable CD5.

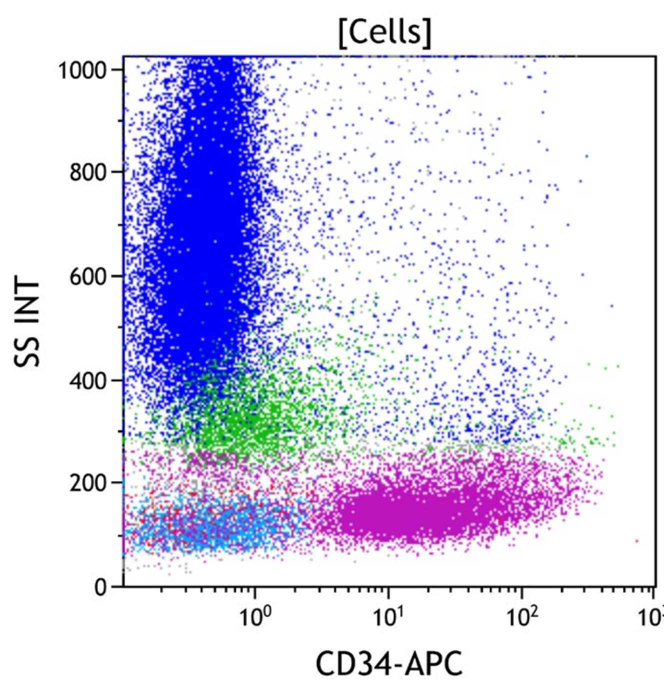


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The expanded progenitors (purple) express CD34.

T Cell Tube

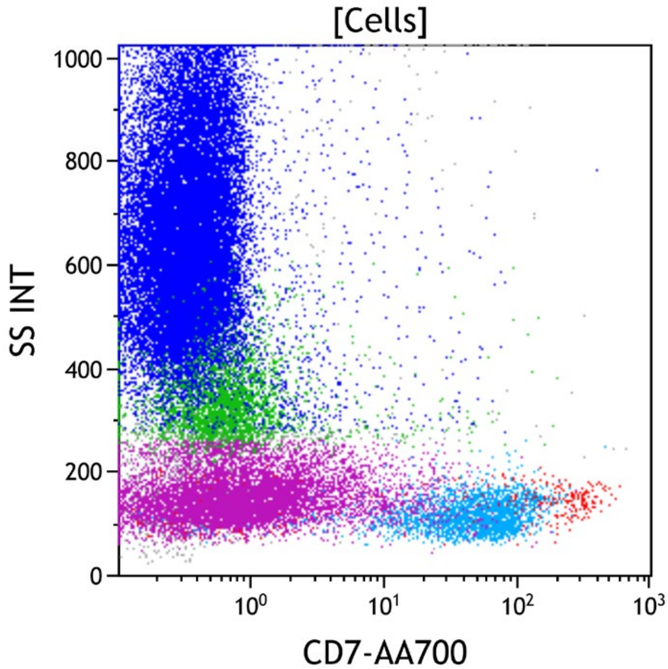


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The expanded progenitors (purple) do not express CD7.

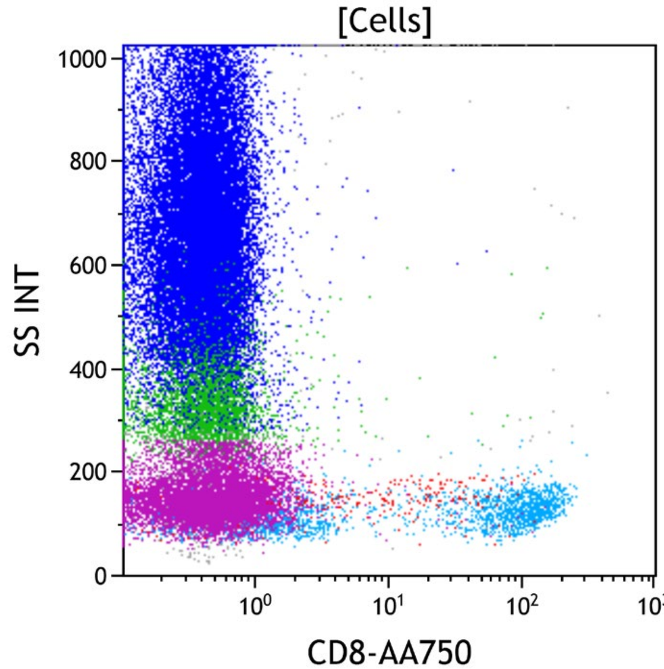


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The expanded progenitors (purple) do not express CD8.

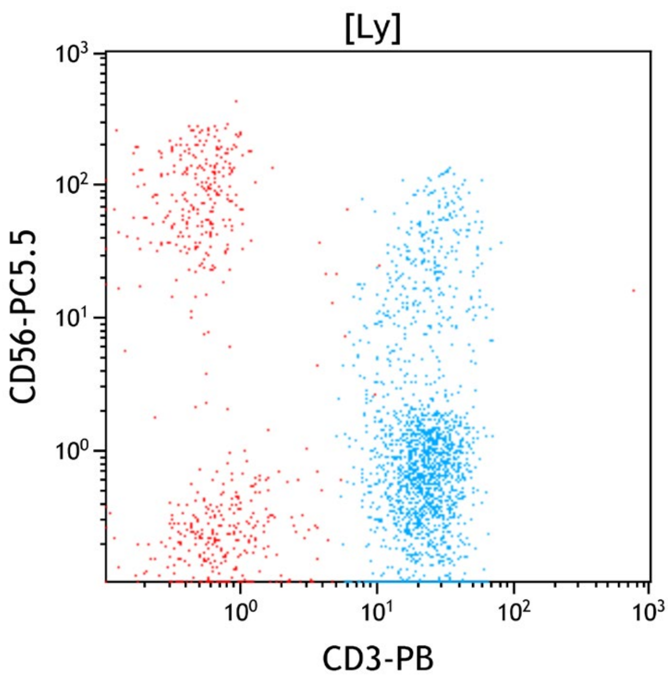


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

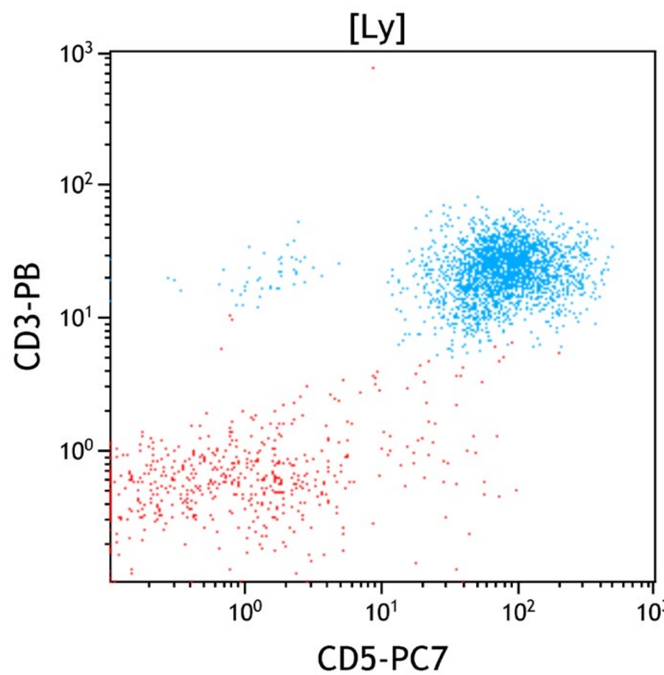


Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

T Cell Tube

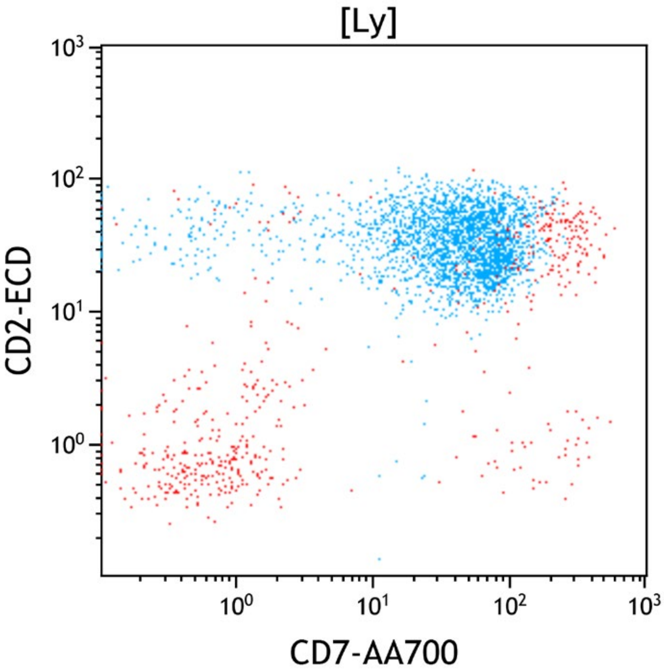


Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

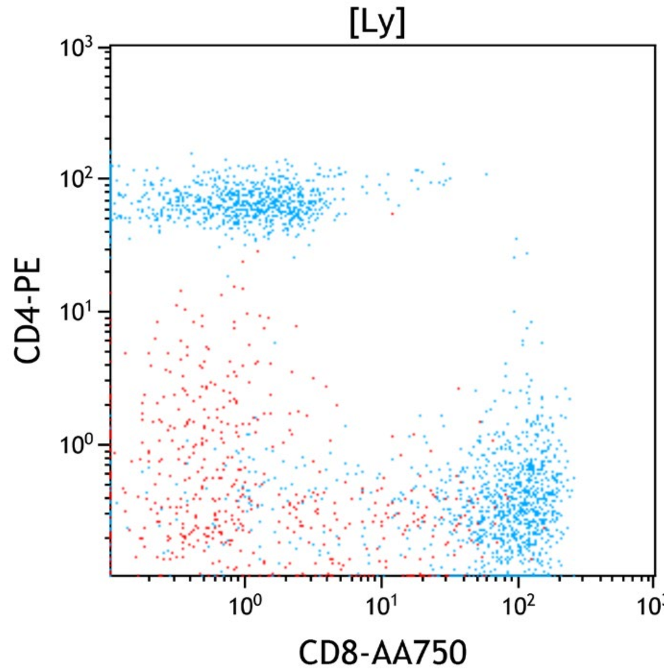


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

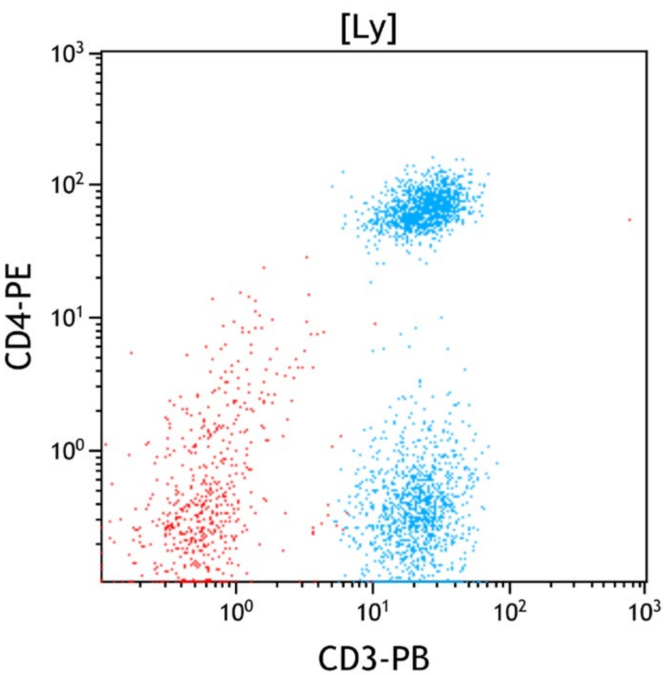


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

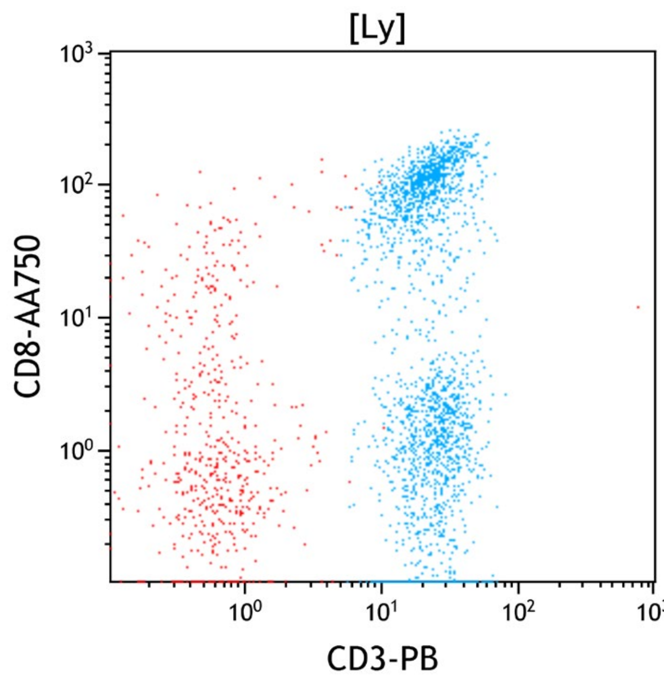


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) without CD3.

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T Cell Tube

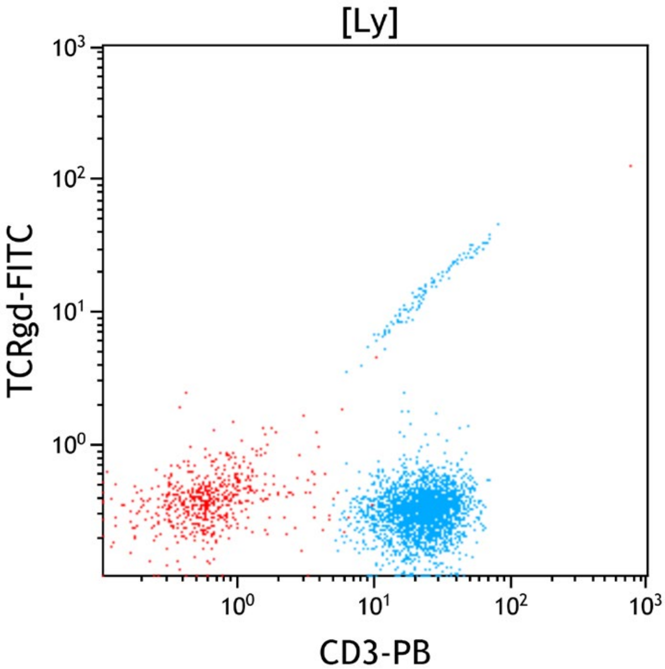

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

M1 Cell Tube

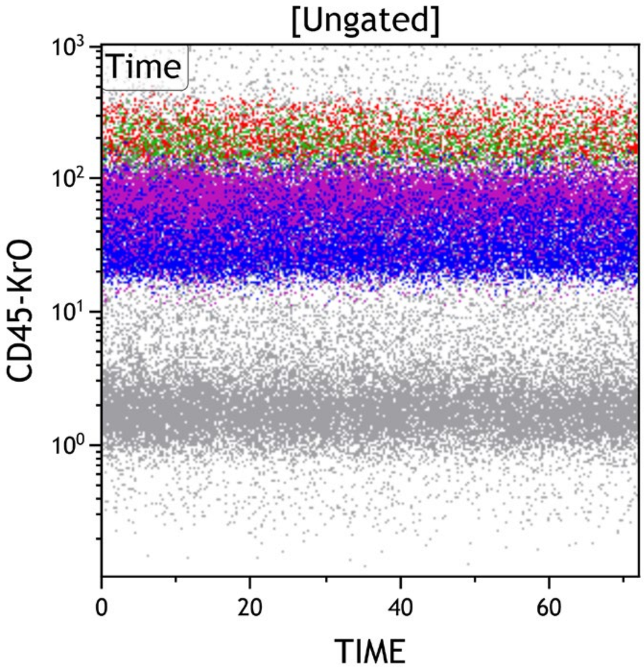

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

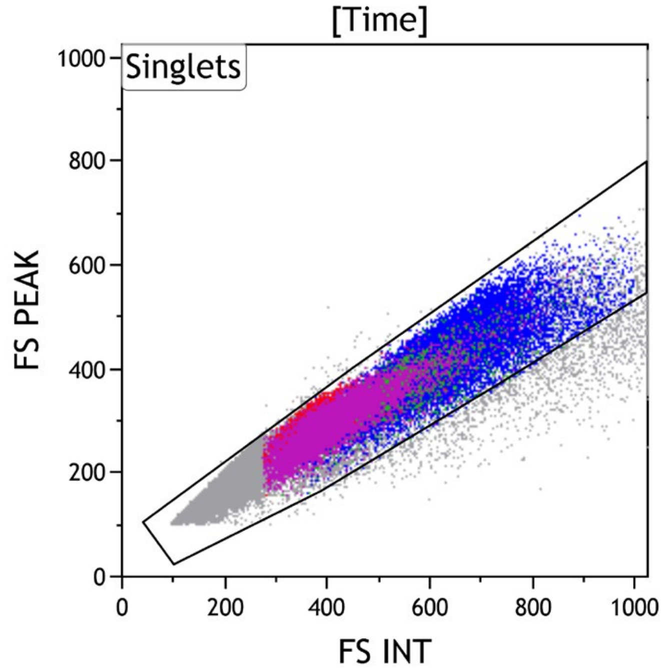

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

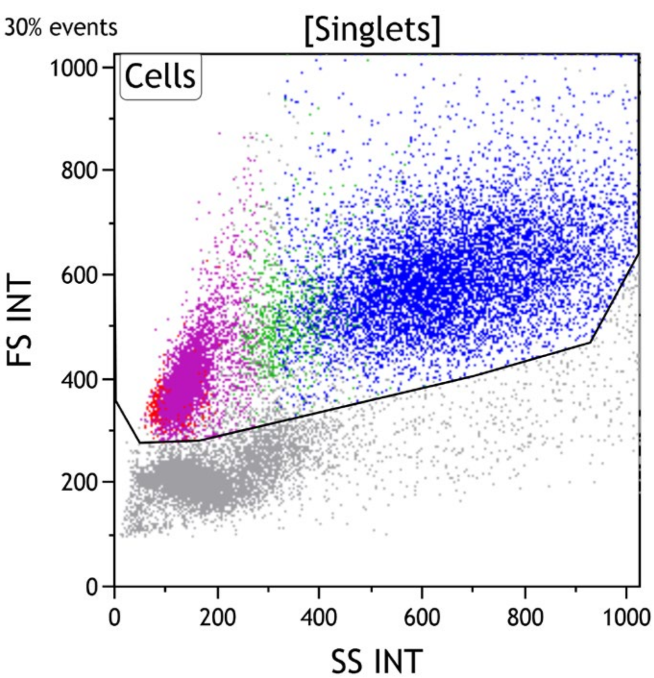

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

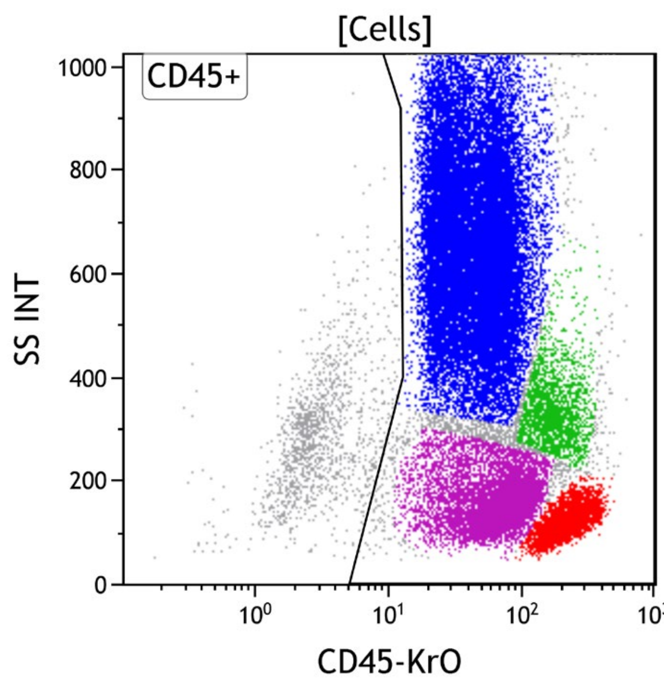

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M1 Cell Tube

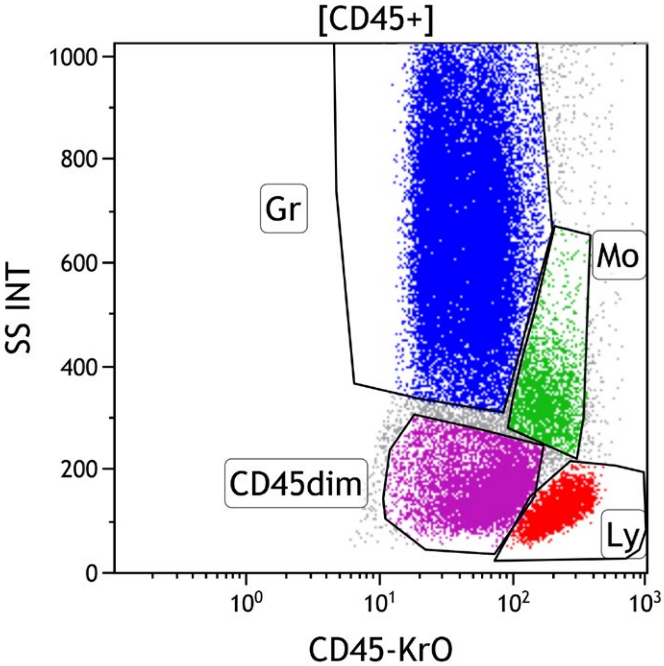

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased number of progenitors (purple)

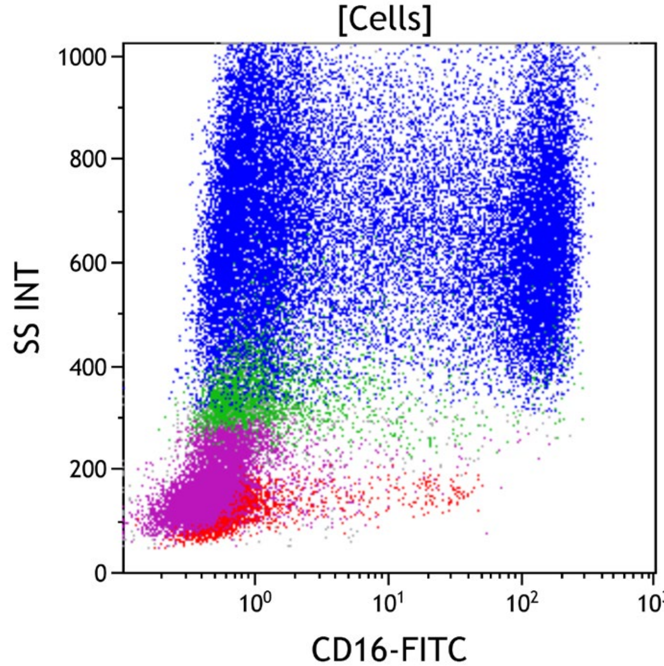

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green). The expanded progenitors (purple) do not express CD16.

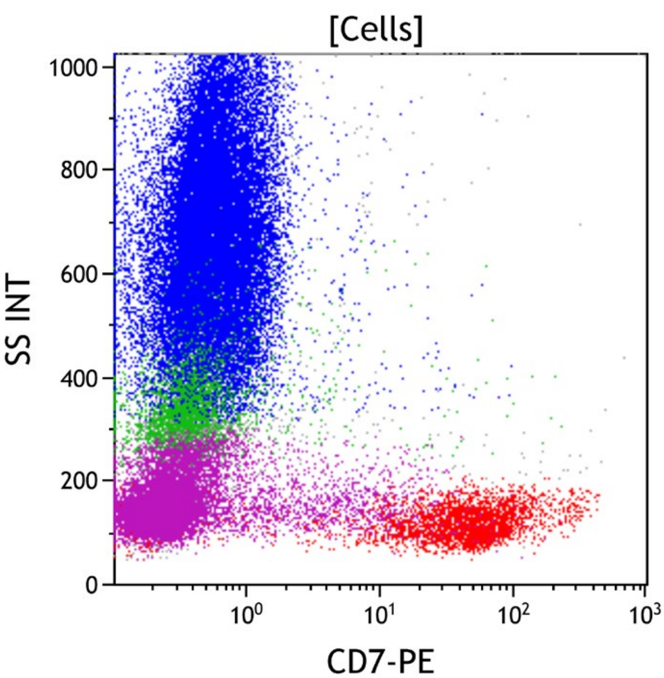

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. Most of the expanded progenitors (purple) do not express CD7.

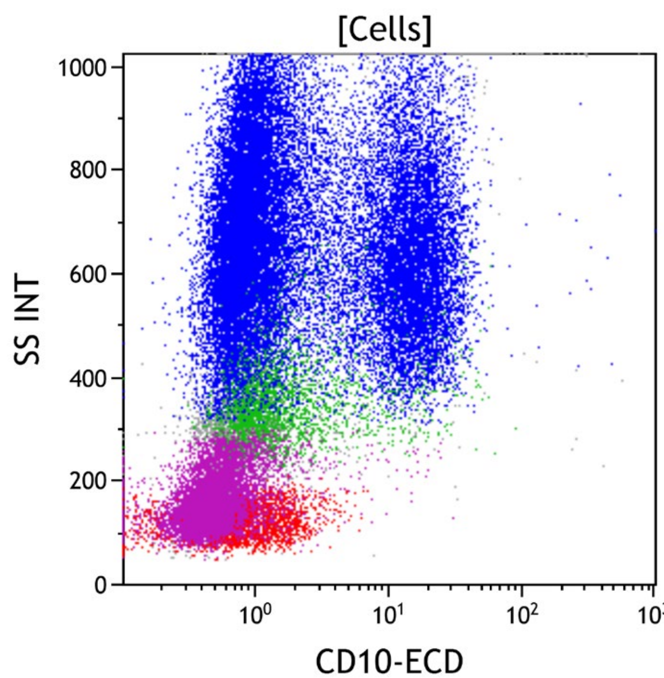

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells mature germinal center B cells, and mature granulocytes. The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The expanded progenitors (purple) do not express CD10.

