ClearLLab 10C Panels

IVD Antibody Combinations for Leukemia / Lymphoma* Analysis

CASEBOOK

* Non-Hodgkin's Lymphoma only

ACCELERATING *answers*

BECKMAN COULTER
Life Sciences

Cases

Introduction

This casebook has been designed to assist in the analysis of flow cytometric immunophenotyping data generated using Beckman Coulter's ClearLLab 10C Panels CE-IVD marked reagent for Leukemia and Lymphoma analysis on the Beckman Coulter DxFLEX flow cytometers. The ClearLLab 10C Panels are currently the only marketed flow cytometry IVD solutions. The panels improve laboratory efficiency and performance through reduced hands-on time; thereby, resulting in a reduction of laboratory errors.

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 encompasses a clinical vignette describing the patient demographics and clinical history, case-specific listmode data files for casebook user reanalysis, ClearLLab 10C specific analysis protocols utilizing listmode data, and a report showing the analysis with provided protocols. Analysis notes highlighting the immunophenotypic findings as well as potential pitfalls are also included in each report.

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 individual cells present in the specimen. When combined, 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. The steps involved for evaluating patient samples with suspected hematolymphoid malignancies are as follows[1]:

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

Consensus Recommendations for Immunophenotyping

Consensus recommendations for flow cytometric immunophenotyping of patient samples with known or suspected hematolymphoid malignancies have emerged over the last two decades; moreover, 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 various cell populations by multiparameter immunophenotyping on the DxFLEX flow cytometer. 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's lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). The reagents can be used with peripheral whole blood (collected in K2EDTA, Acid Citrate Dextrose (ACD) or Heparin), bone marrow (collected in K2EDTA, 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 casebook were 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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Analysis Protocols:
Download the ClearLLab 10C analysis protocol.

Analysis:
Download case specific Kaluza C analysis files.

Related Documents

- DxFLEX & ClearLLab 10C System Guide, PN C83032
- Kaluza C Flow Cytometry Software Instructions For Use, PN C10993
- DxFLEX Flow Cytometer, PN C44326 and C78500
- DxFLEX Flow Cytometer Instructions For Use, PN C44966 and PN C79288
- 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

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's 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.

References

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
6. 2006 Bethesda International Consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: medical indications. Davis BH, Holden JT, Bene MC, Borowitz MJ, Braylan RC, Cornfield D, Gorczyca W, Lee R, Maiese R, Orfao A, Wells D, Wood BL, Stetler-Stevenson M. Cytometry B Clin Cytom. 2007;72 Suppl 1:S5-13
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

CASE OVERVIEW

Case #	**Case 1 LHS-TC4-001**
Diagnosis	**Mature B cell lymphoma**
Clinical Vignette	This 77-year-old male presents with anemia, thrombocytopenia, lymphocytosis, and monocytosis. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased forward and side scatter and expression of CD19, bright CD20, intermediate CD38, bright CD45, variable CD200, bright HLA-DR, and surface kappa light chain restriction without CD5, CD10, or other T or myeloid antigens. Compared with normal B cells, the increased forward and side scatter, increased expression of CD20 and CD38, and the kappa light chain restriction are aberrant. Taken together, the immunophenotype of the aberrant population is consistent with mature B cell lymphoma but does not allow specific subclassification. While the increased forward and side scatter raises the possibility of large B cell lymphoma, morphologic and genetic correlation is required for definitive diagnosis and subclassification.

BACK TO CASE LISTINGS

B Cell Tube

B01

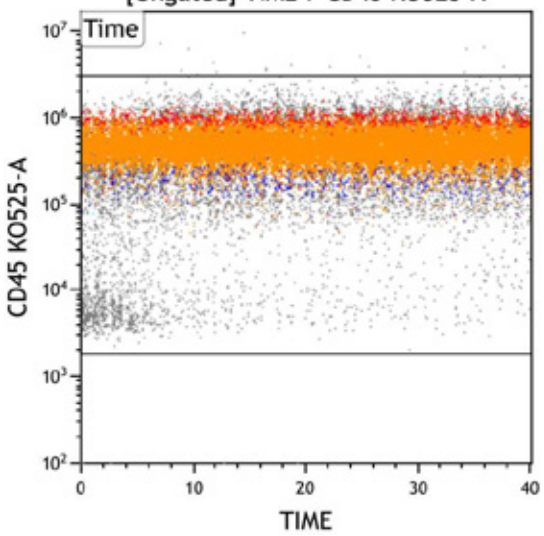

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

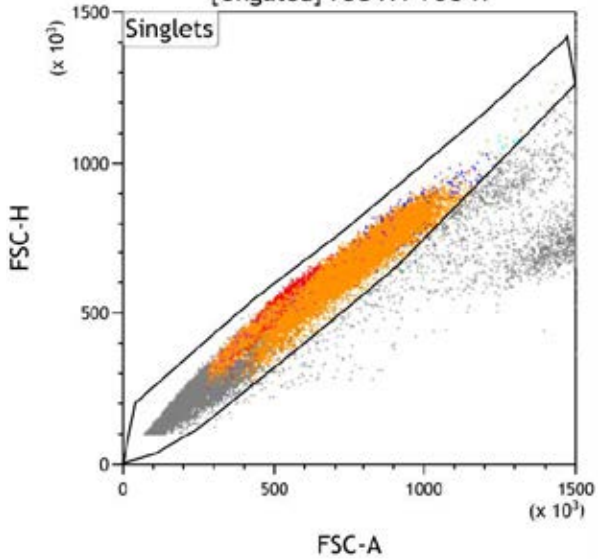

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

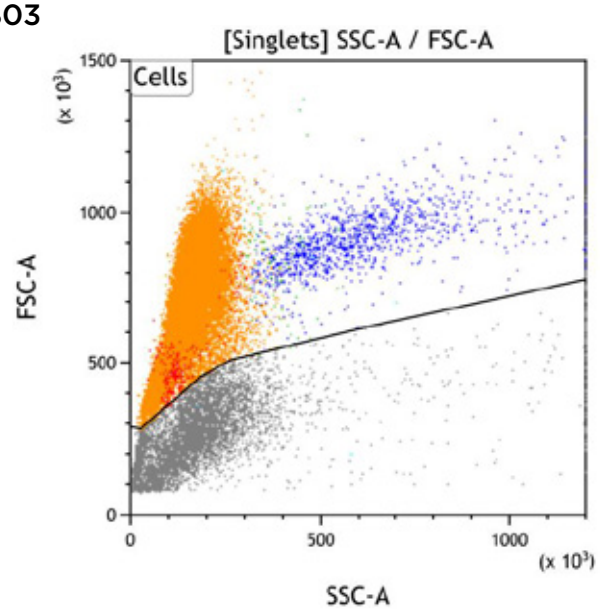

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

B04

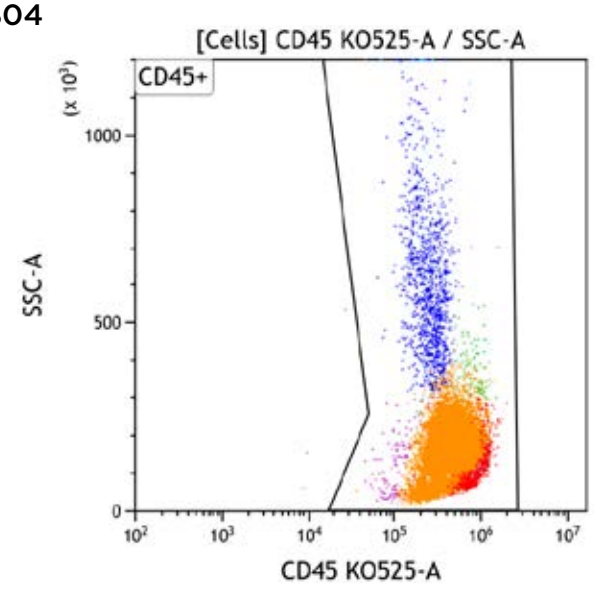

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

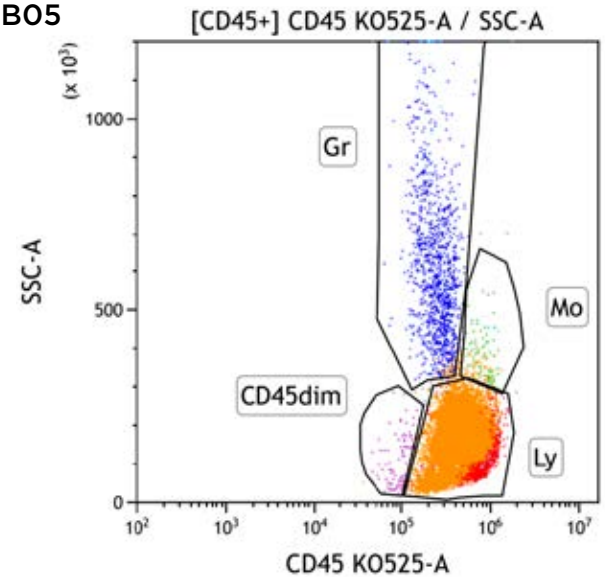

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 followed throughout the analysis. In this case, the white blood cells are composed predominantly of lymphocytes.

B06

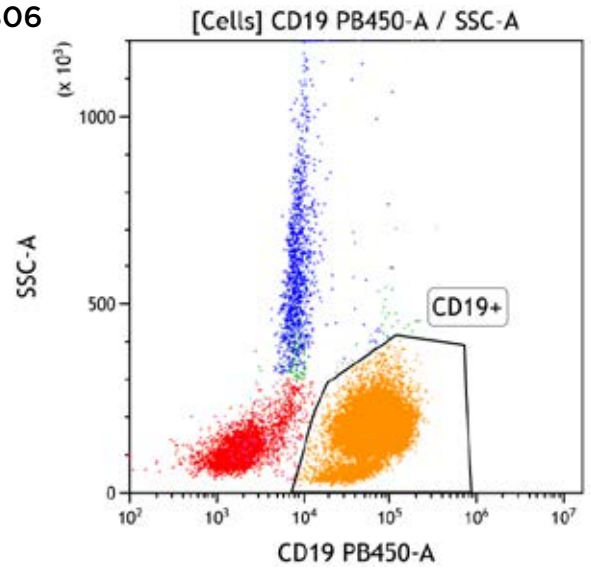

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. Note CD19 positive B cells are proportionally increased and show increased side scatter.

B07

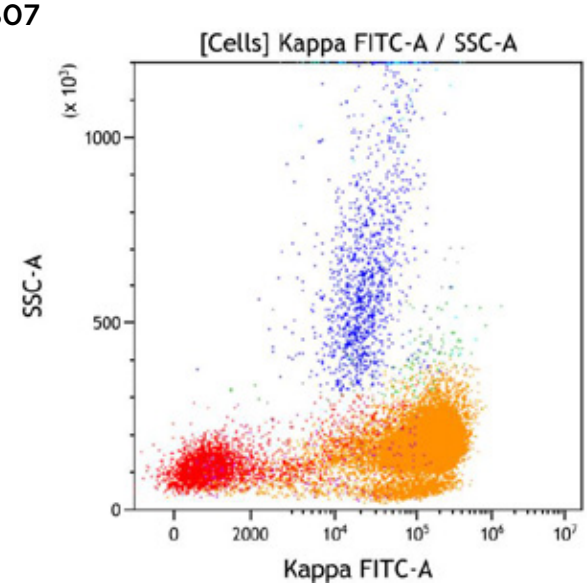

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The majority of the CD19 positive cells (orange) have surface kappa light chain expression.

B08

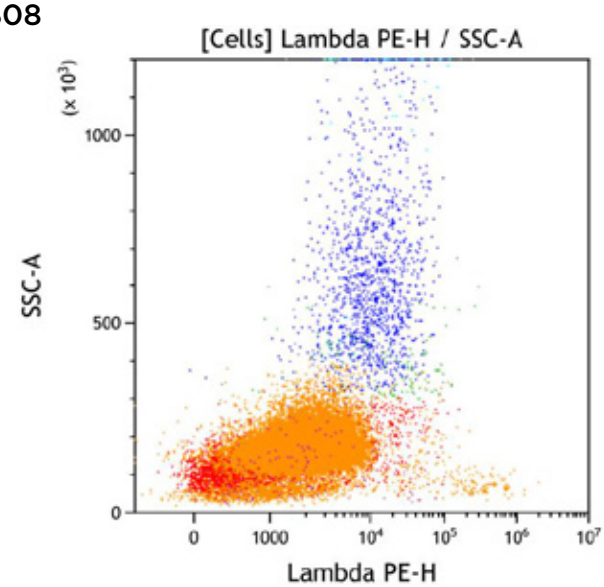

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The majority of the CD19 positive cells (orange) lack surface lambda light chain expression.

BACK TO CASE OVERVIEW

B09

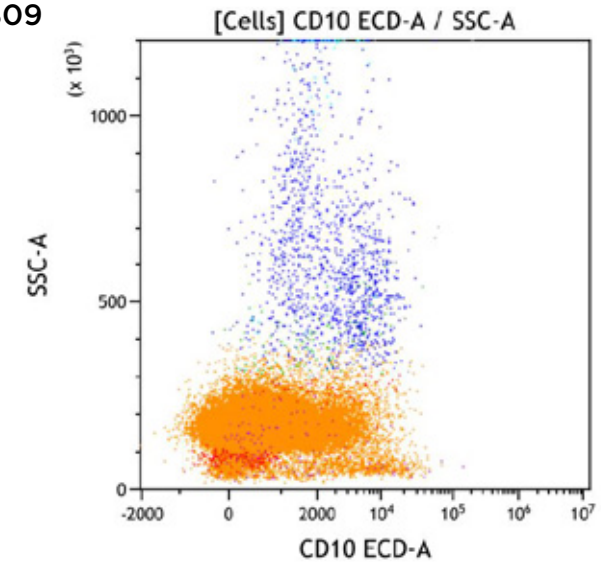

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange)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

B10

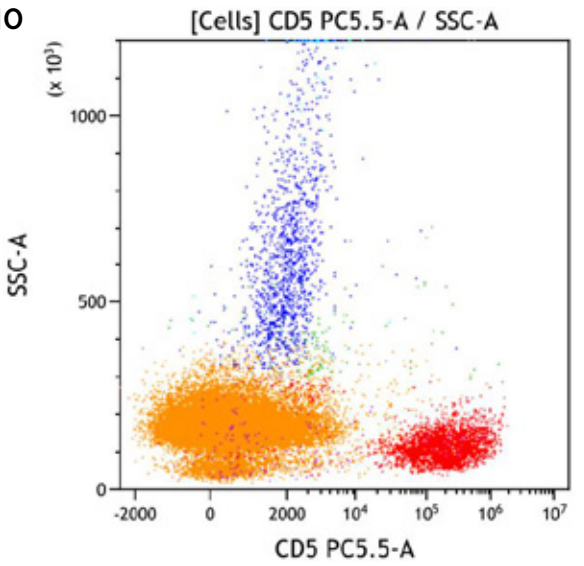

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red), as well as dimly on a subset of mature B cells. The CD19 positive population (orange) is negative for CD5.

B11

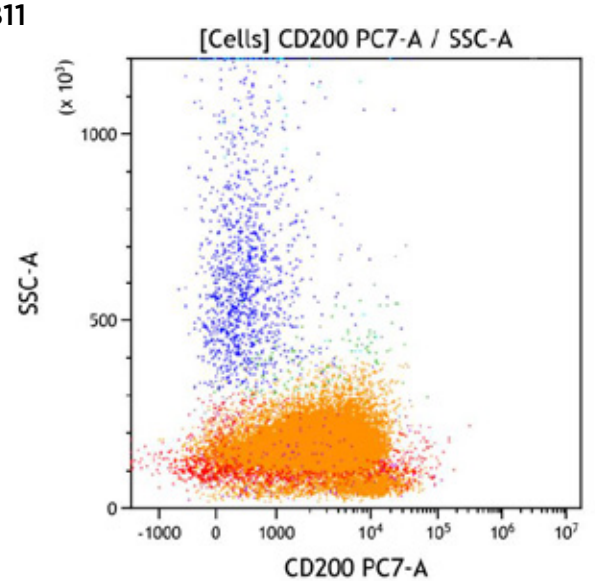

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

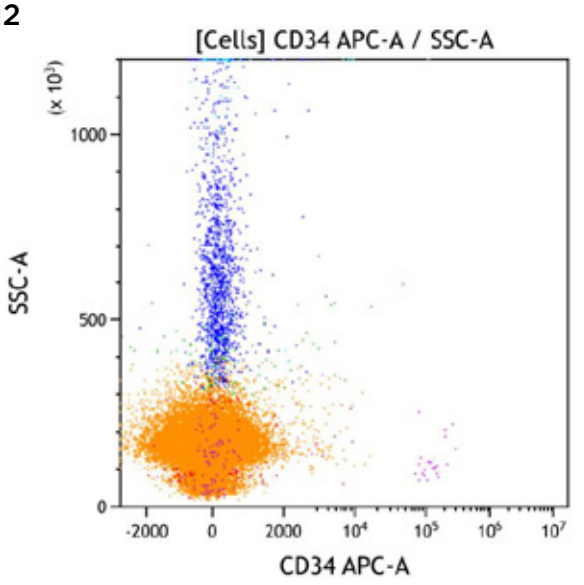

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors (purple, lower middle)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

BACK TO CASE OVERVIEW

B13

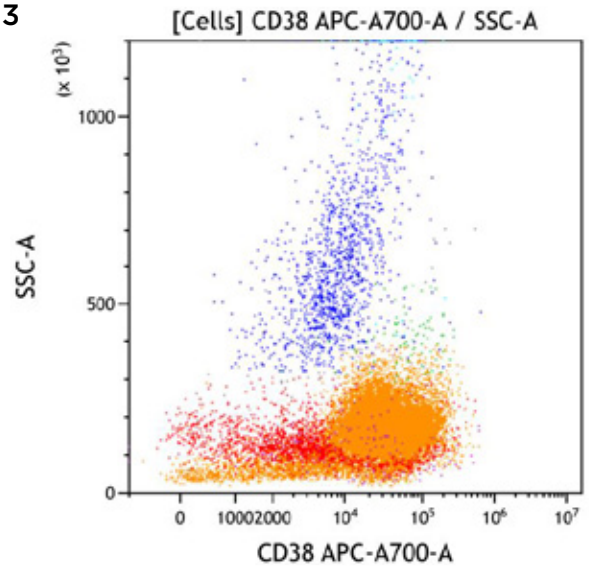

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variable level on activated mature lymphocytes.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

B14

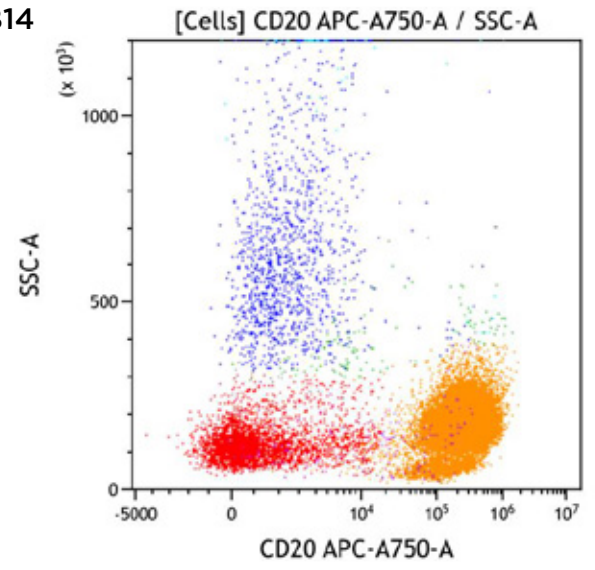

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.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

B15

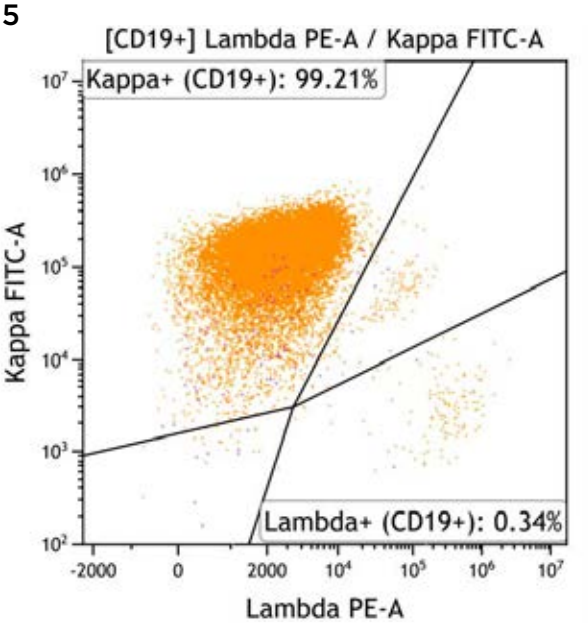

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio between 1 to 2. The CD19 positive population (orange) predominantly has surface kappa light chain expression, indicating a clonal B cell population.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

B16

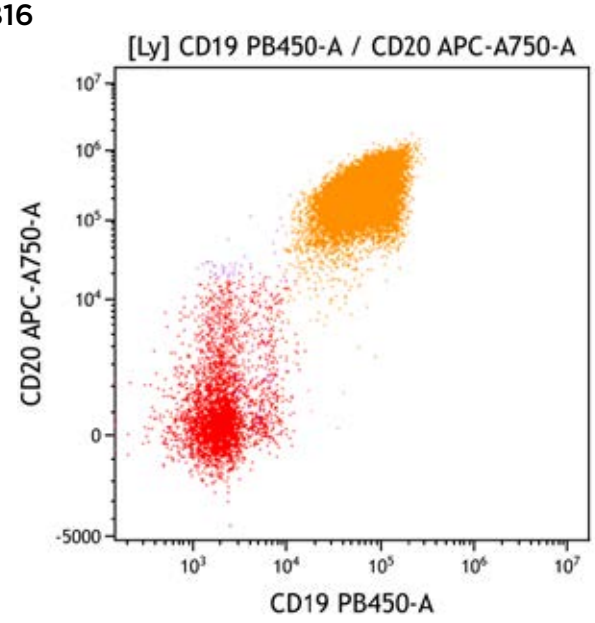

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cell population (orange) expresses CD19 and CD20.

BACK TO CASE OVERVIEW

B17

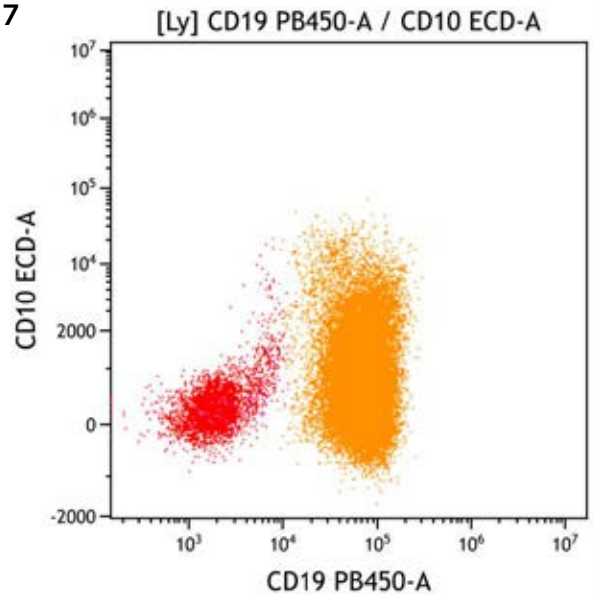

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature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

B18

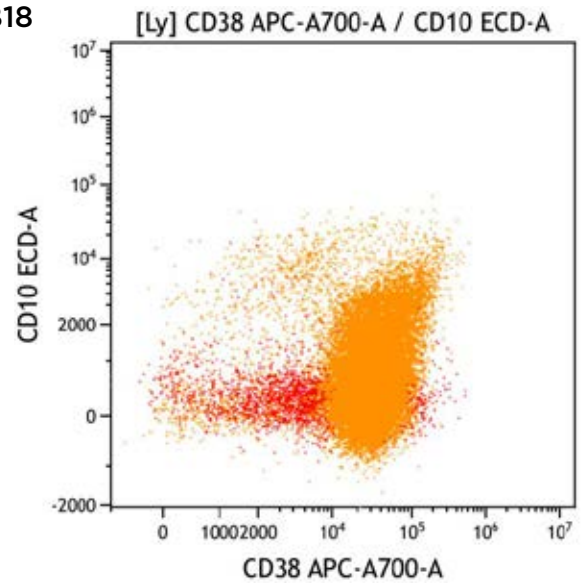

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells display low level expression of CD38. T cells (red) show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

B19

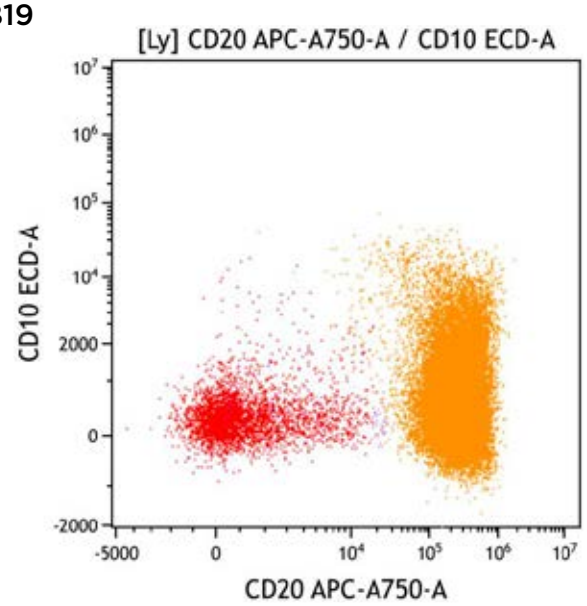

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The clonal B cell population (orange) expresses CD20 and is largely negative for CD10.

B20

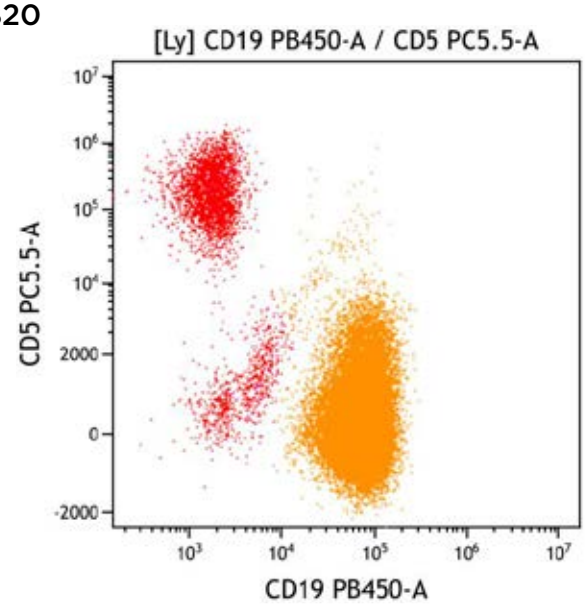

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) expresses CD19 and isnegative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

BACK TO CASE OVERVIEW

B21

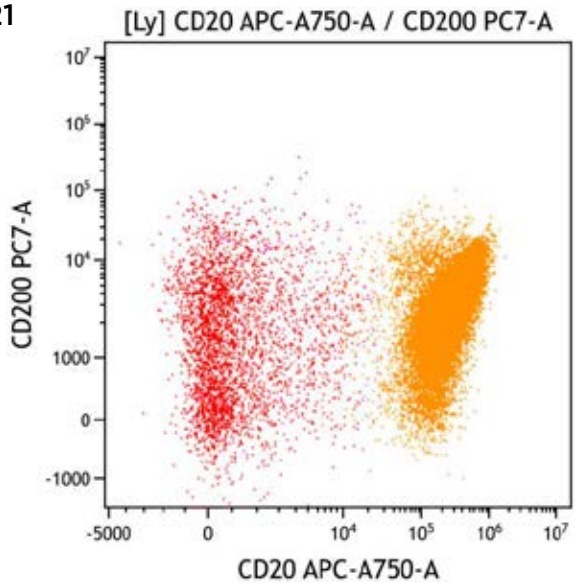

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The clonal B cell population (orange) expresses CD20 and dim CD200.

B22

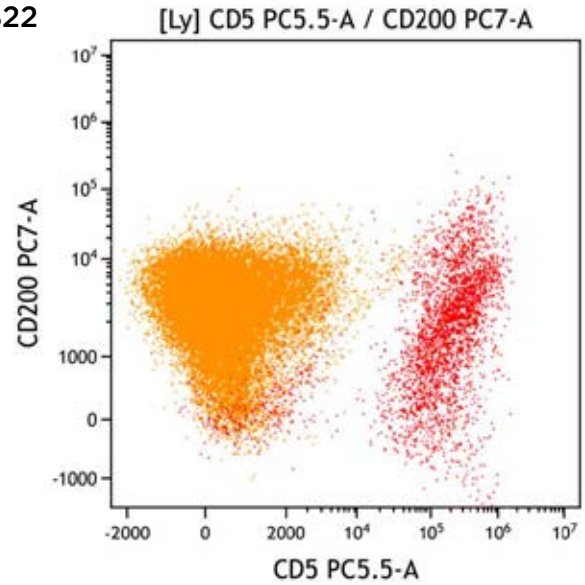

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/ small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma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

BACK TO CASE OVERVIEW

T Cell Tube

T01

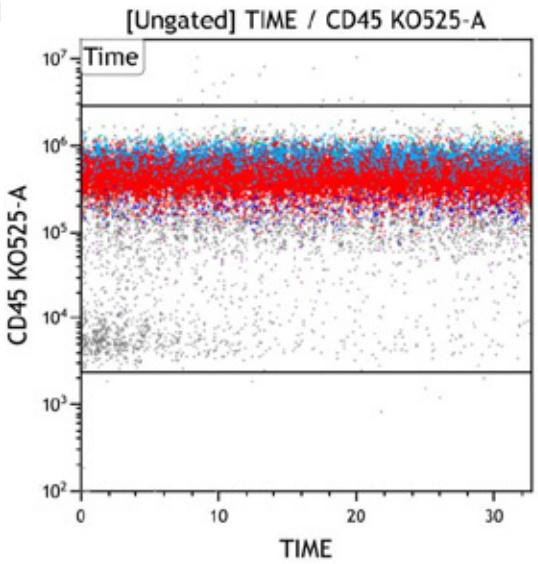

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

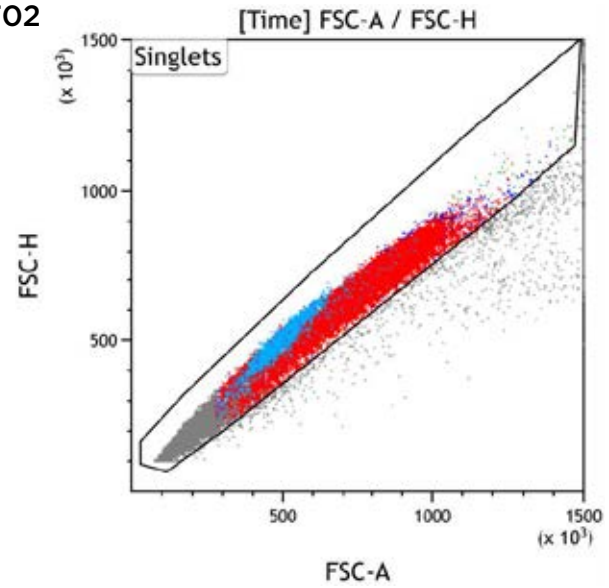

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

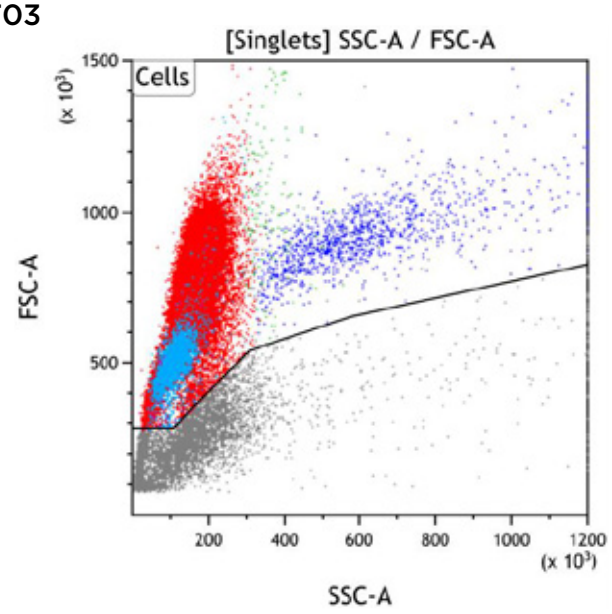

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

T04

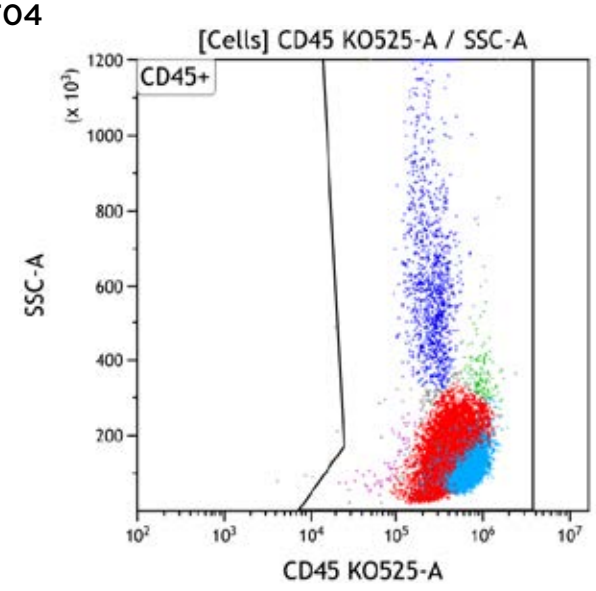

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

BACK TO CASE OVERVIEW

T05

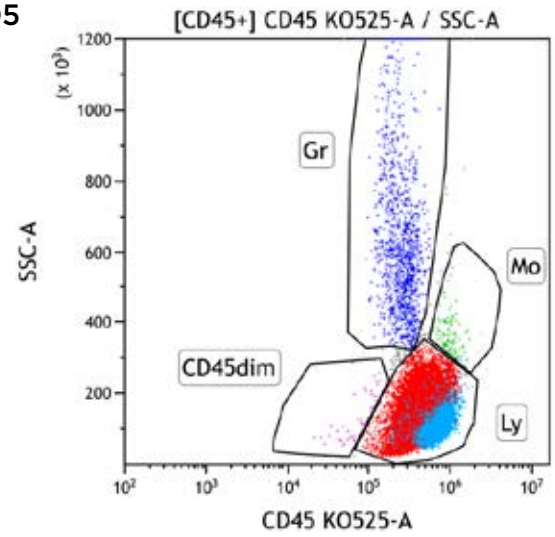

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., myeloblasts and immature 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.

T06

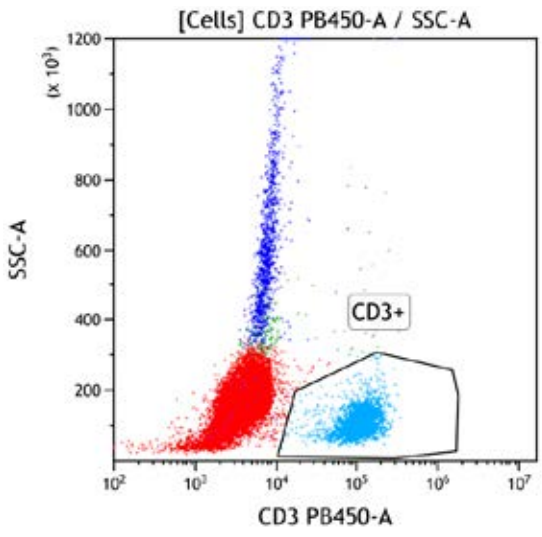

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

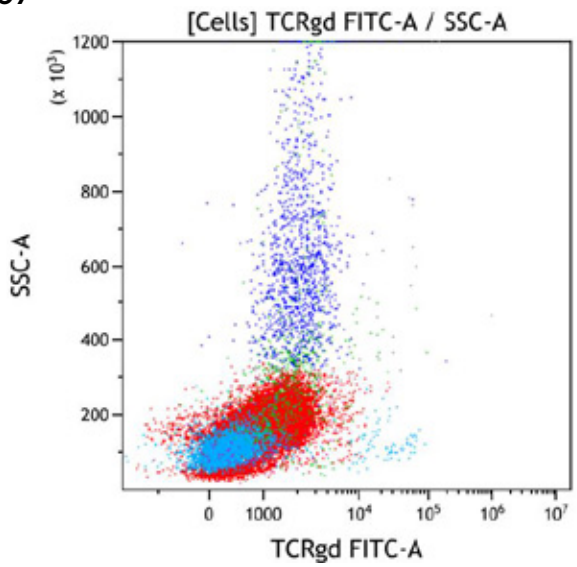

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

T08

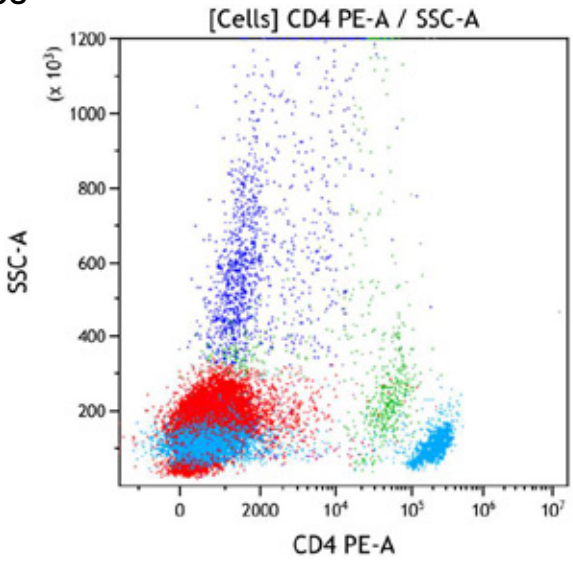

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells.

BACK TO CASE OVERVIEW

T09

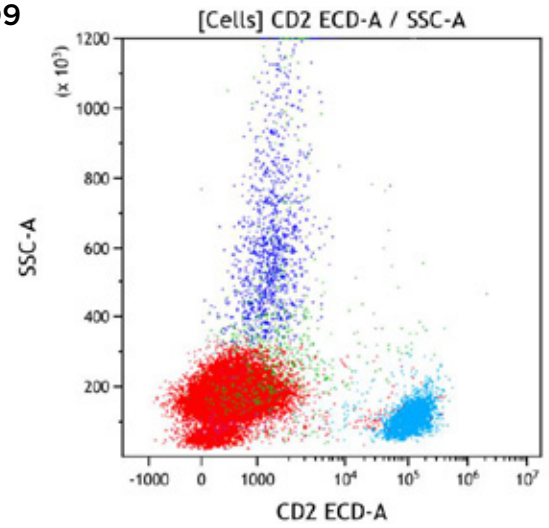

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red).

T10

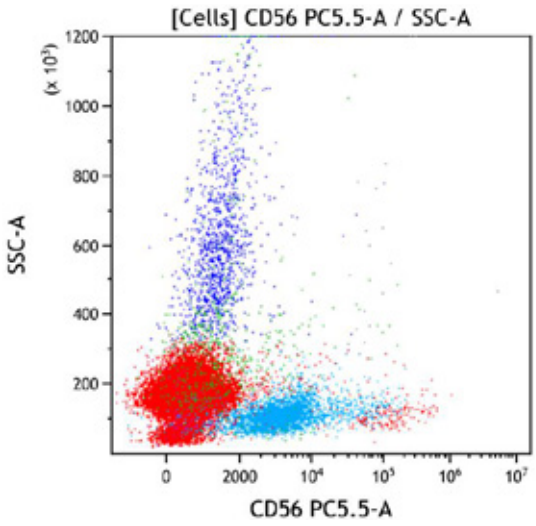

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua).

T11

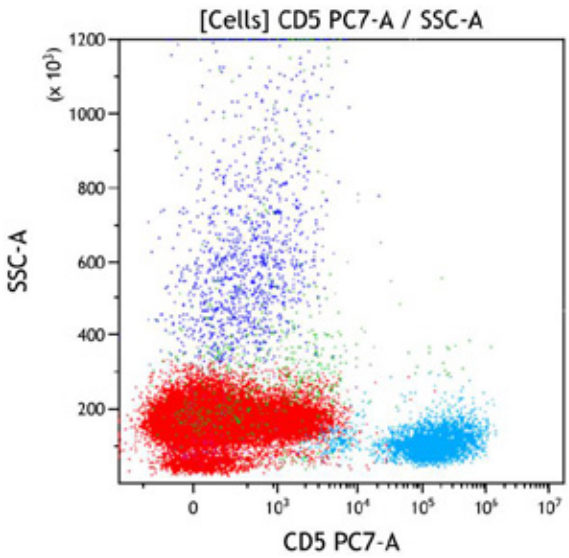

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

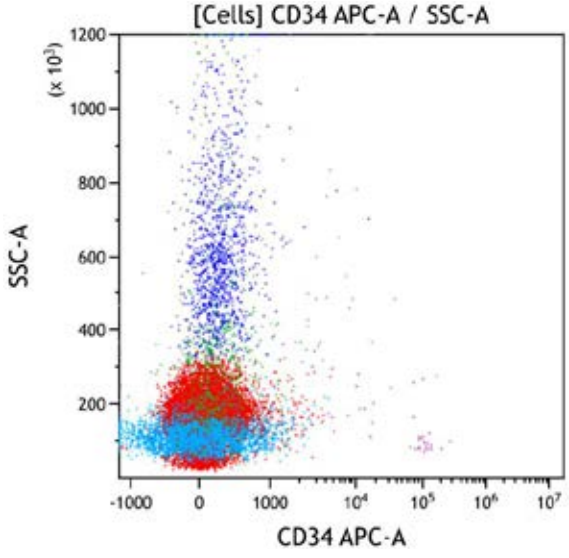

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors (purple, low middle) typically have low to intermediate side scatter. Mature granulocytes (blue), monocytes (green), and lymphocytes (red/aqua) are negative for CD34.

BACK TO CASE OVERVIEW

T13

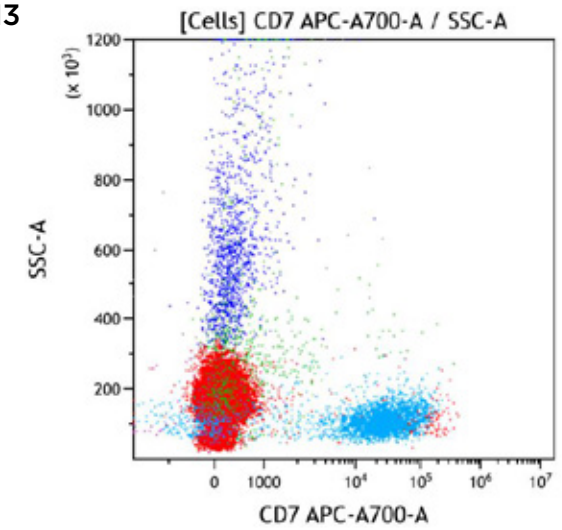

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

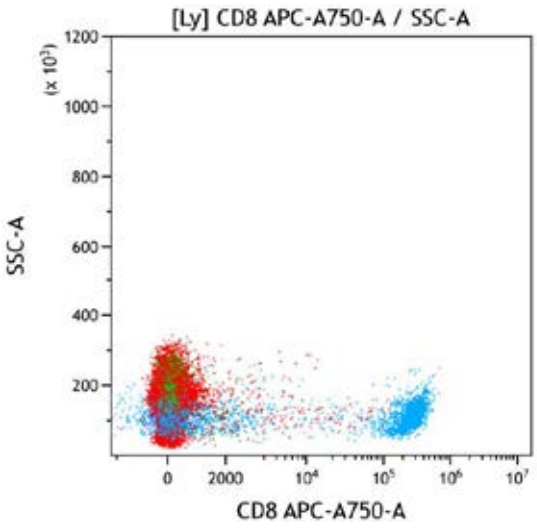

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells.

T15

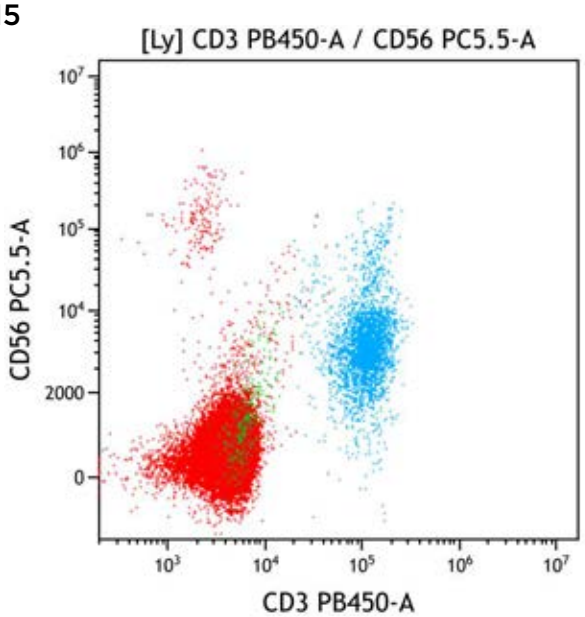

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

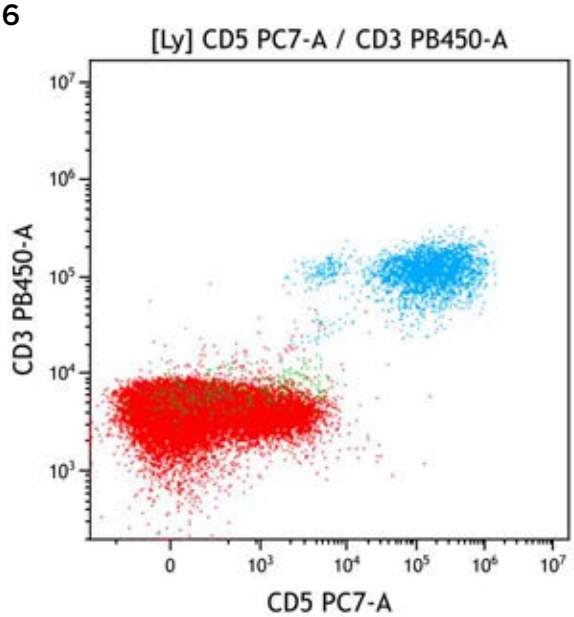

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

BACK TO CASE OVERVIEW

T17

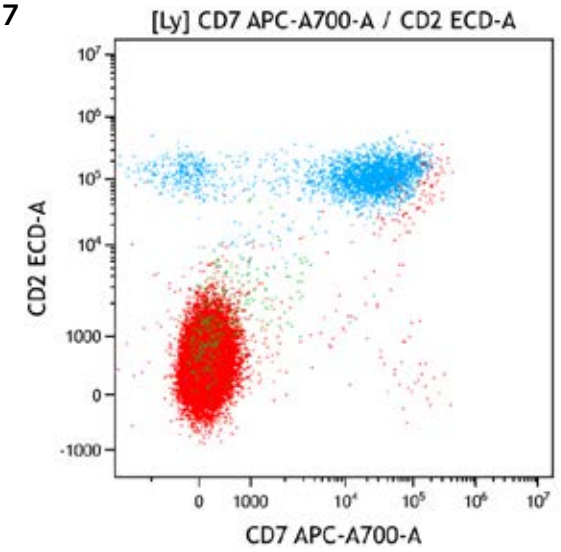

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

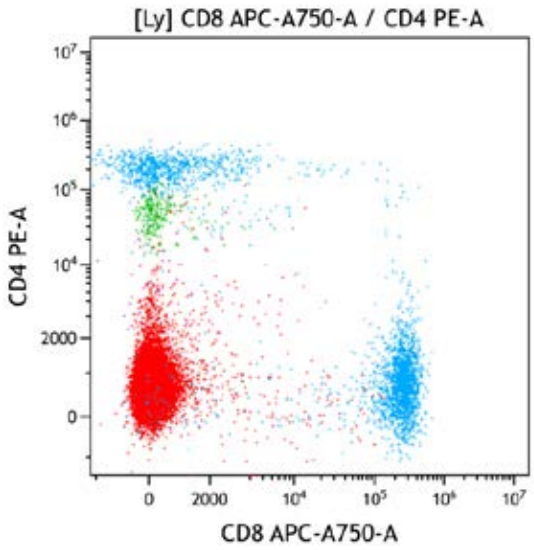

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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 (green, middle left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

T19

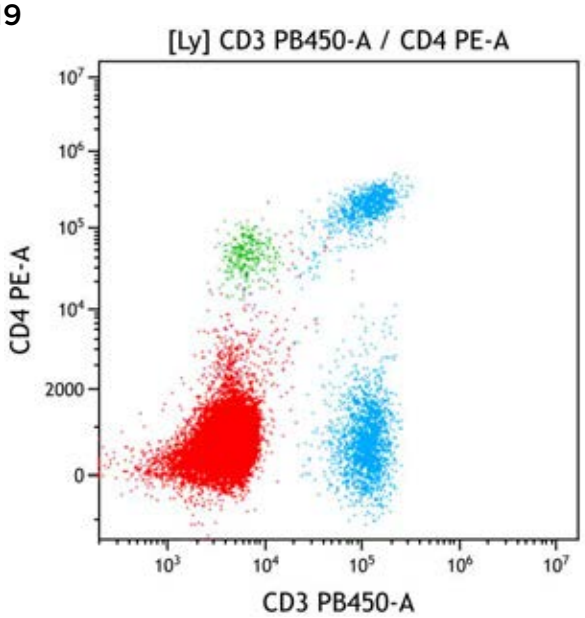

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells (aqua) express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. Of note,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

T20

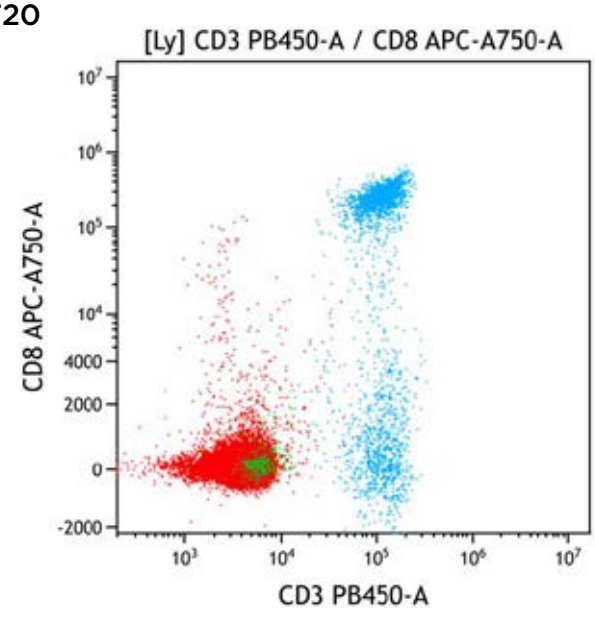

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

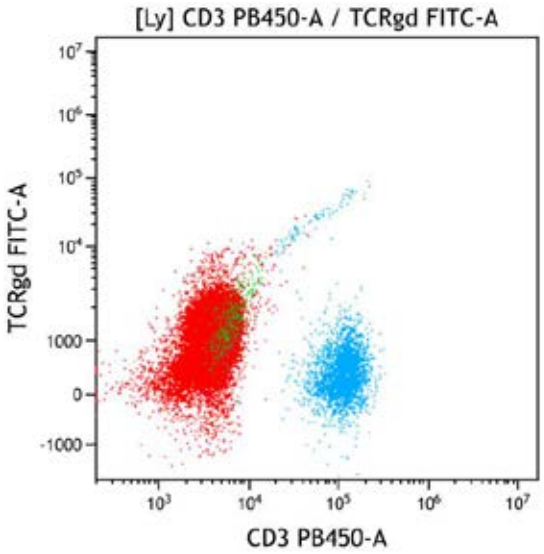

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/delta, which is coexpressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

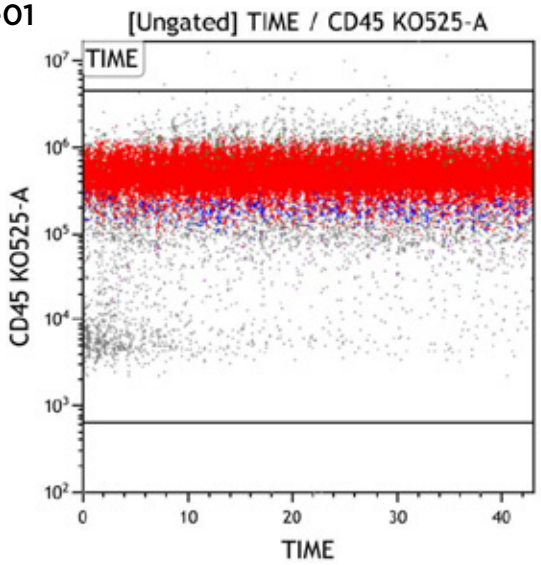

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

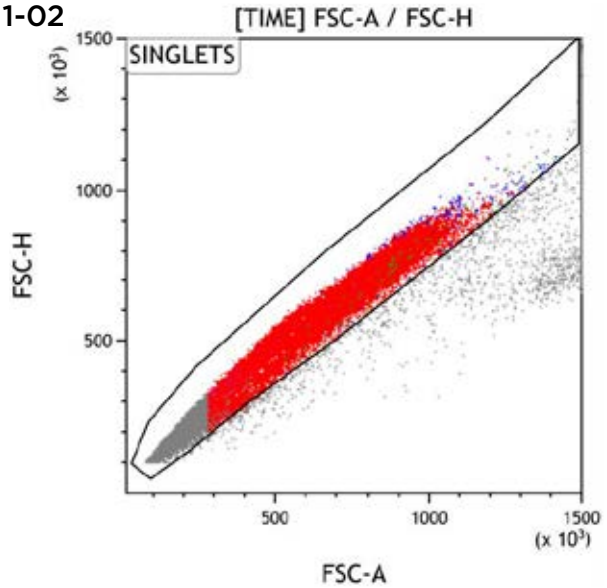

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

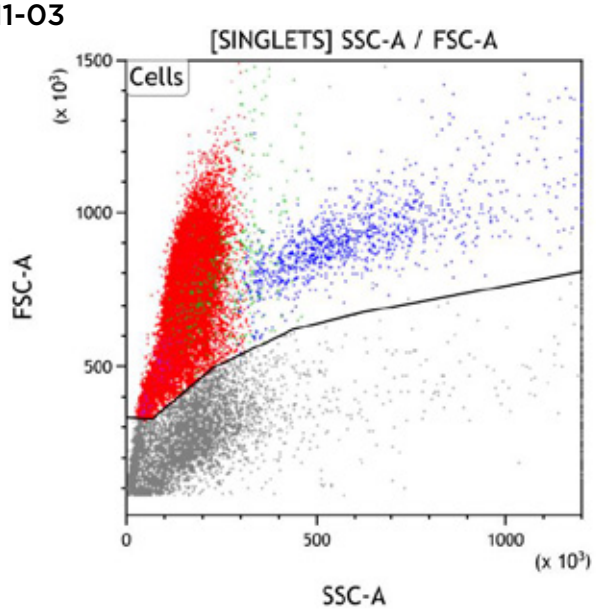

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M1-04

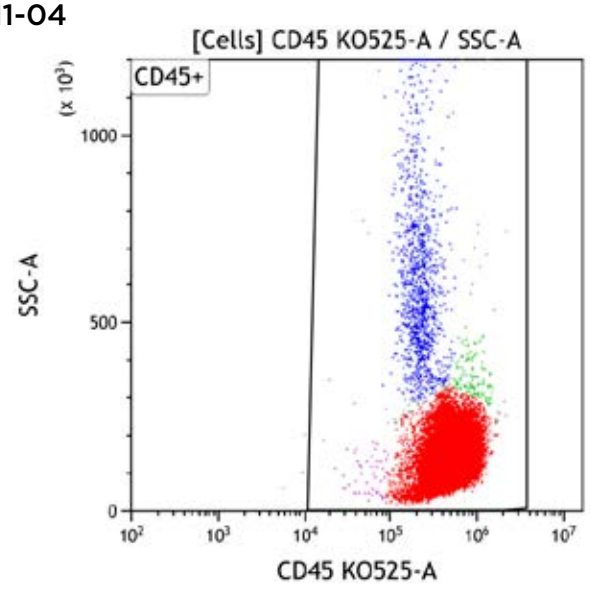

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

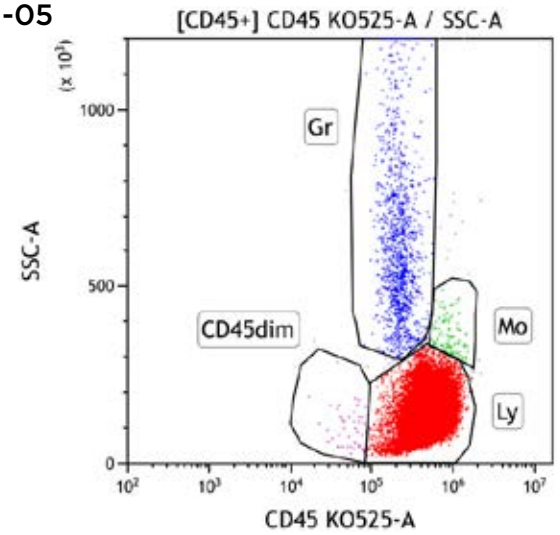

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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.

M1-06

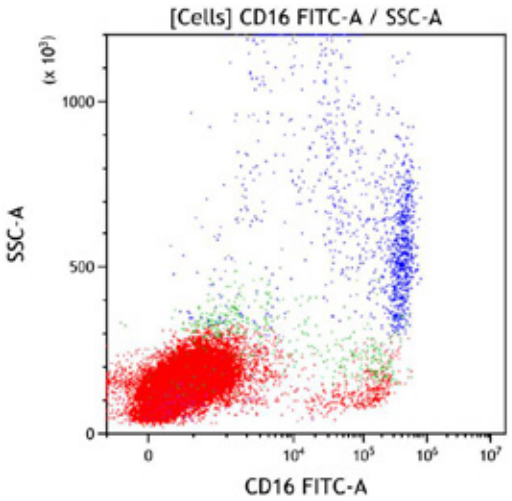

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue) and at a variably low level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

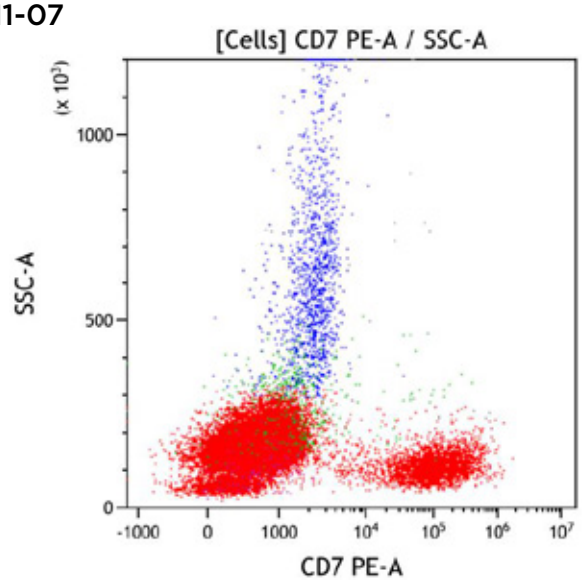

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed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

M1-08

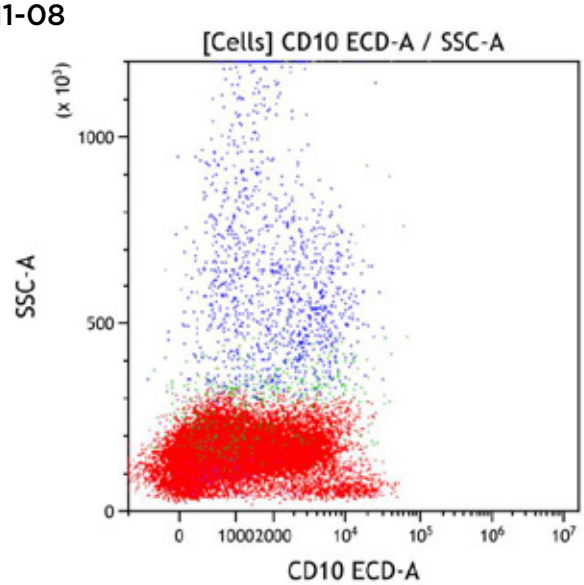

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

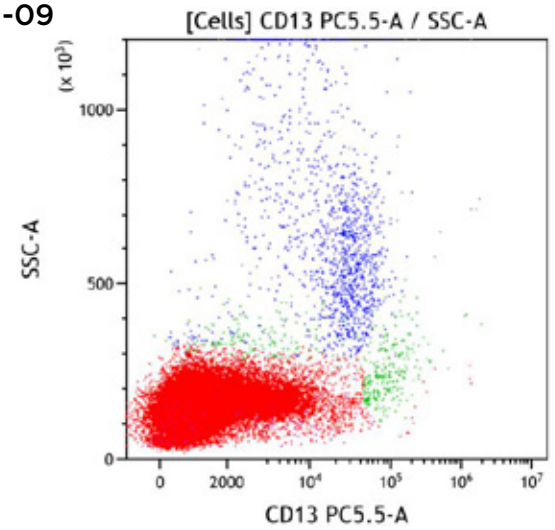

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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.

M1-10

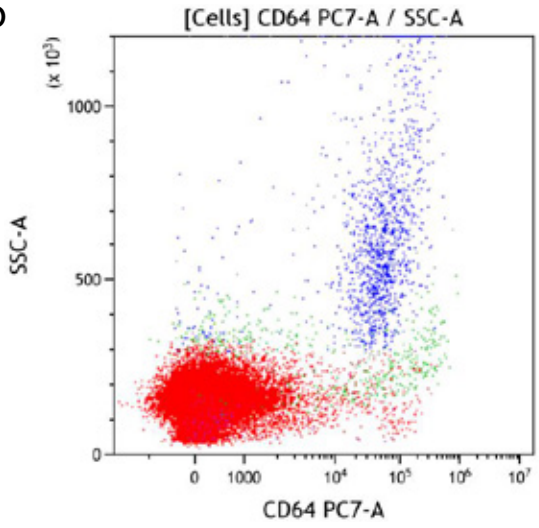

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

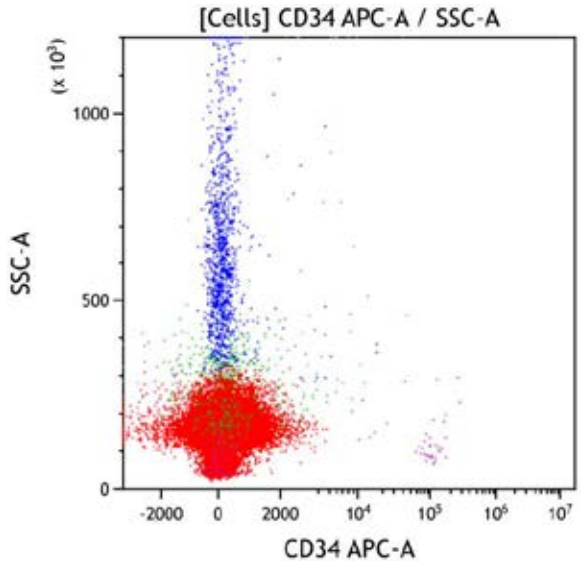

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors (purple, lower middle)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

M1-12

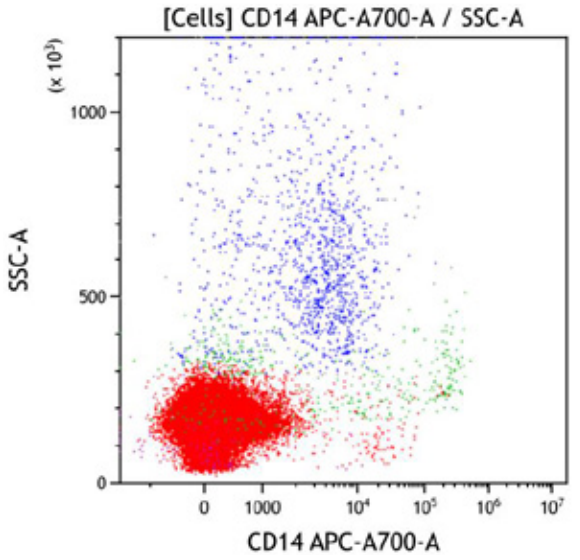

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes (blue) at a lower level.

BACK TO CASE OVERVIEW

M1-13

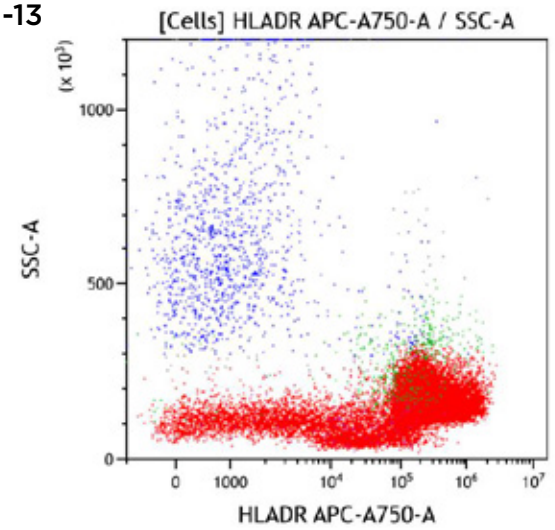

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 progenitors, immature and mature B cells (red, lower right), and activated T cells (red).

M1-14

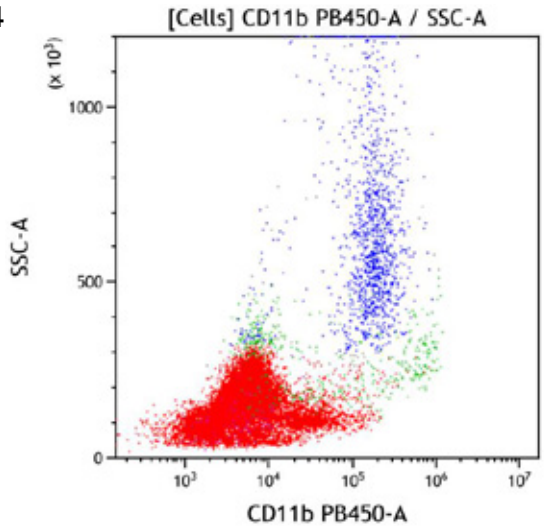

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils.

M1-15

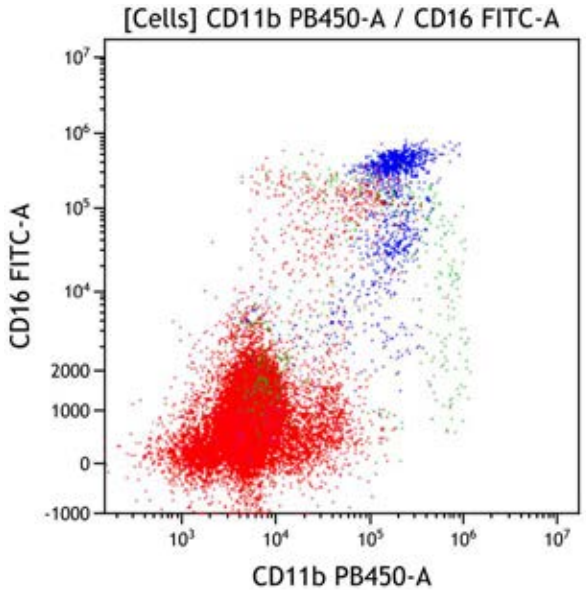

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue) and a subset of NK cells (red). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red).

M1-16

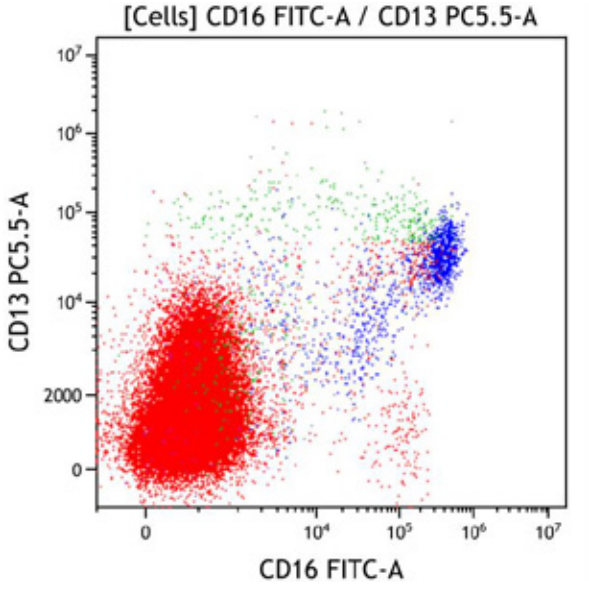

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). Mature granulocytes (blue) have high expression of both CD13 and CD16.

BACK TO CASE OVERVIEW

M1-17

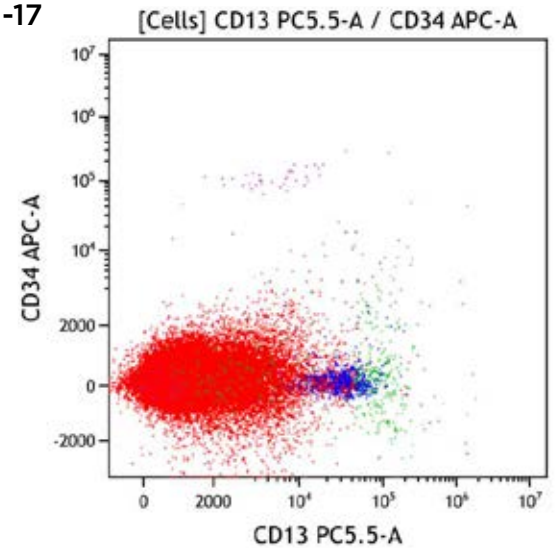

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple, upper left). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors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

M1-18

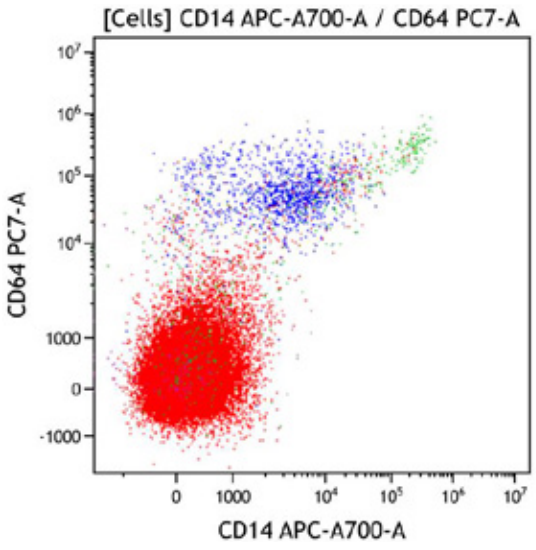

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes.

M1-19

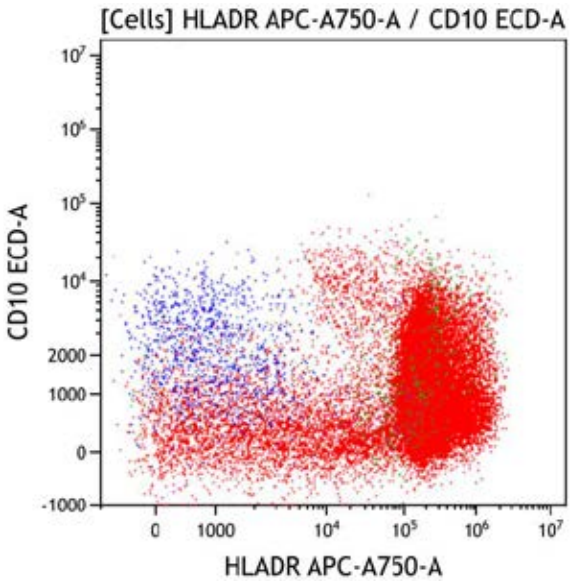

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on mature granulocytes (blue).

M1-20

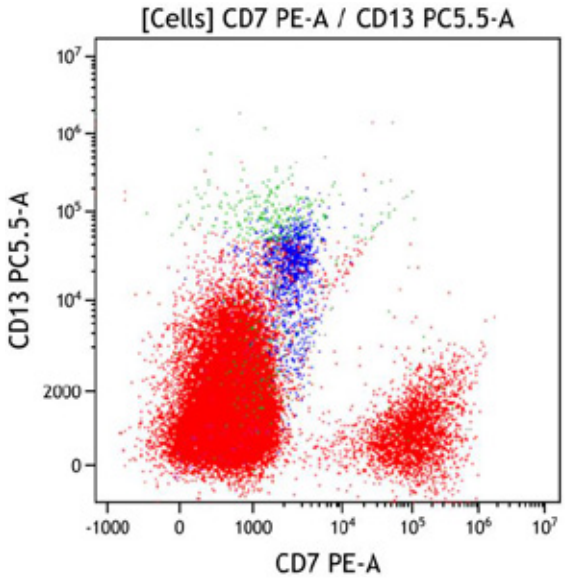

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Co-expression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

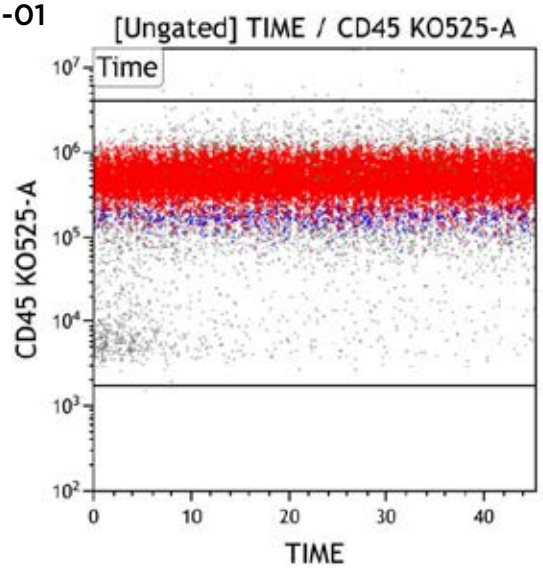

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

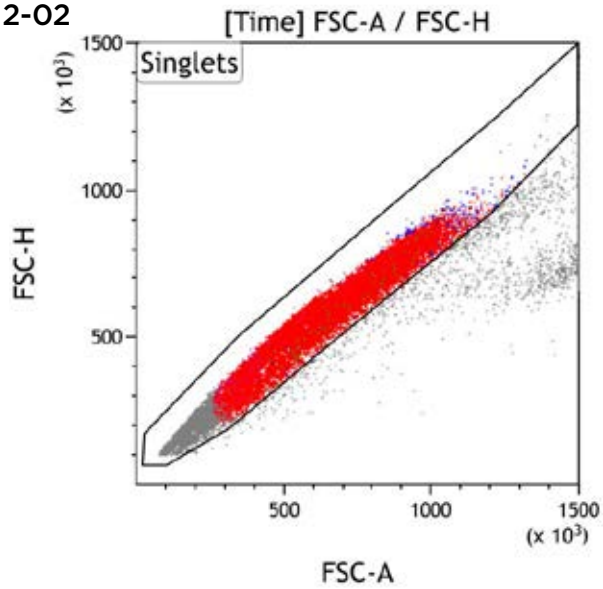

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

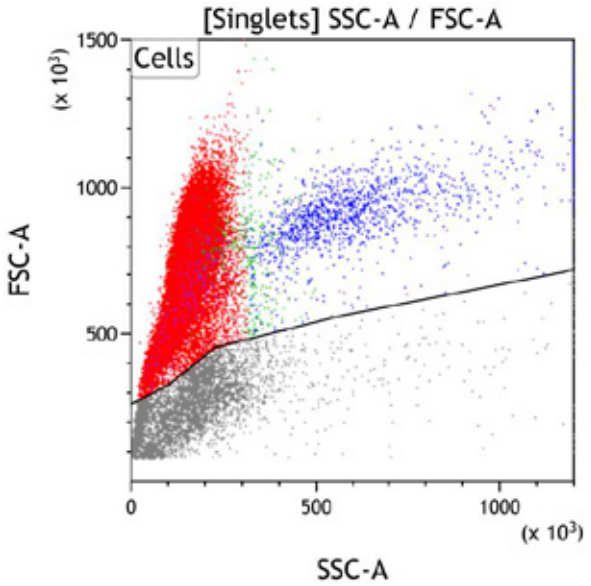

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M2-04

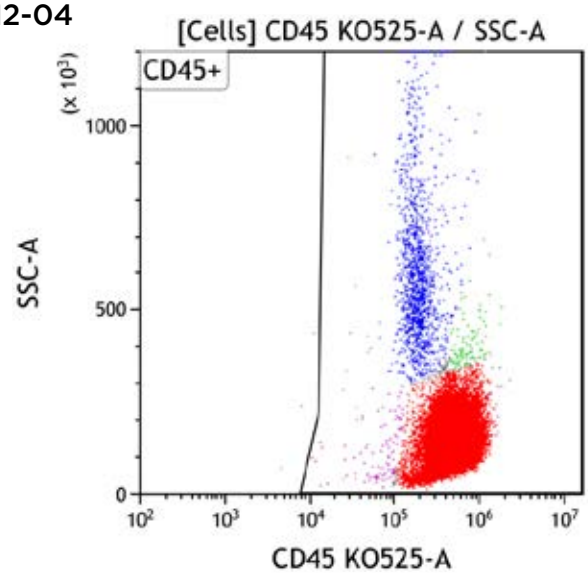

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or nonhematopoietic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

BACK TO CASE OVERVIEW

M2-05

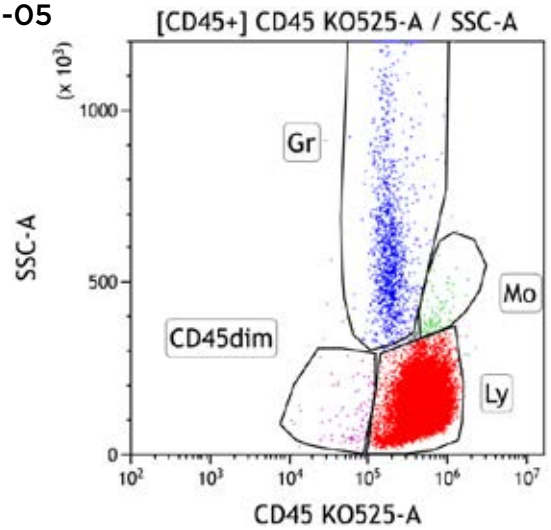

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M2-06

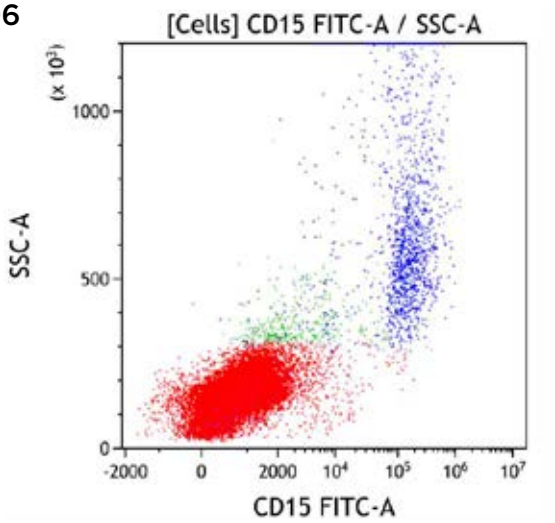

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

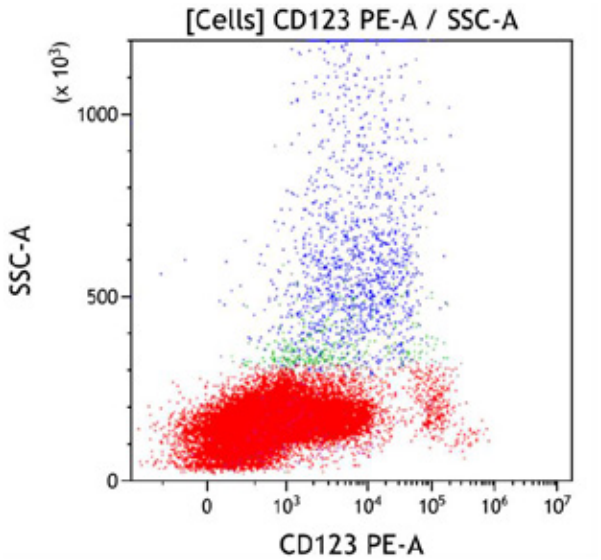

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes.

M2-08

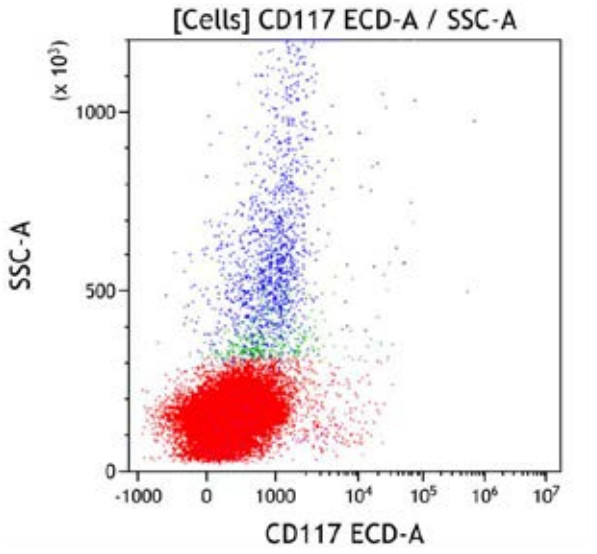

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

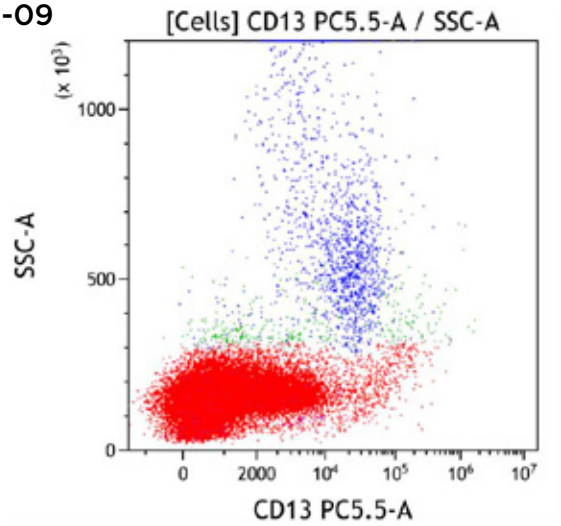

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue) with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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.

M2-10

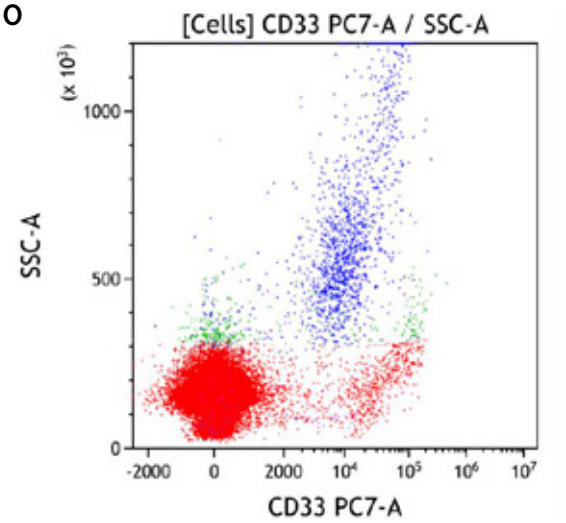

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on immature and mature monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors.

M2-11

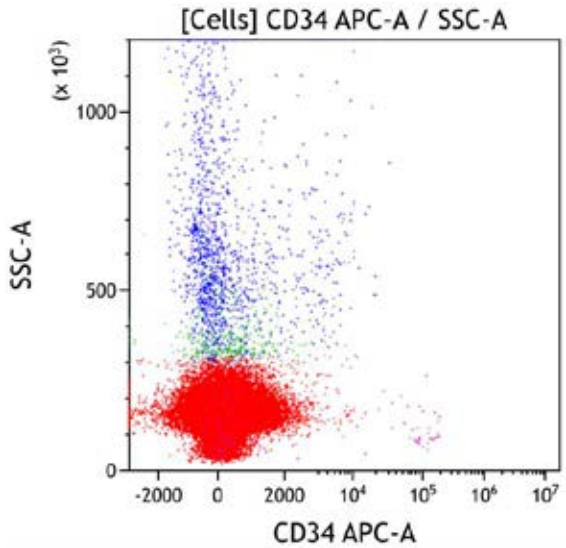

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have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

M2-12

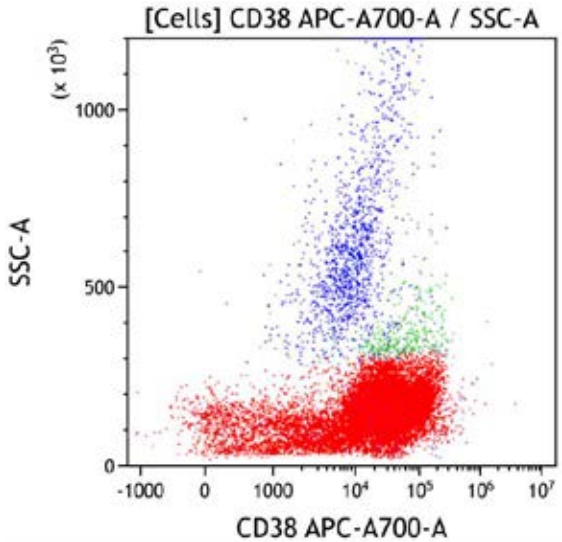

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and at a low level on monocytes (green), and at a variably low level on activated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

BACK TO CASE OVERVIEW

M2-13

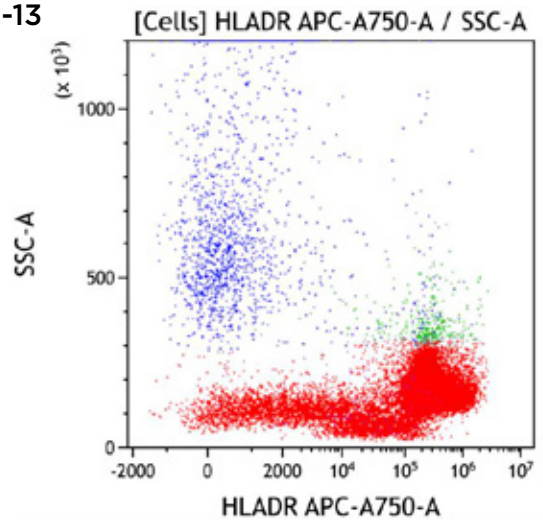

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red, lower right), and activated T cells (red).

M2-14

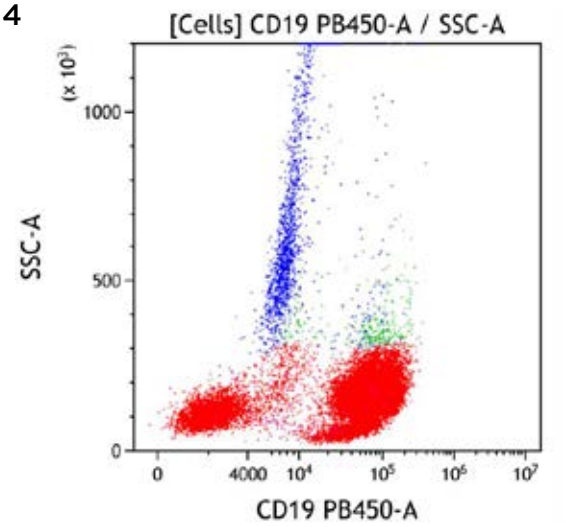

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red), as well as most plasma cells. These cells typically have low to moderate side scatter.