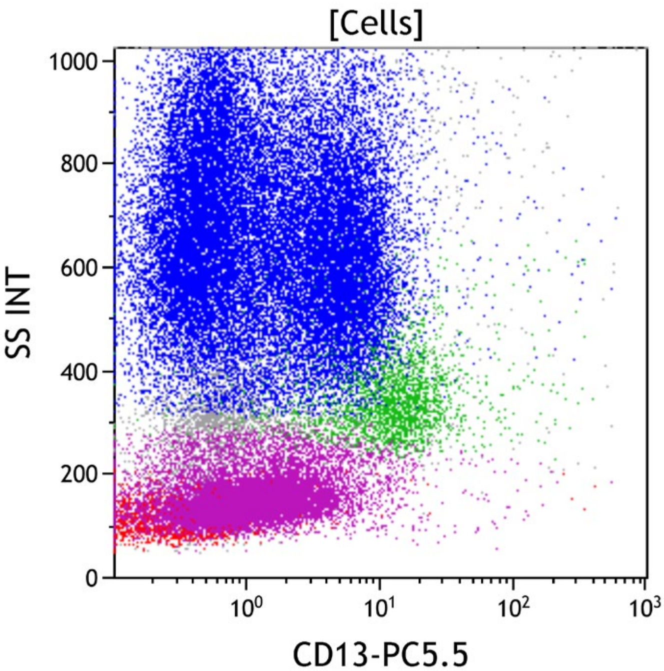

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The expanded progenitors (purple) express dim CD13.

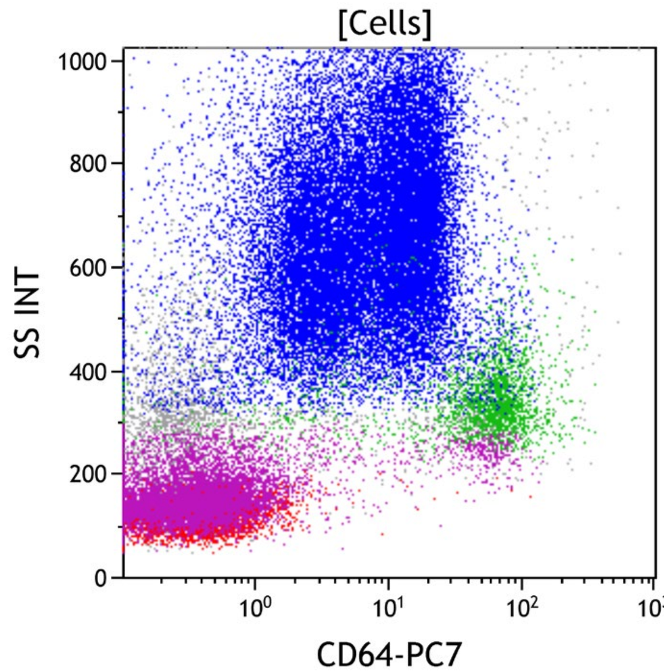

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The expanded progenitors (purple) do not express CD64.

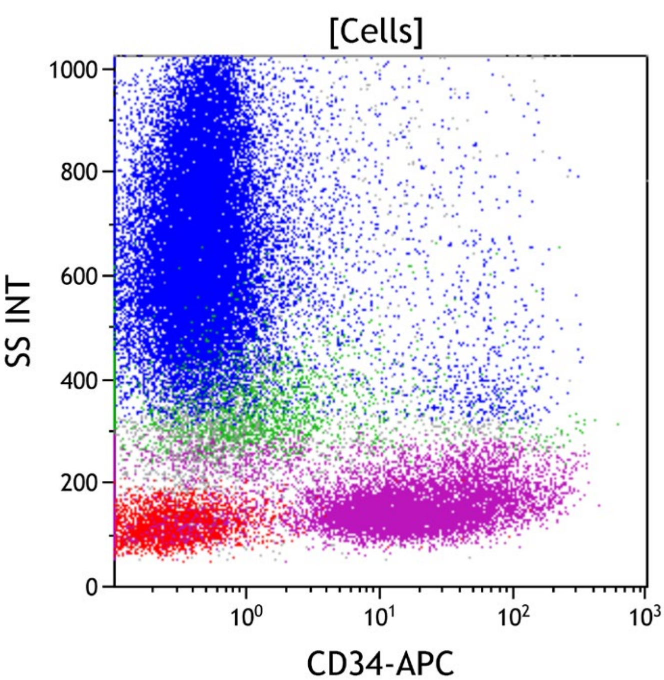

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The expanded progenitors (purple) express CD34.

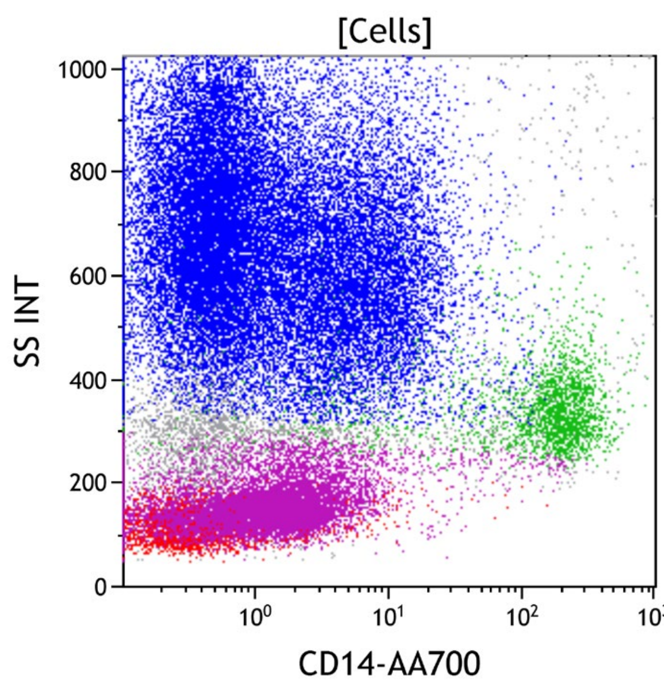

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The expanded progenitors (purple) do not express CD14..

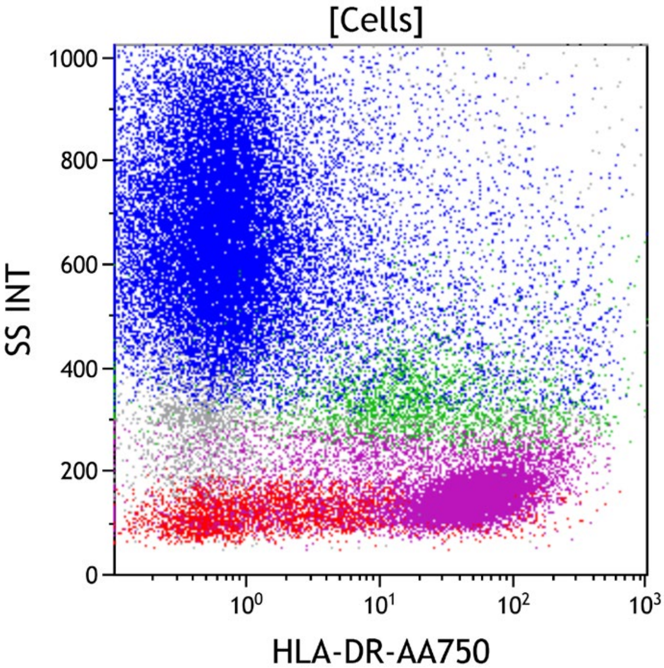

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells. The expanded progenitors (purple) express bright HLA-DR.

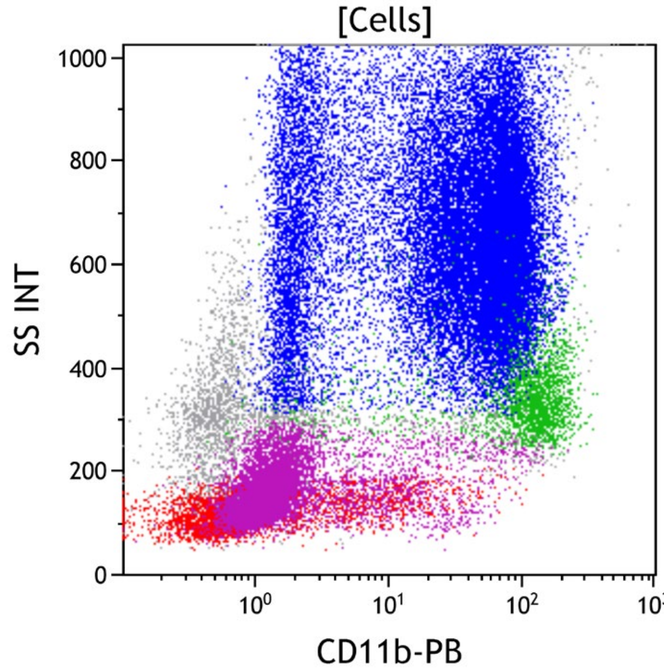

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils. The expanded progenitors (purple) do not express CD11b.

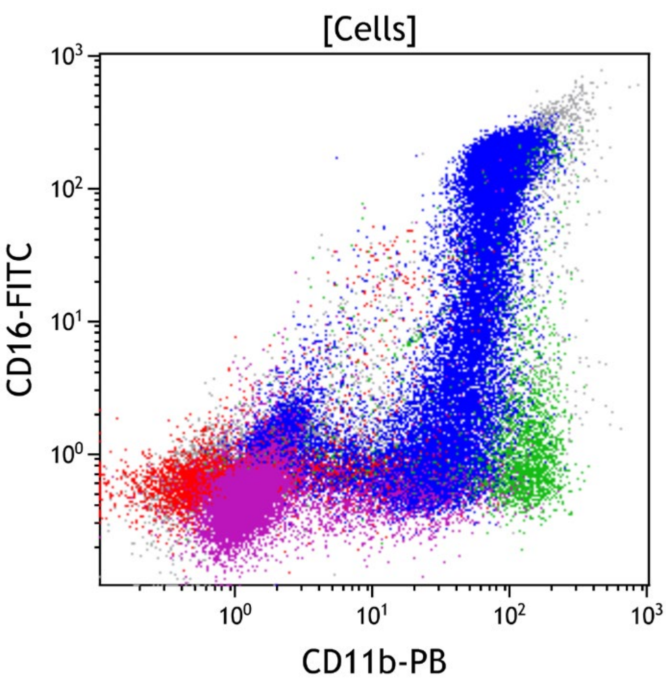

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. The expanded progenitors (purple) do not express CD11b or CD16.

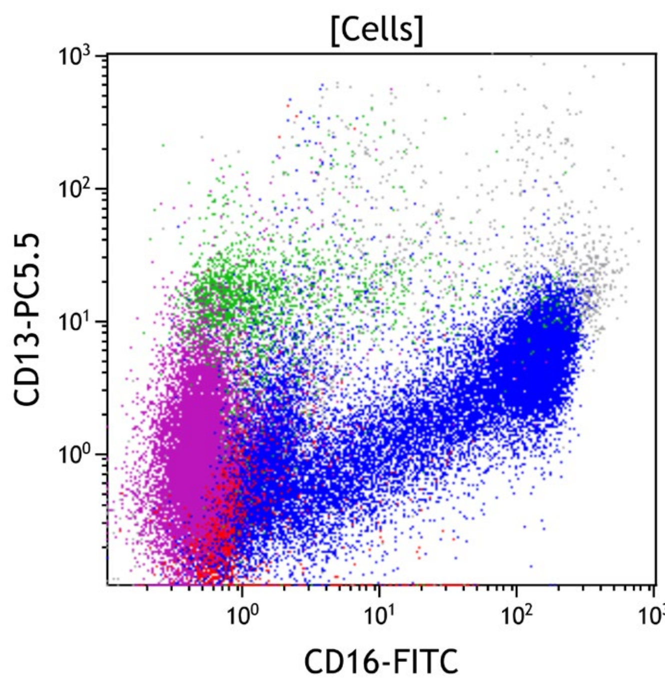

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. The expanded progenitors (purple) express dim CD13 without CD16.

M1 Cell Tube

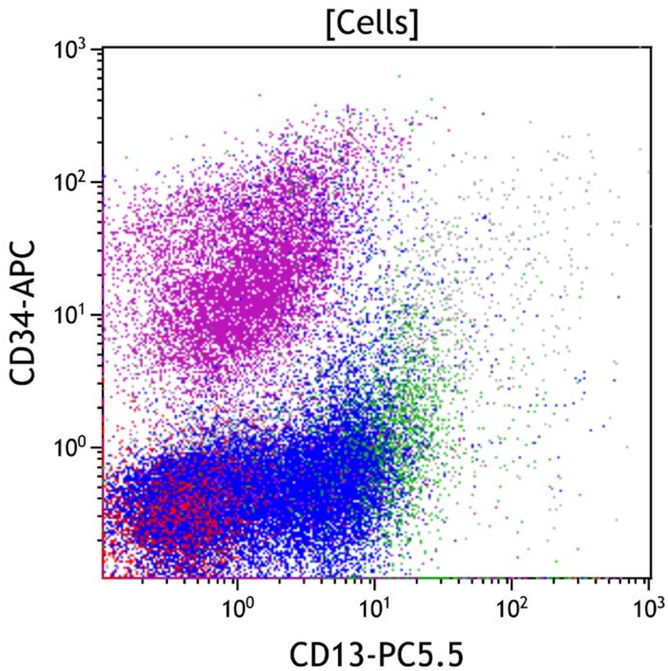

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells. Most of the progenitors (purple) express CD34 and dim CD13.

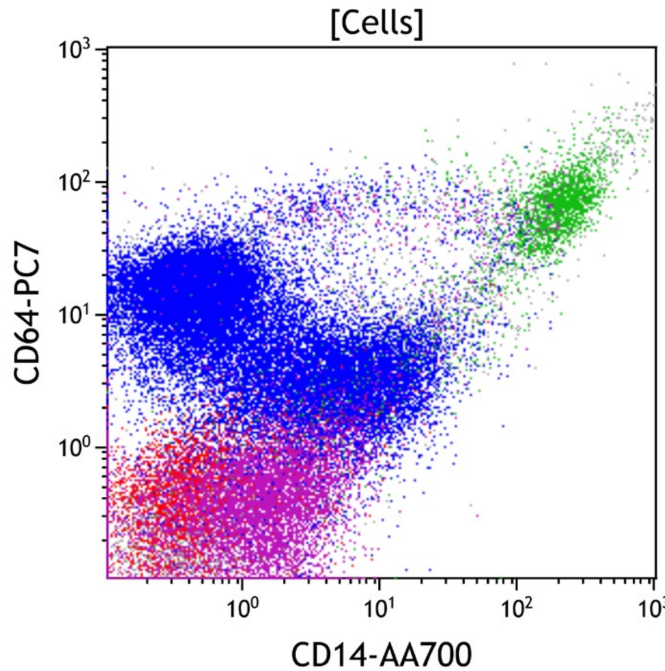

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. The expanded progenitors (purple) do not express CD14 or CD64.

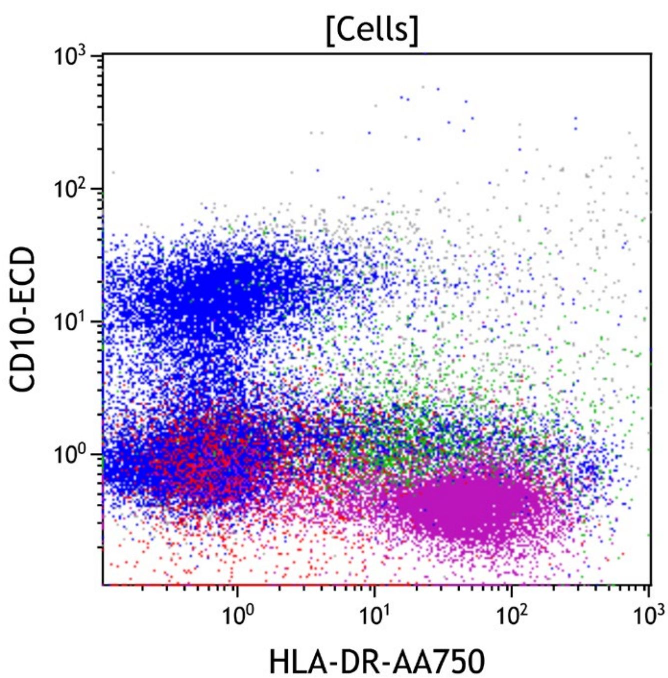

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes and immature B cells. Immature B cells express both CD10 and HLA-DR. The expanded progenitors (purple) express bright HLA-DR without CD10.

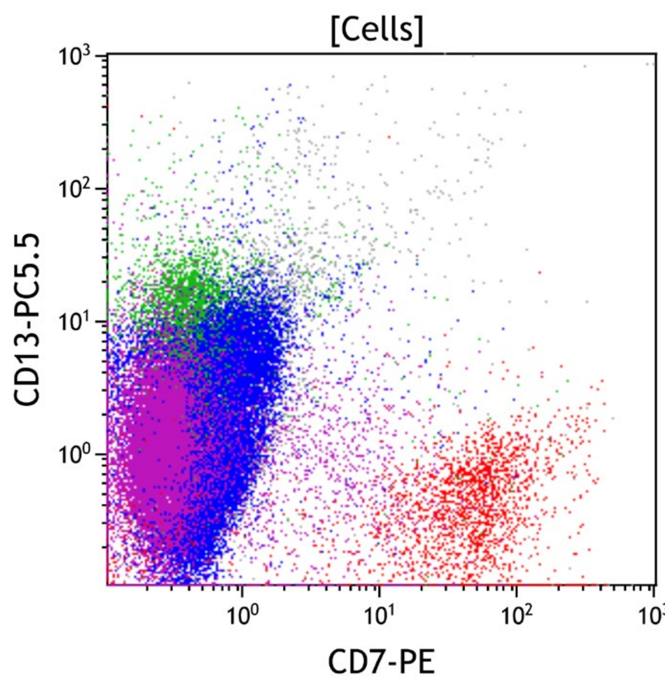

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The expanded progenitors (purple) no not express CD7 and express dim CD13.

M2 Cell Tube

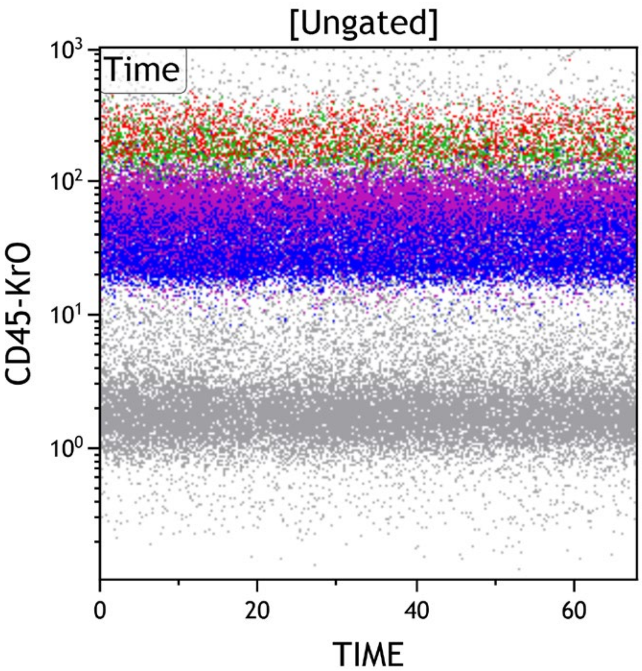

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

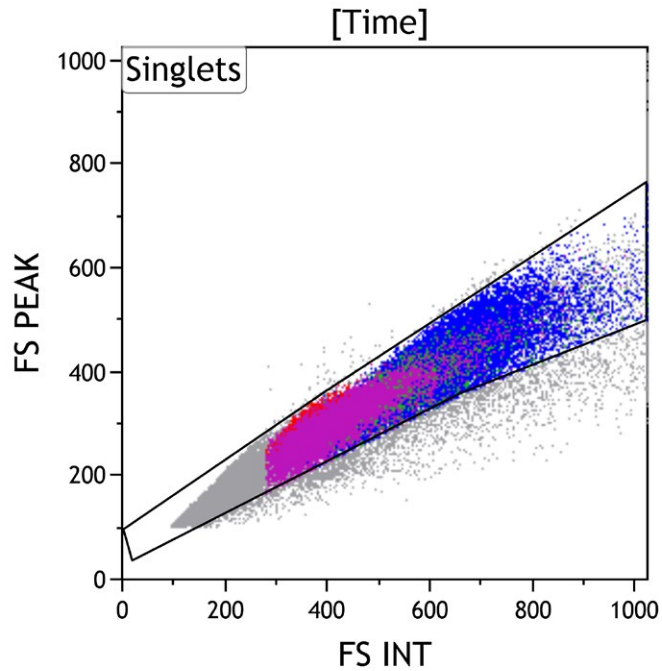

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

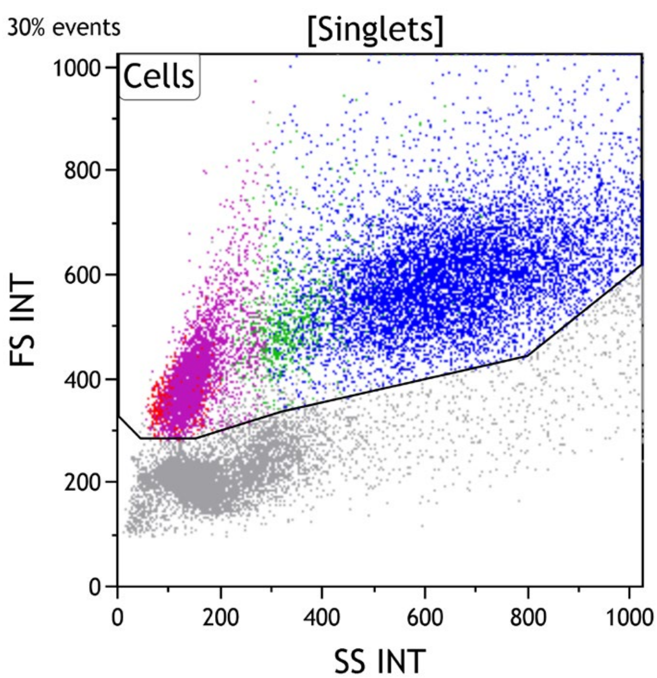

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

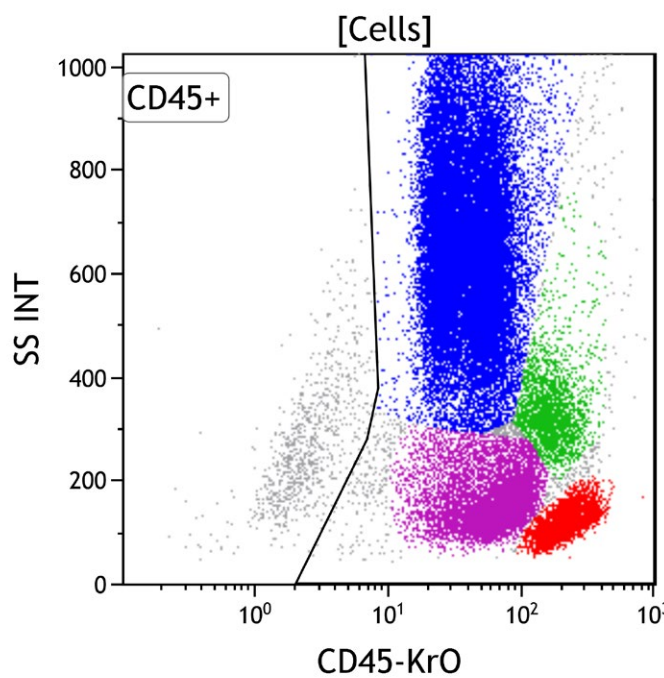

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

M2 Cell Tube

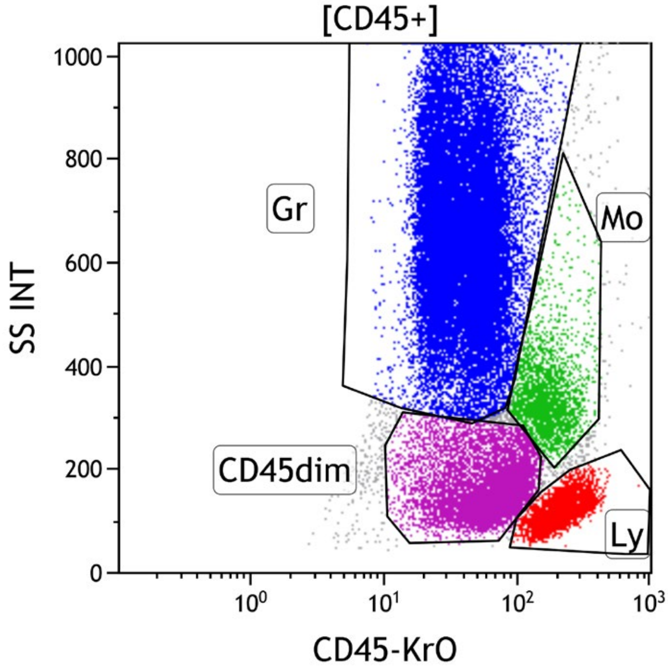

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the relatively increased number of progenitors (purple)

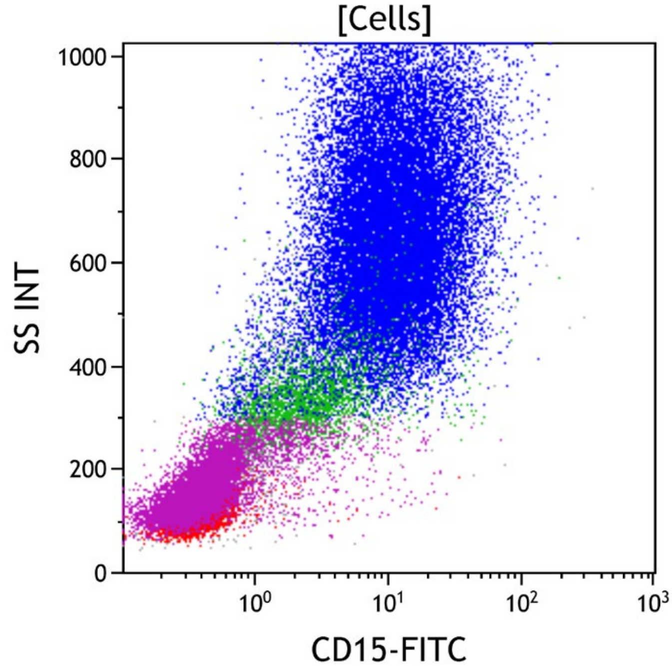

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The expanded progenitors (purple) do not express CD15.

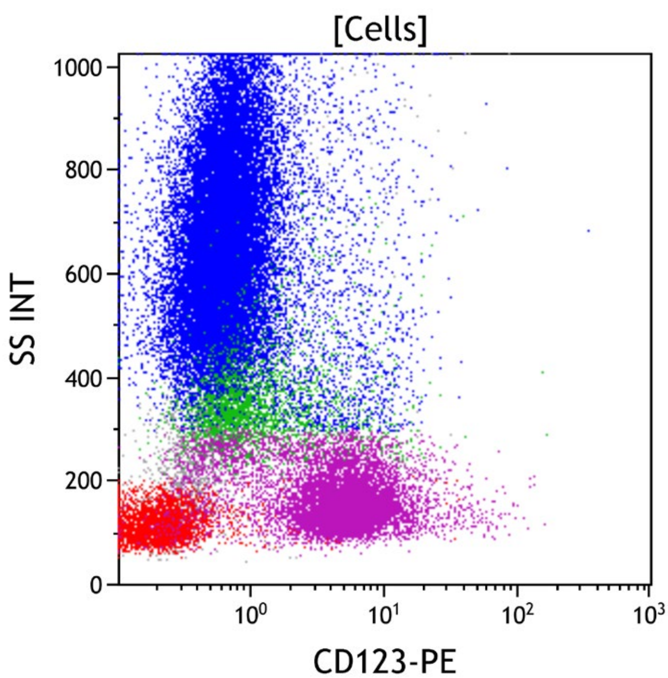

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The expanded progenitors (purple) express intermediate CD123.

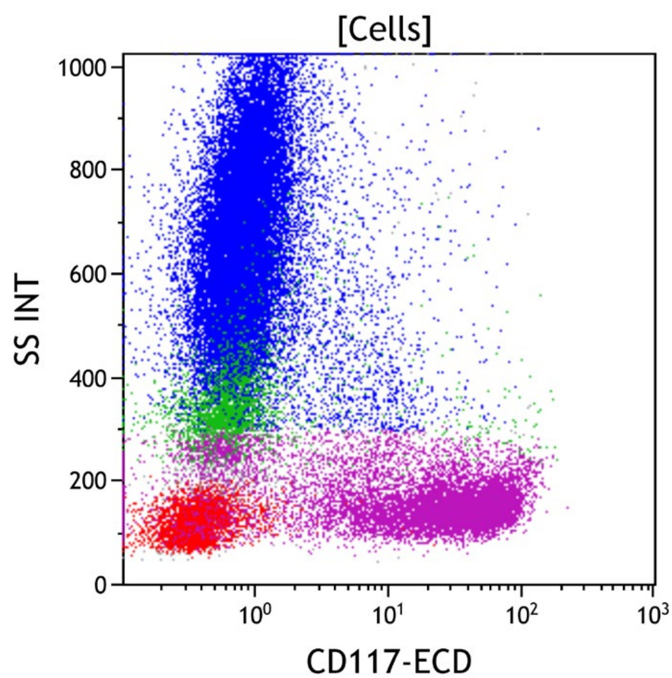

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The expanded progenitors (purple) express CD117.

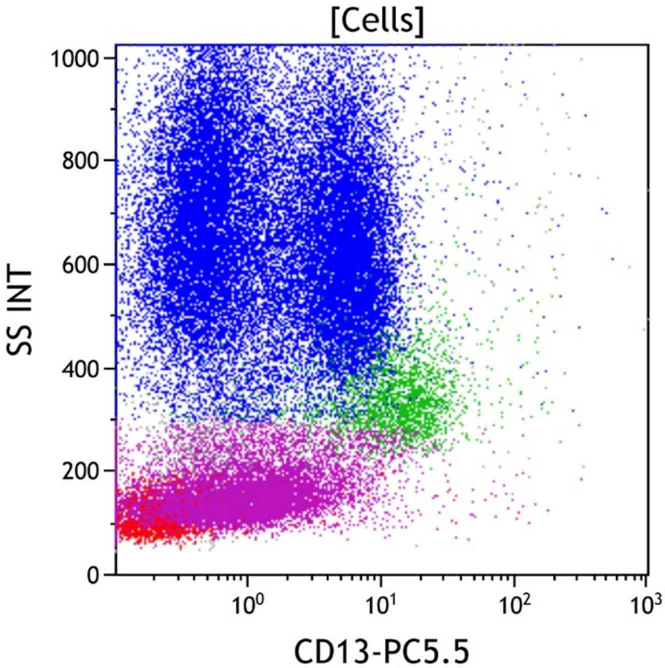

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. The expanded progenitors (purple) express dim CD13.

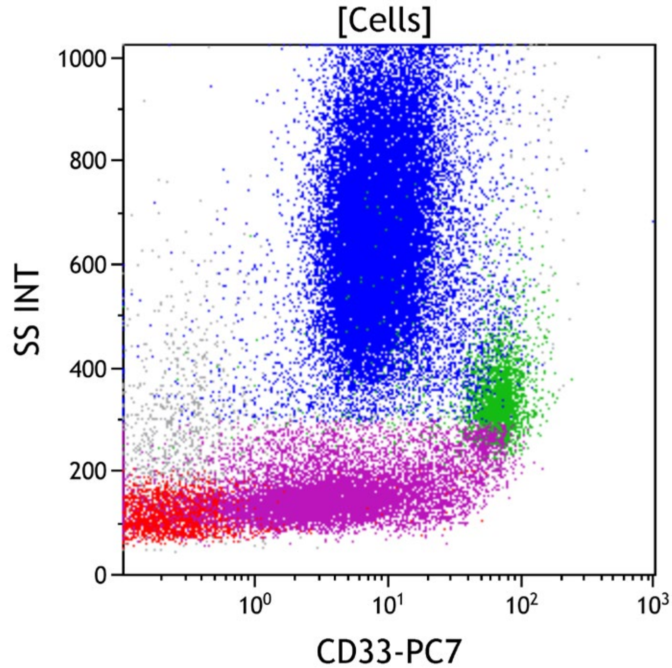

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The expanded progenitors (purple) express dim CD33.

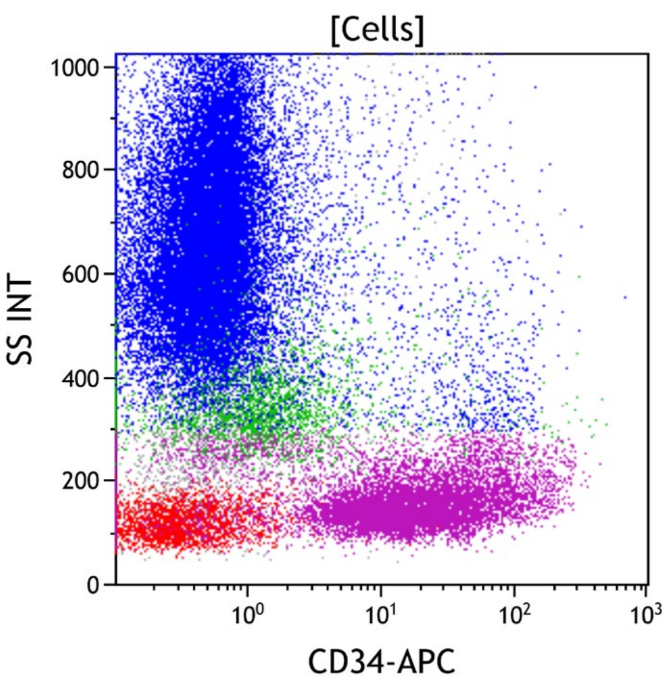

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The expanded progenitors (purple) express CD34.

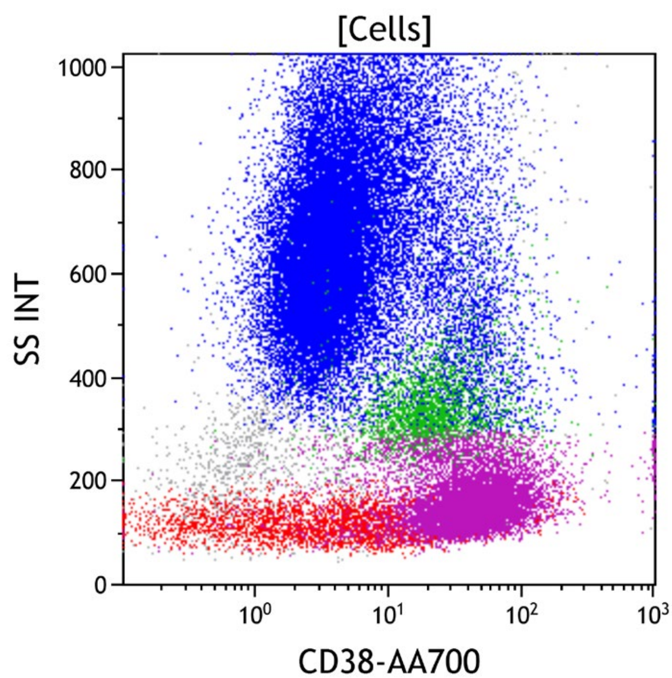

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The expanded progenitors (purple) express intermediate CD38.

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M2 Cell Tube

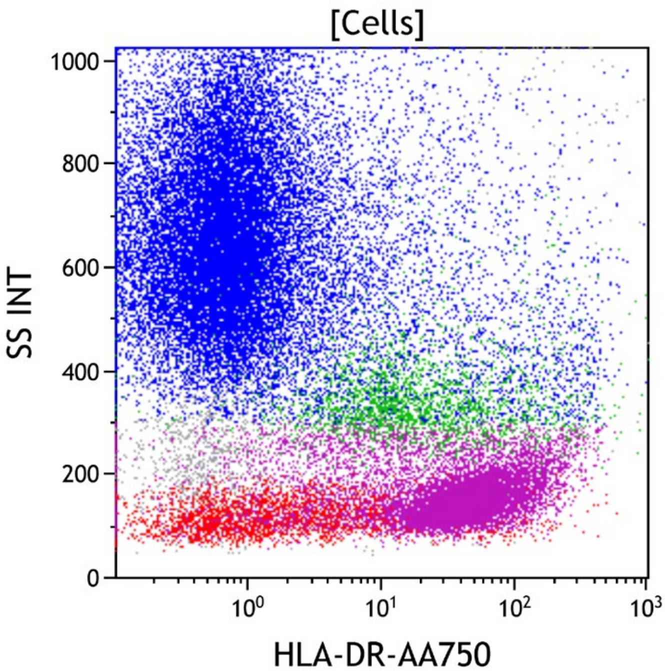

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The expanded progenitors (purple) express bright HLA-DR.

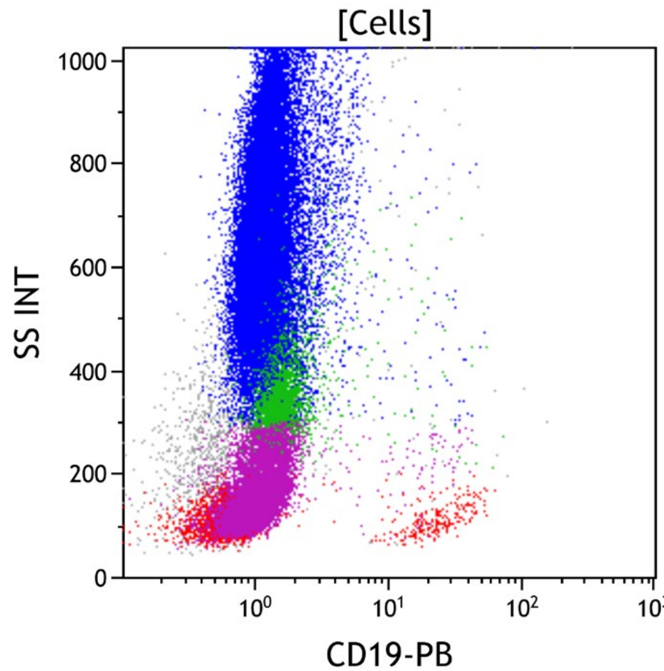

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The expanded progenitors (purple) do not express CD19.

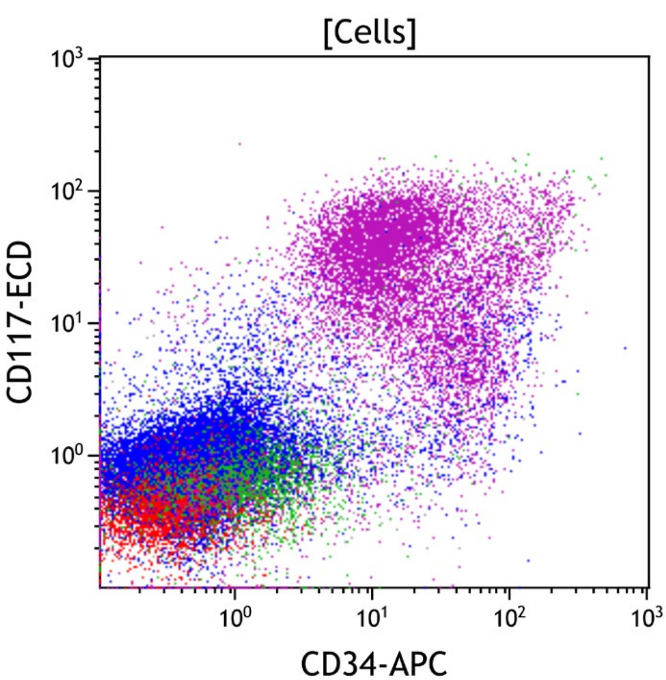

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The expanded progenitors (purple) express CD34 and CD117.

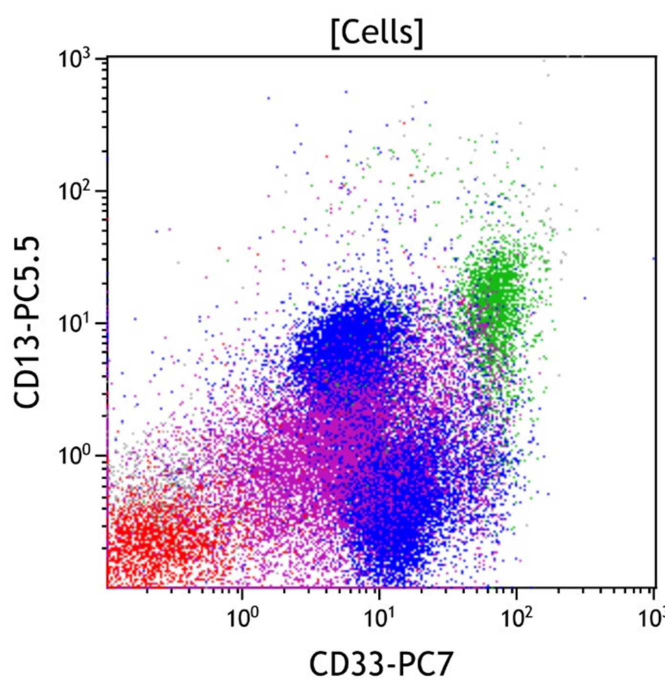

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33. The expanded progenitors (purple) express dim CD13 and dim CD33.

M2 Cell Tube

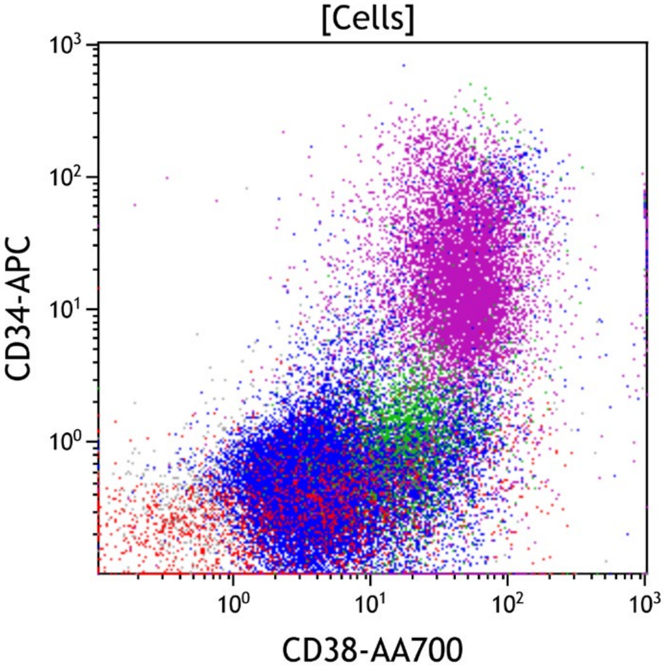

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38. The apparent variable CD34 expression by plasma cells (purple extreme right) is a compensation artifact due to the extremely high level of CD38 that extends beyond the visible scale. The expanded progenitors (purple) express CD34 and intermediate CD38.

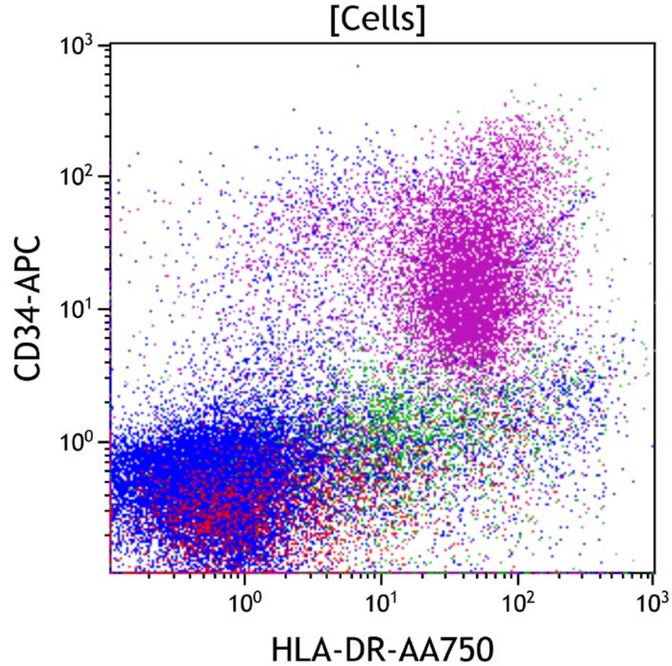

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The expanded progenitors (purple) express CD34 and bright HLA-DR.

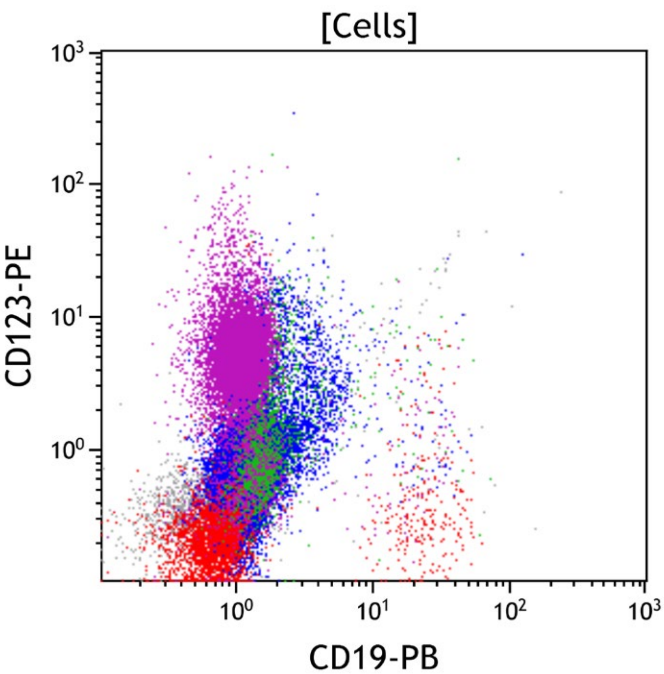

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The expanded progenitors (purple) express intermediate CD123 without CD19.

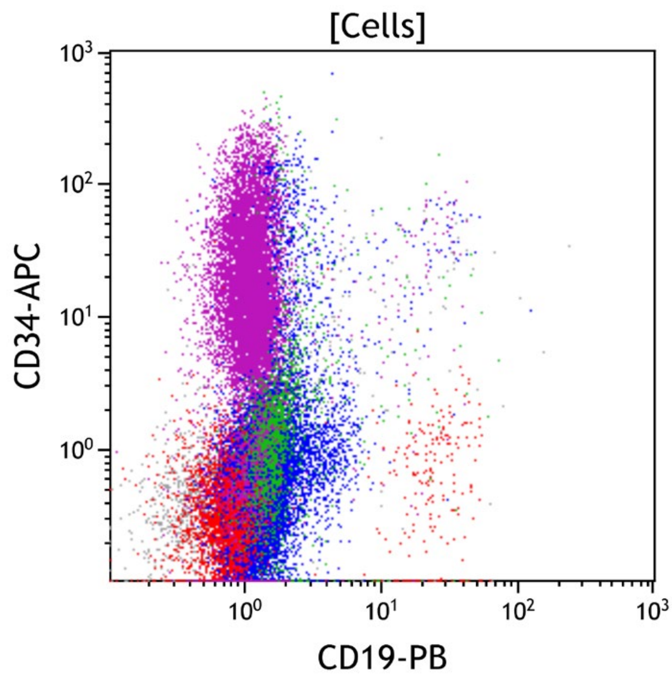

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. The expanded progenitors (purple) express CD34 without CD19.

M2 Cell Tube

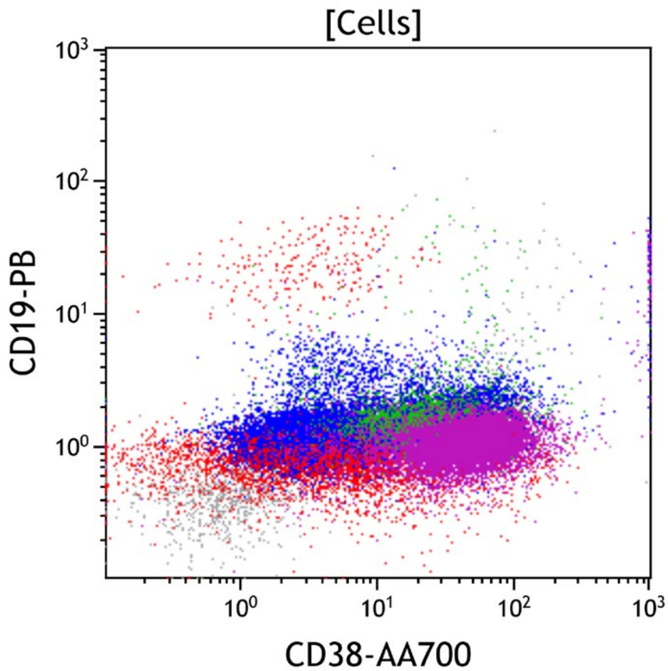

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38. Plasma cells show extremely high CD38 expression that is largely off scale (purple extreme right), but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The expanded progenitors (purple) express intermediate CD38 without CD19.

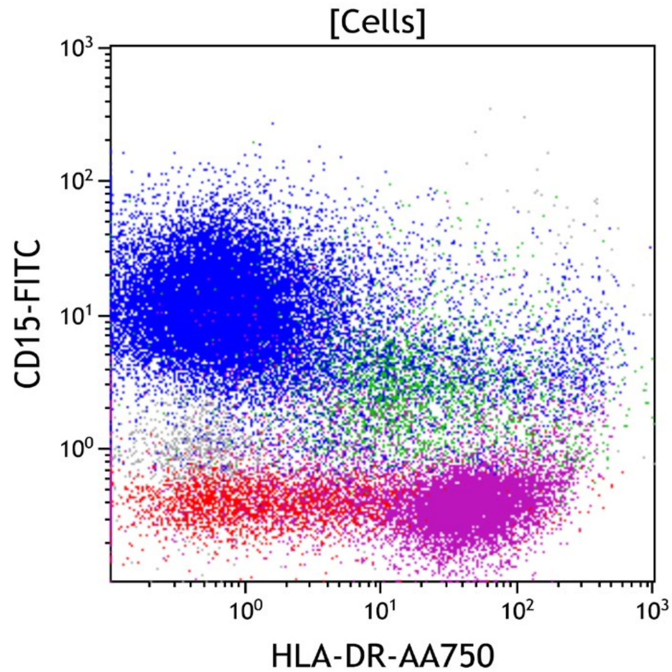

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR, except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. The expanded progenitors (purple) express intermediate CD38 without CD15.

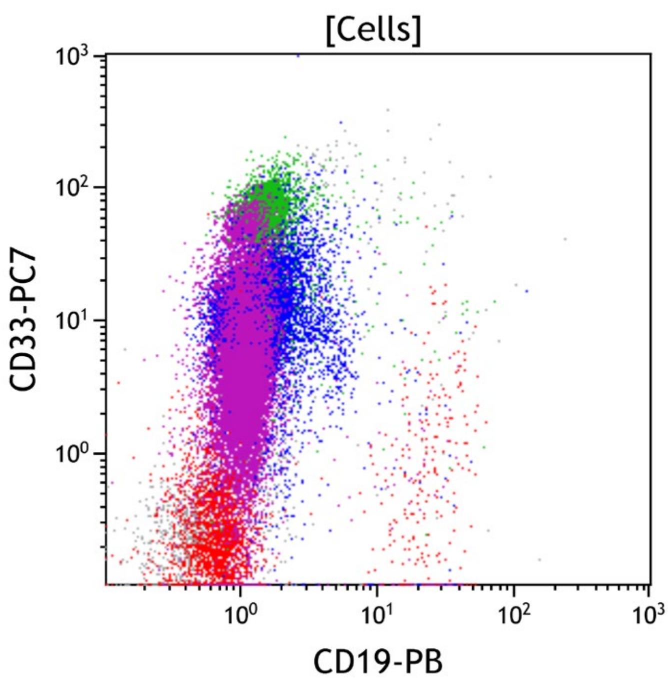

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The expanded progenitors (purple) express dim CD33 without CD19.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of variable CD5, dim CD13, intermediate CD33, intermediate CD34, intermediate CD38, dim CD45, intermediate CD117, intermediate CD123 and bright HLA-DR without CD14, CD15, CD64 and other lymphoid or myeloid antigens. Compared with normal CD34-positive progenitors, the expression of CD5 and decreased expression of CD13 is aberrant.