M2-15

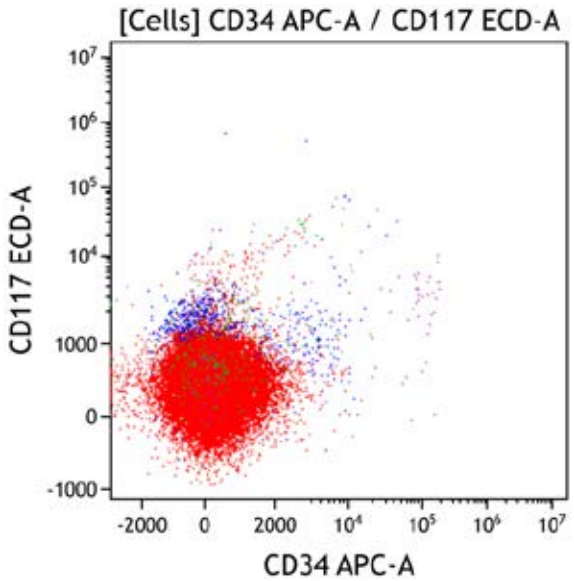

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors.

M2-16

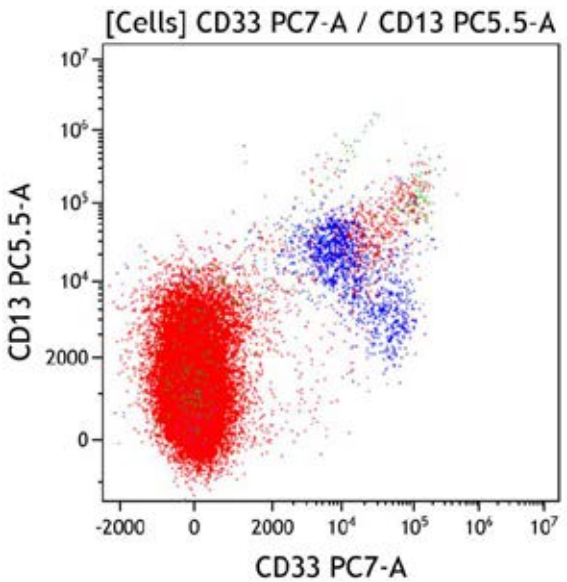

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and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

BACK TO CASE OVERVIEW

M2-17

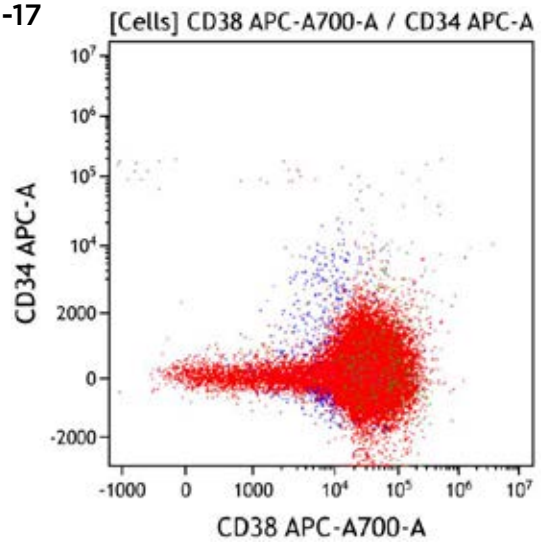

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes (blue), monocytes, and lymphocytes (red) are negative for CD34 with variable CD38.

M2-18

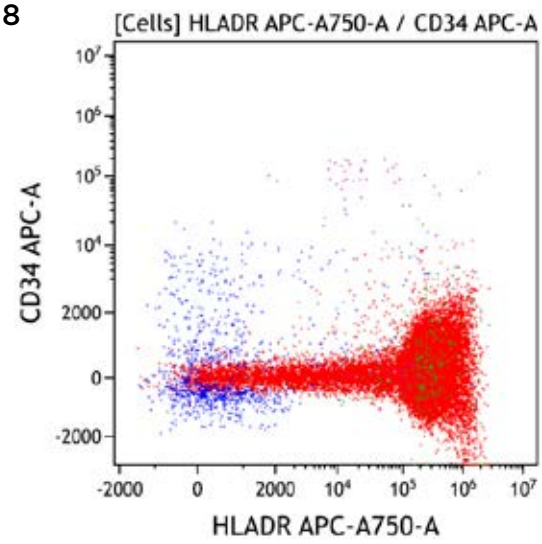

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors (purple, upper middle) variably express both CD34 and HLA-DR.

M2-19

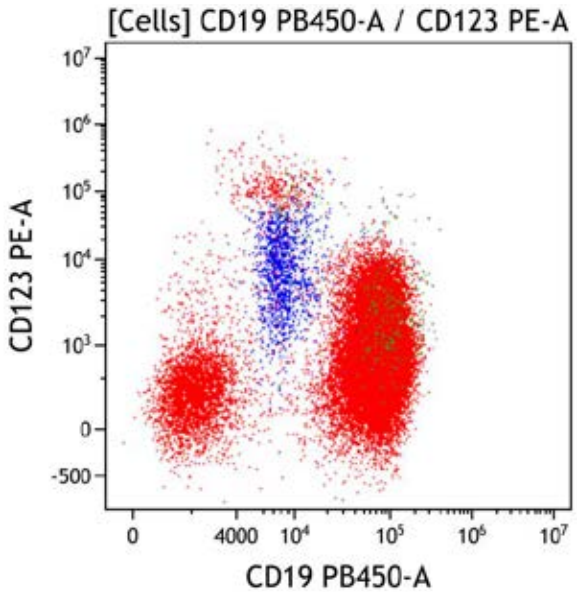

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes, and CD34 positive progenitors. CD19 positive B cells (red) express variable, dim CD123. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence, largely from eosinophils.

M2-20

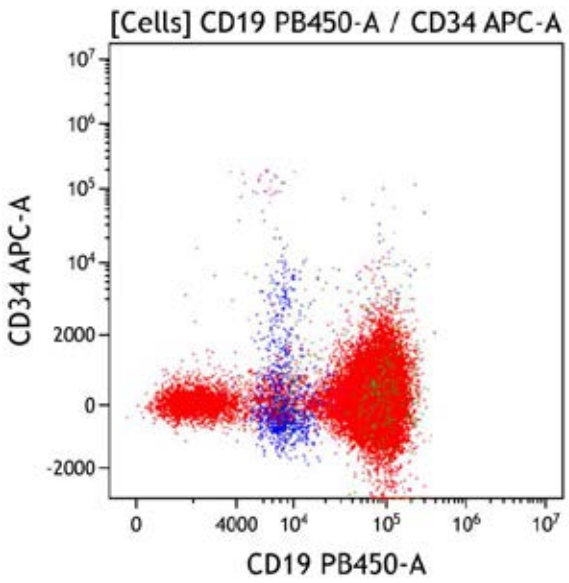

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors (purple, upper left). A large subset of lymphocytes (red) are CD19 positive B cells without expression of CD34.

BACK TO CASE OVERVIEW

M2-21

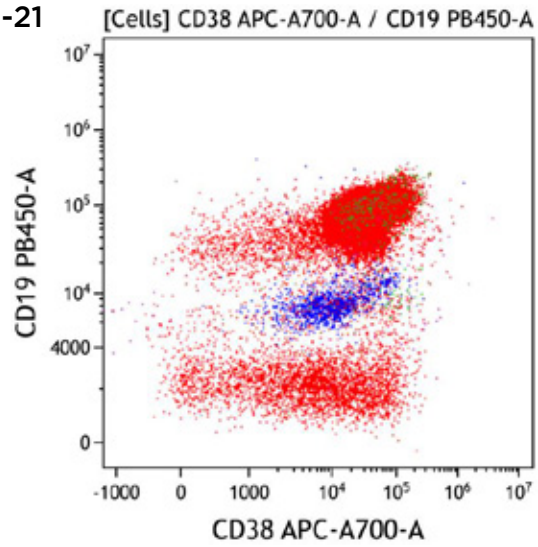

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show intermediate expression of CD38. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence,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

M2-22

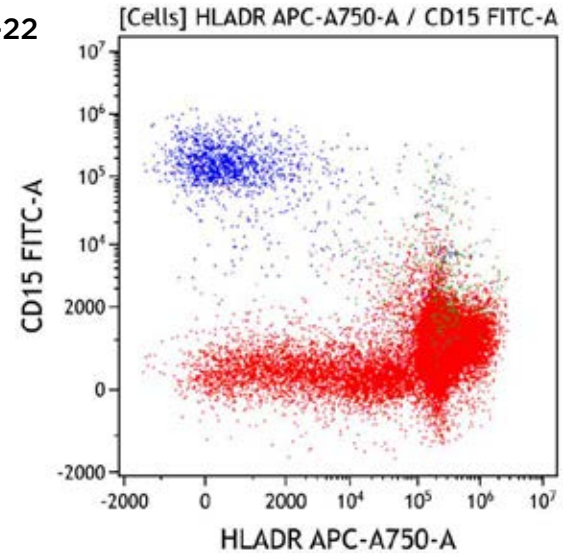

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes. HLA-DR is expressed on B cells (red), monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR.

M2-23

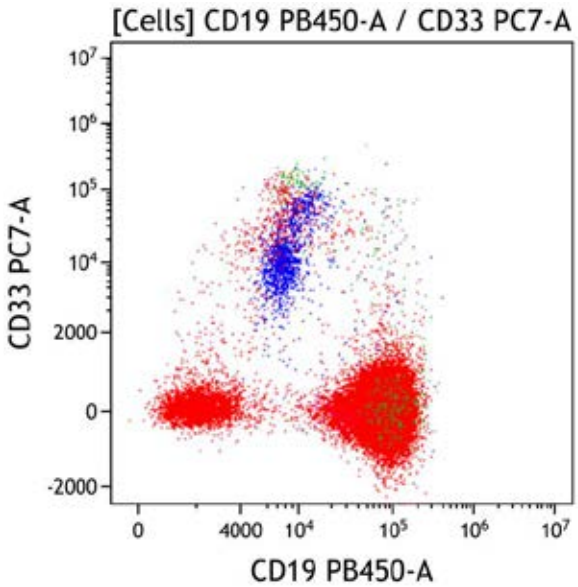

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not normally express significant CD33. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence, largely from eosinophils. A large subset of lymphocytes (red) are CD19 positive B cells without expression of CD33.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 2 LHS-TC4-041**
Diagnosis	**Chronic lymphocytic leukemia/small lymphocytic lymphoma**
Clinical Vignette	This 68-year-old male presents with anemia and lymphocytosis. A peripheral blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	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 cell or myeloid antigens. 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). 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.

BACK TO CASE LISTINGS

B Cell Tube

B01

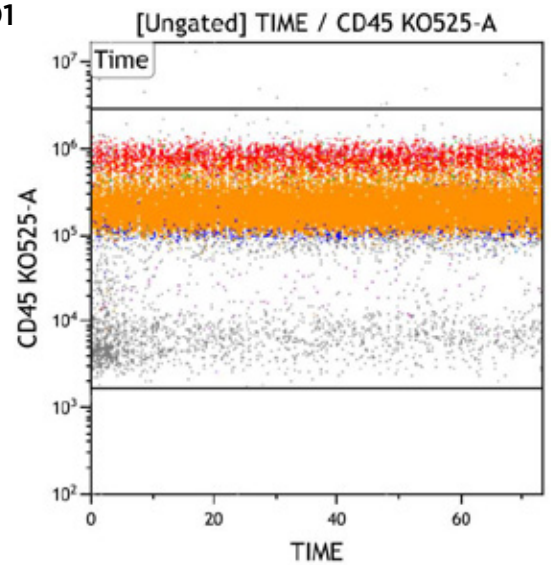

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

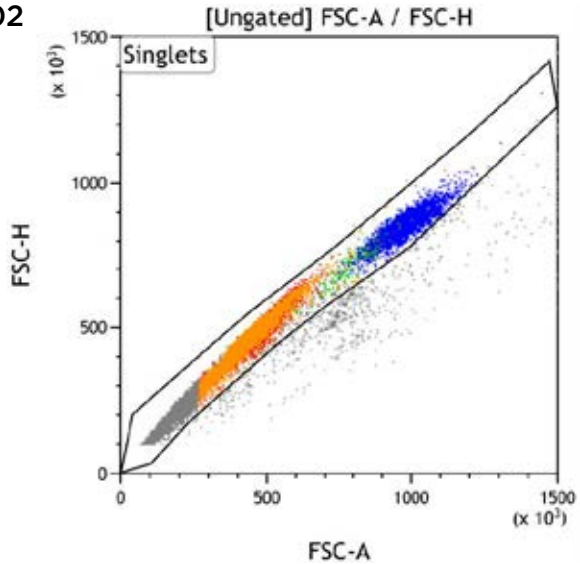

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

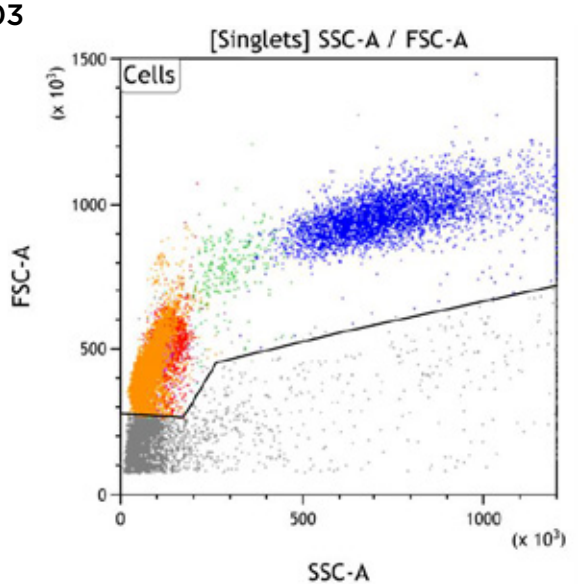

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

B04

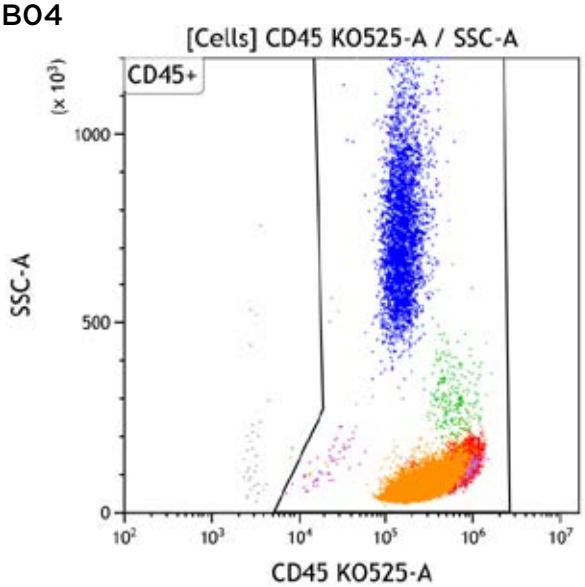

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

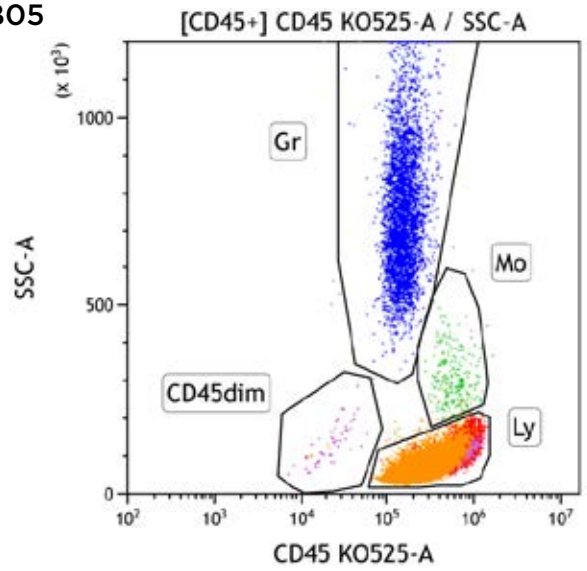

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 followed throughout the analysis.

B06

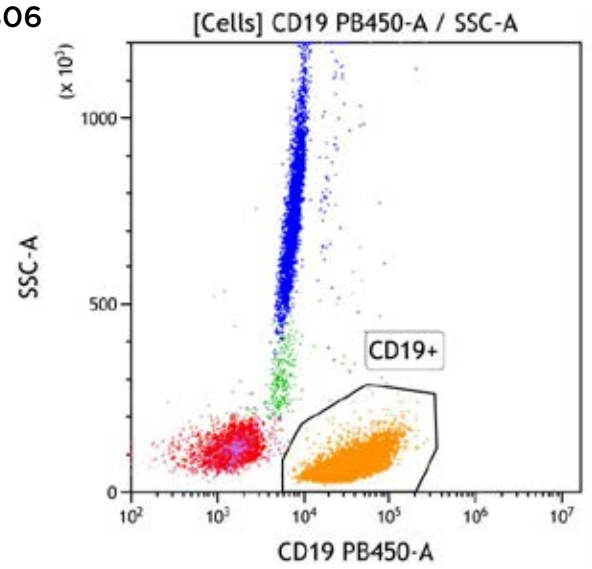

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note the relatively increased number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

B07

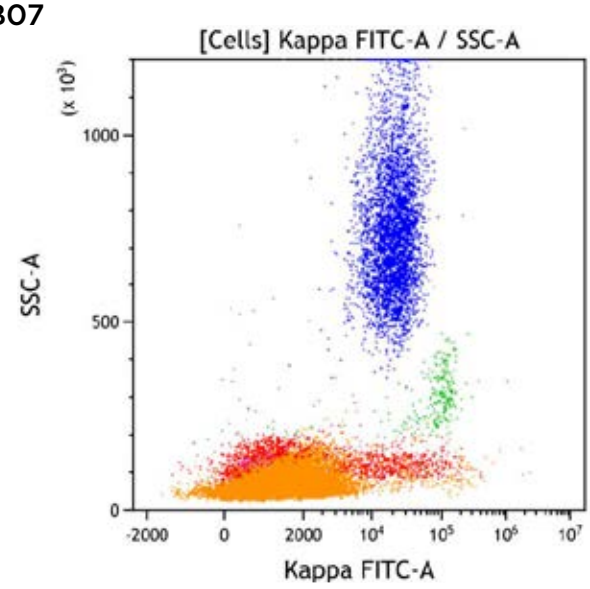

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen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

B08

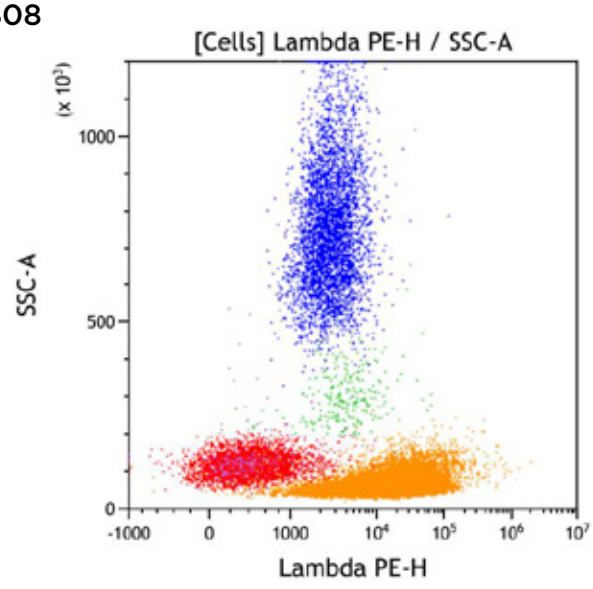

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen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

BACK TO CASE OVERVIEW

B09

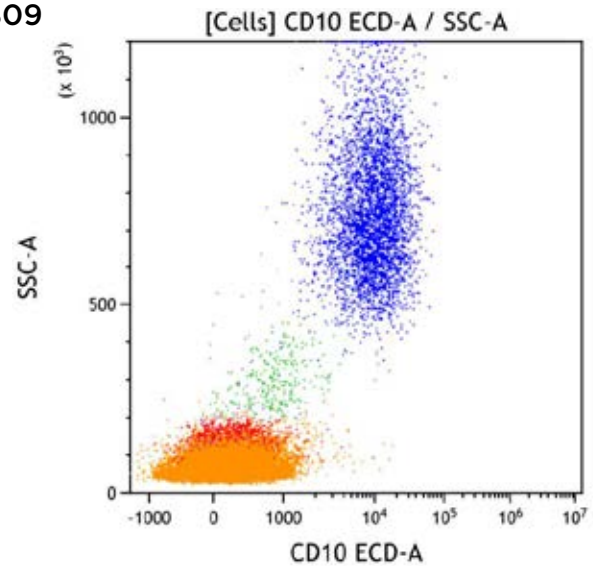

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange)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

B10

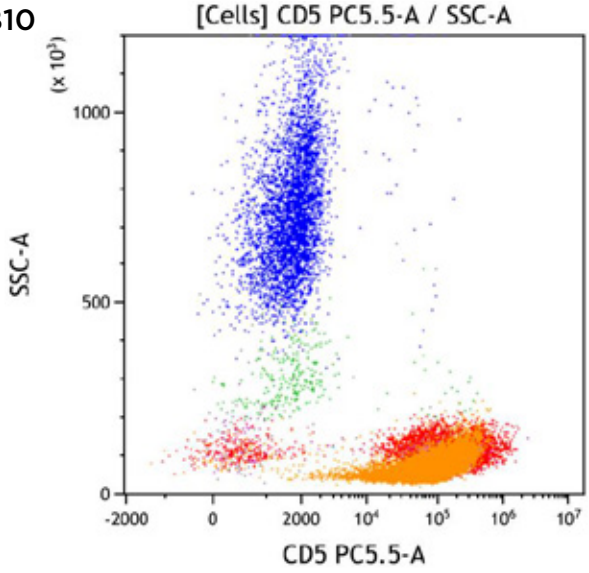

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red), as well as dimly on a subset of mature B cells. These lymphoid cells typically have low side scatter. The CD19 positive population (orange) expresses CD5 at a level similar to T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

B11

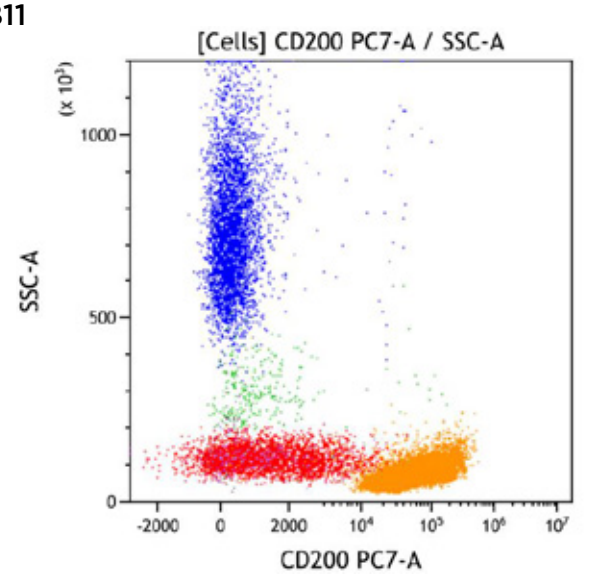

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

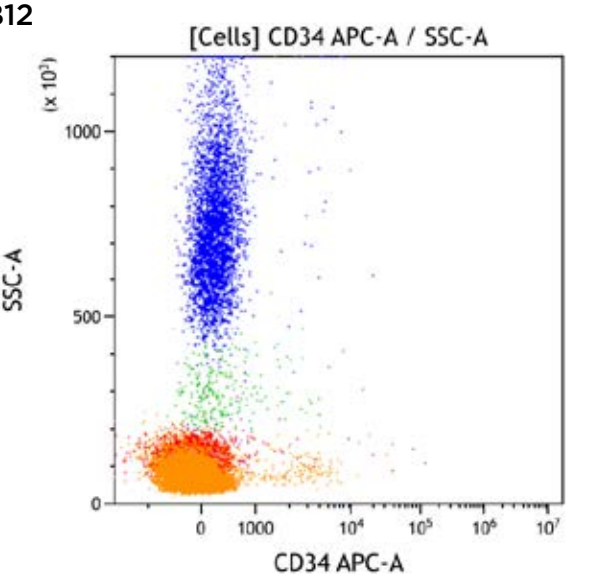

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The CD19 positive population (orange) is negative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

BACK TO CASE OVERVIEW

B13

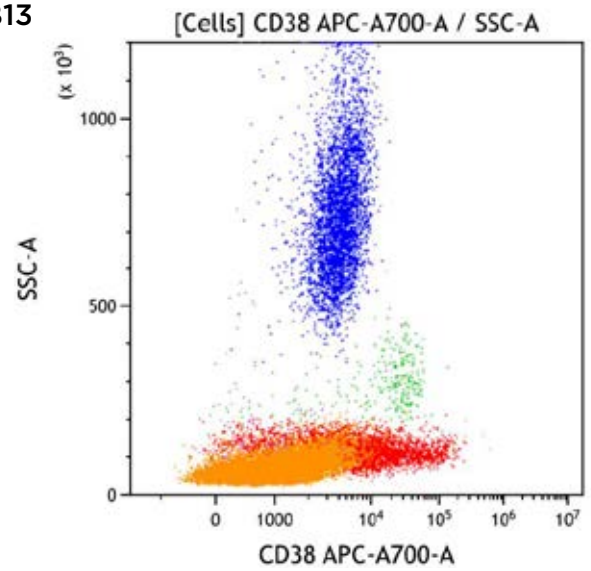

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature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

B14

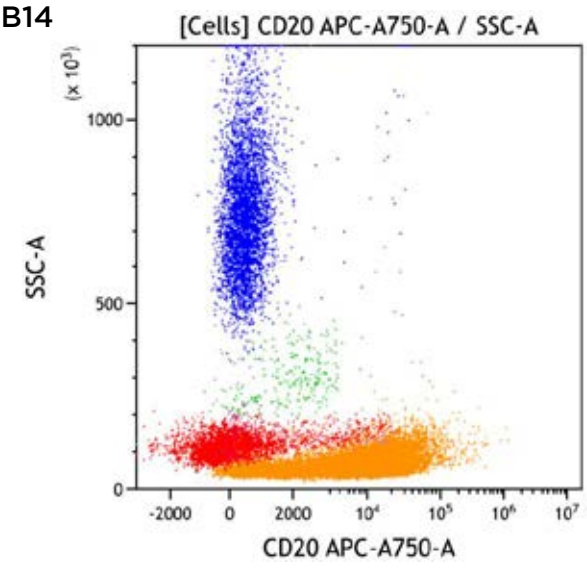

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.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

B15

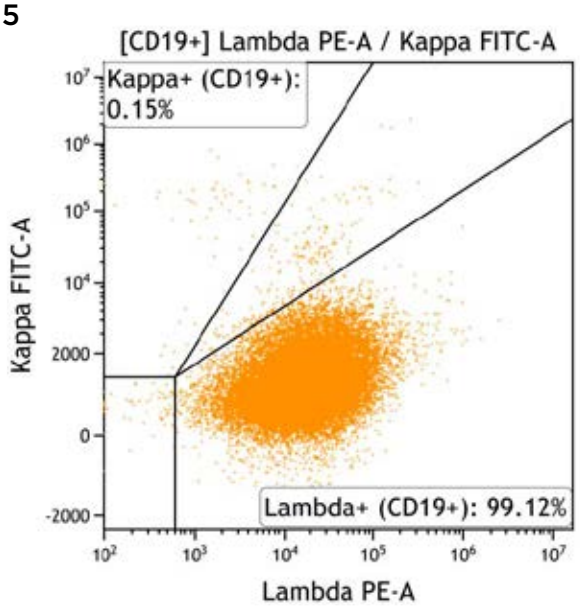

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. The CD19 positive cells (orange) predominantly have surface lambda light chain expression 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.

B16

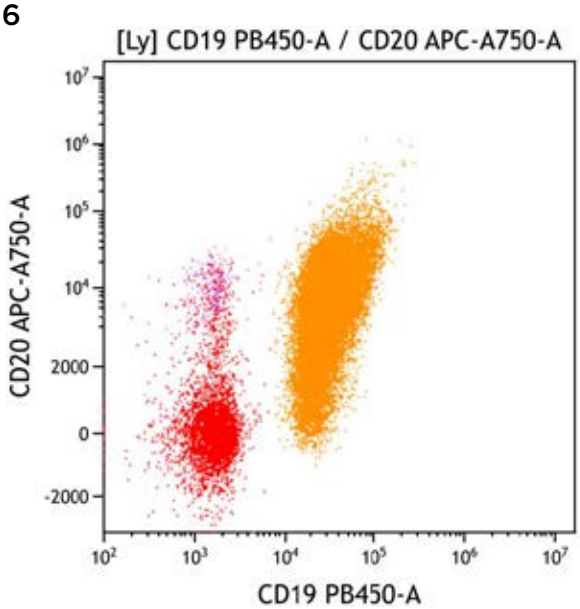

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cells (orange) display decreased CD19 and CD20 expression compared with the higher level seen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

BACK TO CASE OVERVIEW

B17

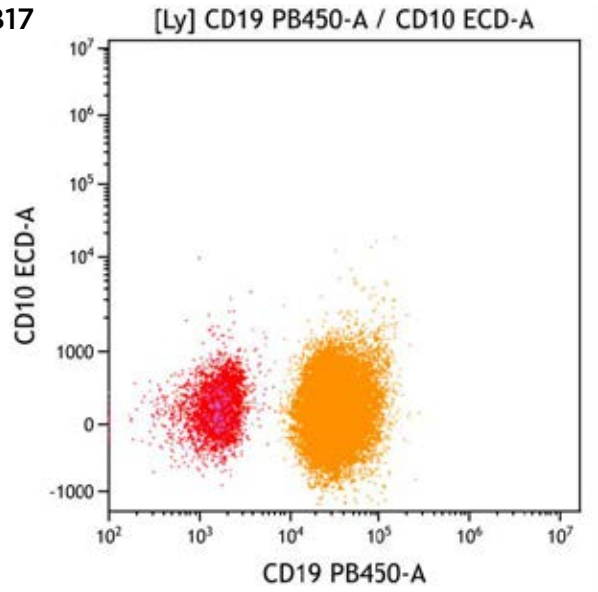

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature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

B18

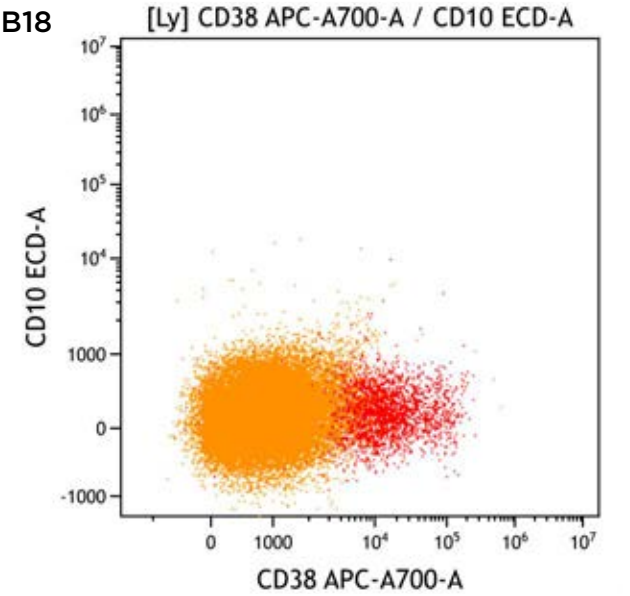

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable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

B19

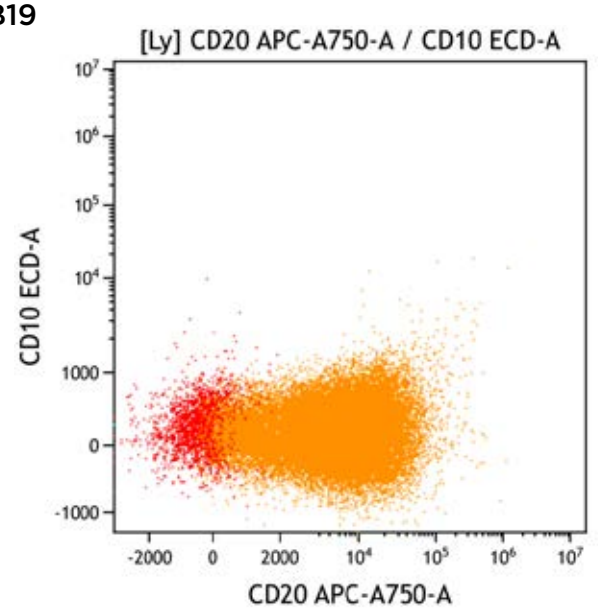

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The clonal B cell population (orange) displays decreased CD20 expression and is negative for CD10. The small population with normal CD20 expression (also in orange,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

B20

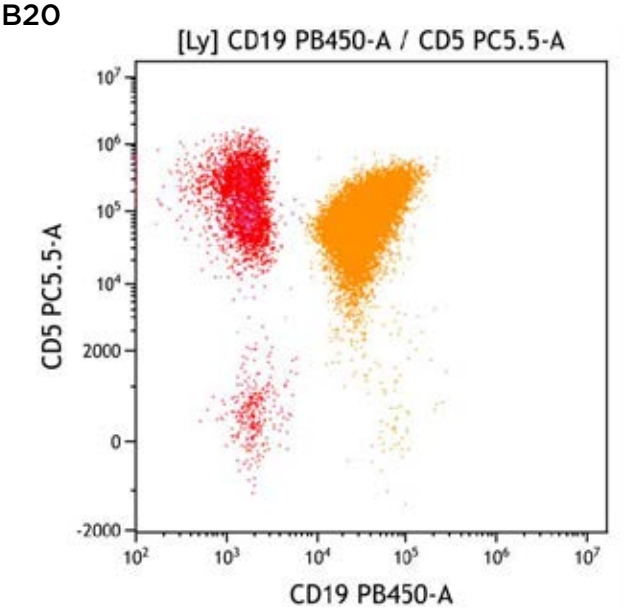

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) expresses CD5 and CD19.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

BACK TO CASE OVERVIEW

B21

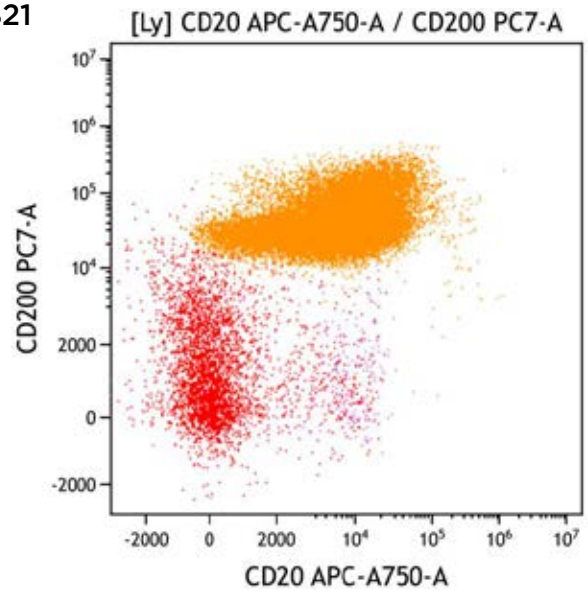

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level. The clonal B cell population (orange) expresses CD200 and variably decreased CD20. The small population with normal CD20 and CD200 expression (also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

B22

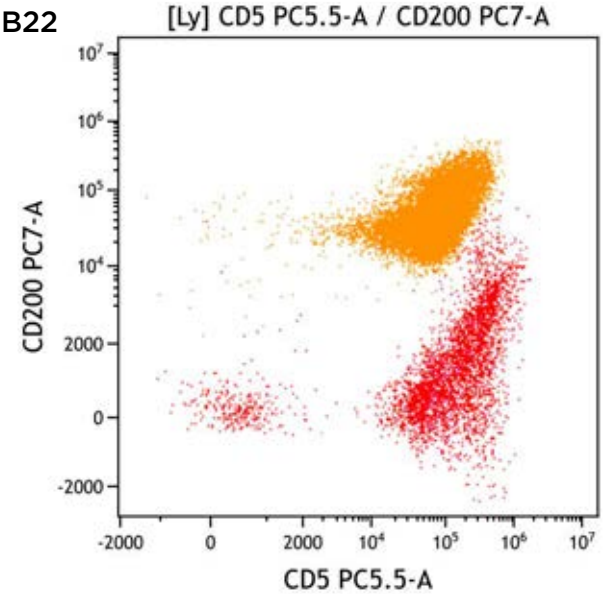

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/ small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma 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.

BACK TO CASE OVERVIEW

T Cell Tube

T01

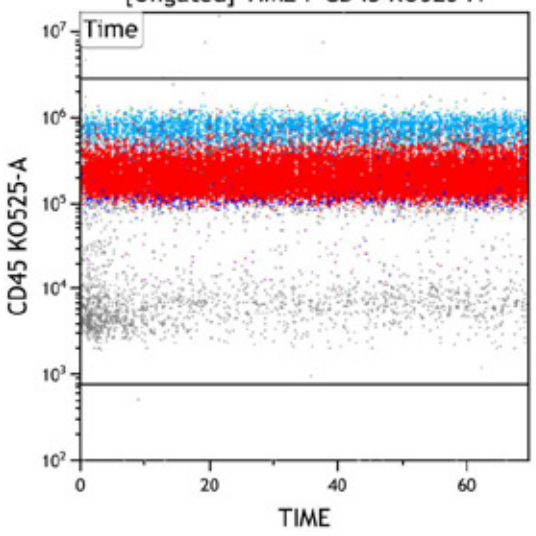

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

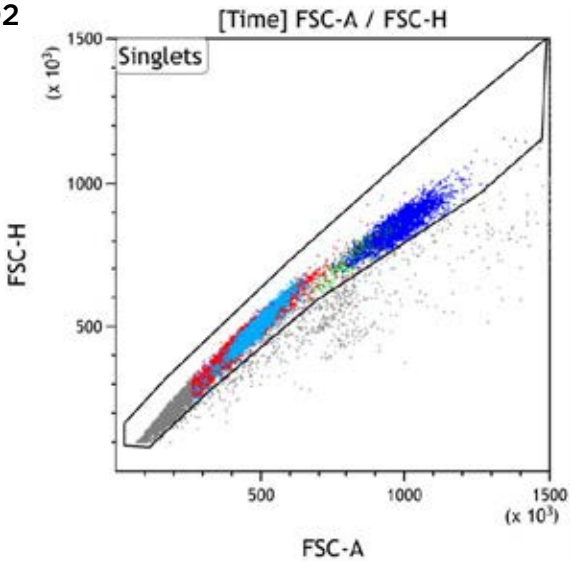

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

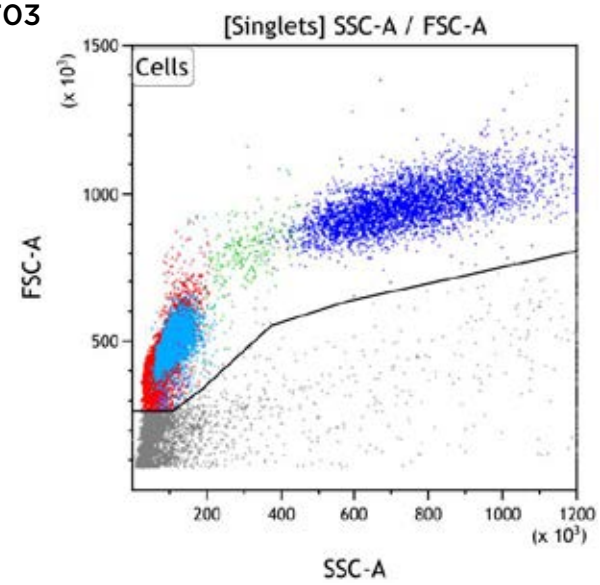

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

T04

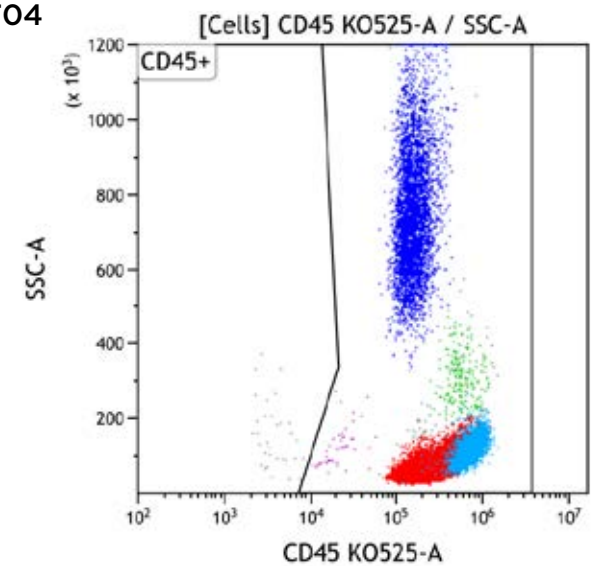

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

T05

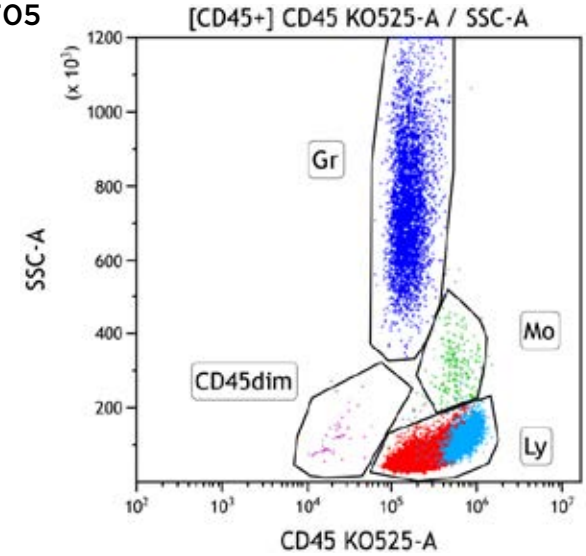

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

T06

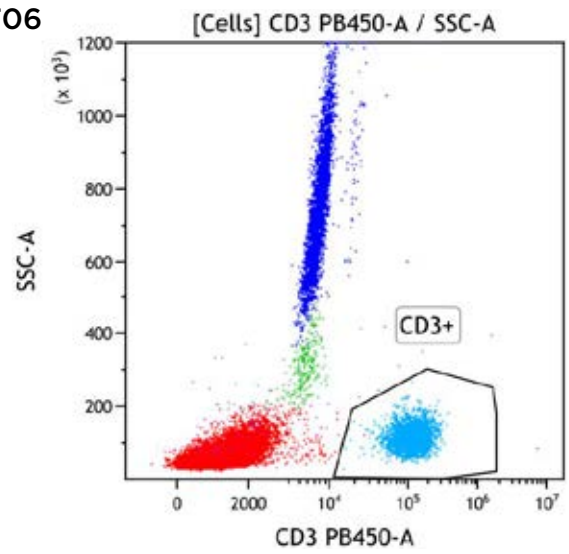

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

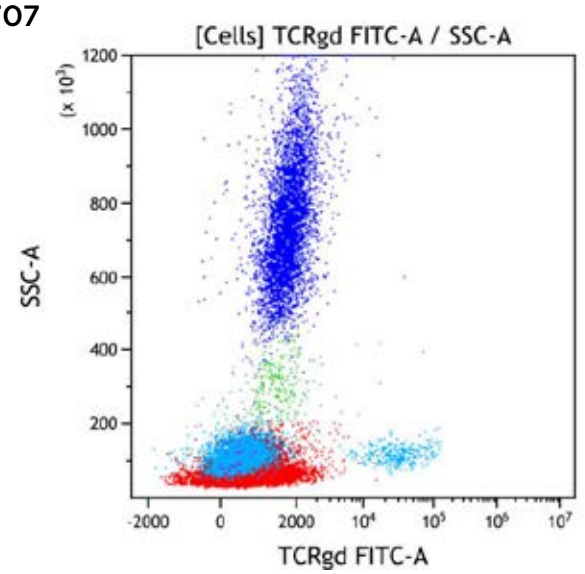

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

T08

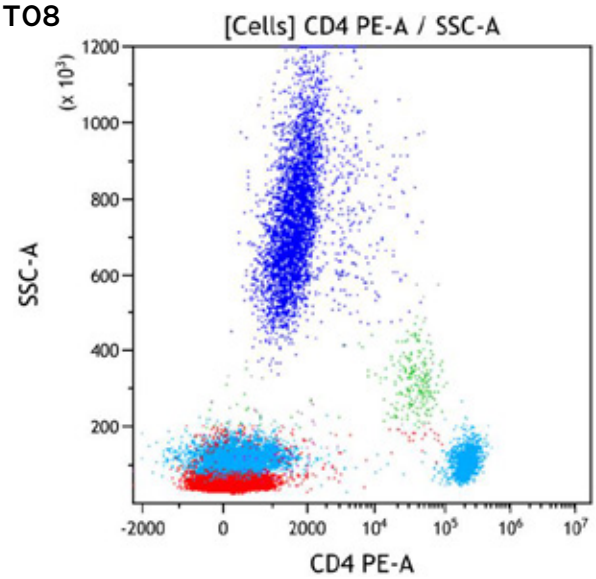

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells.

BACK TO CASE OVERVIEW

T09

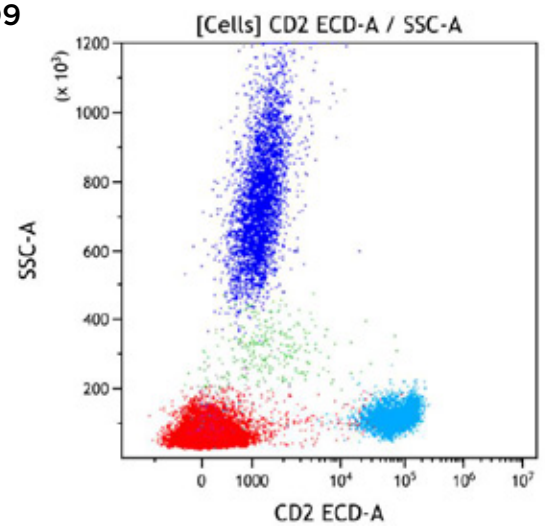

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells and at a low level on monocytes (green).

T10

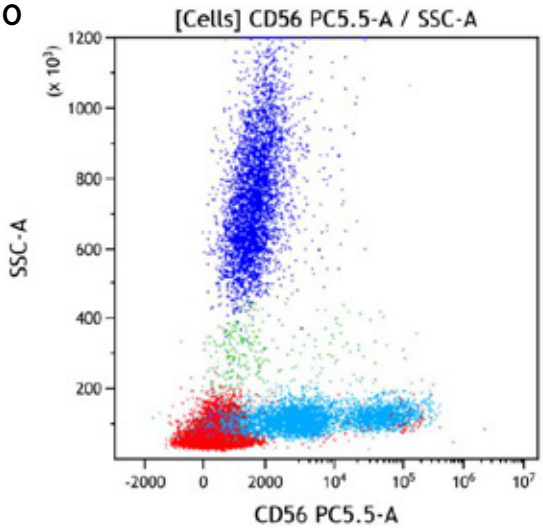

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells, T cells with natural killer activity (NK/T cells), and many gamma/ delta T cells (aqua). CD56 is also partially expressed on monocytes (green) in both reactive and neoplastic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

T11

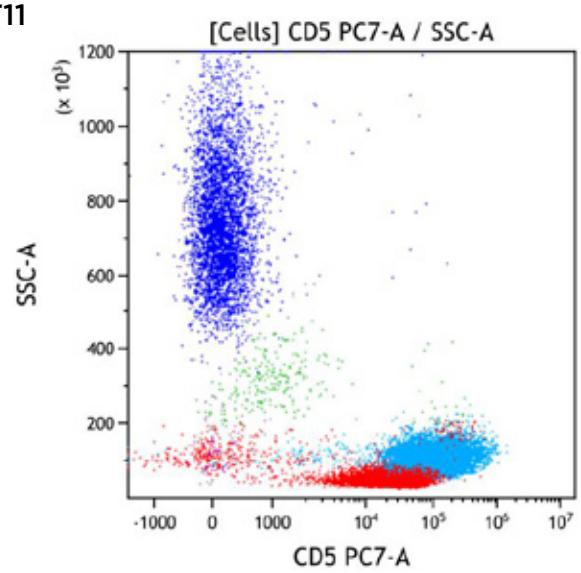

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

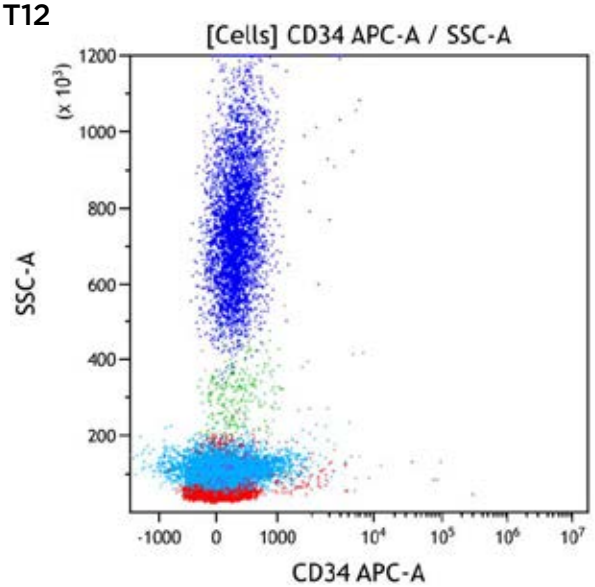

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and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

BACK TO CASE OVERVIEW

T13

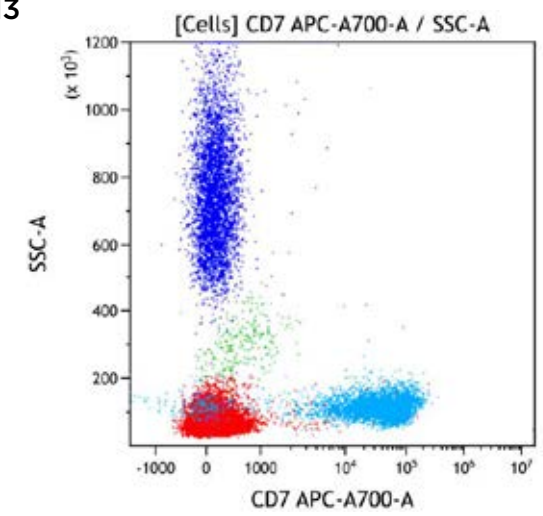

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells, and dimly expressed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

T14

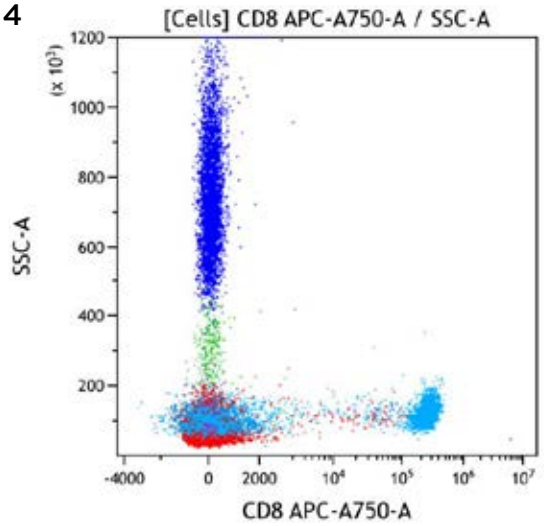

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells.

T15

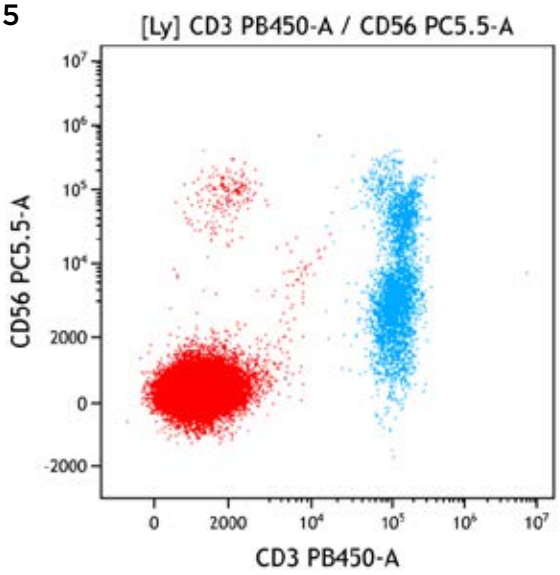

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

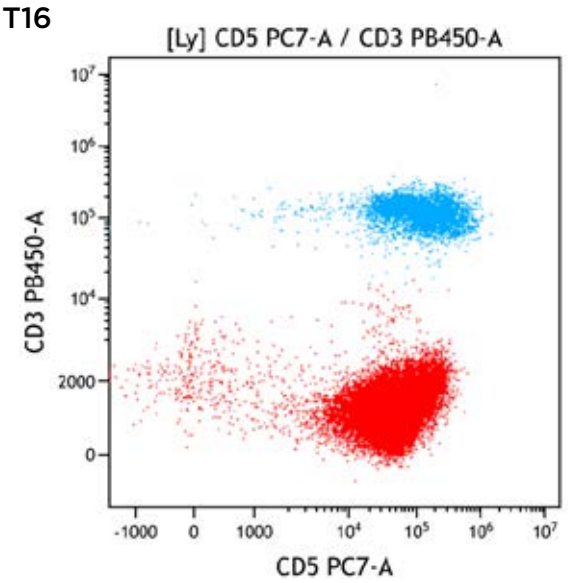

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

BACK TO CASE OVERVIEW

T17

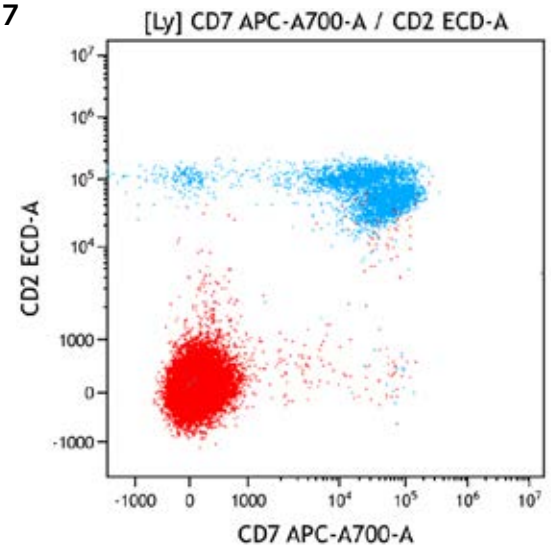

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells.

T18

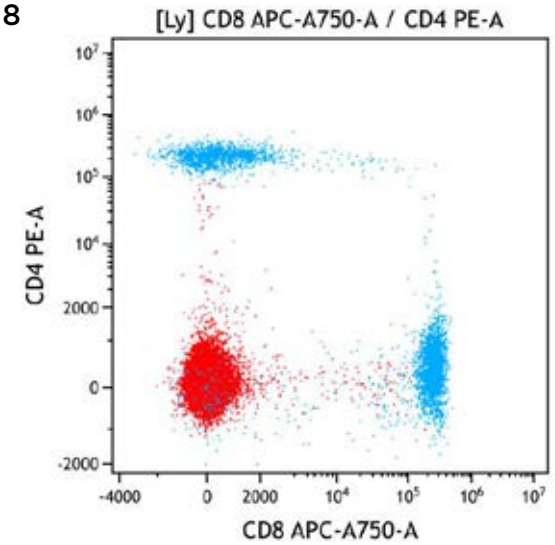

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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 cell

T19

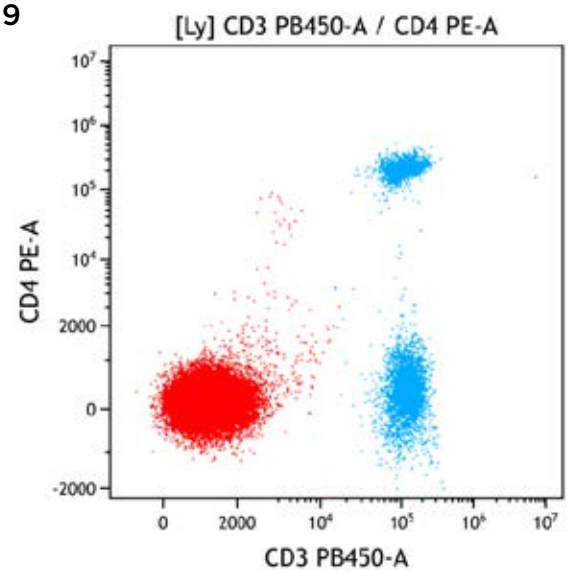

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. Of note, the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

T20

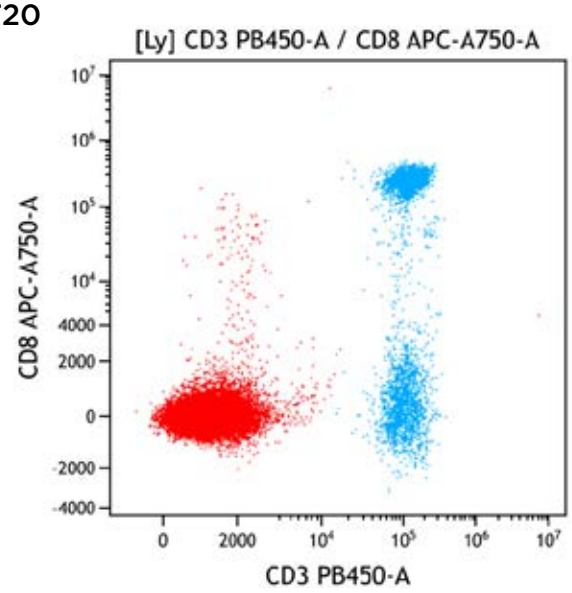

Figure 20. This CD3 vs CD8 dot plot Nshows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

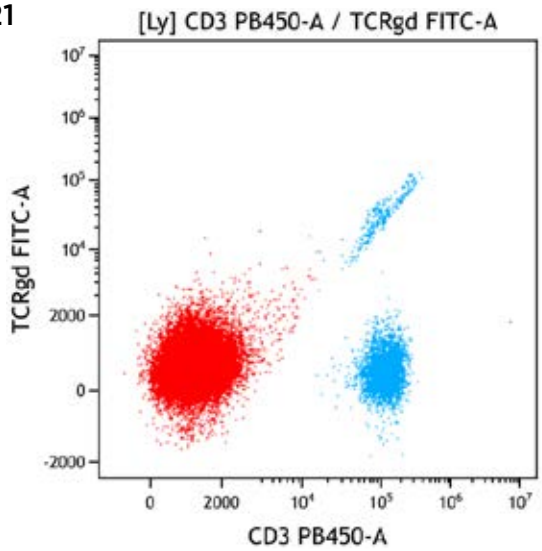

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/ delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

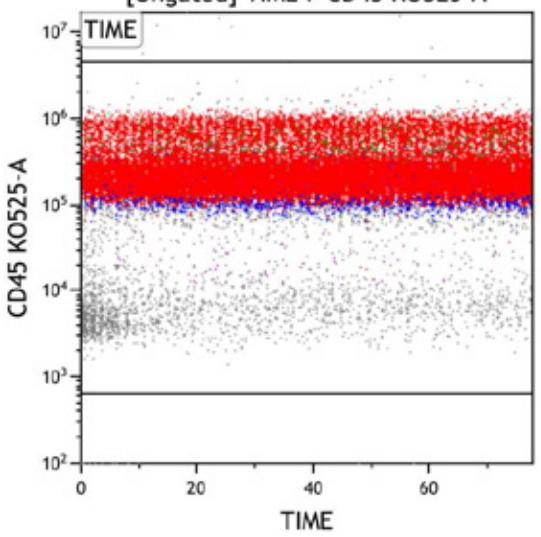

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

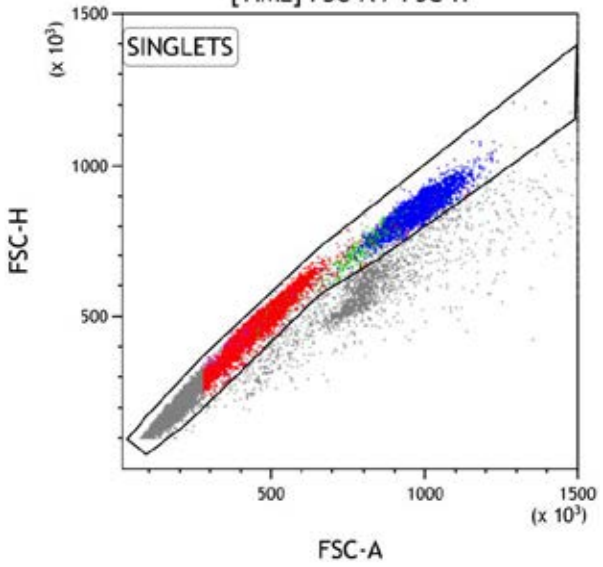

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship

M1-03

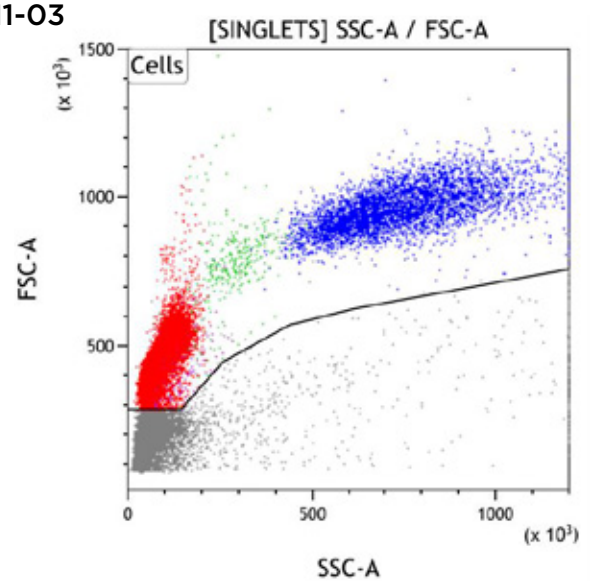

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M1-04

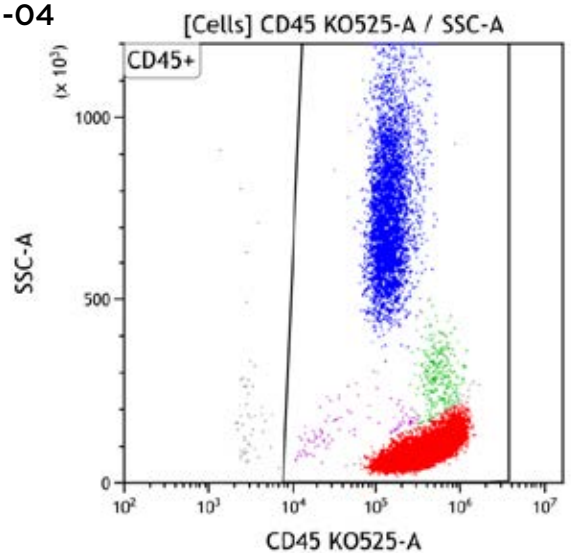

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, or non-hematopoietic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

BACK TO CASE OVERVIEW

M1-05

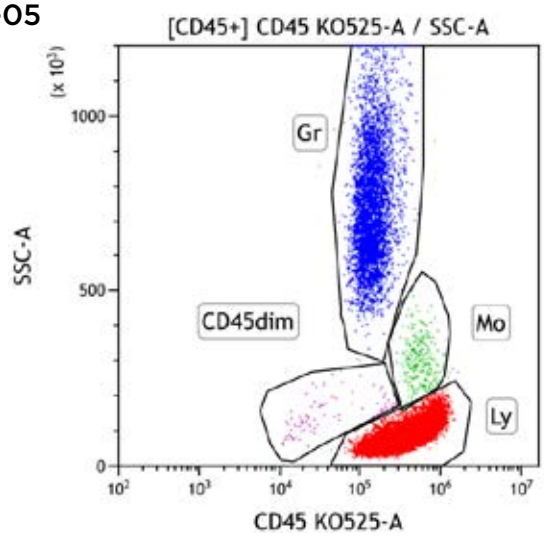

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M1-06

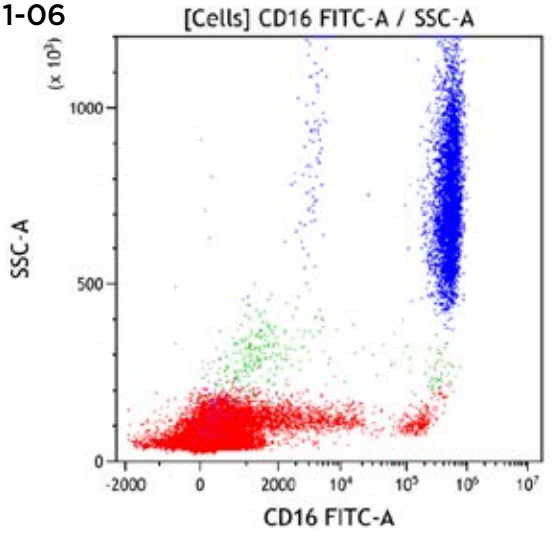

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

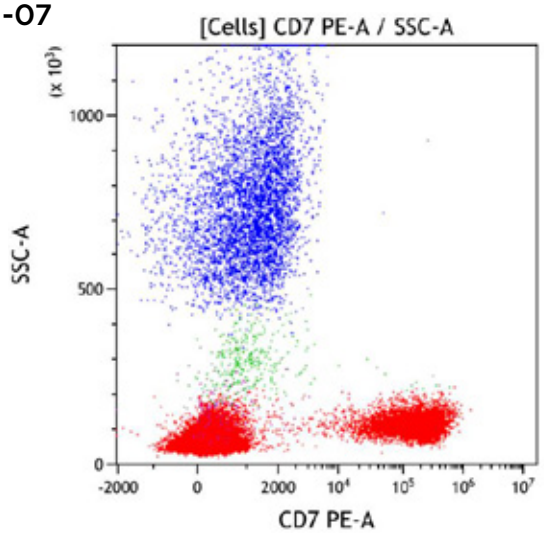

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed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

M1-08

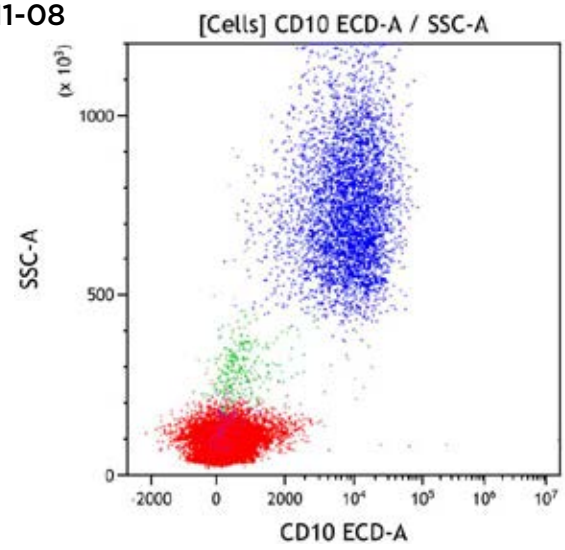

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

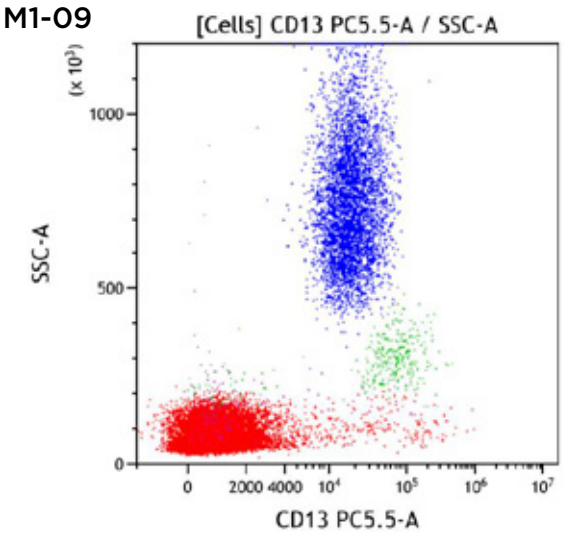

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature granulocytes (blue) and mature monocytes (green).

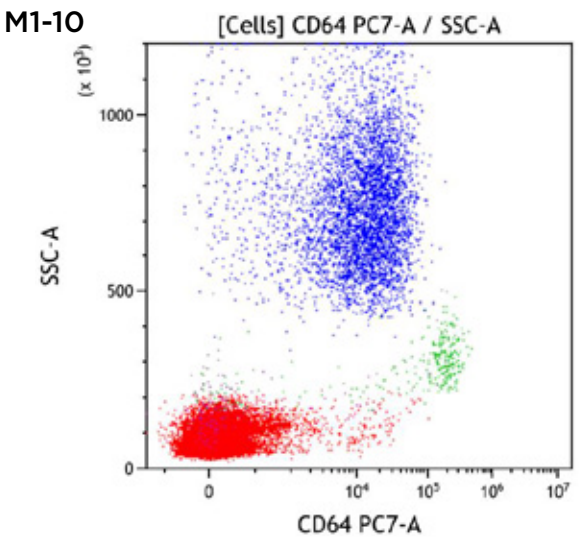

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on monocytes (green). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphocytes (red) or most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

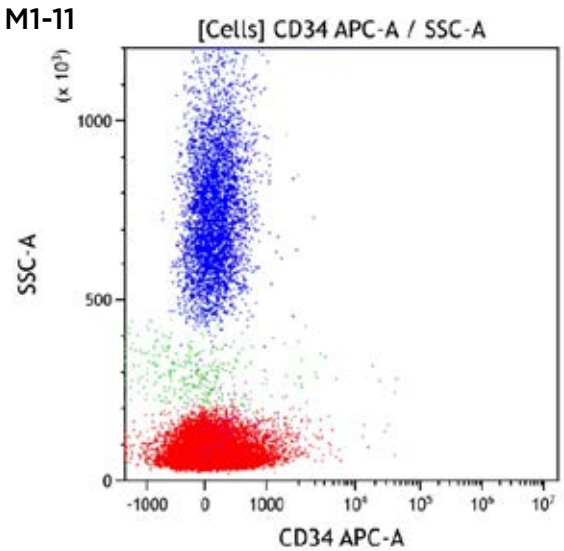

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and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

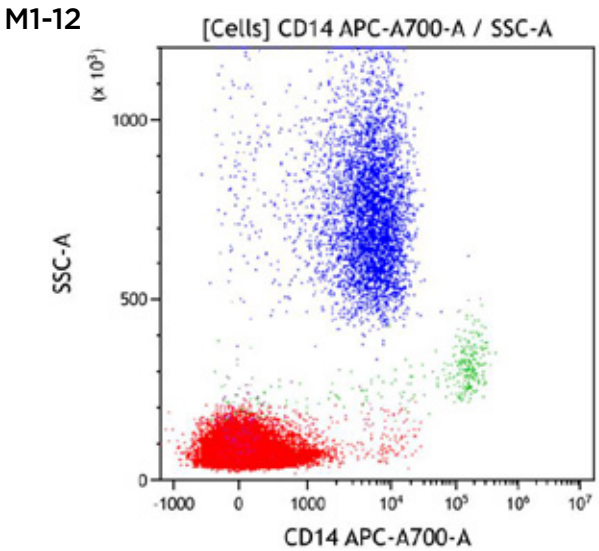

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably low level on immature monocytes. CD14 is also expressed on granulocytes (blue) at a low level.

BACK TO CASE OVERVIEW

M1-13

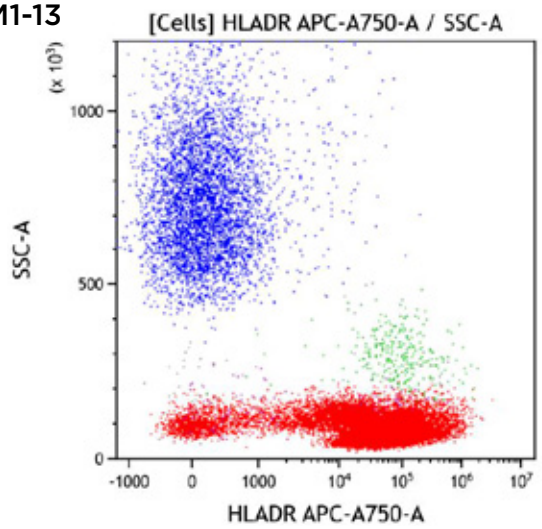

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 progenitors, immature and mature B cells (red), and activated T cells (red).

M1-14

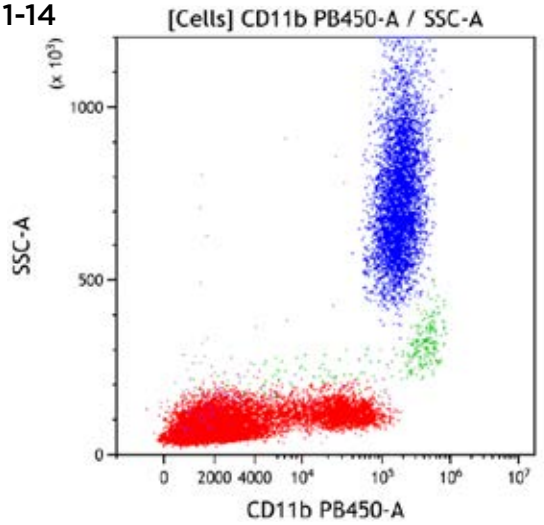

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes (blue) and monocytes (green). CD11b is also expressed on NK cells (red) and basophils.

M1-15

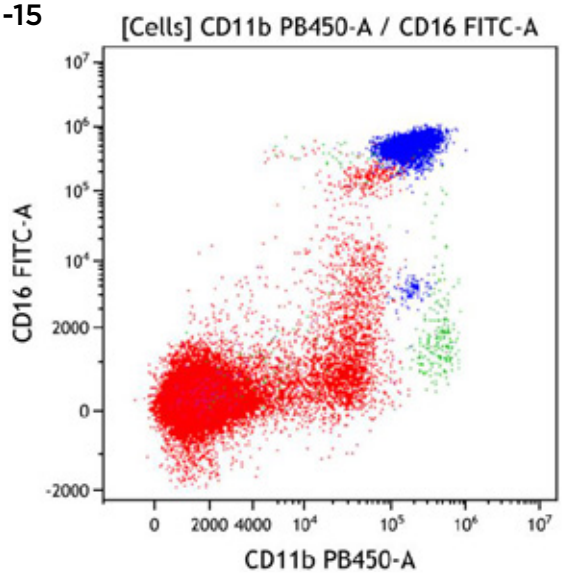

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue) and a subset of NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red).

M1-16

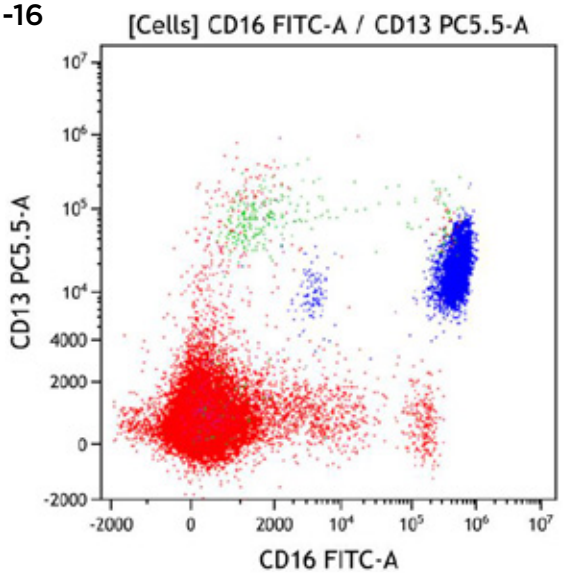

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red).

BACK TO CASE OVERVIEW

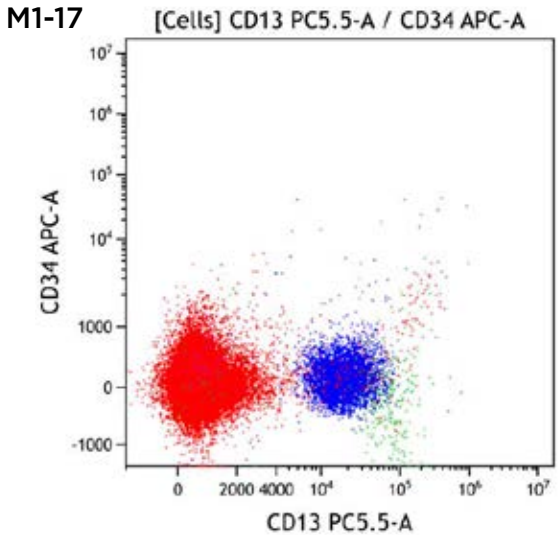

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphocytes.

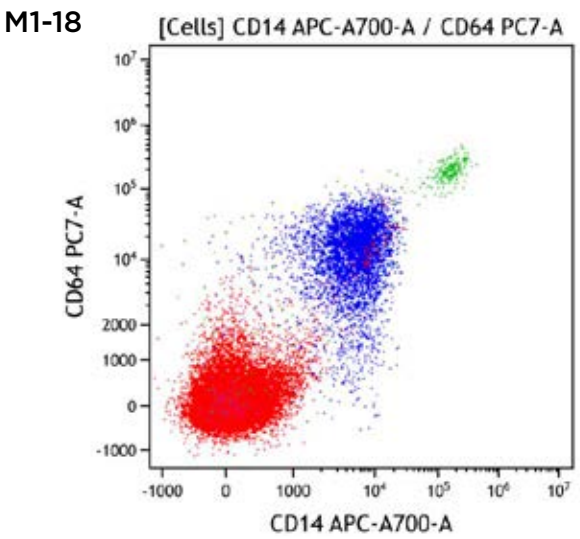

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on granulocytes.

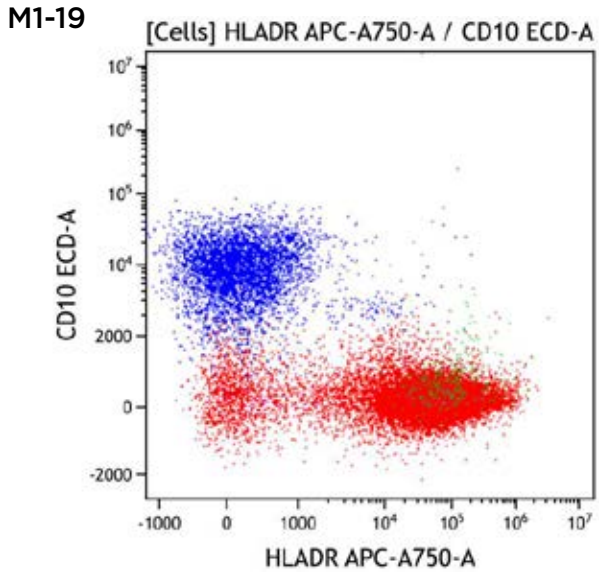

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells (red), plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on granulocytes (blue).

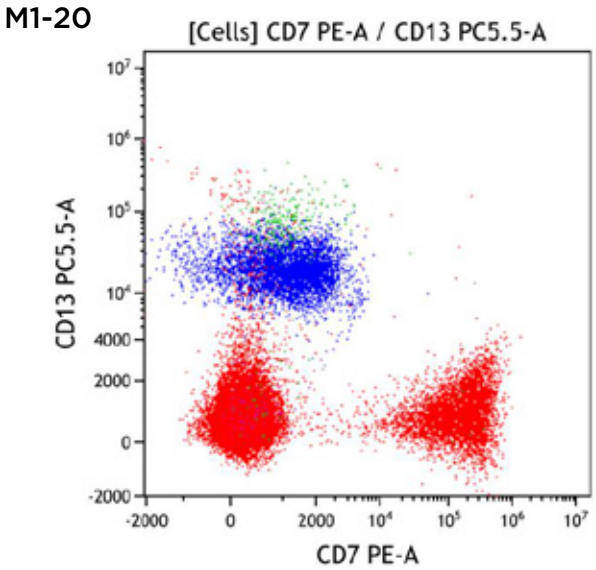

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

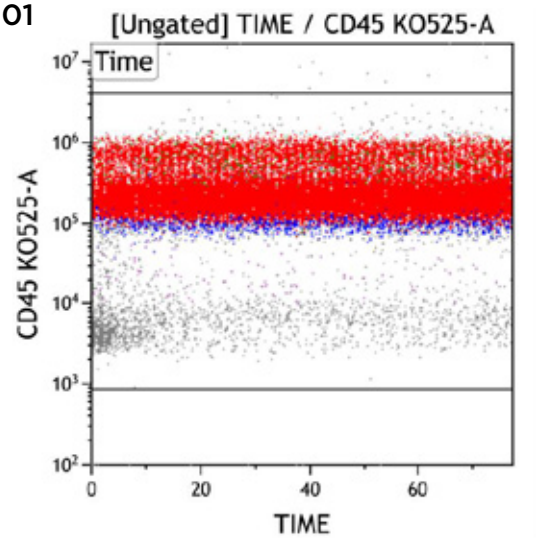

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

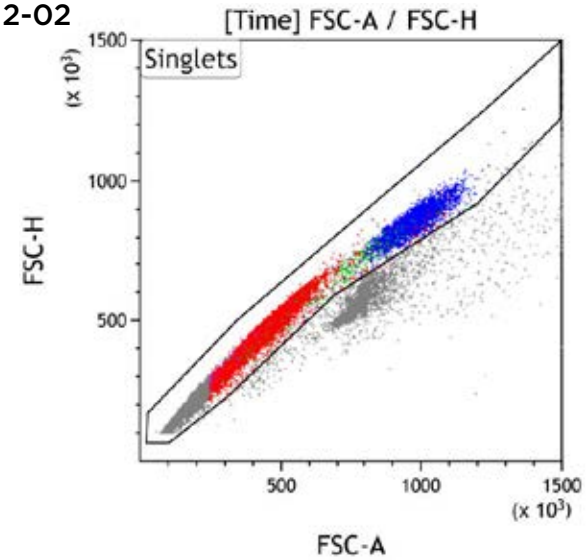

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship

M2-03

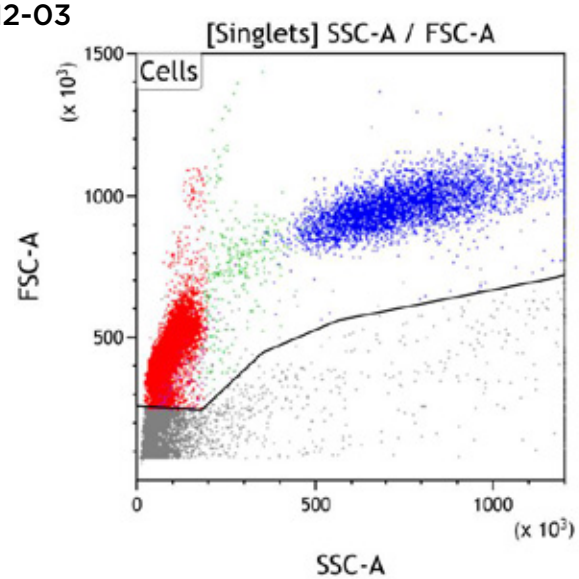

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M2-04

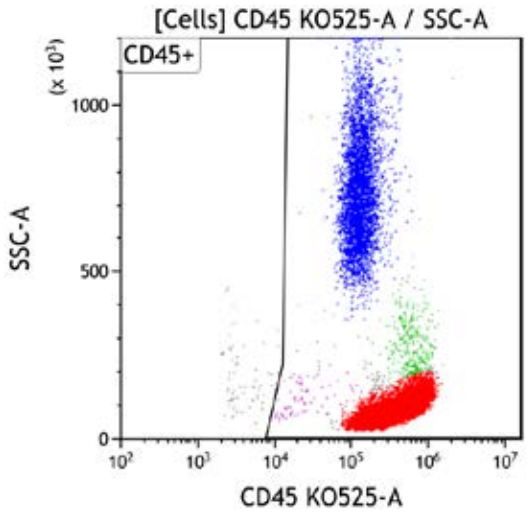

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M2-05

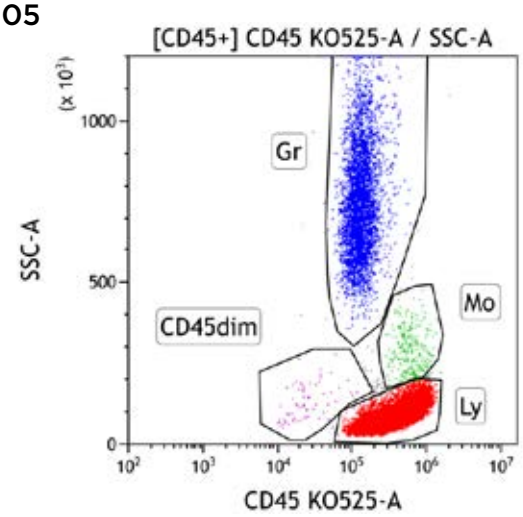

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M2-06

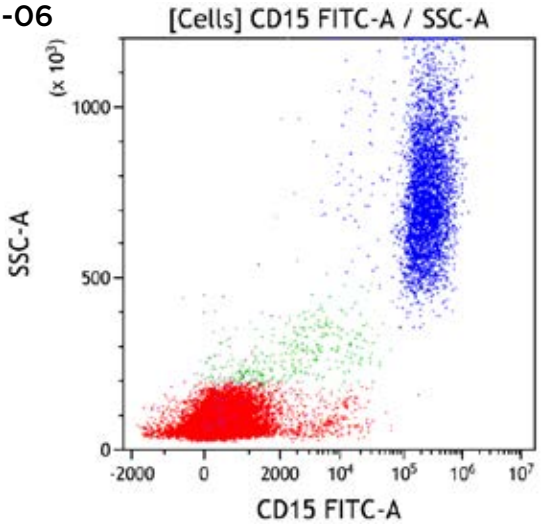

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes (green).

M2-07

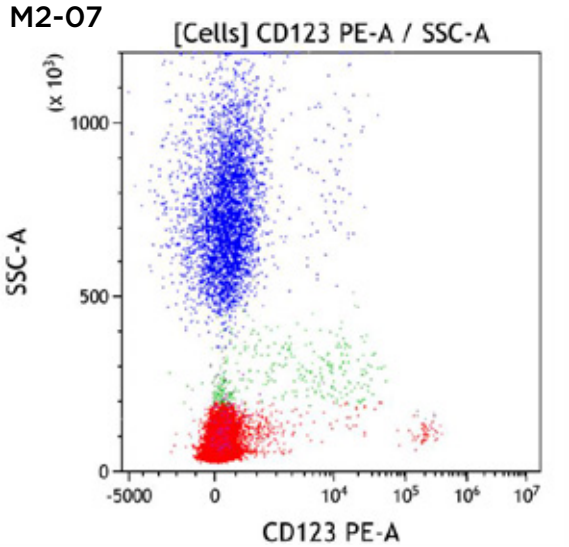

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

M2-08

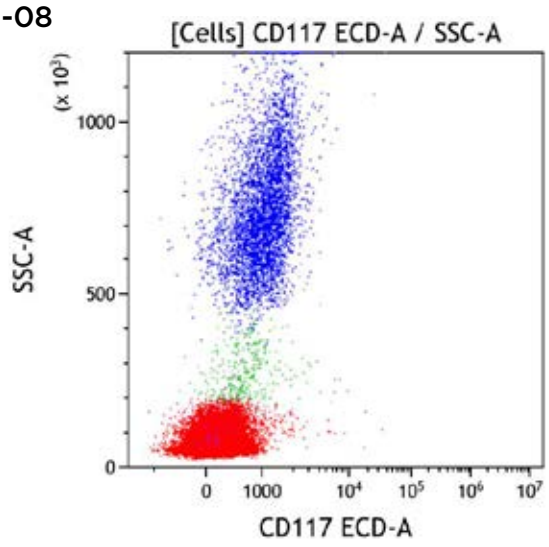

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

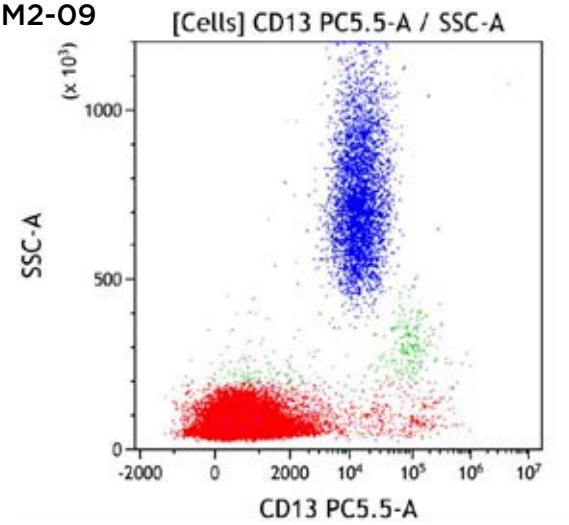

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and on mature monocytes (green).