The immunophenotype of the abnormal population is that of modestly expanded abnormal CD34-positive progenitors. This finding in the context of an elderly patient with cytopenias is consistent with a myeloid stem cell disorder, in particular a myelodysplastic syndrome. Additional testing results from this subject show morphology of 10-12% blasts, multilineage dysplasia, and ringed sideroblasts, and a 12p deletion is seen on karyotype, findings indicative of a myelodysplastic syndrome best classified as MDS with excess blasts-2 (MDS-EB-2) in the WHO classification.

Case #24: Myelodysplastic Syndrome

Clinical Vignette

This 75-year-old male presents with thrombocytopenia and anemia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

Flow Cytometric Immunophenotyping

B Cell Tube

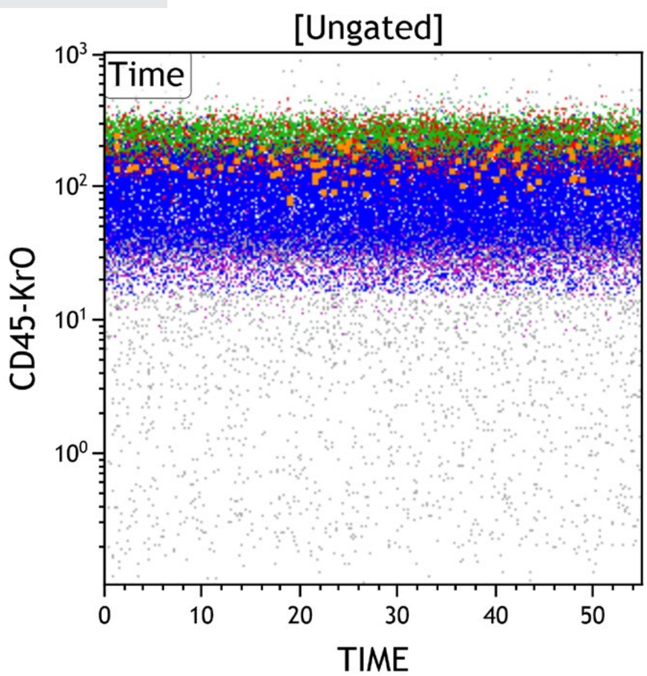

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

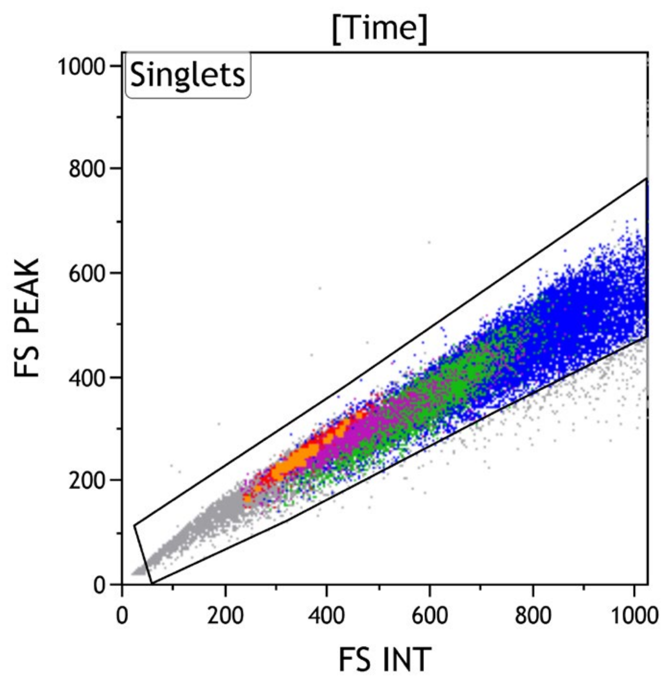

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

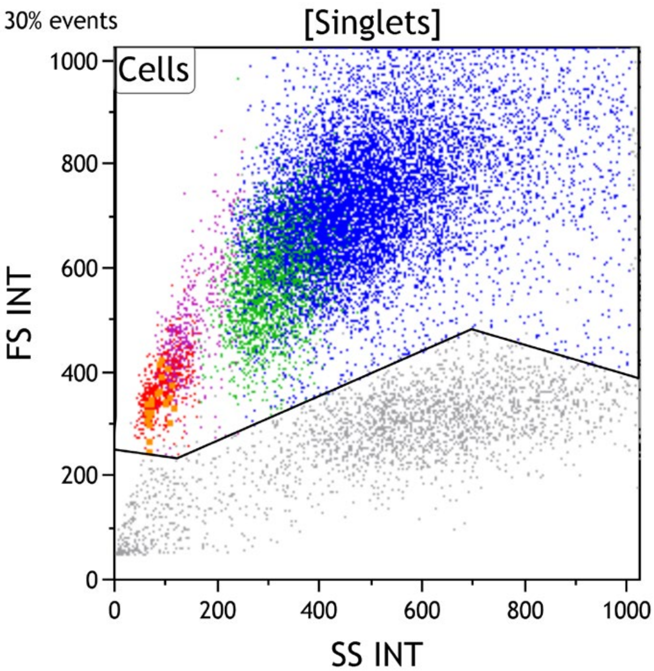

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

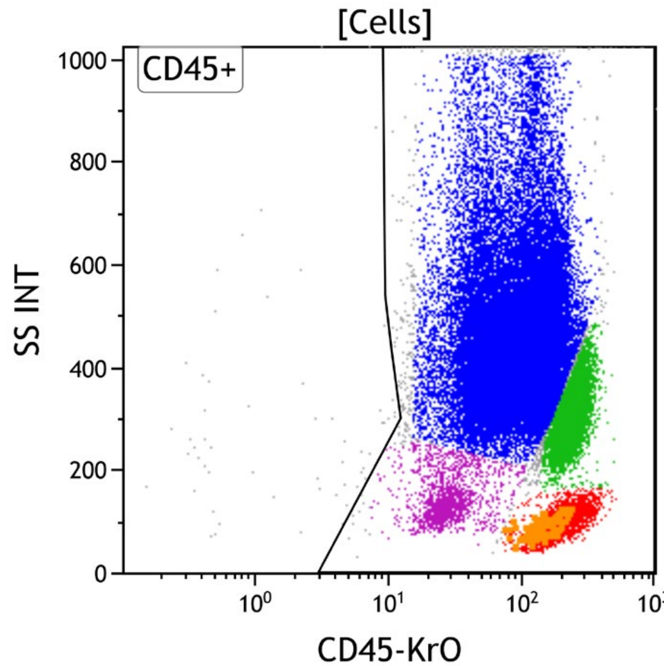

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

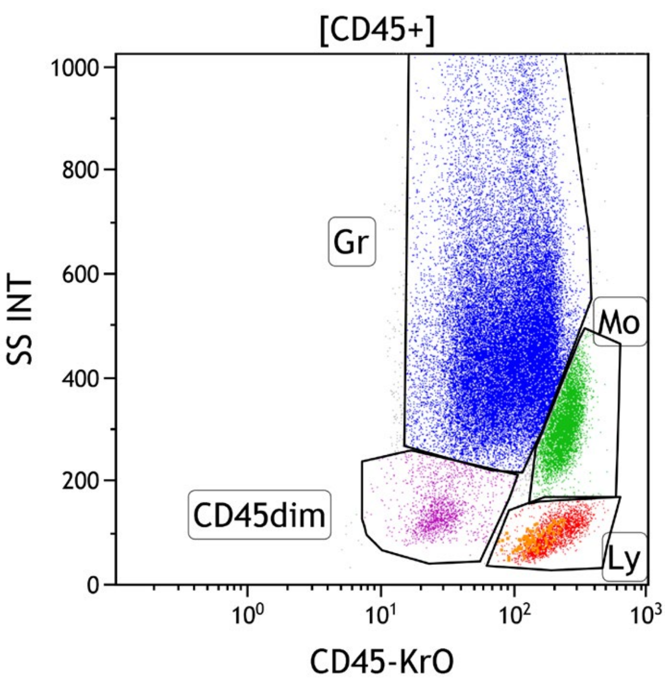

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors, i.e. myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also appear in this area. By applying different colors to the events comprised by each gate, the various populations may be identified throughout the analysis. Note the granulocytic population (blue) has decreased side scatter and a discrete progenitor (purple) population is present.

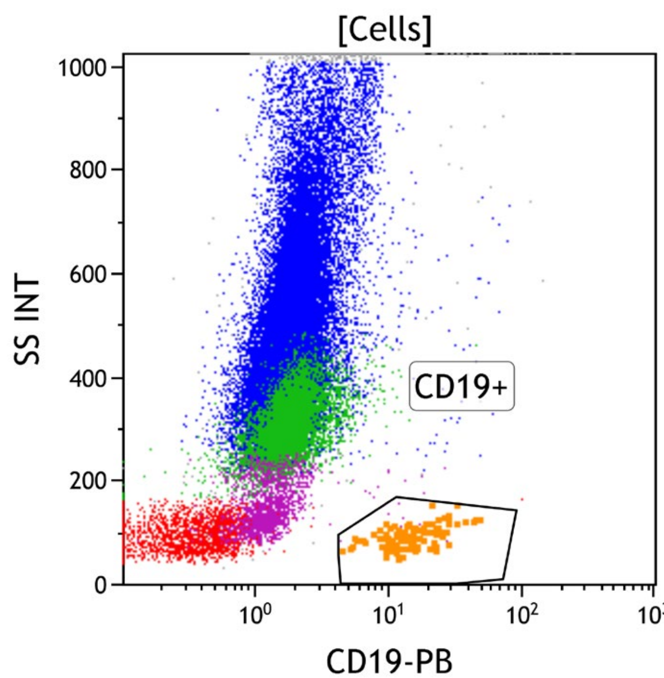

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The progenitors (purple) do not express CD19.

B Cell Tube

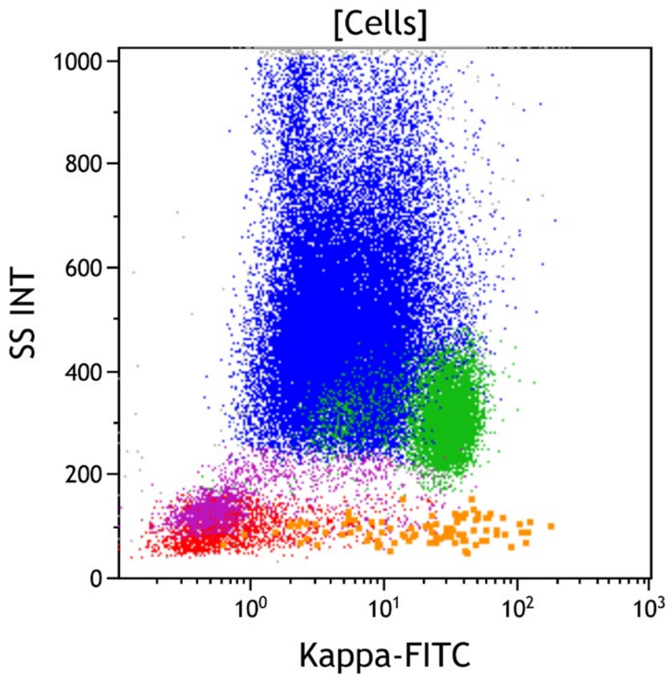

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. The progenitors (purple) do not express kappa.

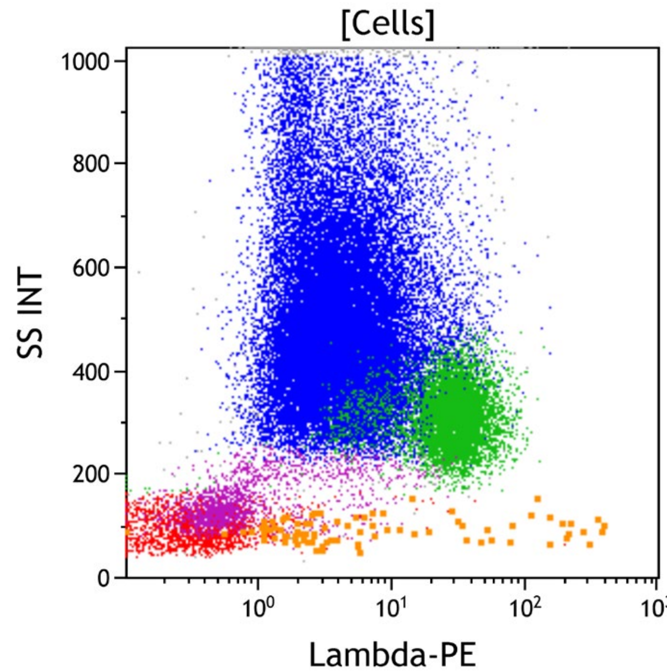

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. The progenitors (purple) do not express lambda.

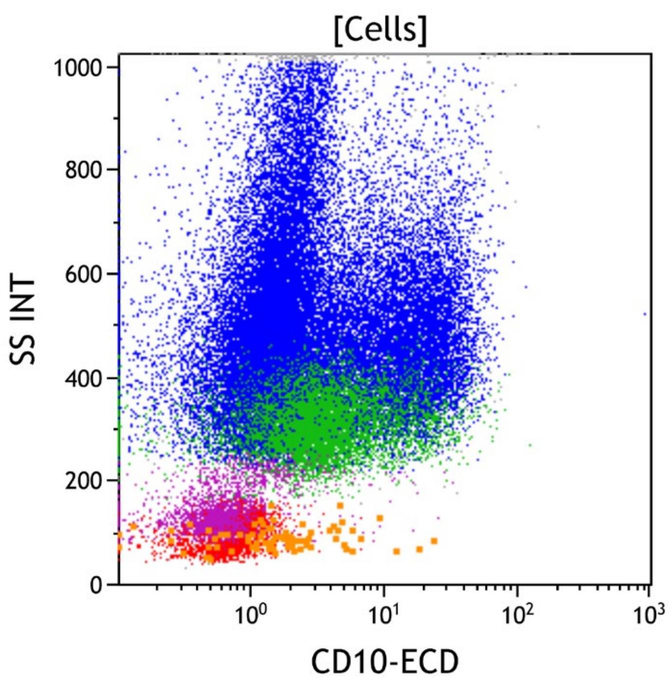

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells mature germinal center B cells, and mature granulocytes. The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The progenitors (purple) do not express CD10. A subset of granulocytes (blue) express CD10.

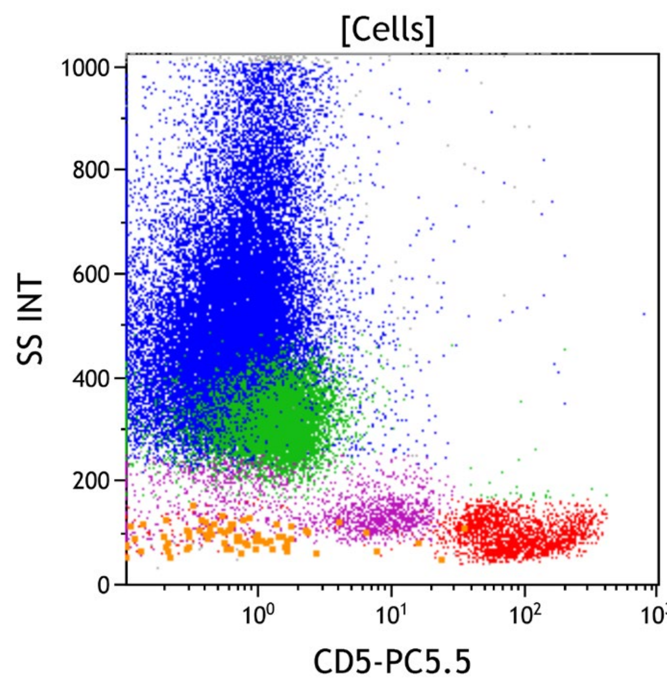

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The progenitors (purple) express intermediate CD5.

B Cell Tube

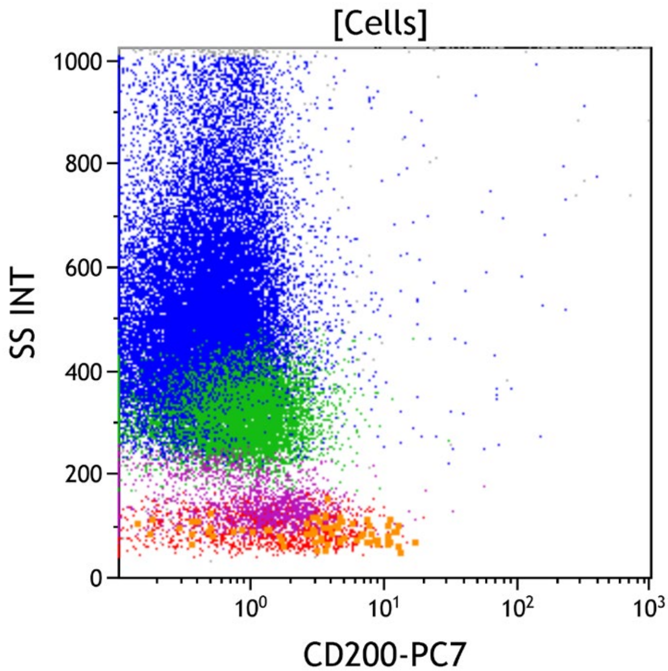

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). The progenitors (purple) do not express CD200.

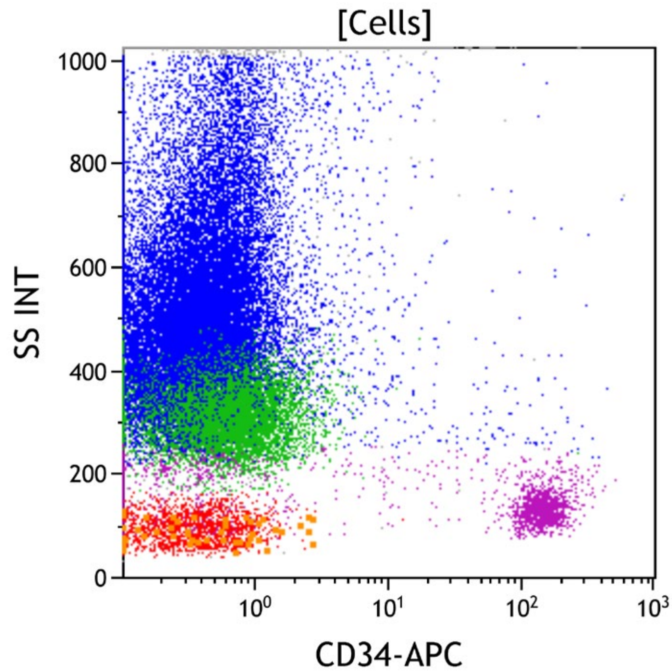

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The progenitors (purple) express bright CD34.

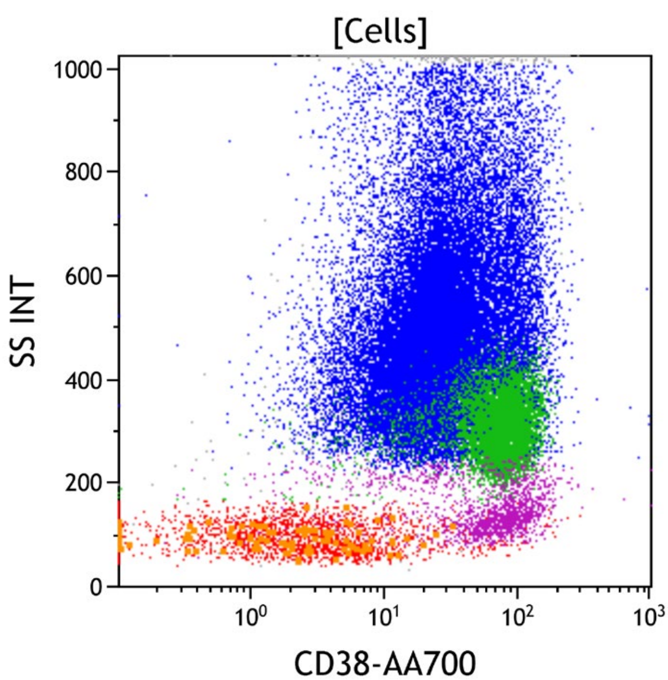

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The progenitors (purple) and granulocytes (blue) express intermediate CD38.

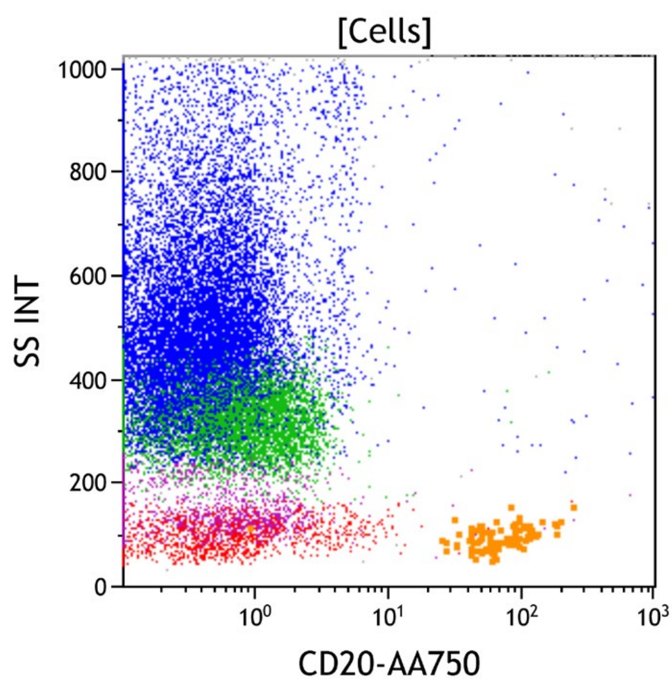

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The progenitors (purple) do not express CD20.

B Cell Tube

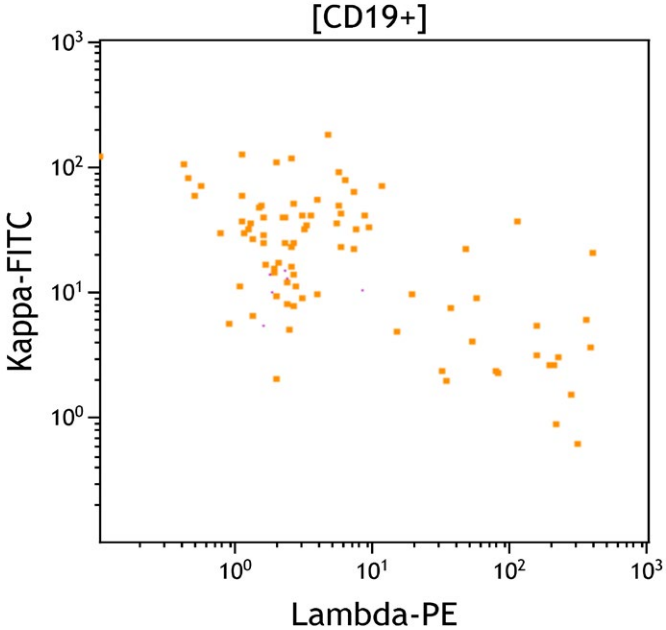

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

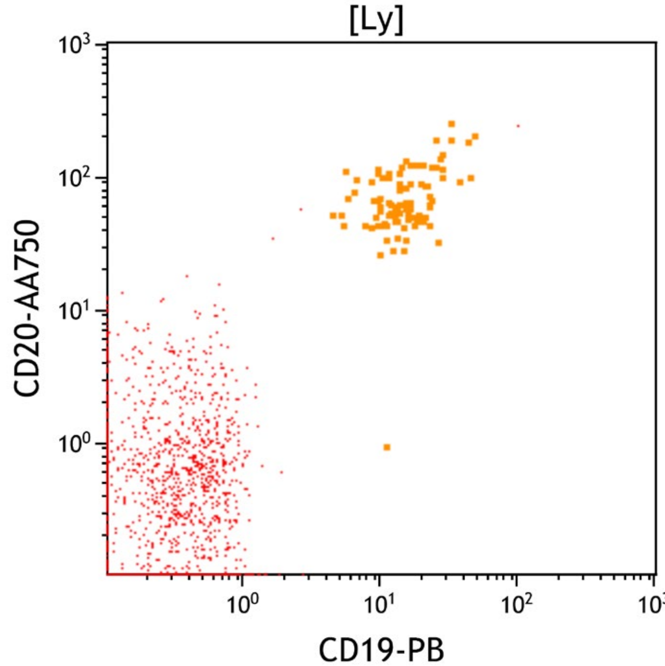

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

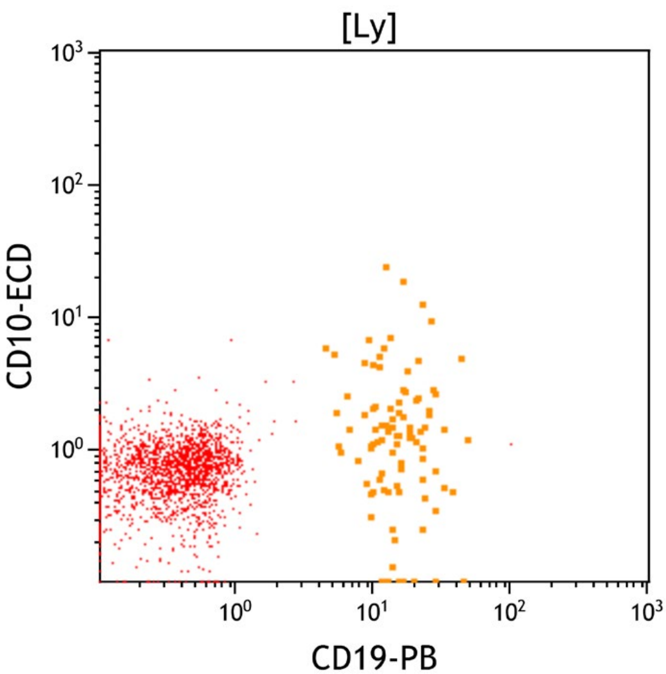

Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

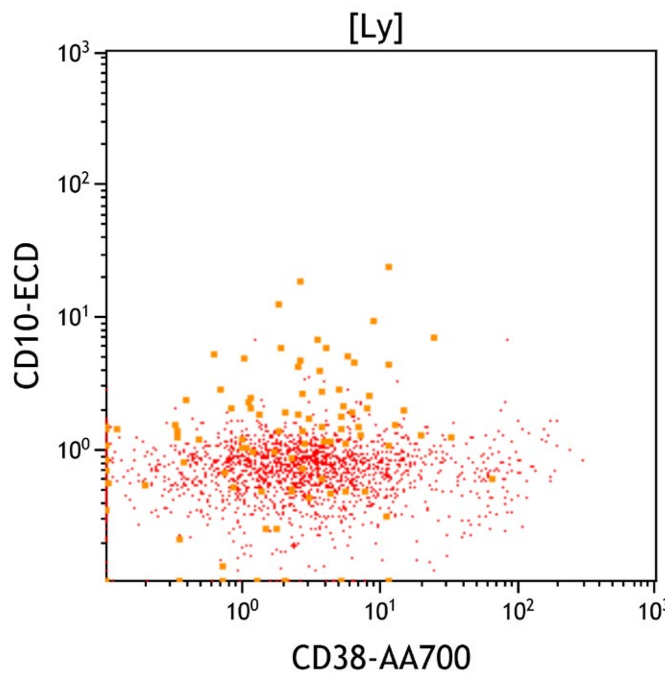

Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The few CD10 positive and CD38 moderate cells represent late stage immature B cells in the lymphocyte gate, though most immature B cells are in the CD45 dim gate.