M2-10

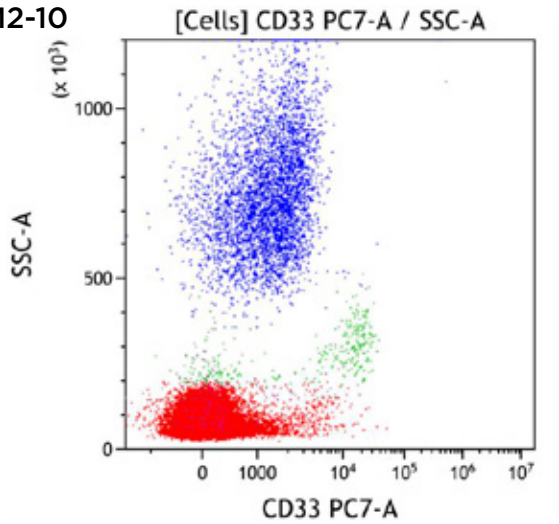

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors.

M2-11

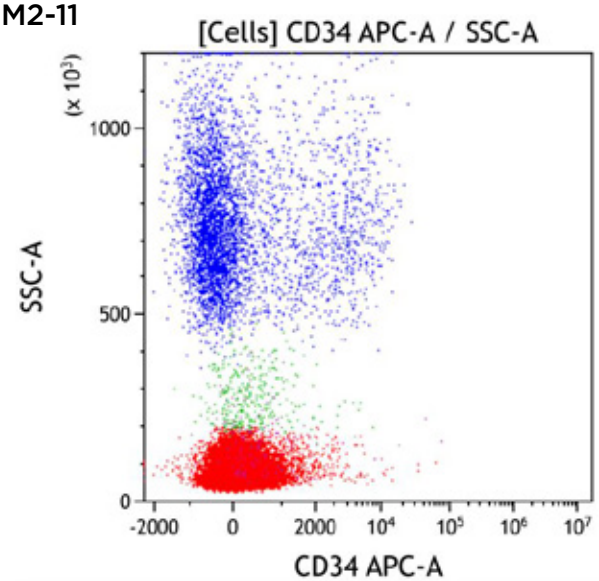

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and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

M2-12

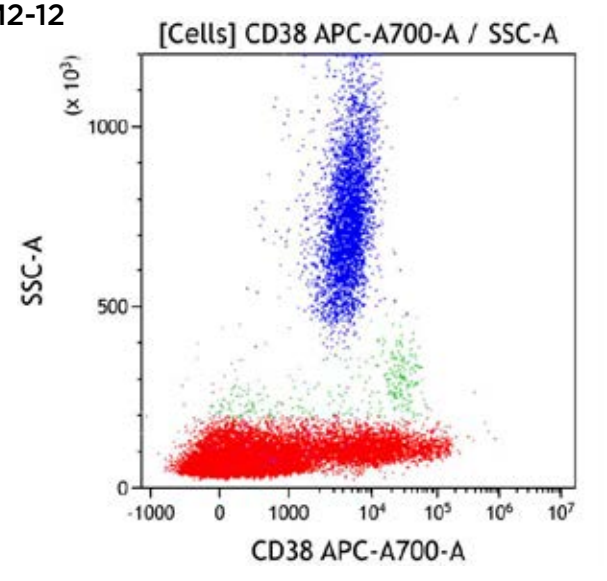

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature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

BACK TO CASE OVERVIEW

M2-13

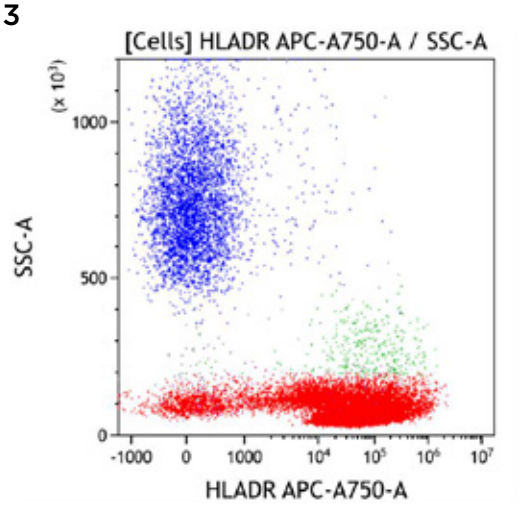

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red), and activated T cells (red).

M2-14

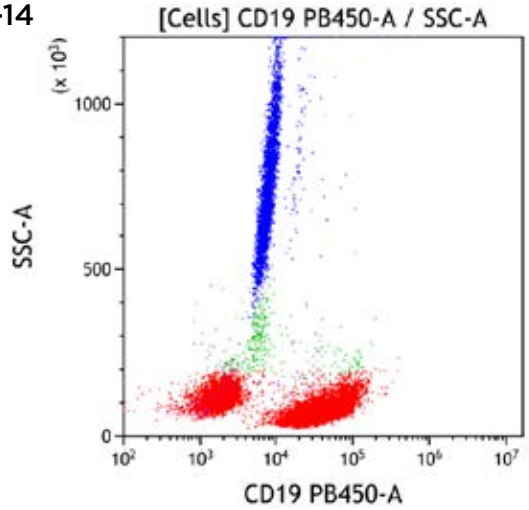

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red), as well as most plasma cells. These cells typically have low to moderate side scatter. The CD19 positive B cell population (red) is relatively expanded compared with normal.

M2-15

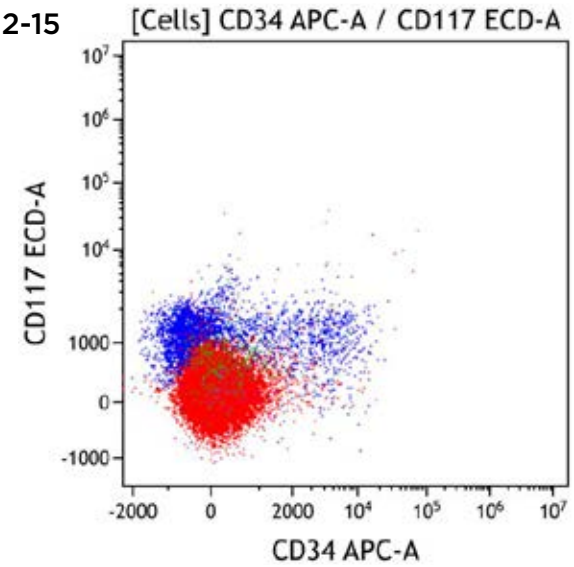

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors.

M2-16

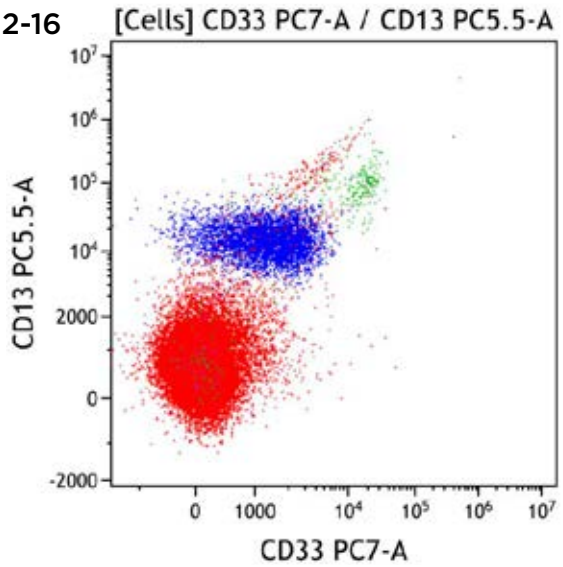

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Lymphocytes (red) largely do not express either CD13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

BACK TO CASE OVERVIEW

M2-17

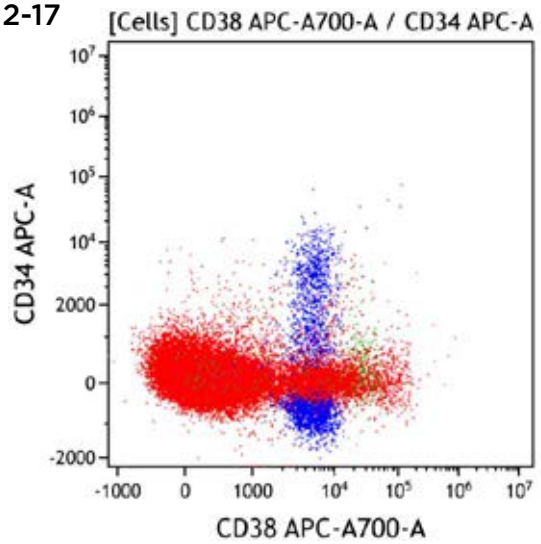

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes (blue), monocytes, and lymphocytes (red) are negative for CD34.

M2-18

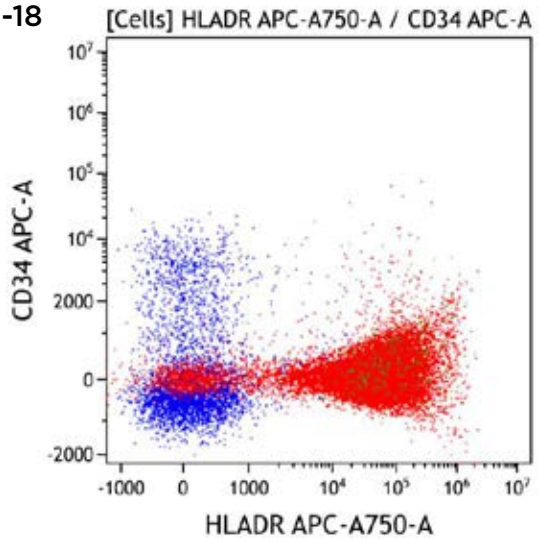

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells (red, lower middle), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors.

M2-19

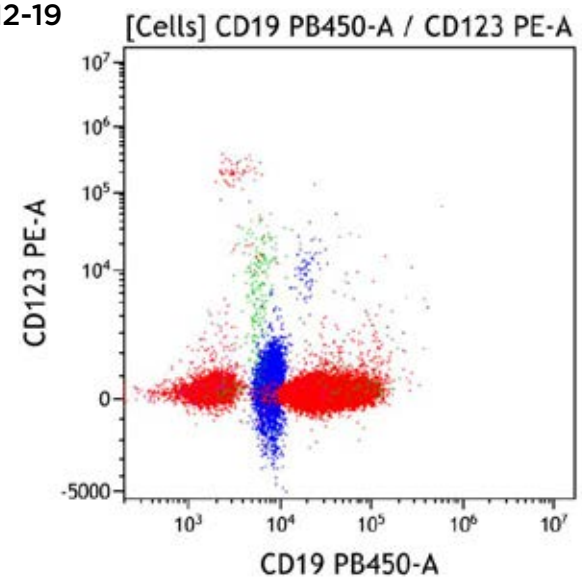

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. Mature CD19 positive B cells (red) normally do not express significant CD123. The apparent CD19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

M2-20

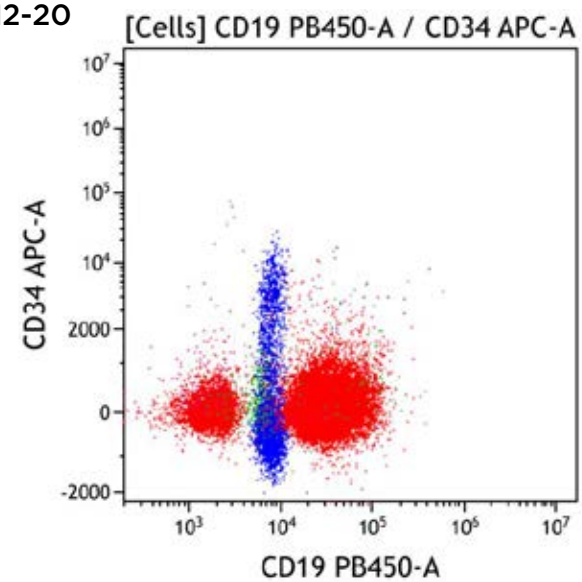

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. A large subset of lymphocytes (red) are CD19 positive B cells without expression of CD34.

BACK TO CASE OVERVIEW

M2-21

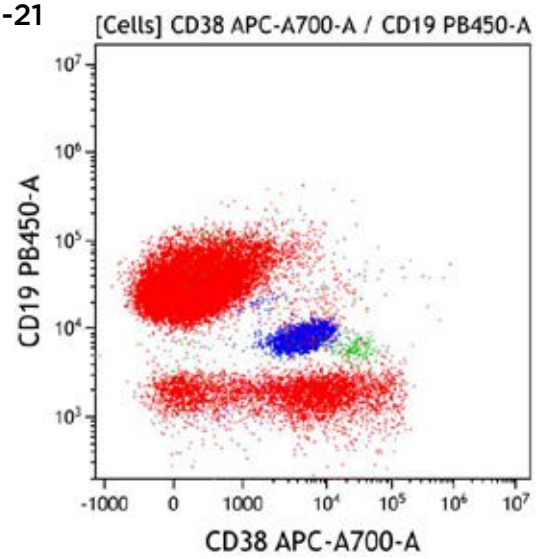

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) have low to absent CD38 expression.

M2-22

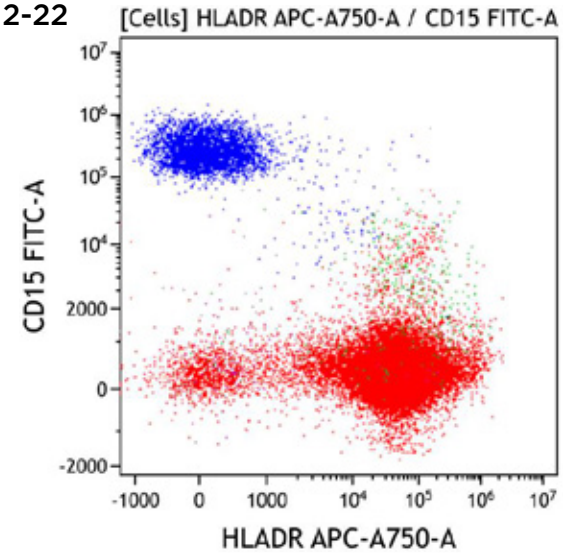

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells (red), monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes do not express HLA-DR.

M2-23

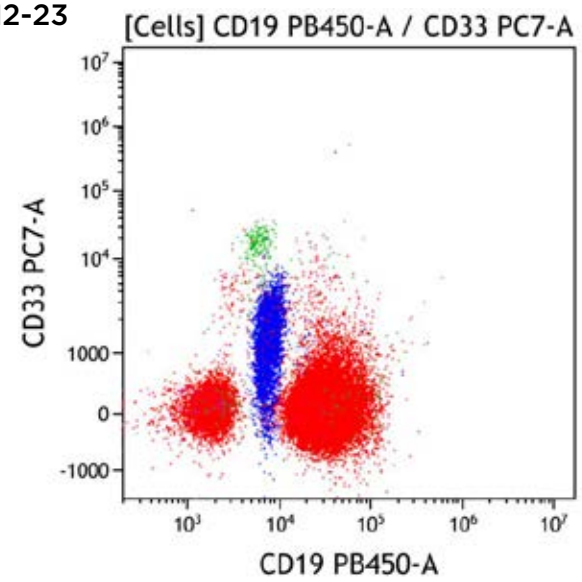

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells. CD33 is expressed by monocytes (green) and granulocytes (blue). Mature CD19 positive B cells (red) normally do not express significant CD33. A large subset of lymphocytes (red) are CD19 positive B cells without expression of CD33.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 3 LHS-TC4-064**
Diagnosis	**B-lymphoblastic leukemia/lymphoma**
Clinical Vignette	This 4-year-old male presents with pancytopenia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells that express bright CD10, dim CD13, intermediate CD19, variable CD20, dim CD33, CD34 (subset), intermediate CD38, dim CD45, intermediate CD123, bright CD200, and bright HLA-DR without significant expression of other T cell or myeloid markers. Compared with normal B cell precursors, the expression of increased CD10, dim CD13, dim CD33, CD34 on a subset, and CD123 are aberrant. 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.

BACK TO CASE LISTINGS

B Cell Tube

B01

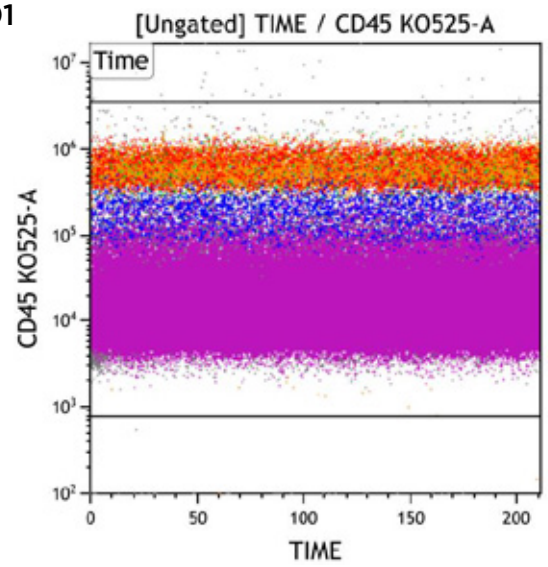

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

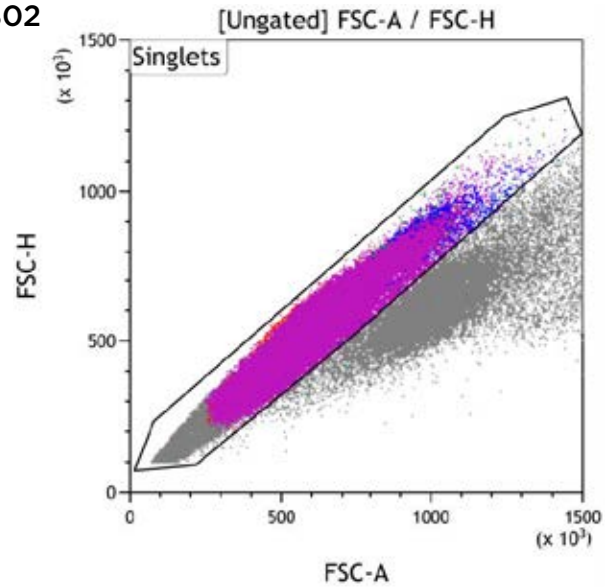

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

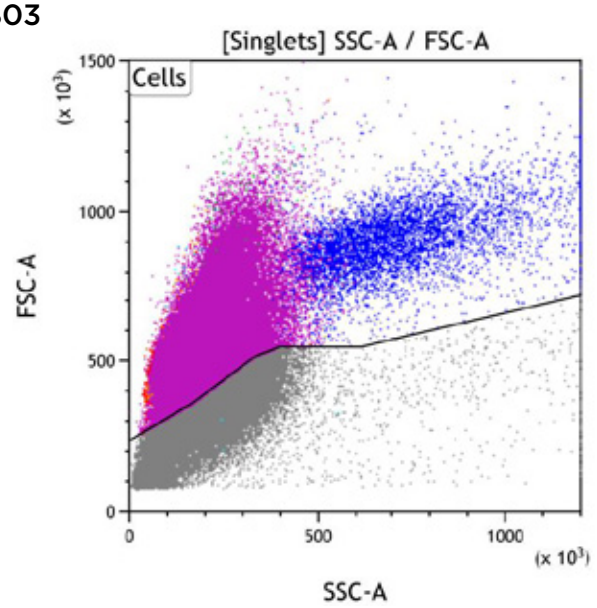

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

B04

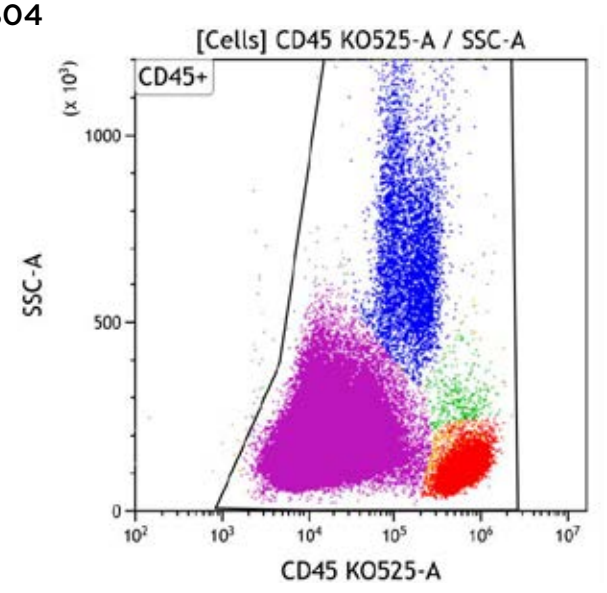

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

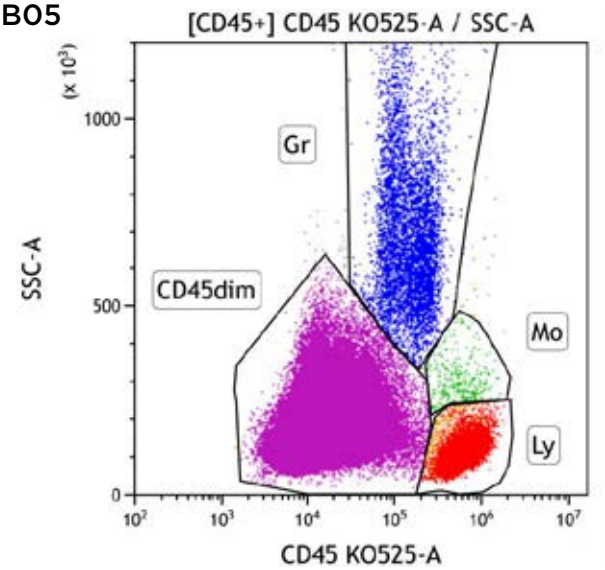

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 followed throughout the analysis. The progenitor population in the CD45dim gate (purple) is expanded.

B06

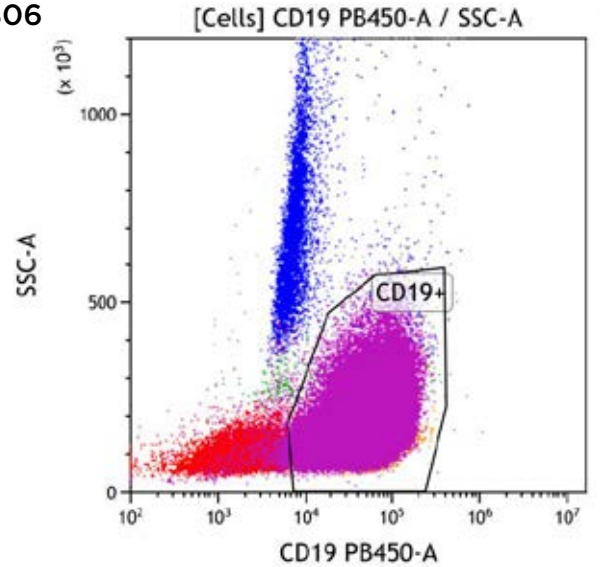

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple)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

B07

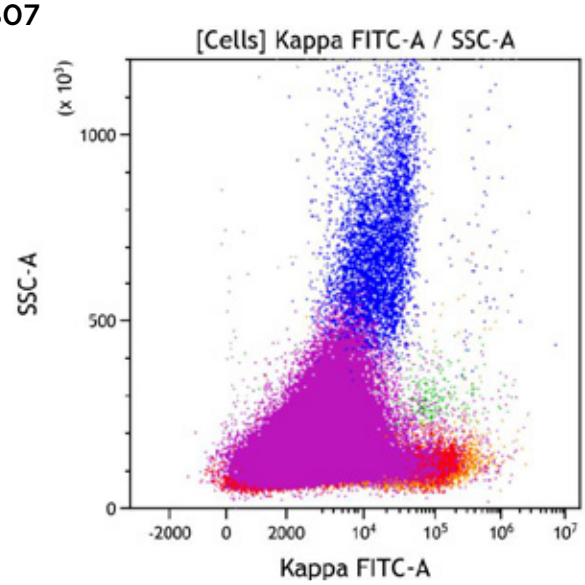

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The Kappa light chain positive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

B08

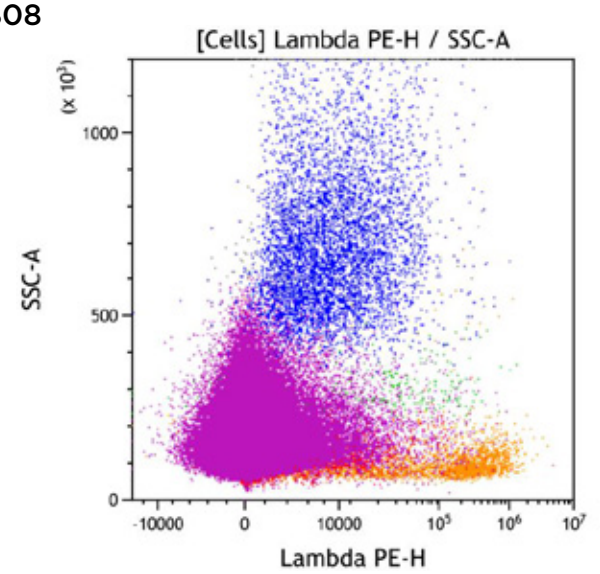

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The lambda light chain positive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

BACK TO CASE OVERVIEW

B09

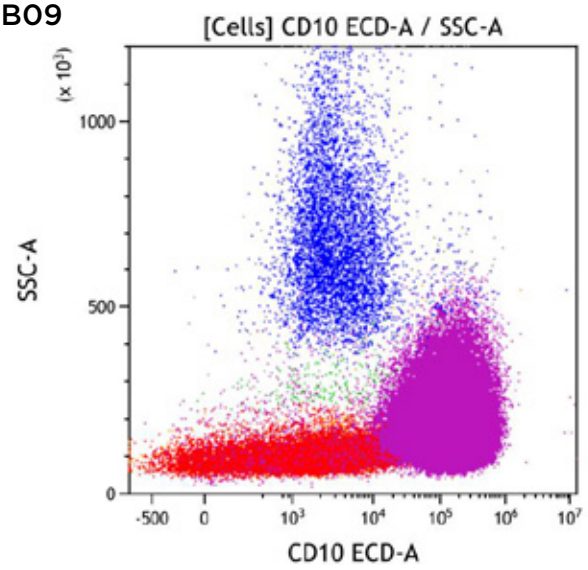

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

B010

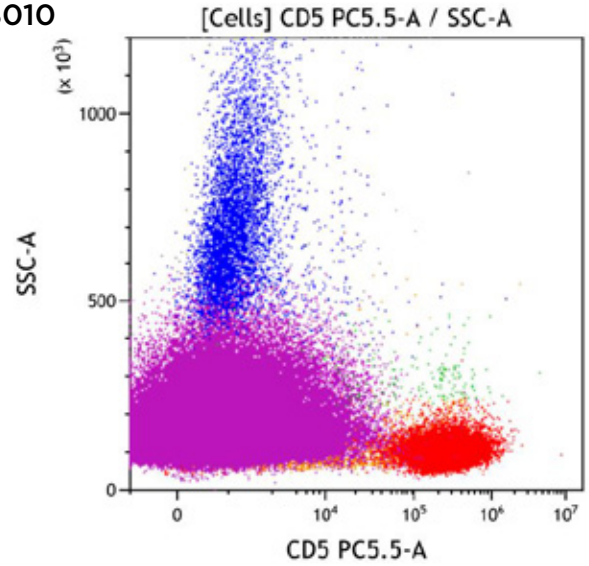

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The aberrant population (purple) is negative for CD5.

B011

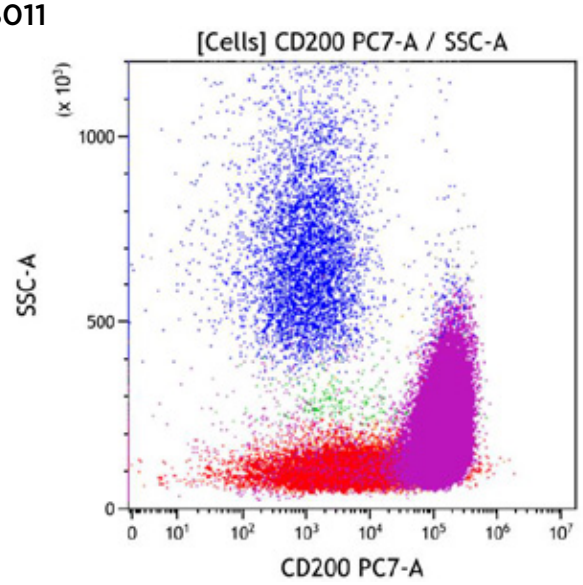

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B012

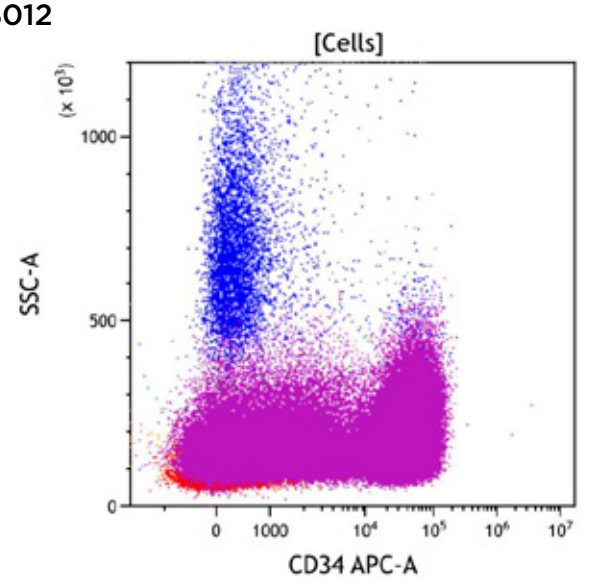

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant populatio (purple) expresses CD34 on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

BACK TO CASE OVERVIEW

B013

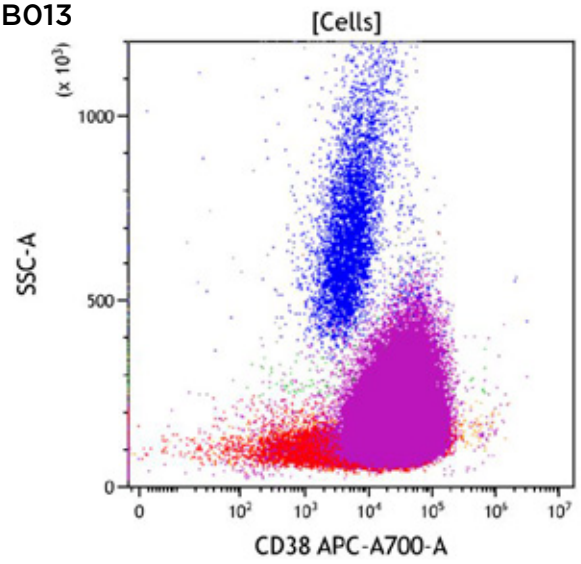

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderat level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variable level on activated mature lymphocytes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

B014

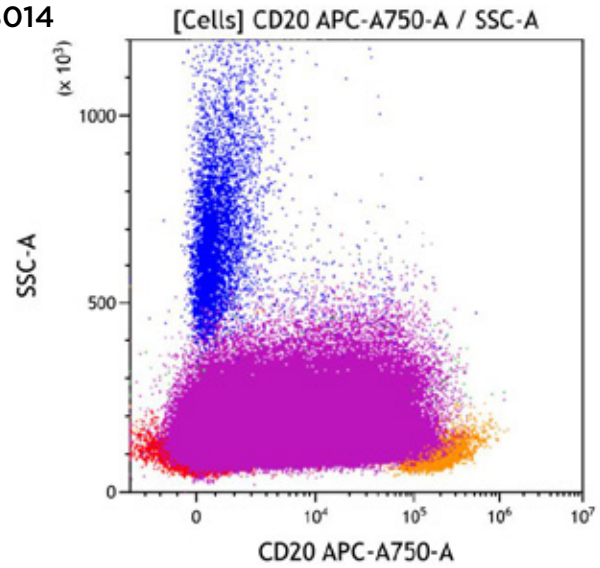

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side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

B015

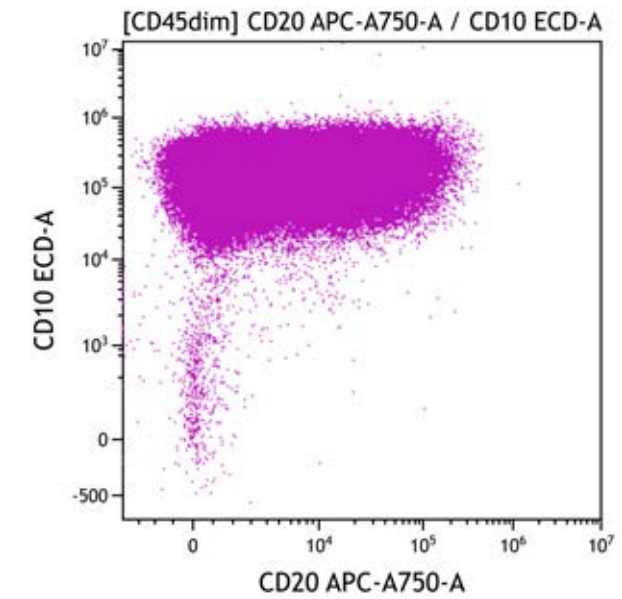

Figure 15. This CD20 vs CD10 dot plot shows the aberrant population in the CD45dim gate. The aberrant population (purple) displays uniform CD10 expression with variable CD20 expression.

B016

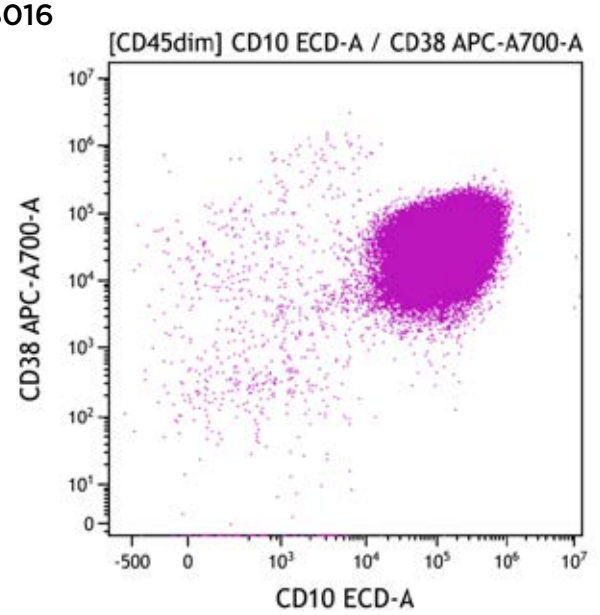

Figure 16. This CD10 vs CD38 dot plot shows the aberrant population in the CD45dim gate. The aberrant population (purple) displays uniform CD10 and CD38 expression.

BACK TO CASE OVERVIEW

B017

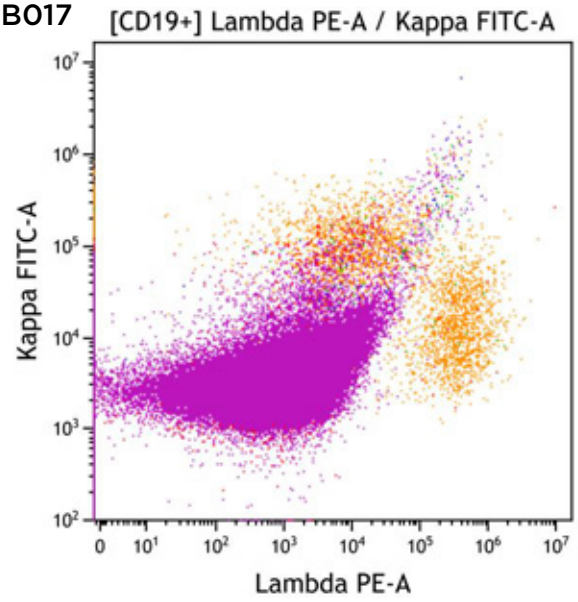

Figure 17. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. Increased background due to adheren plasma immunoglobulin is common. The aberrant population (purple) lacks kappa or lambda light chain expression.

B018

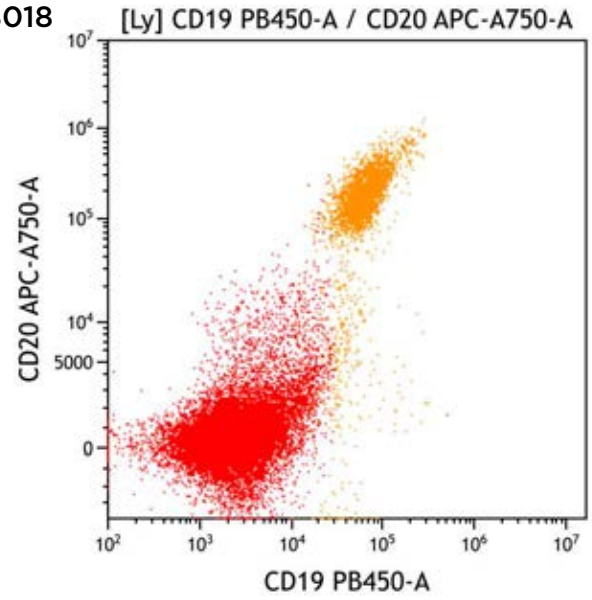

Figure 18. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression. CD19 or CD20 expression.

B019

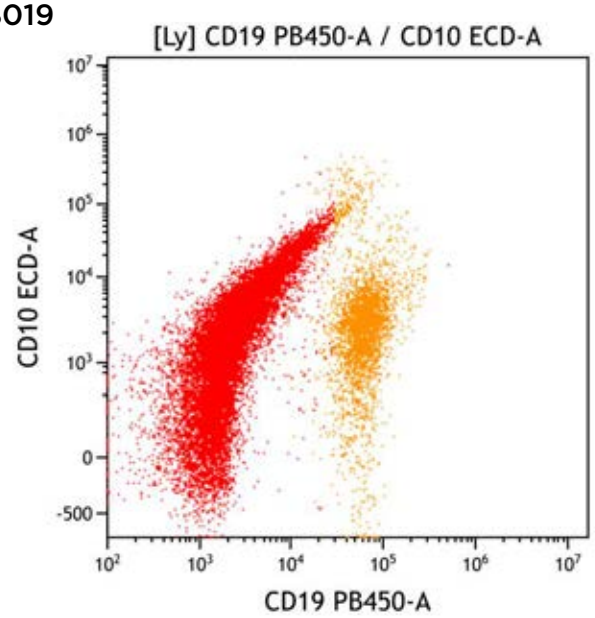

Figure 19. This CD19 vs CD10 dotplot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of latestagem immature B cells is present in peripheral blood and bone marrow aspirates.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

B020

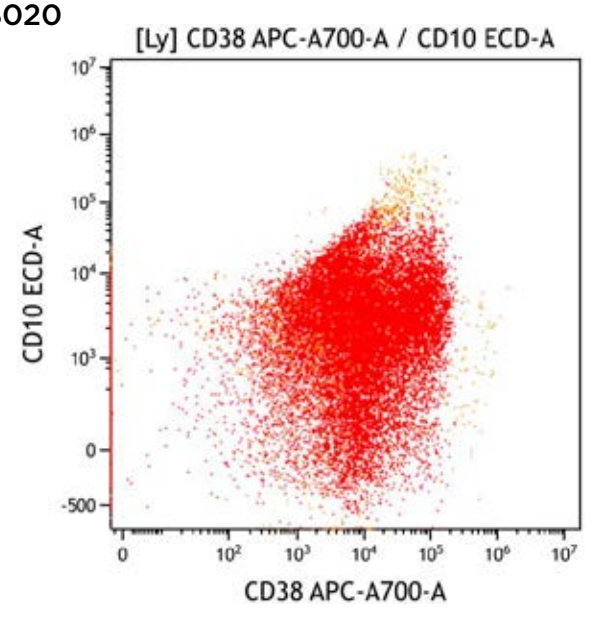

Figure 20. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low level expression of CD38. T cells (red) show variable CD38 expression dependent on activation state.

BACK TO CASE OVERVIEW

B021

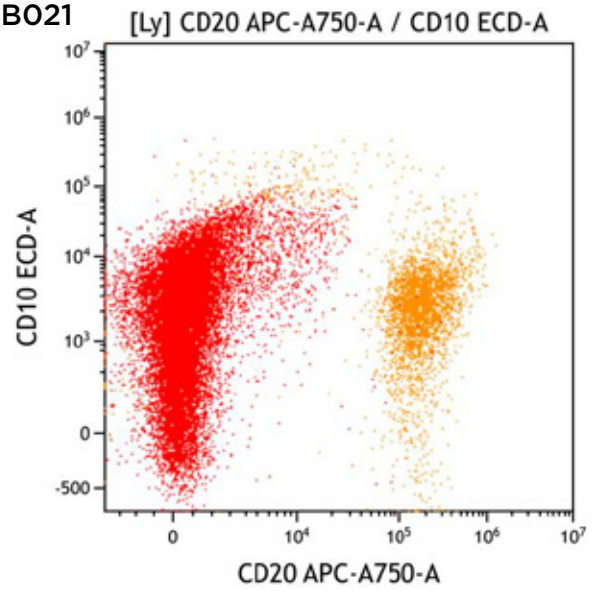

Figure 21. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20 without significant CD10.

B022

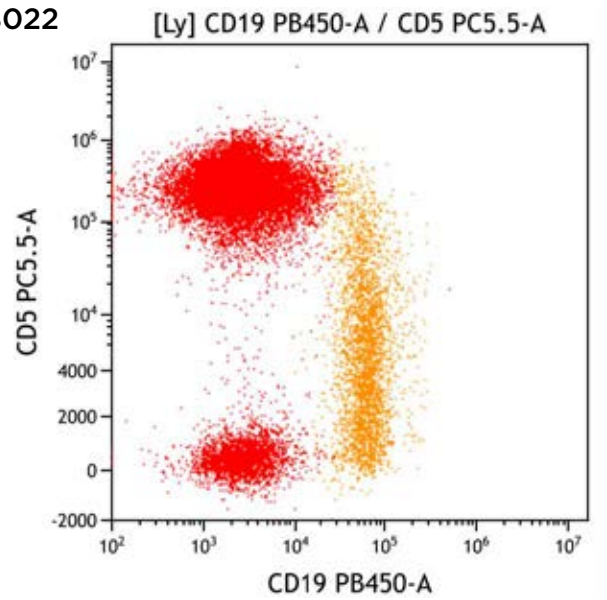

Figure 22. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

B023

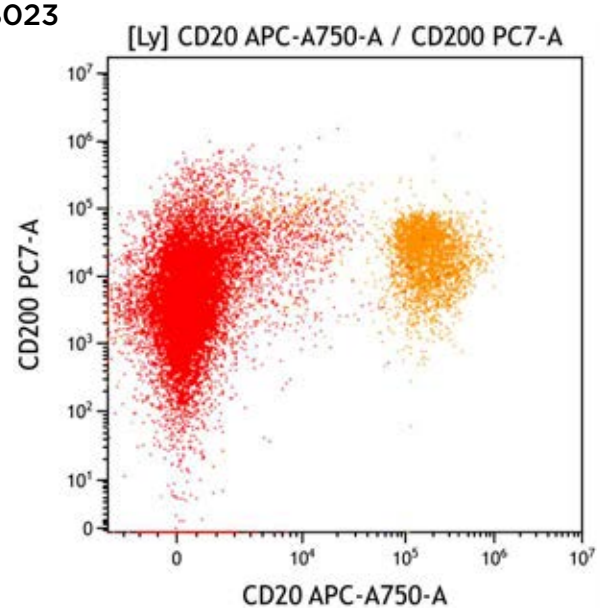

Figure 23. This CD20 vs CD200 dot plot shows all cells in the lymphocytem gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

B024

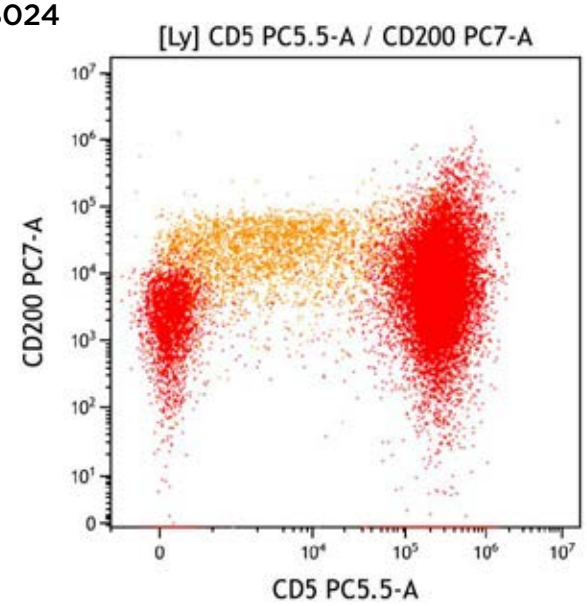

Figure 24. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell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

BACK TO CASE OVERVIEW

T Cell Tube

T01

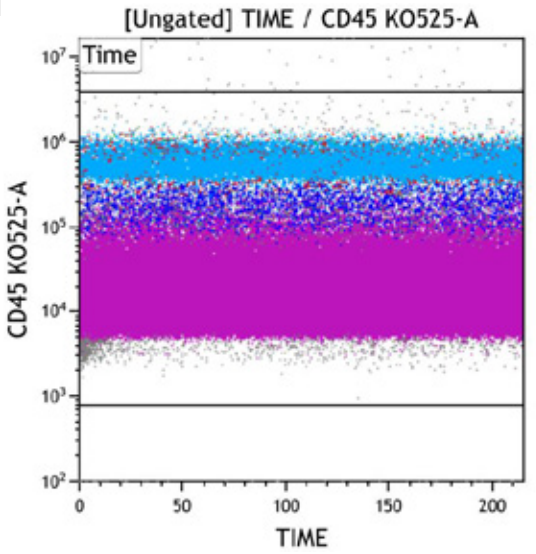

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

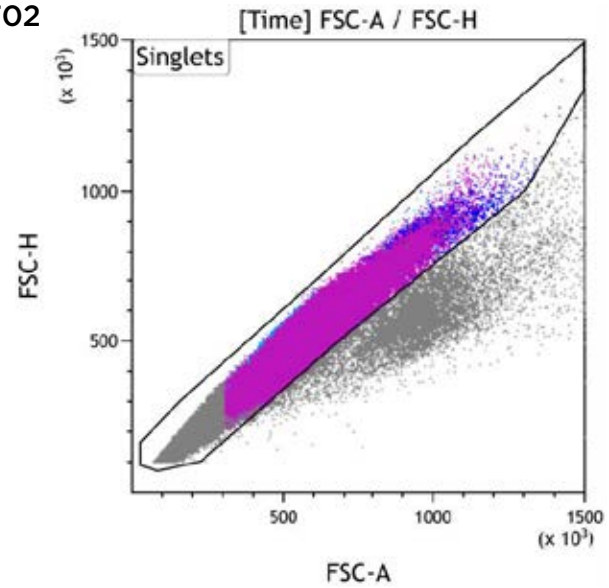

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, whileM doublets lie outside the linear relationship.

T03

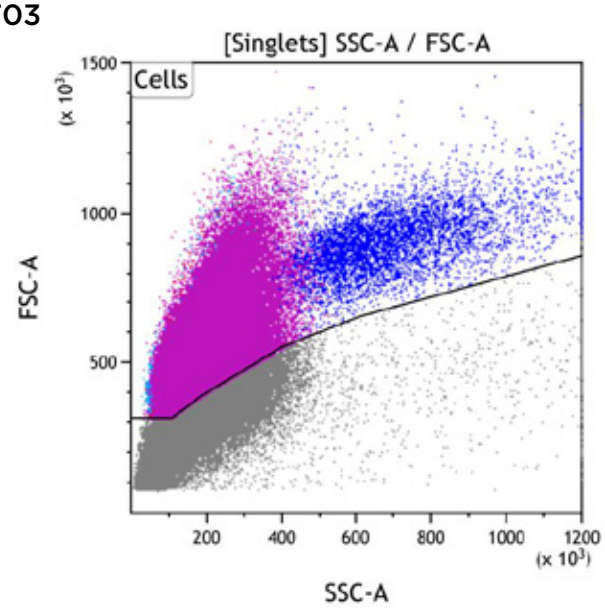

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

T04

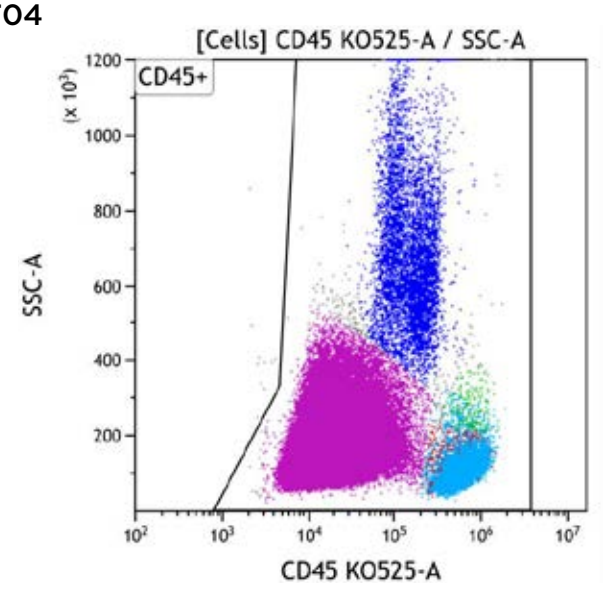

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

BACK TO CASE OVERVIEW

T05

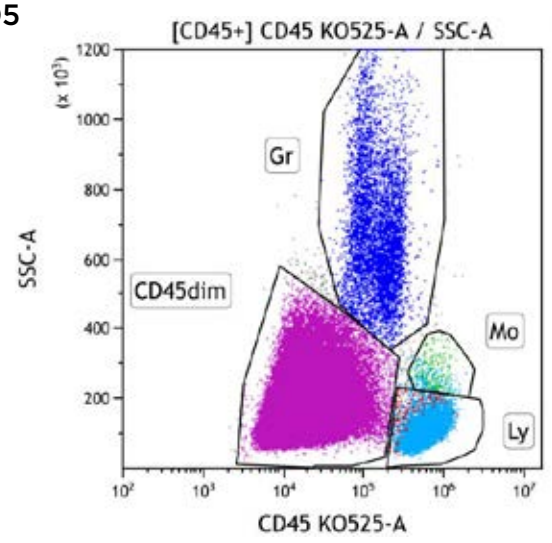

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes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

T06

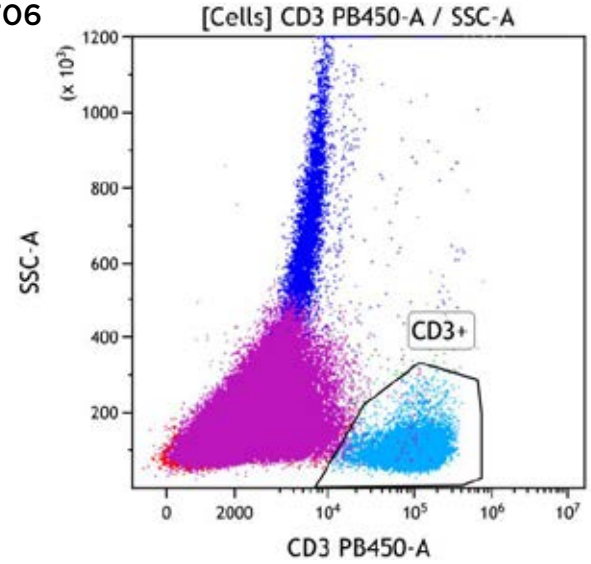

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

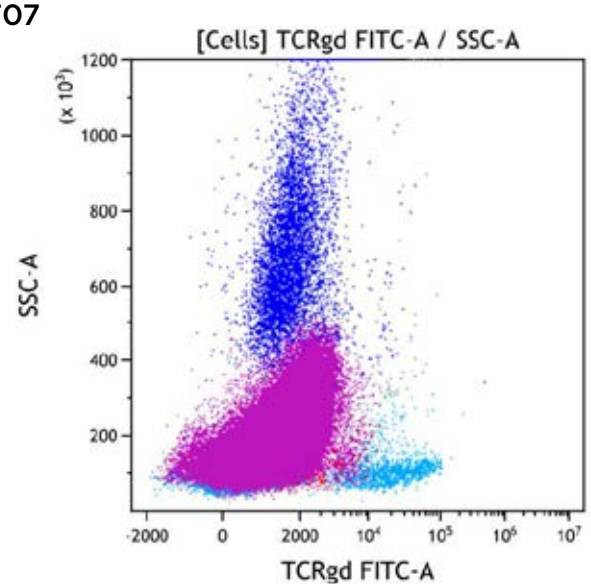

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). The aberrant population (purple) is negative for TCRγδ.

T08

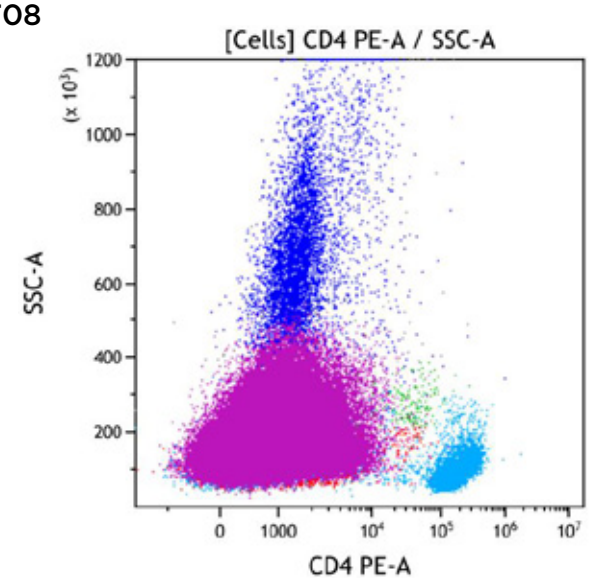

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

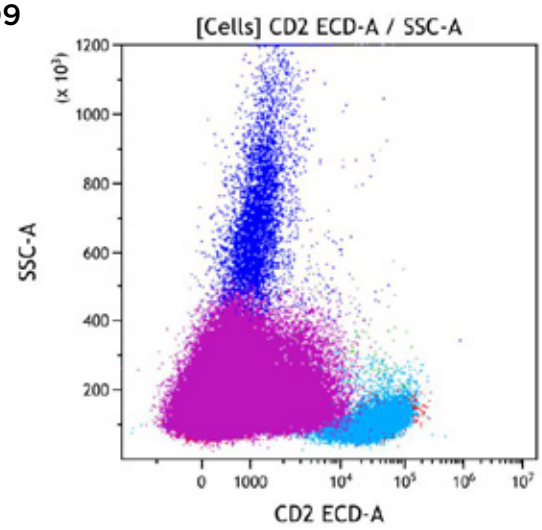

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells.CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red). The aberrant population (purple) is negative for CD2.

T10

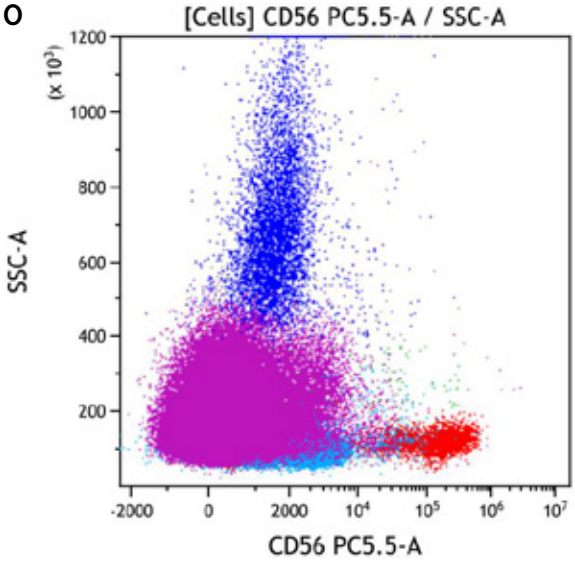

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red) and T cells with natural killer activity (NK/T cells). The aberrant population (purple) is negative for CD56.

T11

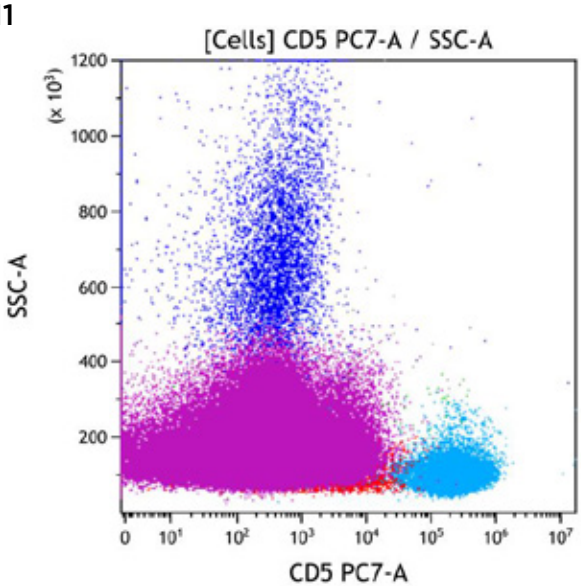

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. The aberrant population (purple)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

T12

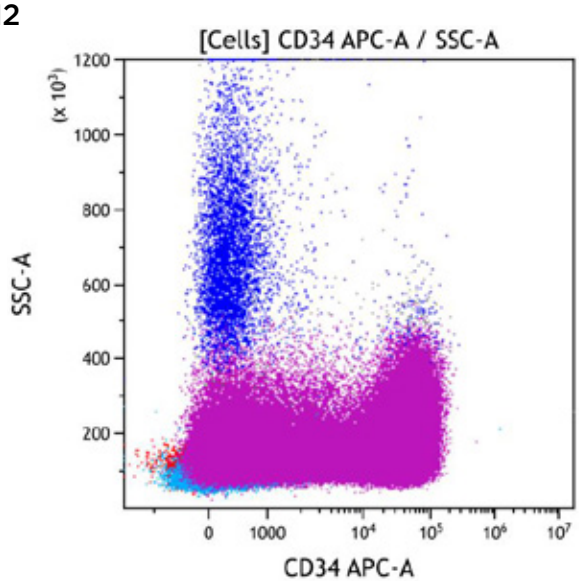

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) expresses CD34 on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

BACK TO CASE OVERVIEW

T13

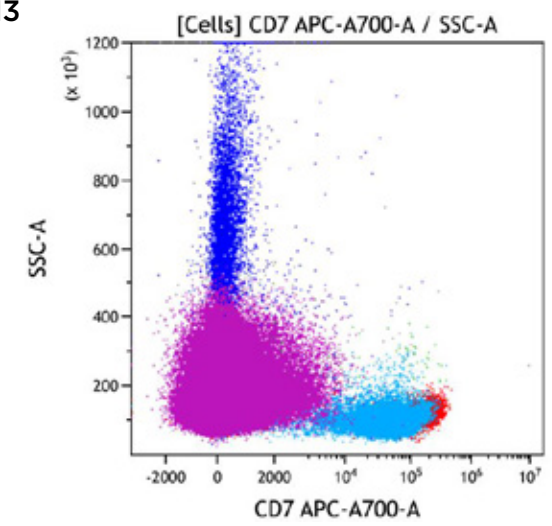

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

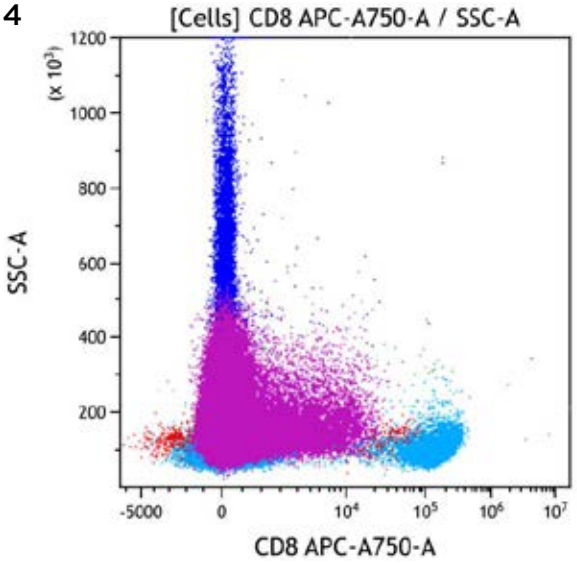

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells. The aberrant population (purple) is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

T15

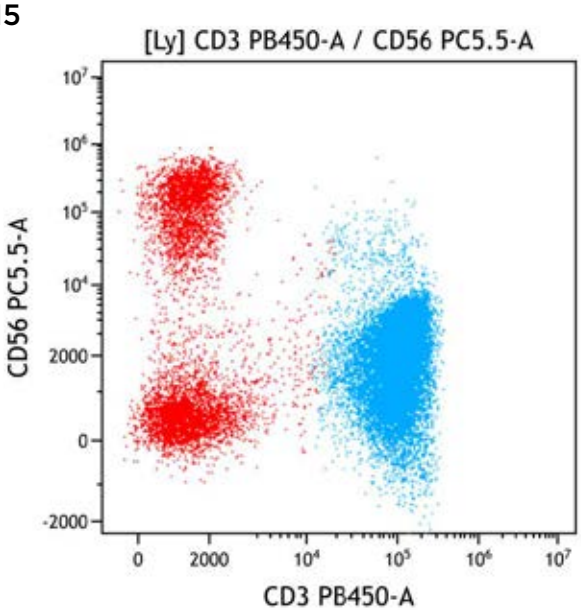

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

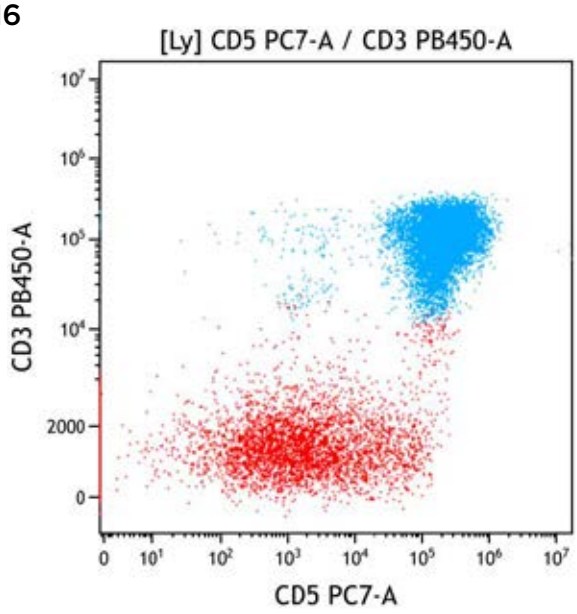

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

BACK TO CASE OVERVIEW

T17

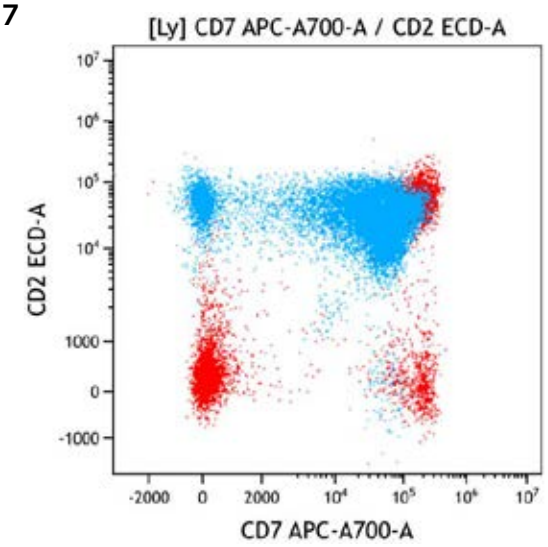

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

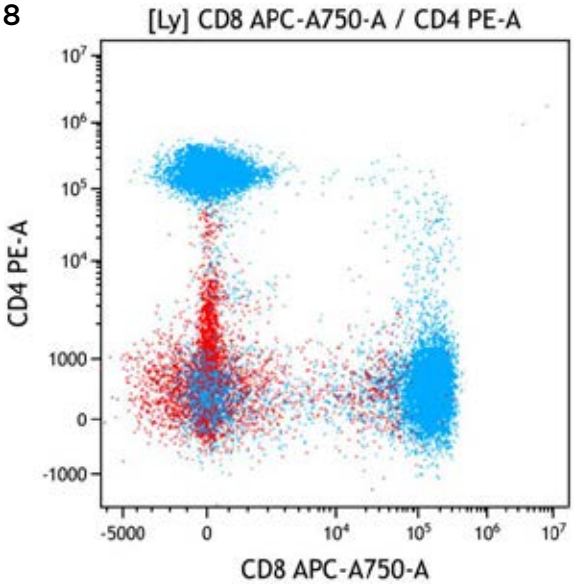

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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).

T19

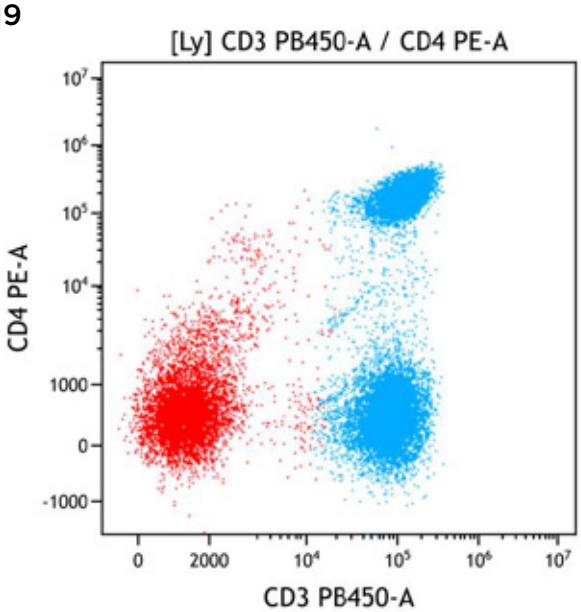

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells (red) lack expression of both CD3 and CD4.

T20

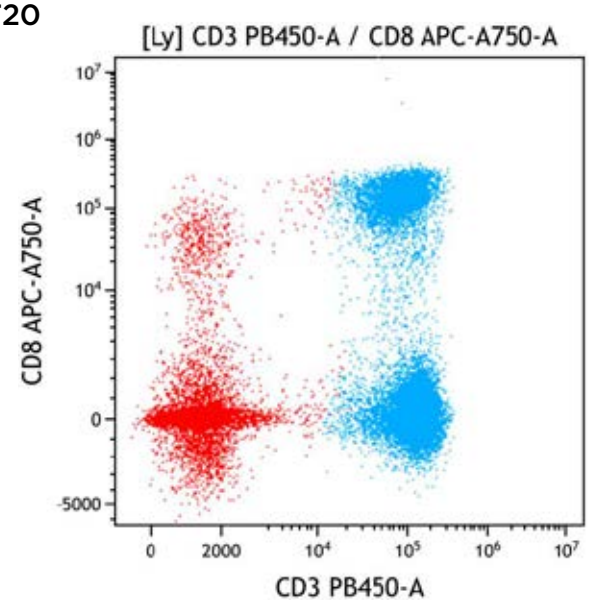

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

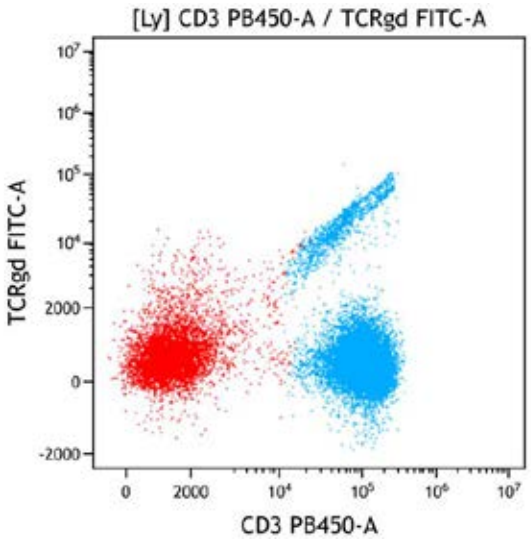

Figure 20. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/ delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

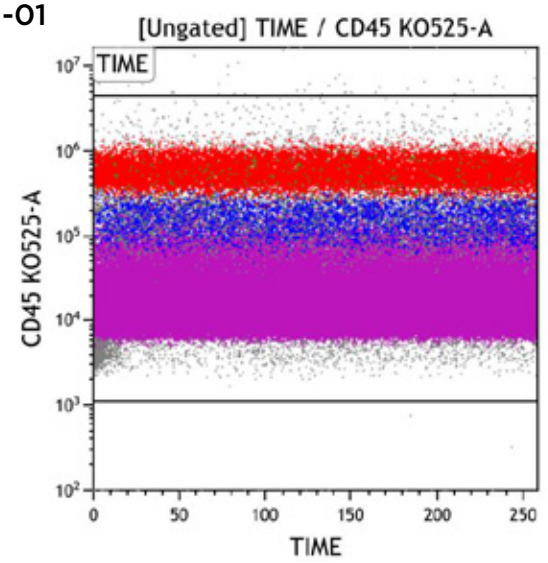

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

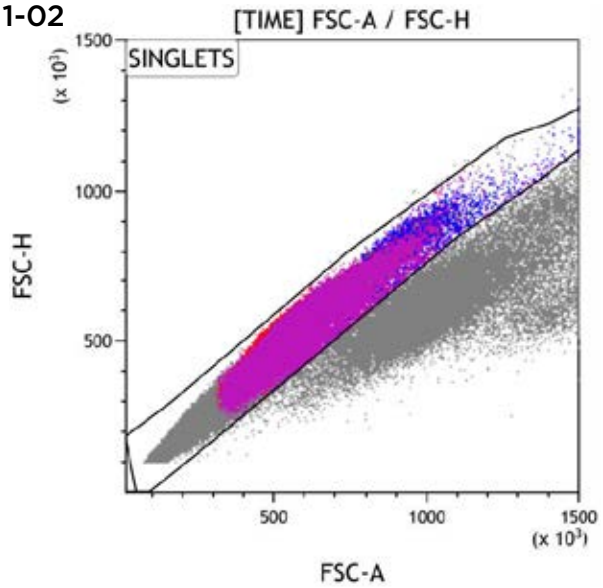

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

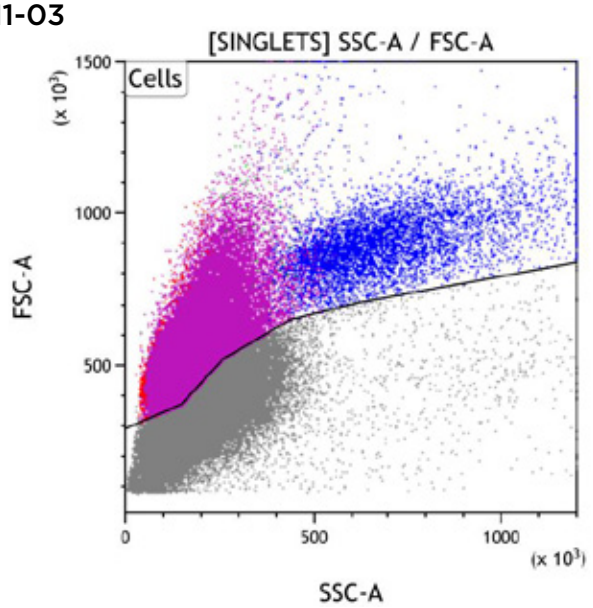

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necroticN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

M1-04

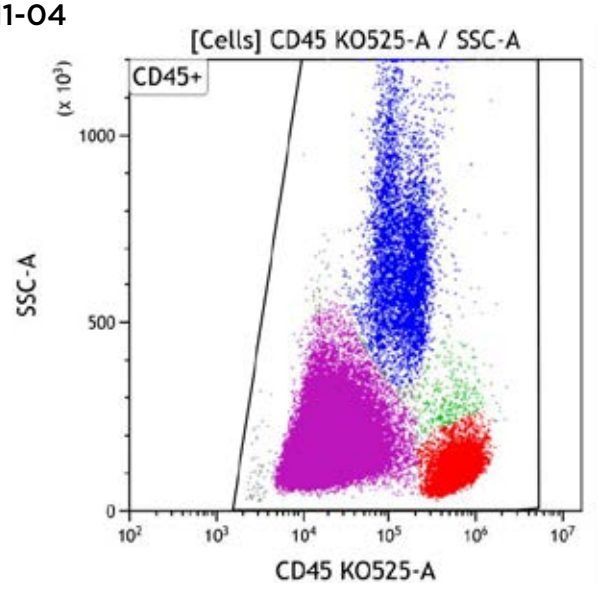

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

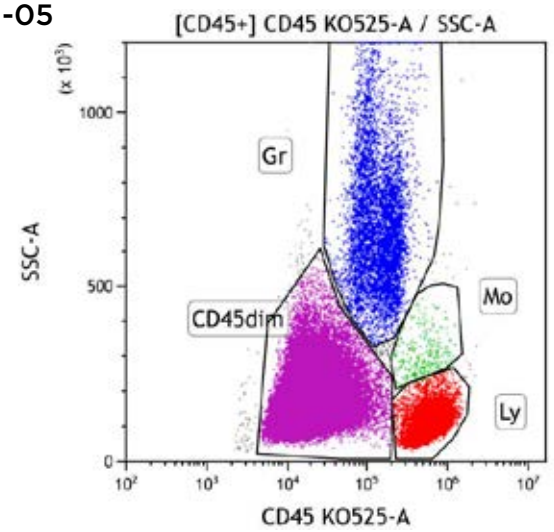

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 in the CD45dim gate (purple) is expanded.

M1-06

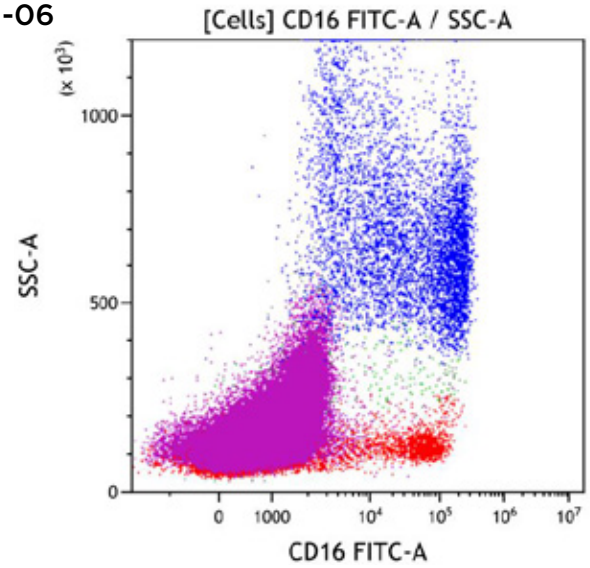

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red). The aberrant population (purple) is negative for CD16.

M1-07

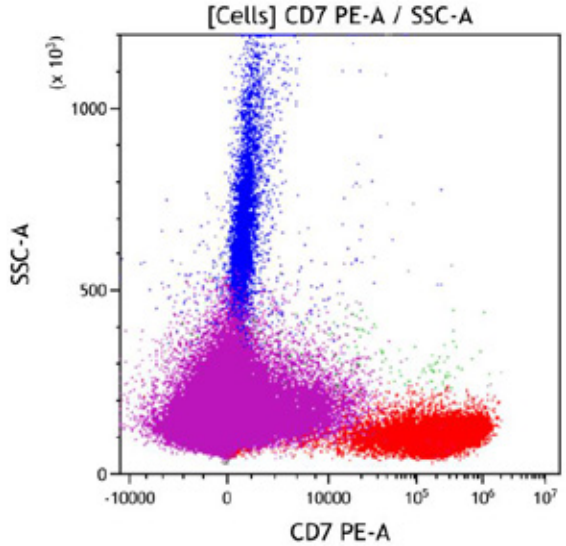

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed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

M1-08

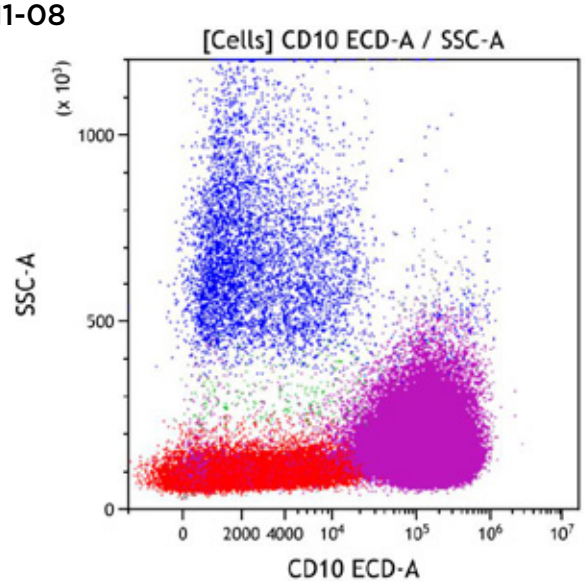

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

BACK TO CASE OVERVIEW

M1-09

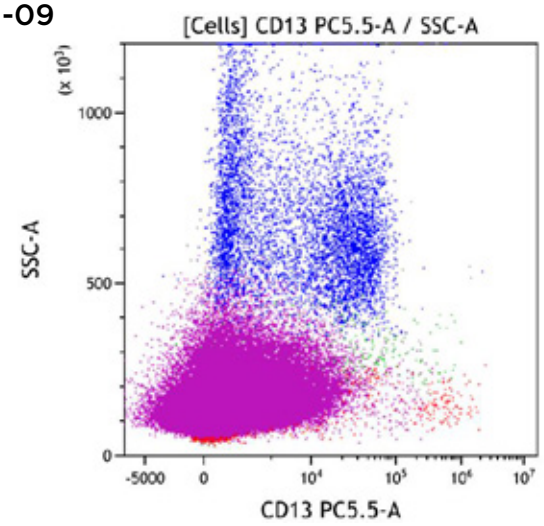

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue) with variable intensity dependent on maturation stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature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

M1-10

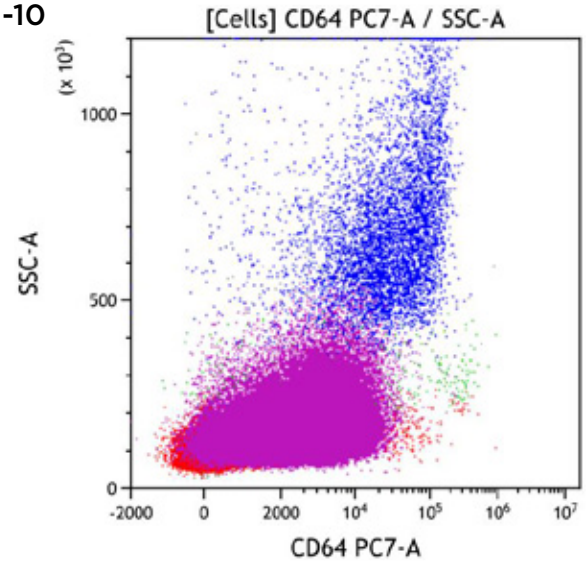

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

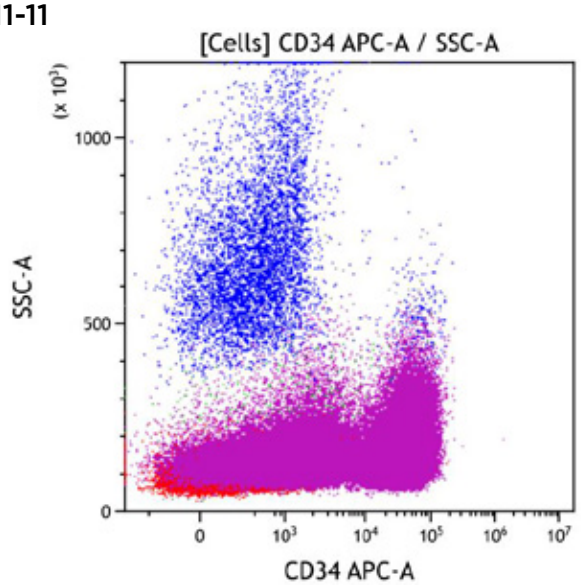

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) expresses CD34 on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

M1-12

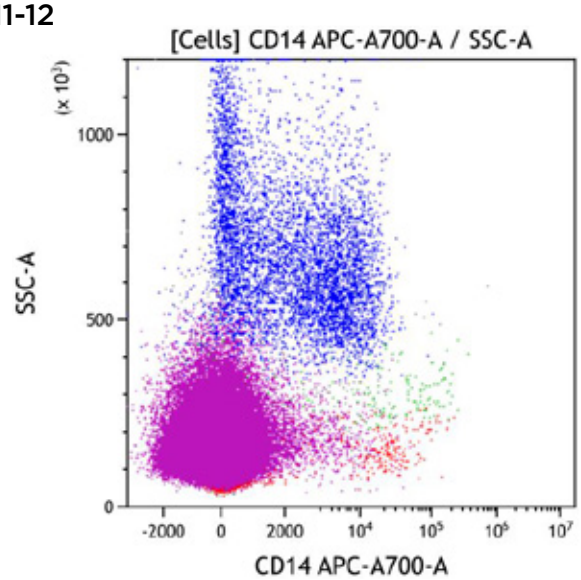

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes (blue) at a low level. The aberrant population (purple) is negative for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

BACK TO CASE OVERVIEW

M1-13

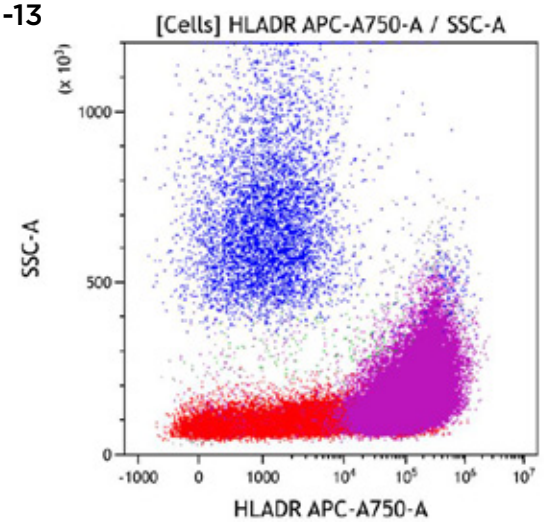

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 progenitors, immature and mature B cells (red), and activated T cells (red). The aberrant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

M1-14

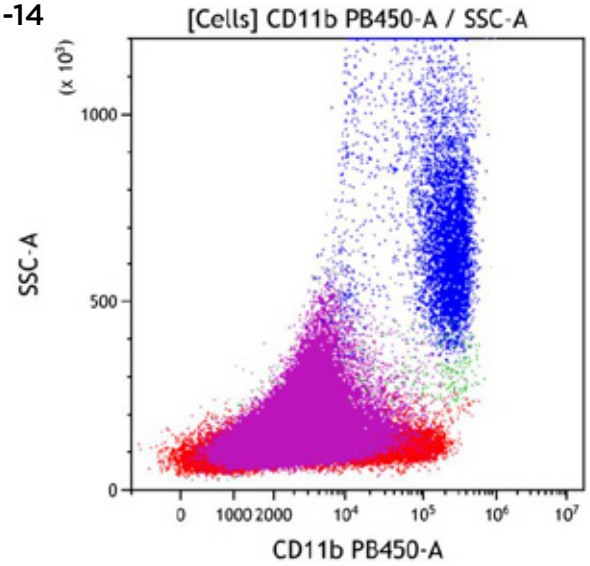

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes (blue), monocytes, basophils, and NK cells (red). The aberrant population (purple) is negative for CD11b.

M1-15

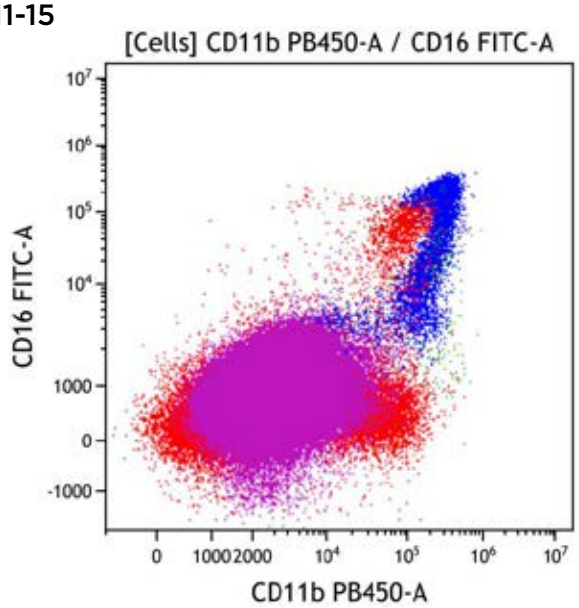

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes (blue), and a subset of NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower middle). 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. The aberrant population (purple) is negative for CD11b and CD16.

M1-16

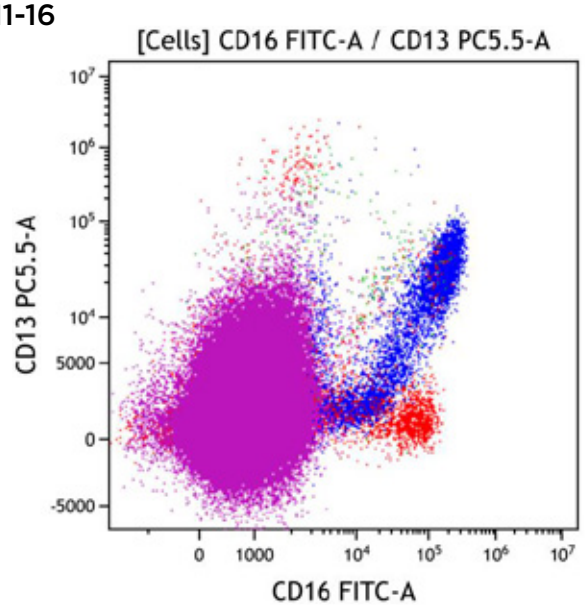

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, 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). The aberrant population (purple) expresses dim CD13 and is negative for CD16.

BACK TO CASE OVERVIEW

M1-17

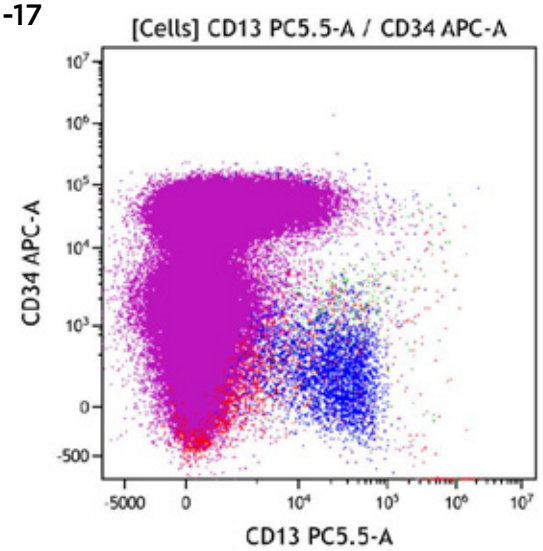

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphocytes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

M1-18

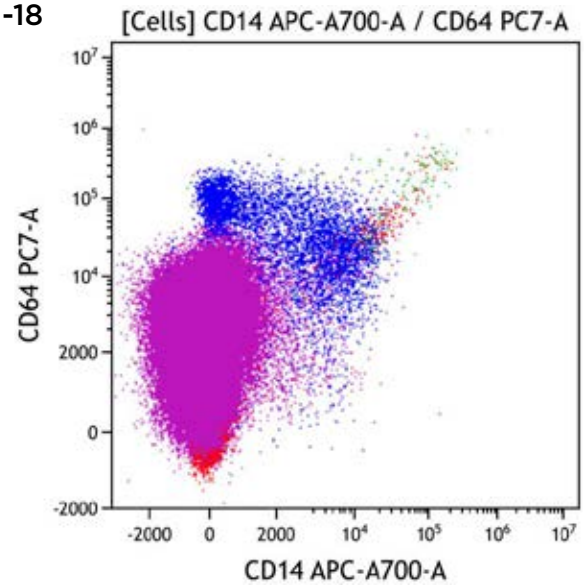

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left). The aberrant population (purple) is negative for CD14 and CD64.

M1-19

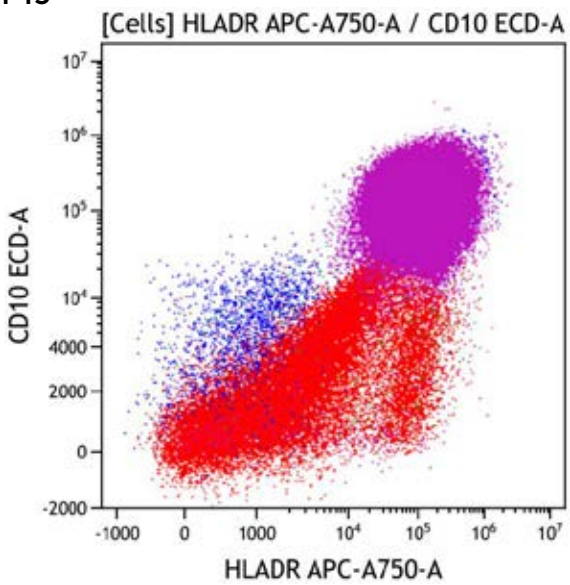

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on mature granulocytes (blue) and immature B cells. The aberrant population (purple) expresses uniform CD10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

M1-20

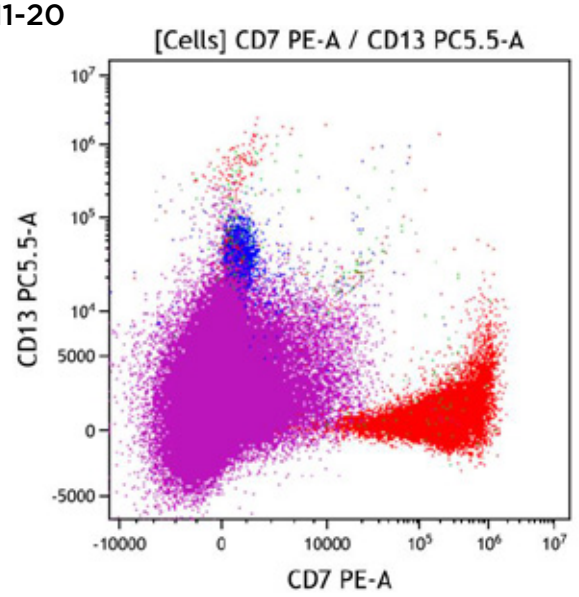

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The aberrant population (purple) expresses dim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

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

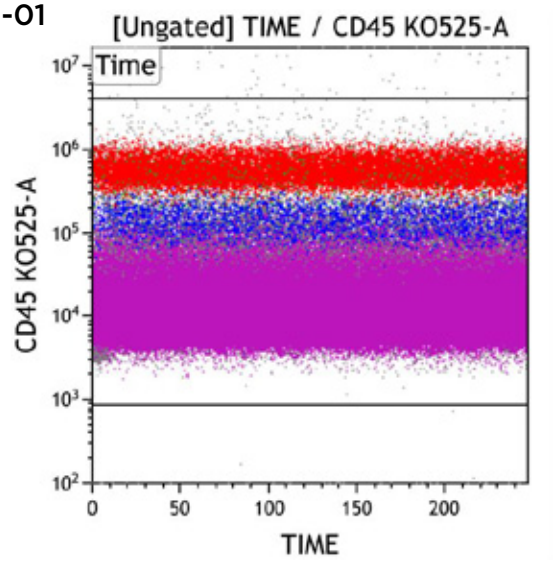

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

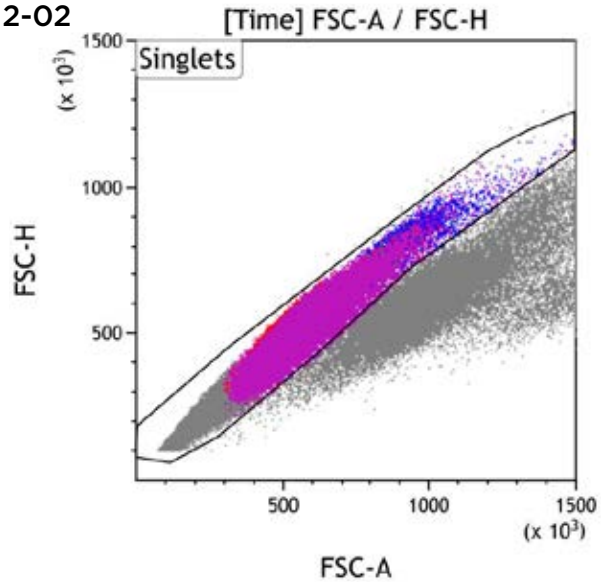

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

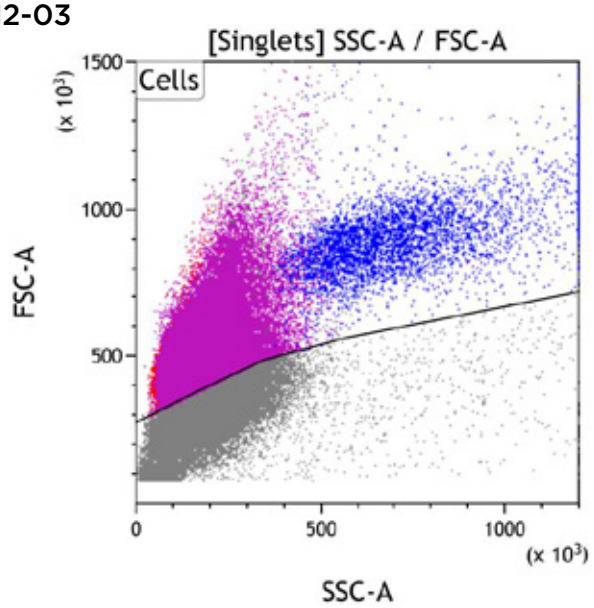

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M2-04

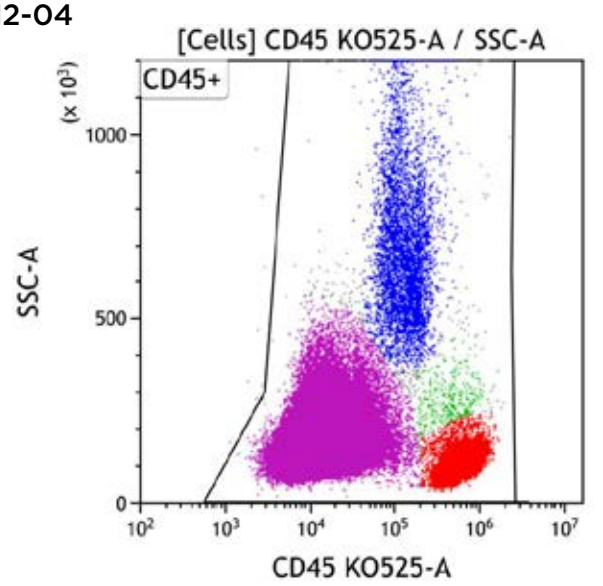

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

BACK TO CASE OVERVIEW

M2-05

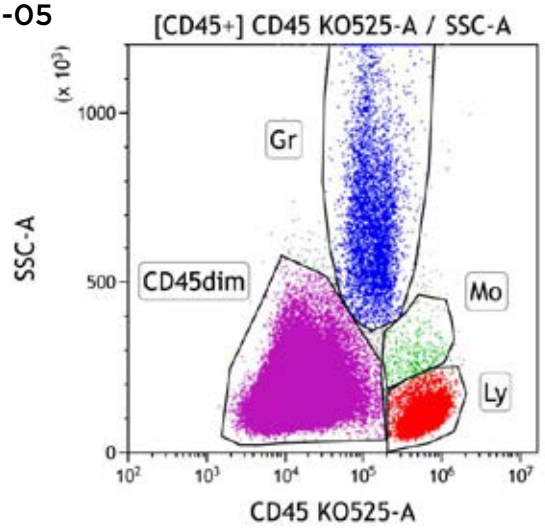

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 in the CD45dim gate (purple) is expanded.

M2-06

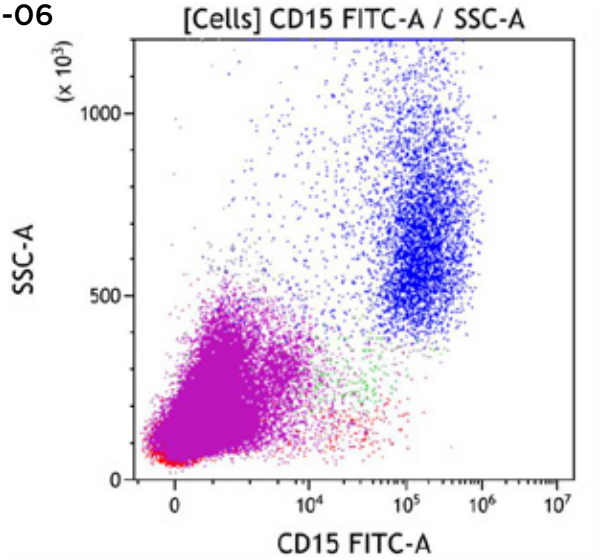

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes. The aberrant population (purple) is negative for CD15.

M2-07

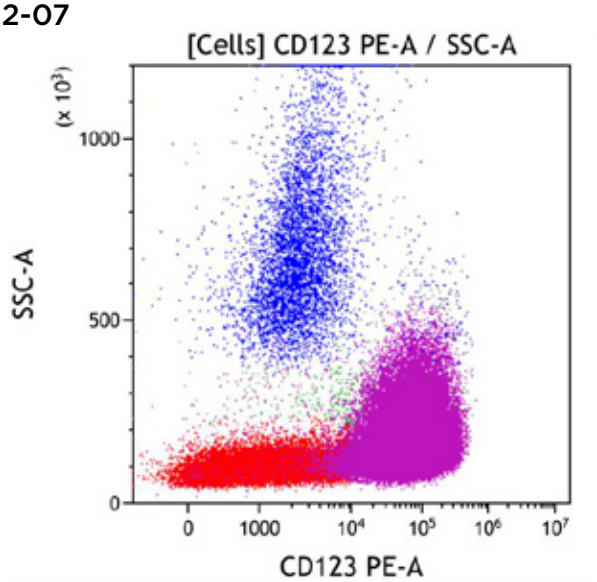

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. The aberrant population (purple) expresses CD123.

M2-08

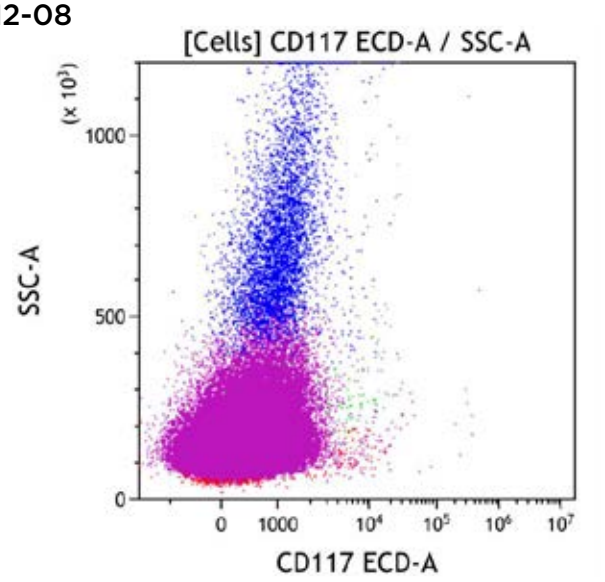

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

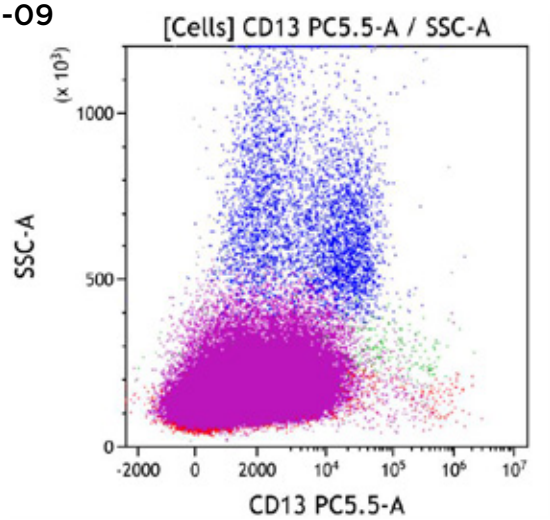

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue) with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature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

M2-10

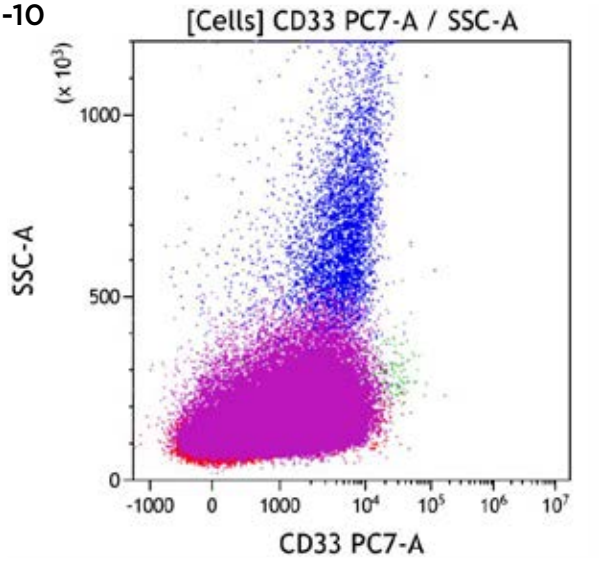

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on immature and mature monocytes, at a slightly low level on immature granulocytes (blue), and at the lowest level on matureB granulocytes (blue). CD33 is also expressed on basophils, a subset of NK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

M2-11

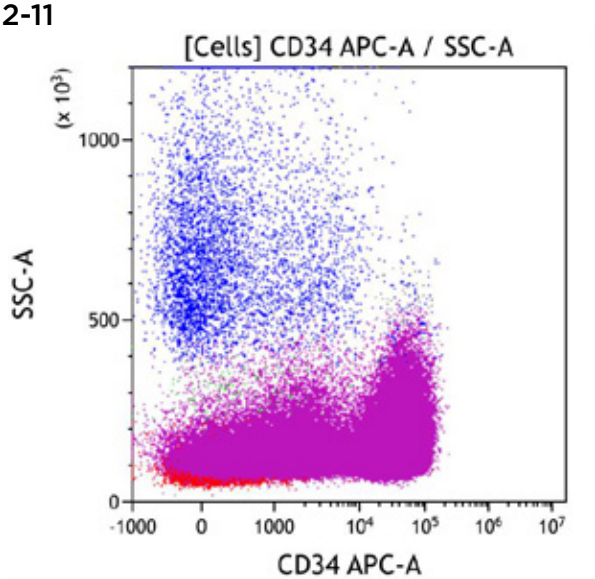

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45dim gate. The aberrant population (purple) expresses CD34 on a subset.

M2-12

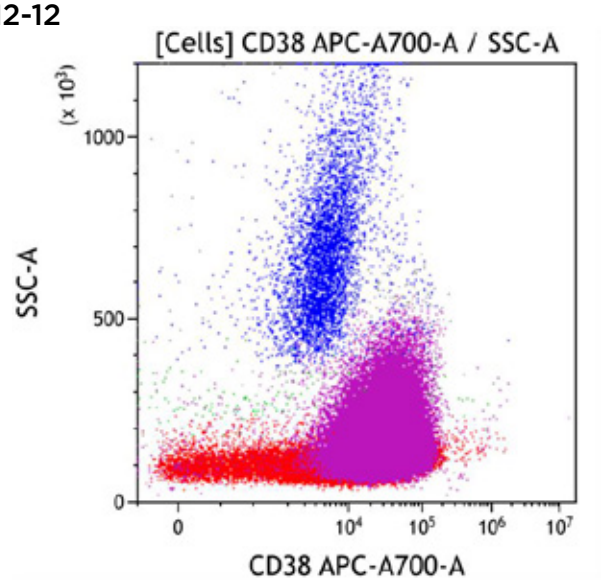

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and at a low level on monocytes, and at a variably low level on activated mature lymphocytes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

BACK TO CASE OVERVIEW

M2-13

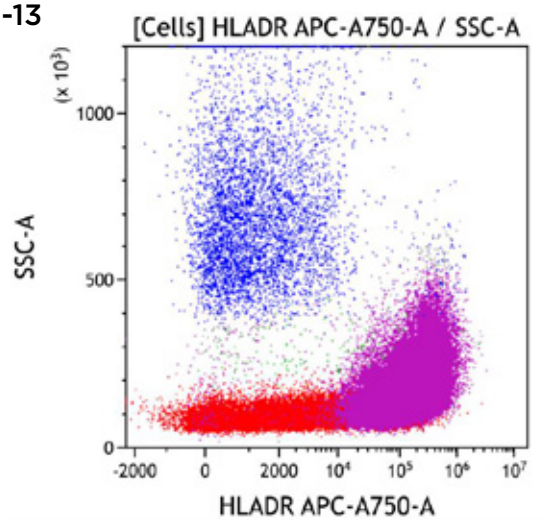

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), immature and mature B cells (red), and activated T cells (red). The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

M2-14

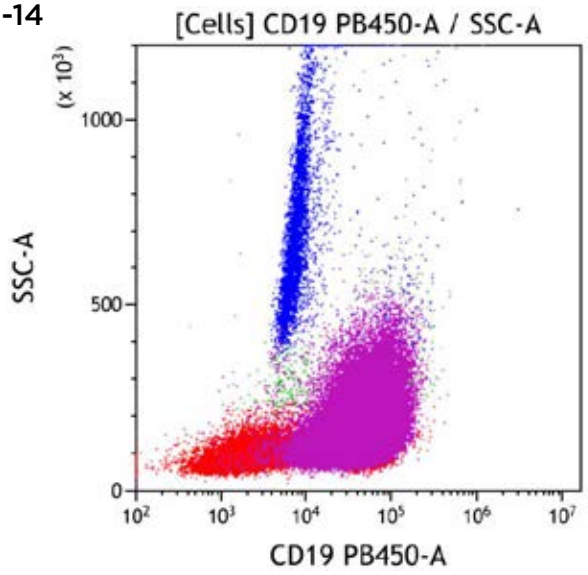

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red), as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) expresses CD19.

M2-15

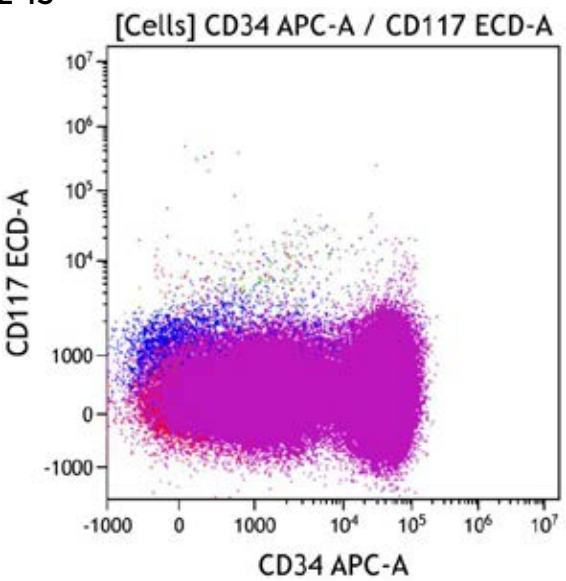

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The aberrant population (purple) expresses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

M2-16

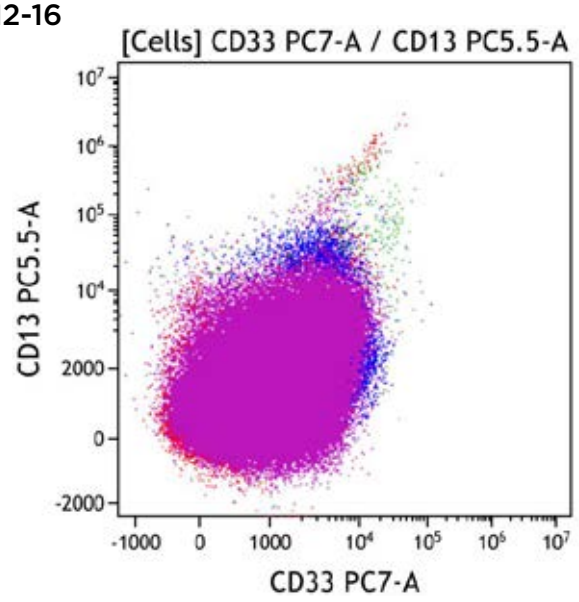

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes largely do not express either CD13 or CD33 (red). The aberrant population (purple) expresses dim CD13 and dim CD33.

BACK TO CASE OVERVIEW

M2-17

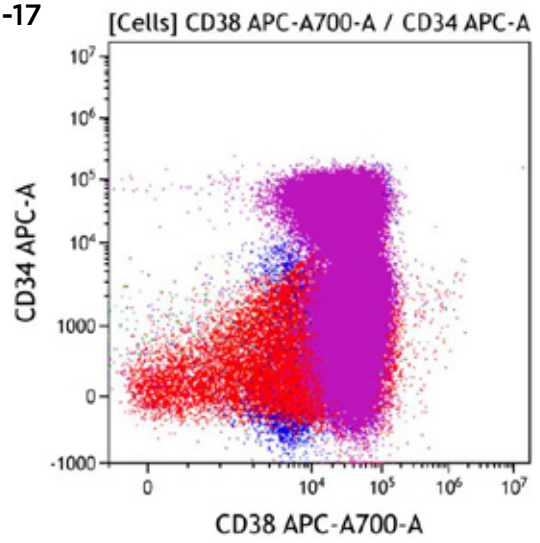

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. The aberrant population (purple) expresses CD34 on a subset and uniformN CD38.

M2-18

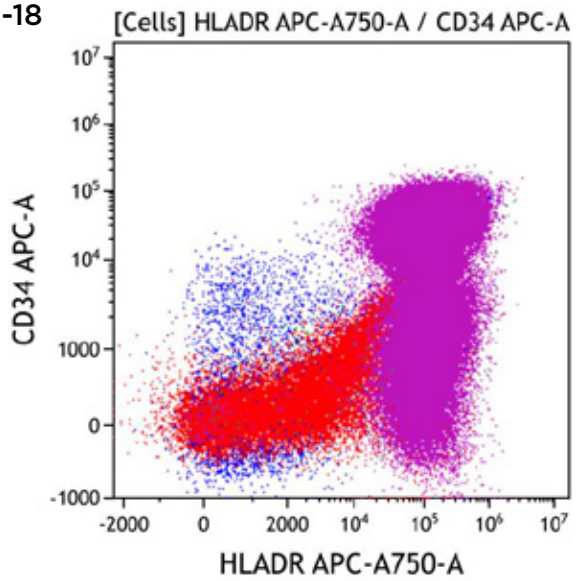

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. The aberrant population (purple) expresses CD34 on a subset and HLA-DR.

M2-19

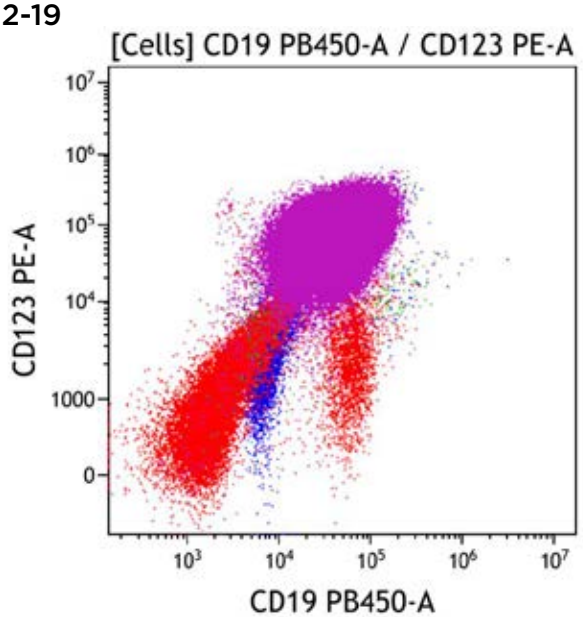

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes, and CD34 positive progenitors. CD19 positive B cells (red) normally do not express significant CD123. The aberrant population (purple)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

M2-20

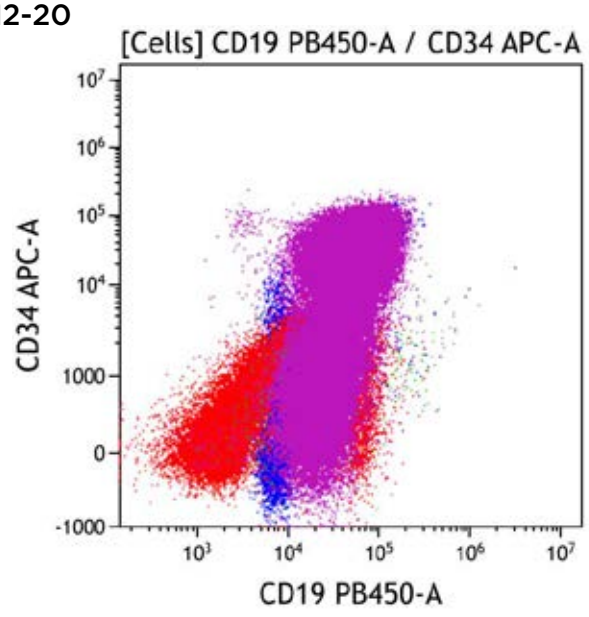

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red) do not express CD34. The aberrant population (purple) express CD34 on a subset and CD19.

BACK TO CASE OVERVIEW

M2-21

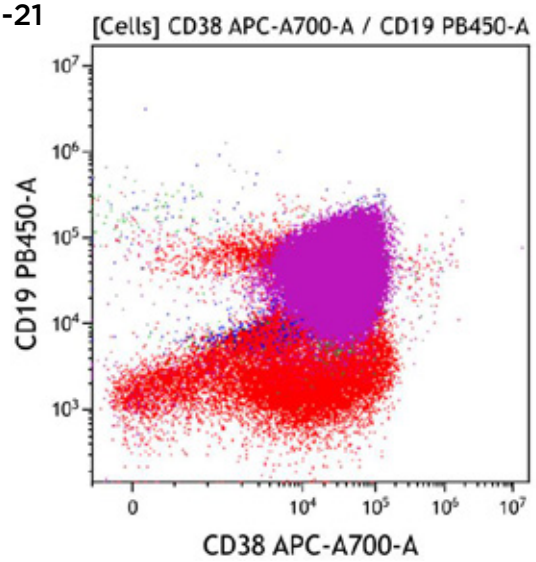

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show low level expression of CD38 (red). The aberrant population (purple) expresses CD19 and CD38.

M2-22

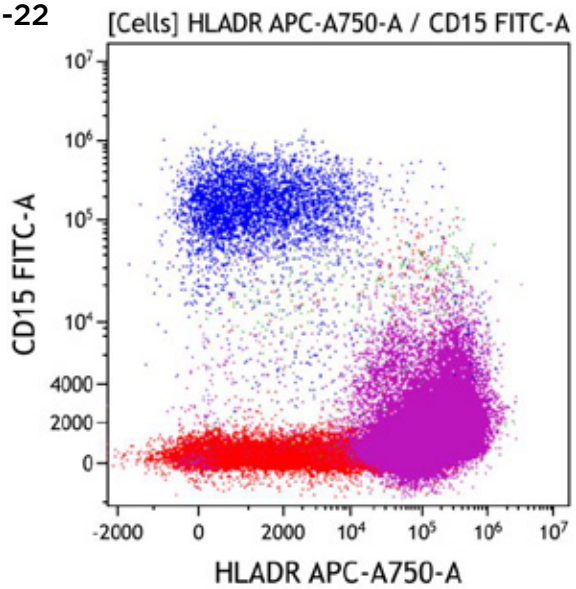

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR. The aberrant population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

M2-23

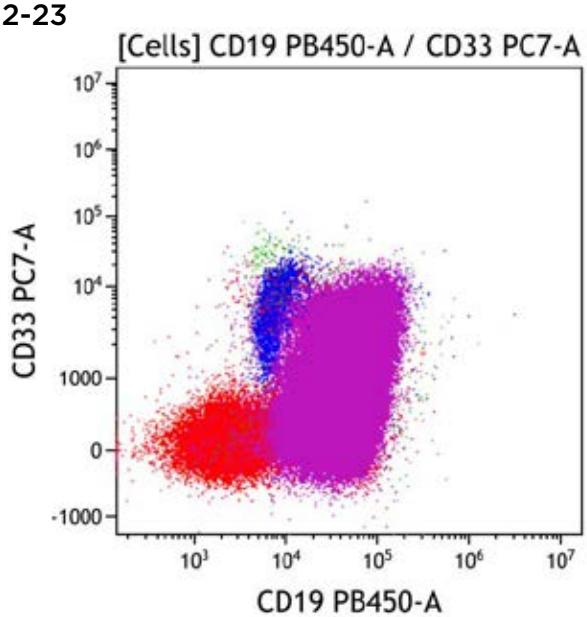

Figure 24. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes and granulocytes (blue). Mature CD19 positive B cells normally do not express significant CD33. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (purple) expresses CD19 and dim CD33.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 4 LHS-TC4-078**
Diagnosis	**Plasma cell neoplasm**
Clinical Vignette	This 64-year-old female presents with anemia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with increased forward and side scatter, absent CD19, bright CD38, slightly decreased CD45, CD56, CD200, and surface kappa light chain without CD5, CD10, surface lambda light chain, or other T cell or myeloid markers. The immunophenotype of the aberrant population is consistent with a plasma cell neoplasm and could be consistent with monoclonal gammopathy, plasmacytoma or plasma cell myeloma. Compared with normal plasma cells, the absence of CD19, CD56 expression, and surface kappa light chain restriction are aberrant. Correlation with clinical, morphologic and laboratory data is required for definitive subclassification of this plasma cell neoplasm.

BACK TO CASE LISTINGS

B Cell Tube

B01

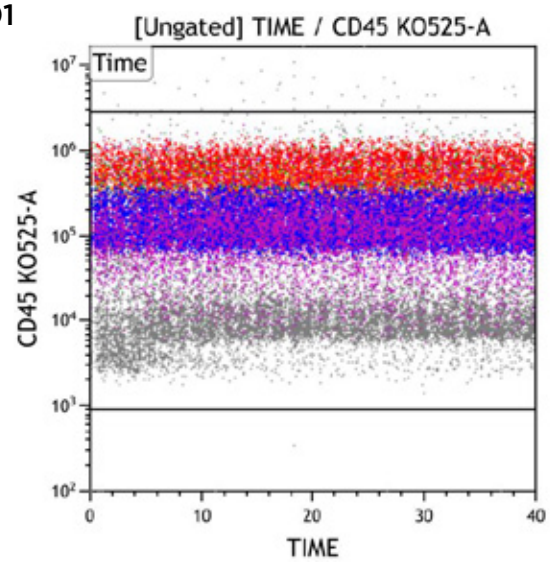

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

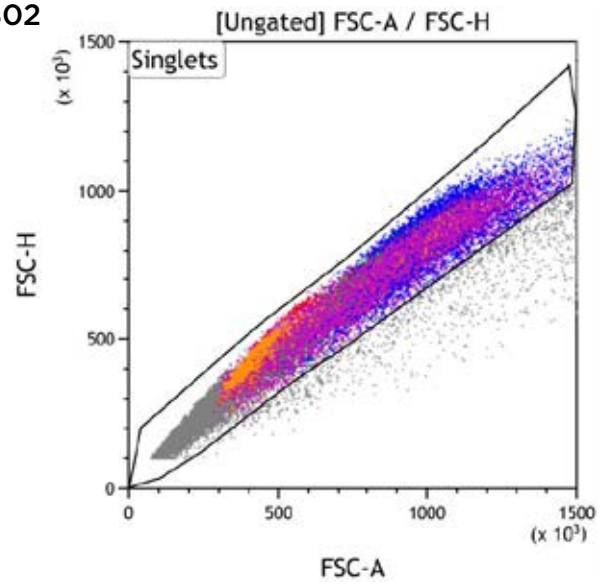

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

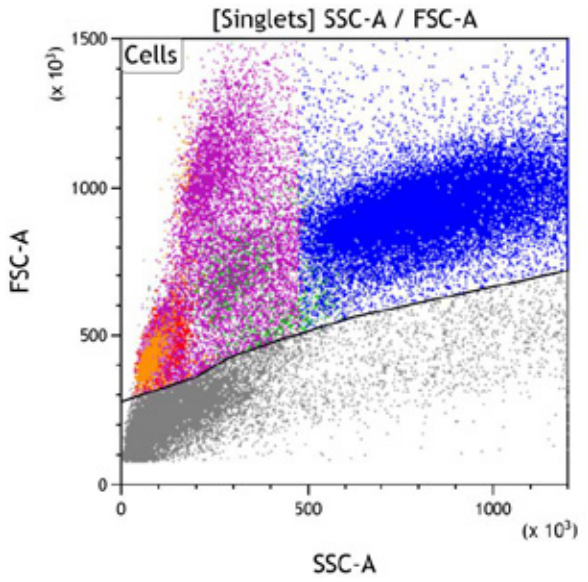

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

B04

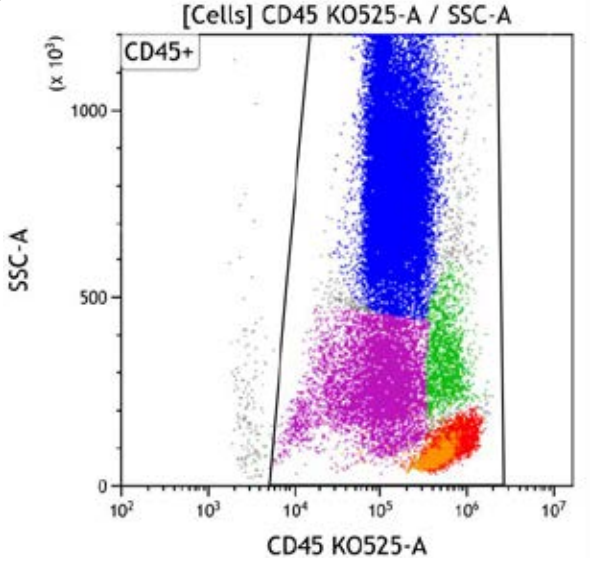

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

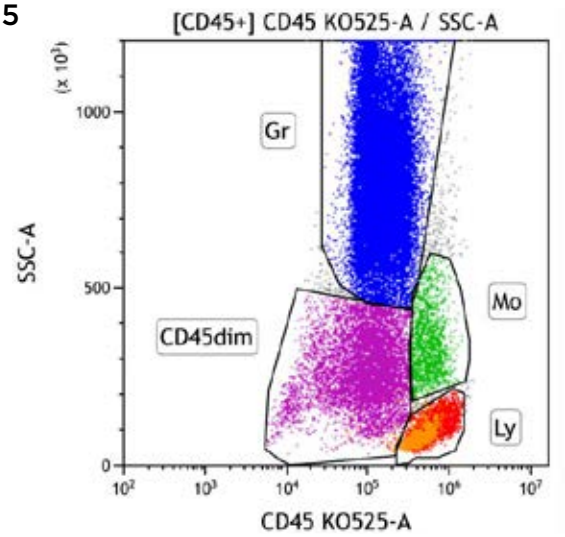

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 followed throughout the analysis. The aberrant population (purple) has slightly decreased expression of CD45.

B06

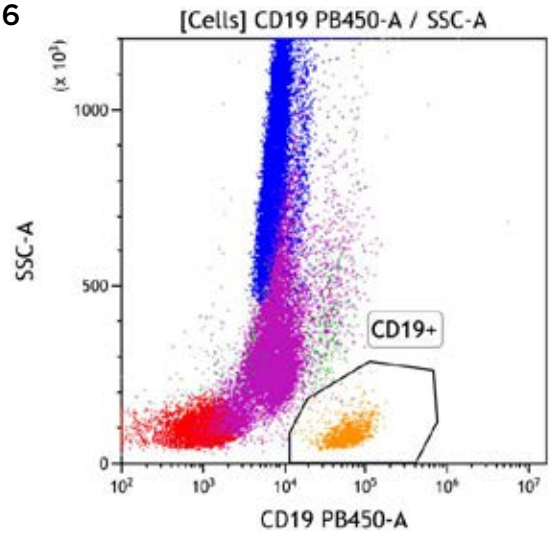

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple)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

B07

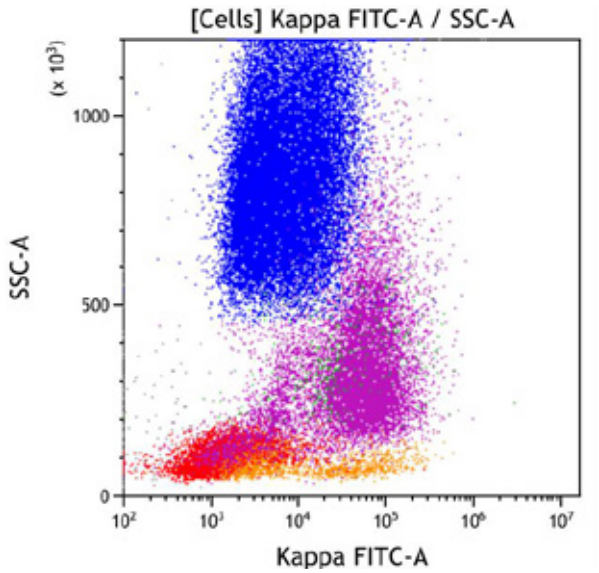

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The Kappa light chain positive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

B08

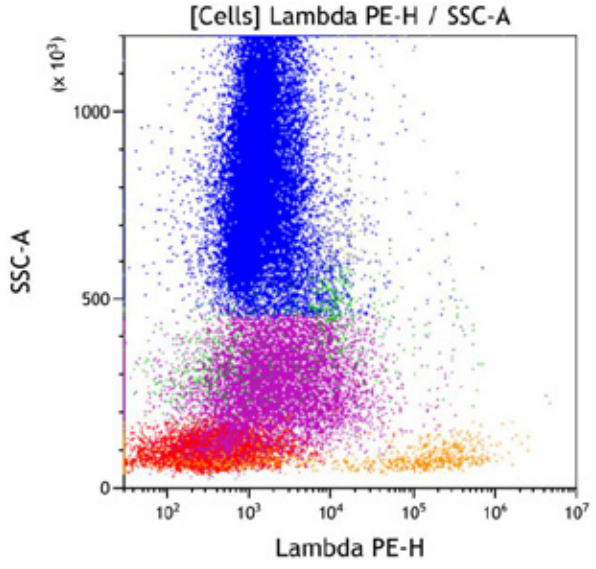

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The lambda light chain positive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

BACK TO CASE OVERVIEW

B09

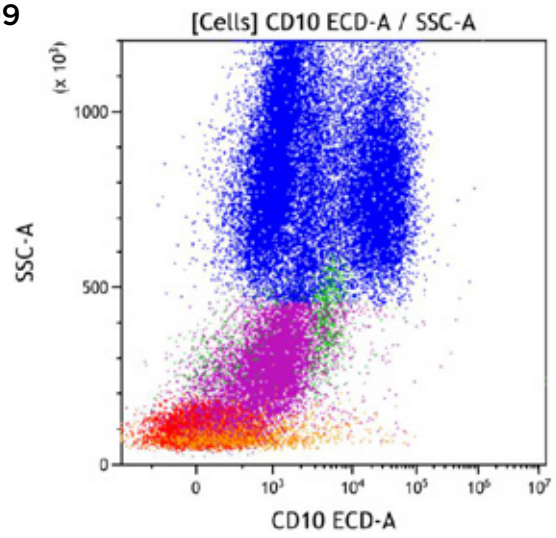

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

B10

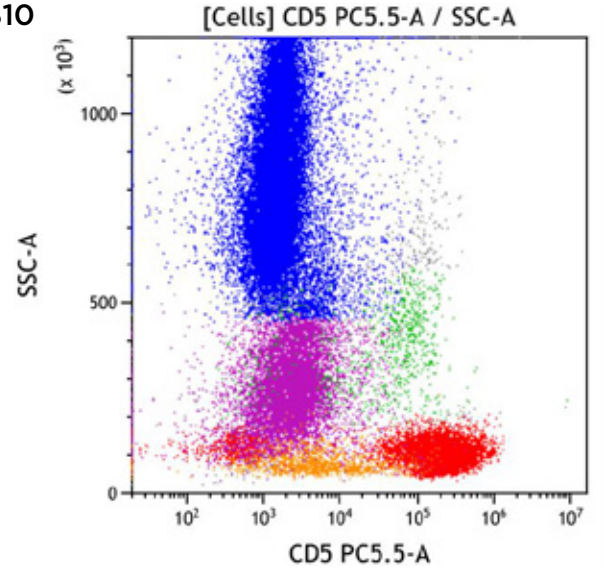

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The aberrant population (purple) is negative for CD5.

B11

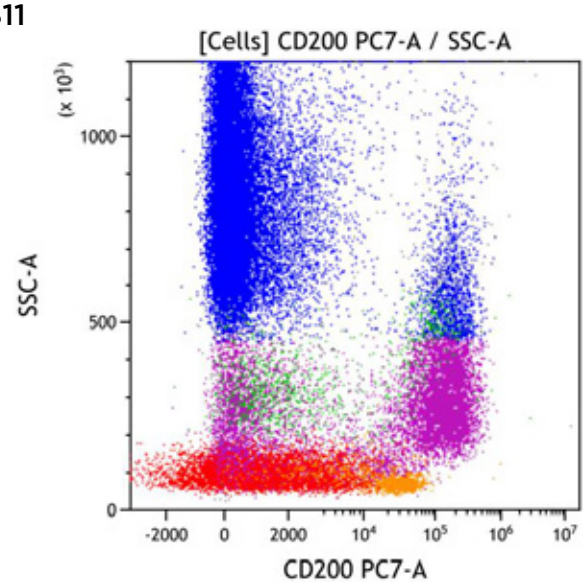

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

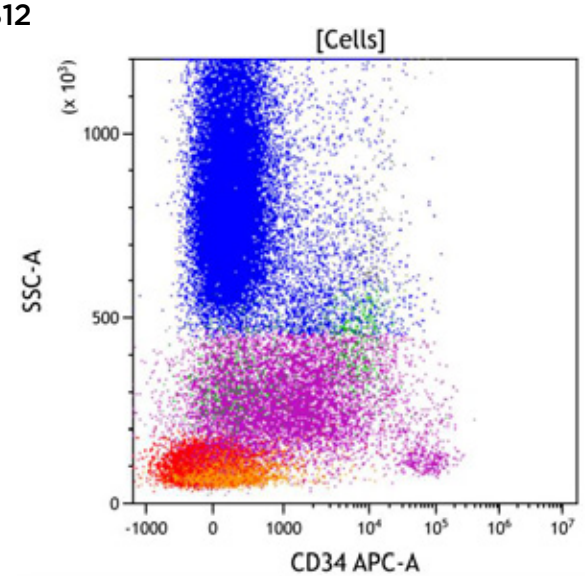

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

B13

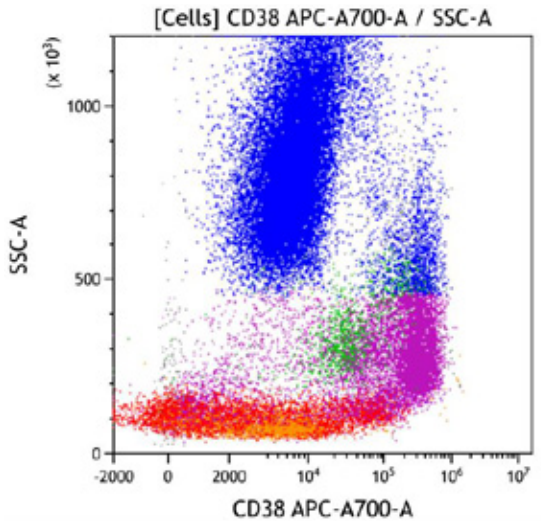

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably level on activated mature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

B14

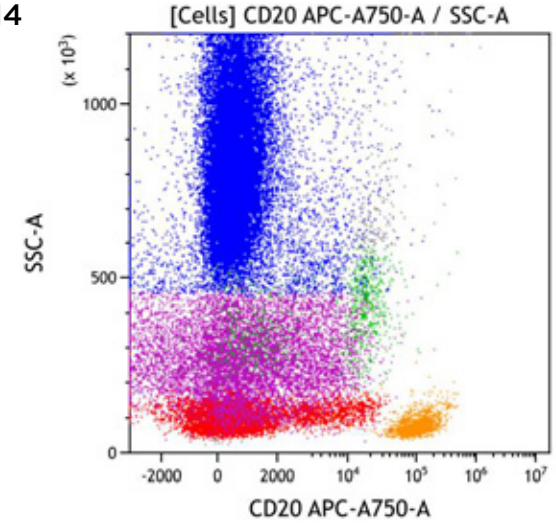

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side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

B15

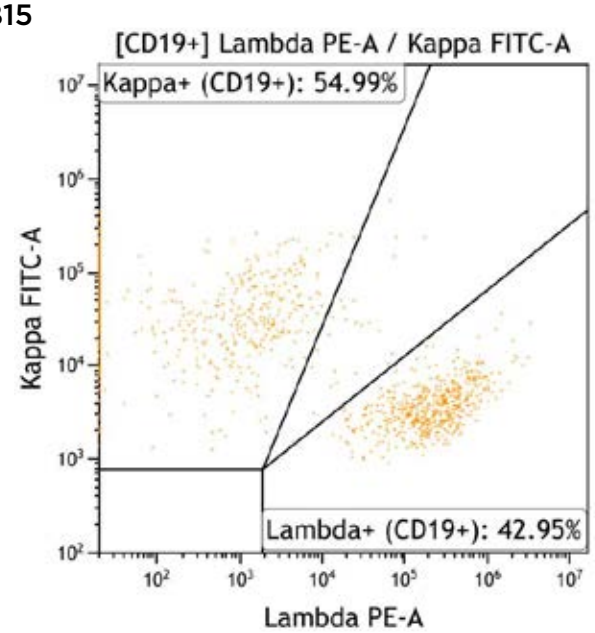

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

B16

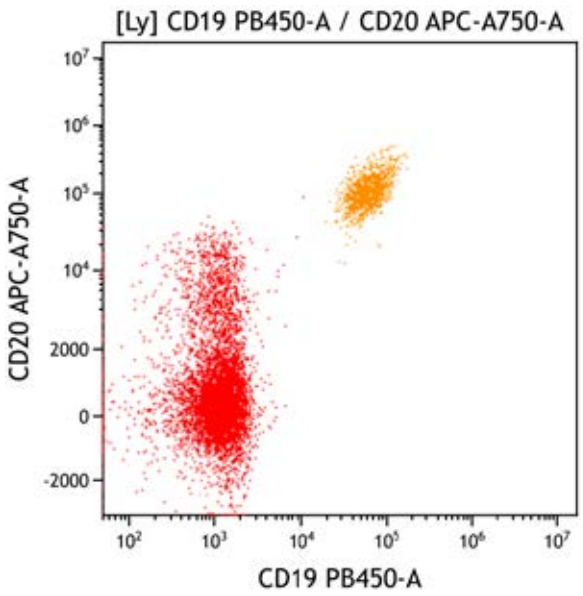

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

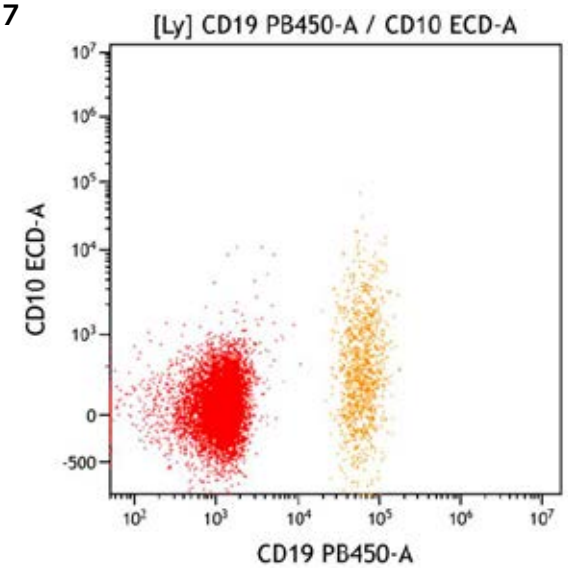

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow aspirates.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

B18

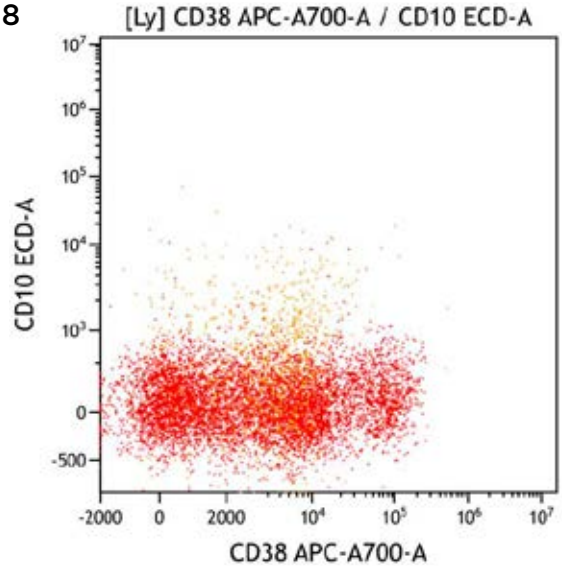

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low level expression of CD38. T cells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

B19

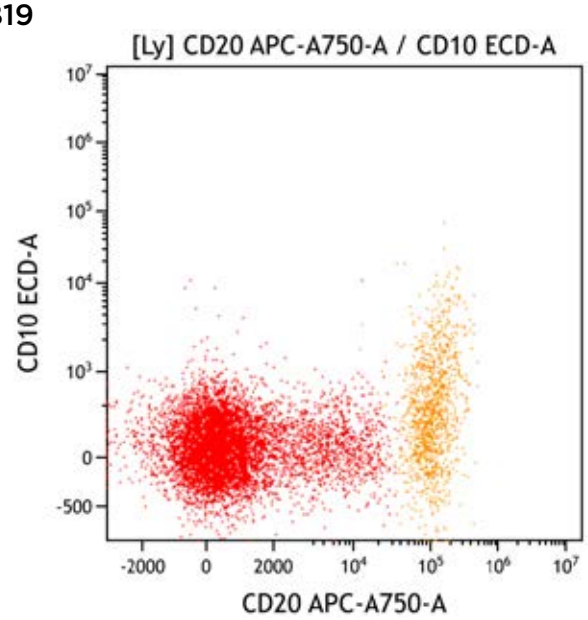

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20 without significant CD10. The few CD10 positive B cells represent late-stage immature B cells.

B20

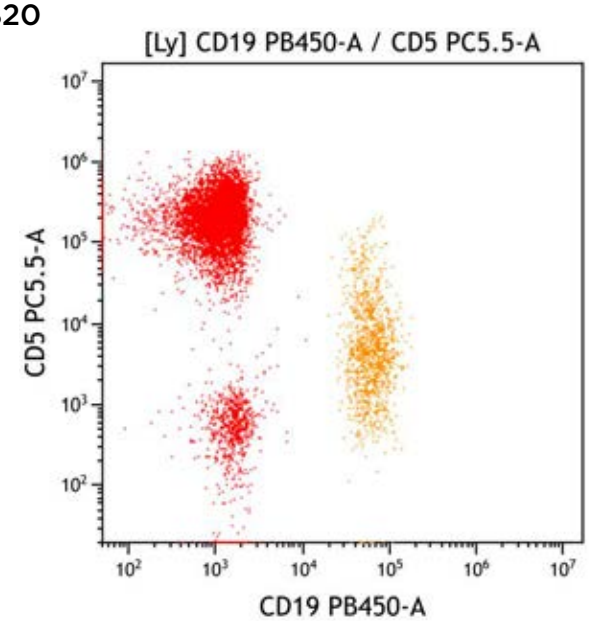

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

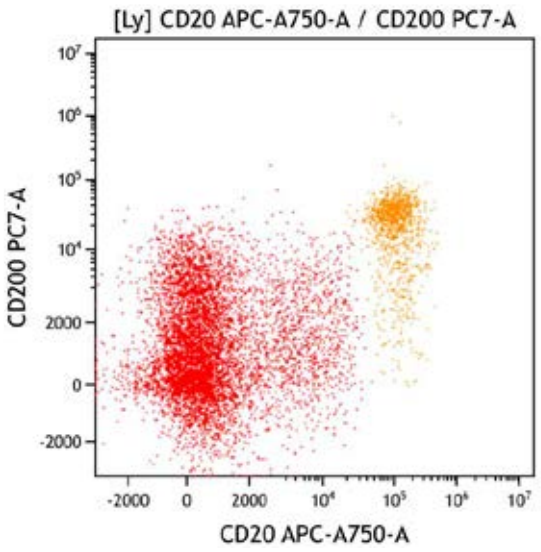

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

B22

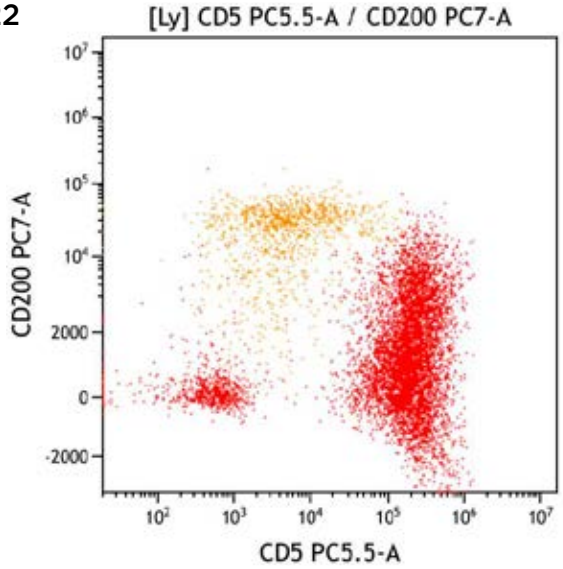

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell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

BACK TO CASE OVERVIEW

T Cell Tube

T1

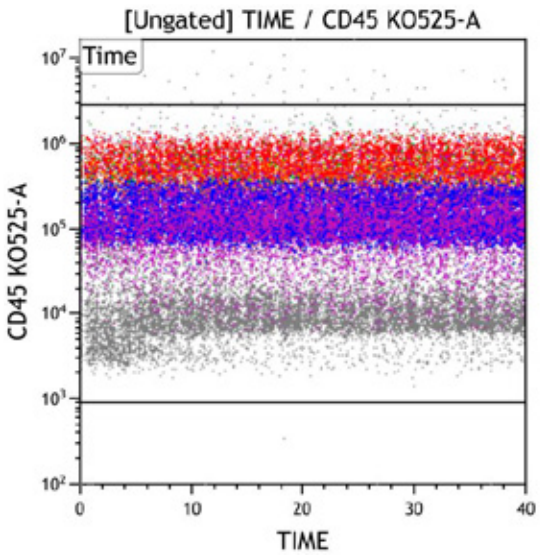

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T2

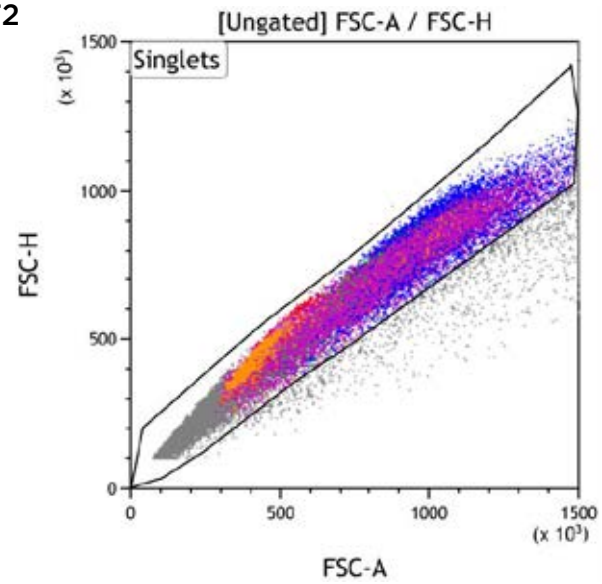

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T3

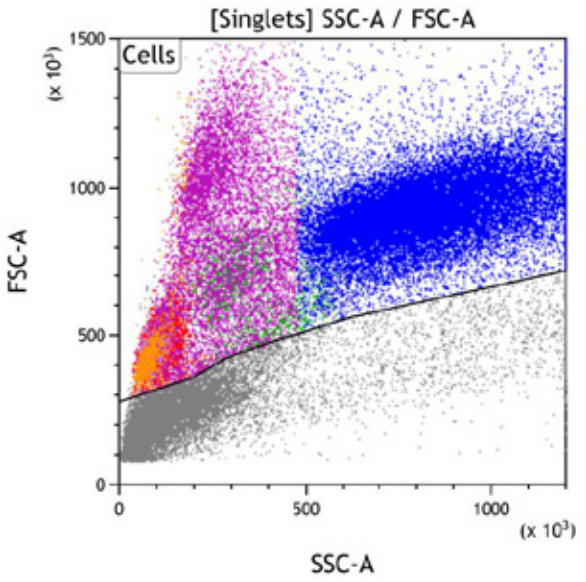

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

T4

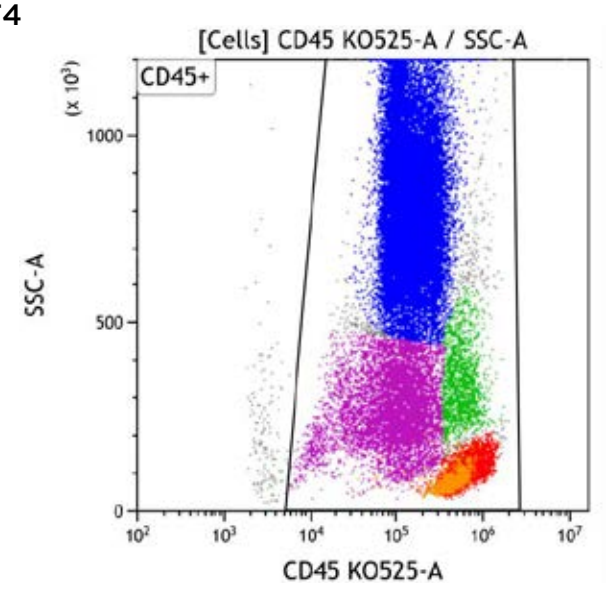

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T5

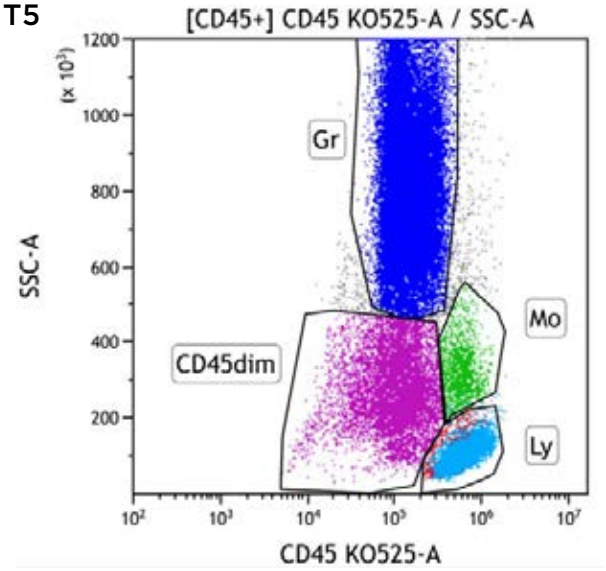

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 slightly decreased expression of CD45.

T6

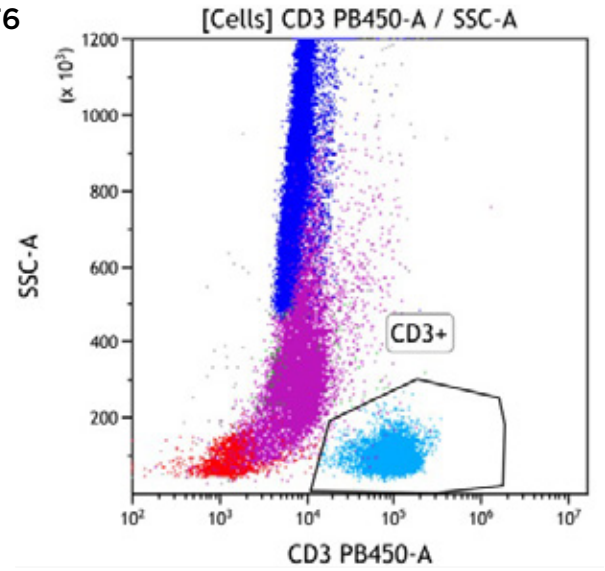

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T7

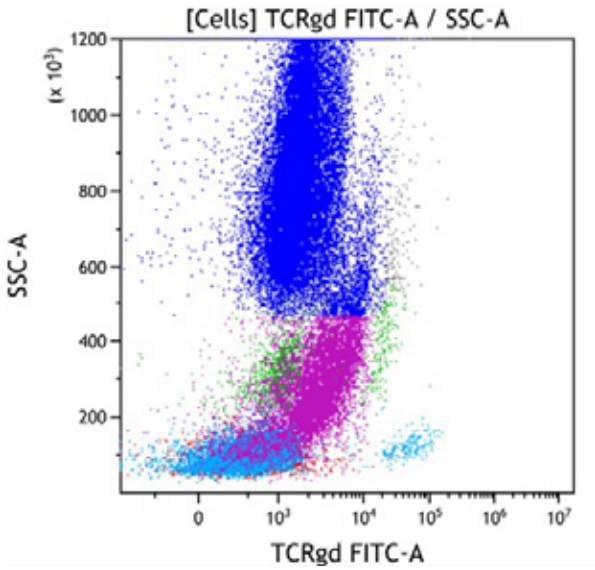

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). The aberrant population (purple) is negative for TCRγδ.

T8

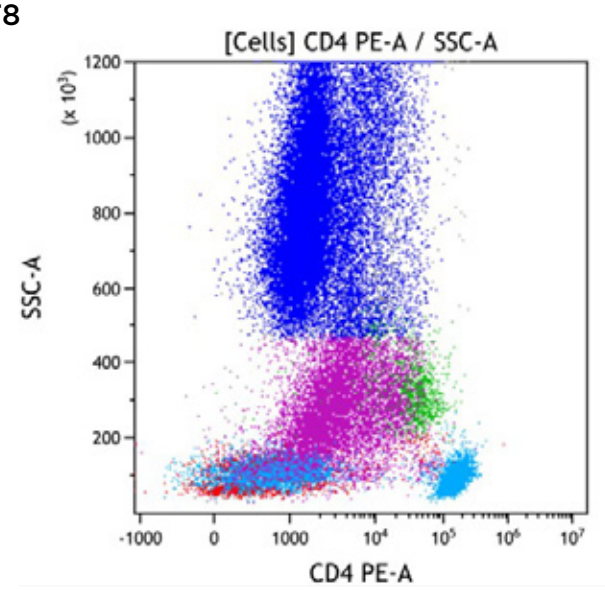

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T9

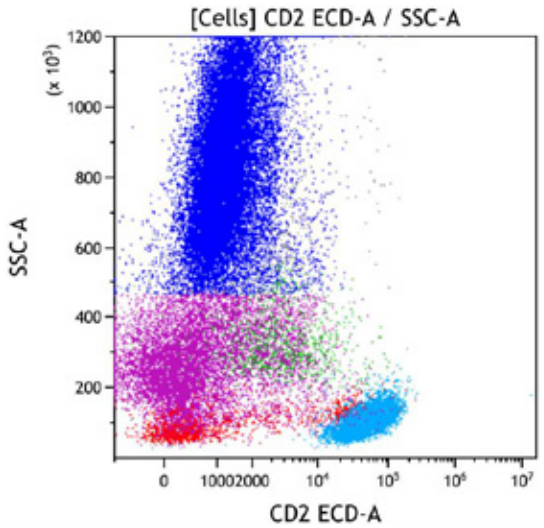

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red, lower middle) and at a low level on monocytes (green). The aberrant population (purple) is negative for CD2.

T10

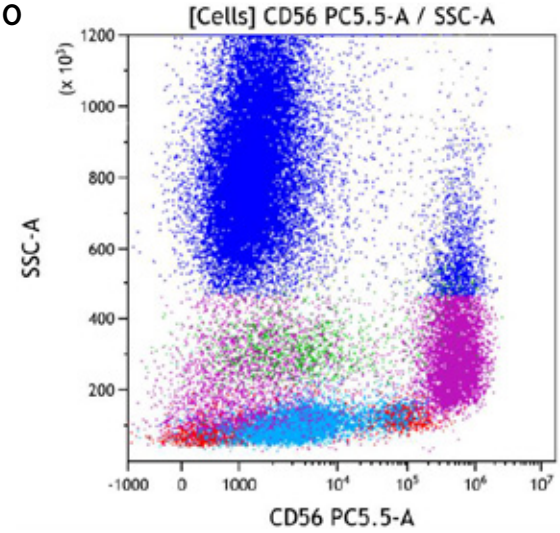

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytes (green) in both reactive and neoplastic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

T11

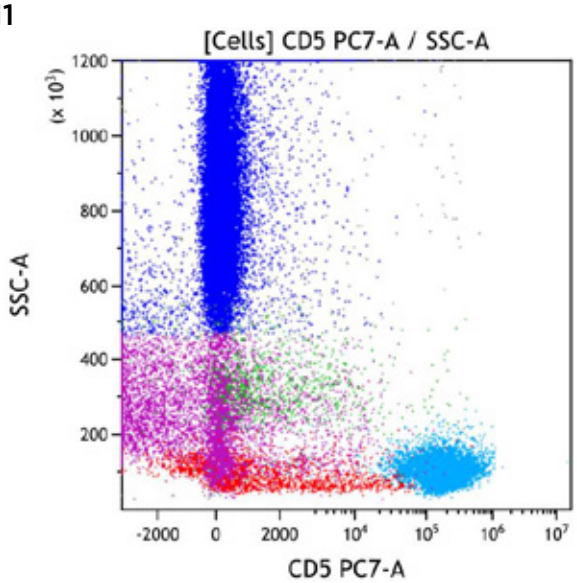

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

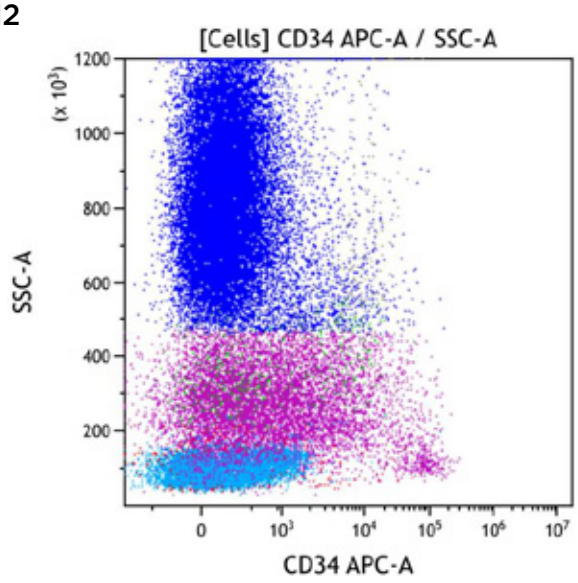

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and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

BACK TO CASE OVERVIEW

T13

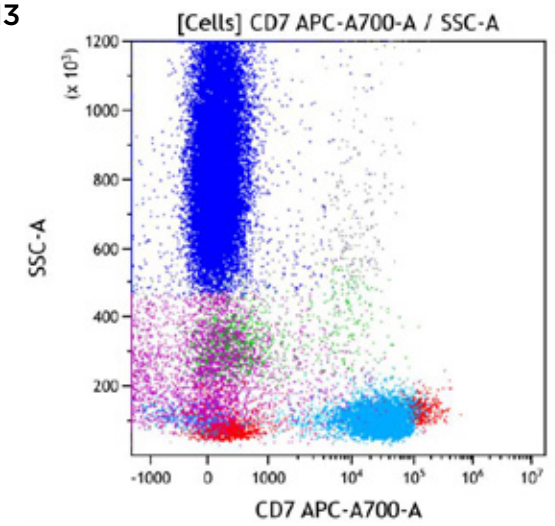

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

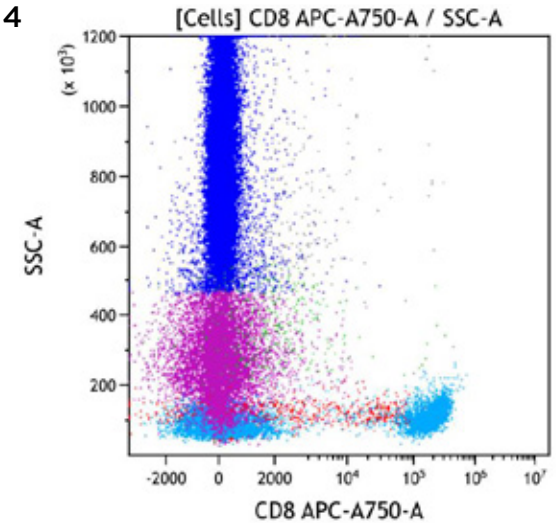

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells. The aberrant population (purple) is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

T15

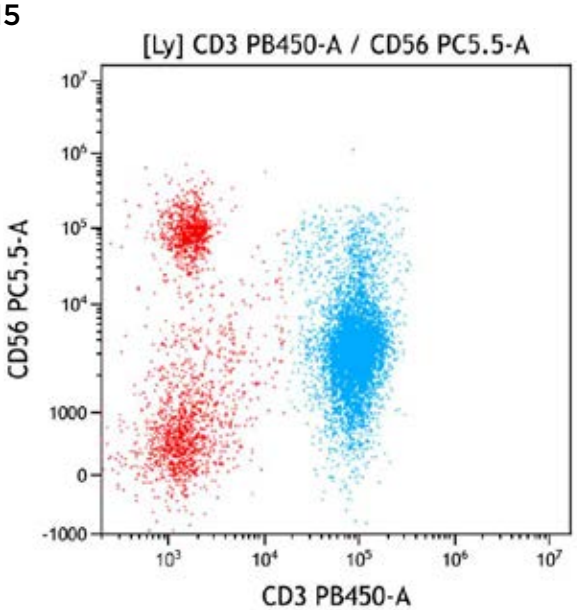

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

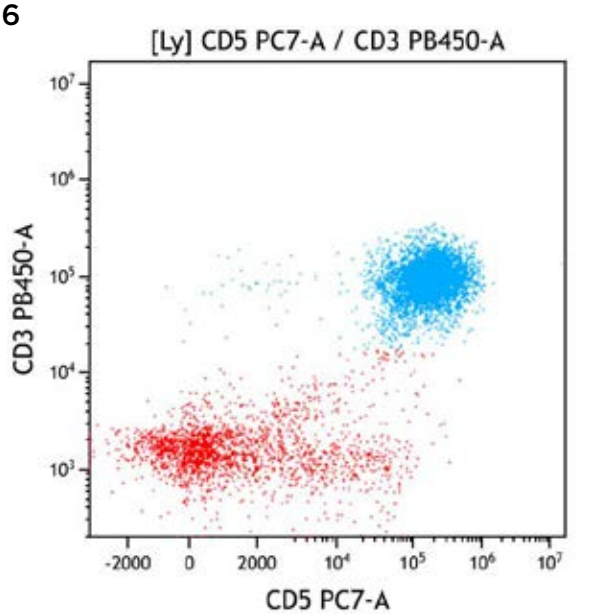

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

BACK TO CASE OVERVIEW

T17

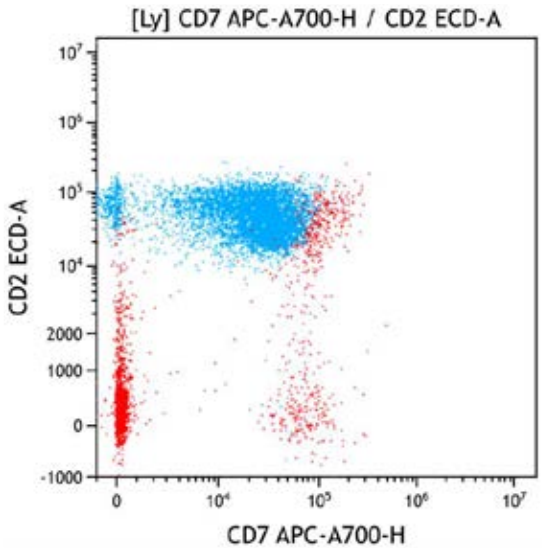

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

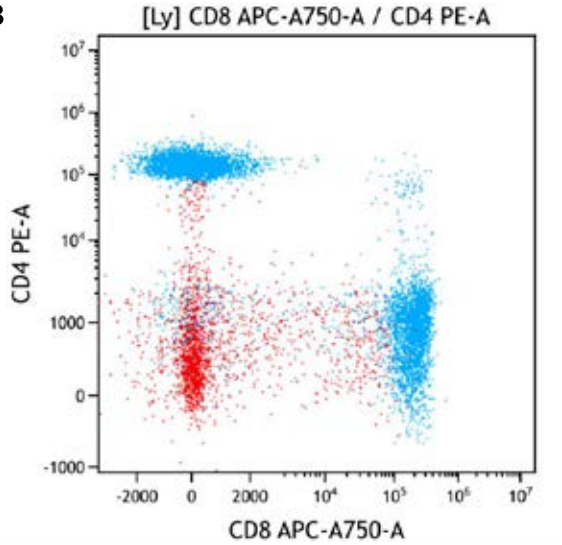

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets.CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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).

T19

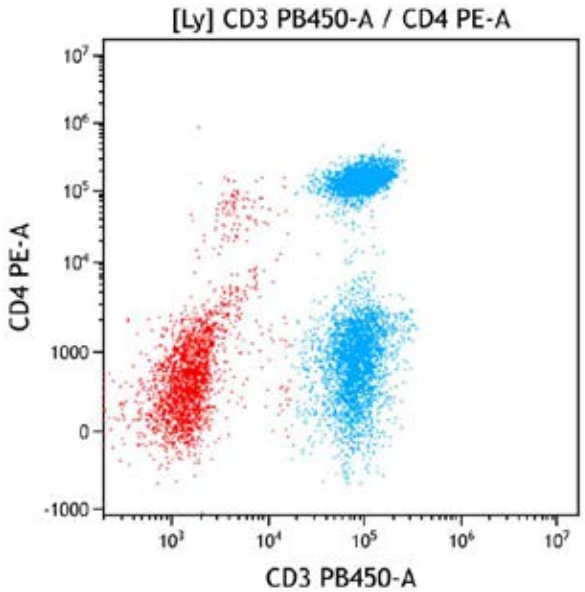

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells (red) lack expression of both CD3 and CD4. Of note,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

T20

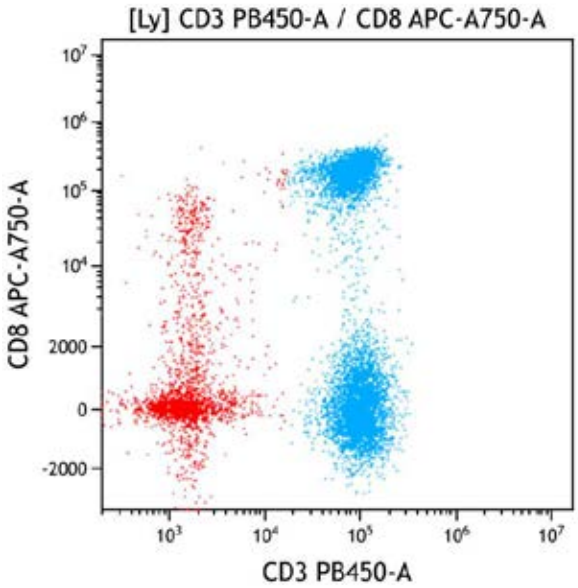

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

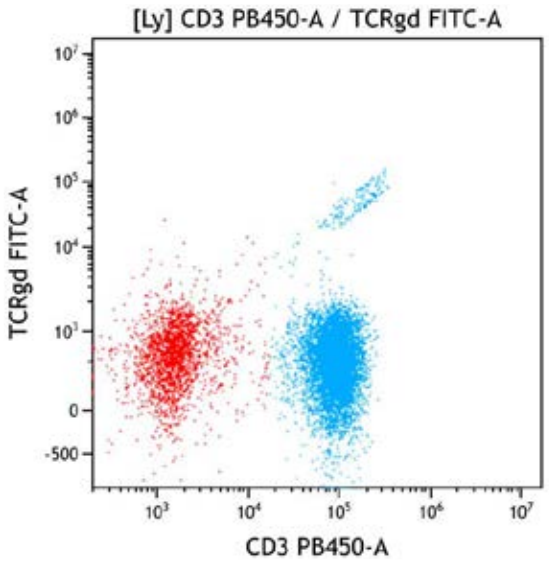

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

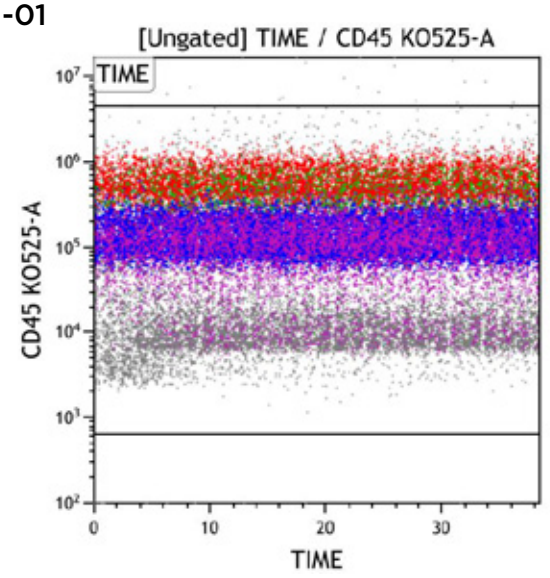

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

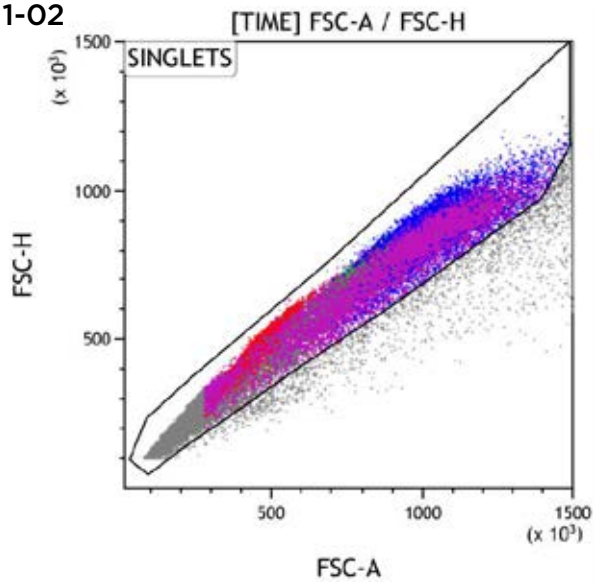

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSCH and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

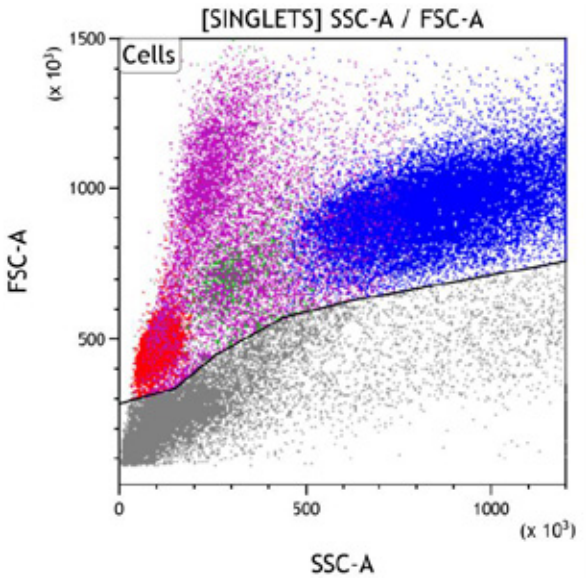

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M1-04

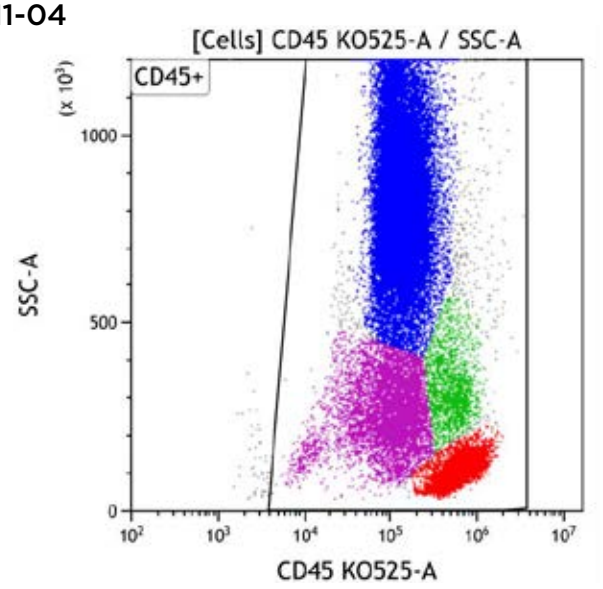

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

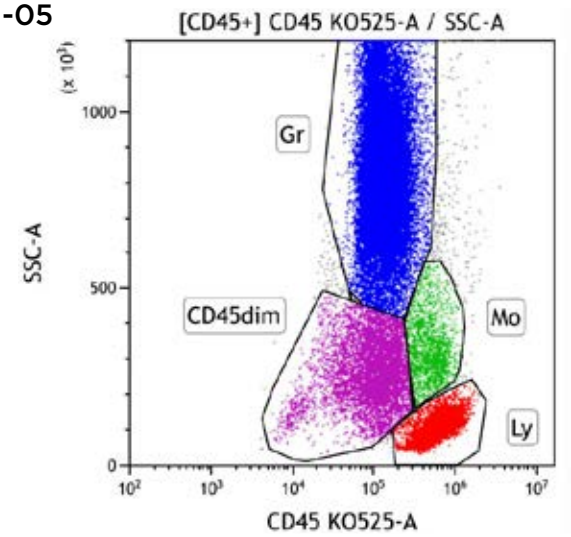

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr,blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 population may be followed throughout the analysis. The aberrant population (purple) has slightly decreased expression of CD45.

M1-06

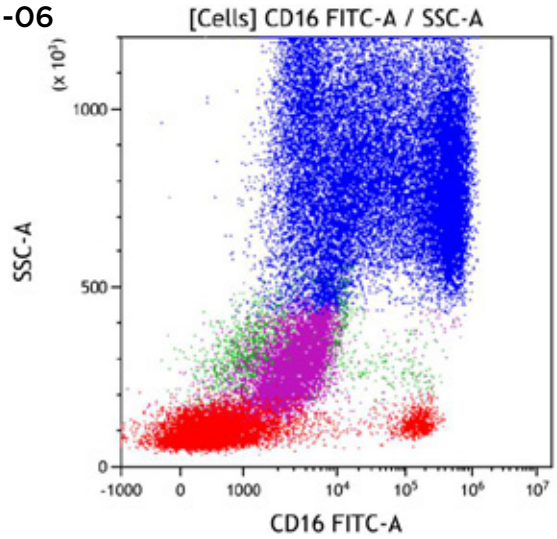

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The aberrant population (purple) is negative for CD16.

M1-07

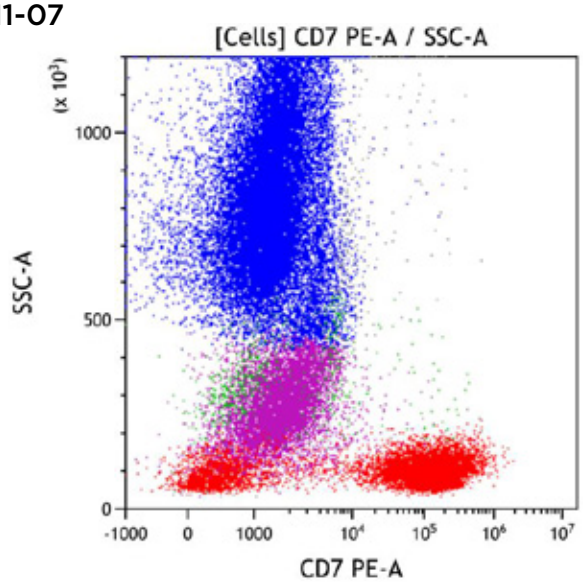

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed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

M1-08

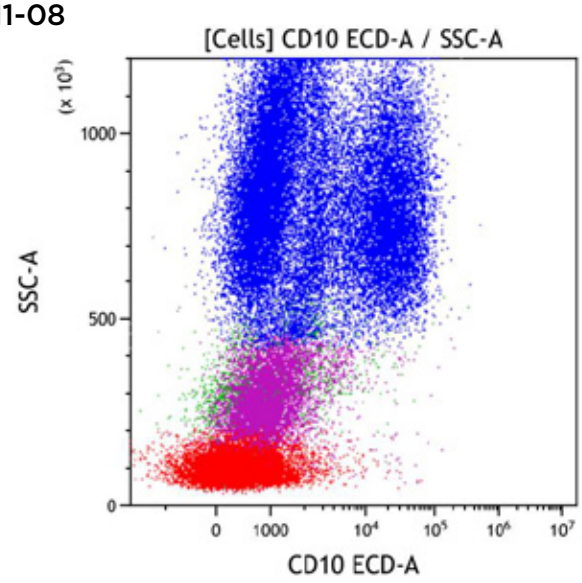

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

BACK TO CASE OVERVIEW

M1-09

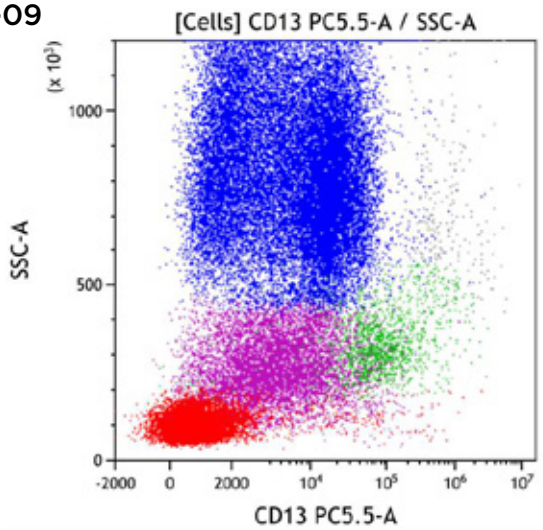

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue) with variable intensity dependent on maturation stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature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

M1-10

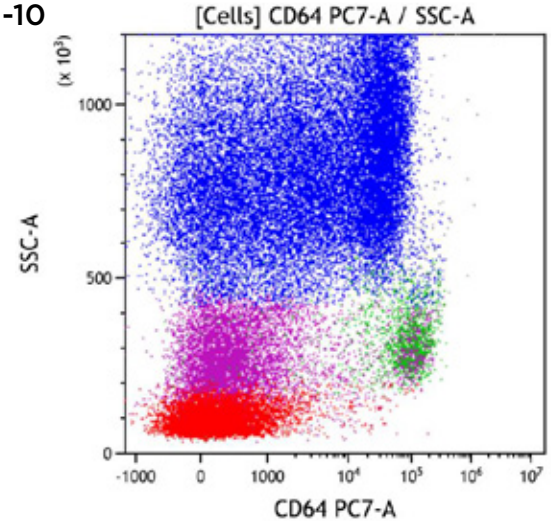

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

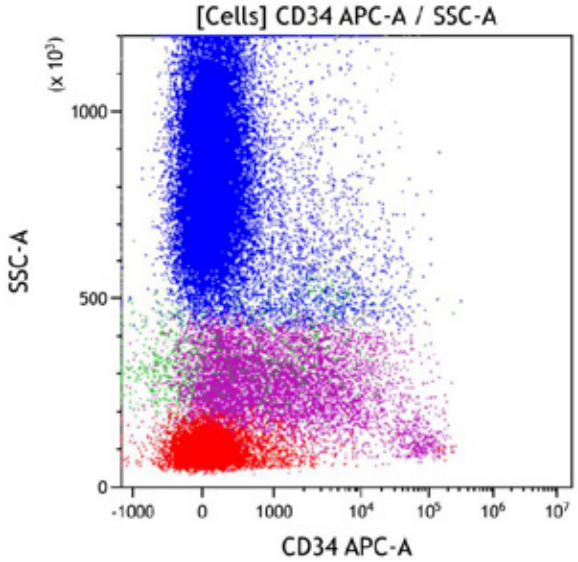

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45dim gate 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.