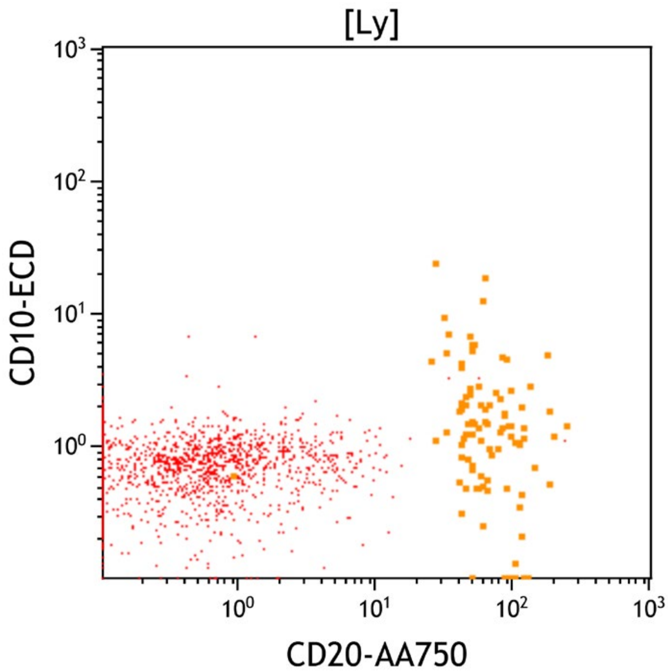

Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells.

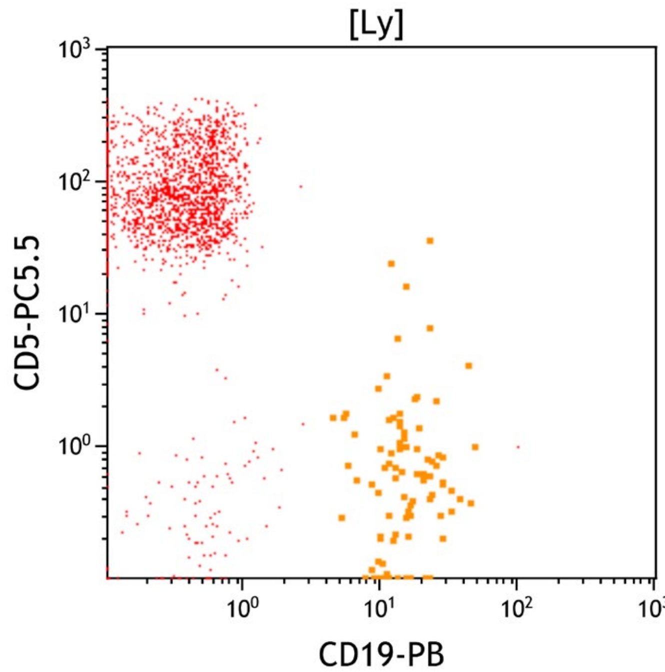

Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

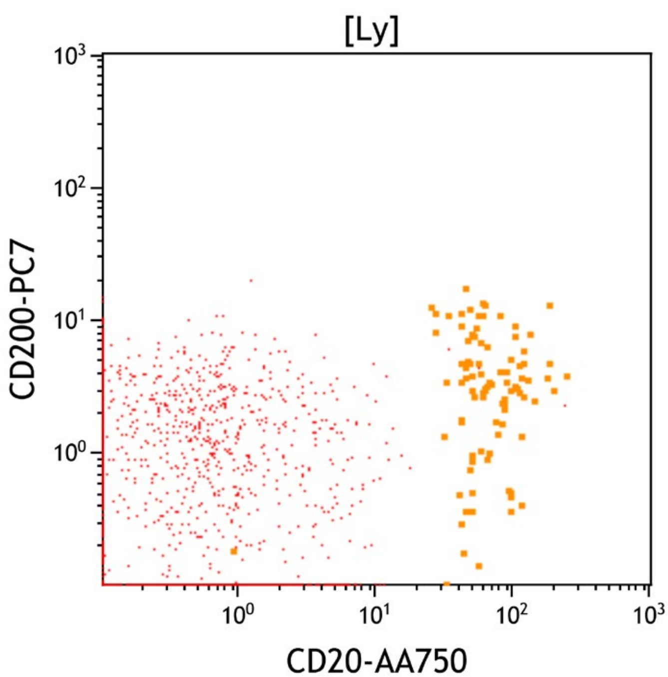

Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

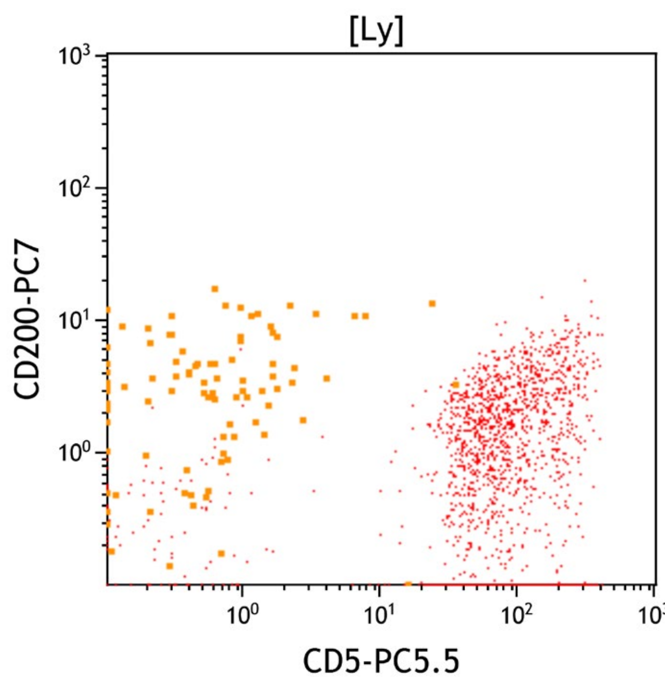

Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

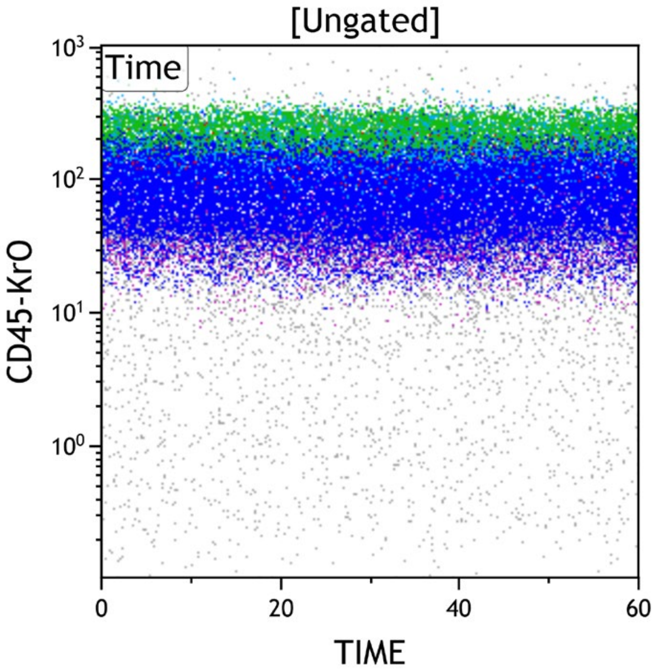

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

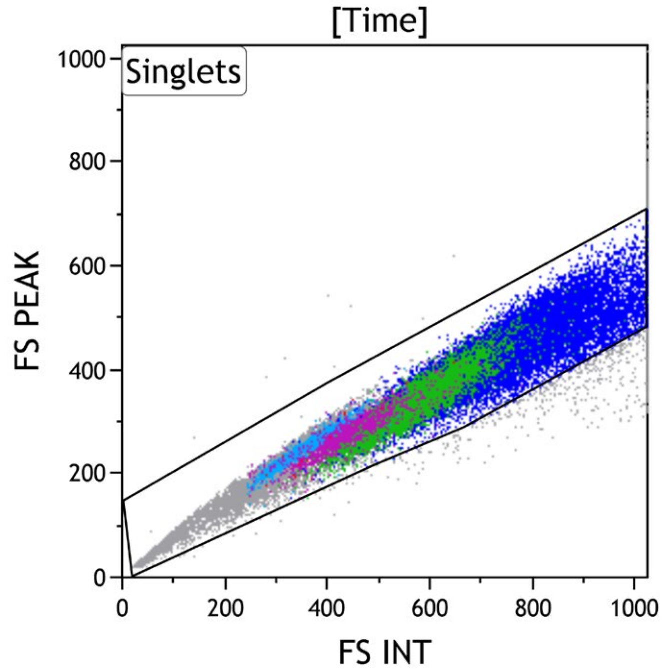

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

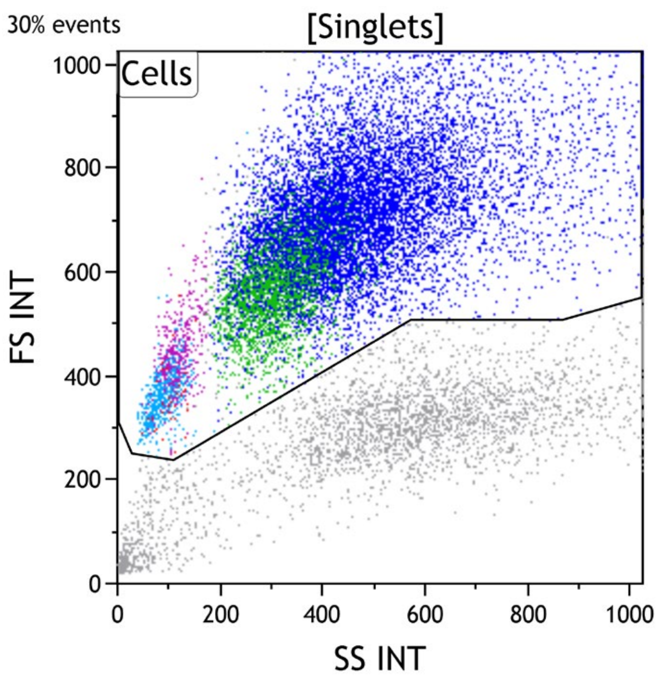

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

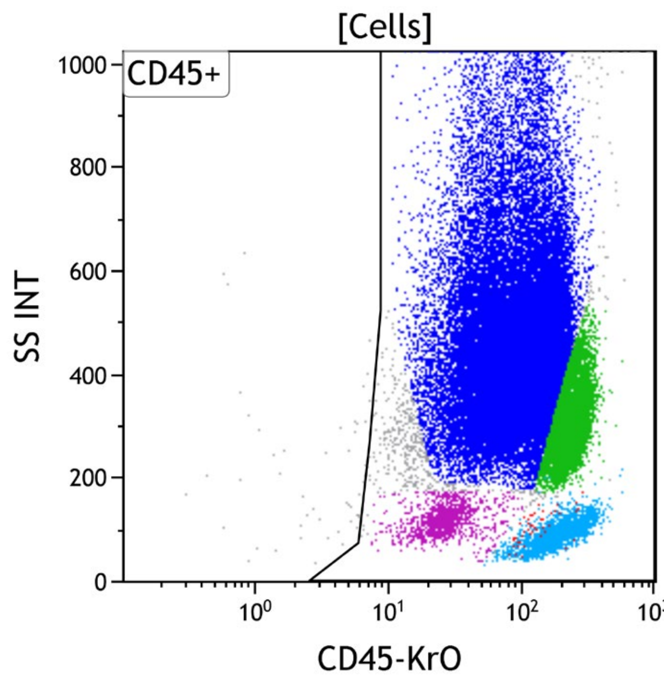

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various types of white blood cells, which are CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates or non-hematopoietic cells.

T Cell Tube

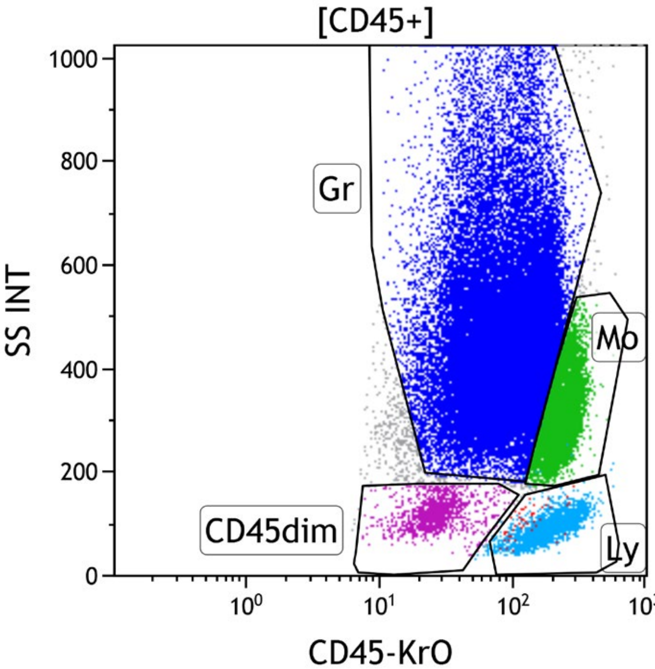

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the granulocytic population (blue) has decreased side scatter and a discrete progenitor (purple) population is present.

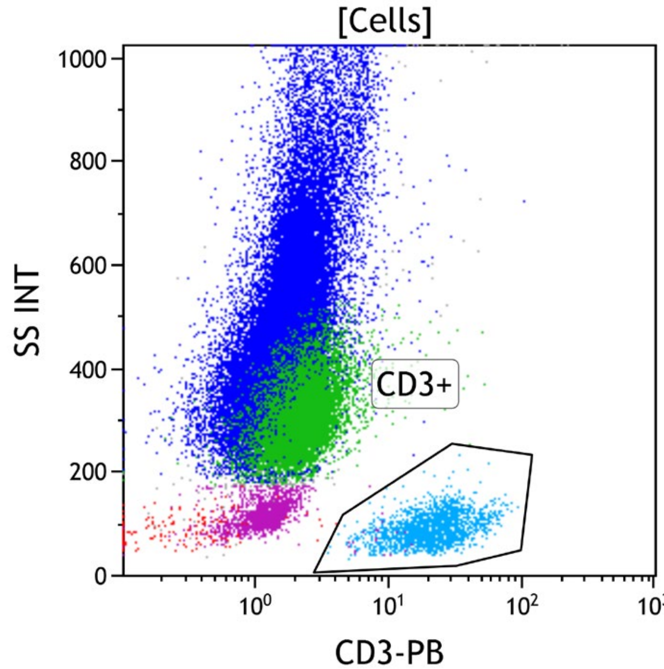

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. The progenitors (purple) do not express CD3.

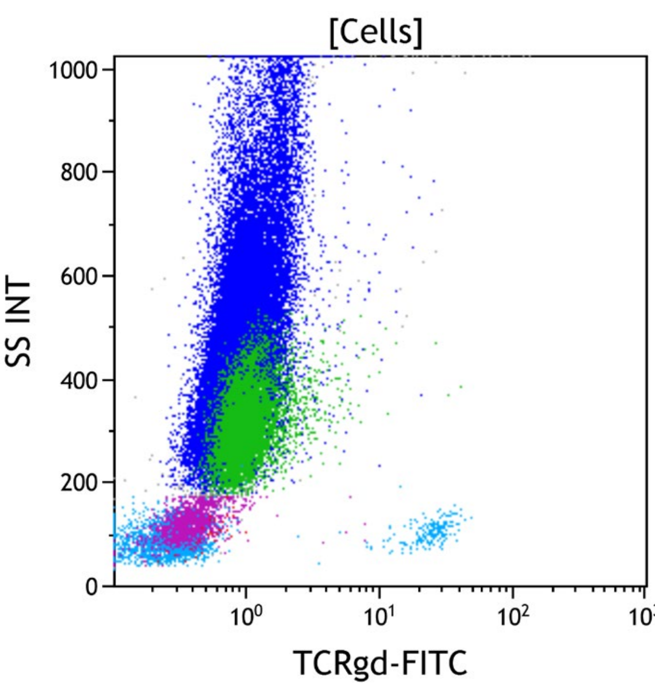

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The progenitors (purple) do not express TCRγδ.

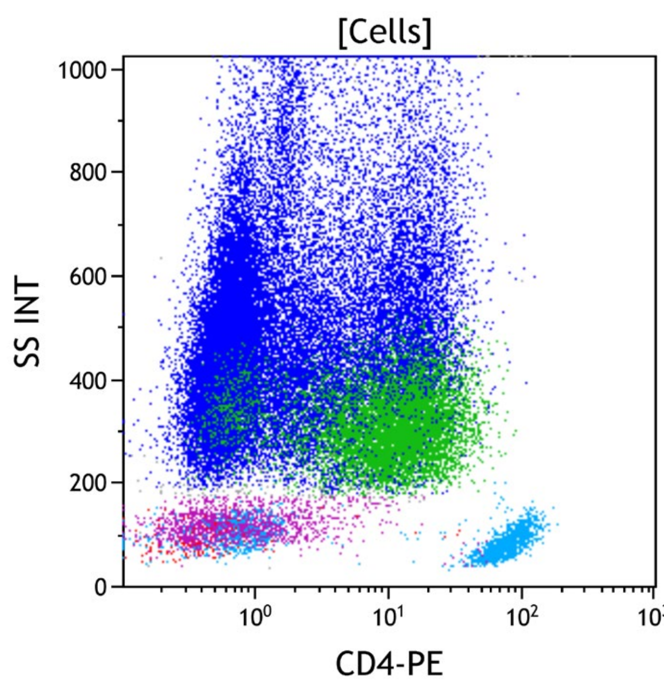

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. The progenitors (purple) do not express CD4.

T Cell Tube

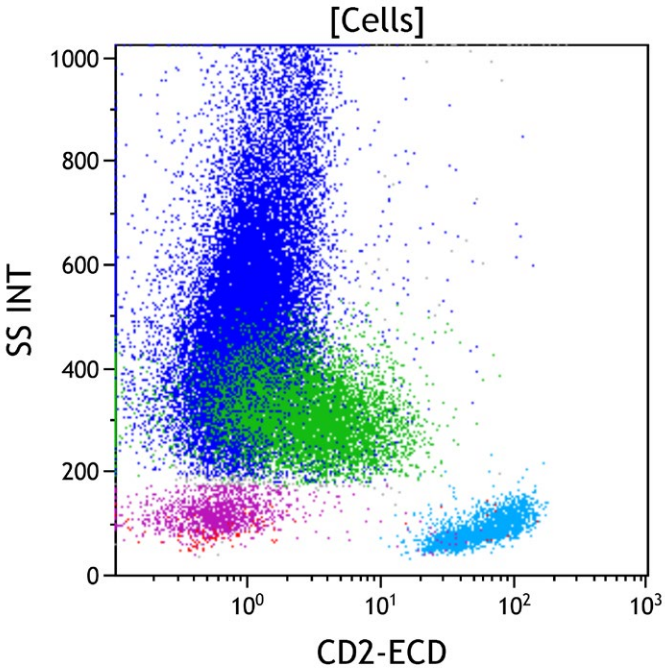

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The progenitors (purple) do not express CD2.

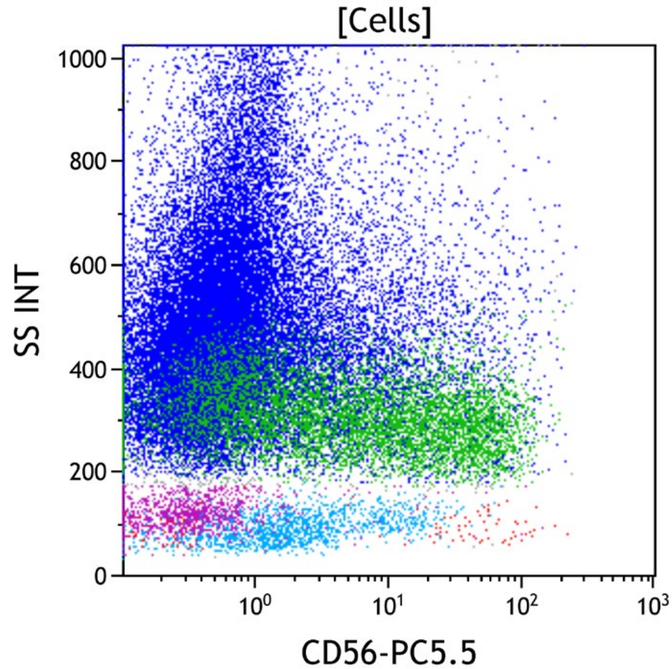

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. The progenitors (purple) do not express CD56. Most monocytes (green) express dim to intermediate CD56.

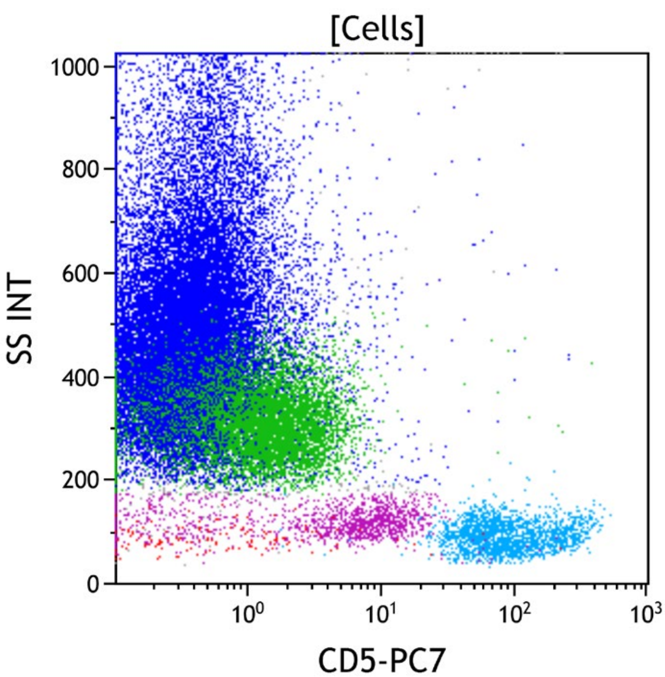

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. The progenitors (purple) express CD5.

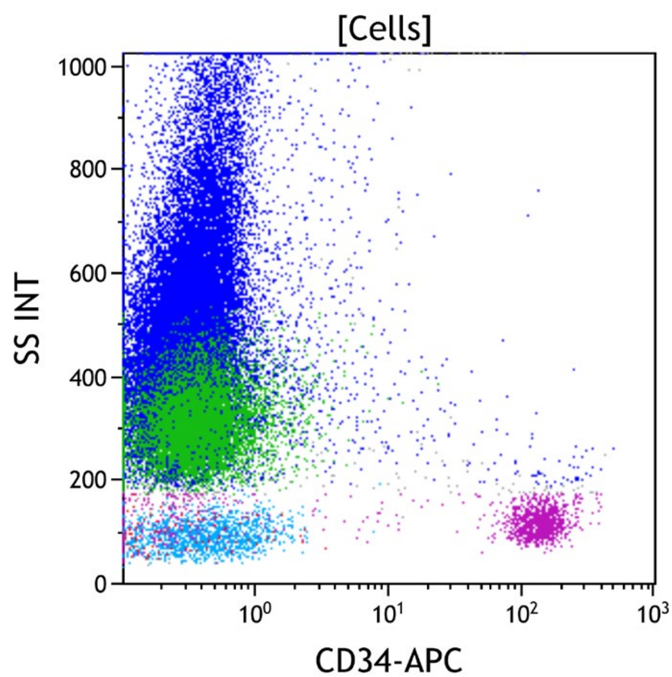

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The progenitors (purple) express bright CD34.