M1-12

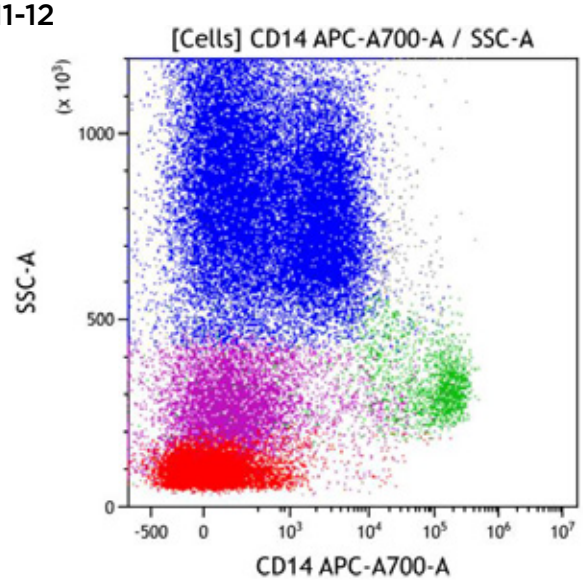

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes (blue) at a low level. The aberrant population (purple) is negative for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

BACK TO CASE OVERVIEW

M1-13

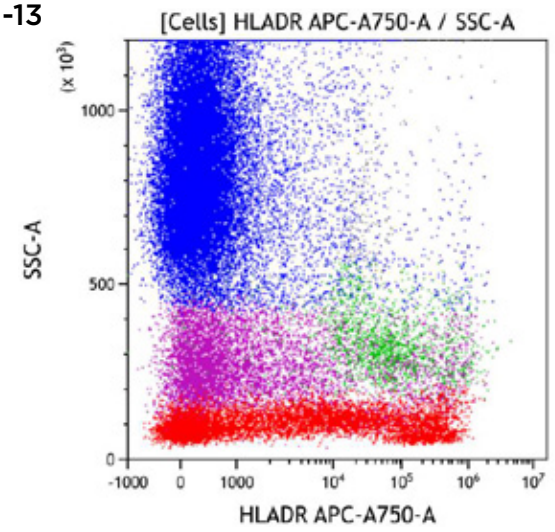

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 progenitors, immature and mature B cells (red), and activated T cells (red). The aberrant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

M1-14

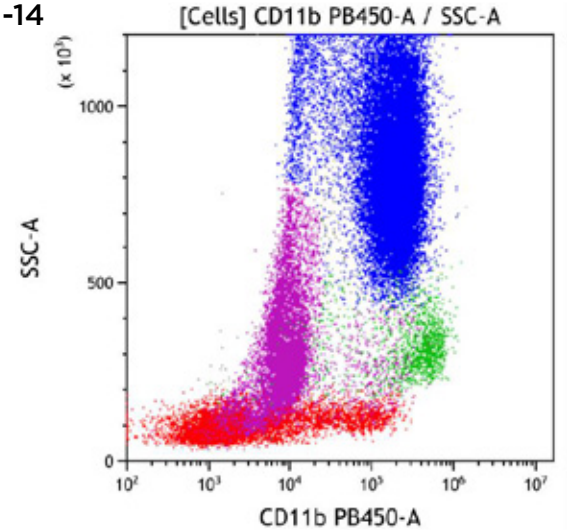

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes (blue) beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The aberrant population (purple) is negative for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

M1-15

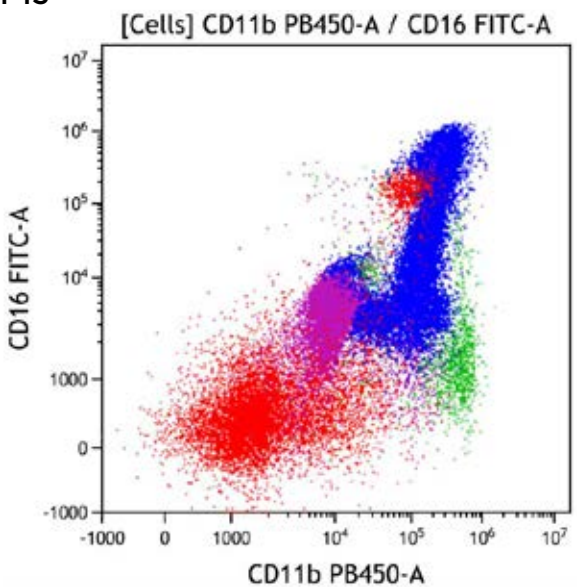

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), immature and mature granulocytes (blue) and a subset of NK cells (red). CD16 is expressed on immature and mature granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes lack CD11b and CD16 (blue, lower left) and acquire CD11b as they mature toward myelocytes (blue, lower middle). 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.

M1-16

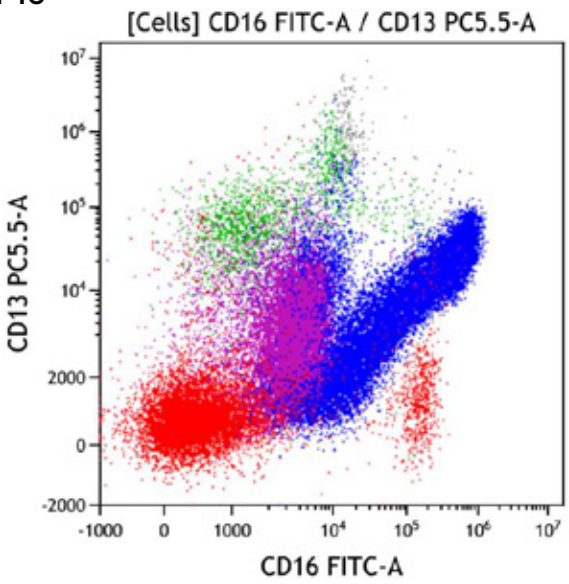

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 (blue, 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). The aberrant population (purple) expresses dim CD13 and is negative for CD16.

BACK TO CASE OVERVIEW

M1-17

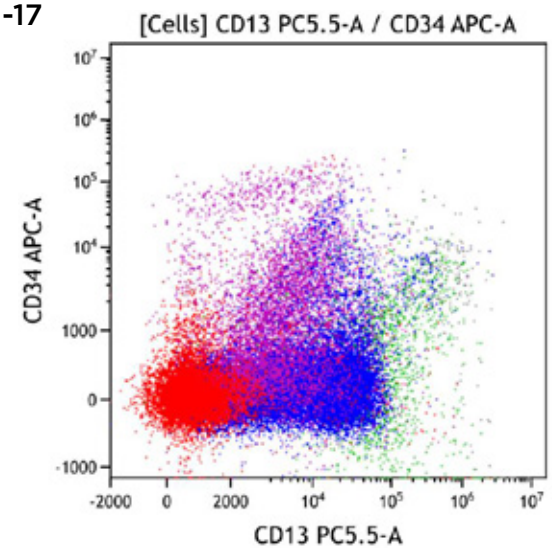

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple, upper left). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors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

M1-18

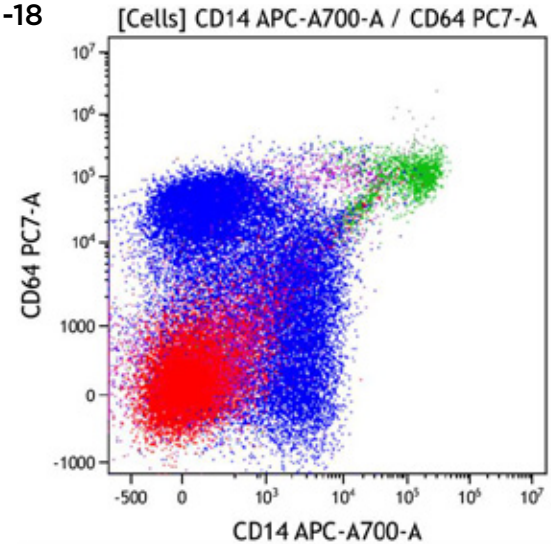

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature granulocytes express moderate CD64 without CD14 (blue, upper left) and acquire CD14 and lose CD64 at transition to mature granulocytes (blue, lower left). The aberrant population (purple)

M1-19

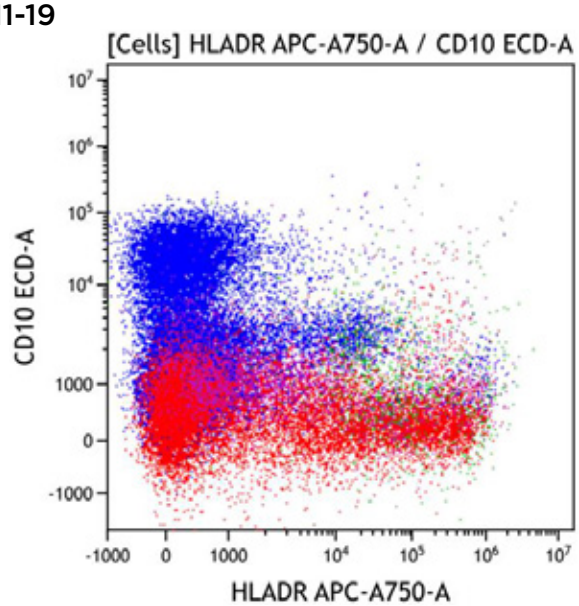

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on mature granulocytes (blue) and immature B cells.

M1-20

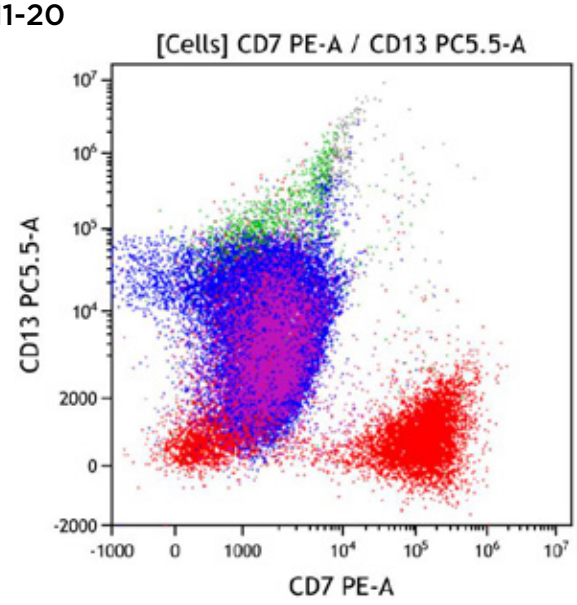

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Co-expression of CD13 and CD7 is generally not seen. The aberrant population (purple) expresses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

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

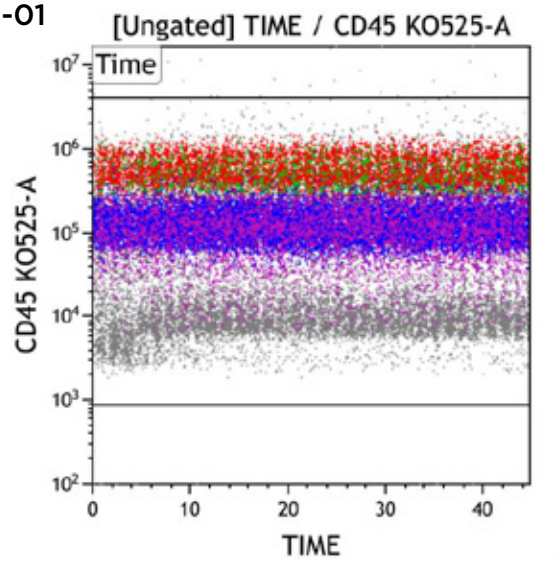

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

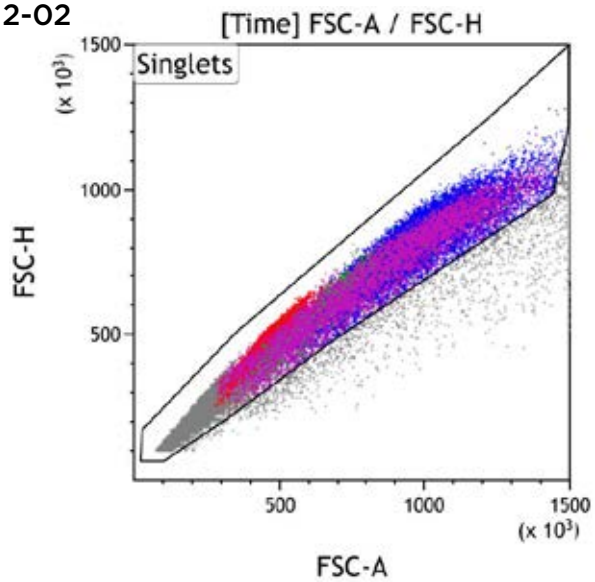

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSCH and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

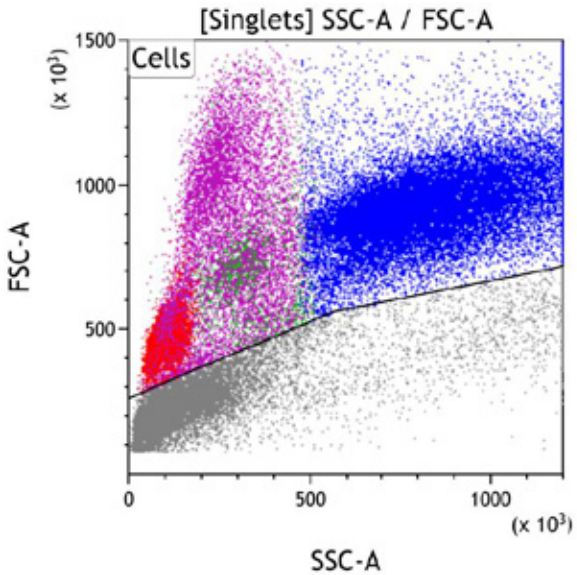

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M2-04

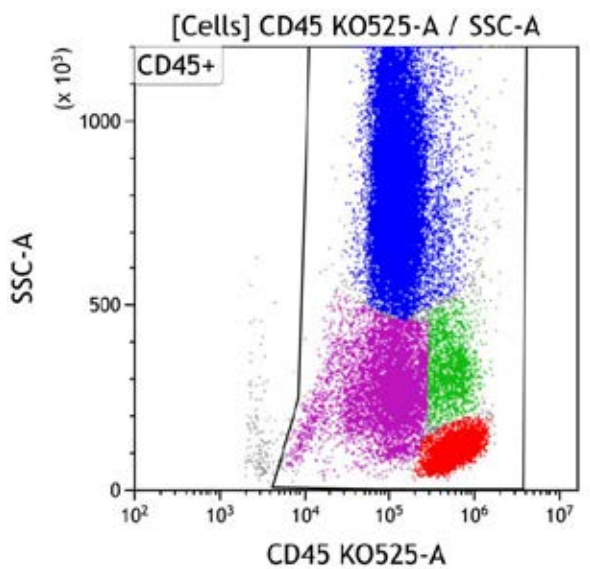

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

BACK TO CASE OVERVIEW

M2-05

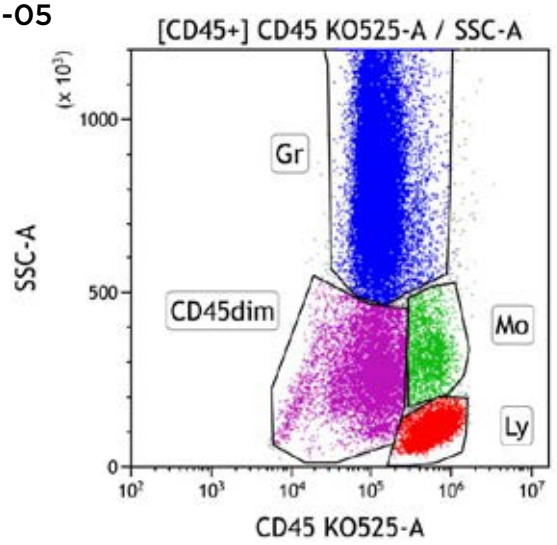

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 slightly decreased expression of CD45.

M2-06

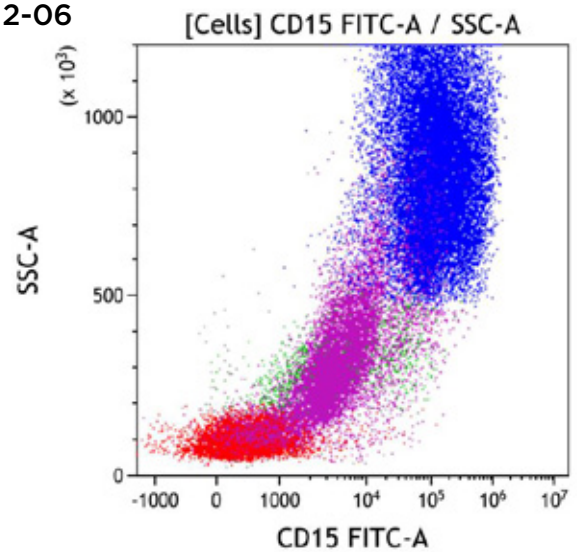

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes (green). The aberrant population (purple) is negative for CD15.

M2-07

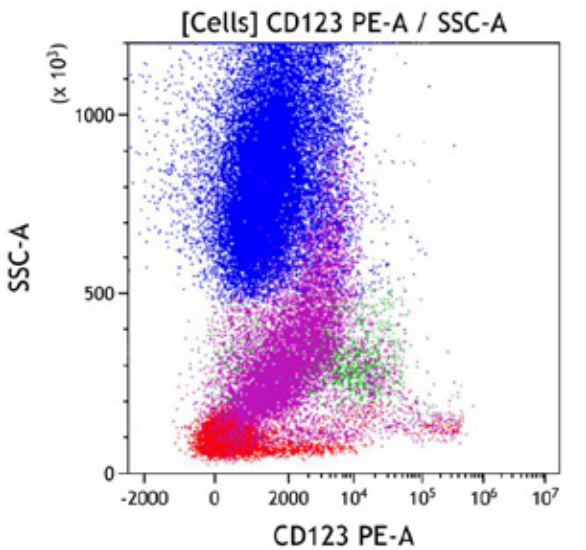

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The aberrant population (purple) is negative for CD123.

M2-08

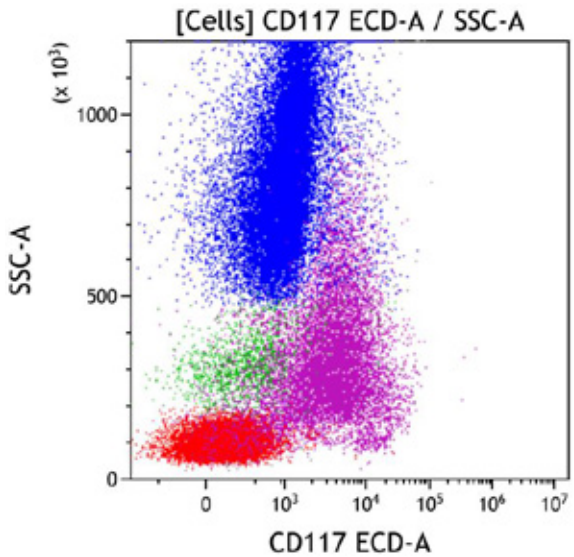

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

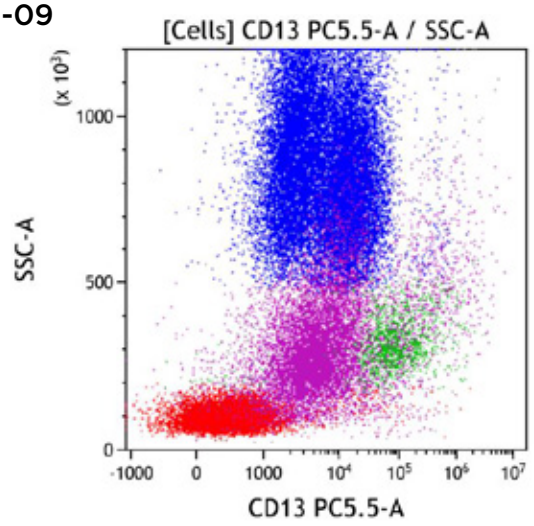

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue) with variable intensity dependent on maturational stage. It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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 aberrant population (purple) expresses dim CD13.

M2-10

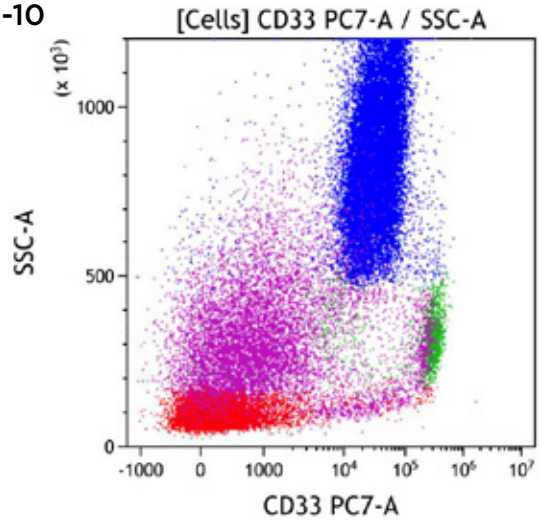

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on immature and mature monocytes (green), at a slightly low level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset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

M2-11

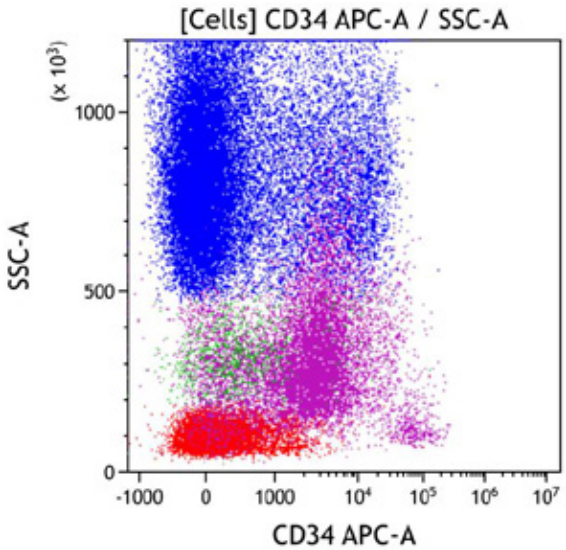

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45dim gate (purple, lower middle).

M2-12

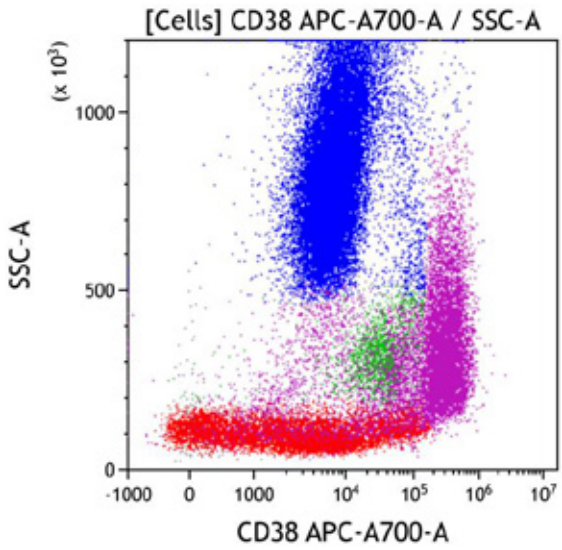

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and on monocytes (green), and at a variably level on activated mature lymphocytes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

BACK TO CASE OVERVIEW

M2-13

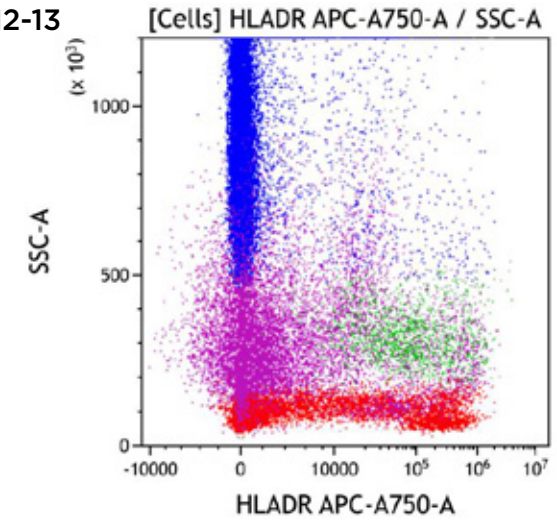

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red). The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

M2-14

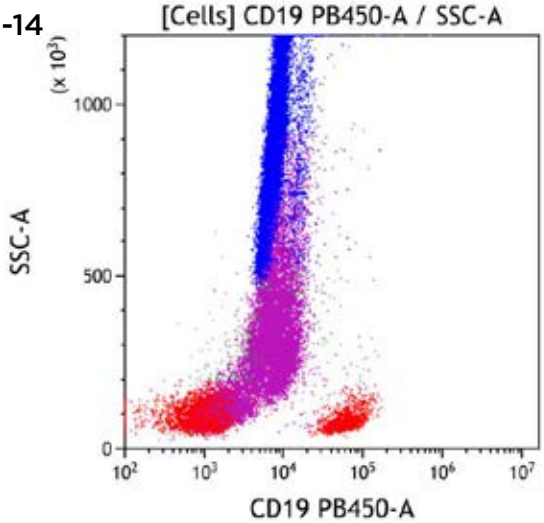

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red, lower middle), as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is negative for CD19.

M2-15

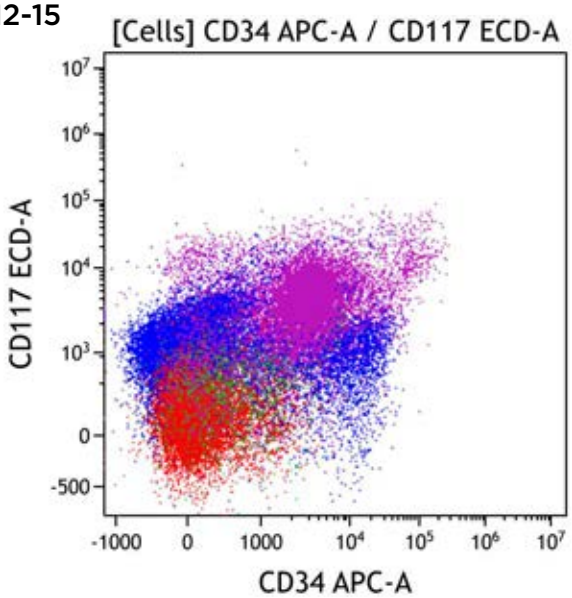

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts (purple, middle right), promyelocytes (blue), and early erythroid precursors, but negative on early B cell precursors. The apparent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

M2-16

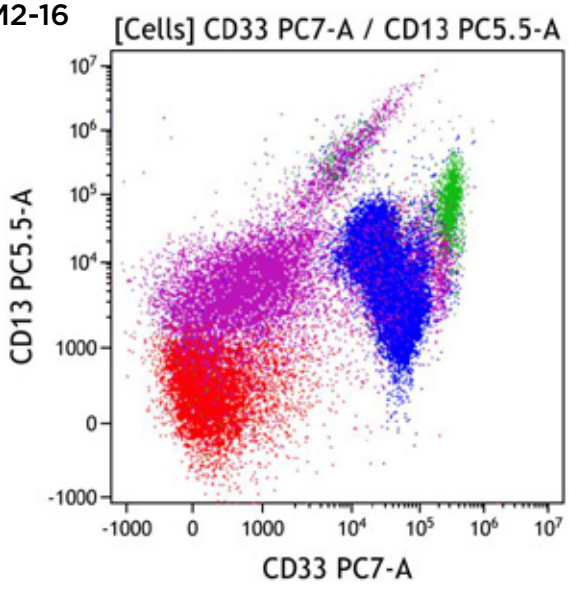

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and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

BACK TO CASE OVERVIEW

M2-17

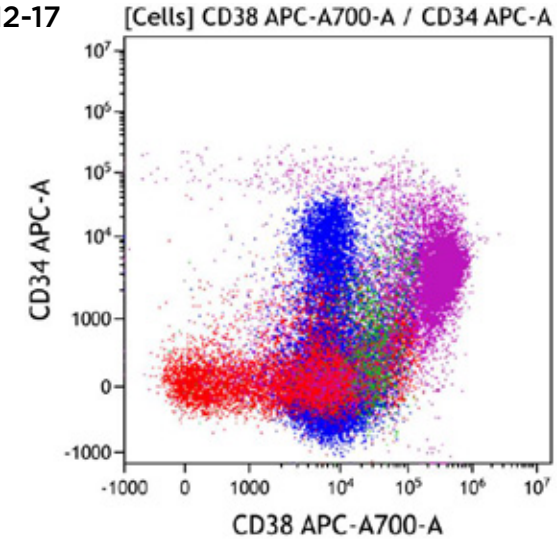

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, upper). The aberrant population (purple) has bright CD38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

M2-18

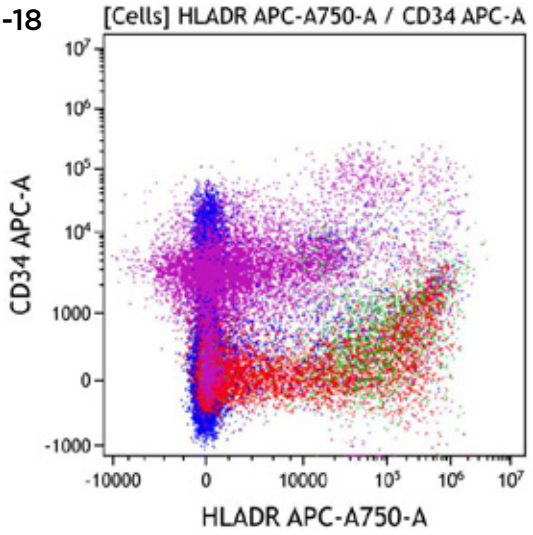

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors (purple, upper). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest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

M2-19

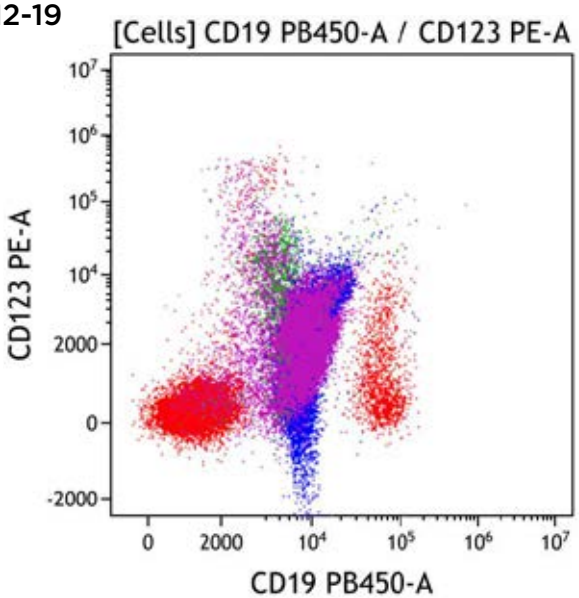

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red) normally do not express significan CD123. CD123 positive basophilsand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

M2-20

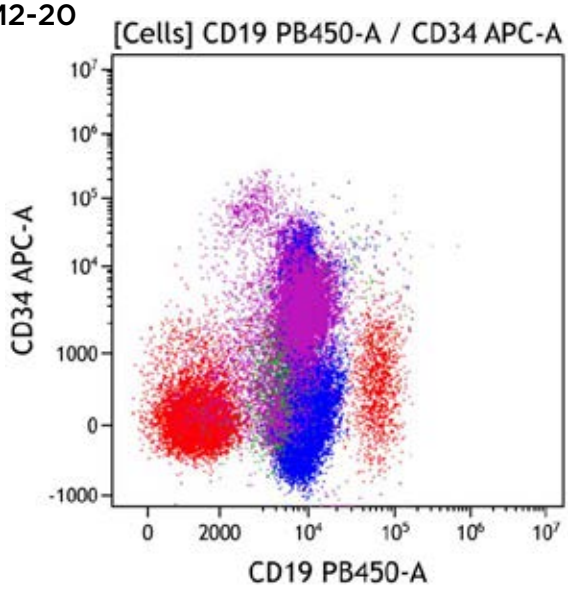

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors (purple, upper left). CD34 positive progenitors do not express CD19. A subset of lymphocytes (red) are CD19 positive B cells without expression of CD34.

BACK TO CASE OVERVIEW

M2-21

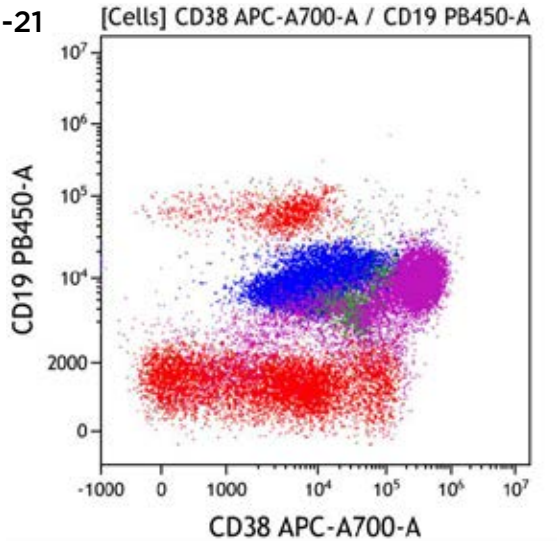

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells show low level expression of CD38 (red). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. The aberrant population (purple) show high CD38 expression without CD19.

M2-22

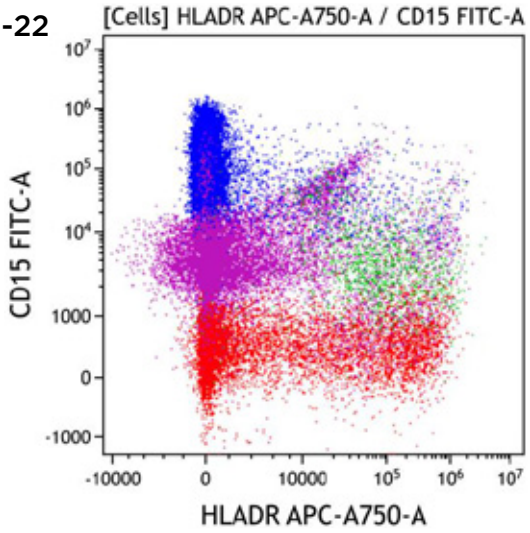

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes (blue) and monocytes (green). HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR. CD34 positive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

M2-23

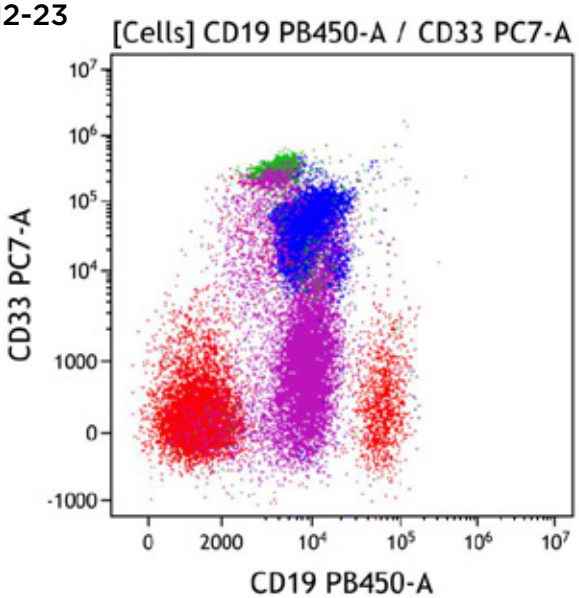

Figure 24. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not normally express significant CD33. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence,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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 5 LHS-TC4-093**
Diagnosis	**T-Lymphoblastic Leukemia/Lymphoma**
Clinical Vignette	This 12-year-old male presents with anemia, thrombocytopenia, lymphocytosis, and blast in peripheral blood. A peripheral blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of CD2, intermediate surface CD3, variable CD4, intermediate CD5, bright CD7, intermediate CD8, variable CD10, intermediate CD38, dim CD45, and variable TCRγδ without CD34, CD56, CD117, or other B cell or myeloid antigens. The immunophenotype of the population is consistent with abnormal immature T cells, i.e. T-lymphoblasts. Compared with normal peripheral blood, the presence of an immature T cell population in the peripheral blood is aberrant, with co-expression of CD4 and CD8, mildly decreased CD5, and dim CD45. This finding supports a diagnosis of T-lymphoblastic leukemia/lymphoma. Additional testing for TdT and CD1a could be performed to confirm the immaturity of the T cells.

BACK TO CASE LISTINGS

B Cell Tube

B01

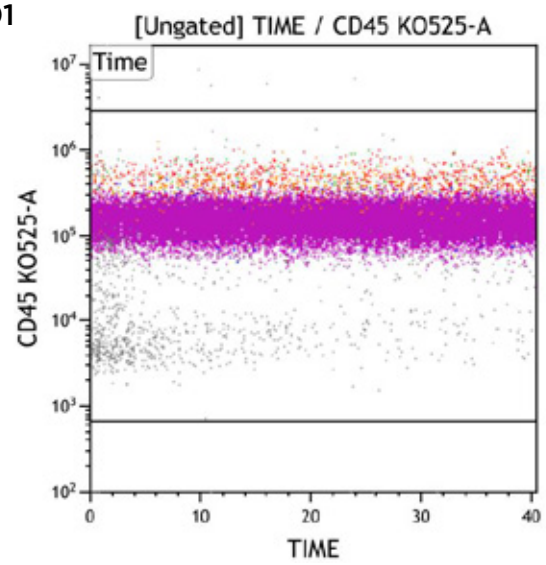

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

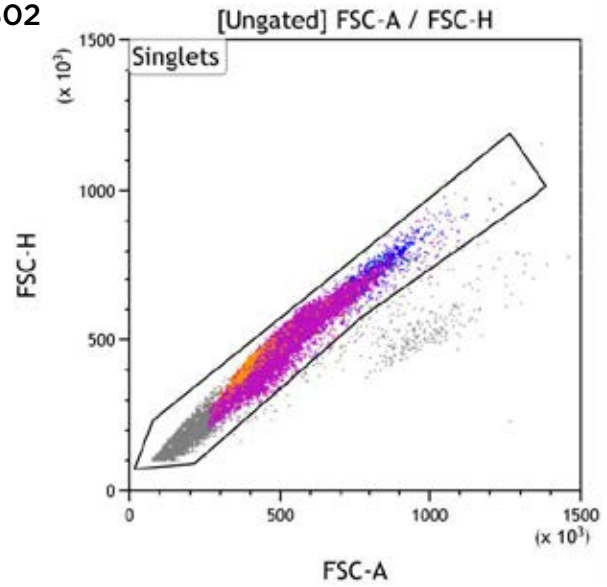

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

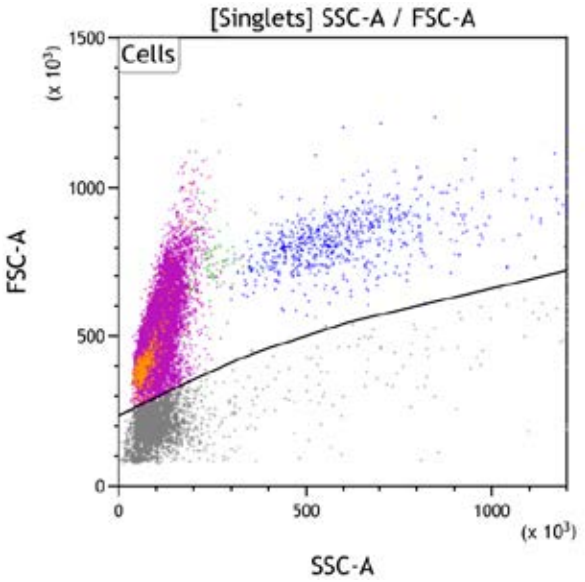

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

B04

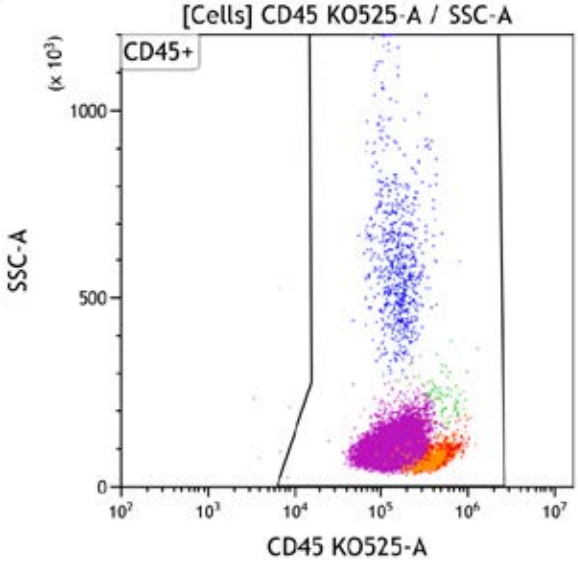

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

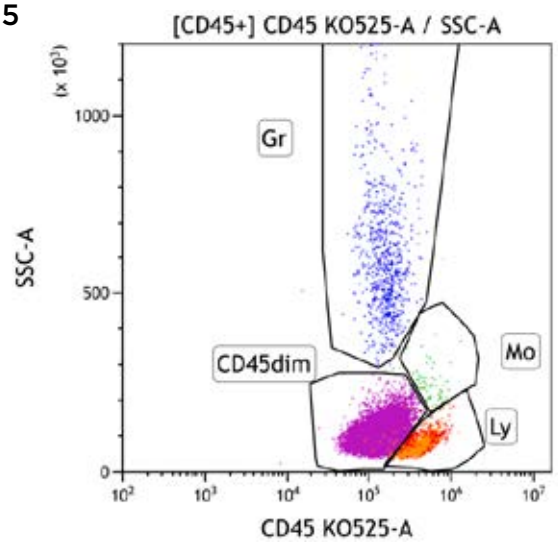

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 followed throughout the analysis. The progenitor population in the CD45dim gate (purple) is expanded.

B06

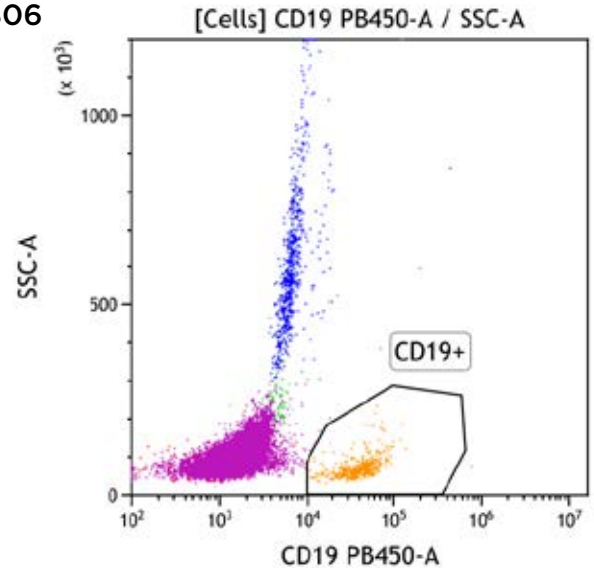

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple)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

B07

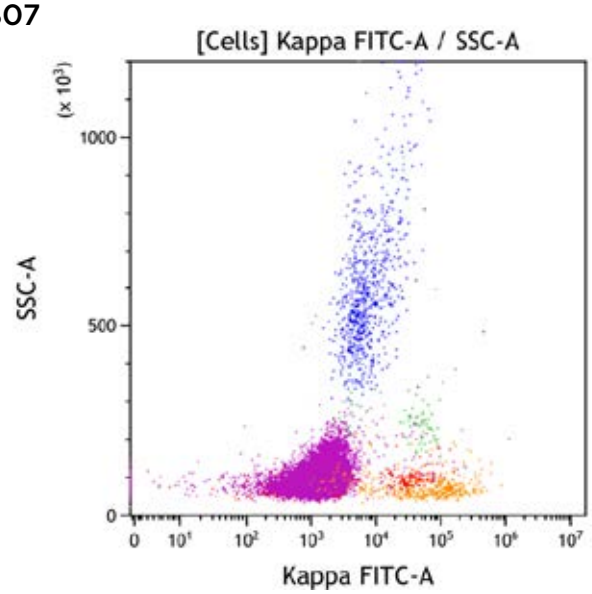

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity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

B08

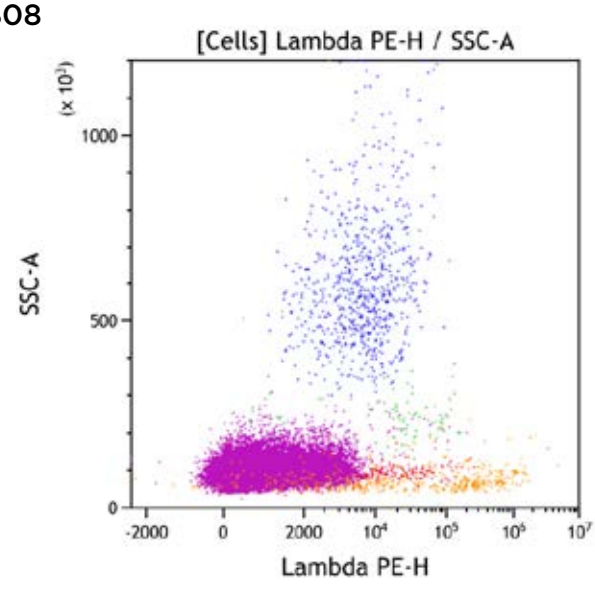

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity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

BACK TO CASE OVERVIEW

B09

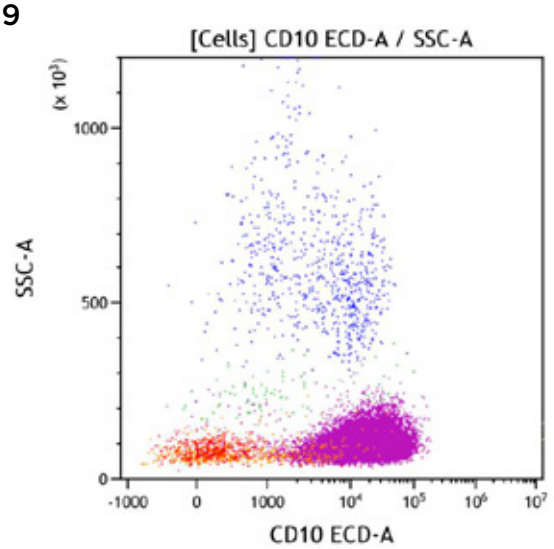

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

B10

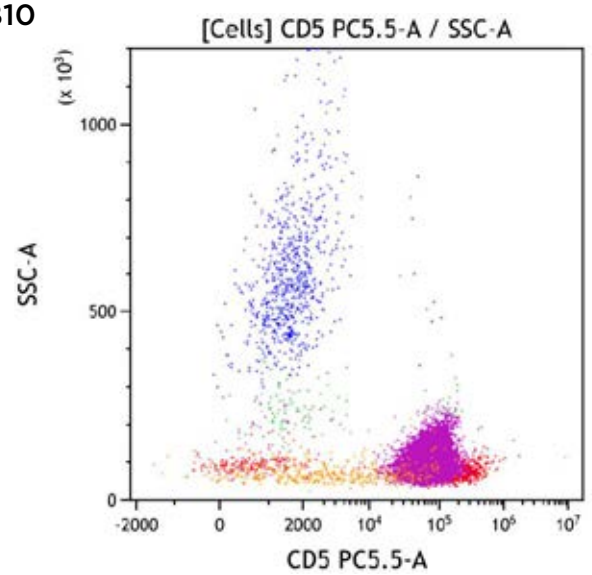

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The aberrant population (purple) expresses CD5 at a level lower than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

B11

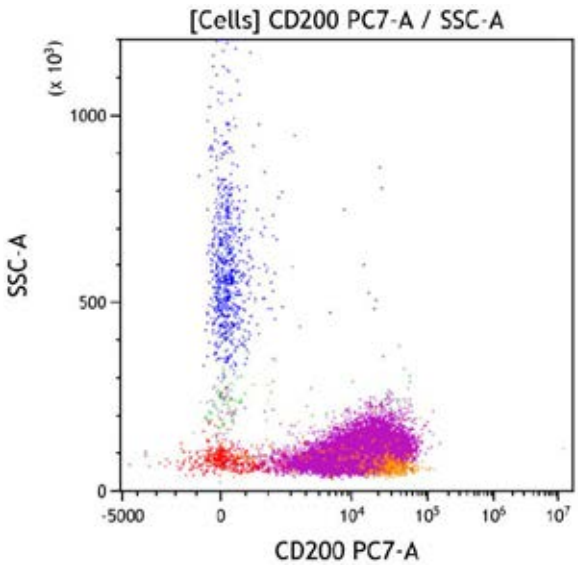

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

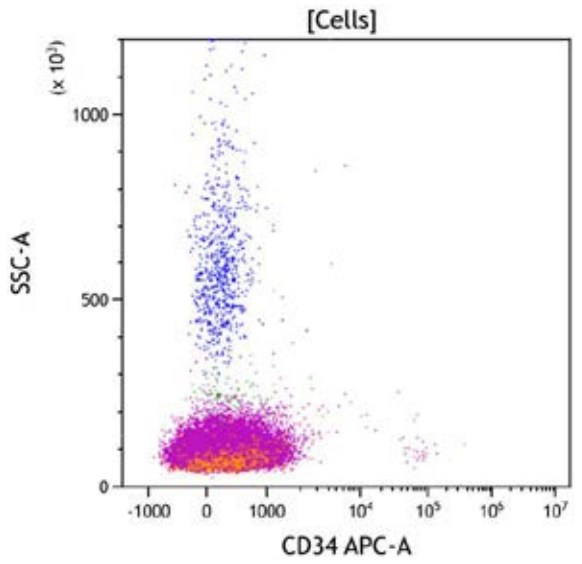

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

B13

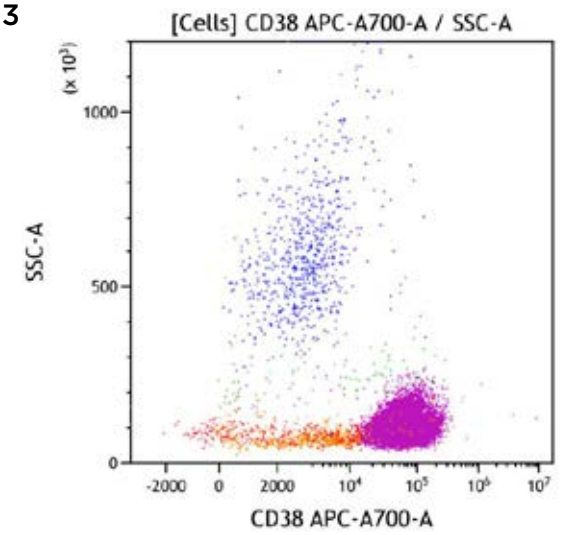

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature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

B14

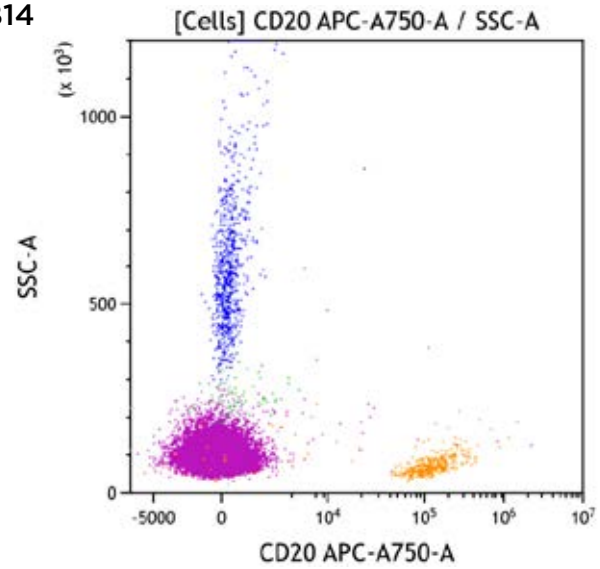

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side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

B15

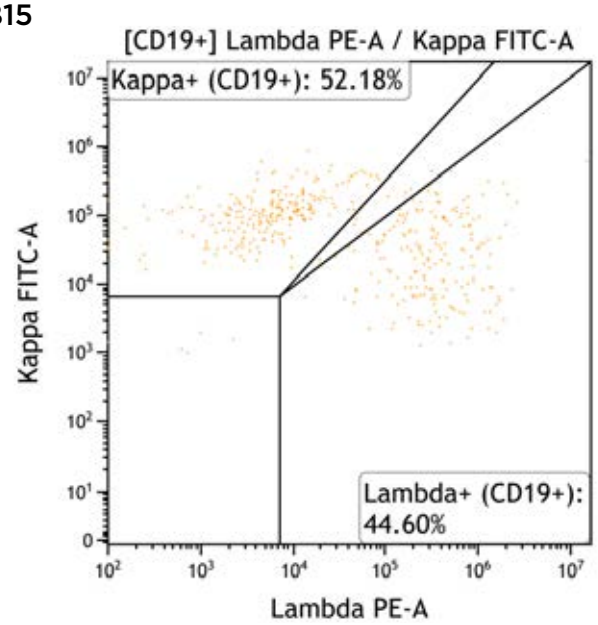

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

B16

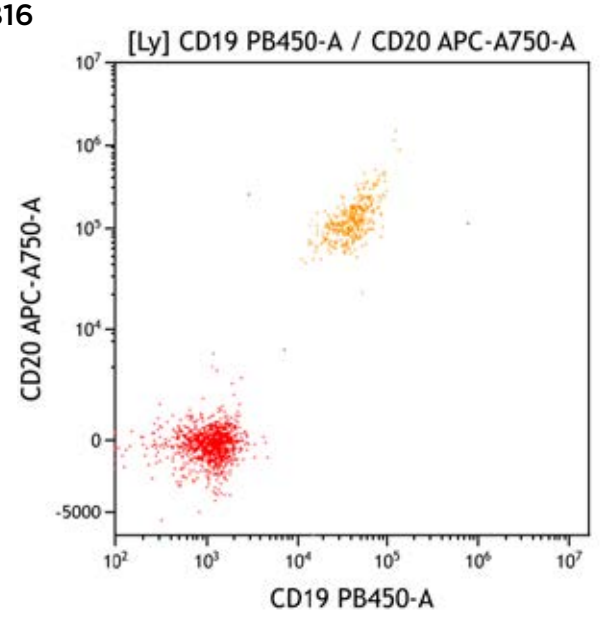

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

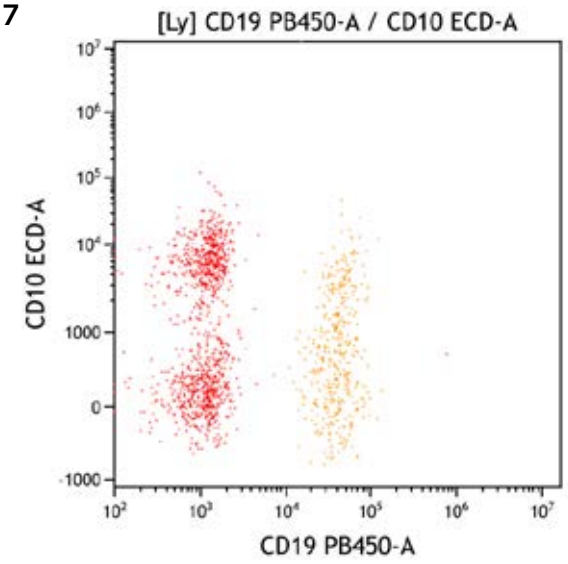

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of latestage immature B cells is present in peripheral blood and bone marrow aspirates.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

B18

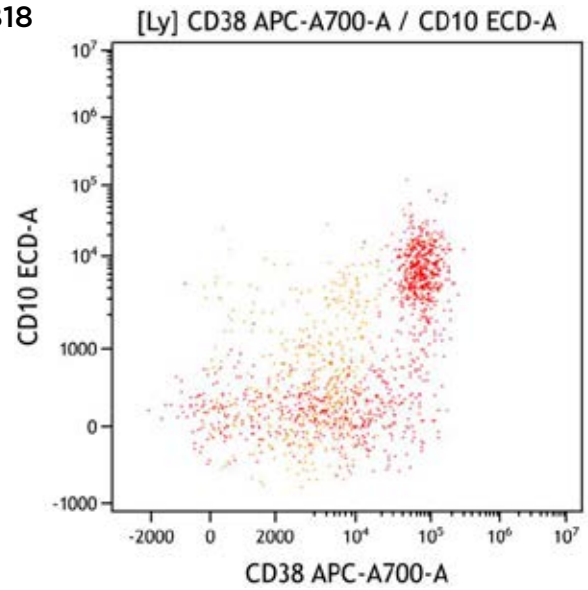

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low level expression of CD38. T cells (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 CD45dim gate.

B19

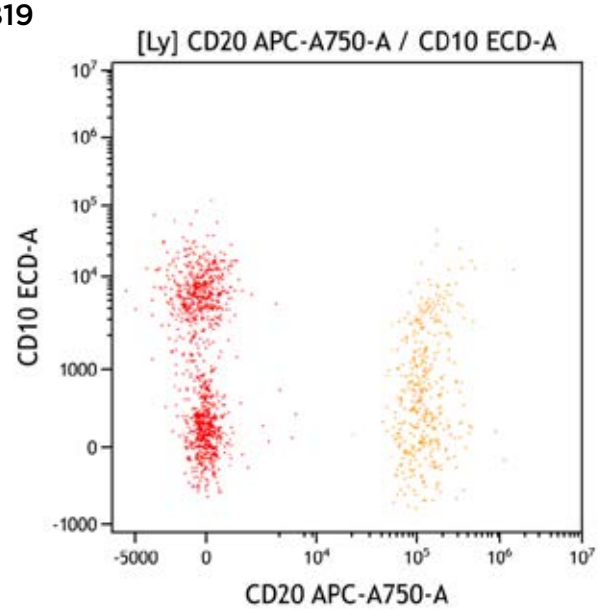

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20 without significant CD10. The few CD10 positive B cells represent late-stage immature B cells.

B20

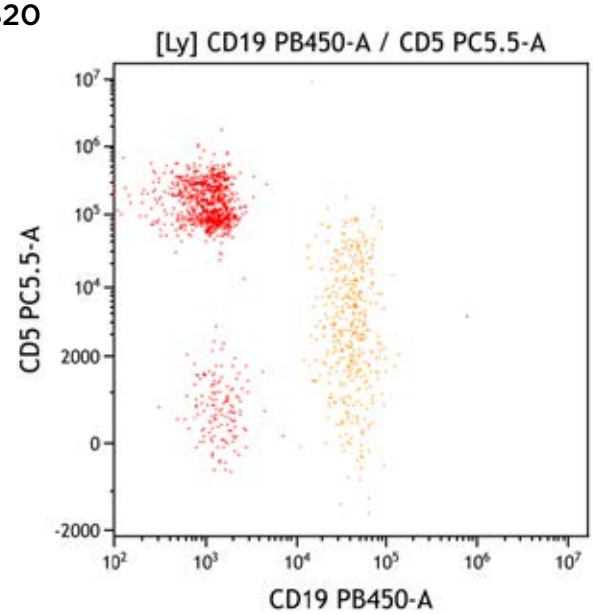

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

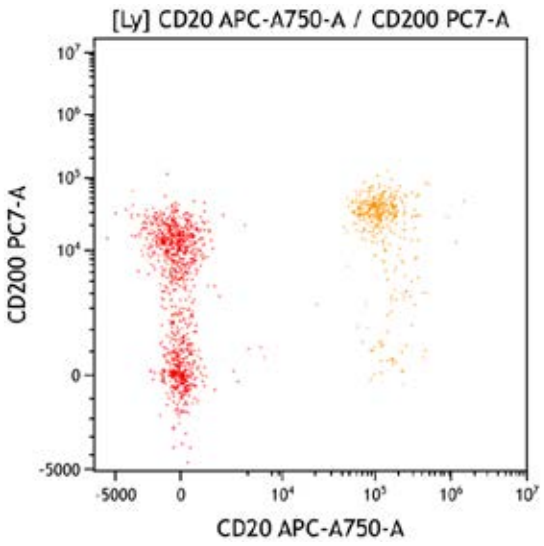

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

B22

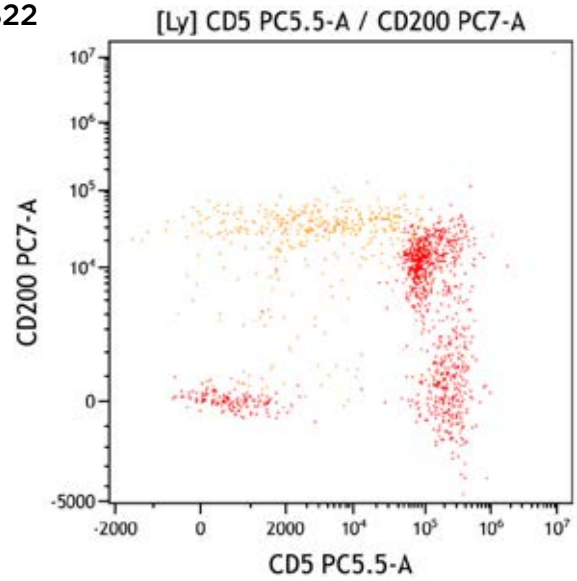

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell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

BACK TO CASE OVERVIEW

T Cell Tube

T01

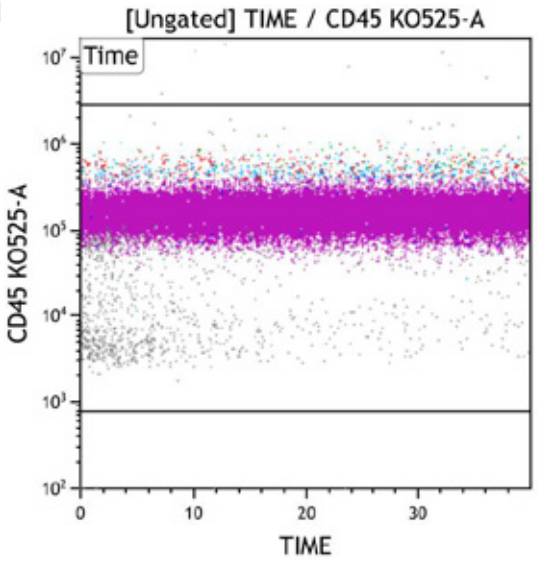

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

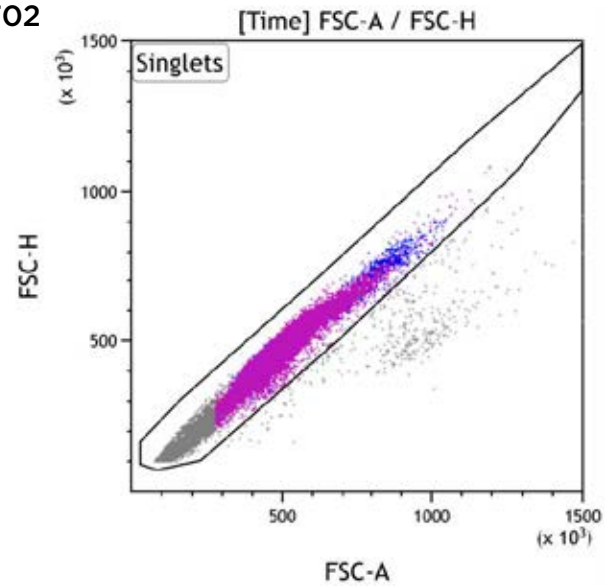

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

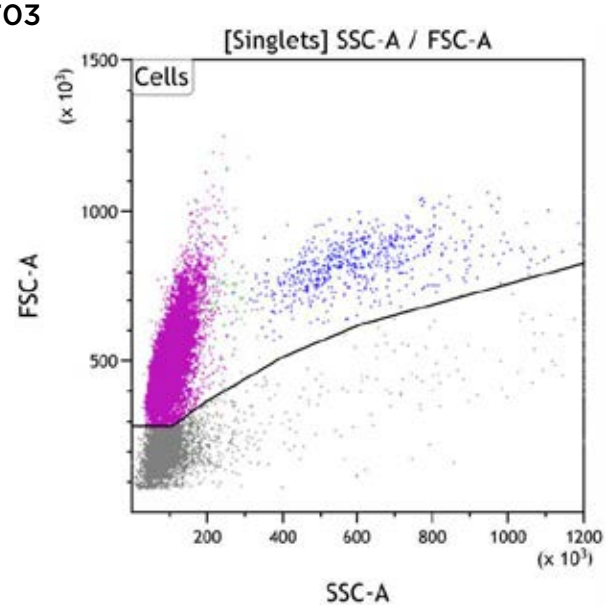

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

T04

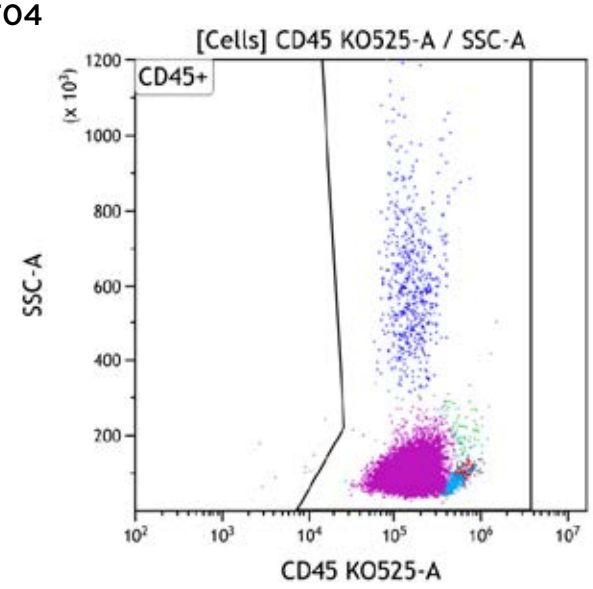

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

T05

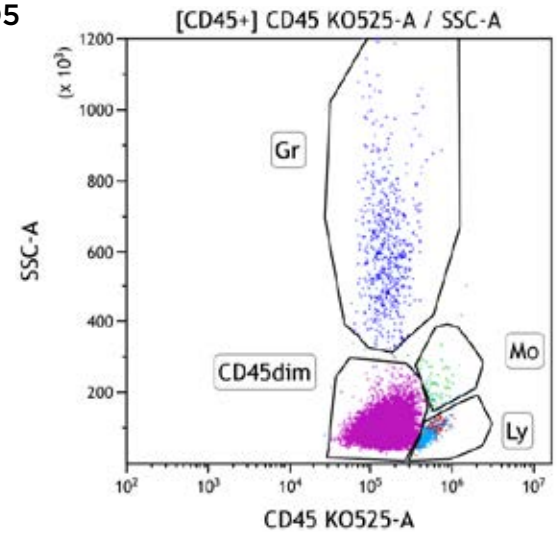

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 in the CD45dim gate (purple) is expanded.

T06

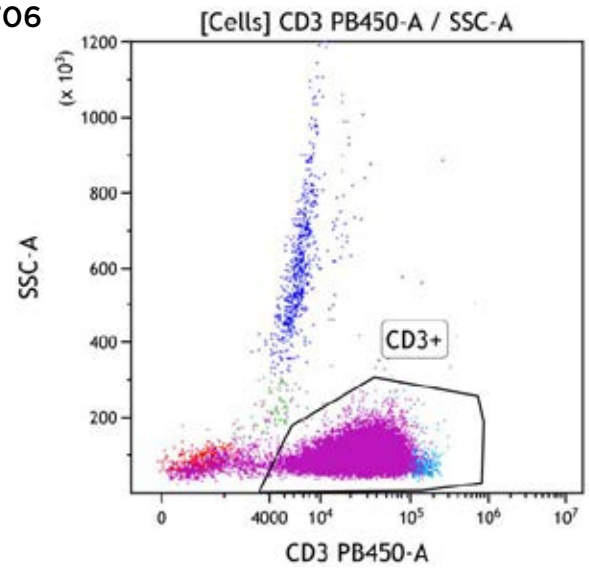

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression. CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.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

T07

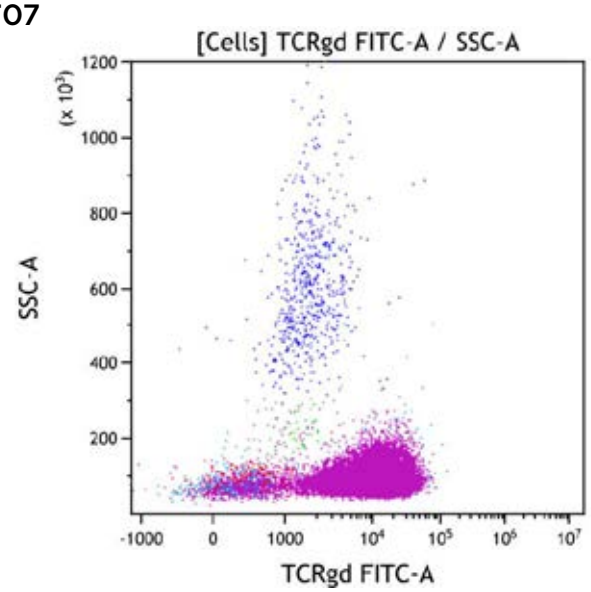

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter. The aberrant population (purple) expresses variable TCRγδ at a level lower than that seen on normal gamma/delta T cells (few aqua events, lower middle).

T08

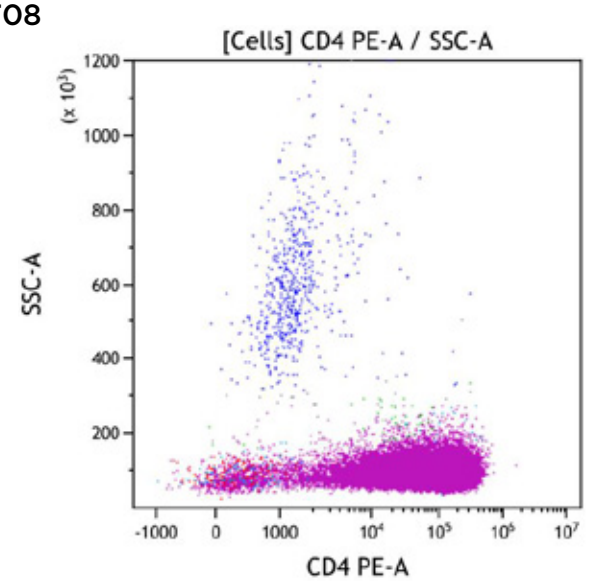

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells. The aberrant population (purple) expresses variable CD4.

BACK TO CASE OVERVIEW

T09

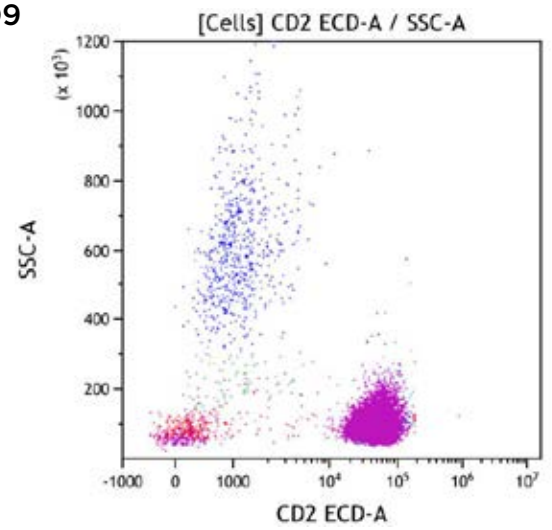

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). The aberrant population (purple) expresses CD2.

T10

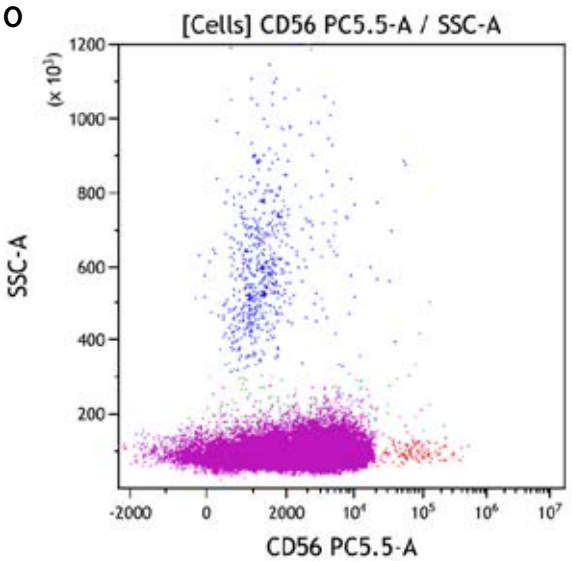

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytes (green) in both reactive and neoplastic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

T11

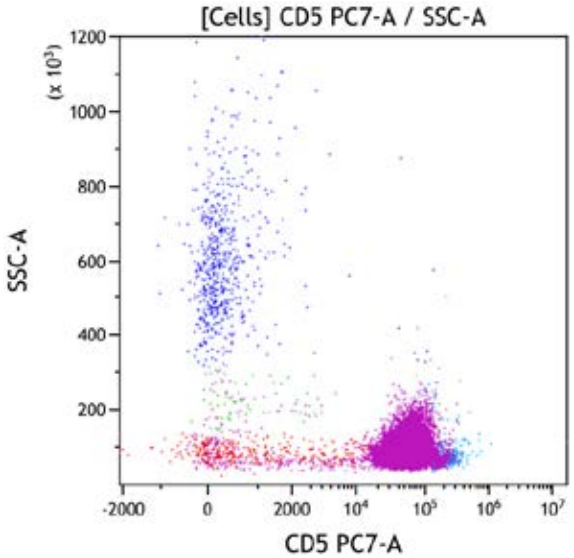

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

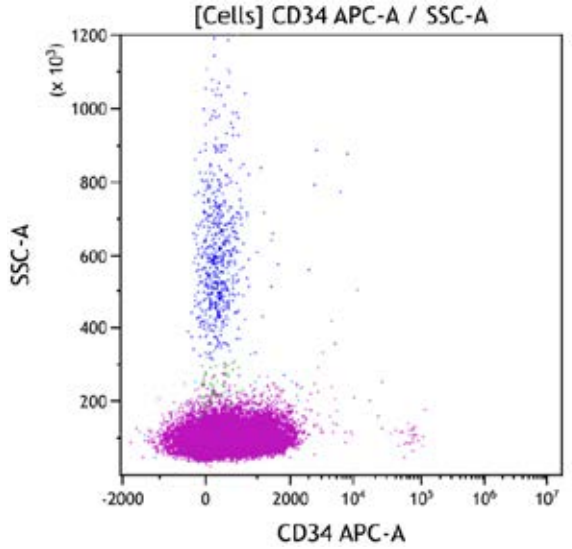

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is negative for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

BACK TO CASE OVERVIEW

T13

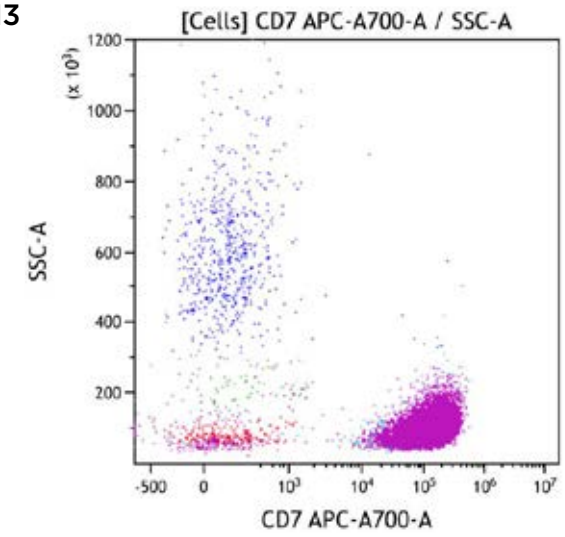

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

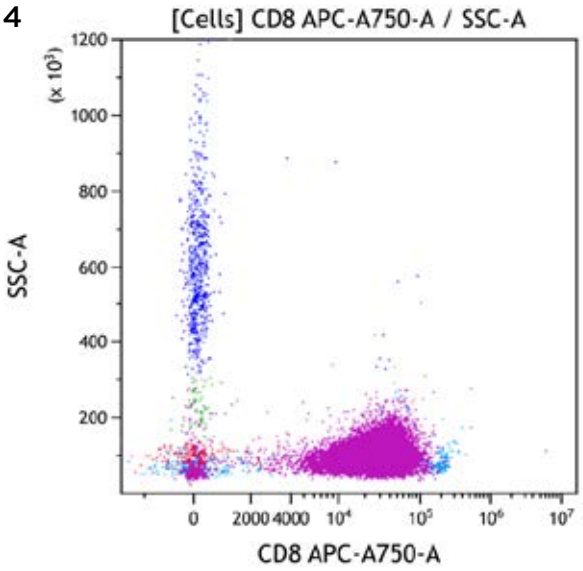

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells. The aberrant population (purple) expresses CD8 at a level lower than that seen on normal CD8 positive T cells (aqua).

T15

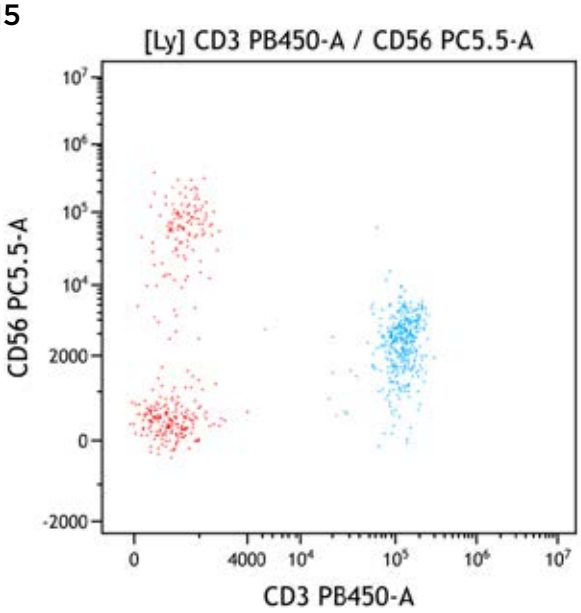

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present Non all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

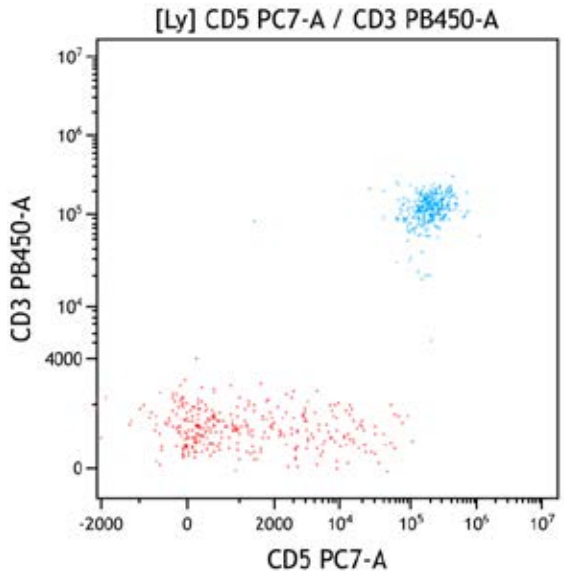

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocytegate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reducedto absent expression of CD5. CD5 is not expressed on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

BACK TO CASE OVERVIEW

B17

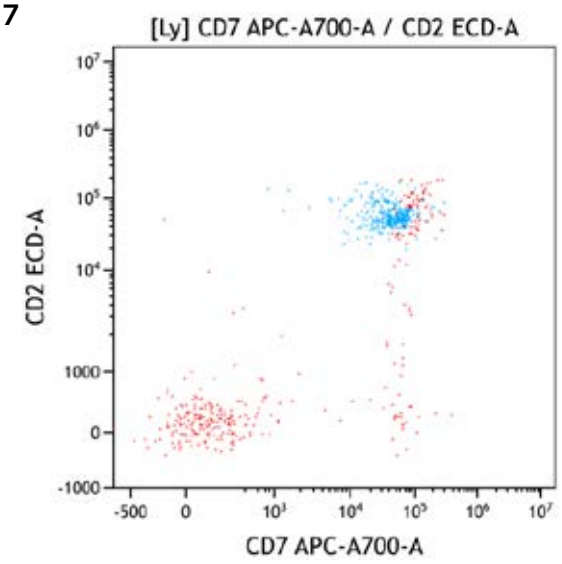

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells (red).

B18

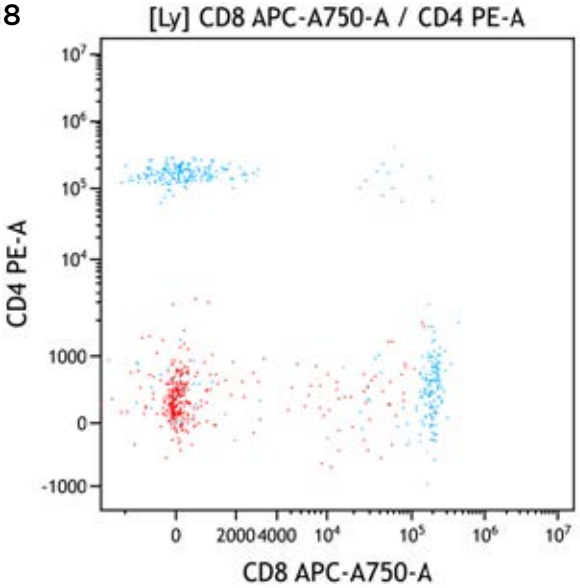

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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.

B19

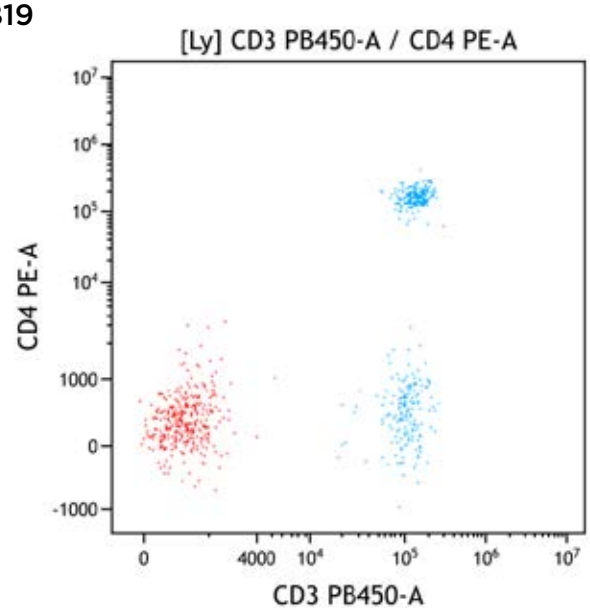

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells (red) lack expression of both CD3 and CD4.

B20

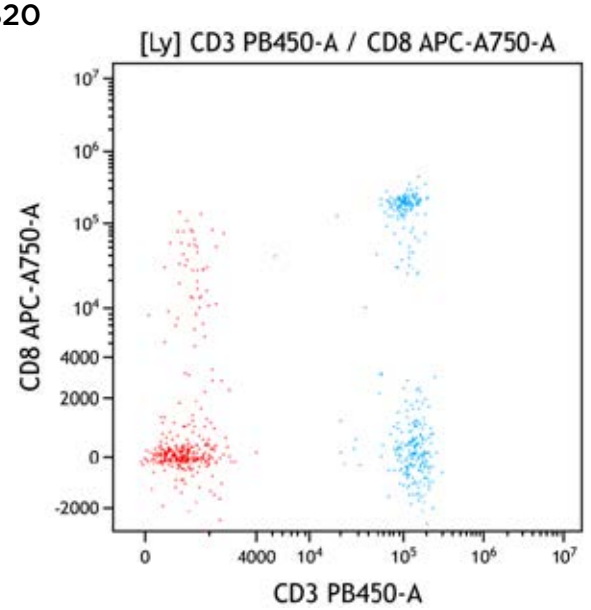

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

B21

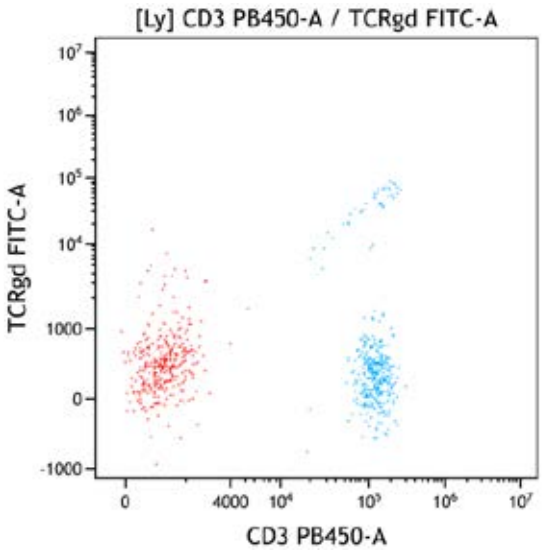

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/ delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

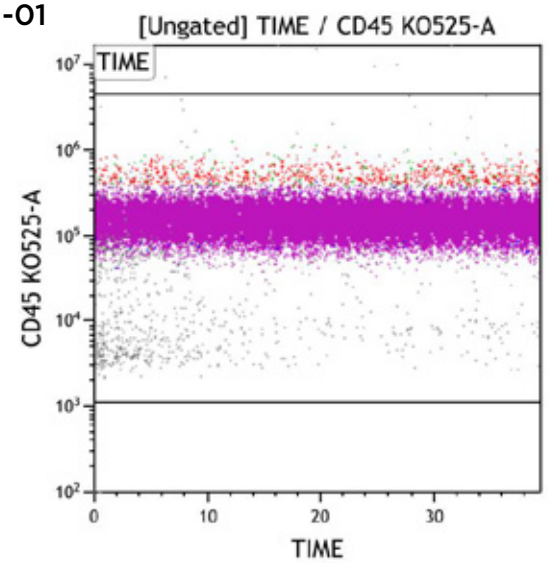

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

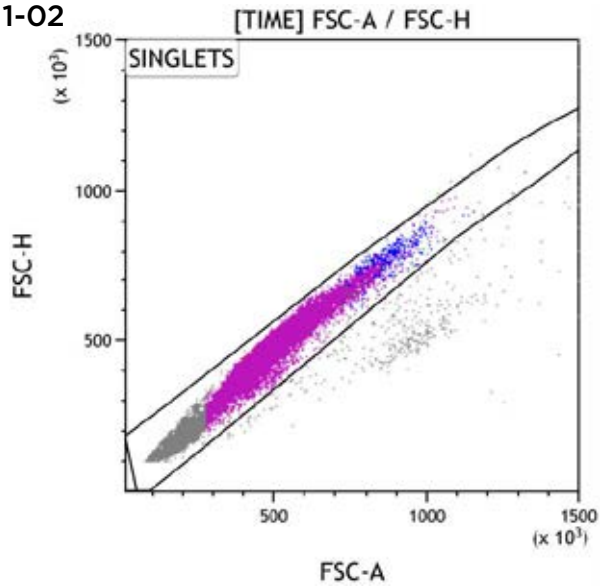

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSCH and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

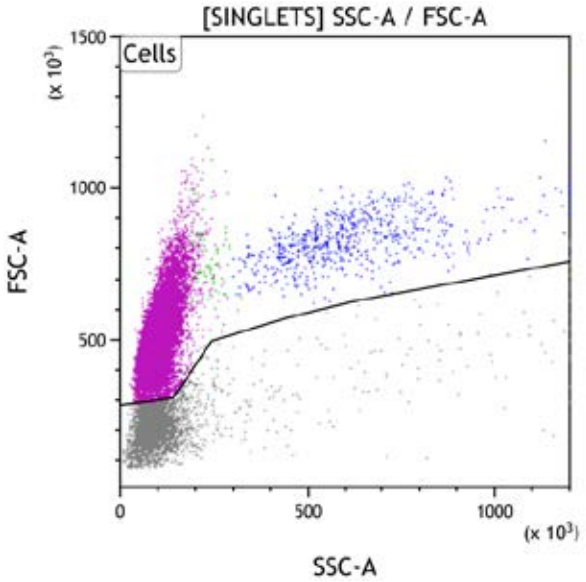

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M1-04

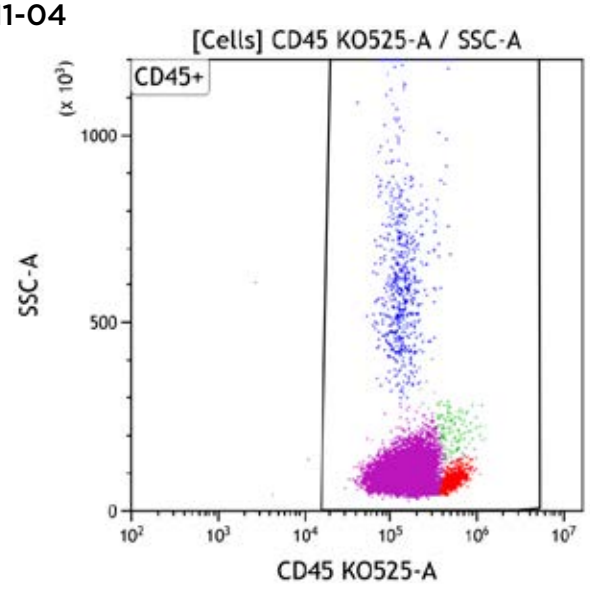

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, or non-hematopoietic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

BACK TO CASE OVERVIEW

M1-05

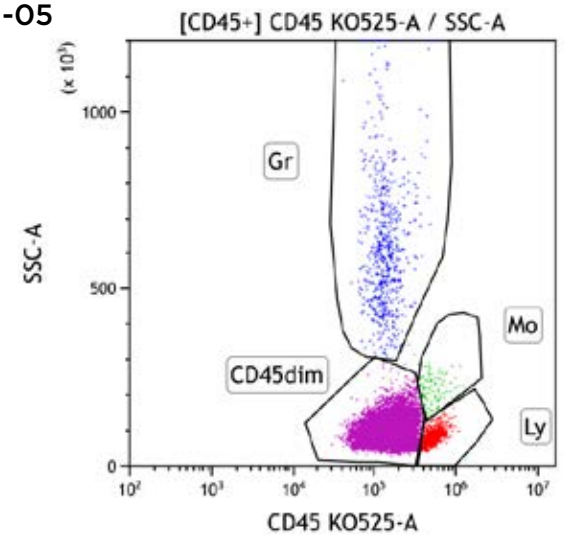

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typicallyoccupied by early progenitors (e.g., 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 in the CD45dim gate (purple) is expanded.

M1-06

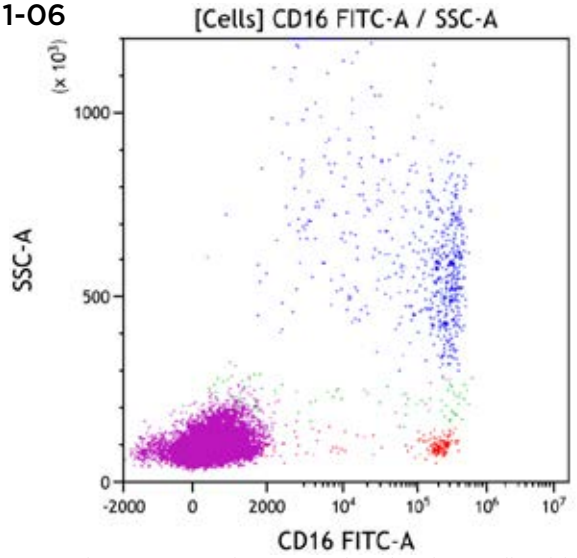

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). The aberrant population (purple) is negative for CD16.

M1-07

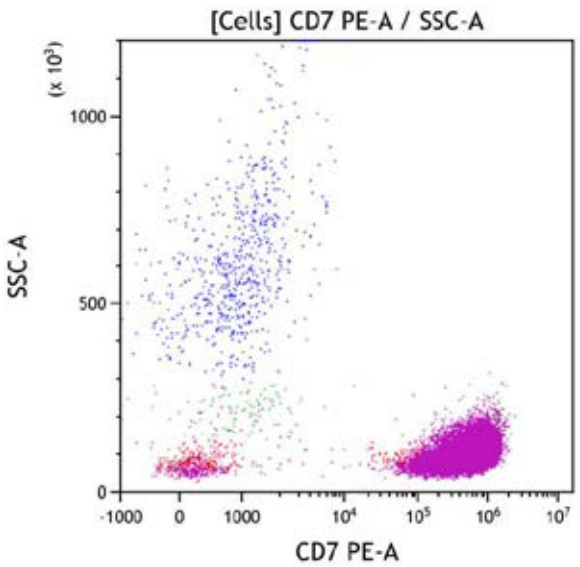

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed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

M1-08

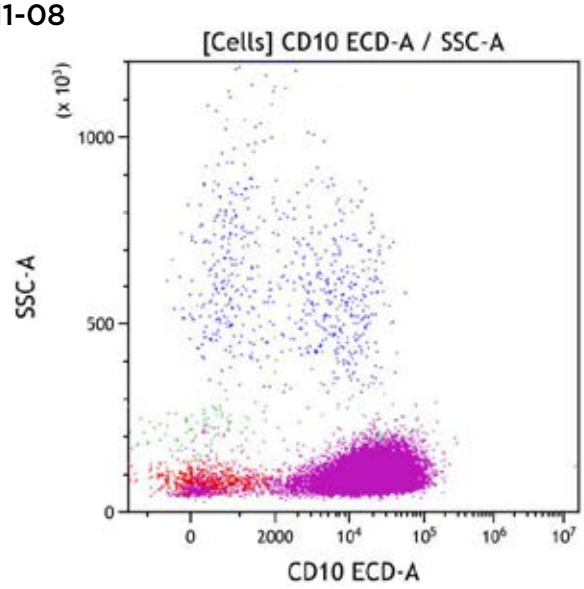

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

BACK TO CASE OVERVIEW

M1-09

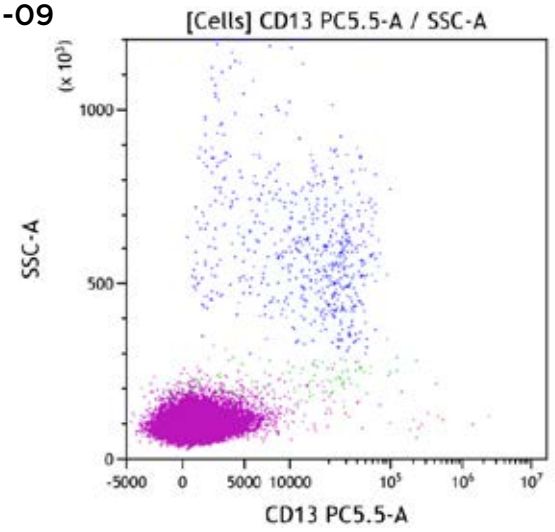

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature granulocytes (blue), mature monocytes (green), and myeloid progenitors. The aberrant population (purple) is negative for CD13.

M1-10

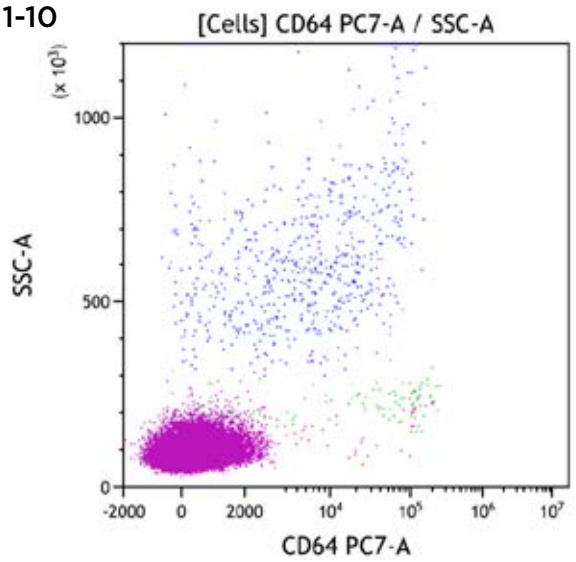

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on monocytes (green). Activated mature monocytes express CD64 at lower level and have lower side scatter. CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression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

M1-11

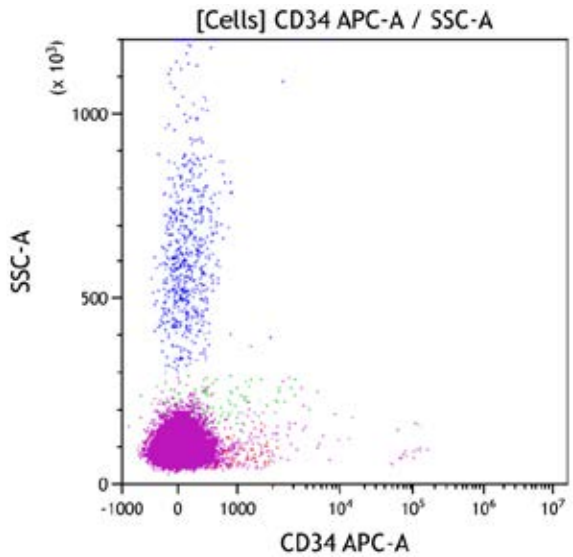

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is negative for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

M1-12

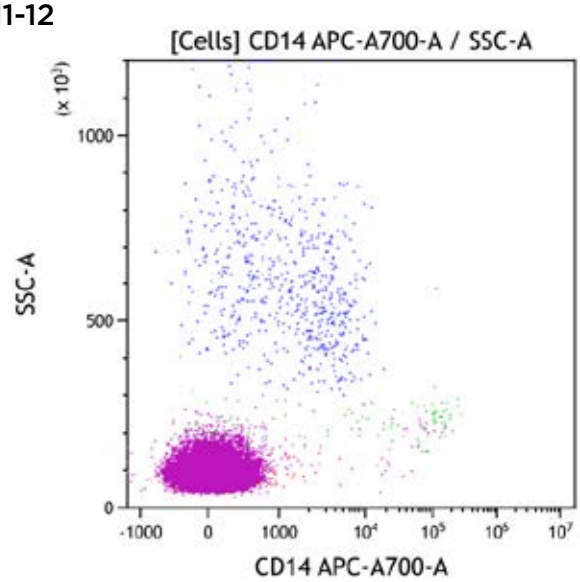

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a low level on mature granulocytes (blue). The aberrant population (purple) is negative for CD14.

BACK TO CASE OVERVIEW

M1-13

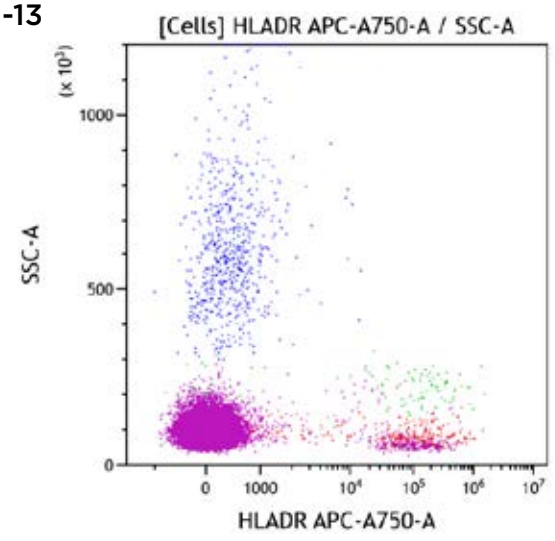

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. The aberrant population (purple) is negative for HLADR.

M1-14

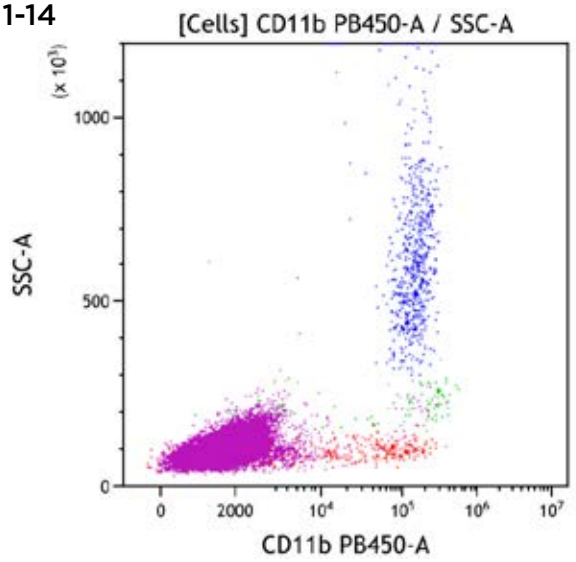

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes (blue) and monocytes (green). CD11b is also expressed on NK cells (red) and basophils. The aberrant population (purple) is negative for CD11b.

M1-15

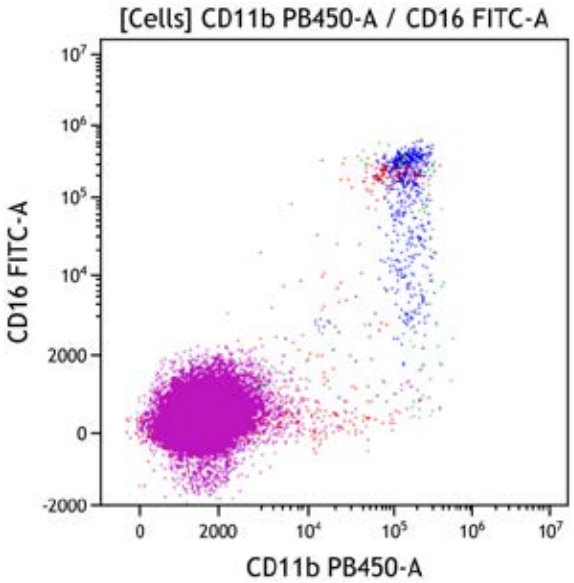

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, granulocytes (blue) and a subset of NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). Activated mature monocytes express CD16 at a variable level and are CD11b positive.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

M1-16

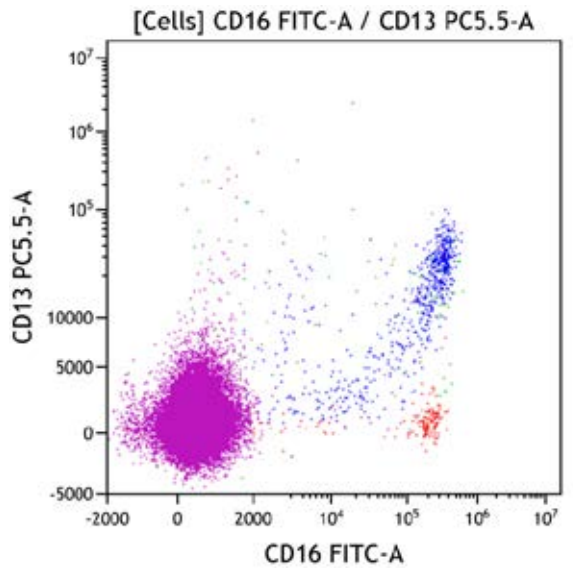

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is negative for CD16 and CD13.

BACK TO CASE OVERVIEW

M1-17

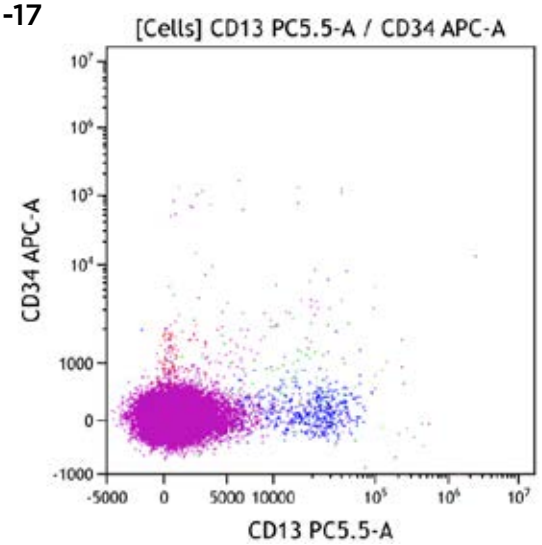

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive progenitors or mature lymphocytes. The aberrant population (purple) is negative for CD34.

M1-18

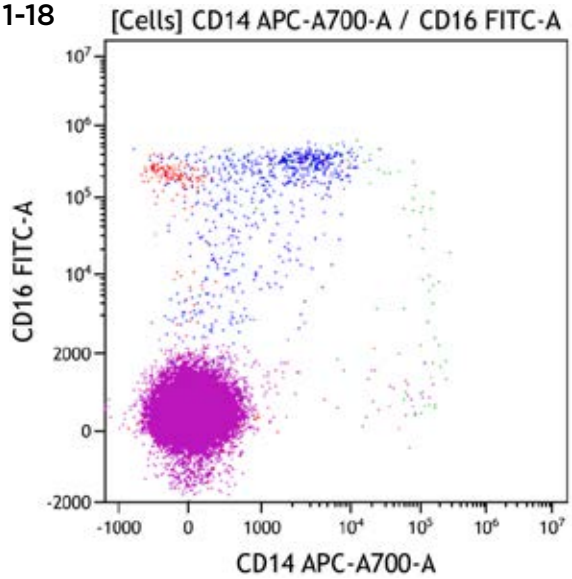

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on granulocytes. Activated mature monocytes express CD14 and CD64 at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

M1-19

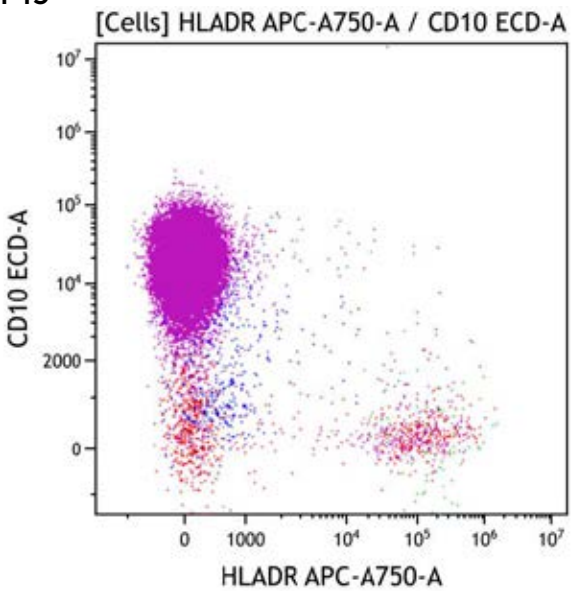

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue). The aberrant population (purple) expresses CD10 and is negative for HLADR.

M1-20

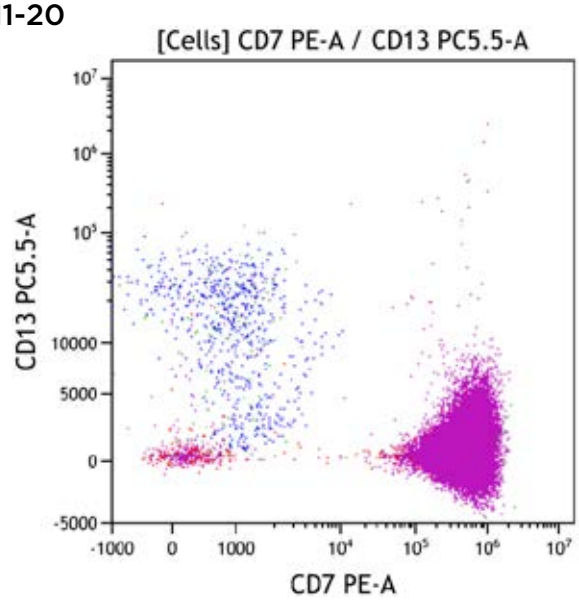

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The aberrant population (purple) expresses bright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

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

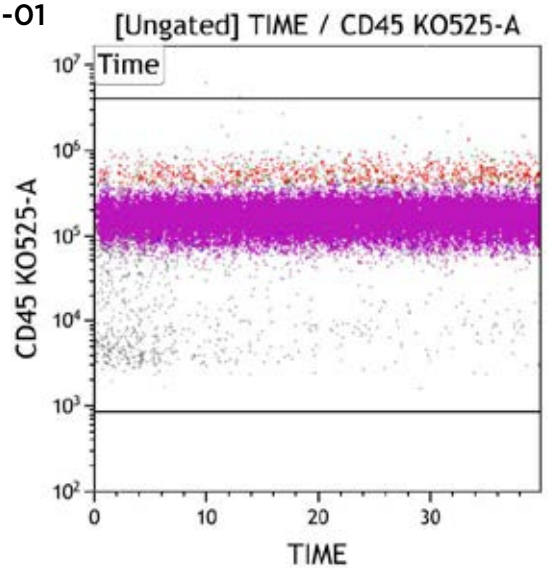

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

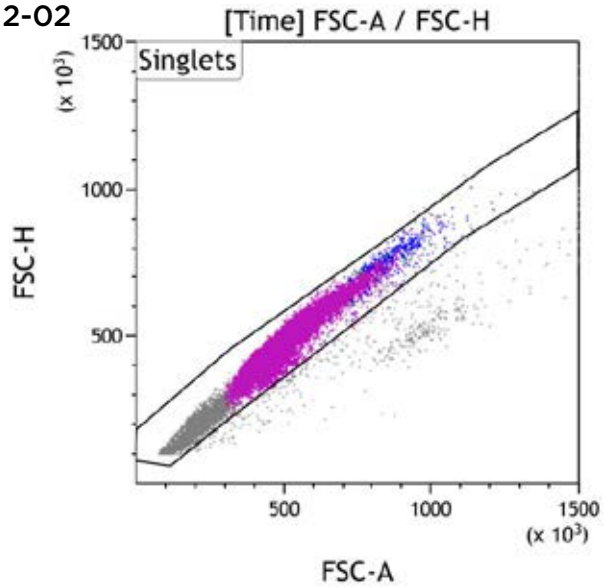

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

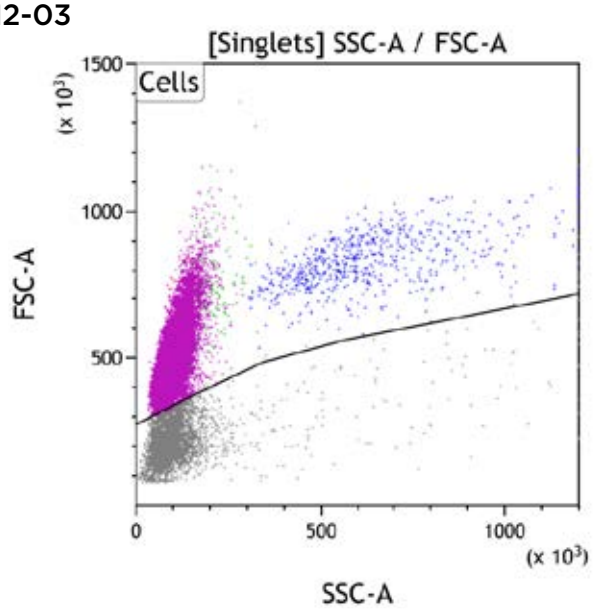

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M2-04

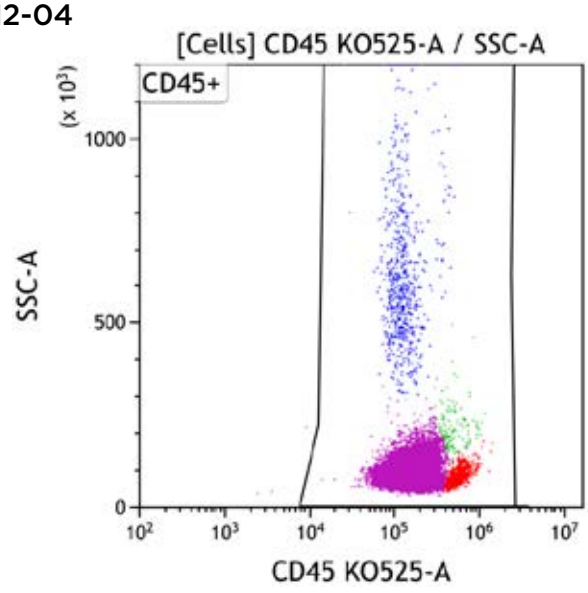

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M2-05

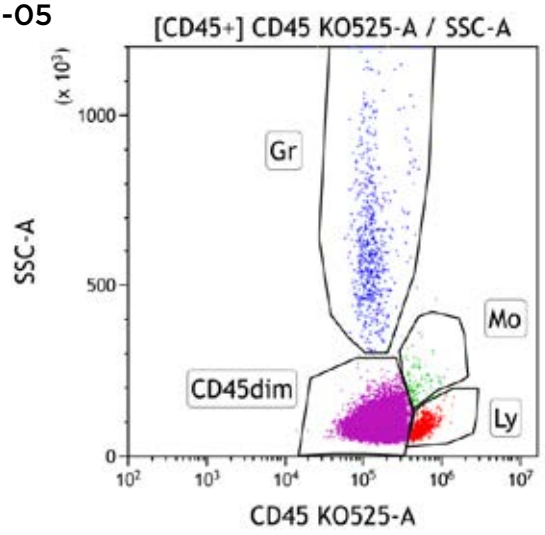

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 in the CD45dim gate (purple) is expanded.

M2-06

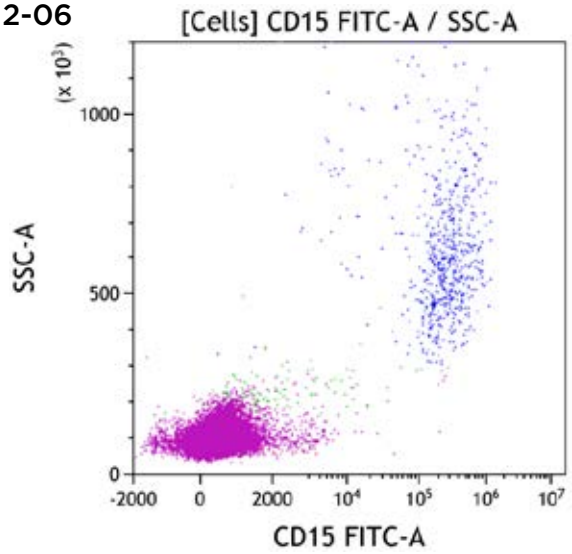

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes. The aberrant population (purple) is negative for CD15.

M2-07

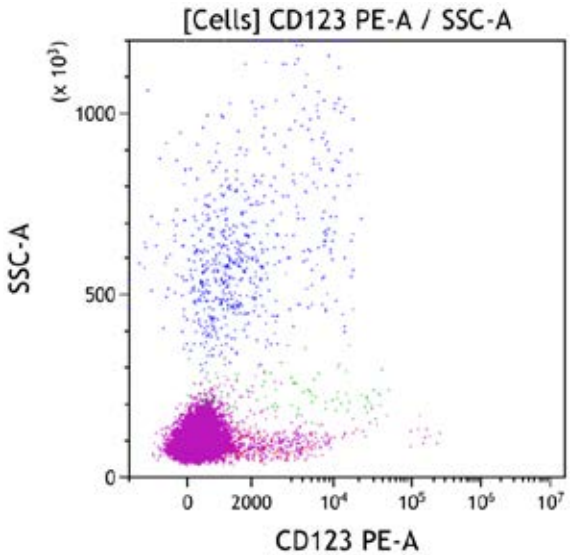

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. The aberrant population (purple) is negative for CD123.

M2-08

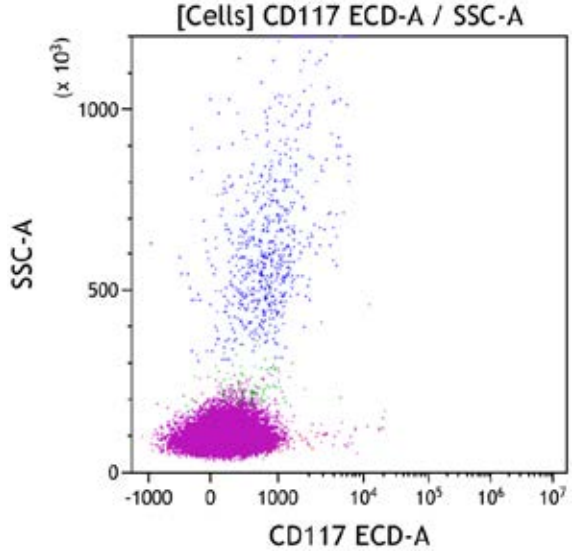

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

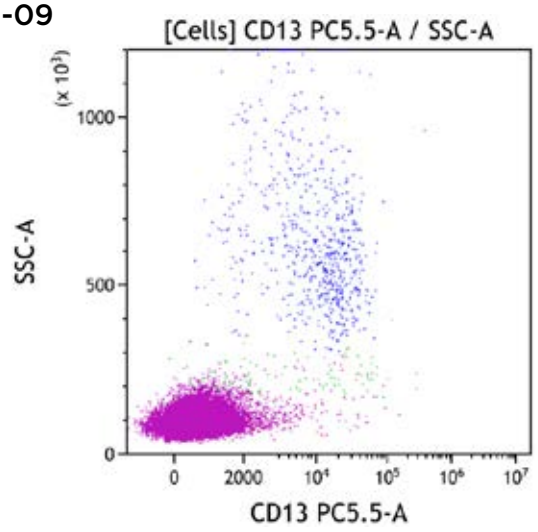

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and variably on myeloid progenitors. The aberrant population (purple) is negative for CD13.

M2-10

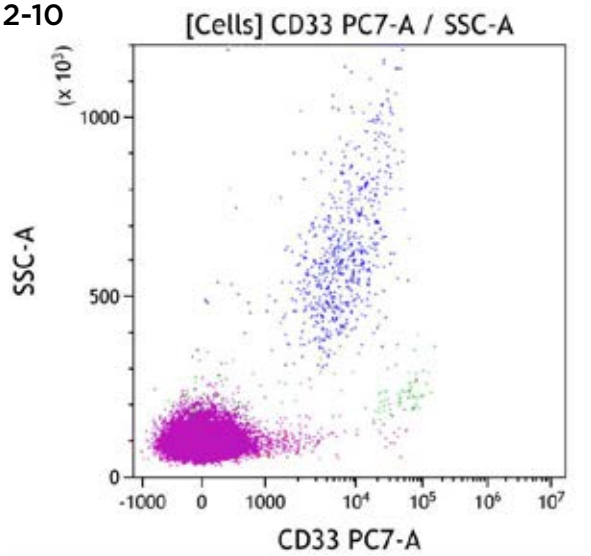

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. The aberrant population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

M2-11

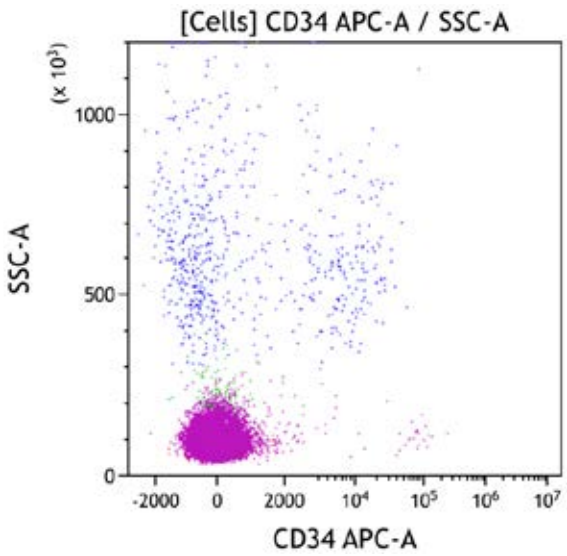

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45dim gate. The aberrant population (purple) is negative for CD34.

M2-12

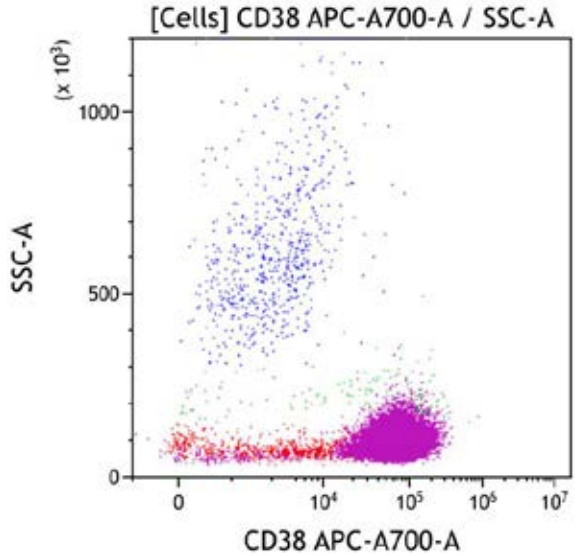

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes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

BACK TO CASE OVERVIEW

M2-13

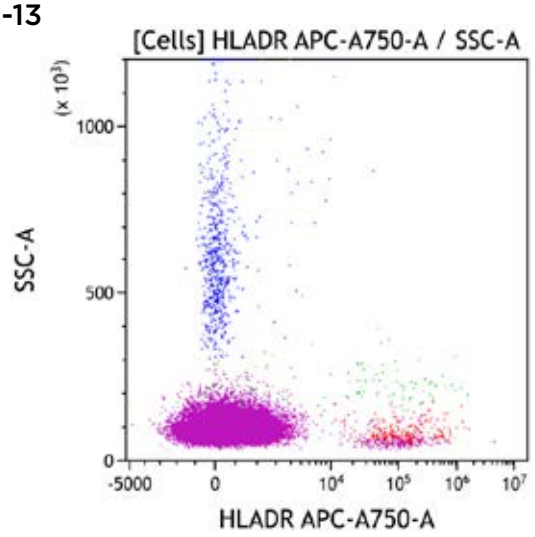

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells, and activated T cells. The aberrant population (purple)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

M2-14

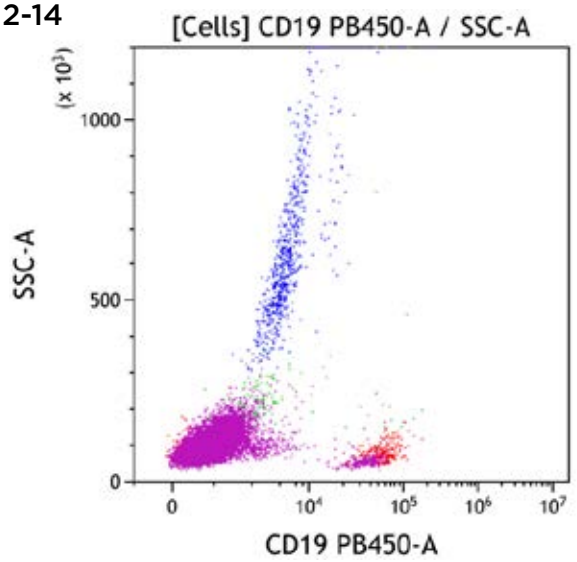

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red), as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is negative for CD19.

M2-15

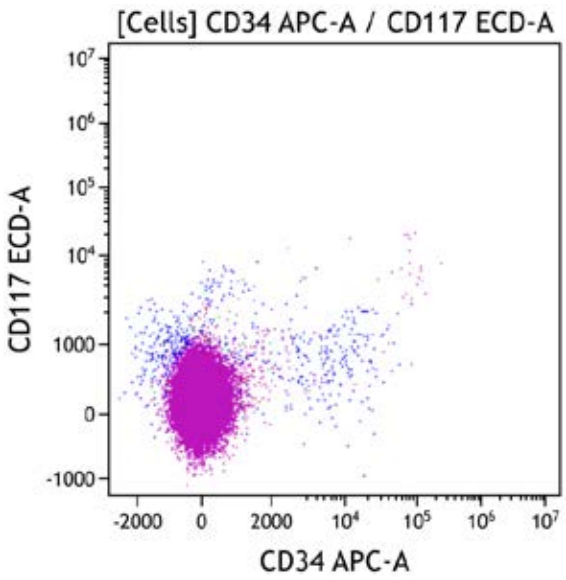

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The aberrant population (purple) is negative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

M2-16

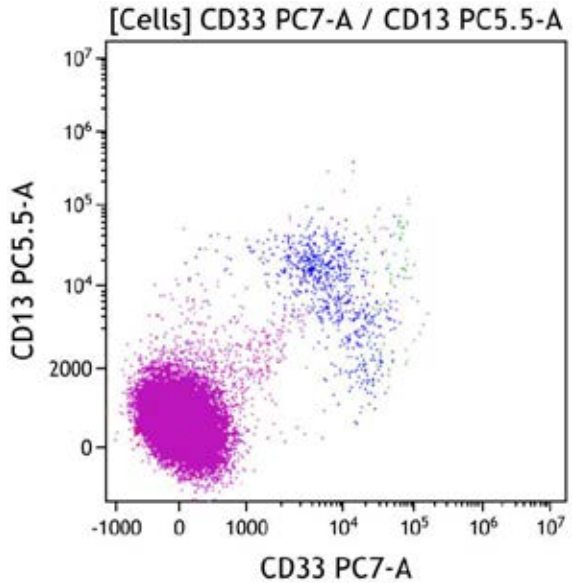

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, granulocytes (blue), basophils, and CD34 positive progenitors. The aberrant population (purple) is negative for CD13 and CD33.

BACK TO CASE OVERVIEW

M2-17

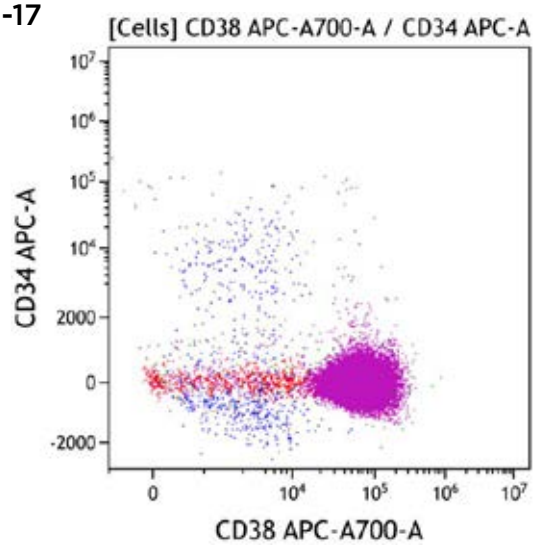

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. The aberrant population (purple) expresses CD38 and is negative for CD34.

M2-18

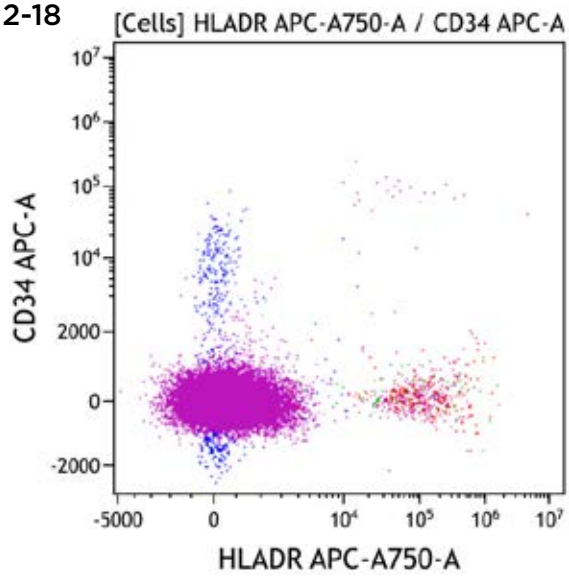

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. The aberrant population (purple) is negative for HLA-DR and CD34.

M2-19

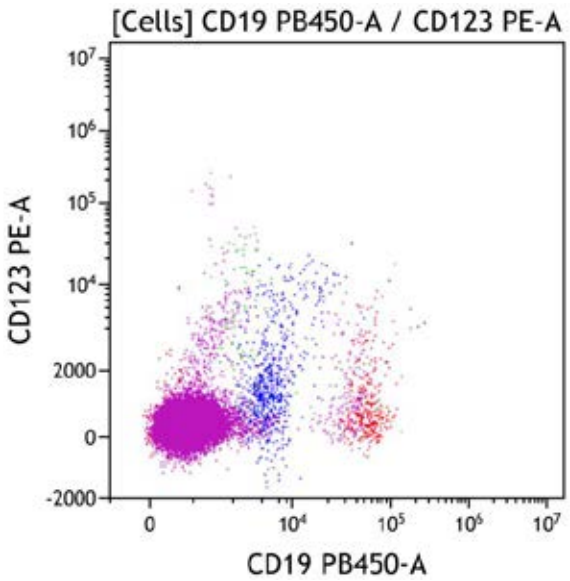

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red) normally do not express significant CD123. The apparent CD19 positivity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

M2-20

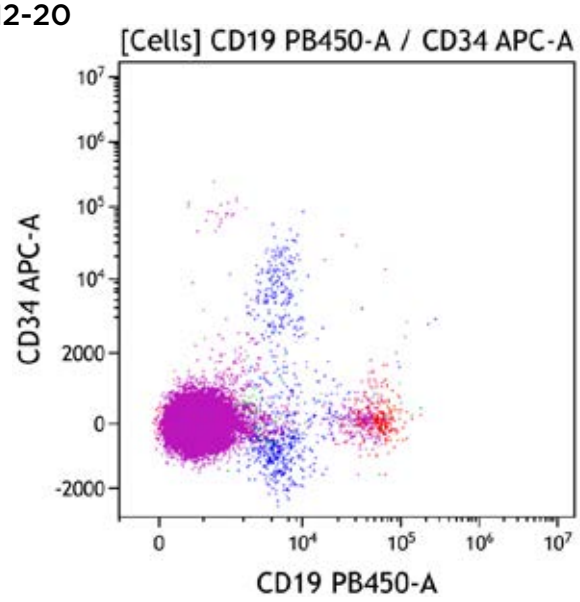

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. CD34 positive progenitors do not express CD19. The aberrant population (purple) is negative for CD19 and CD34.

BACK TO CASE OVERVIEW

M2-21

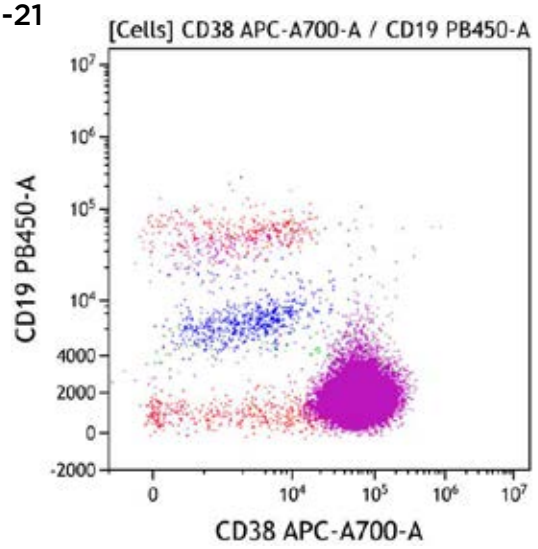

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show low level expression of CD38. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence,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

M2-22

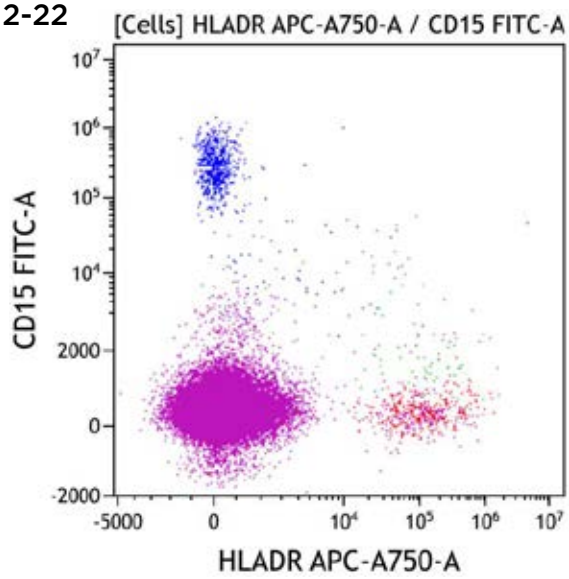

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do no express HLA-DR. CD34 positive myeloid progenitors express HLA-DR. The aberrant population (purple) is negative for HLA-DR and CD15.

M2-23

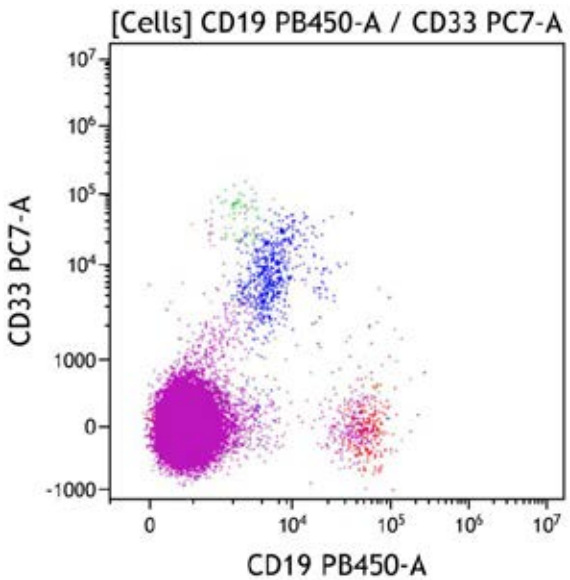

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not normally express significant CD33. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence,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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 6 MLL-TC4-008**
Diagnosis	**T cell lymphoproliferative disorder**
Clinical Vignette	This 58-year-old female presents with atypical cells. A peripheral blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of variable CD2, intermediate CD3, variable CD5, variable CD7, bright CD8, and bright CD45 without CD56. 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 is aberrant. The immunophenotype of the abnormal population is consistent with expanded abnormal T-cell large granular lymphocytes. In the appropriate clinical context of prolonged cytopenias without other identifiable etiologies, this finding would be consistent with T-cell large granular lymphocytic leukemia.

BACK TO CASE LISTINGS

B Cell Tube

B01

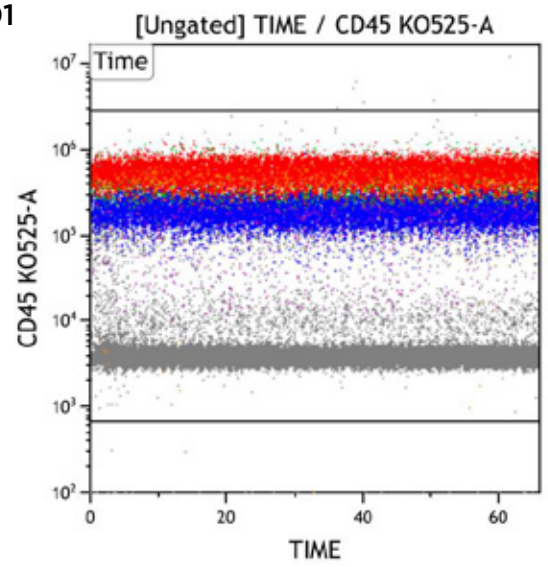

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

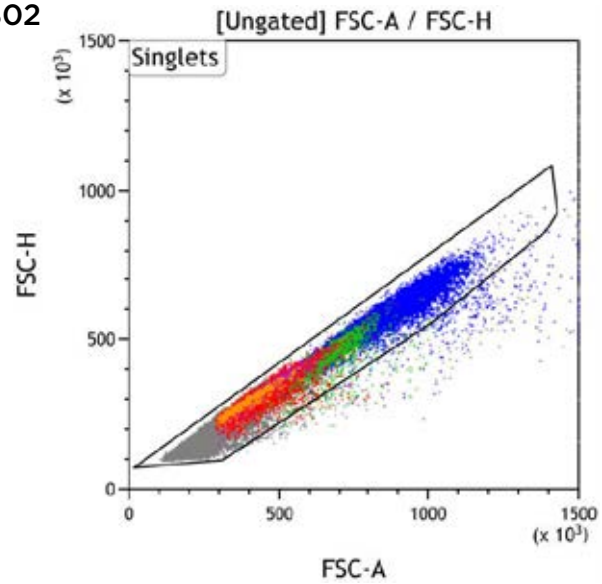

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended ton exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

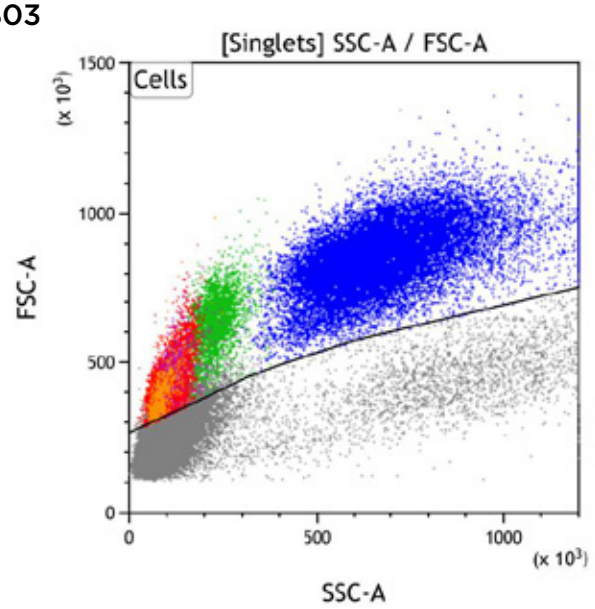

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

B04

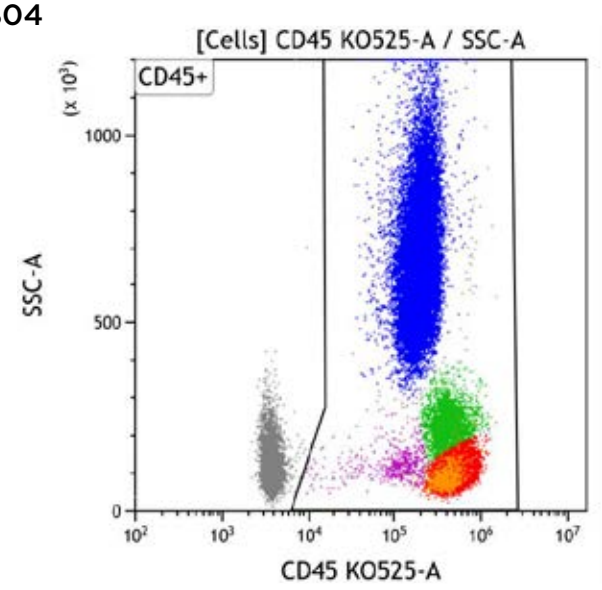

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, or non-hematopoietic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

BACK TO CASE OVERVIEW

B05

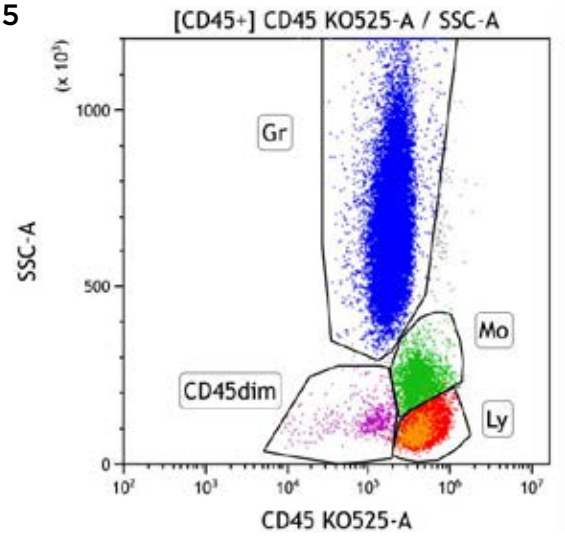

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 followed throughout the analysis.

B06

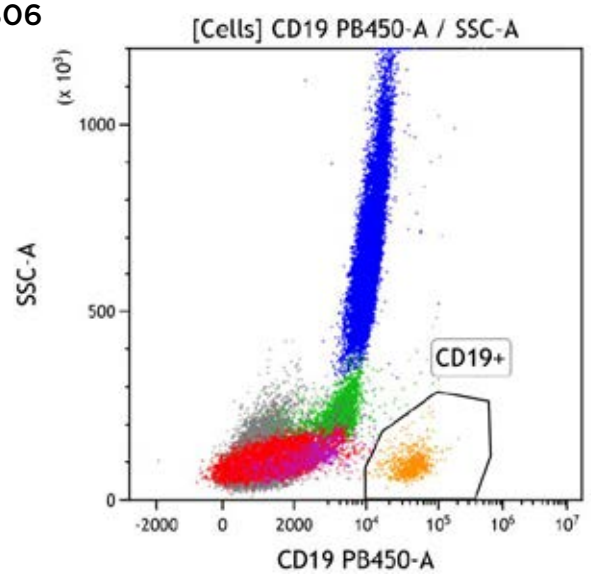

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Most lymphocytes (red) are negative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

B07

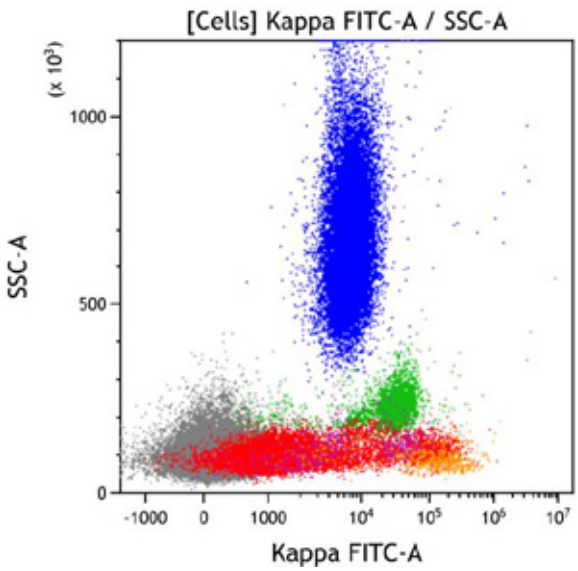

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity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

B08

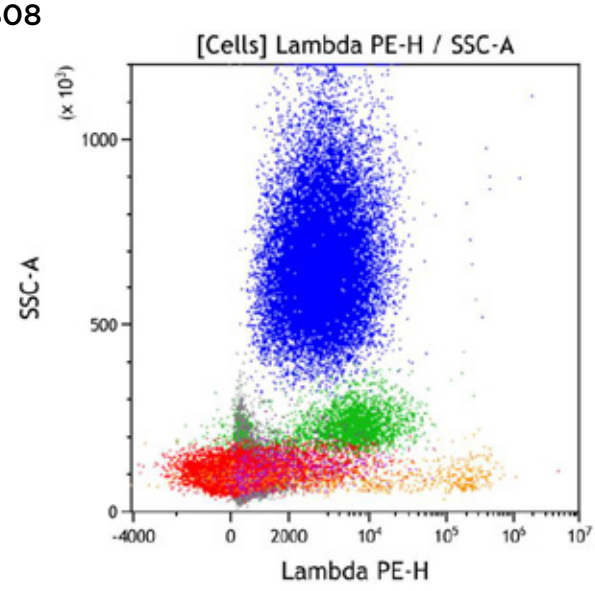

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity 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 (red) are negative for surface lambda light chain.

BACK TO CASE OVERVIEW

B09

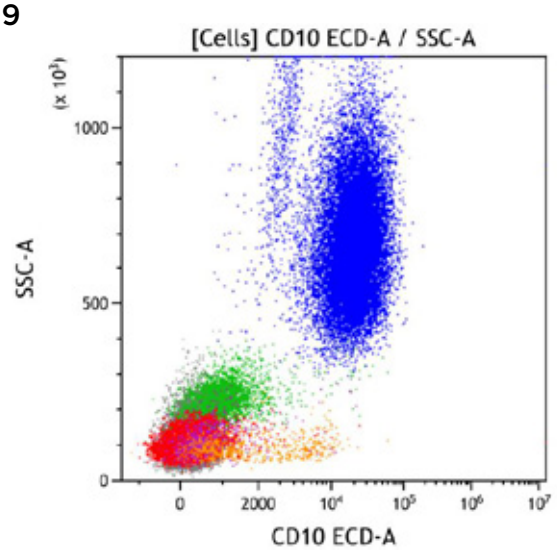

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Most lymphocytes (red) are negative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

B10

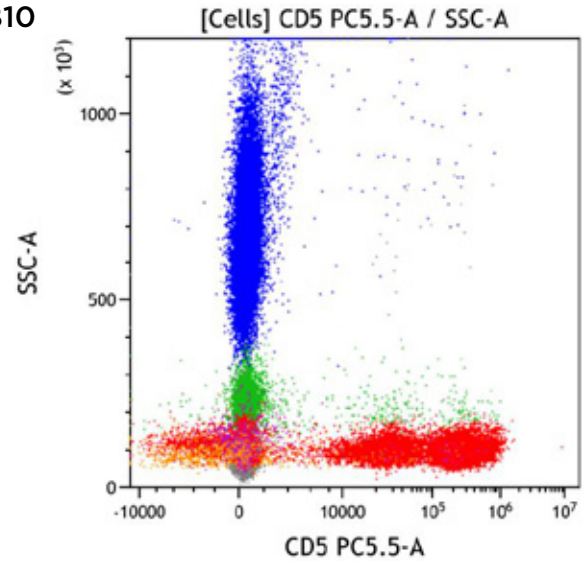

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cellsM (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. Most lymphocytes (red) express variable CD5.

B11

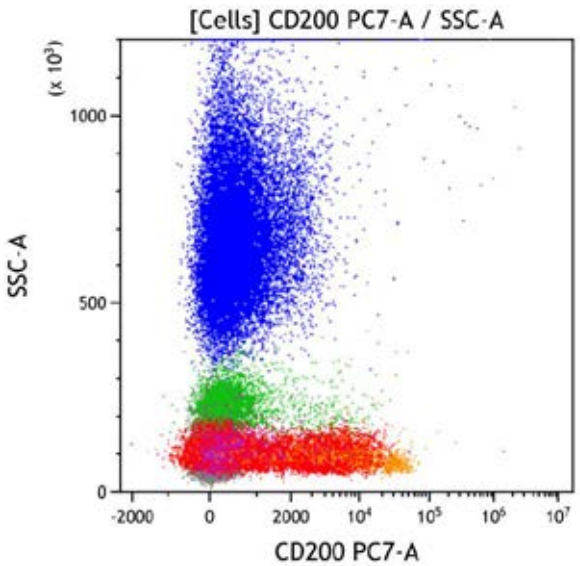

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

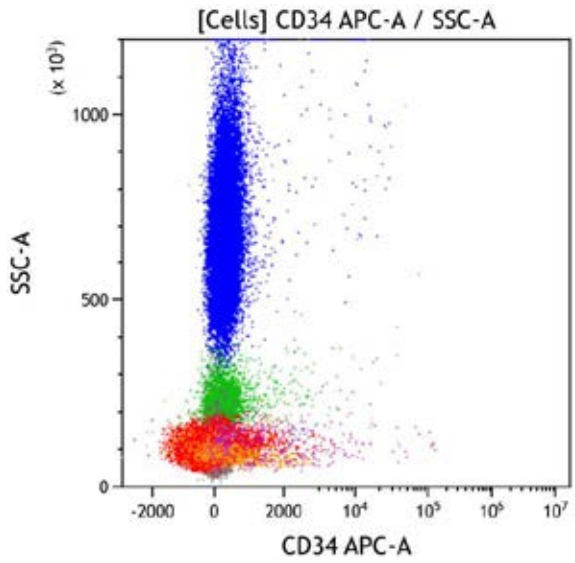

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45dim gate. Most lymphocytes (red) are negative for CD34.

BACK TO CASE OVERVIEW

B13

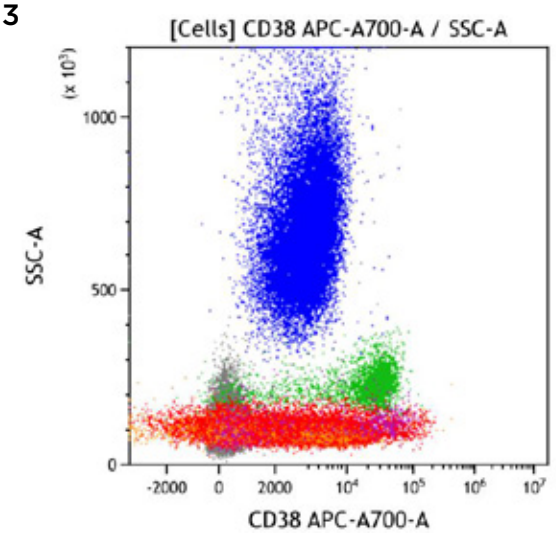

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably level on activated mature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

B14

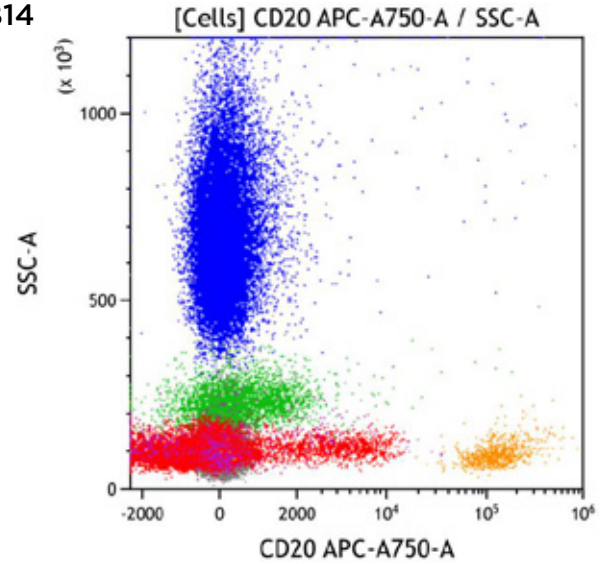

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side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

B15

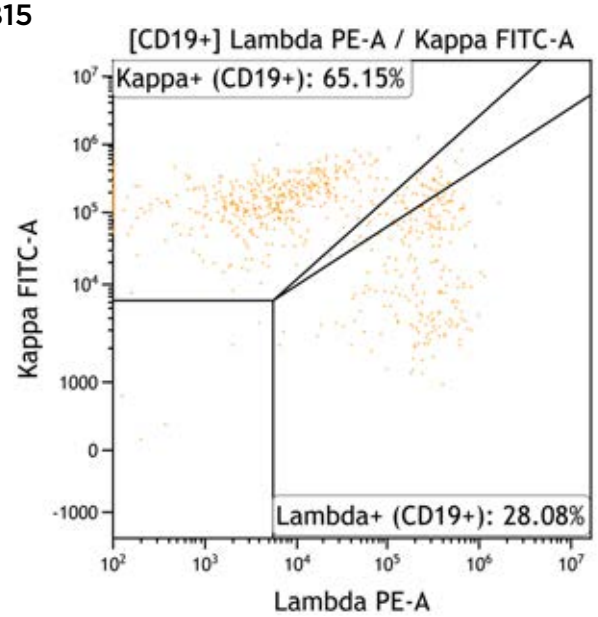

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

B16

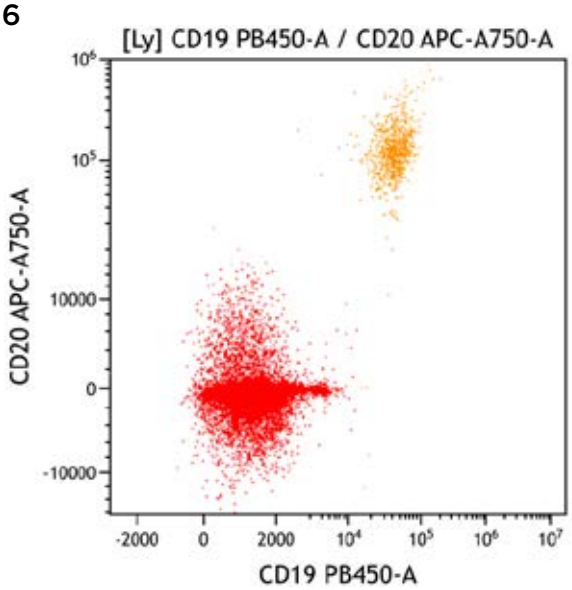

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

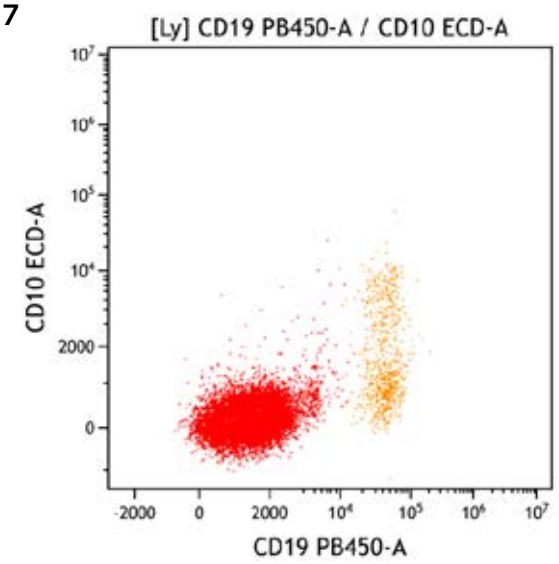

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of latestage immature B cells is present in peripheral blood and bone marrow aspirates.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

B18

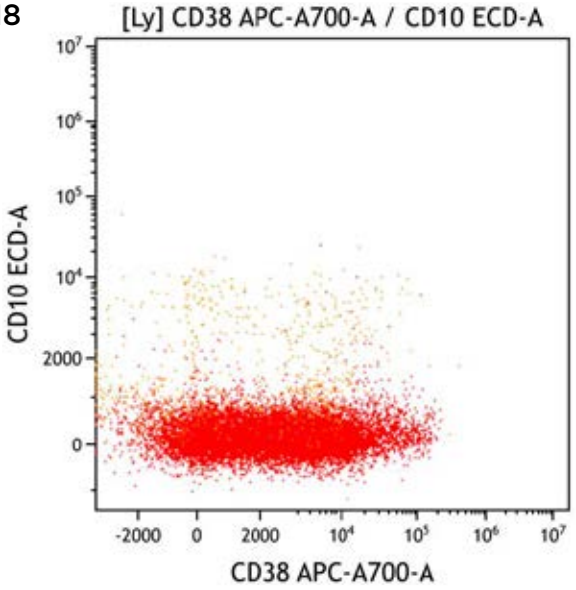

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinalM center B cells. Most mature B cells (orange) display low level expression ofMCD38. T cells (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. Most lymphocytes (red) express dim CD38 but not CD10.

B19

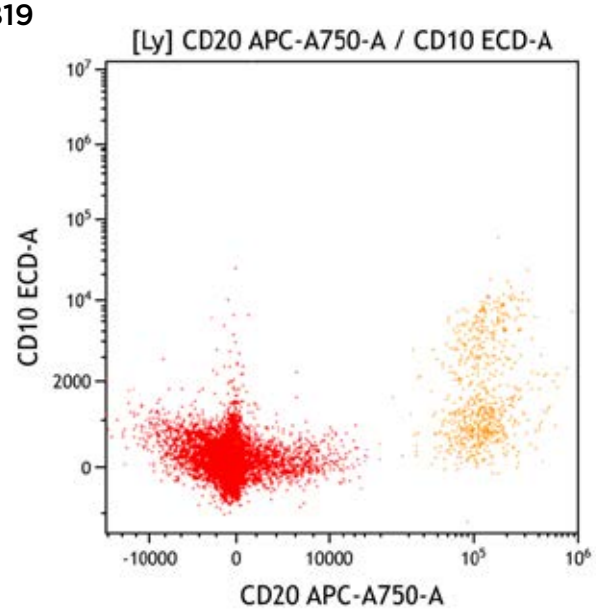

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20 without significant CD10. The few CD10 positive B cells represent late-stage immature B cells.

B20

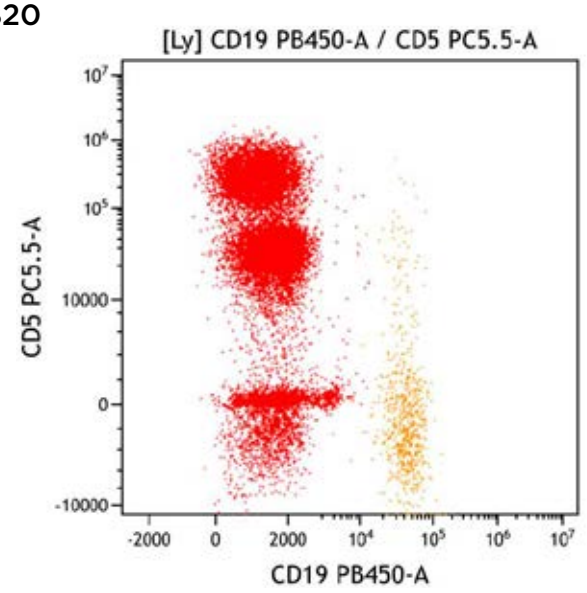

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells. Most lymphocytes (red) express CD5, but two discrete subpopulations with different levels of CD5 are present, the lower level of CD5 being abnormal for mature T cells.

BACK TO CASE OVERVIEW

B21

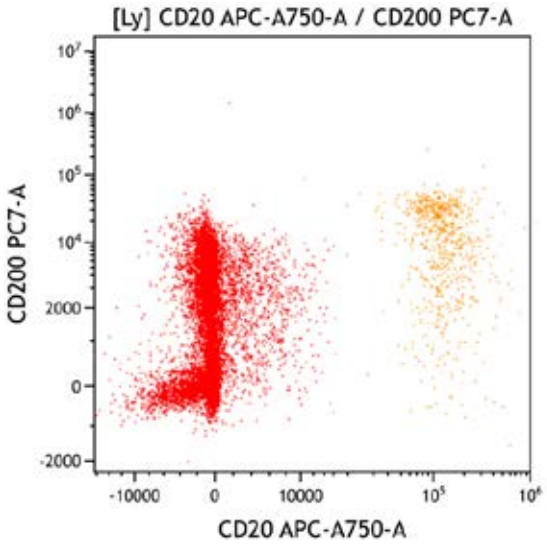

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

B22

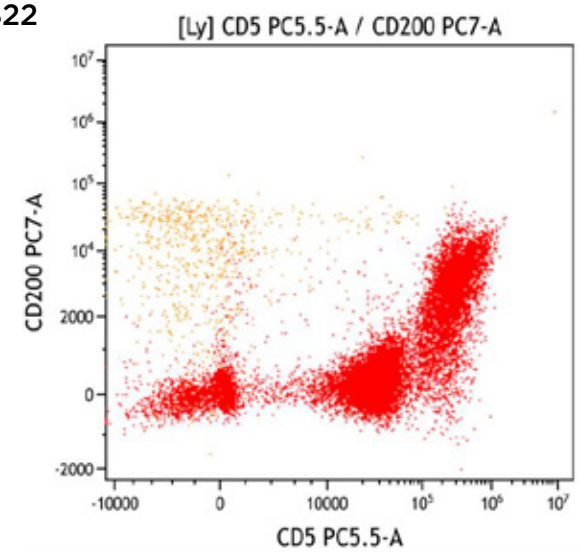

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. Most lymphocytes (red) express CD5, but two discrete subpopulations with different levels of CD5 are present, the lower level of CD5 being abnormal for mature T cells.

BACK TO CASE OVERVIEW

T Cell Tube

T01

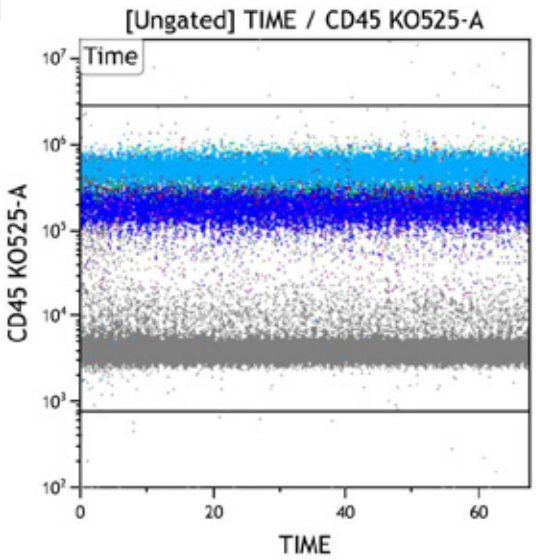

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

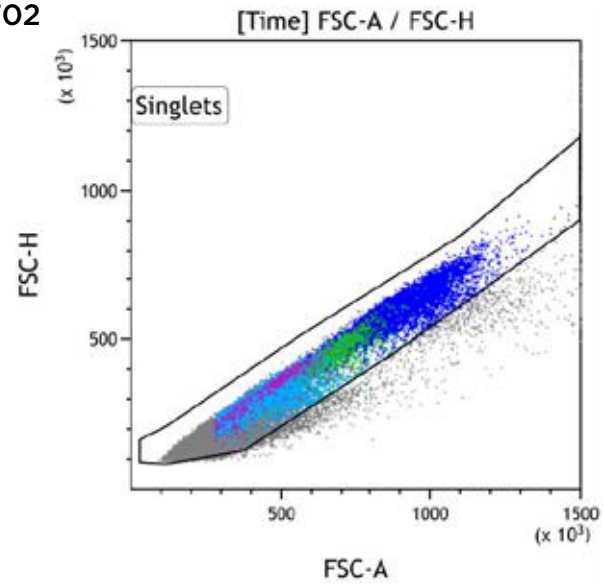

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

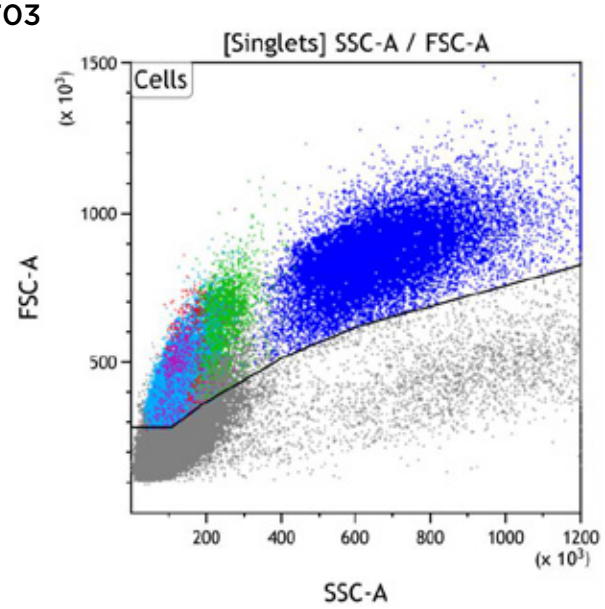

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter withincreased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

T04

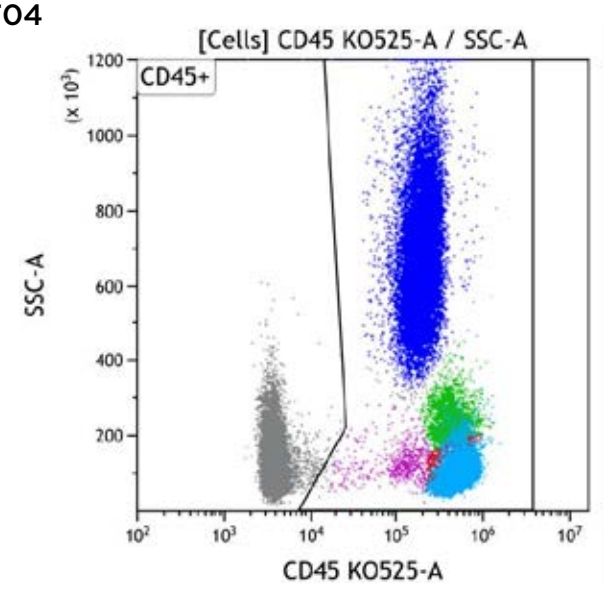

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

T05

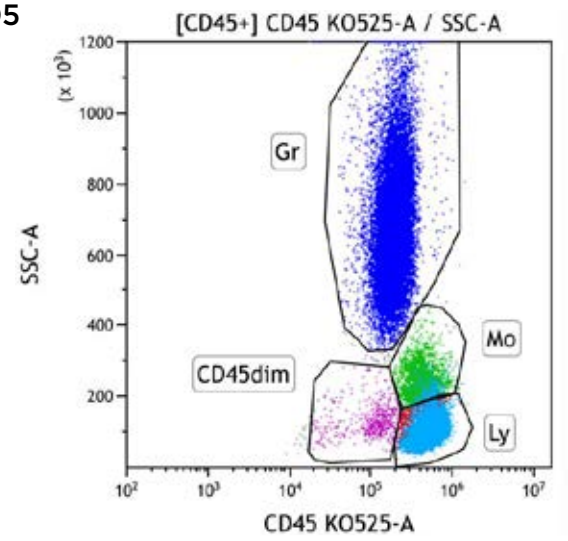

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

T06

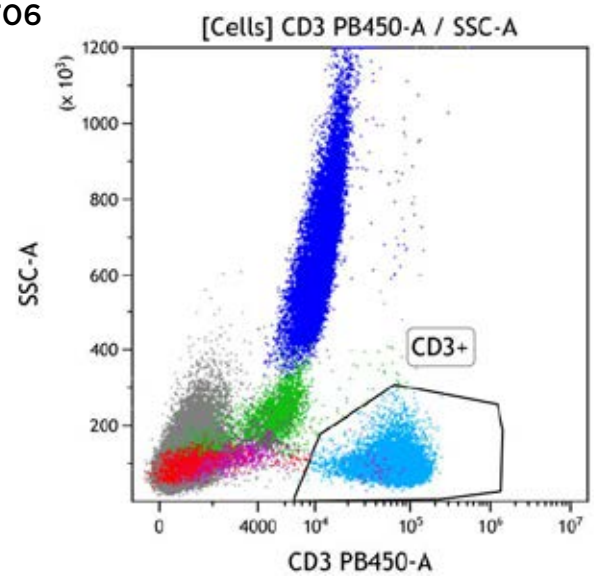

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

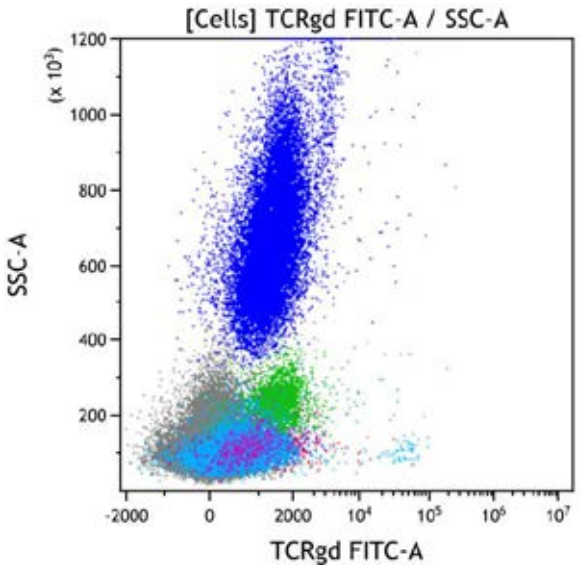

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). Most T cells are negative for TCRγδ.

T08

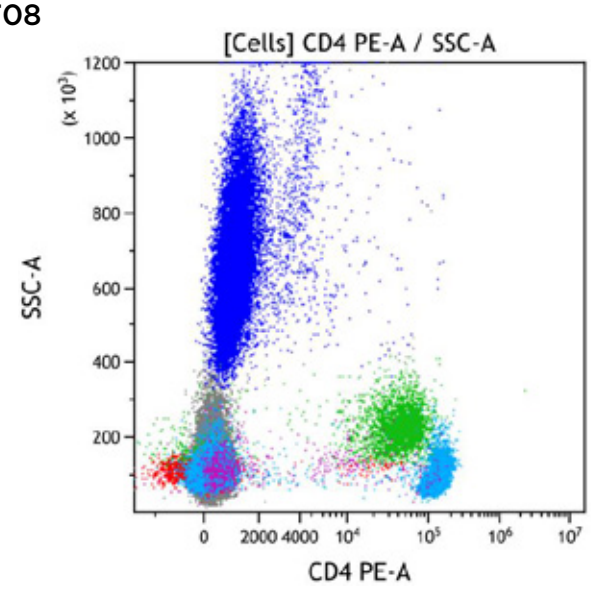

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells.

BACK TO CASE OVERVIEW

T09

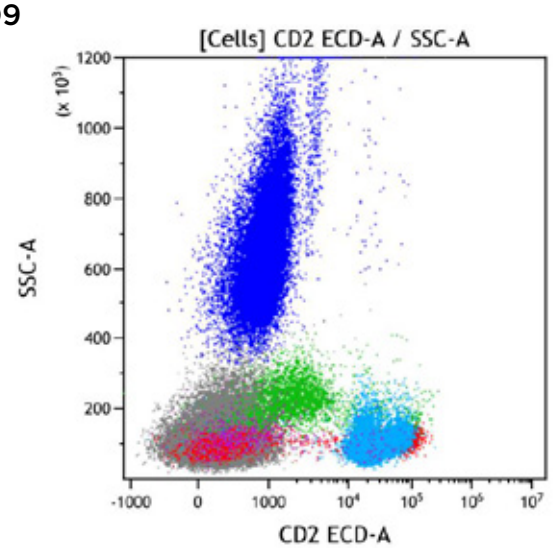

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

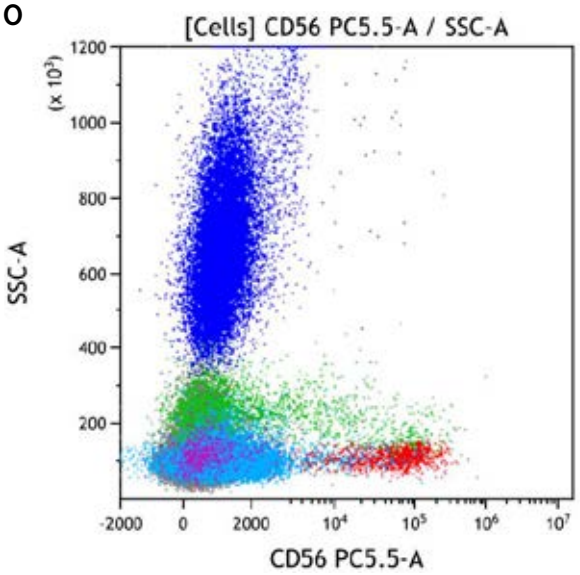

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytes (green) in both reactive and neoplastic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

T11

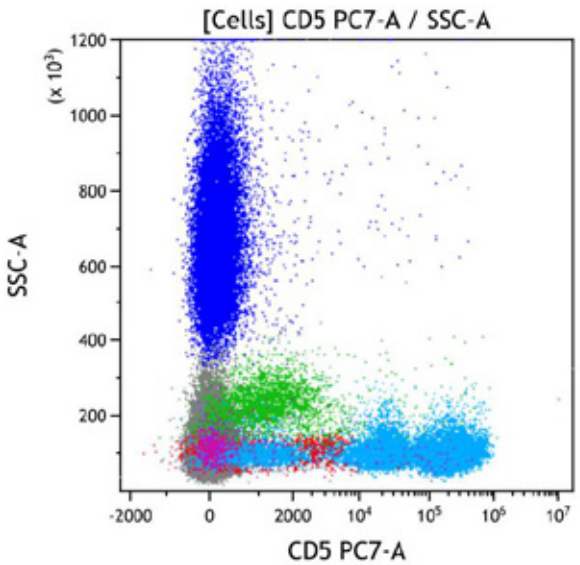

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cellsN (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

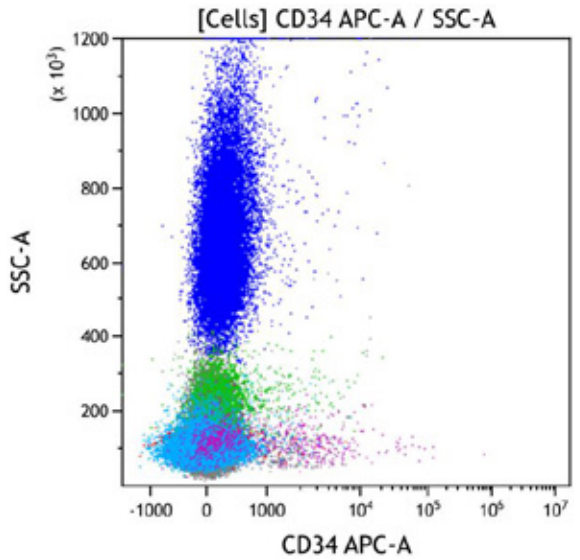

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). T cells (aqua) are negative for CD34.