T Cell Tube

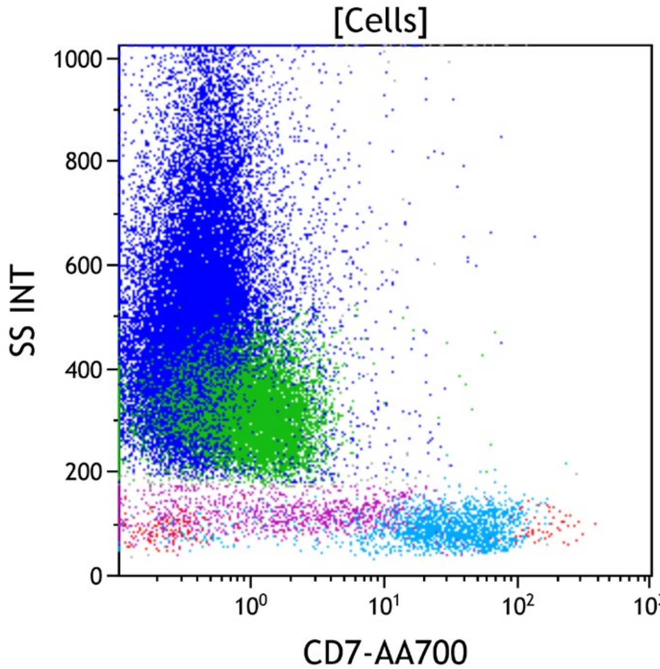

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors. The progenitors (purple) express variable CD7.

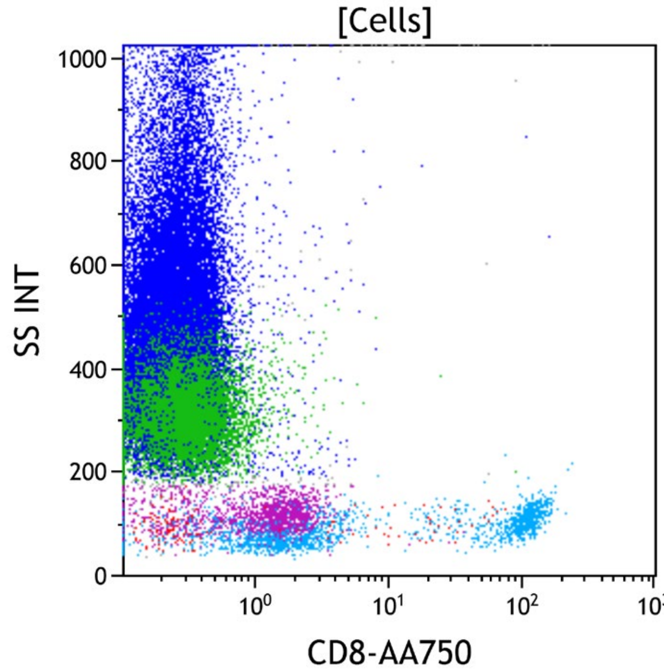

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The progenitors (purple) do not express CD8.

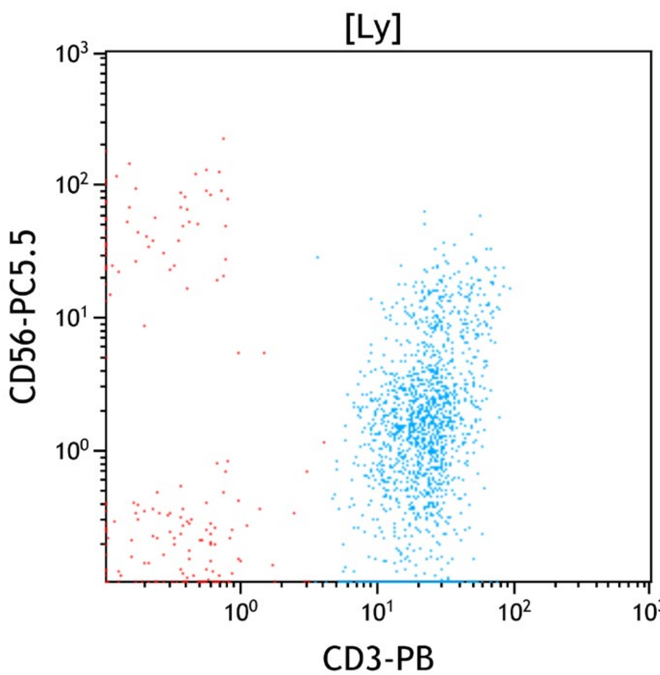

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red, upper left). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

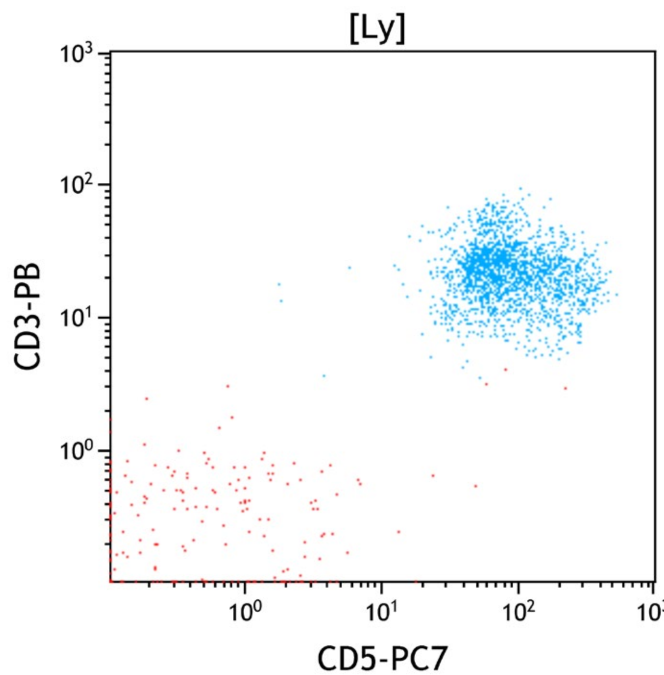

Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells.

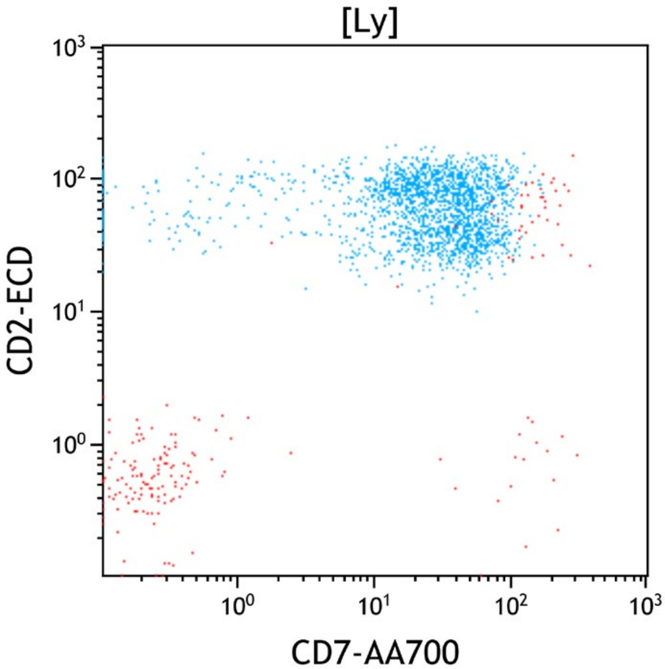

Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

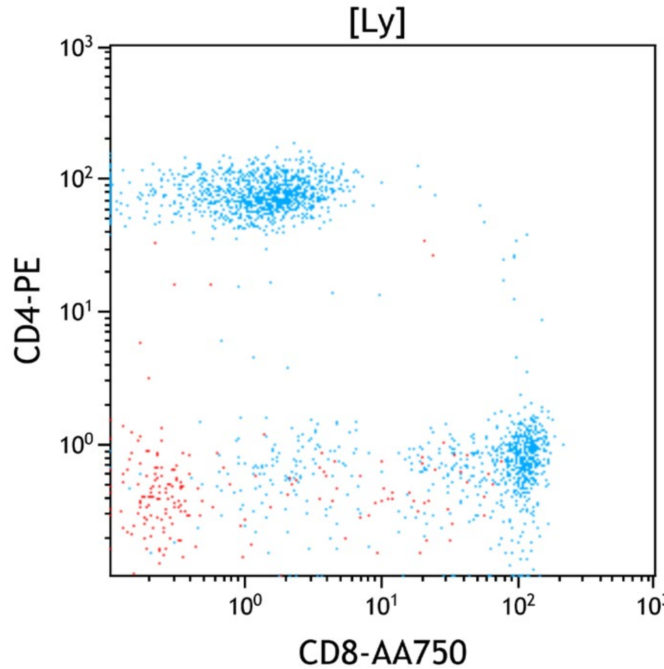

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/delta T cells. Of note, the CD4 positive but CD3 negative cells are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

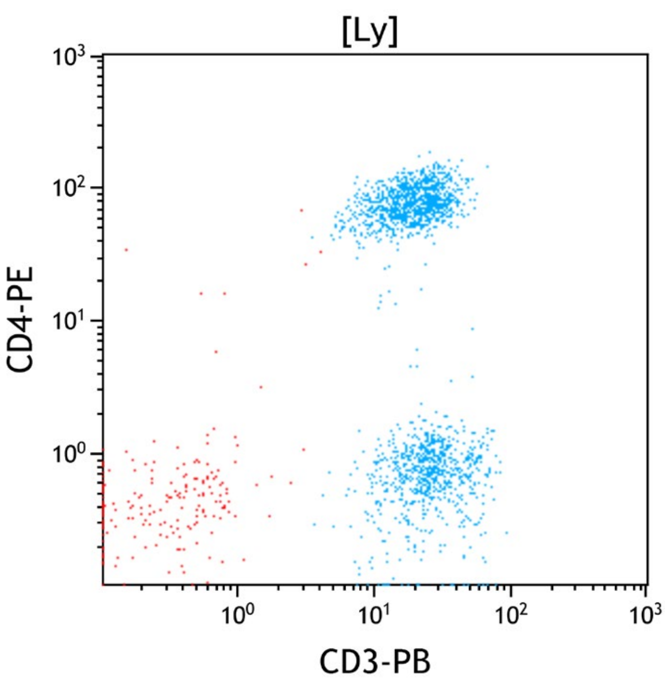

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

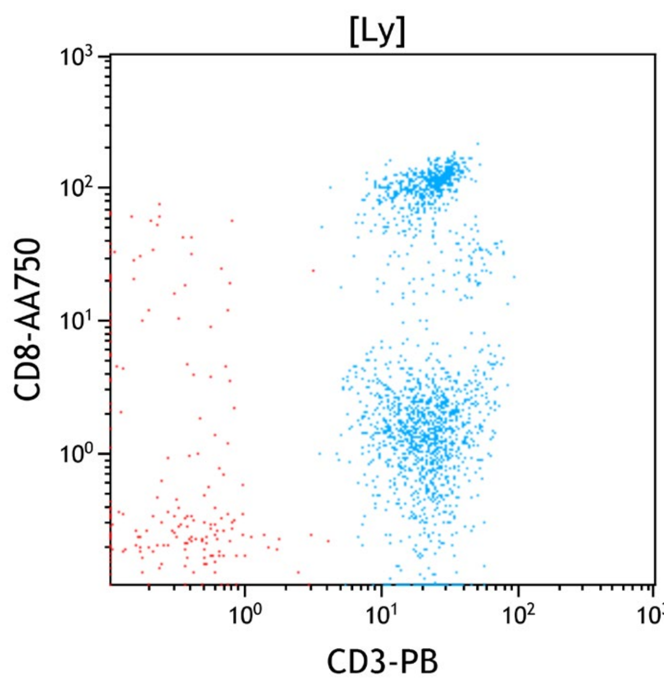

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 without CD3.

T Cell Tube

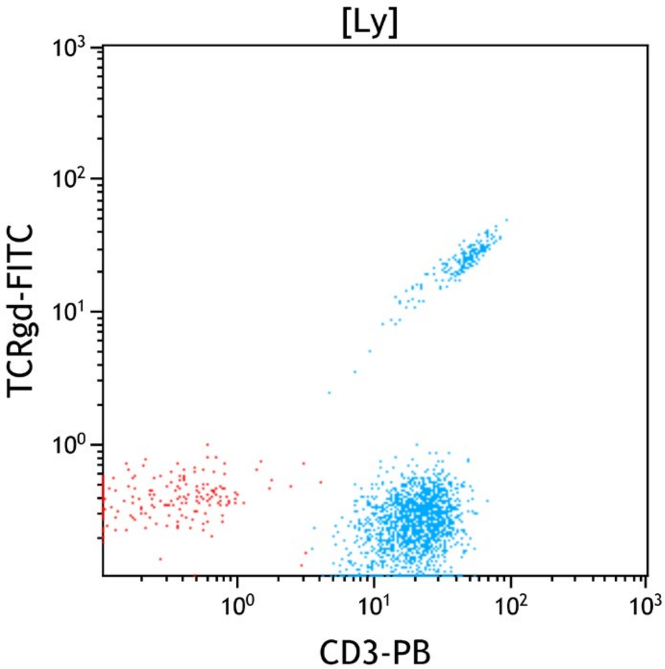

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

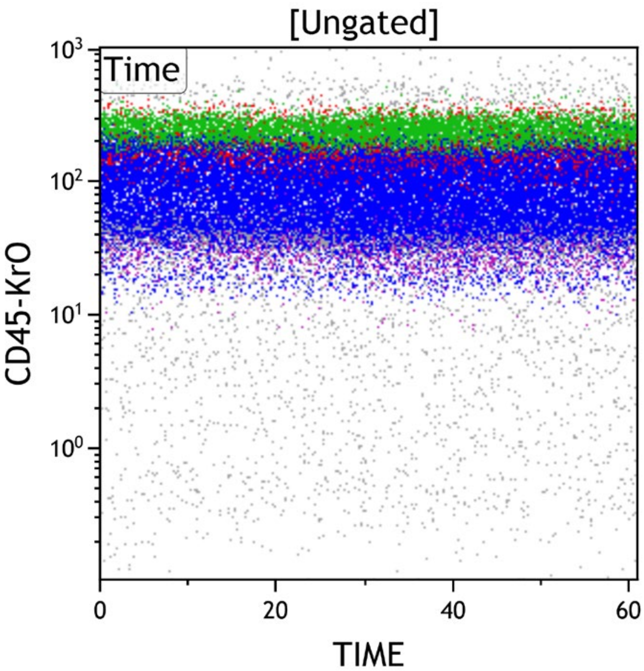

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

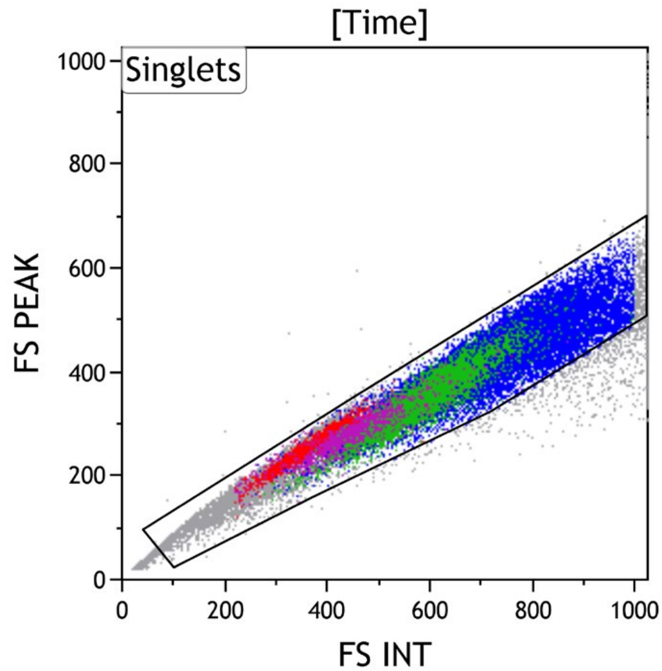

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

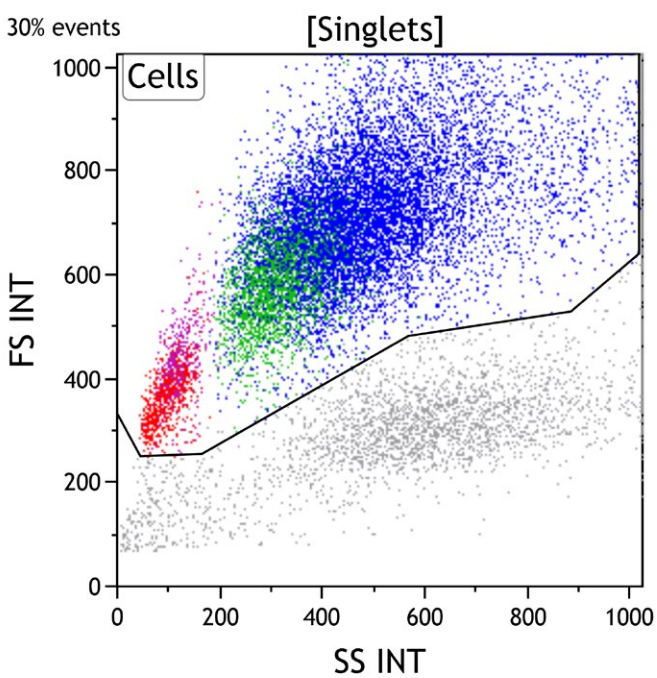

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

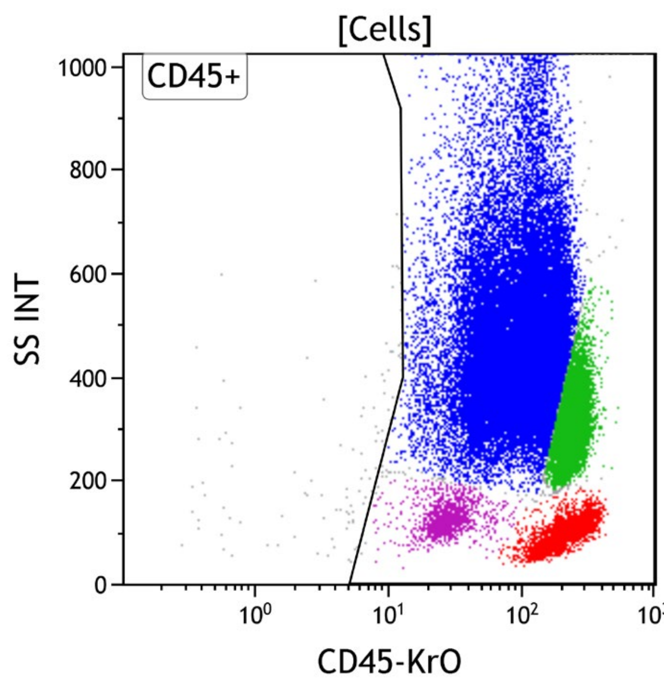

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

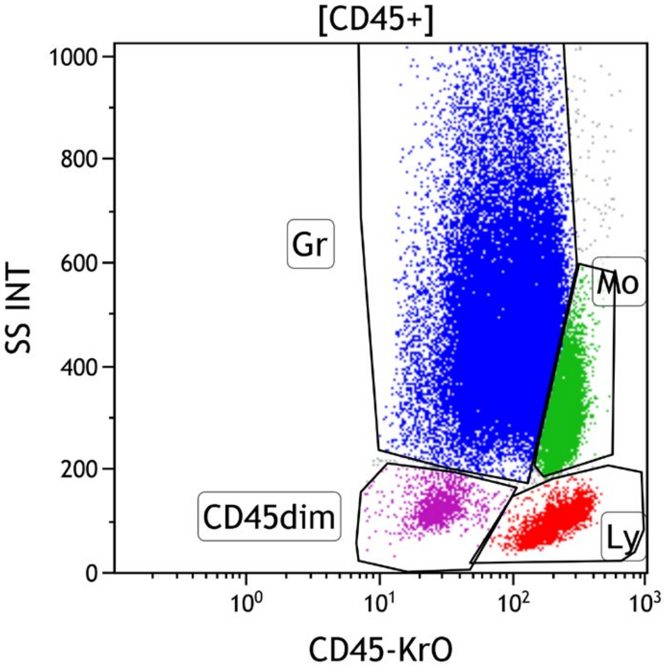

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the granulocytic population (blue) has decreased side scatter and a discrete progenitor (purple) population is present.

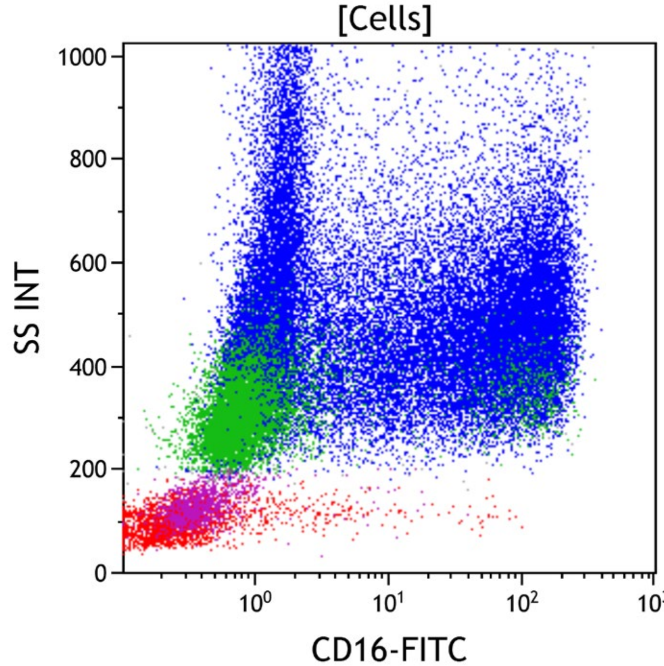

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The progenitors (purple) do not express CD16. A major subset of granulocytes (blue) express CD16.

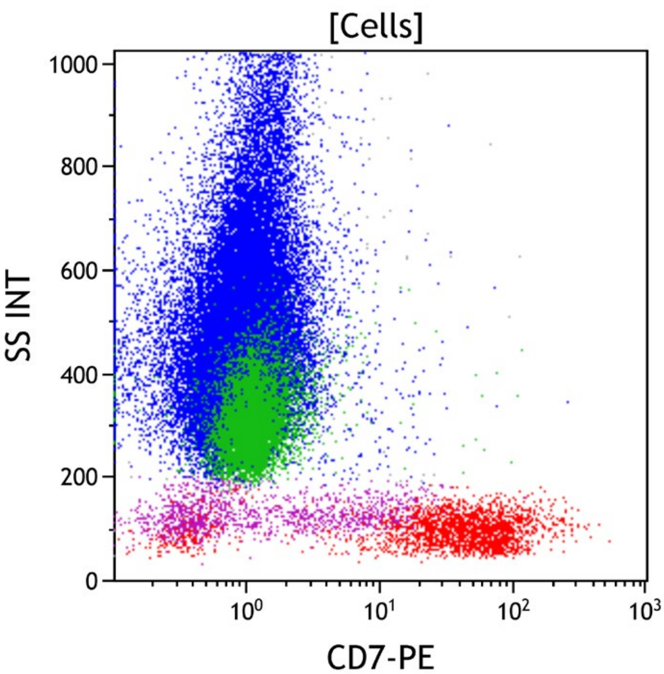

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red, lower right), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed progenitors. The progenitors (purple) express variable CD7.

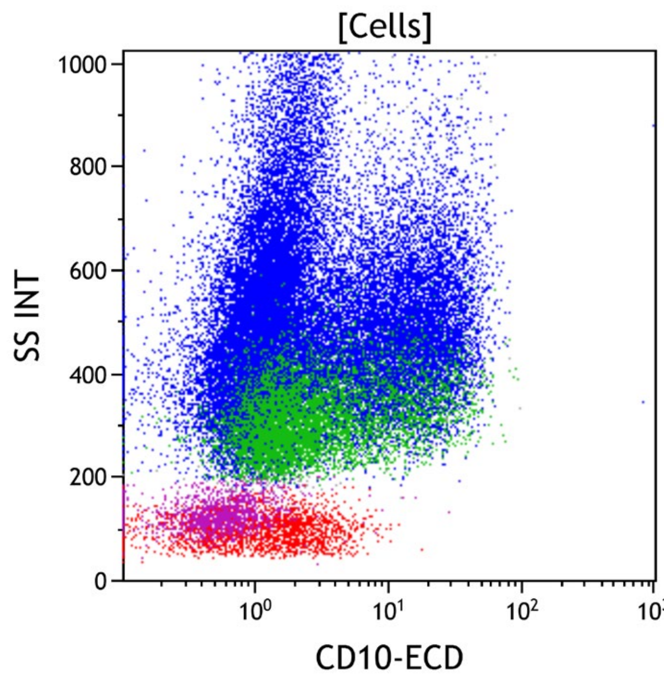

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes. The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The progenitors (purple) do not express CD10. A subset of granulocytes (blue) express CD10.

M1 Cell Tube

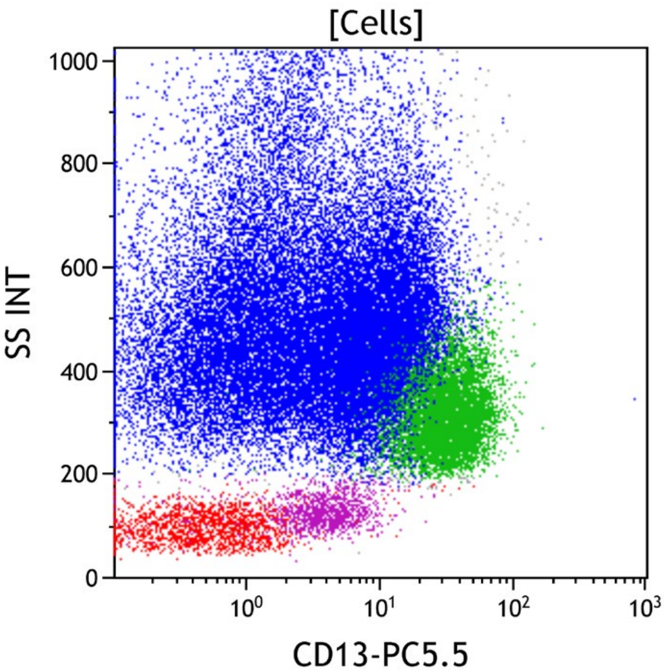

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. The progenitors (purple) express intermediate CD13.