BACK TO CASE OVERVIEW

T13

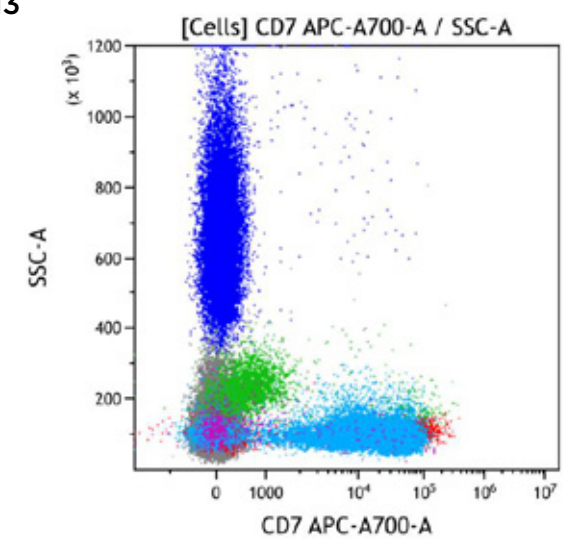

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

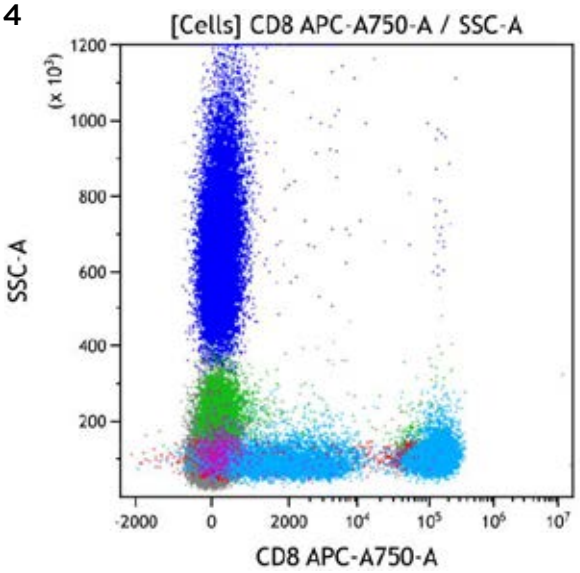

Figure 14. This CD8 vs Side Scatter dot plot shows all viablecells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells. A major subset of T cells (aqua) expresses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

T15

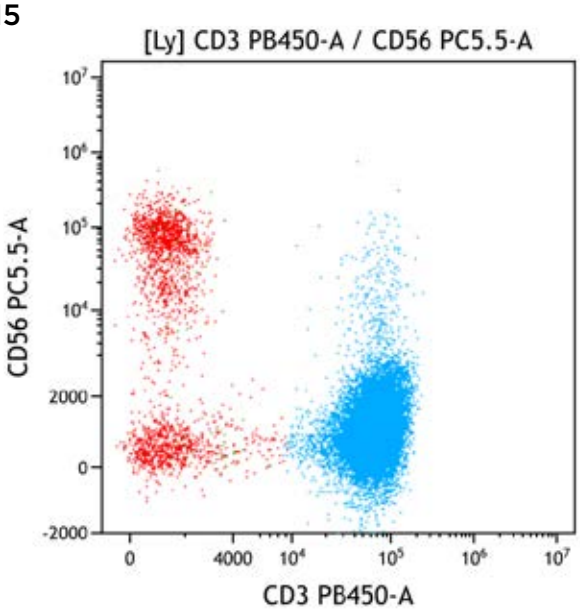

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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. Most T cells (aqua) are negative for CD56.

T16

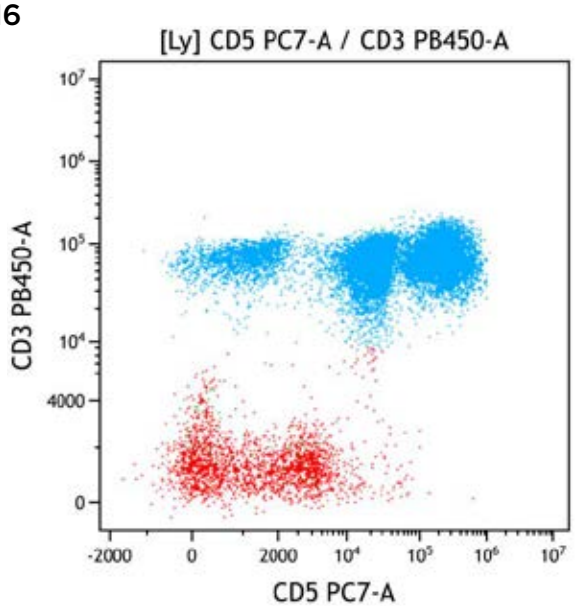

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

BACK TO CASE OVERVIEW

T17

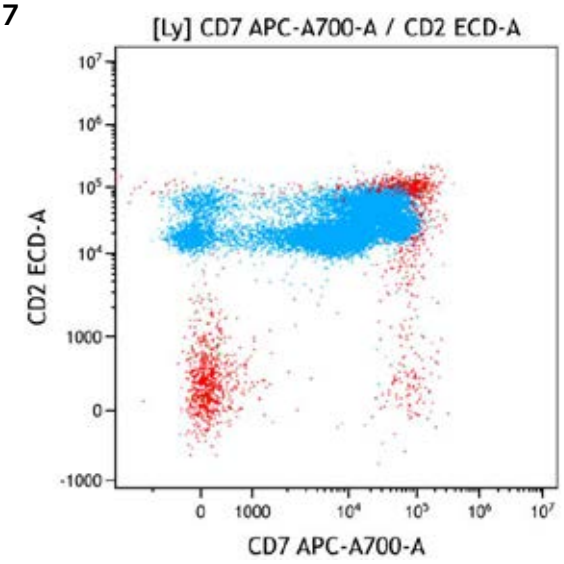

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells (red). CD2 and CD7 are variably decreased on a subset of T cells.

T18

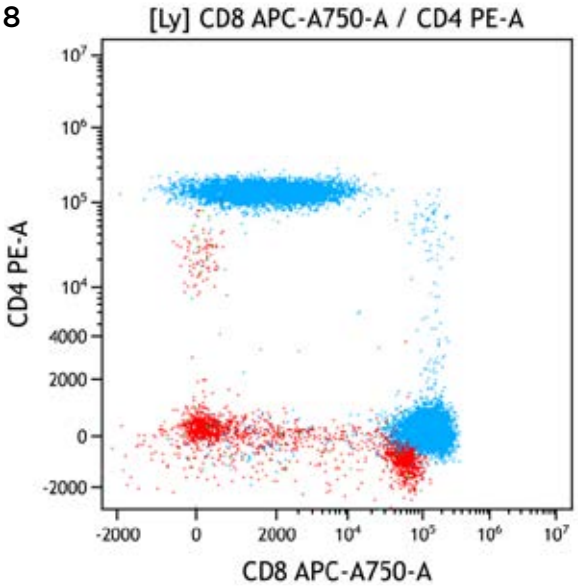

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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. CD8 positive T cells are proportionally increased.

T19

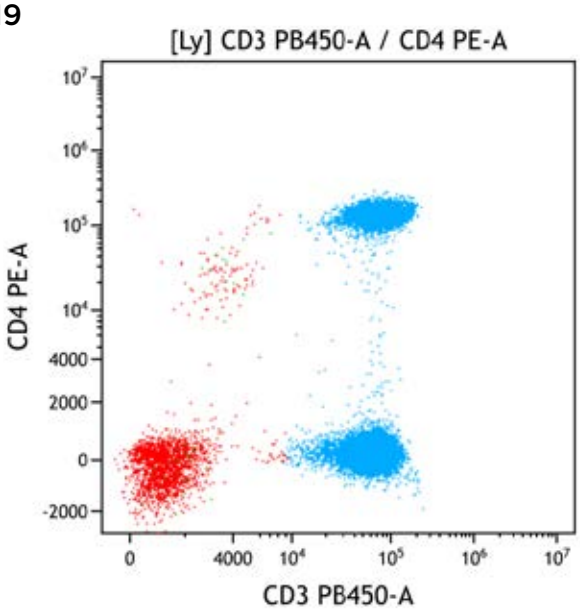

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells (red) lack expression of both CD3 and CD4. Of note, the fewMCD4 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.

T20

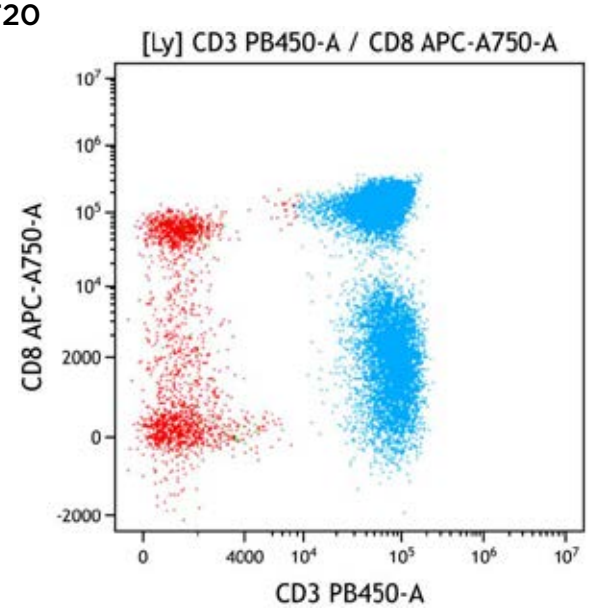

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3. CD8 positive T cells are proportionally increased.

BACK TO CASE OVERVIEW

T21

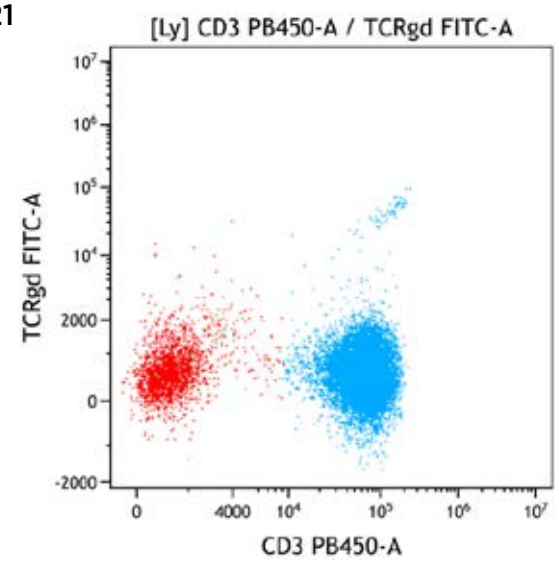

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/ delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other. Most T cells (aqua) do not express TCRγδ, thus are not gamma/ delta T cells.

T22

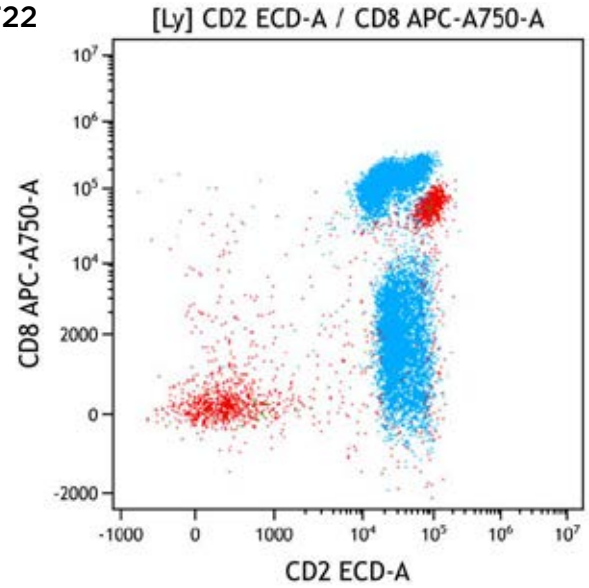

Figure 22. This CD2 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8 negative T cells (aqua, lower middle), a large subset of CD8 positive T cells (aqua, upper left) show aberrantly decreased CD2 expression.

T23

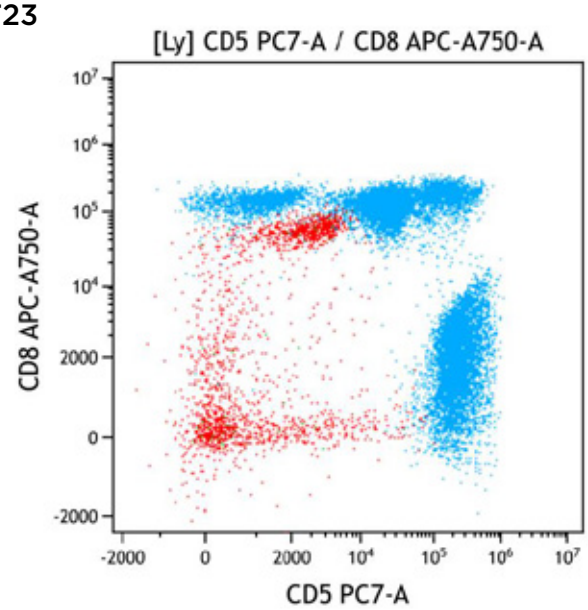

Figure 23. This CD5 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8 negative T cells (aqua, lower right), a large subset of CD8 positive T cells (aqua, upper) show variably decreased CD5 expression.

T24

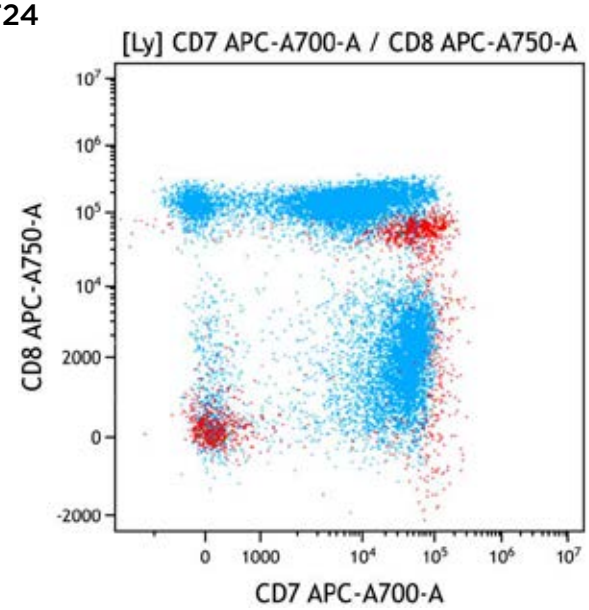

Figure 24. This CD7 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). Compared to the CD8 negative T cells (aqua, lower middle), a large subset of CD8 positive T cells (aqua, upper) show aberrantly decreased CD7 expression.

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

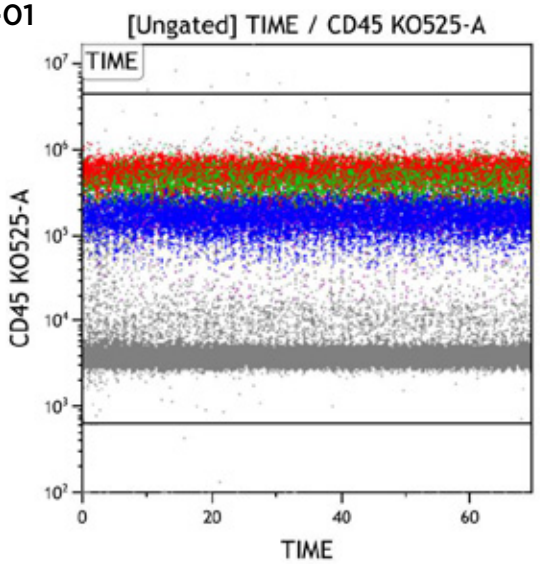

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

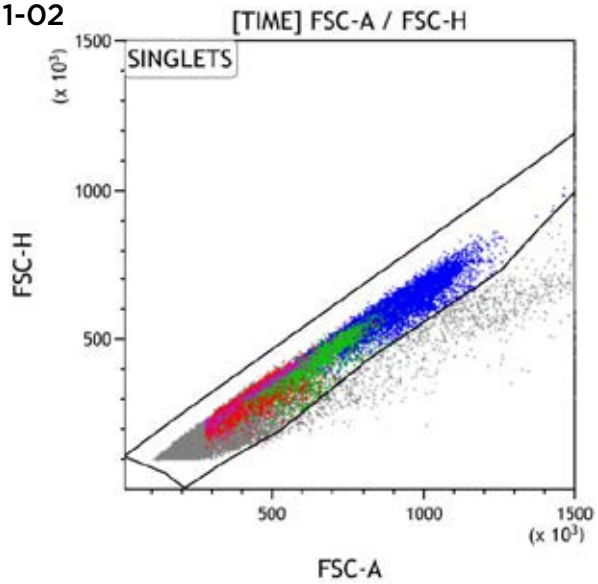

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

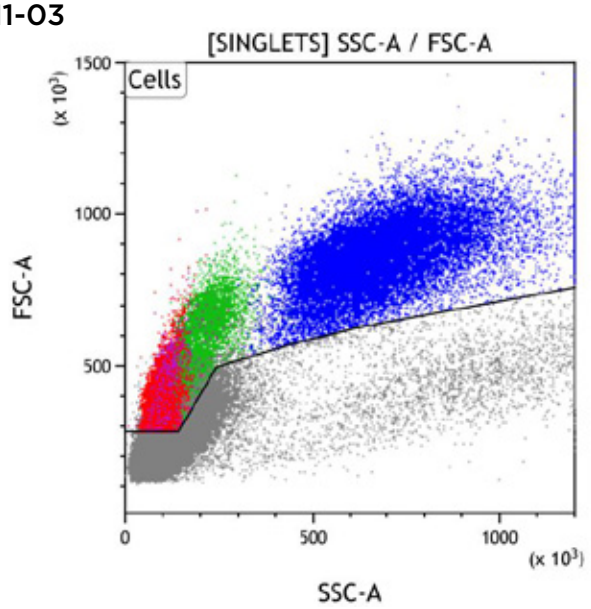

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter withincreased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M1-04

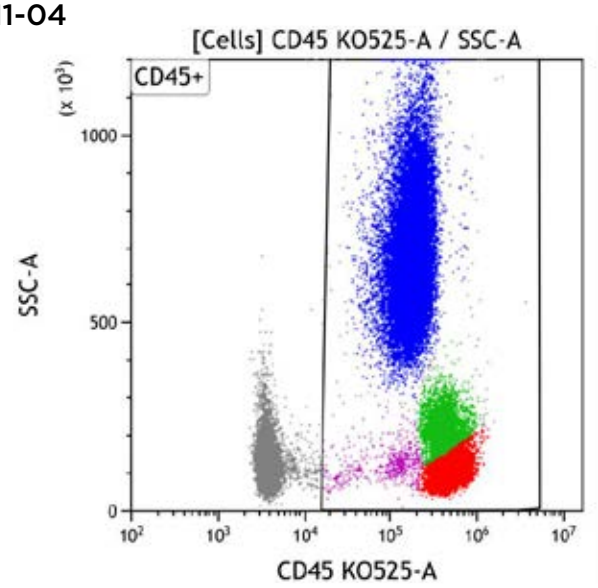

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M1-05

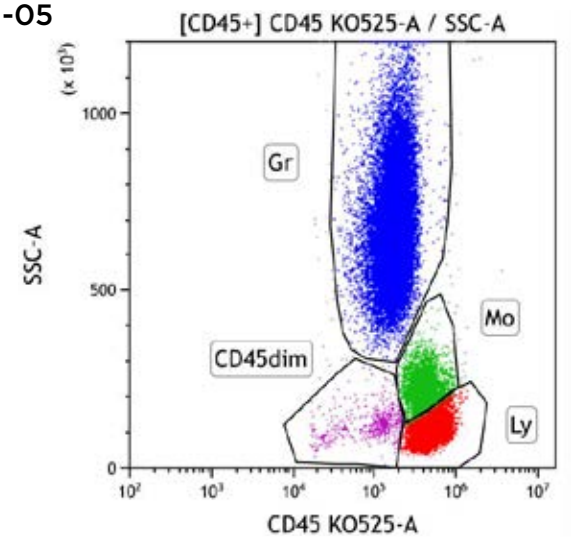

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by by early progenitors (e.g., 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.

M1-06

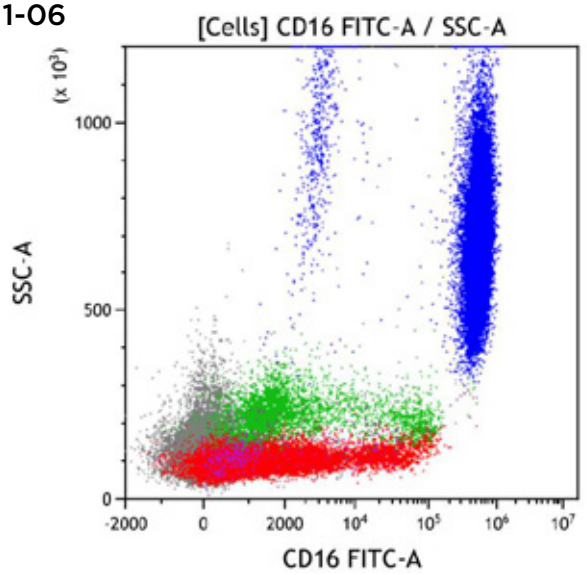

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). CD16 is expressed on a larger subset of lymphocytes (red) than is normal.

M1-07

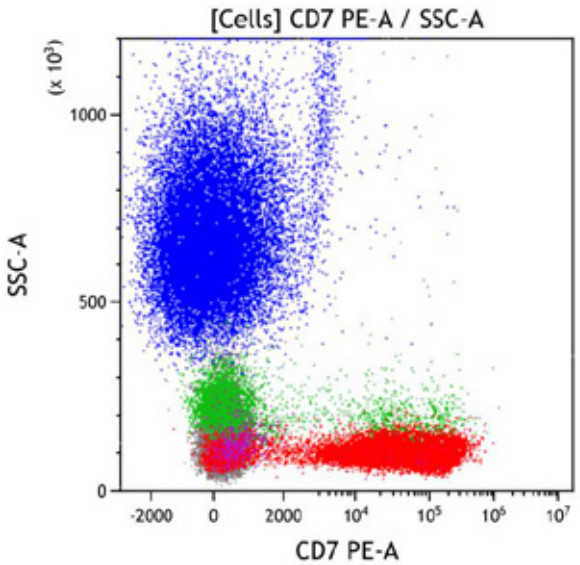

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed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

M1-08

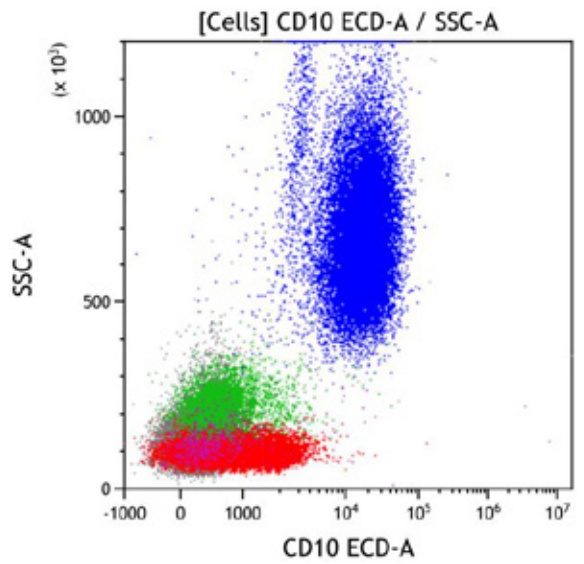

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The lymphocytes (red) are negative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

BACK TO CASE OVERVIEW

M1-09

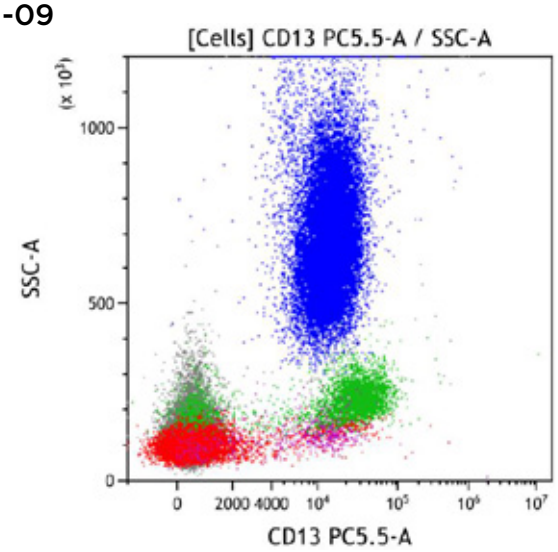

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature granulocytes (blue), mature monocytesB (green), and myeloid progenitors (purple). The lymphocytes (red) are negative for CD13.

M1-10

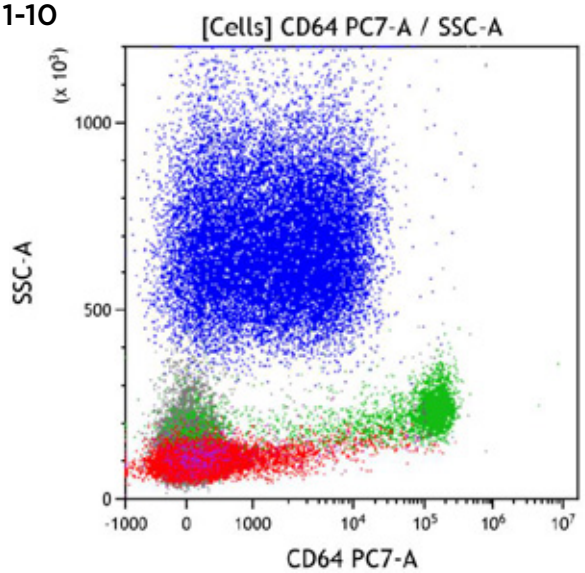

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on monocytes (green). Activated mature monocytes express CD64 at lower level and have lower side scatter. CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression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

M1-11

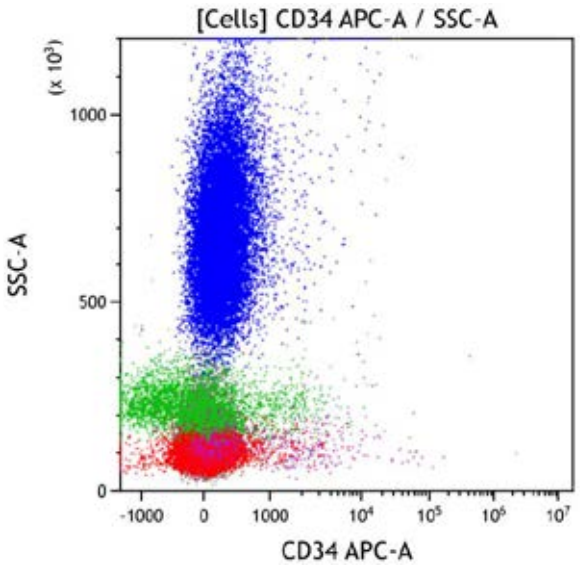

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and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

M1-12

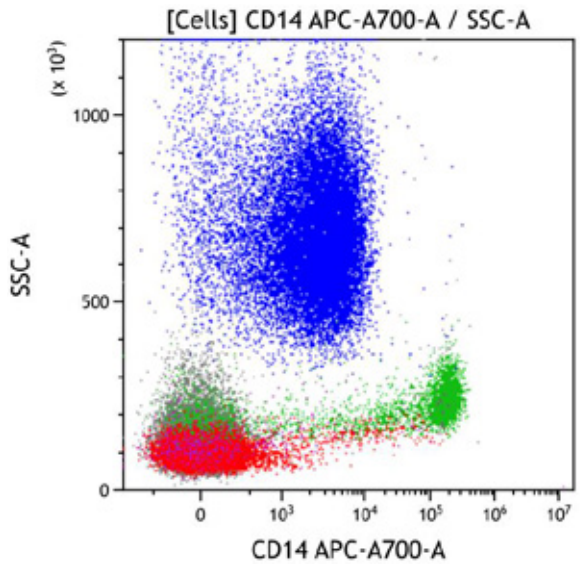

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a low level on mature granulocytes (blue). The lymphocytes (red) are negative for CD14.

BACK TO CASE OVERVIEW

M1-13

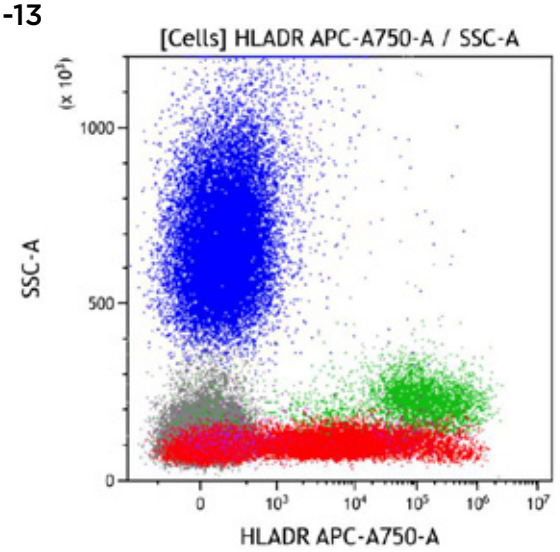

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. HLADR is variably expressed on the lymphocytes (red).

M1-14

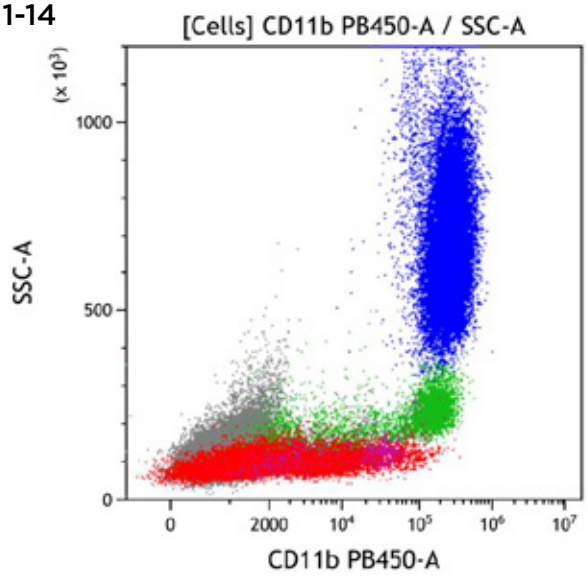

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on monocytes (green). CD11b is also expressed on NK cells (red) and basophils.

M1-15

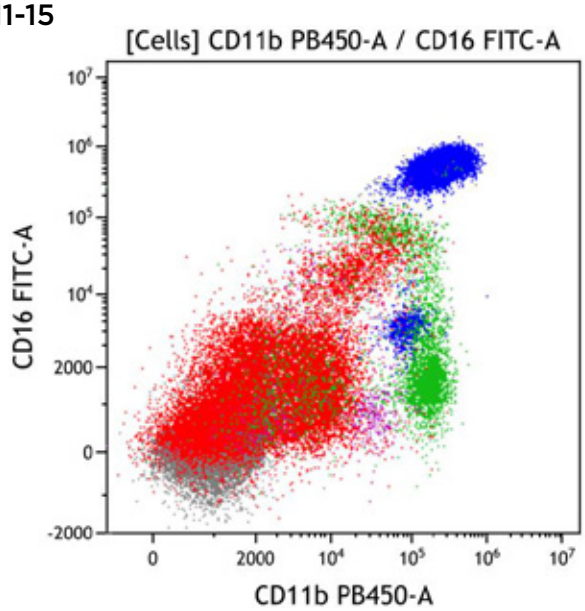

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue) and a subset of NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). Activated mature monocytes express CD16 at a variable level and are CD11b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

M1-16

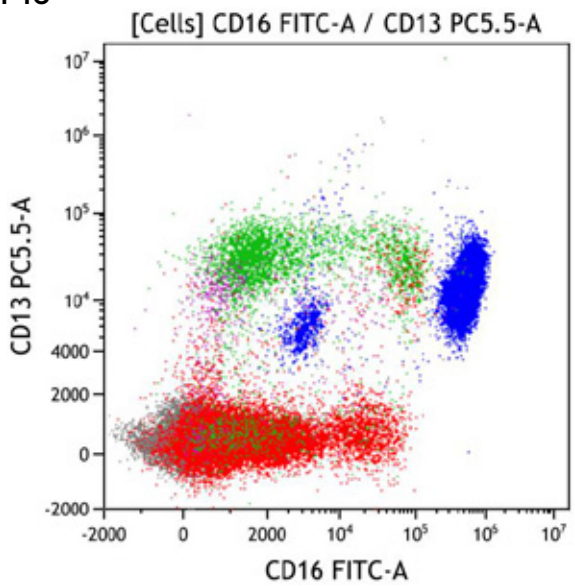

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue), a subset of NK cells (red) and a subset of lymphocytes (red).

BACK TO CASE OVERVIEW

M1-17

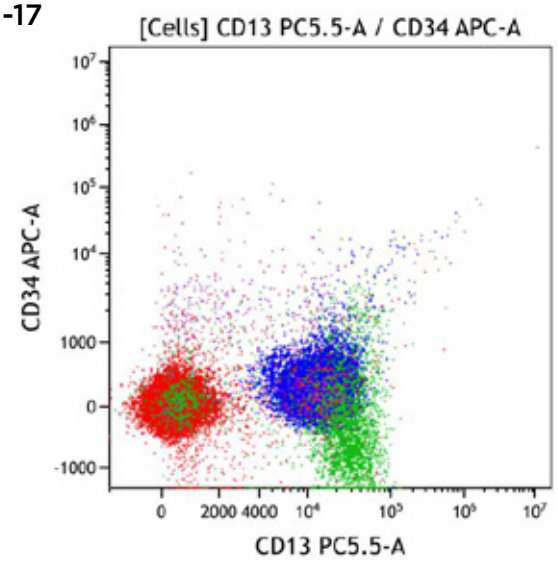

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive progenitors or mature lymphocytes. The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

M1-18

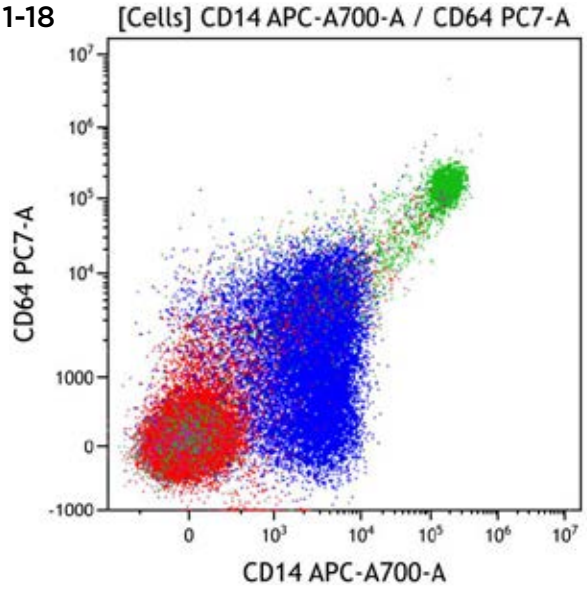

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on granulocytes. Activated mature monocytes express CD14 and CD64 at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

M1-19

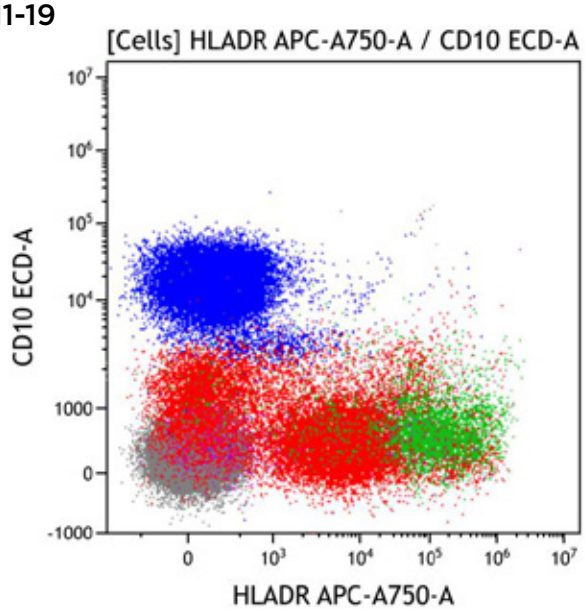

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytesN (green), B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on mature granulocytes (blue). A large subset of lymphocytes (red) expresses HLA-DR.

M1-20

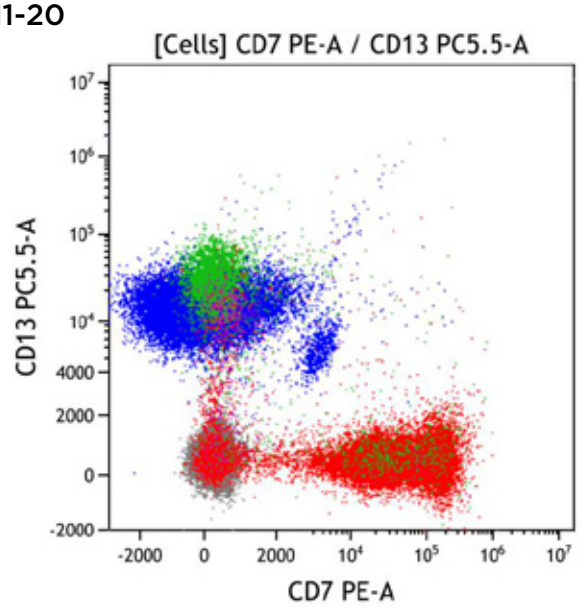

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. A large subset of lymphocytes (red) expresses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

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

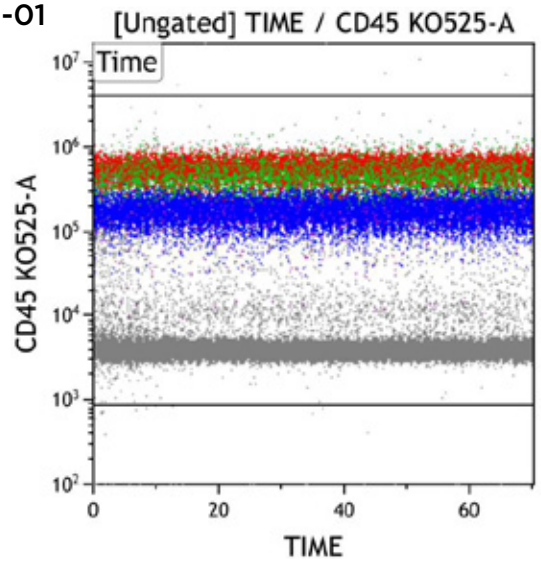

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

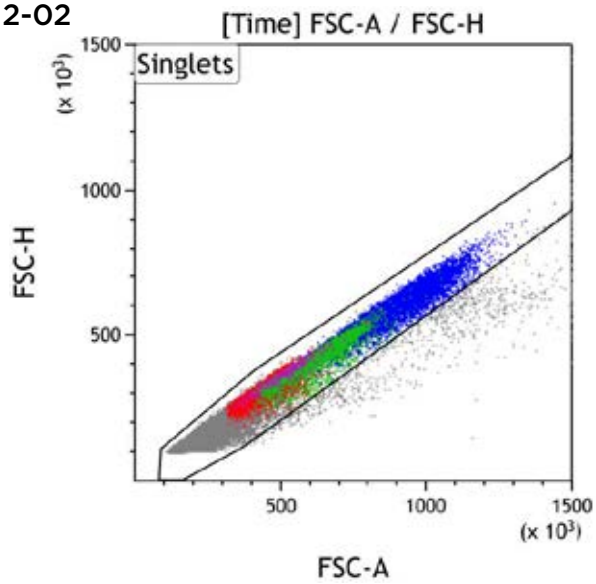

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

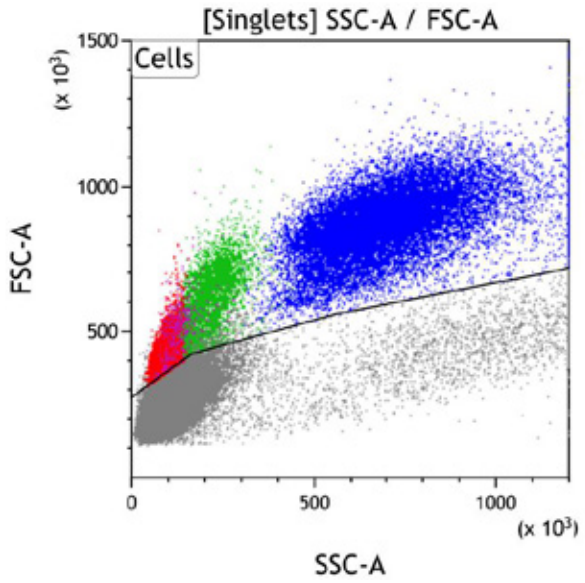

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter withincreased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

M2-04

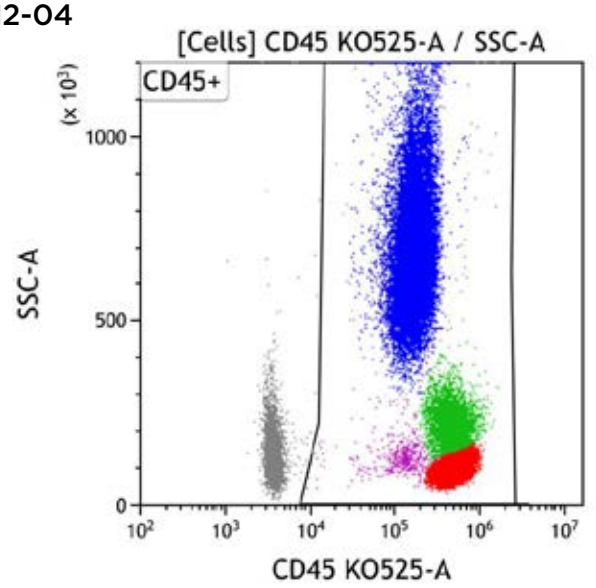

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M2-05

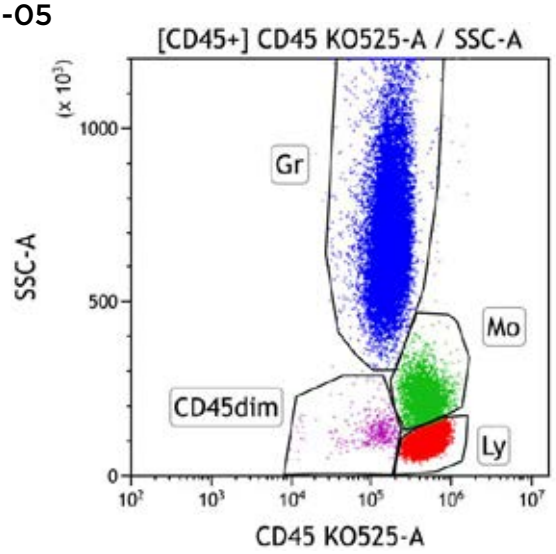

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied b early progenitors (e.g., 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.

M2-06

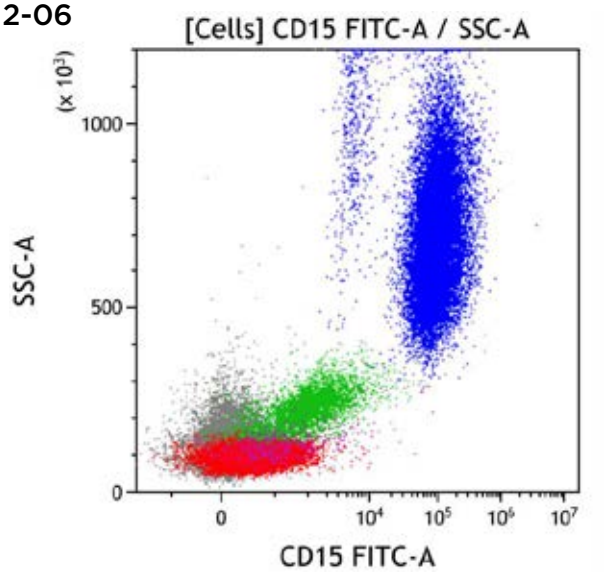

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes (green). The lymphocytes (red) are negative for CD15.

M2-07

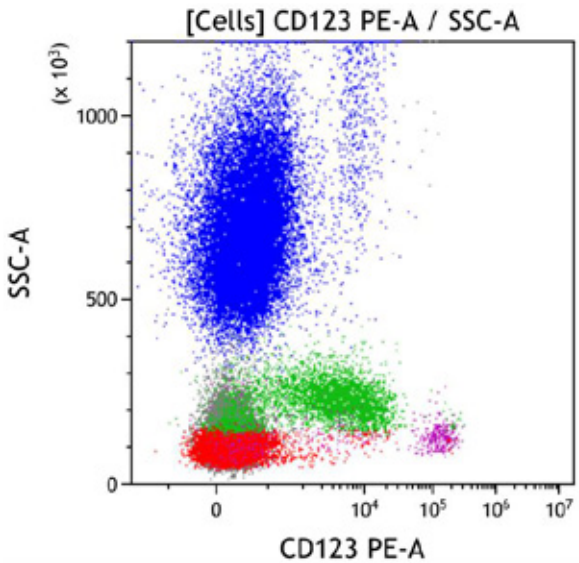

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green). The lymphocytes (red) are negative for CD123.

M2-08

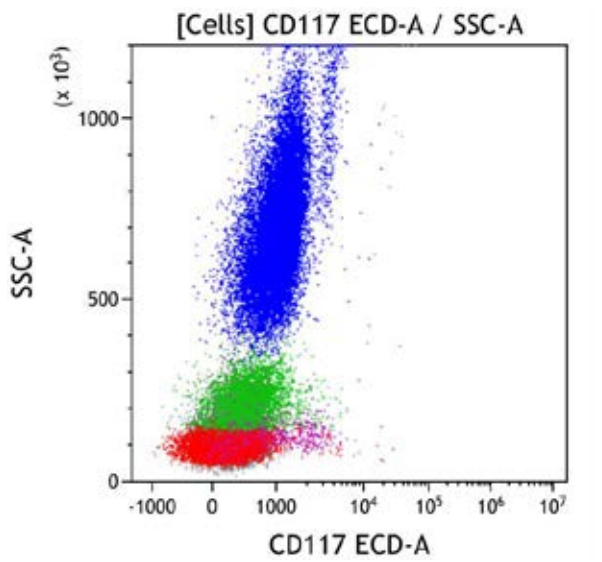

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

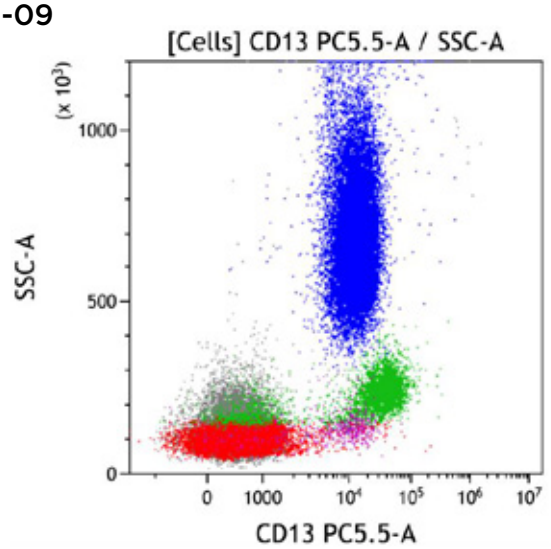

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and variably on myeloid progenitors. The lymphocytes (red) are negative for CD13.

M2-10

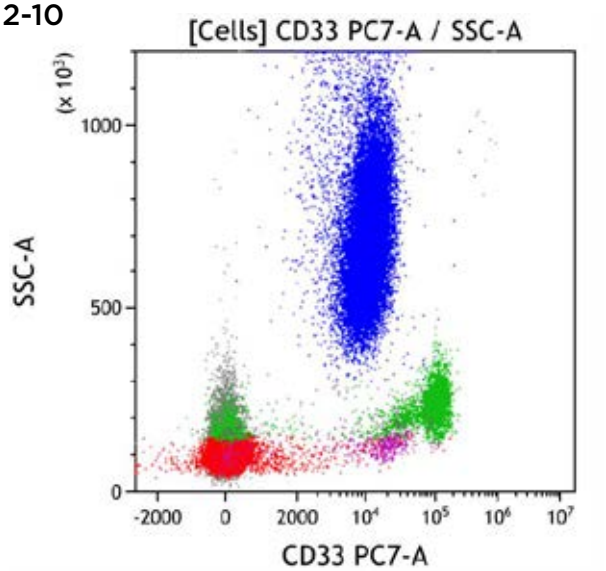

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The lymphocytes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

M2-11

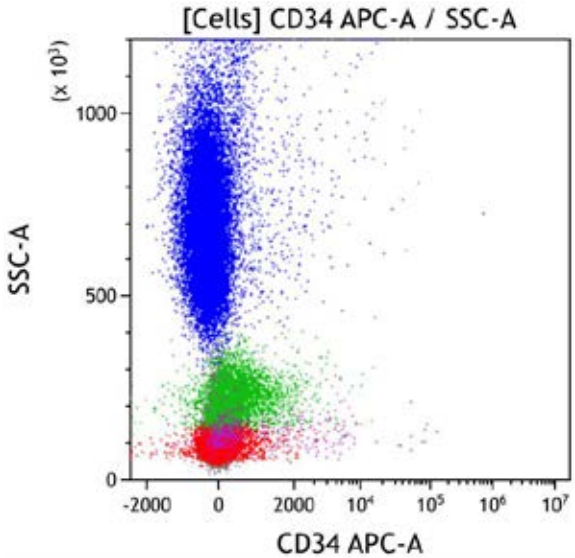

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive blasts typically have low to intermediate side scatter in the CD45dim gate. The lymphocytes (red) are negative for CD34.

M2-12

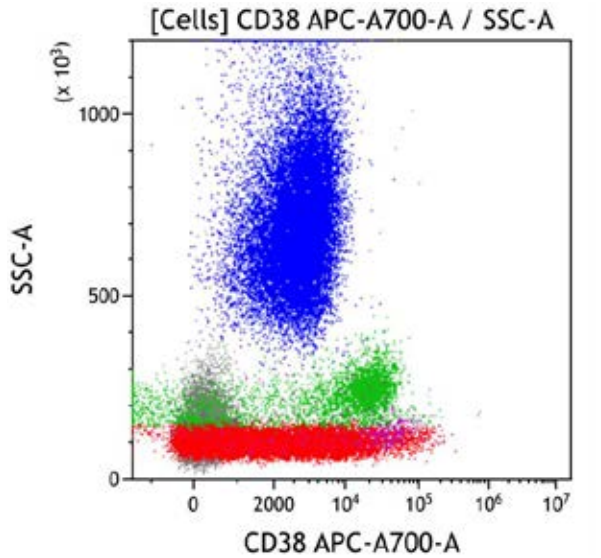

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature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

BACK TO CASE OVERVIEW

M2-13

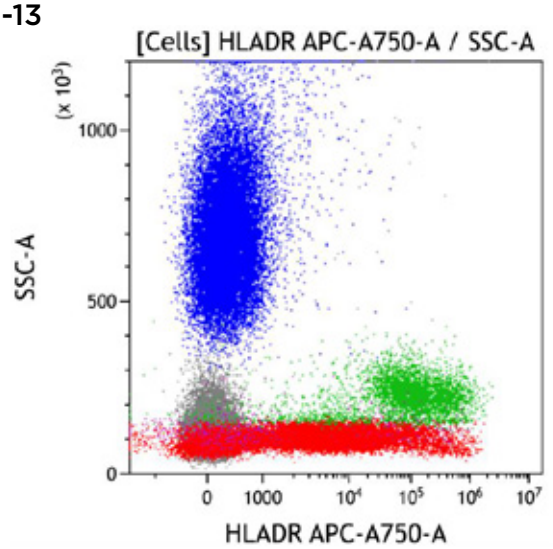

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells, and activated T cells. A subset of lymphocytes (red)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

M2-14

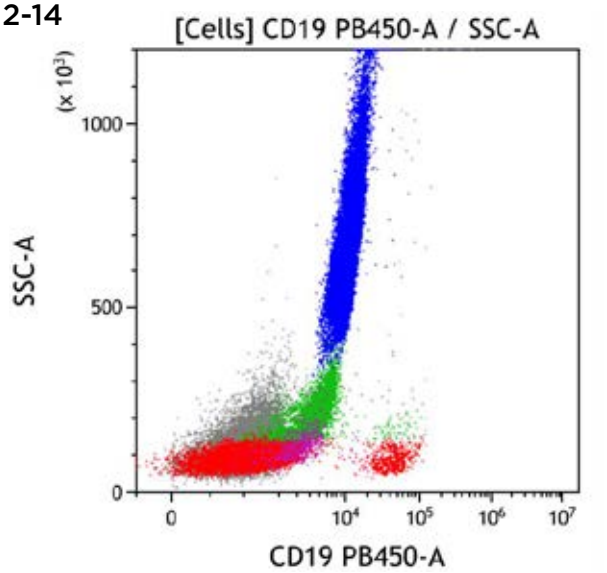

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red), as well as most plasma cells. These cells typically have low to moderate side scatter. Most of lymphocytes (red) are negative for CD19.

M2-15

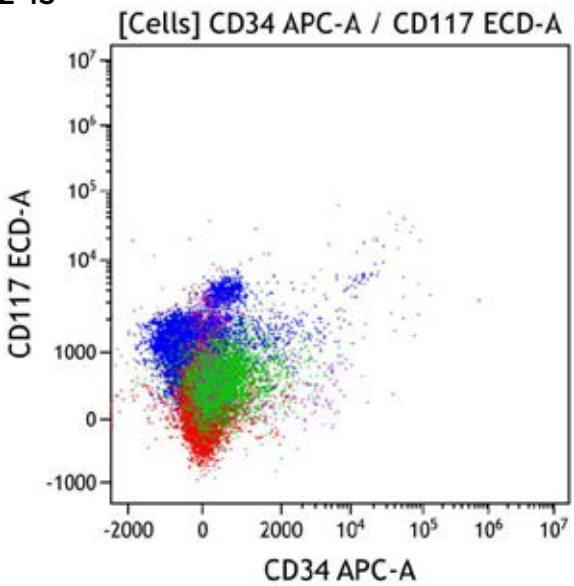

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The lymphocytes (red) are negative for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

M12-16

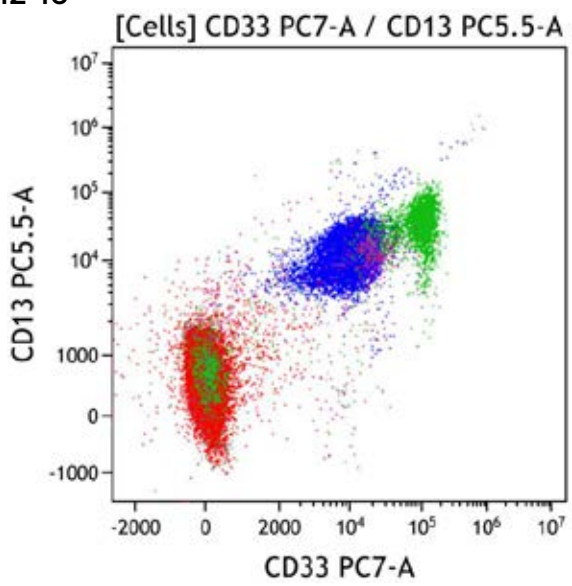

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes (red) are negative for CD13 or CD33.

BACK TO CASE OVERVIEW

M2-17

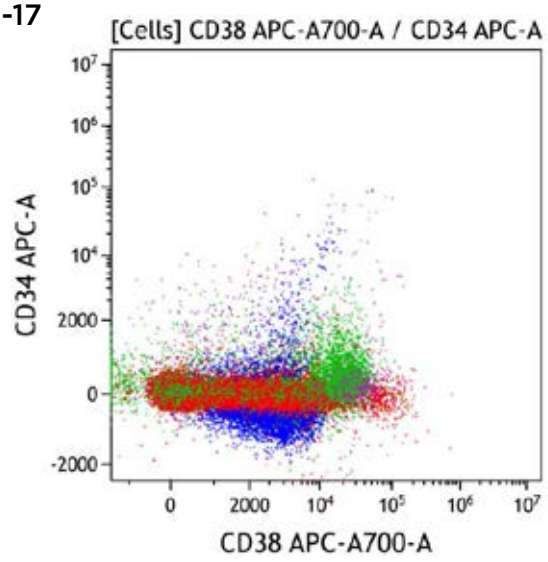

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Most of lymphocytes (red) express dim CD38 but not CD34.

M2-18

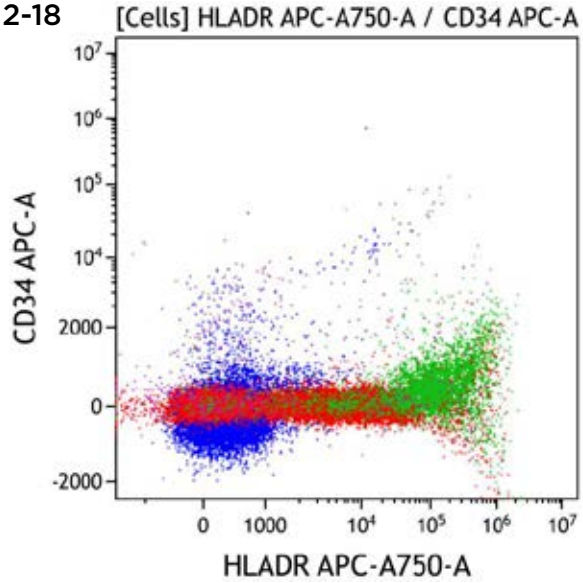

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. A subset of lymphocytes (red) variably expresses HLA-DR.

M2-19

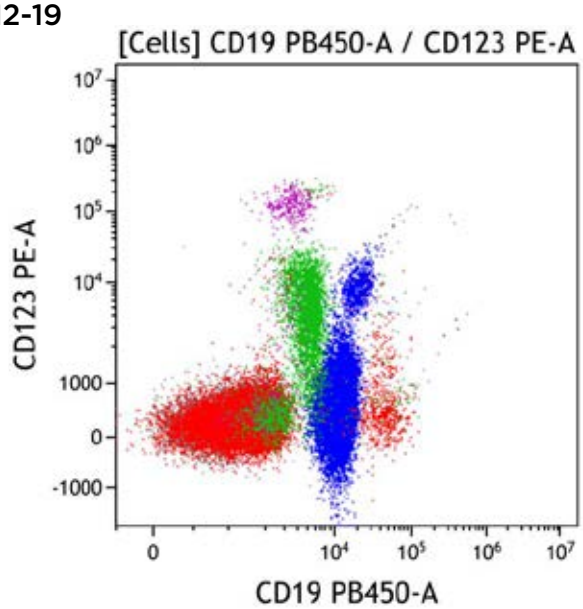

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red) normally do not express significant CD123. The apparent CD19 positivity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

M2-20

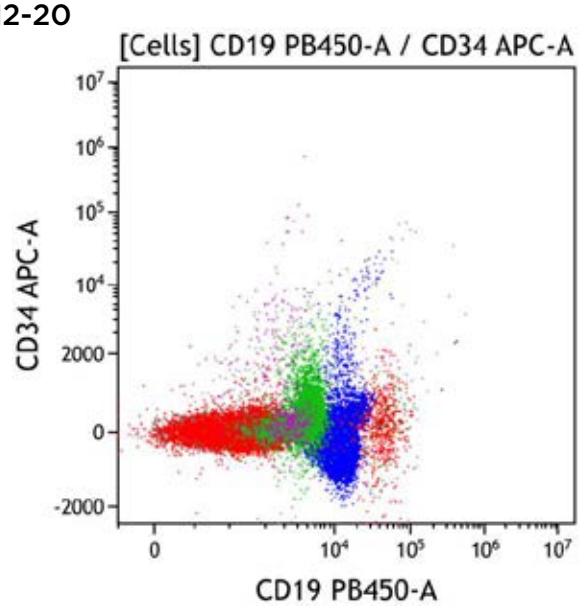

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. CD34 positive progenitors do not express CD19. A small subset of lymphocytes (red) is CD19 positive B cells without expression of CD34.

BACK TO CASE OVERVIEW

M2-21

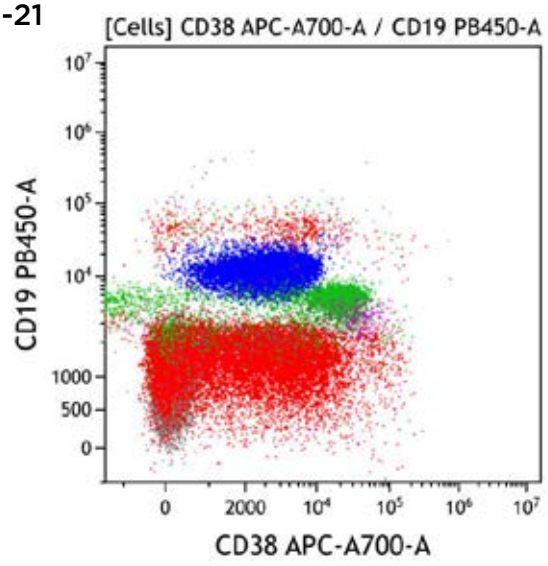

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show low level expression of CD38. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence,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

M2-22

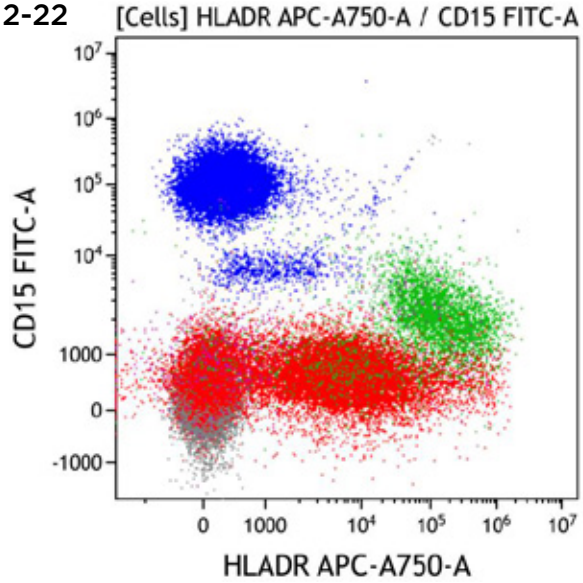

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLADR. CD34 positive myeloid progenitors express HLA-DR but only transiently express CD15. A subset of lymphocytes (red) variably expresses HLA-DR.

M2-23

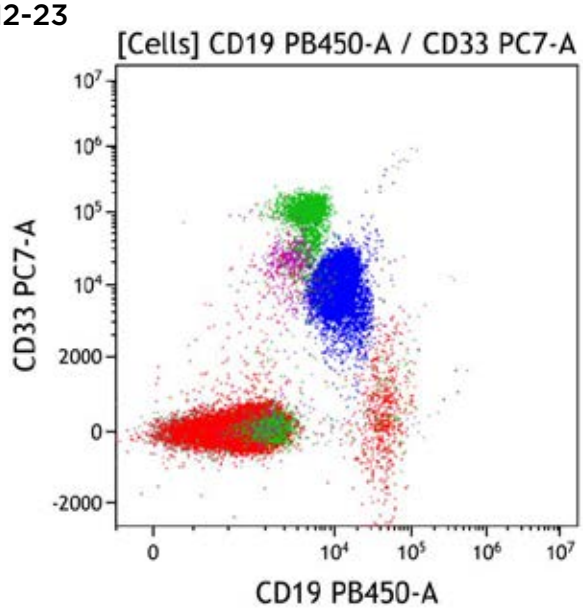

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not normally express significant CD33. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence, largely from eosinophils. A small subset of lymphocytes (red) is CD19 positive B cells without expression of CD33.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 7 MLL-TC4-035**
Diagnosis	**Acute myeloid leukemia**
Clinical Vignette	72-year-old woman with newly developed anemia and unclassified cells on peripheral blood smear. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	The flow cytometry study detected expanded myeloid blasts and monocytes. The blasts are in the CD45dim gate and express CD33, CD34, CD117 and HLA-DR. The blasts also abnormally express CD10 and CD56 (in a subset), while negative for CD19. The expanded monocytes are positive for CD13, CD14 and CD64 with increased CD56 in a subset. Together, the flow cytometry results support acute myelomonocytic leukemia.

BACK TO CASE LISTINGS

B Cell Tube

B01

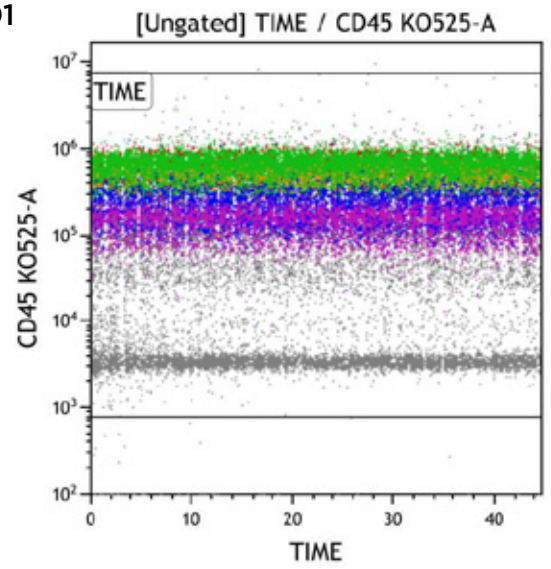

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

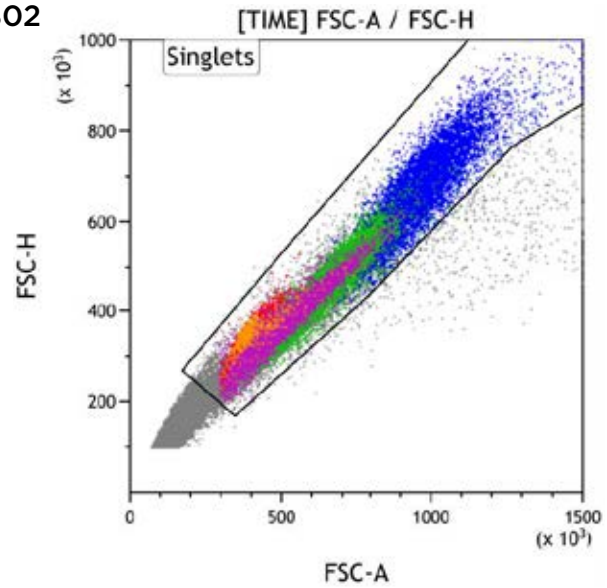

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

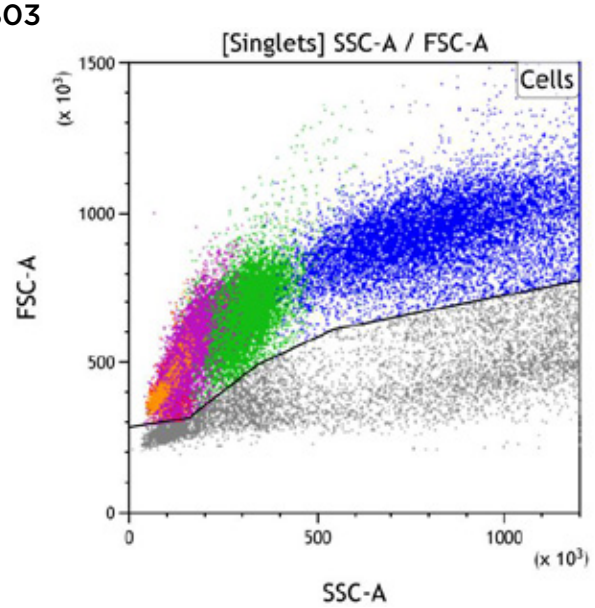

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

B04

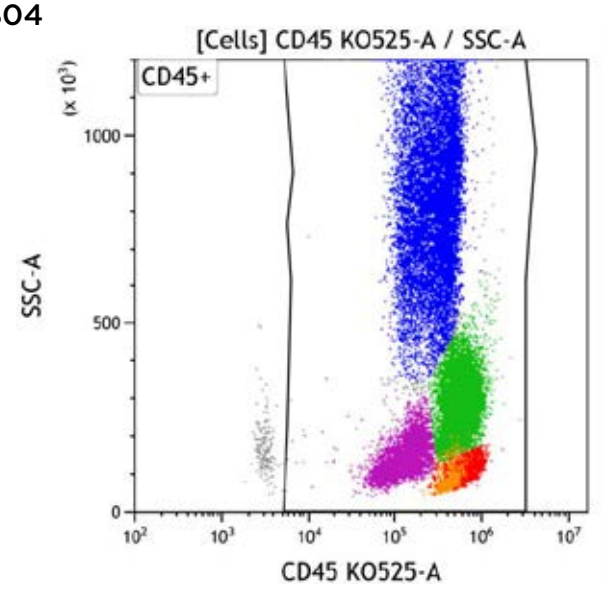

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

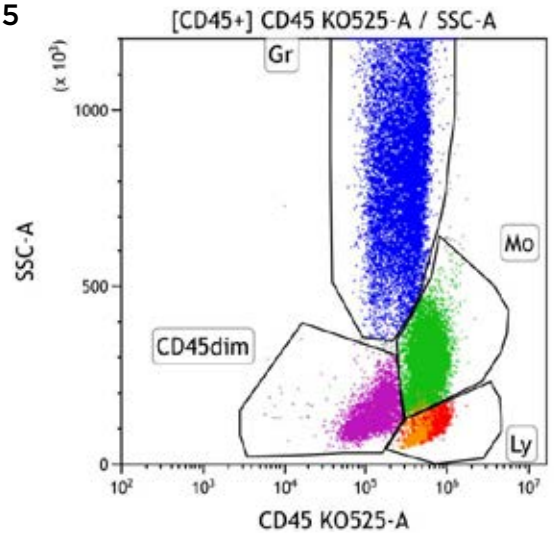

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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. Of note, cells in the CD45dim and monocyte gates are increased.

B06

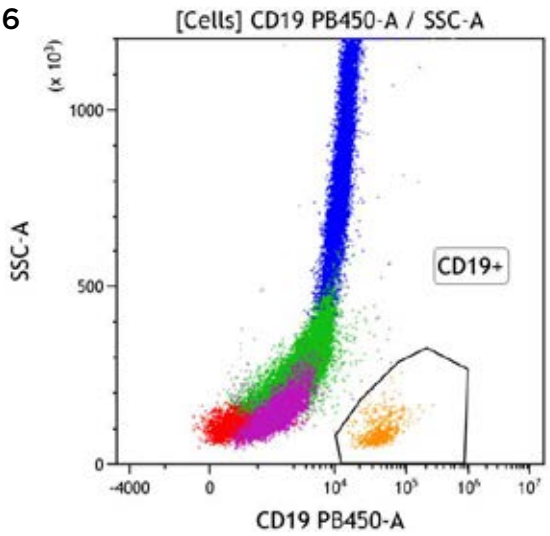

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B07

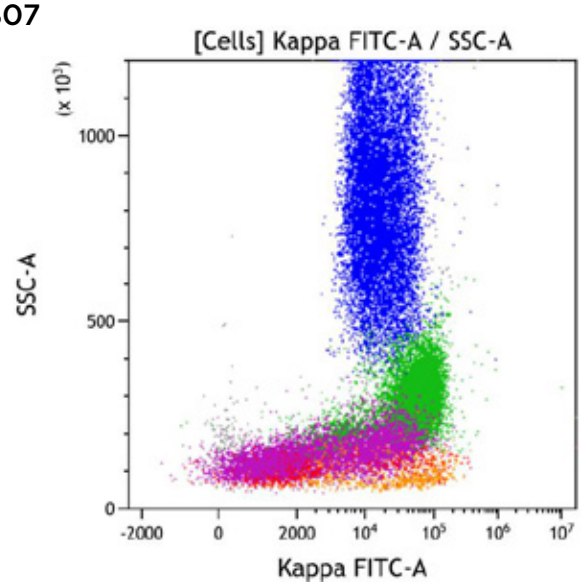

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent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

B08

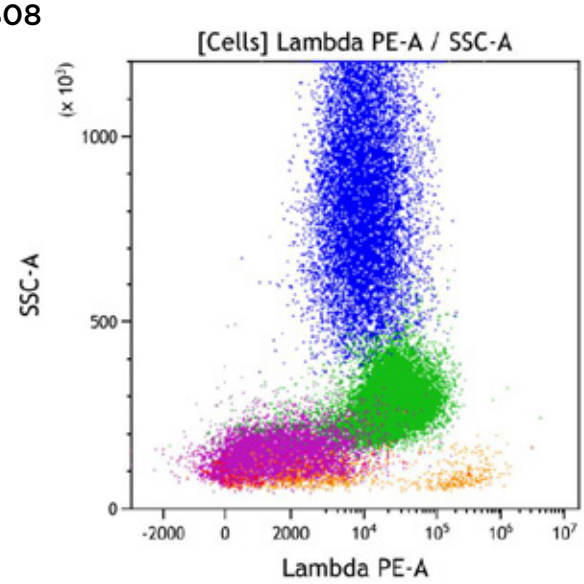

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in yellow) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent 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.

BACK TO CASE OVERVIEW

B09

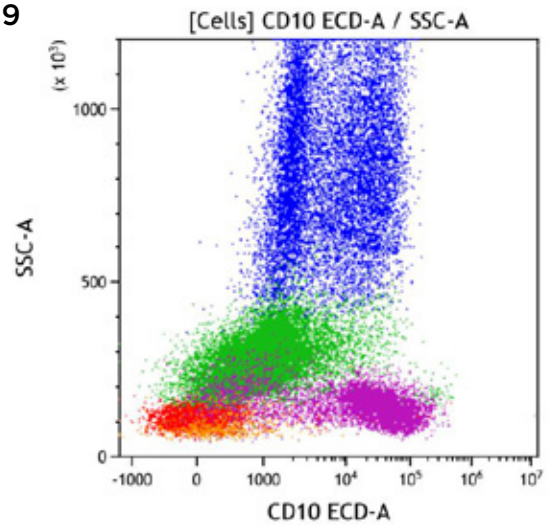

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is normally expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Note most of the blasts (in purple) are CD19-negative but abnormally CD10-positive.

B10

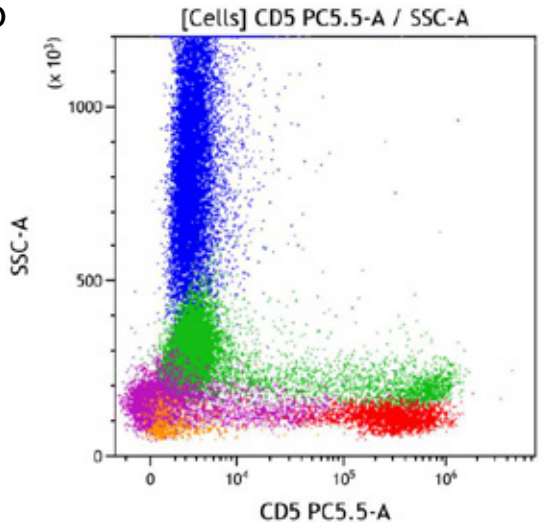

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in yellow). These lymphoid cells typically have low side scatter.

B11

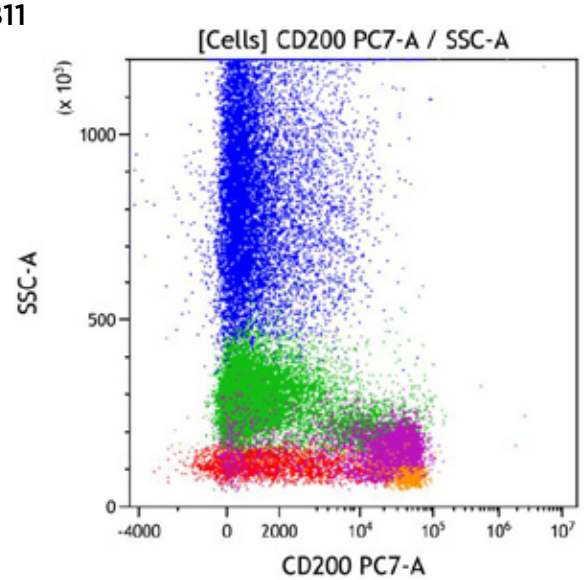

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

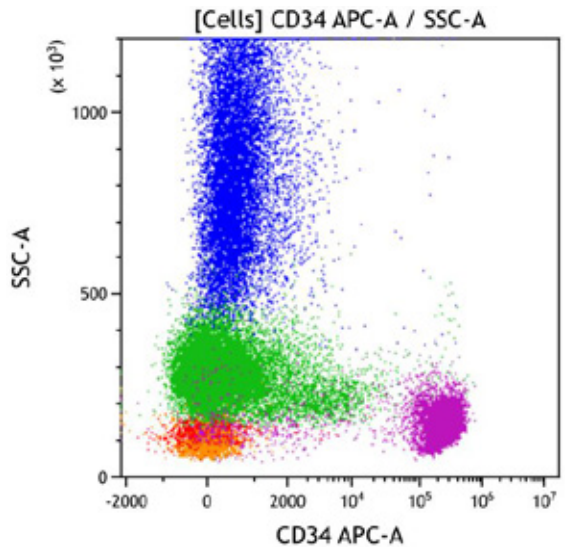

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with im-mature B cell progenitors having lower side scatter than immature myeloid progenitors. Note most of the cells in the ex-panded CD45dim gate are CD34 positive.

BACK TO CASE OVERVIEW

B13

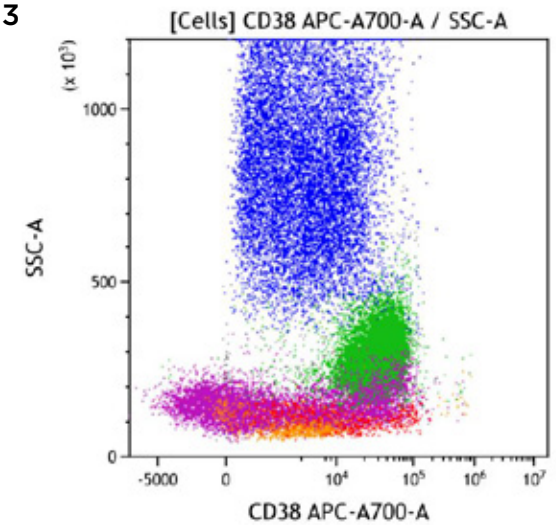

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

B14

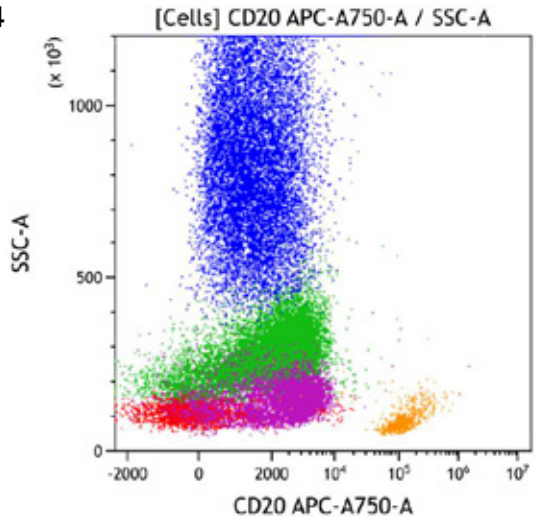

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (highlighted in orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate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

B15

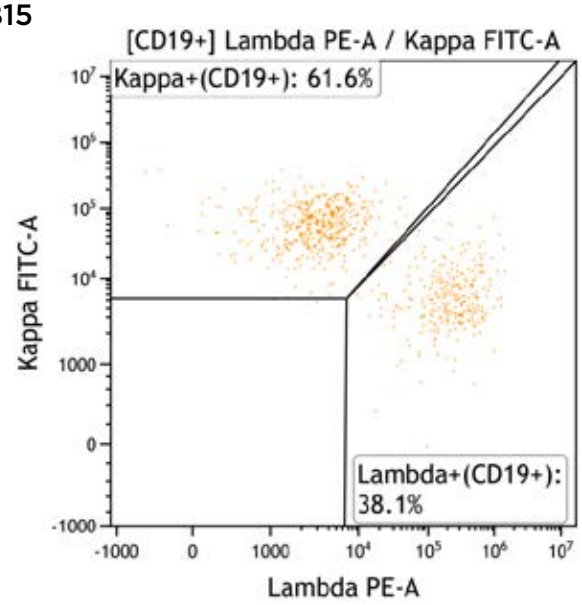

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

B16

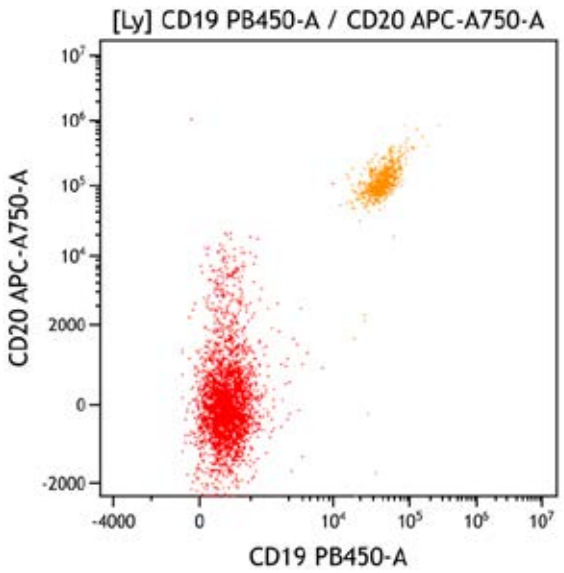

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells express both CD19 and CD20 (highlighted in orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

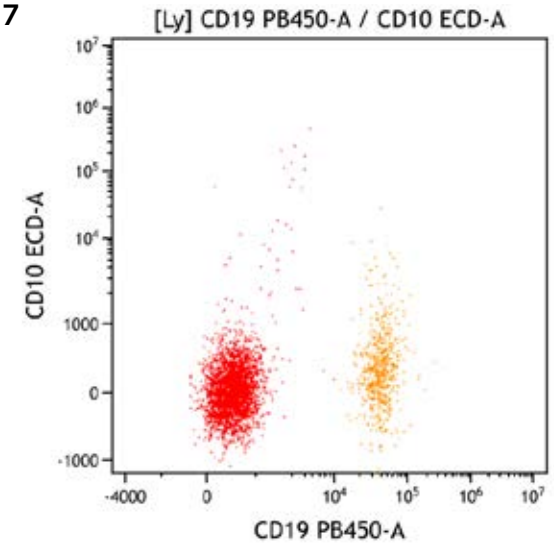

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lympho-cyte gate. B cells are CD19 positive (highlighted in yellow). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow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

B18

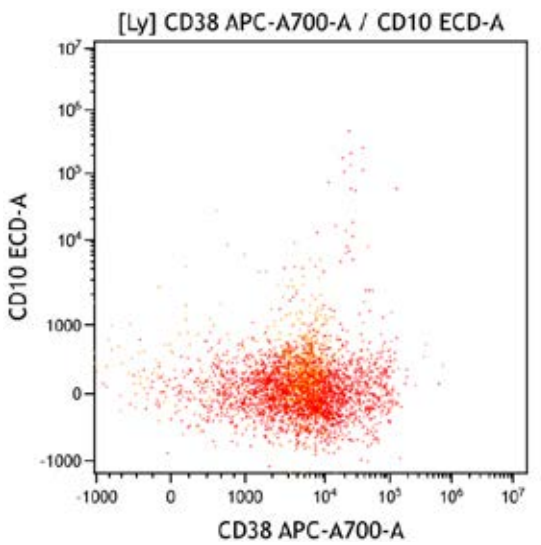

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lympho-cyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

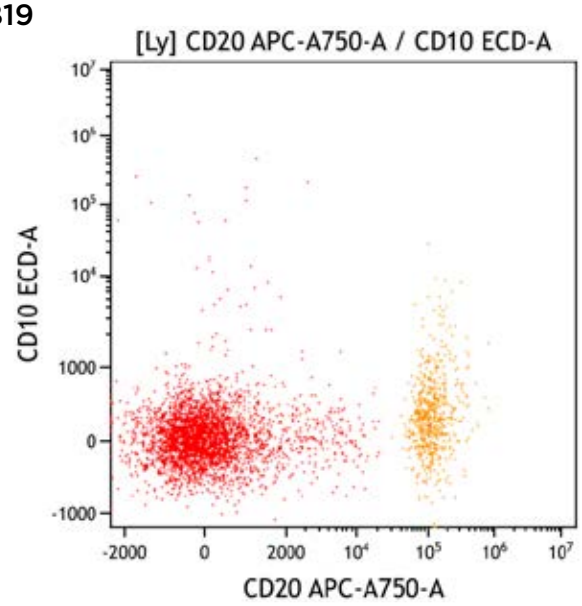

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late-stage immature B cells.

B20

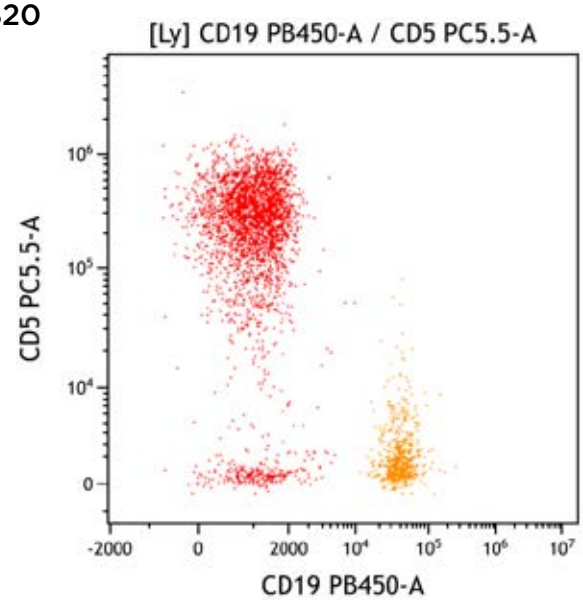

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

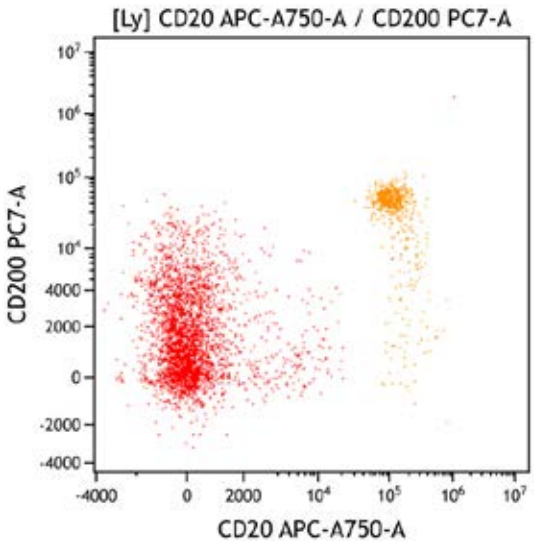

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in orange).