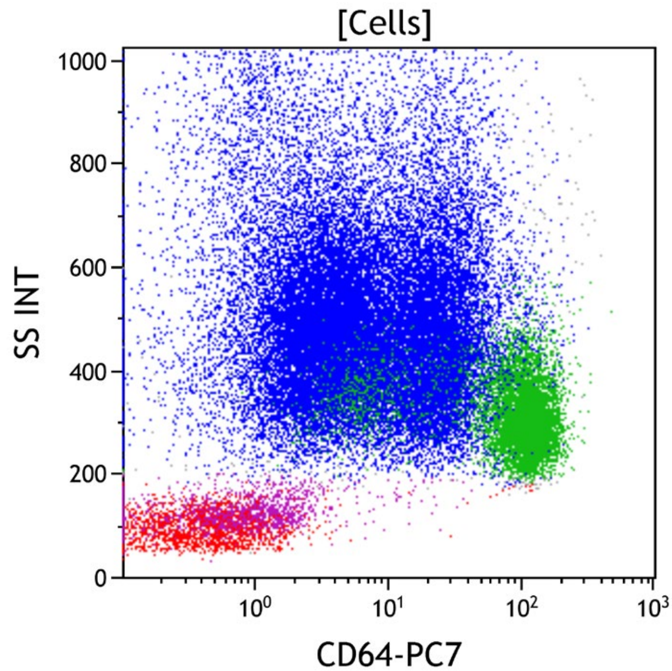

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes and at a slightly lower level on promyelocytes and myelocytes. CD64 is not well expressed on resting mature granulocytes, but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The progenitors (purple) do not express CD64.

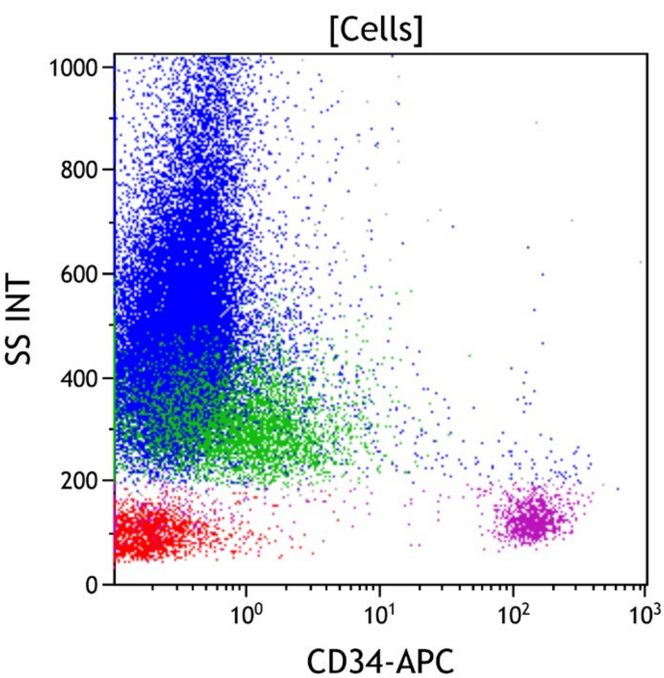

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. The progenitors (purple) express bright CD34.

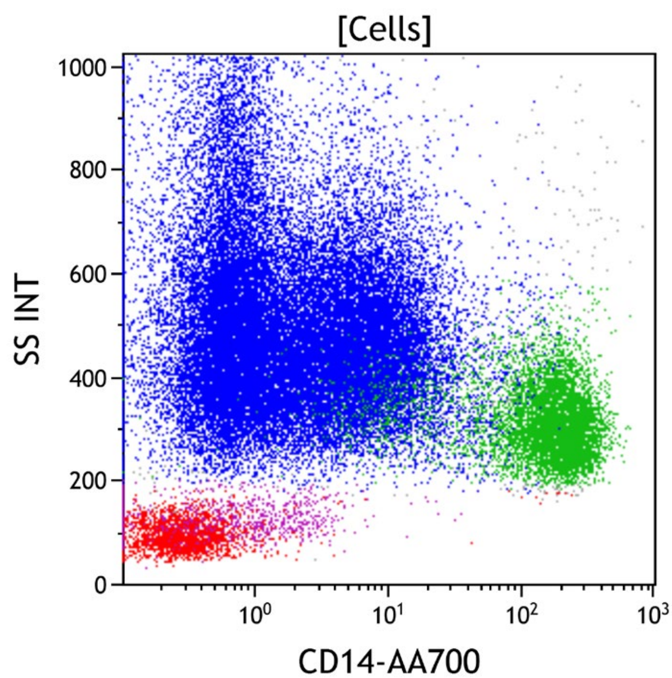

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. The progenitors (purple) do not express CD14.

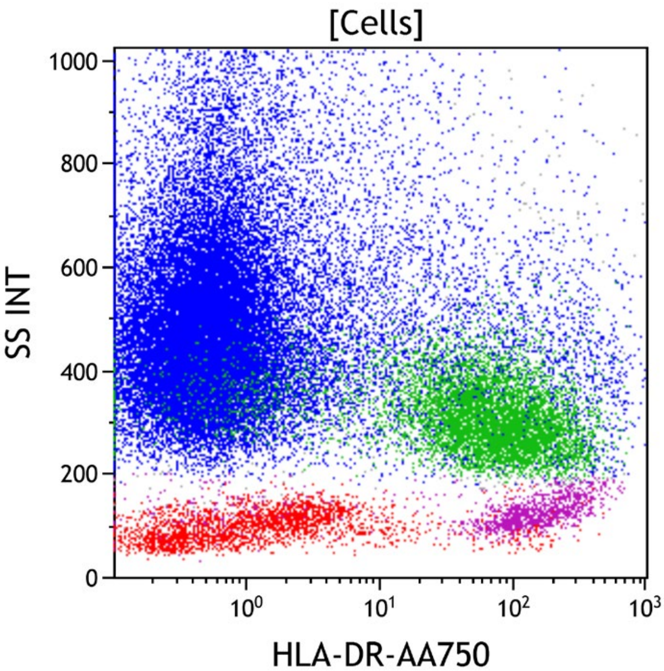

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The progenitors (purple) express bright HLA-DR.

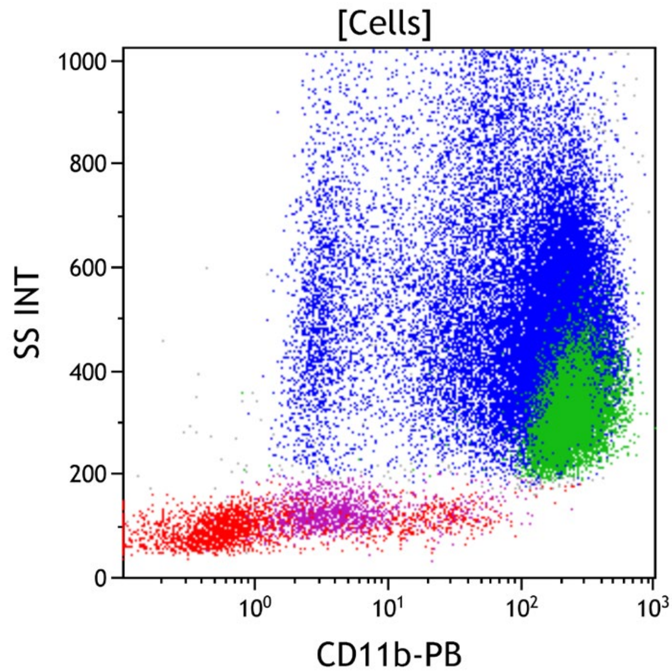

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage, and on monocytes. CD11b is also expressed on NK cells and basophils. The progenitors (purple) do not express CD11b. The granulocytes (blue) express uniform bright CD11b.

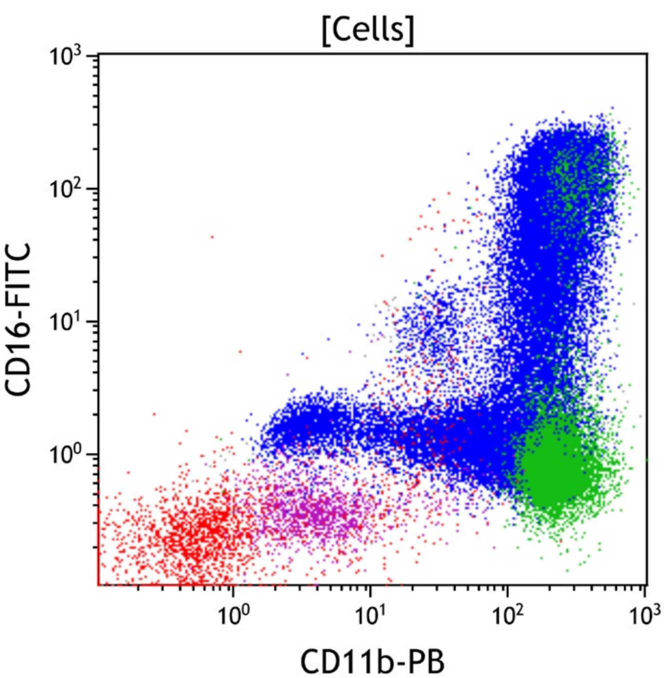

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level.

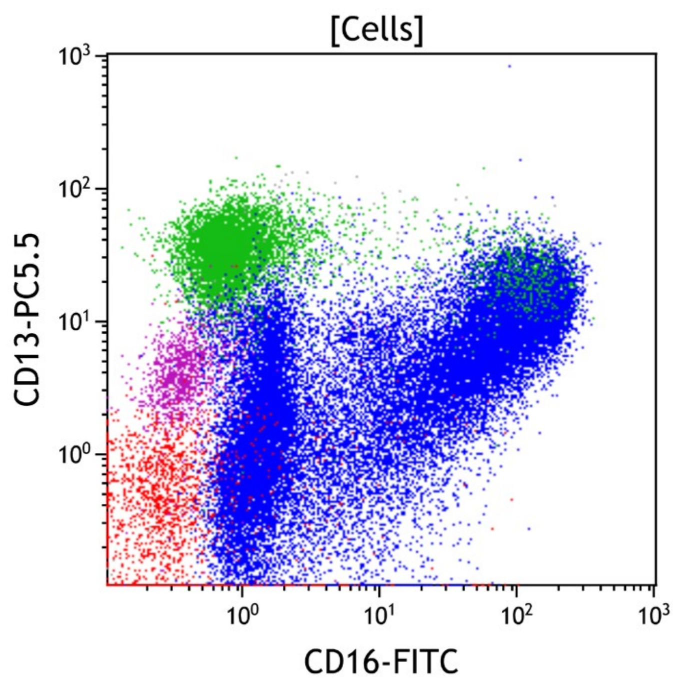

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16.

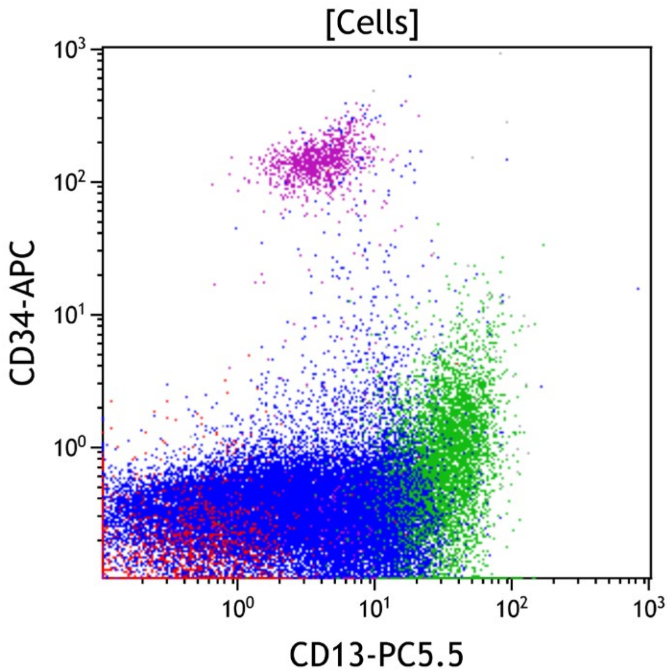

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells.

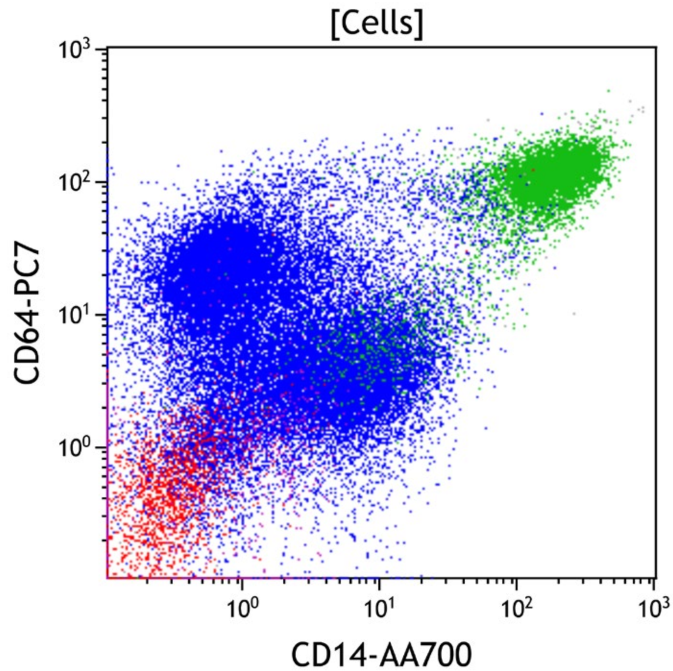

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes.

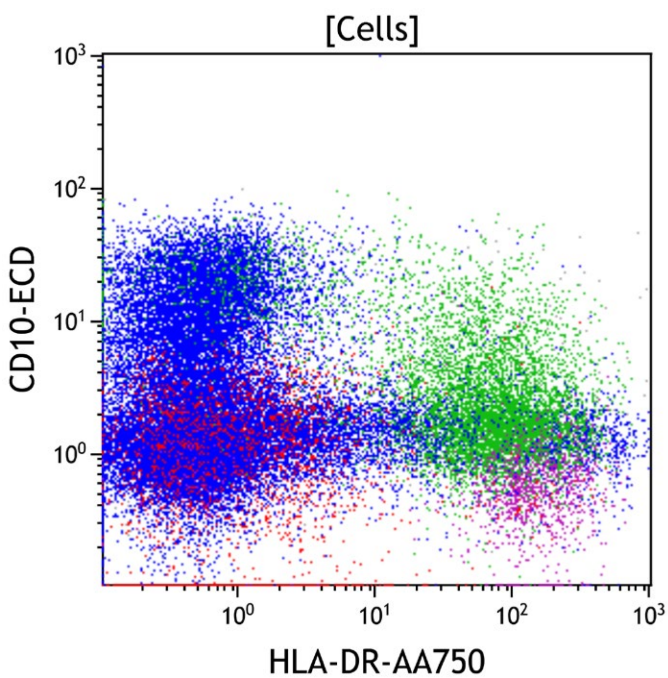

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes and immature B cells. Immature B cells express both CD10 and HLA-DR.

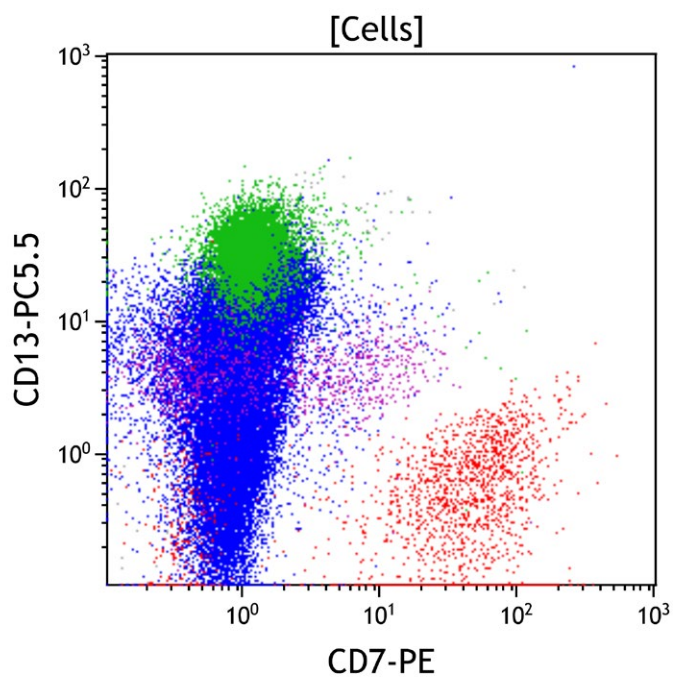

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The progenitors (purple) express intermediate CD13 and variable CD7.

M2 Cell Tube

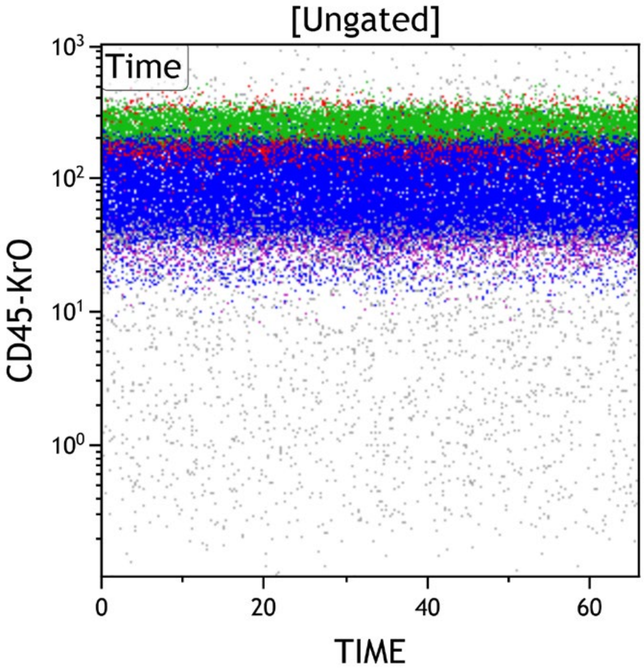

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded from the Time gate.

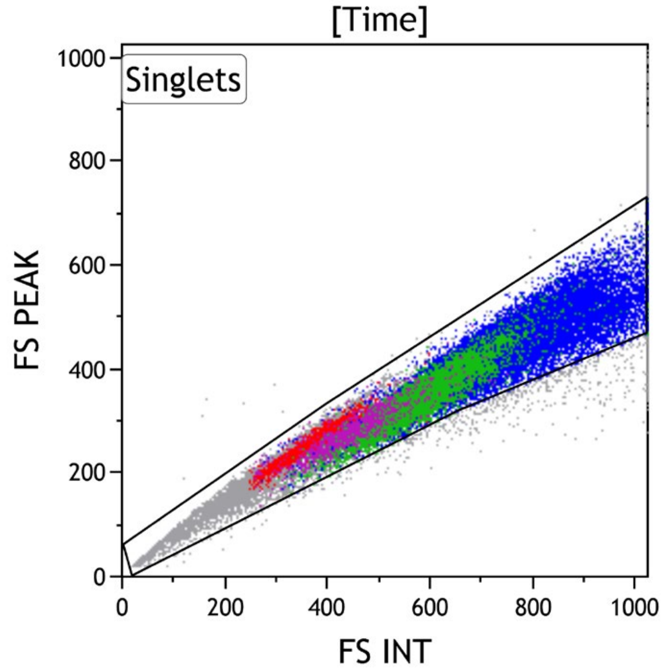

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

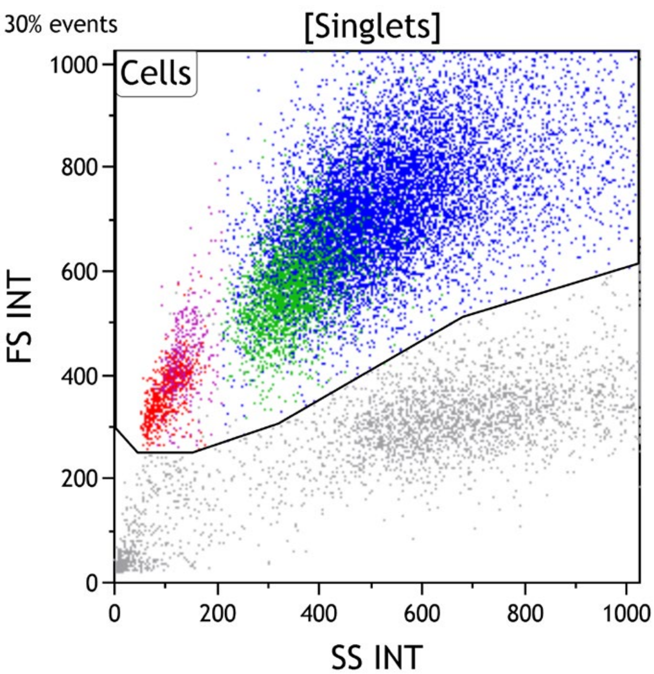

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

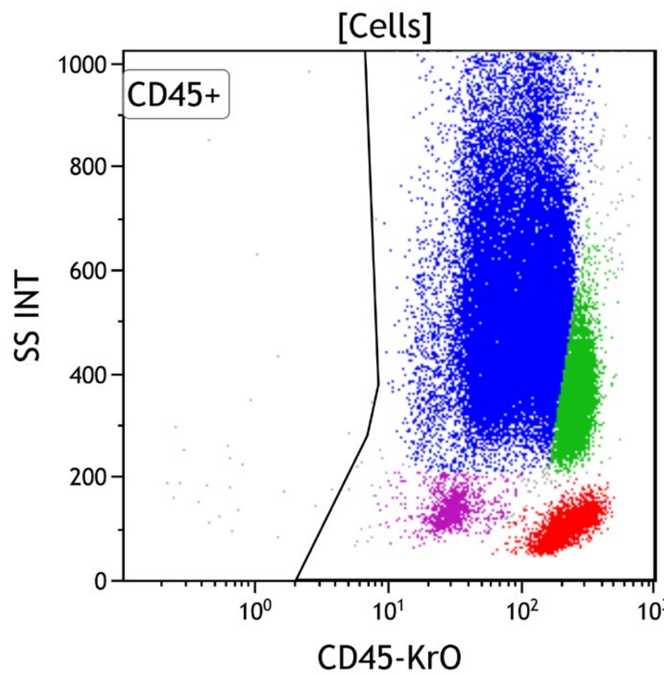

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

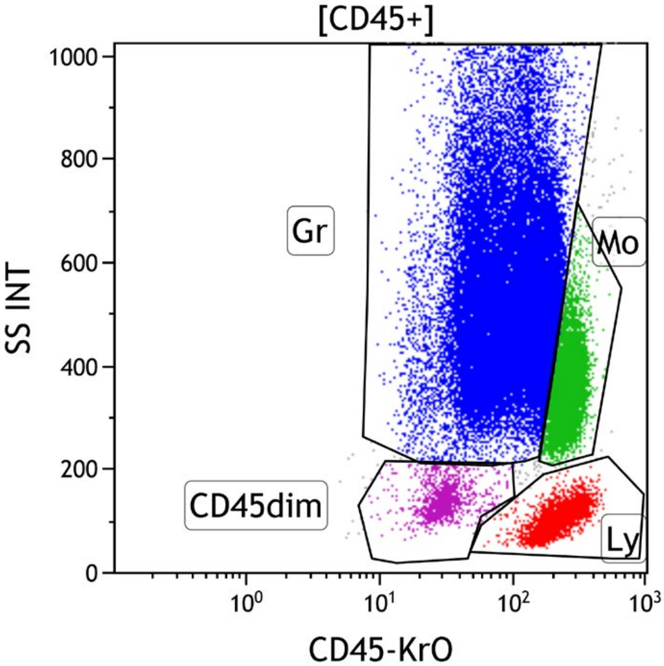

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by myeloblasts and immature B cells. Basophils, plasmacytoid dendritic cells, plasma cells and NK cells may also fall in this area. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Note the granulocytic population (blue) has decreased side scatter and a discrete progenitor (purple) population is present.

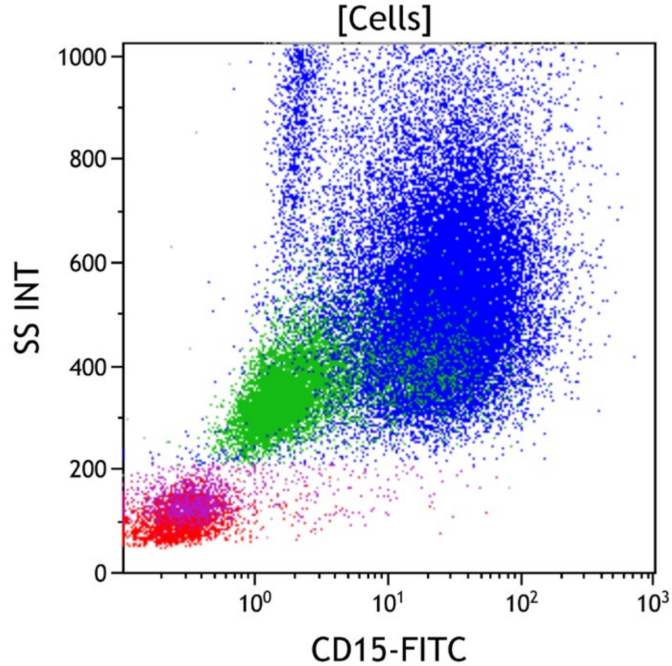

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). The progenitors (purple) do not express CD15.

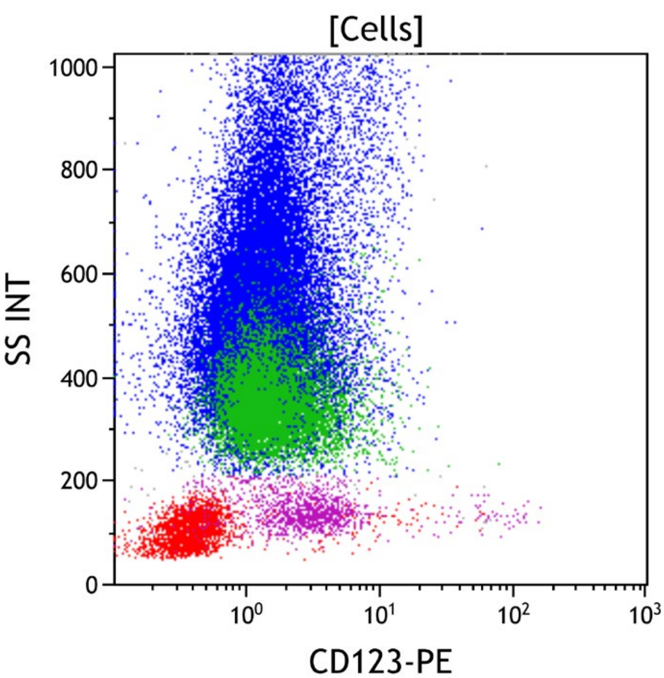

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The progenitors (purple) express dim CD123.

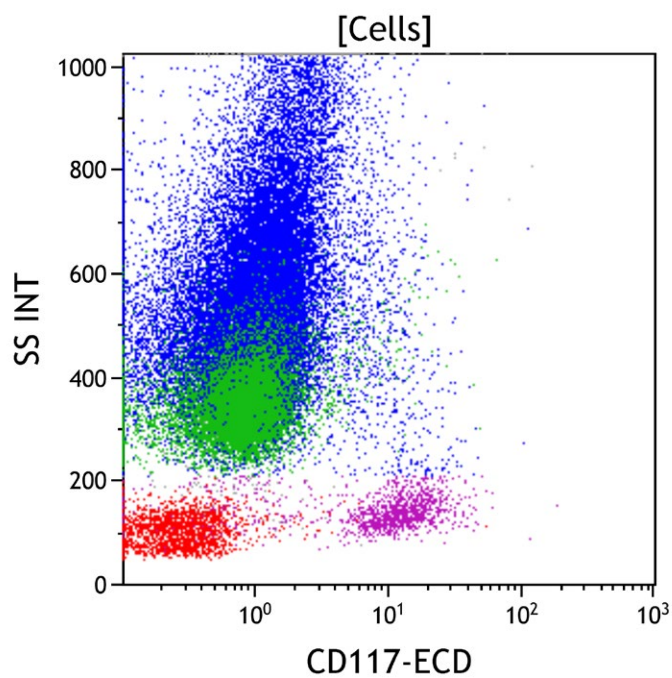

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. The progenitors (purple) express intermediate CD117.

M2 Cell Tube

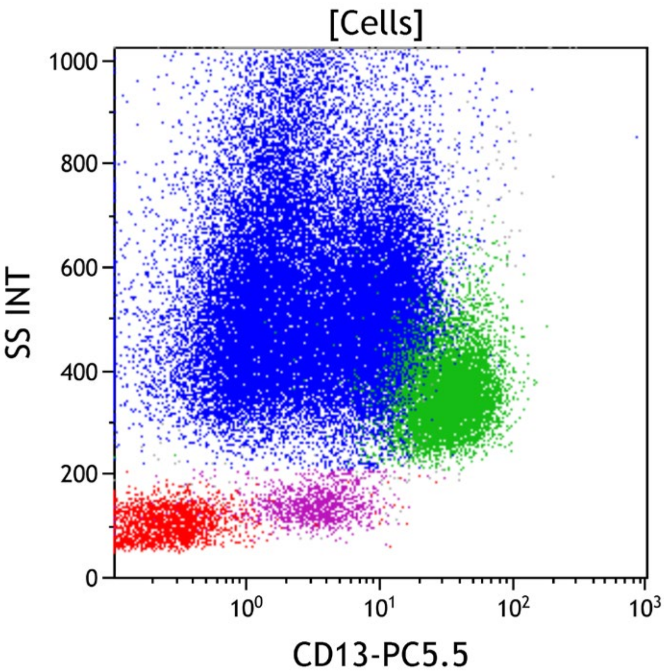

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. The progenitors (purple) express intermediate CD13.

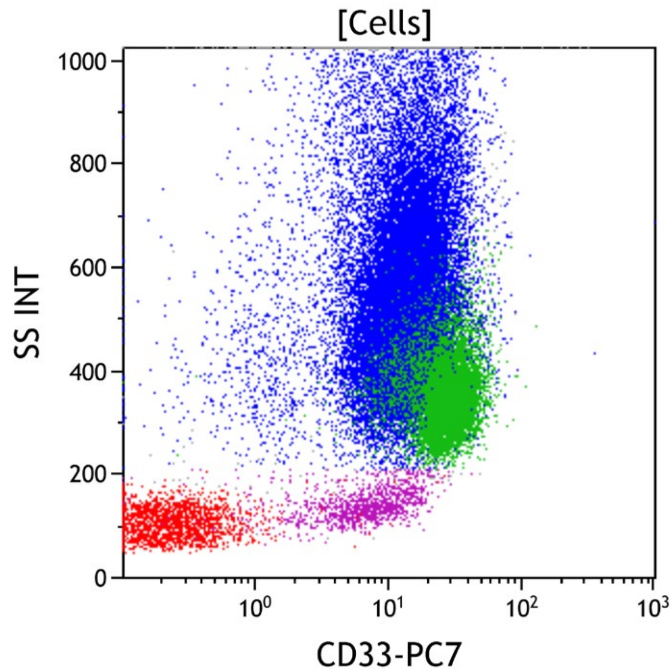

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes, and at the lowest level on mature granulocytes. CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. The progenitors (purple) express intermediate CD33.

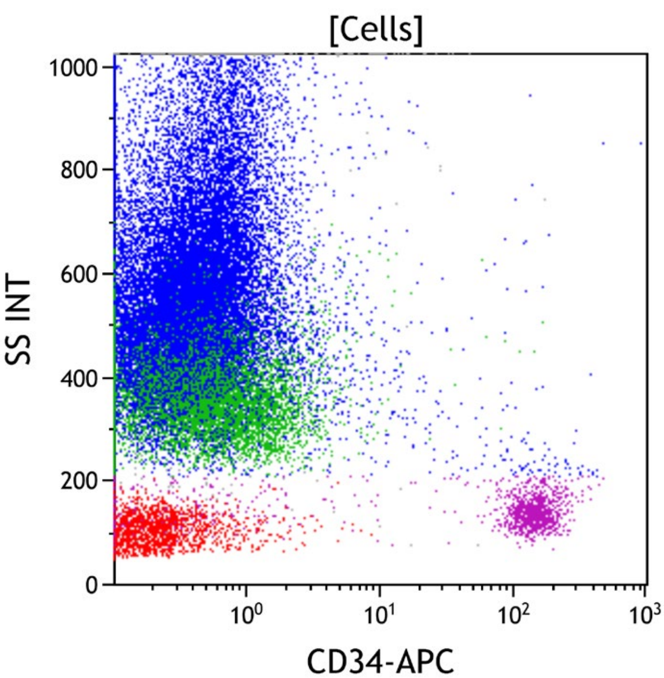

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. The progenitors (purple) express bright CD34.

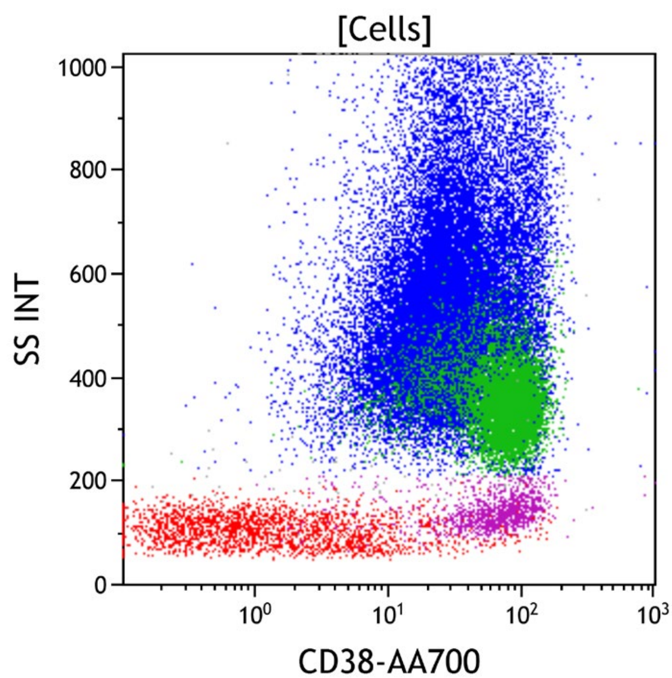

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes. CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. The progenitors (purple) express intermediate CD38.

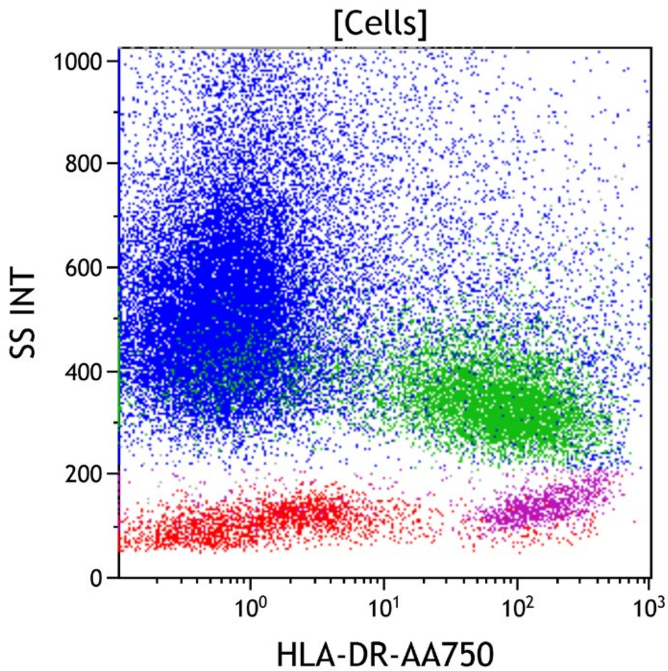

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The progenitors (purple) express bright HLA-DR.

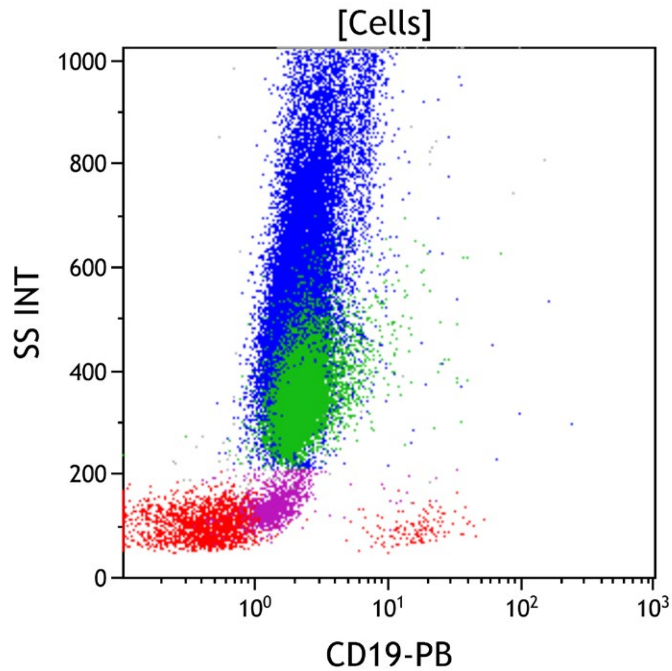

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The progenitors (purple) do not express CD19.

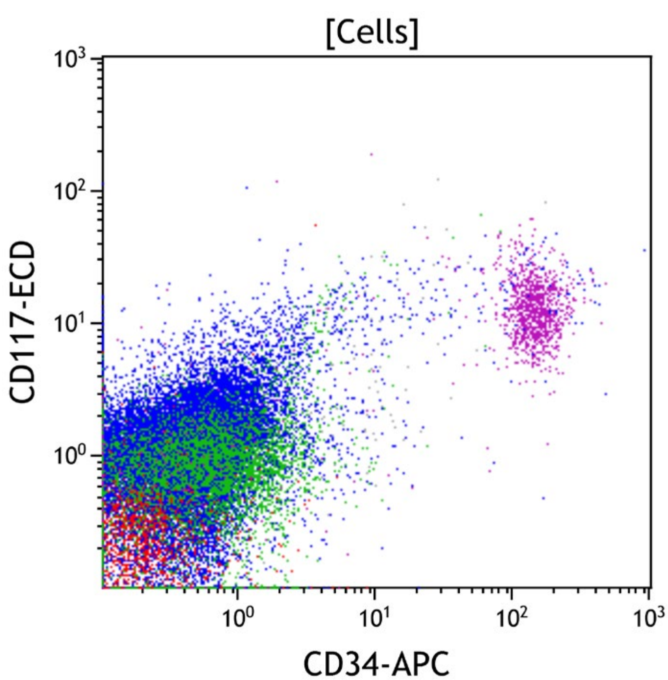

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The progenitors (purple) express CD34 and CD117.

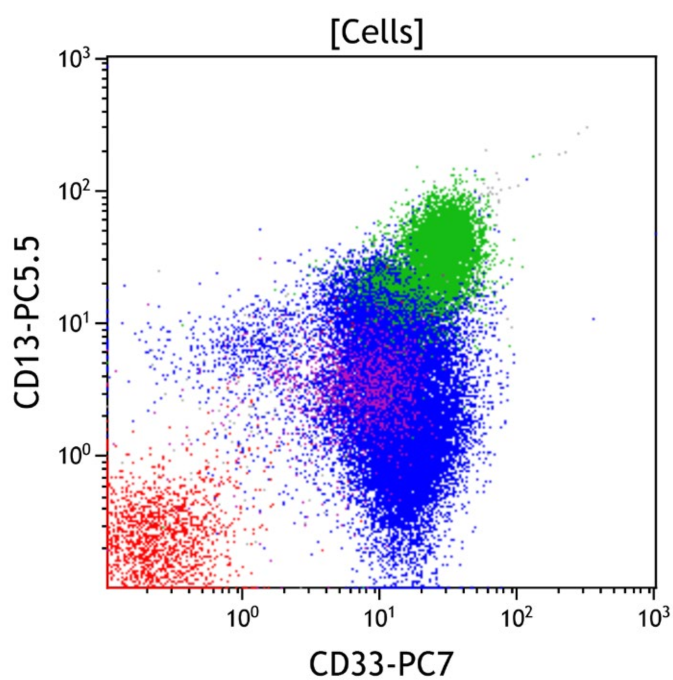

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than more mature granulocytes. Lymphocytes largely do not express either CD13 or CD33. The granulocytes (blue) show increased CD33 expression relative to monocytes (green) with variable CD13.

M2 Cell Tube

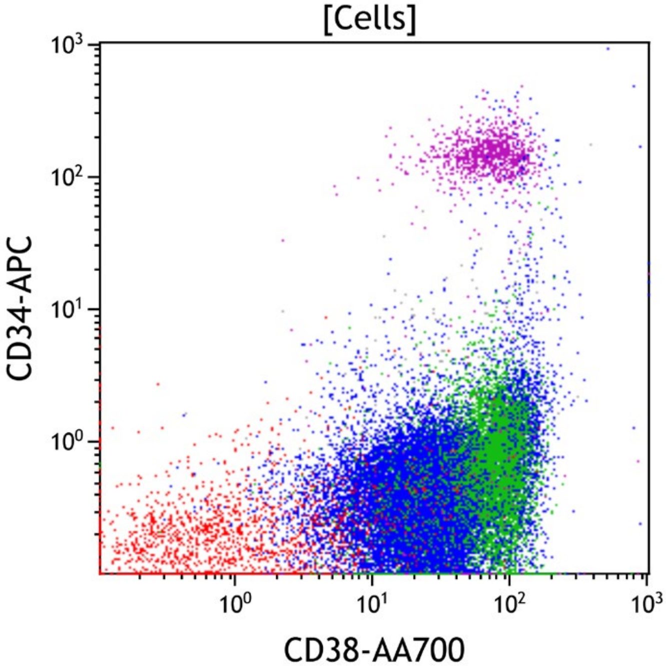

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38. The progenitors (purple) express CD38 and CD34.

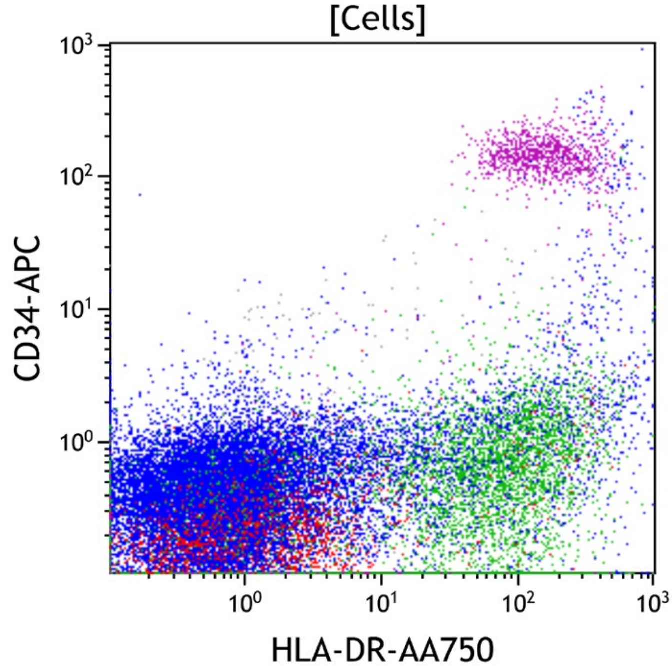

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. The progenitors (purple) express CD34 and bright HLA-DR.

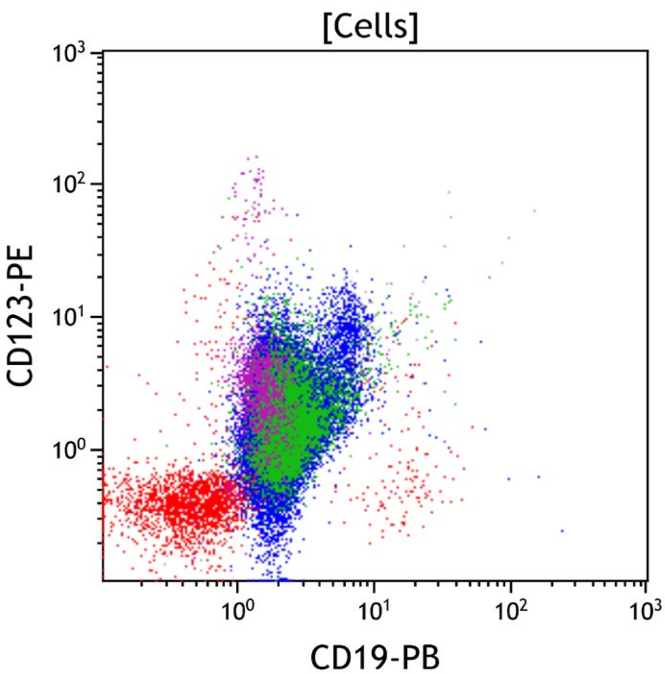

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not express CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The progenitors (purple) express dim CD123.

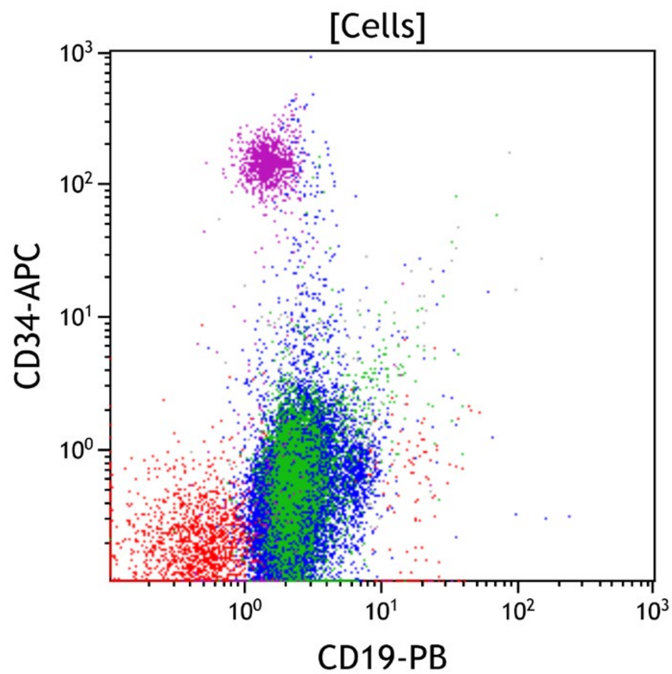

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors (purple) do not express CD19.

M2 Cell Tube

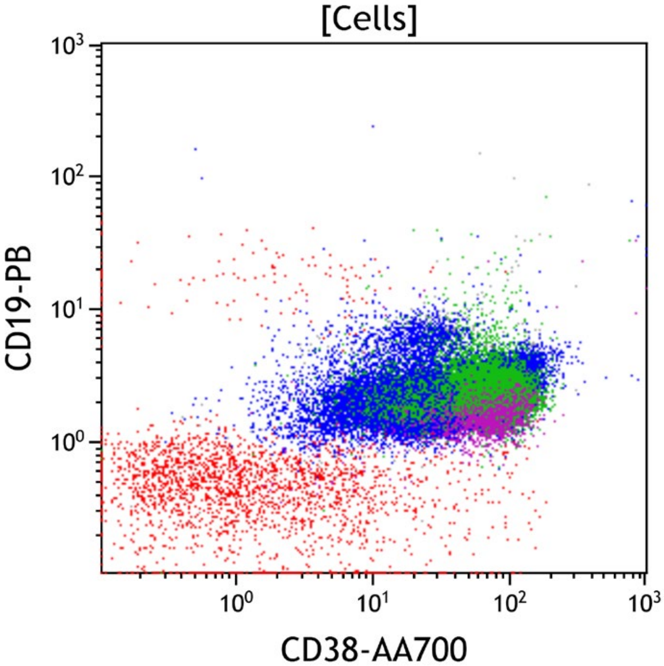

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38. Plasma cells show extremely high CD38 expression but also express variable CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The progenitors (purple) express intermediate CD38.

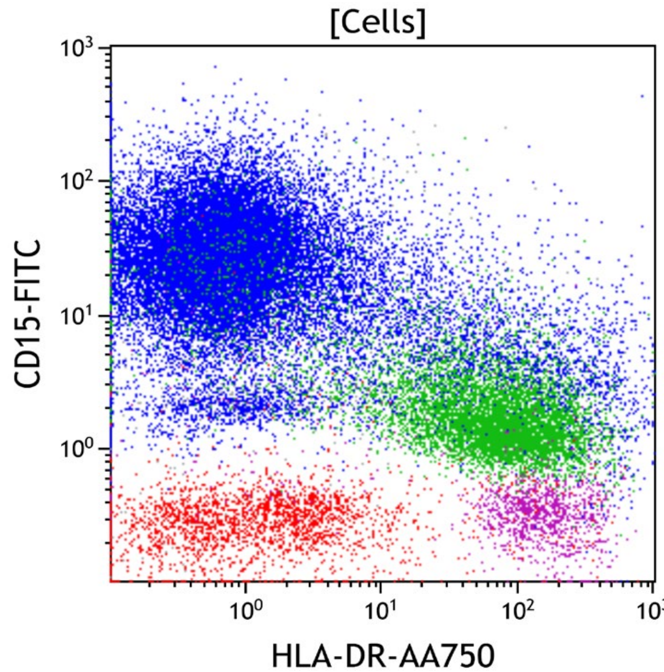

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR, except the earliest forms where CD15 is being acquired. CD34 positive myeloid progenitors (purple) express HLA-DR.

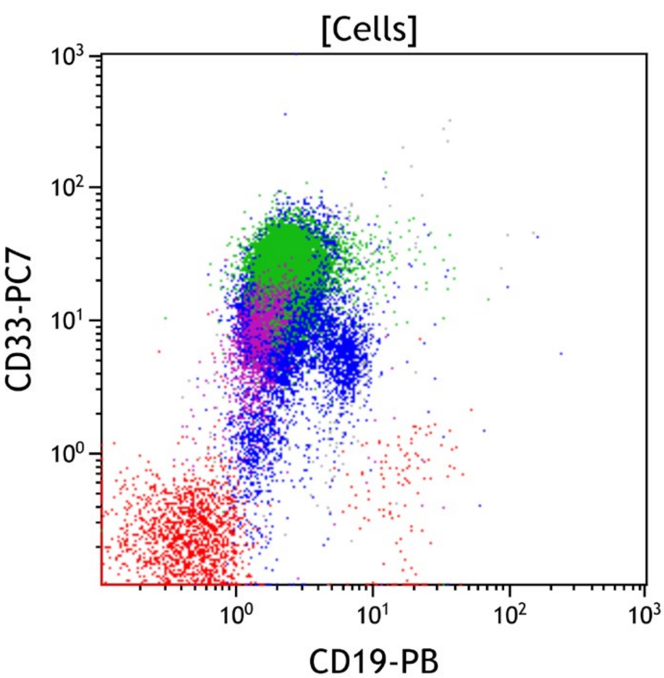

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes and maturing granulocytes. CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of progenitors with expression of intermediate CD5, variable CD7, intermediate CD13, intermediate CD33, bright CD34, intermediate CD38, dim CD45, intermediate CD117, dim CD123 and bright HLA-DR without CD14, CD15, CD64 and other lymphoid or myeloid antigens. Compared with normal CD34-positive progenitors, the expression of CD5 and CD7, increased CD34, and decreased expression of CD45 is aberrant. In addition, the monocytes (green) show aberrant expression of CD56 and granulocytes (blue) show abnormally decreased side scatter suggesting hypogranularity with aberrant expression of increased CD11b and increased CD33.

The immunophenotype of the abnormal progenitor population is that of mildly expanded abnormal CD34-positive progenitors. This finding along with abnormalities of monocytes and granulocytes in the context of an elderly patient with cytopenias is consistent with a myeloid stem cell disorder, in particular a low-grade myelodysplastic syndrome. However, a diagnosis of myelodysplasia requires morphologic and cytogenetic correlation, including a morphologic blast count to exclude acute myeloid leukemia. Additional testing shows the presence of 14% blasts by morphology with 22% monocytes and a normal karyotype which raises the possibility of chronic myelomonocytic leukemia (CMML), but additional laboratory correlation is required for definitive classification.

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