B22

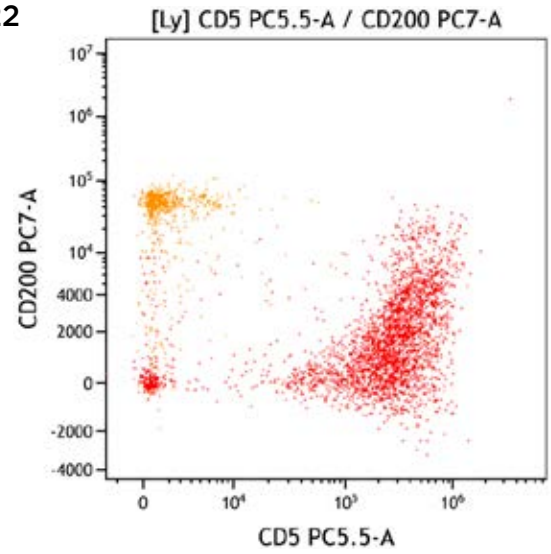

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically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

BACK TO CASE OVERVIEW

T Cell Tube

T01

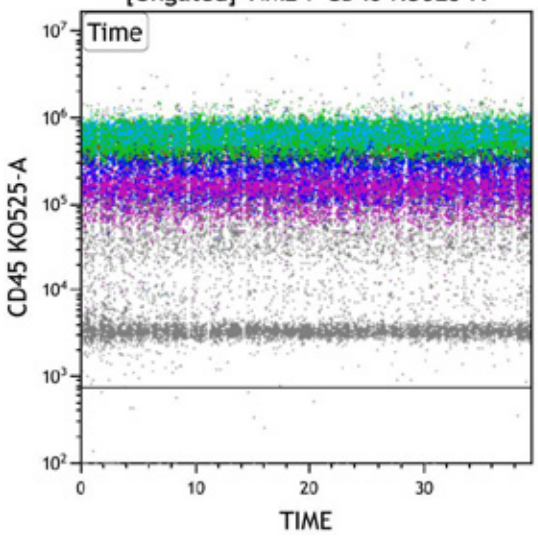

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

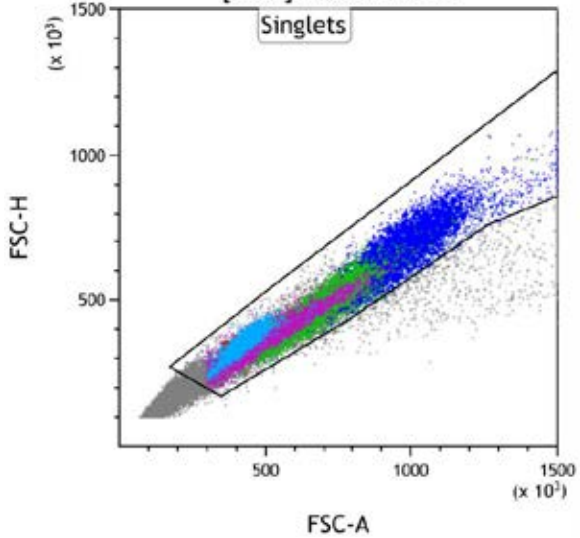

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

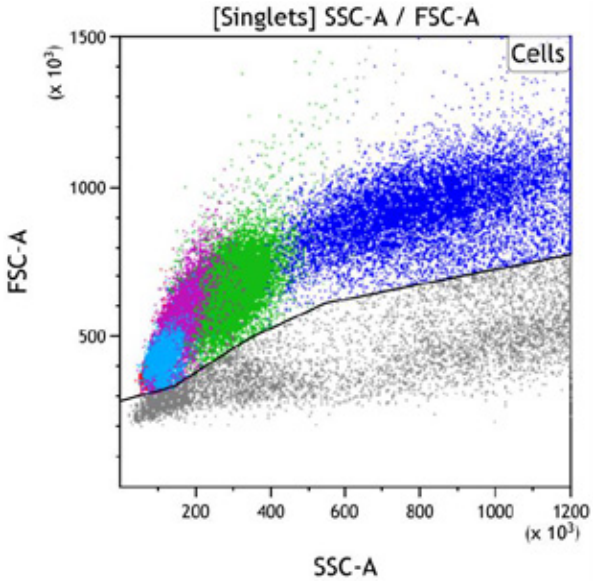

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

T04

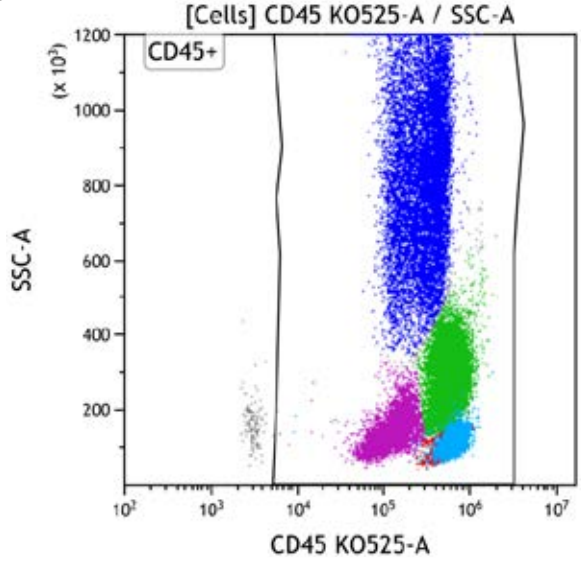

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

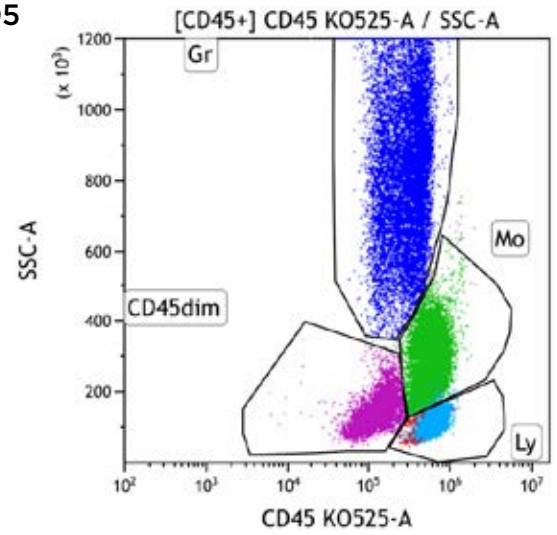

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot per-mits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied my-eloblasts 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 anal-ysis. Of note, cells in the CD45dim and monocyte gates are expanded.

T06

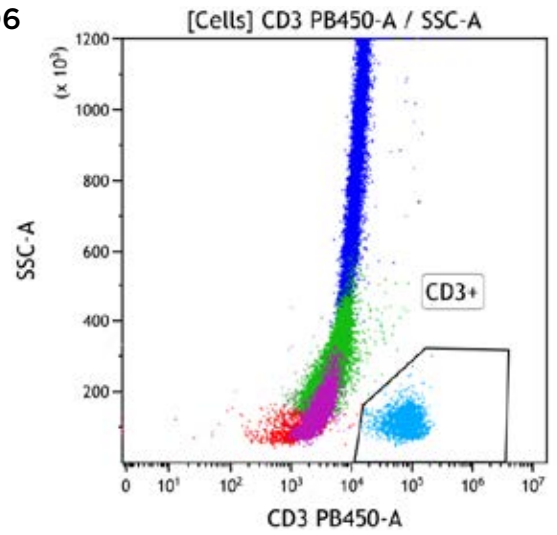

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

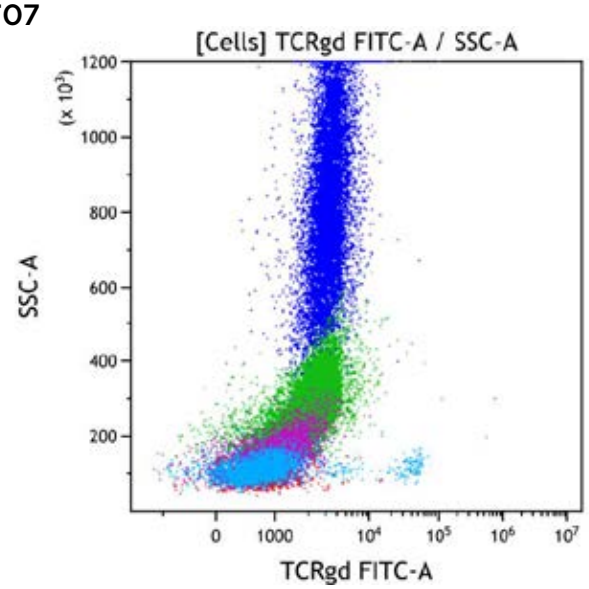

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

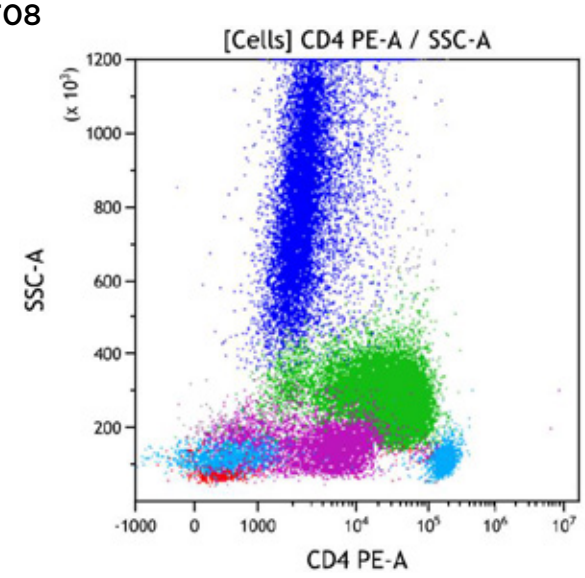

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

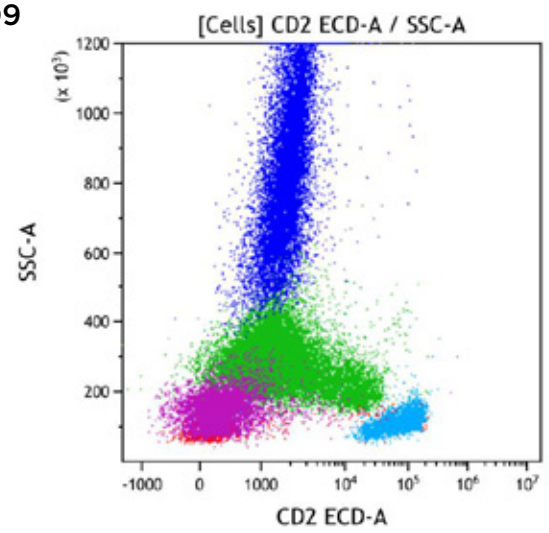

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

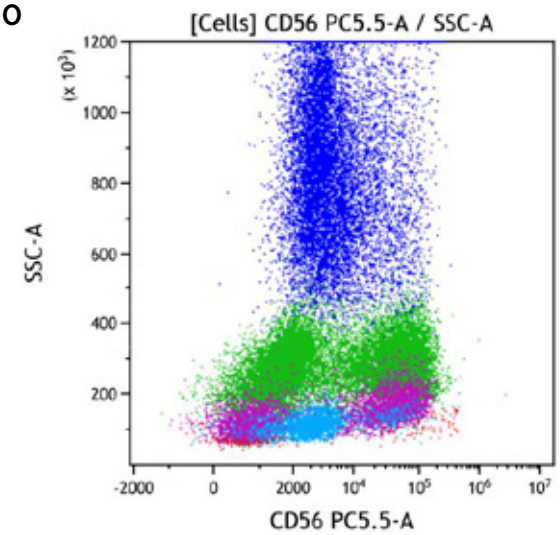

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. Note a subset of CD45 dim blasts (in purple) is also CD56 positive.

T11

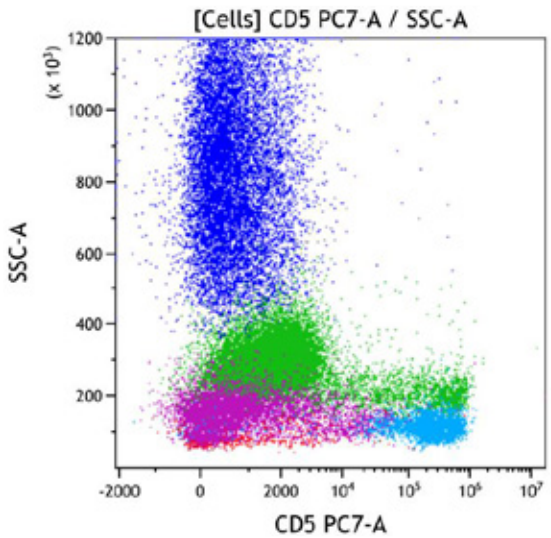

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

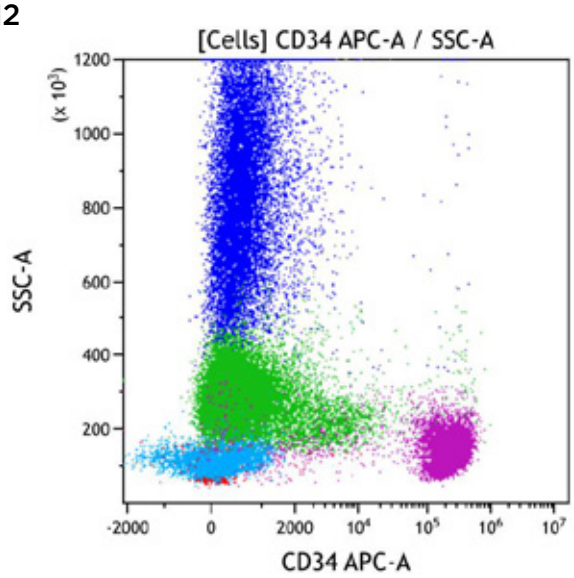

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Note most of cells in the CD45dim gate are CD34 positive.

BACK TO CASE OVERVIEW

T13

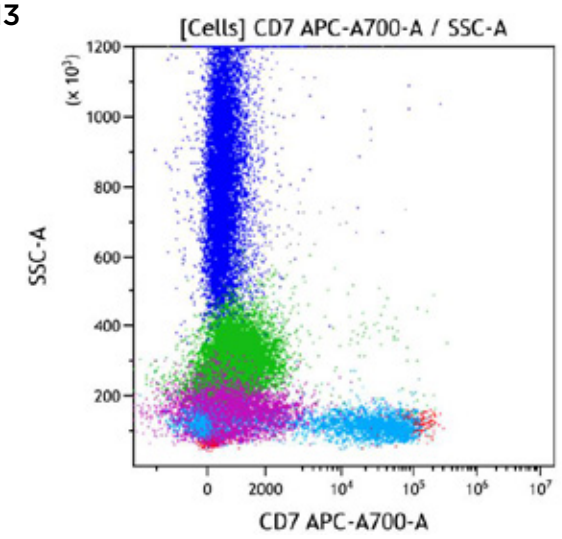

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

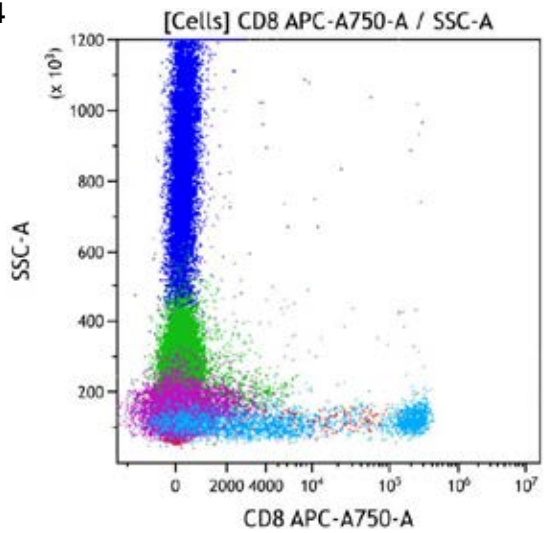

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

T15

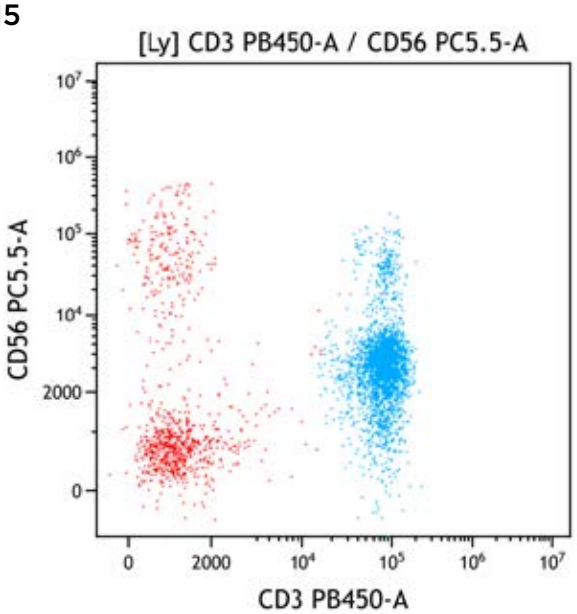

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small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

T16

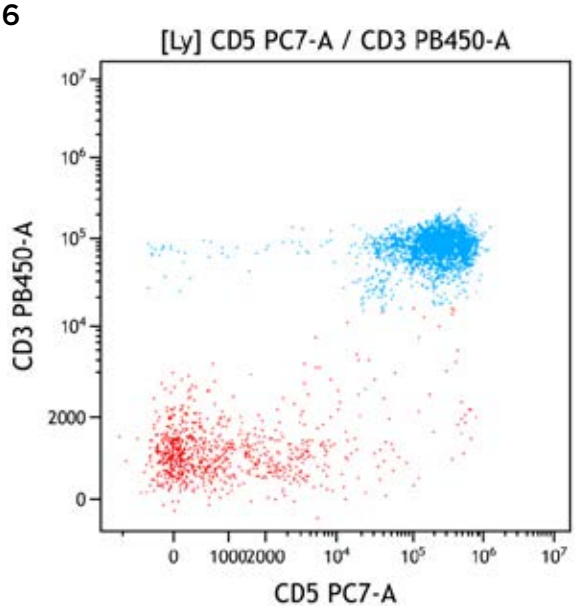

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most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

BACK TO CASE OVERVIEW

T17

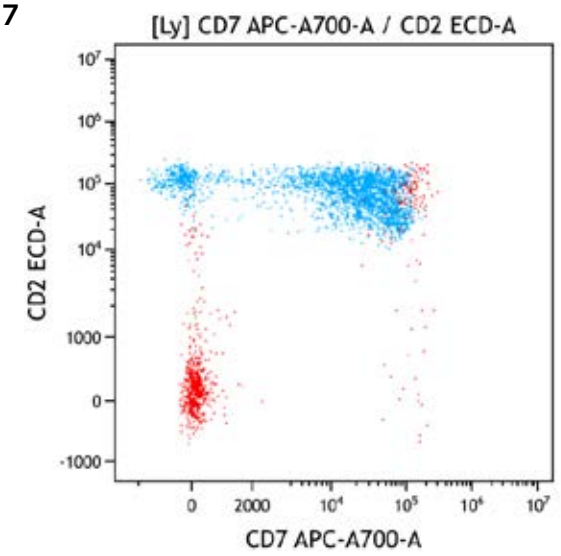

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coex-pressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

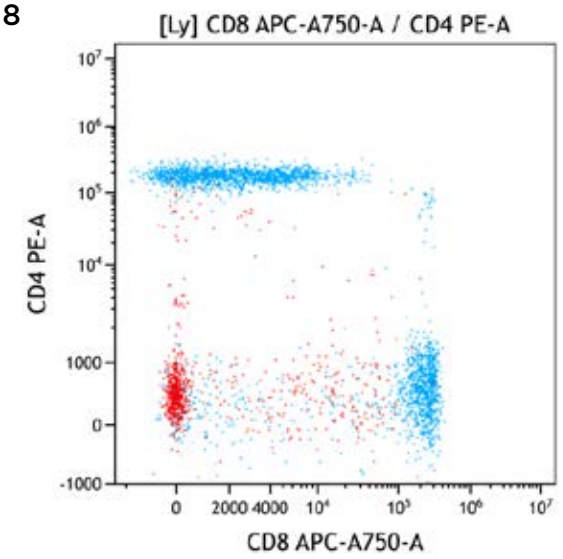

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typical-ly 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 lympho-cyte identification.

T19

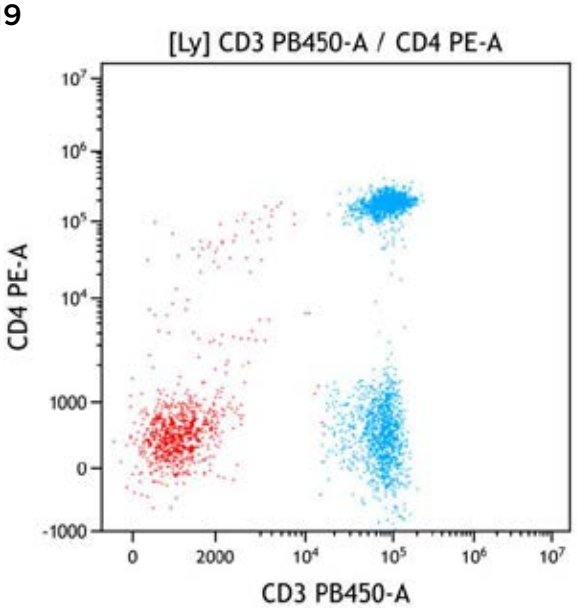

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

T20

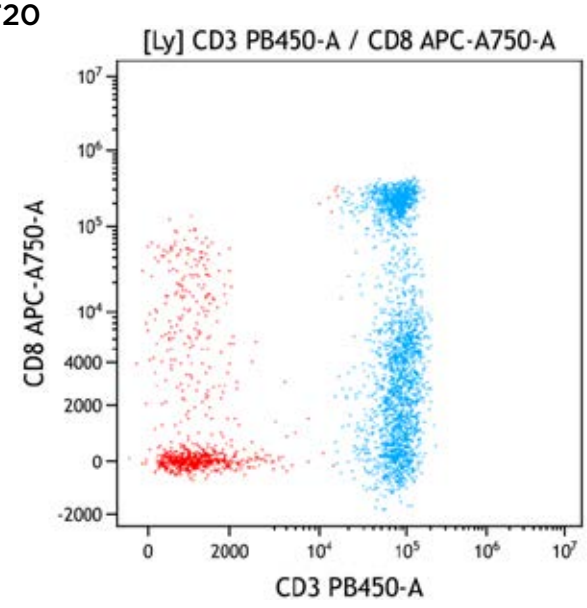

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

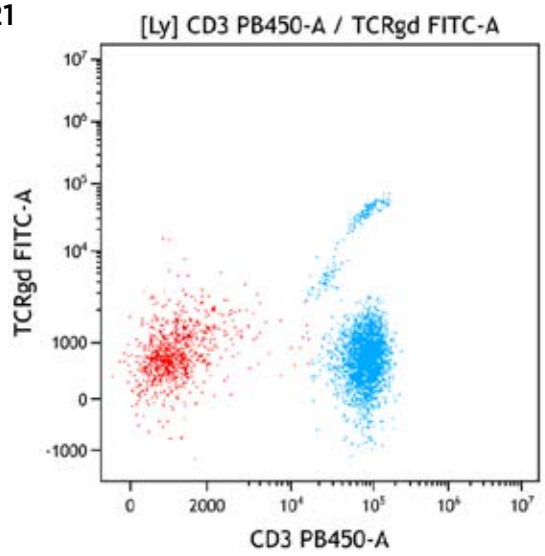

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

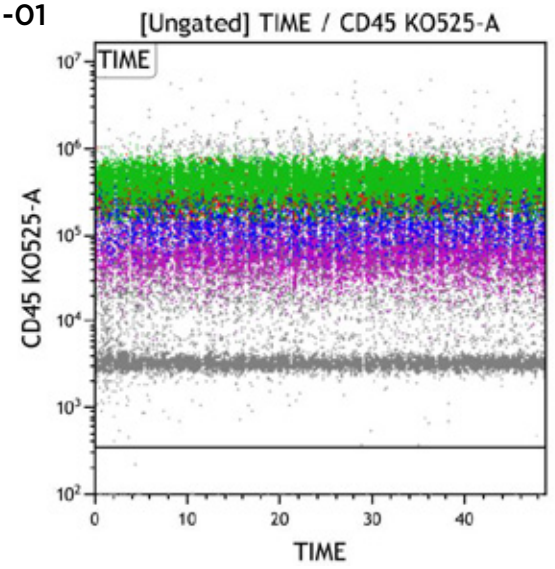

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

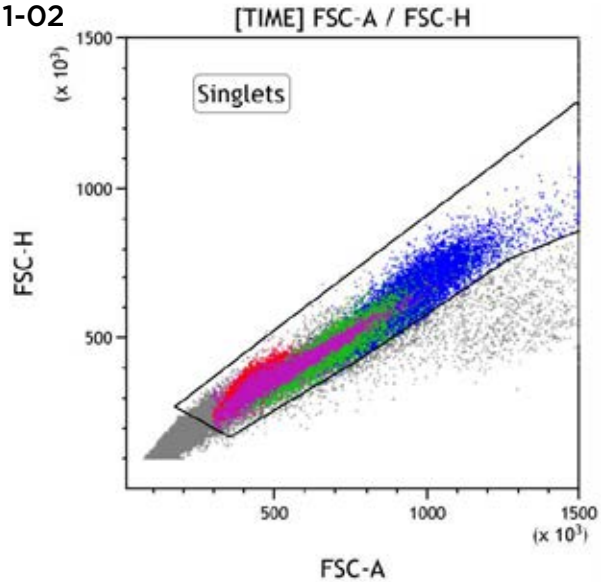

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

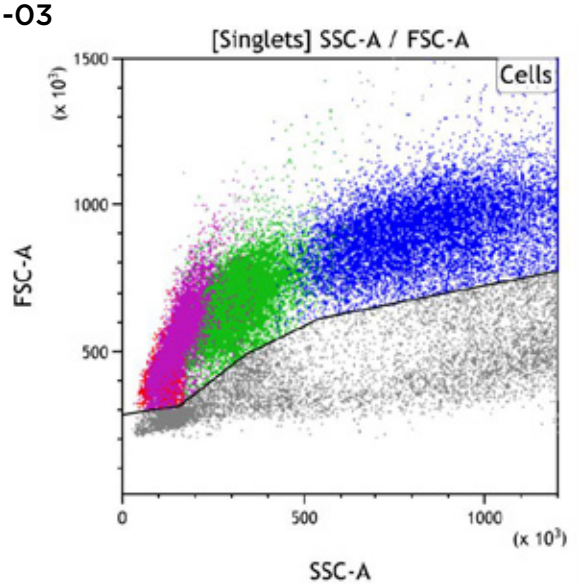

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M1-04

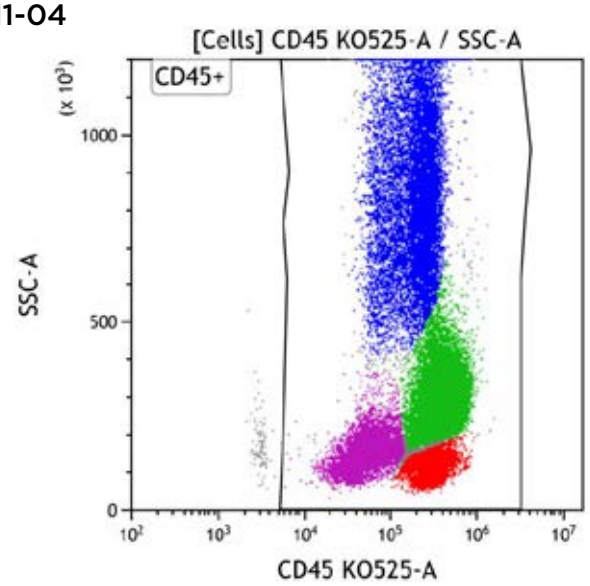

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

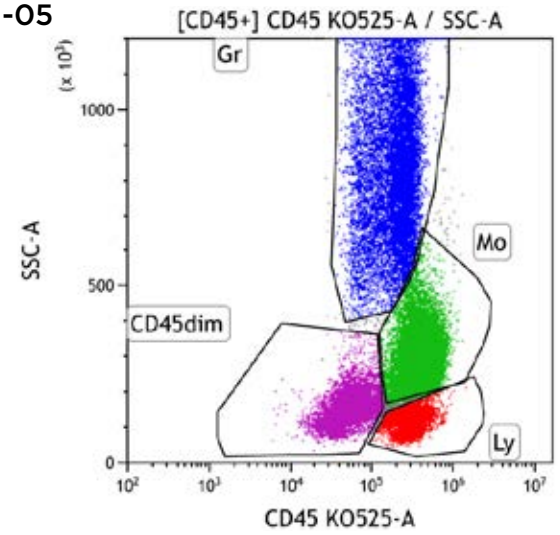

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot per-mits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., myeloblasts and immature B cells). Basophils, plas-macytoid 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. Of note, cells in the CD45dim and monocyte gates are expanded.

M1-06

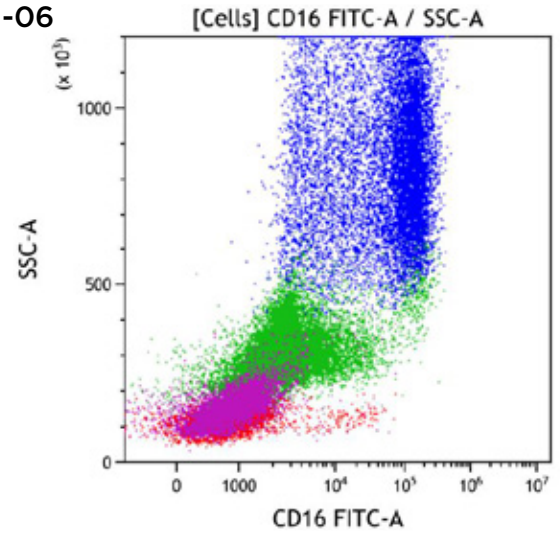

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its high-est level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated mono-cytes (green).

M1-07

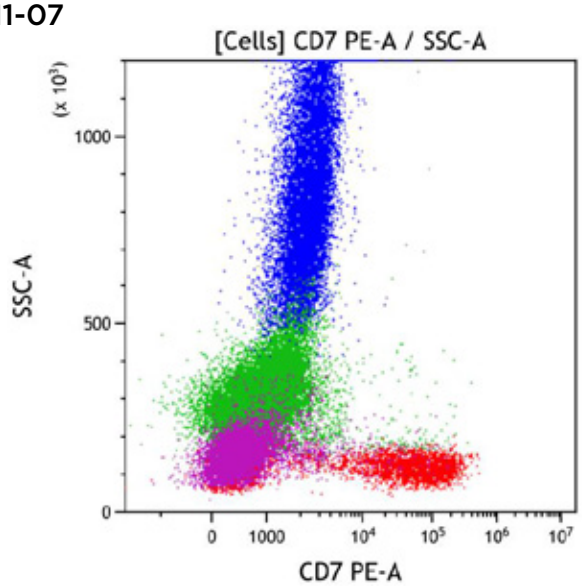

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

M1-08

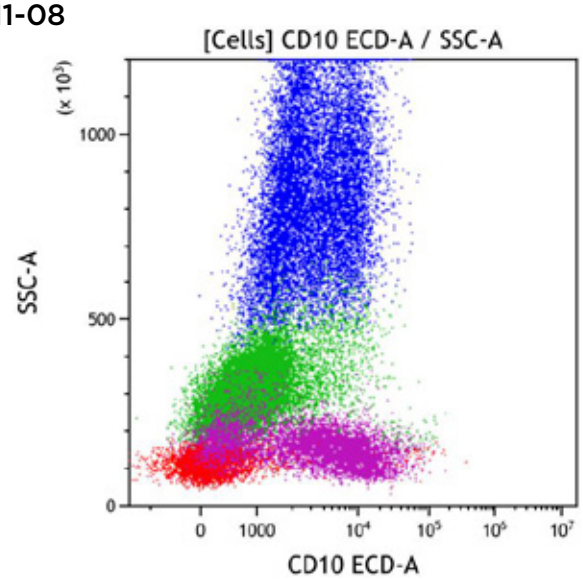

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Note most of the cells in the CD45dim gate are CD10 positive.

BACK TO CASE OVERVIEW

M1-09

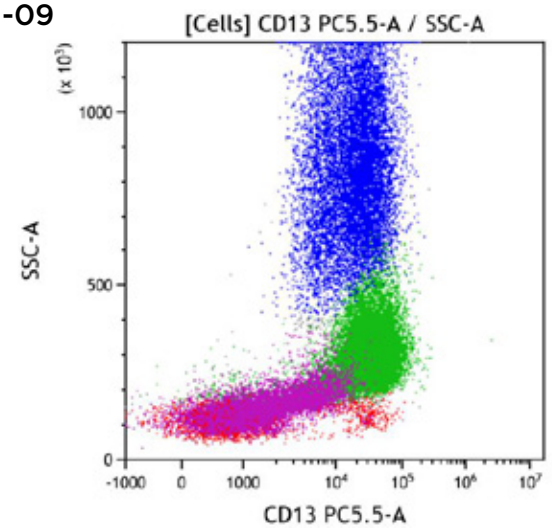

Figure 9. This CD13 vs Side Scatter dot plot shows all vi-able cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature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

M1-10

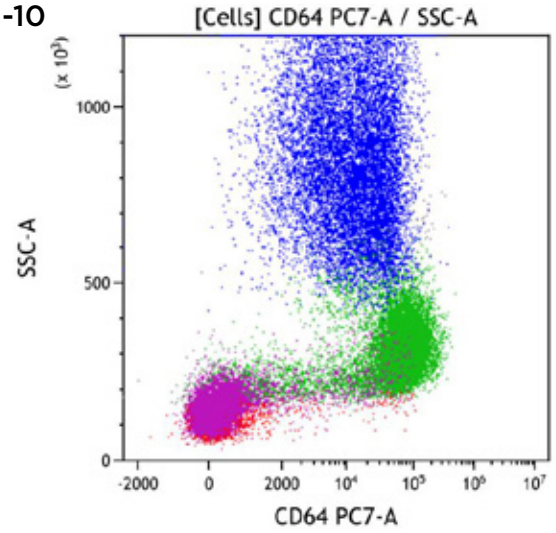

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its high-est level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34+ progenitors. Of note, the cells in the expanded Mo gate are strongly positive for CD64.

M1-11

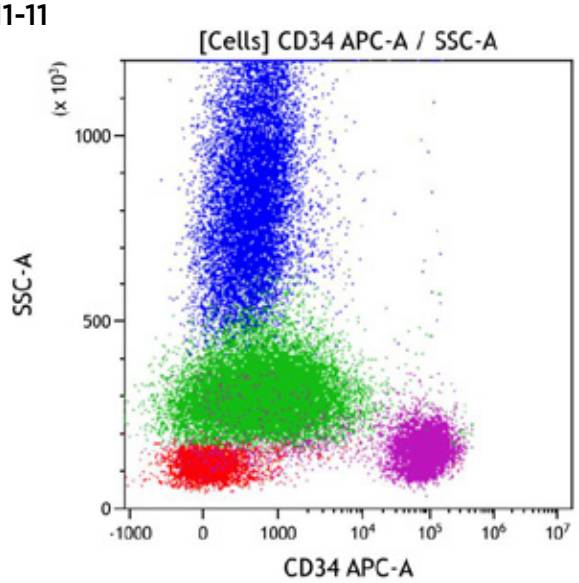

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

M1-12

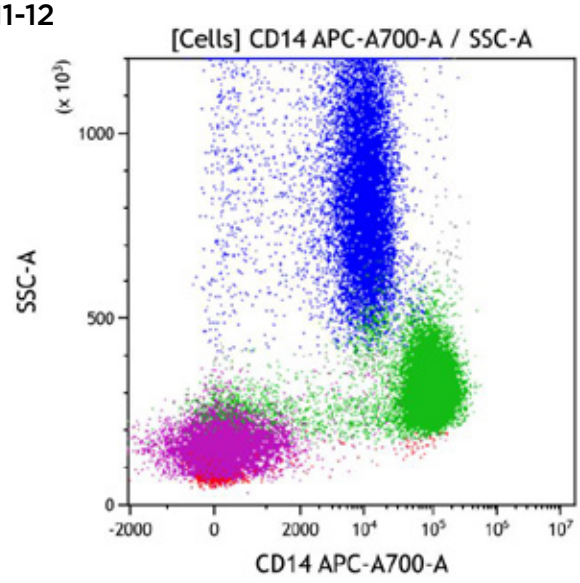

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Of note, the cells in the expanded Mo gate are strongly positive for CD14.

BACK TO CASE OVERVIEW

M1-13

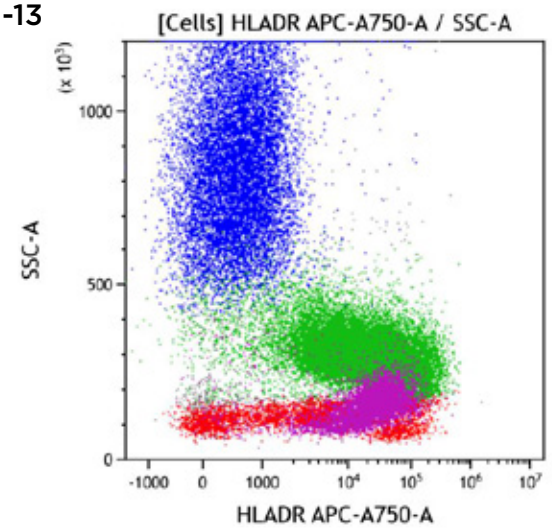

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

M1-14

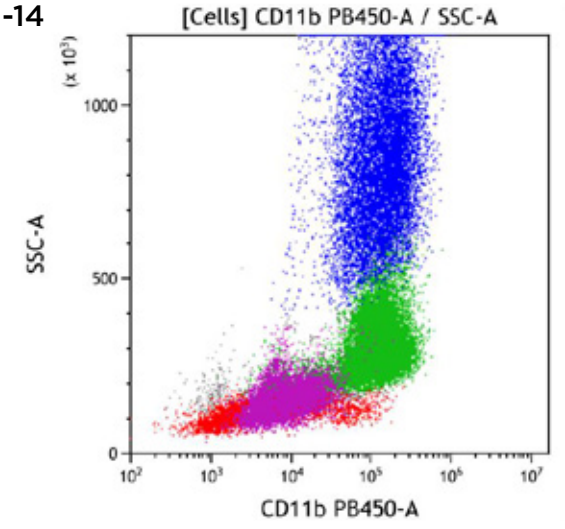

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

M1-15

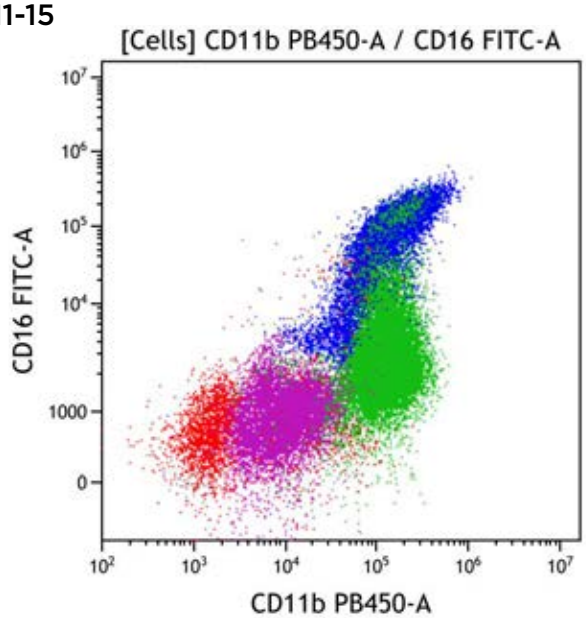

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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 mat-uration to mature granulocytes, where it is expressed at its highest level. Blasts (in purple) are negative for CD11b or CD16.

M1-16

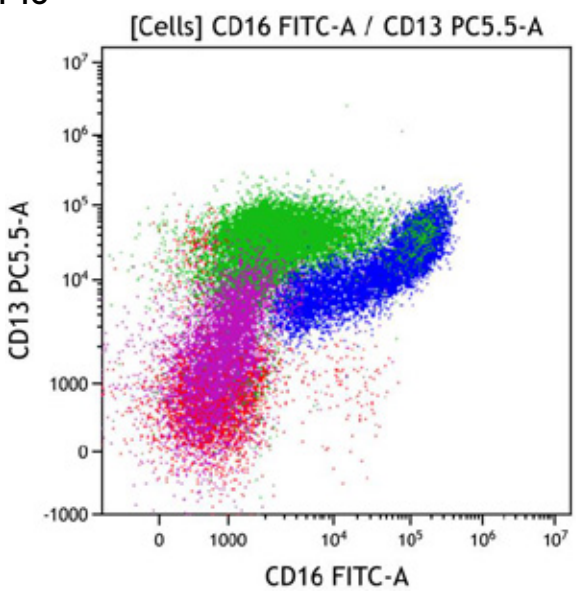

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulo-cytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic matura-tion, CD13 is expressed variably by promyelocytes without CD16 (blue left) and lose CD13 as they mature to myelocytes (blue bot-tom left). Myelocytes then simul-taneously acquire CD13 and CD16 as they mature from met-amyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16 (blue top right) .

BACK TO CASE OVERVIEW

M1-17

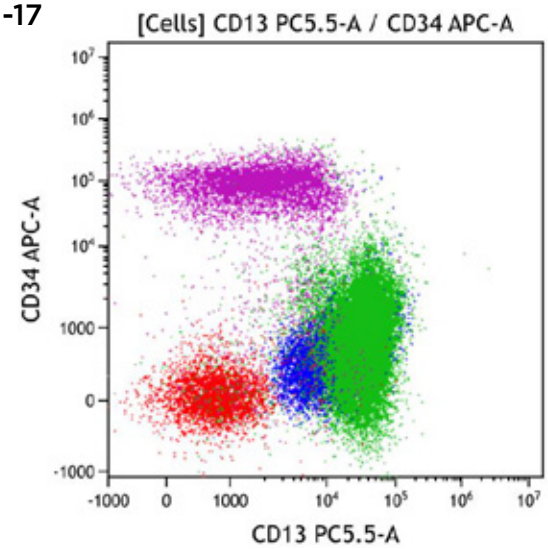

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulo-cytes (blue), monocytes (green), basophils, and CD34 posi-tive progenitors (purple). CD34 is expressed on early he-matopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphoid cells (red). Of note, the CD34 positive blasts are partially positive for CD13.

M1-18

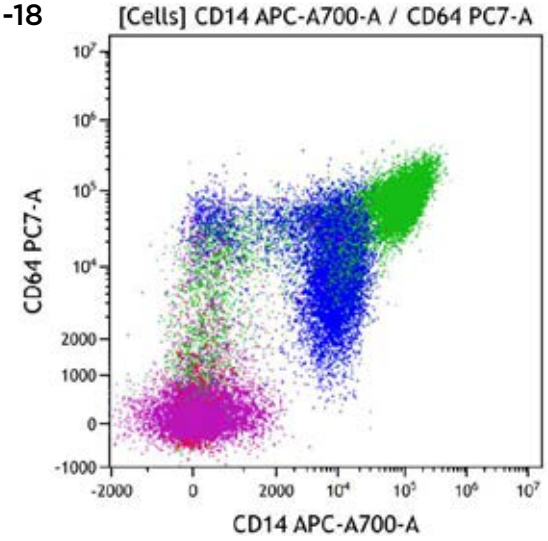

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on mono-cytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulo-cytes (blue). Immature monocytes show high expression of CD64 without CD14 (blue top left) and progressively acquire CD14 during maturation to ma-ture 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). Blasts (in purple) are negative for CD14 or CD64.

M1-19

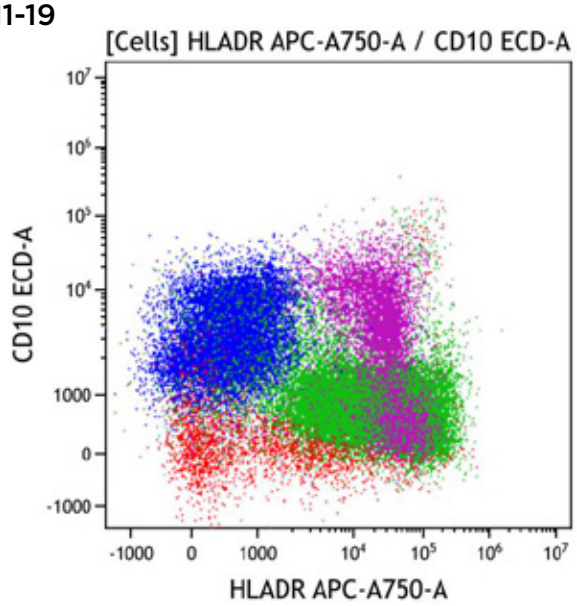

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).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

M1-20

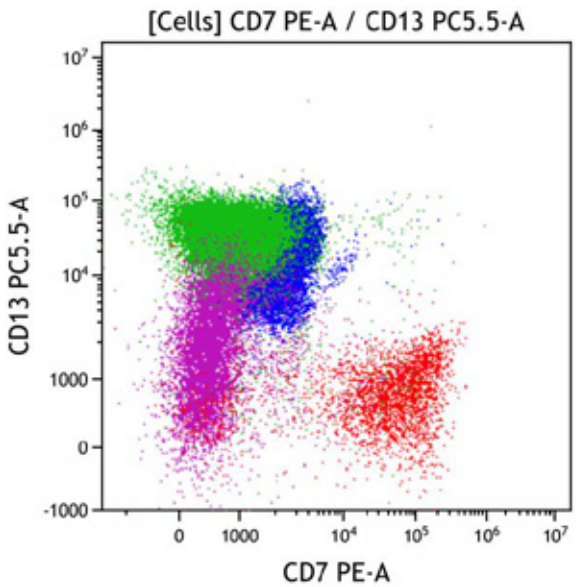

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

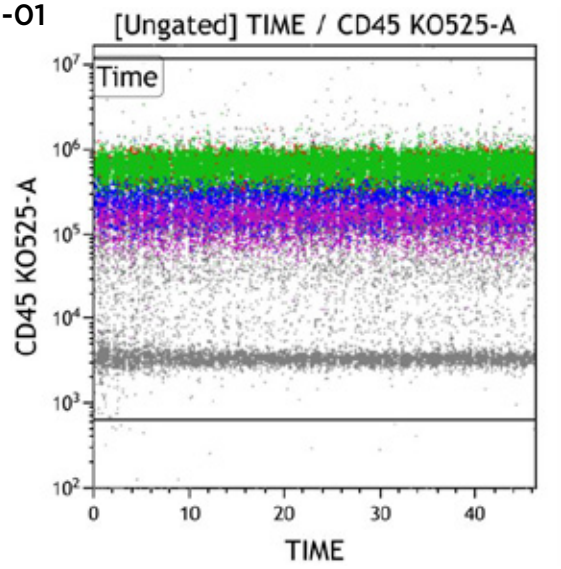

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

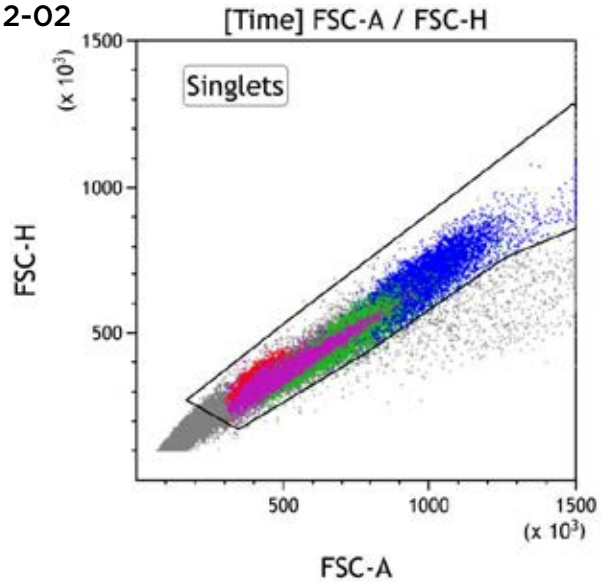

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

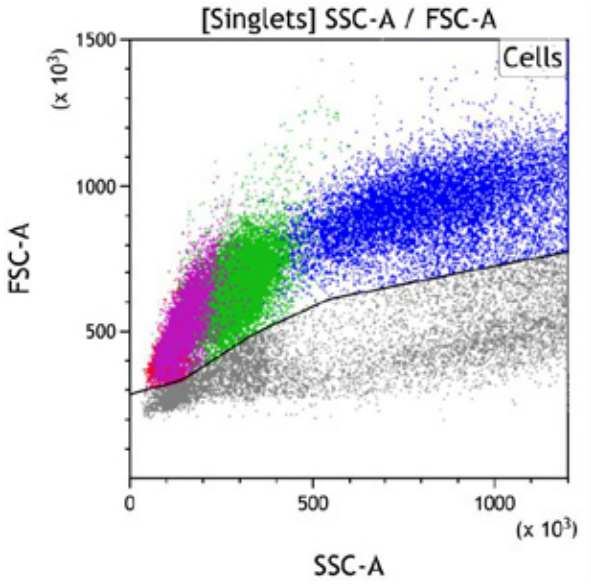

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M2-04

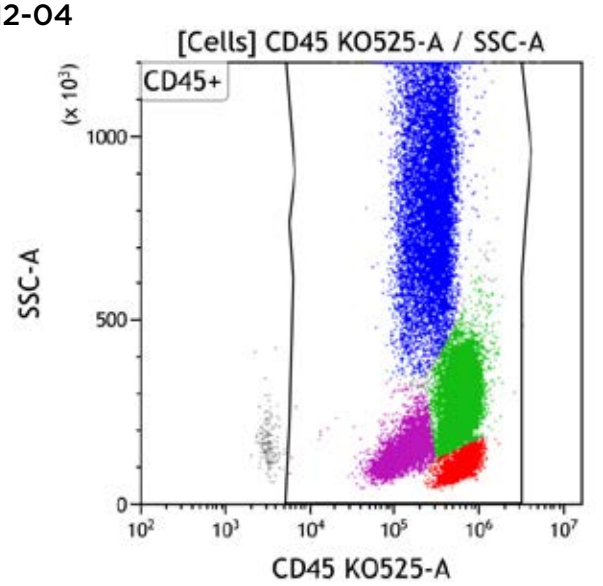

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M2-05

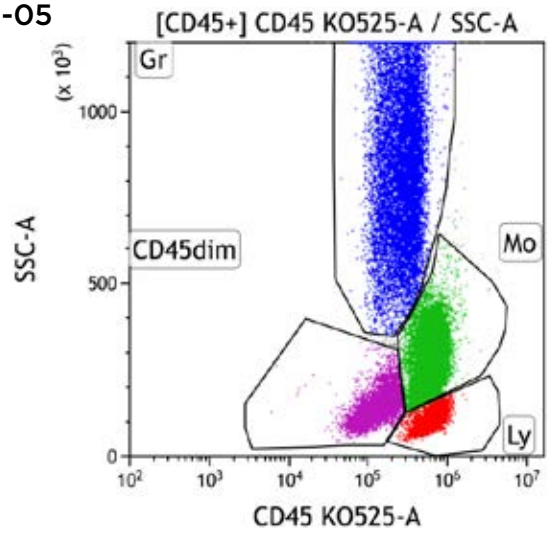

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (GateGr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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. Of note, cells in the CD45dim and monocyte gates are expanded.

M2-06

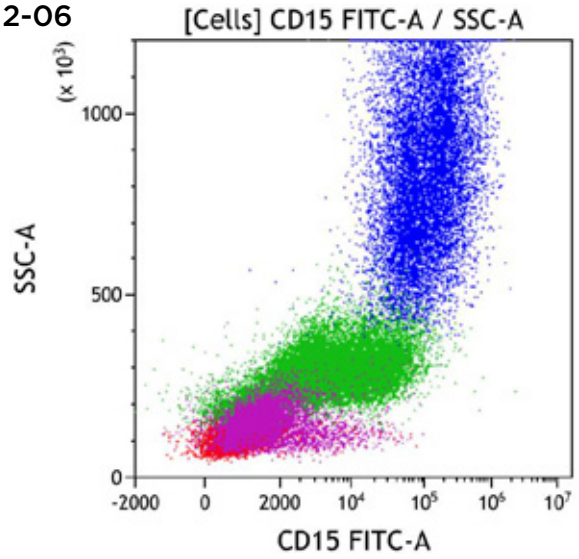

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

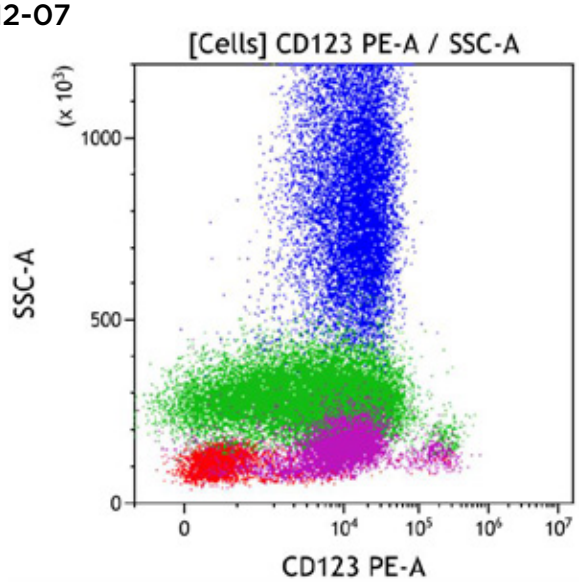

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green). Blasts (in purple) are dimly positive for CD123.

M2-08

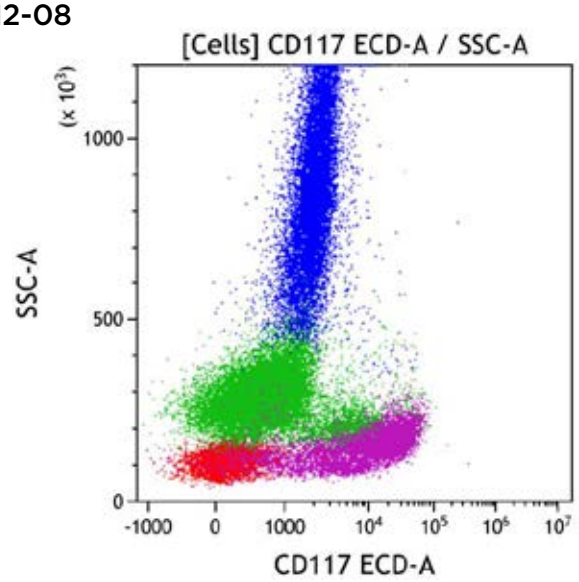

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

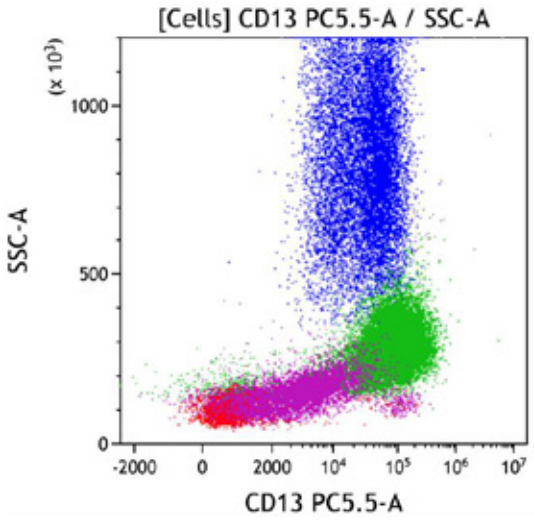

Figure 9. This CD13 vs Side Scatter dot plot shows all vi-able cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature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

M2-10

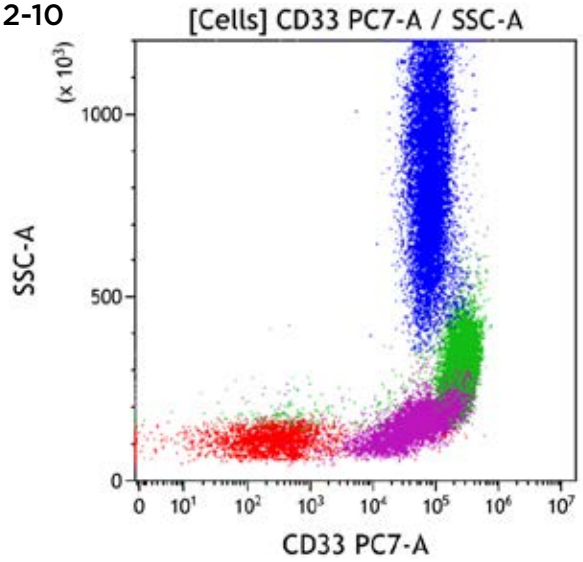

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granu-locytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a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

M2-11

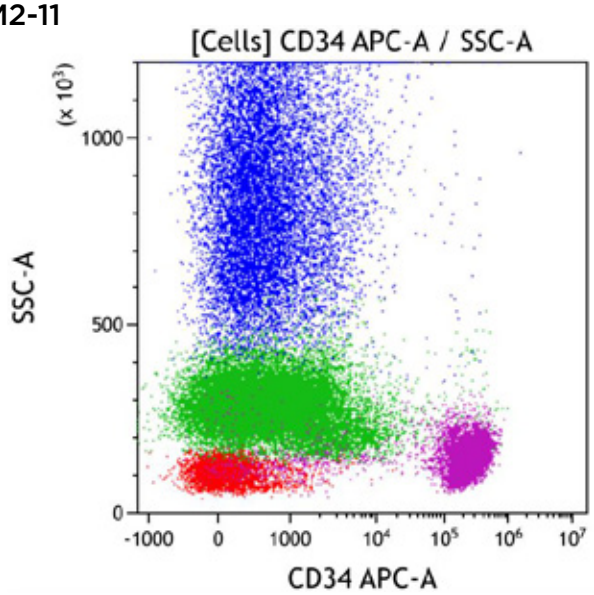

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

M2-12

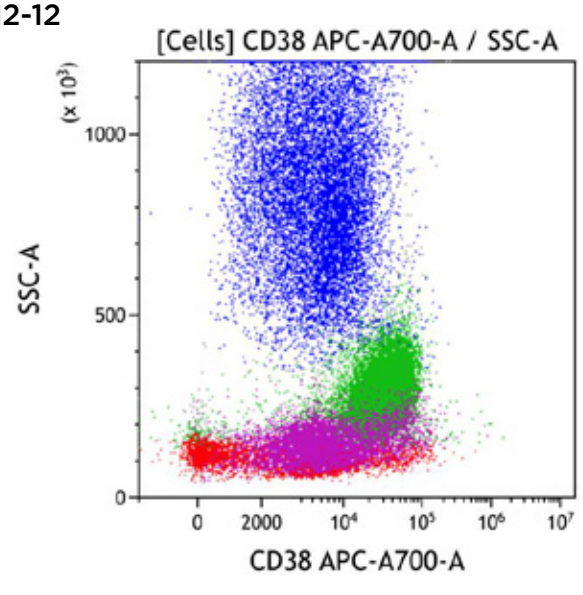

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

BACK TO CASE OVERVIEW

M2-13

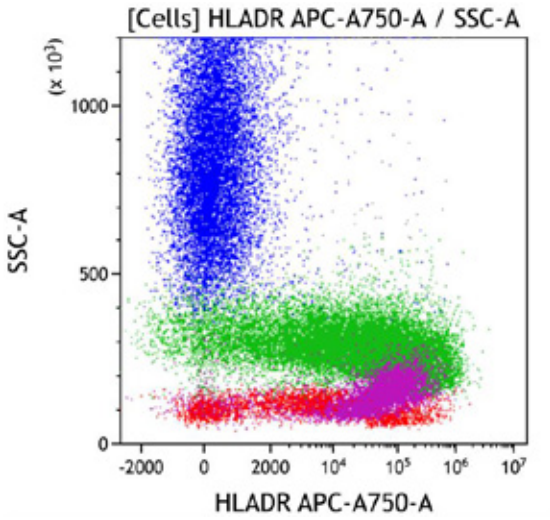

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T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

M2-14

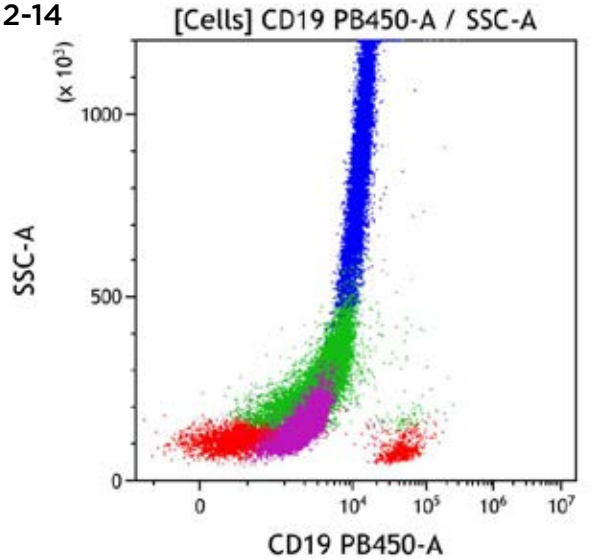

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

M2-15

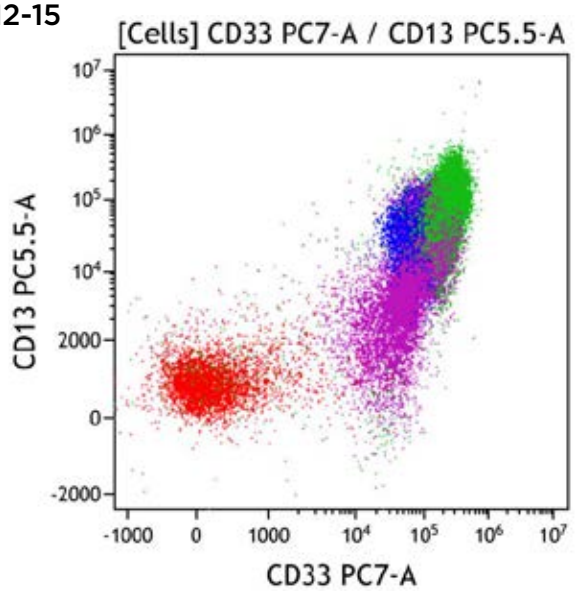

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher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

M2-16

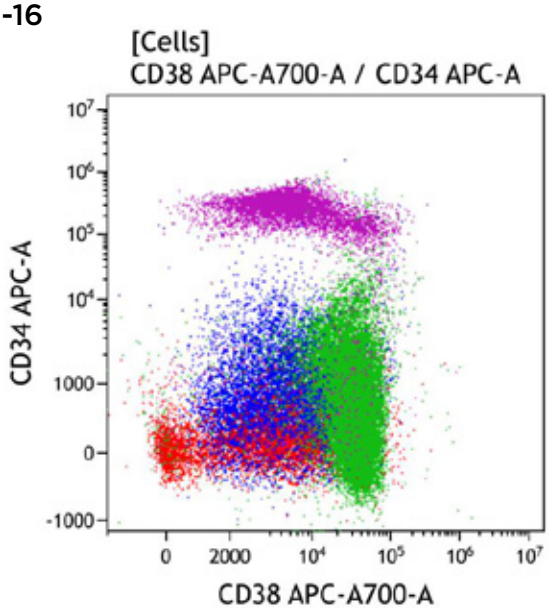

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors hav-ing variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent var-iable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

BACK TO CASE OVERVIEW

M2-17

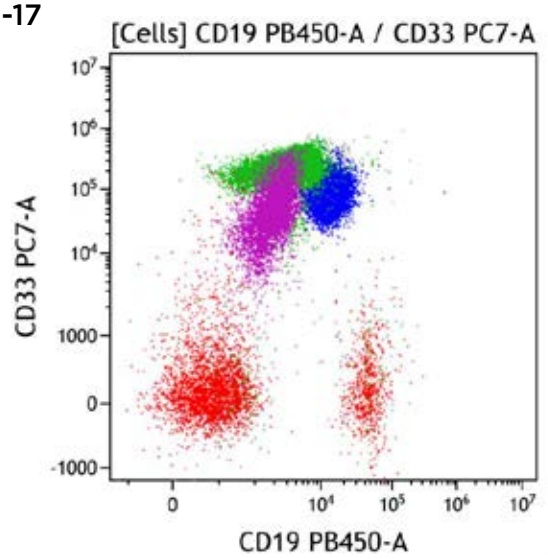

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to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

M2-18

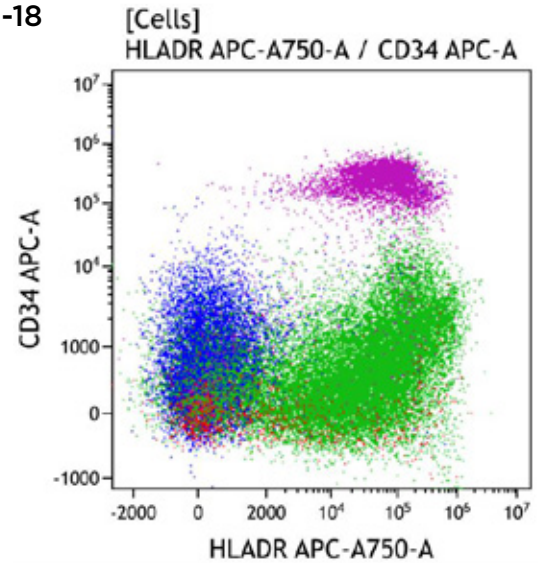

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of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

M2-19

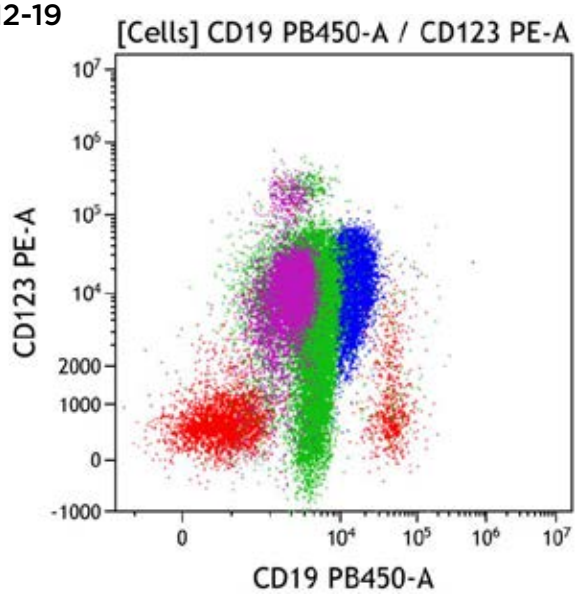

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid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

M2-20

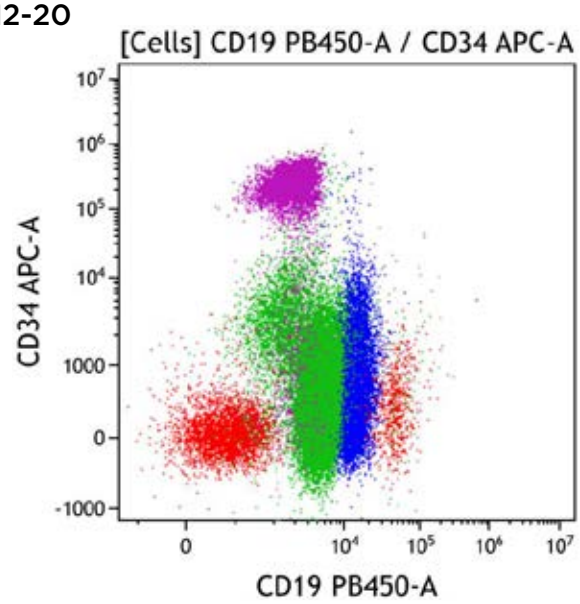

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19. The CD34 positive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

BACK TO CASE OVERVIEW

M2-21

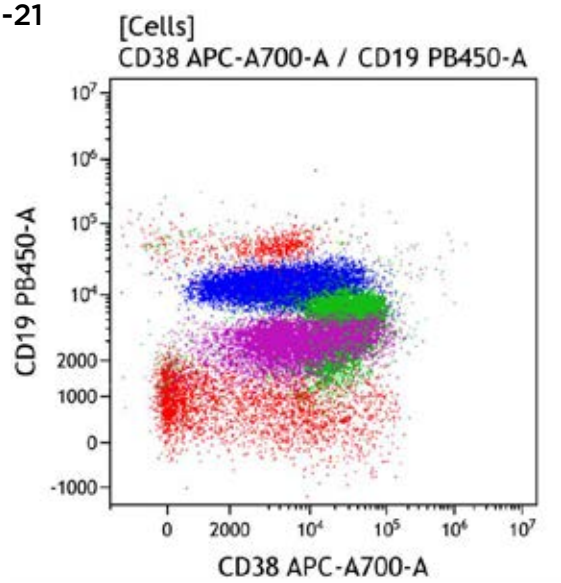

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression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

M2-22

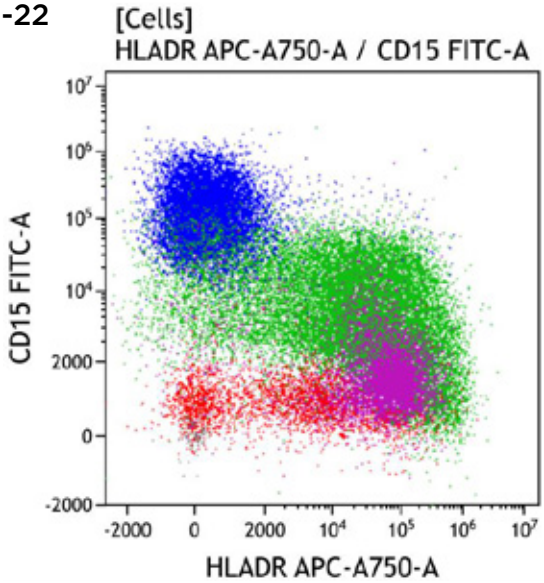

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLADR (blue),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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 8 MLL-TC4-039**
Diagnosis	**Chronic lymphocytic leukemia/small lymphocytic lymphoma**
Clinical Vignette	72-year-old man developed lymphocytosis. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	The flow cytometry study detected an abnormal mature B cell population with kappa light chain restriction. The abnormal population expresses CD5 with variably decreased expression of CD20 and normal expression of CD200. This immunophenotype is characteristic for chronic lymphocytic leukemia/small lymphocytic lymphoma.

BACK TO CASE LISTINGS

B Cell Tube

B01

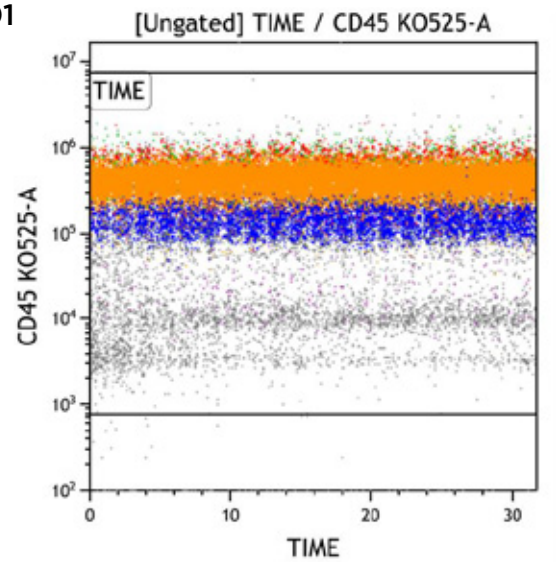

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

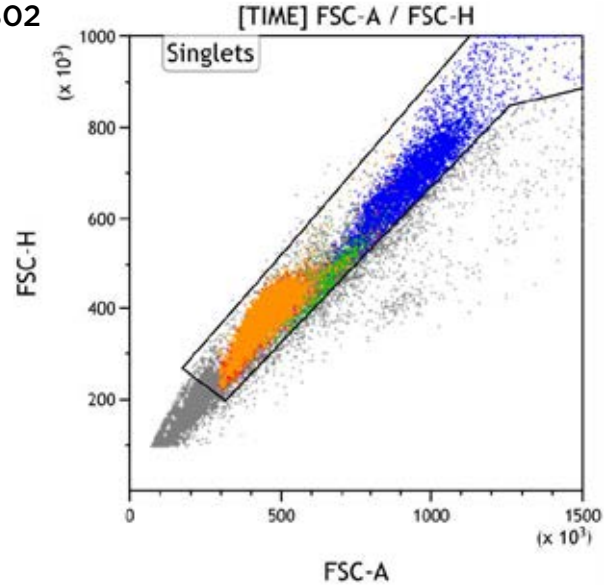

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

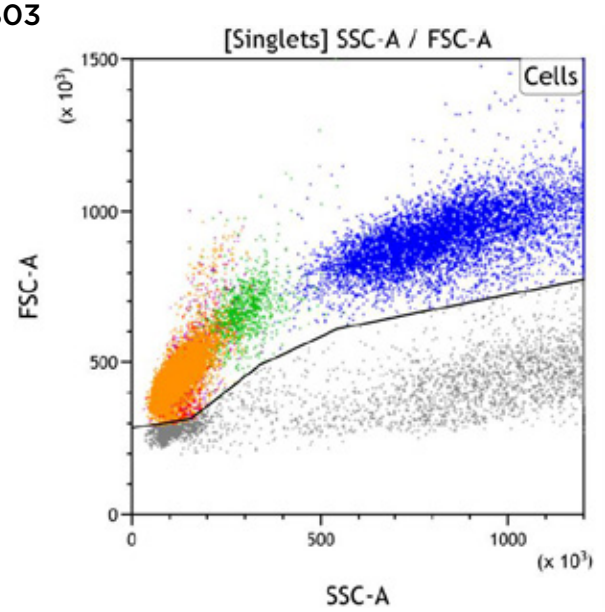

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased orward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

B04

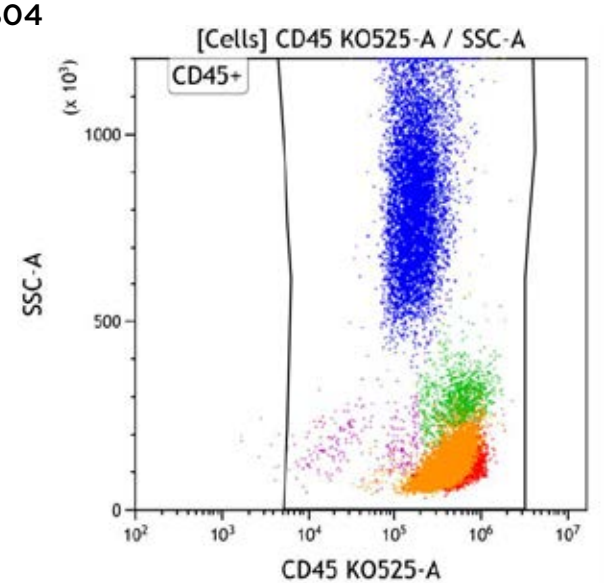

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

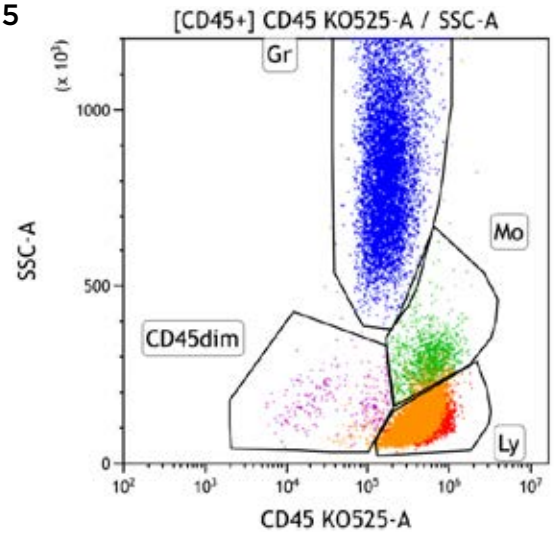

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 cells in the lymphocyte gate are increased.

B06

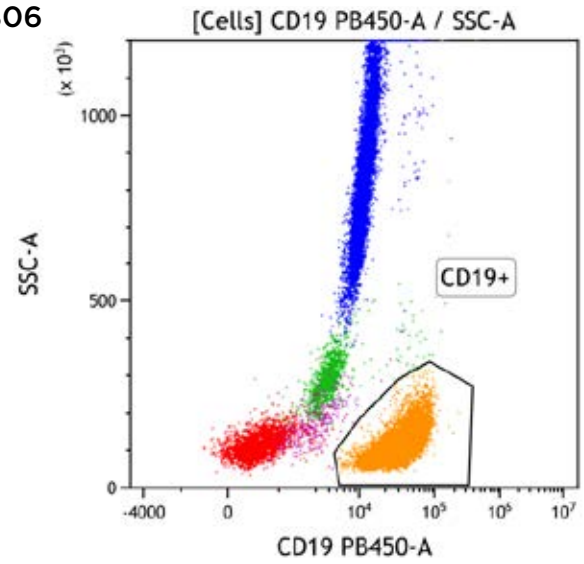

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate (in orange) identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.CD19-positive cells are increased.

B07

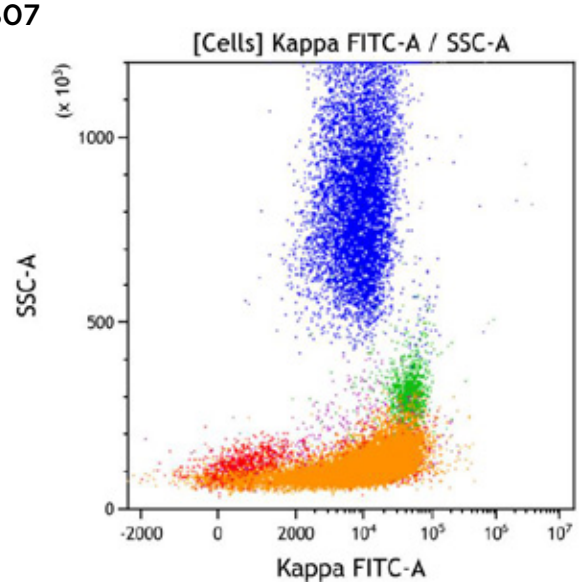

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in orange) and latestage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa 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.

B08

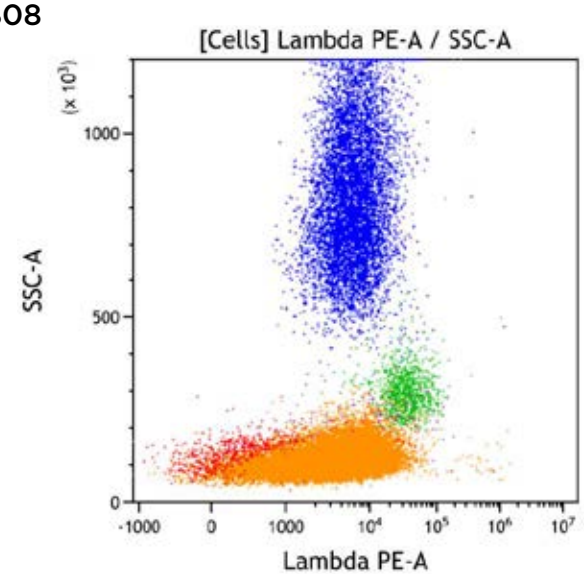

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent 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.

BACK TO CASE OVERVIEW

B09

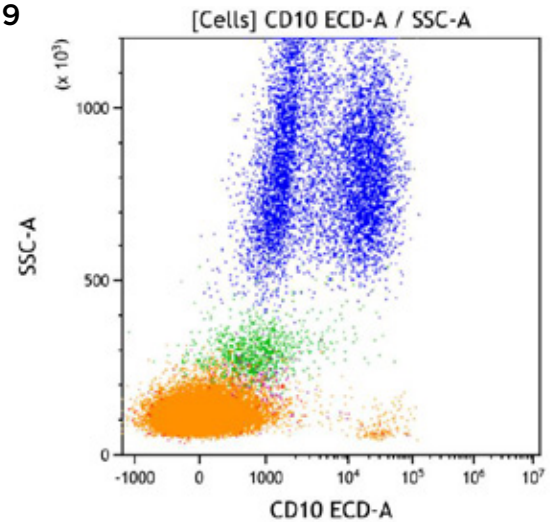

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. A very small subset of B cells is CD10-positive. This subset corresponds to normal B cell precursors.

B10

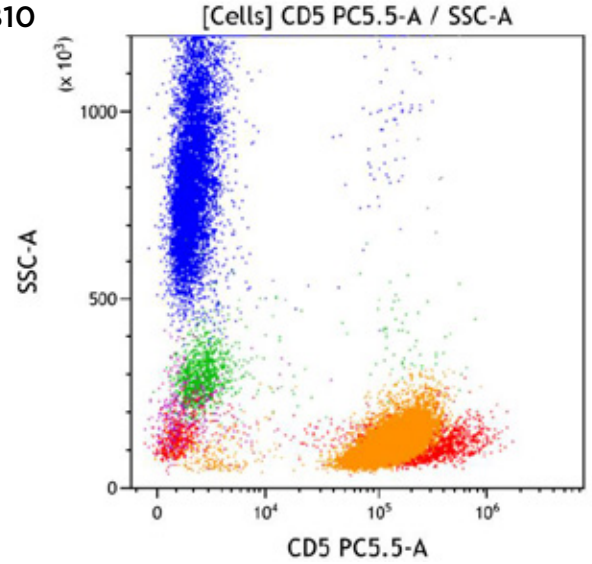

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in orange). These lymphoid cells typically have low side scatter. Note most of B cells are uniformly CD5-positive at a level slightly dimmer than T cells (in red).

B11

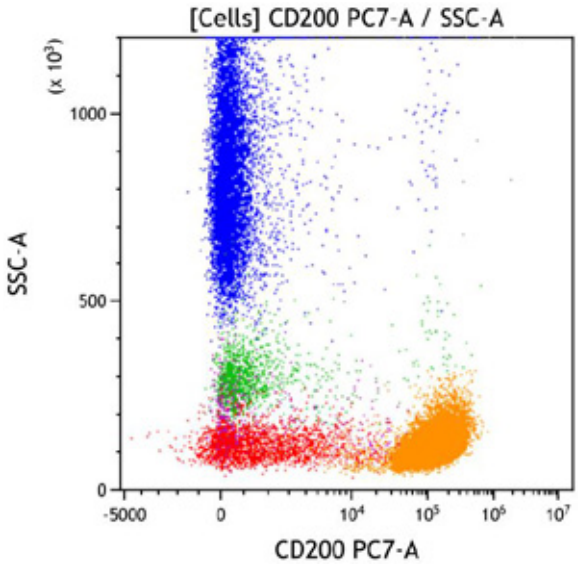

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

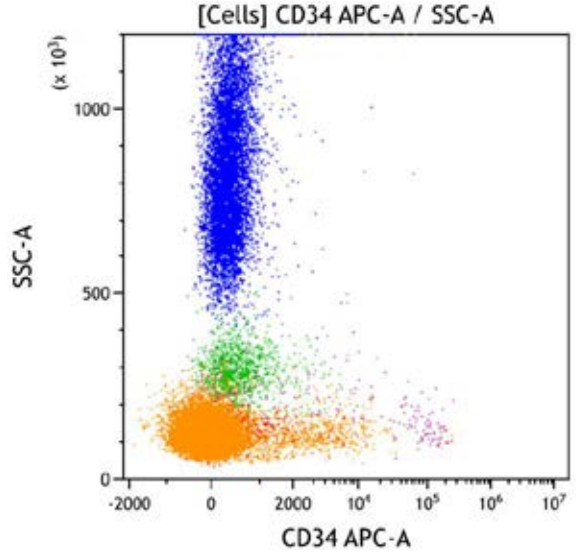

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

B13

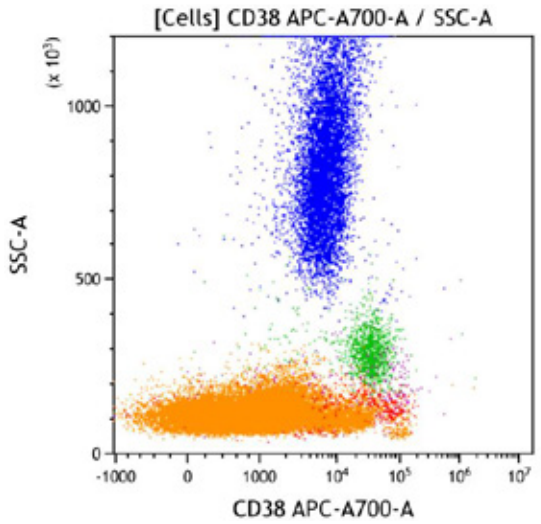

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

B14

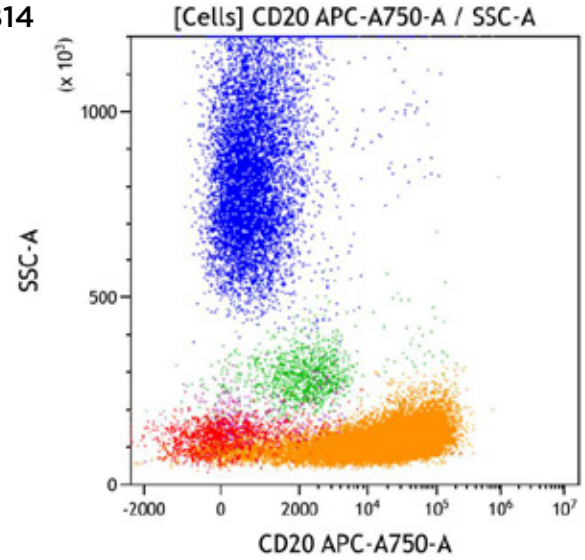

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is uniformly expressed on mature B cells in the lymphocyte gate. Here CD20 is variably expressed (in orange).

B15

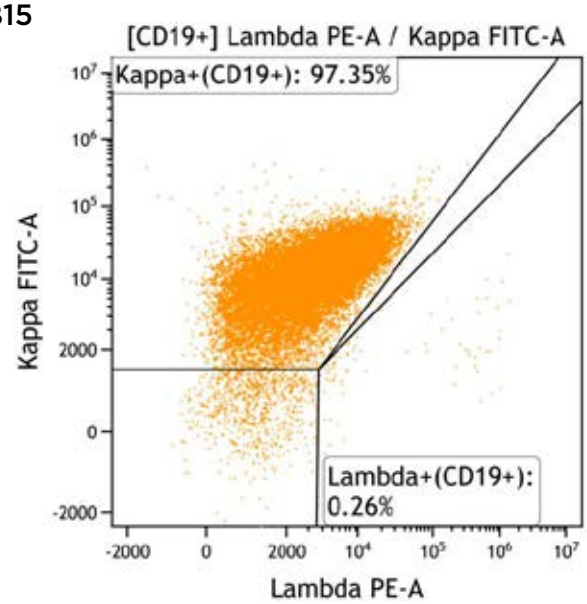

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is 1.4 with a range between 1 to 2. Note most of B cells dimly express kappa light chain, indicative of kappa light chain restriction. Dim surface light chain expression is characteristic in chronic lymphocytic leukemia.

B16

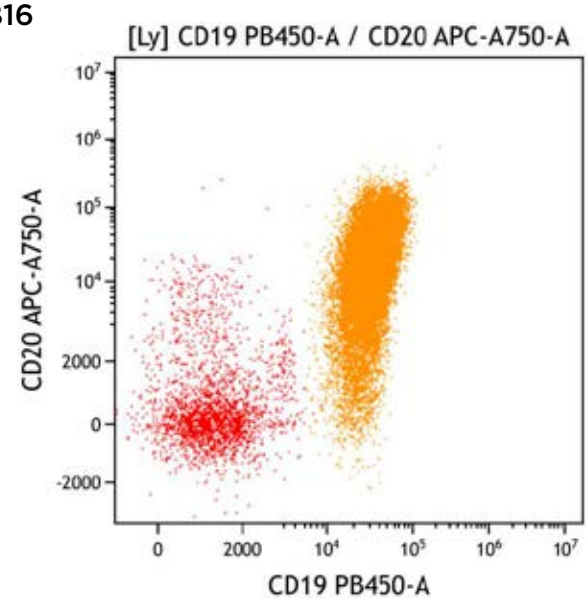

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells uniformly express both CD19 and CD20, whereas immature B cells express CD19 and variably lower CD20. Here, the expanded mature B cells express variably decreased CD20.

BACK TO CASE OVERVIEW

B17

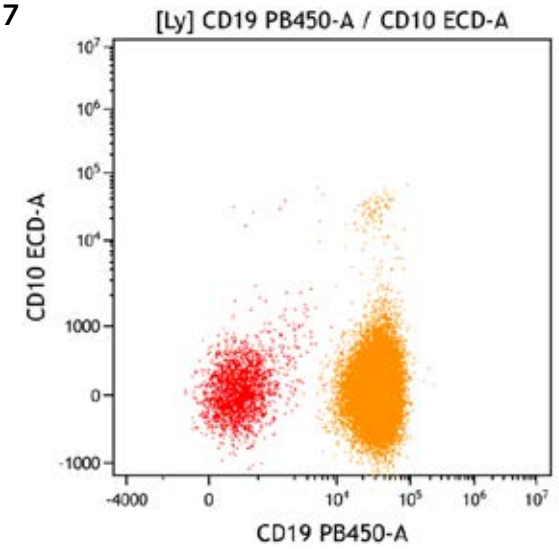

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate. B cells are CD19 positive (in orange). Note a small subset of late-stage immature B cells is present. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate.

B18

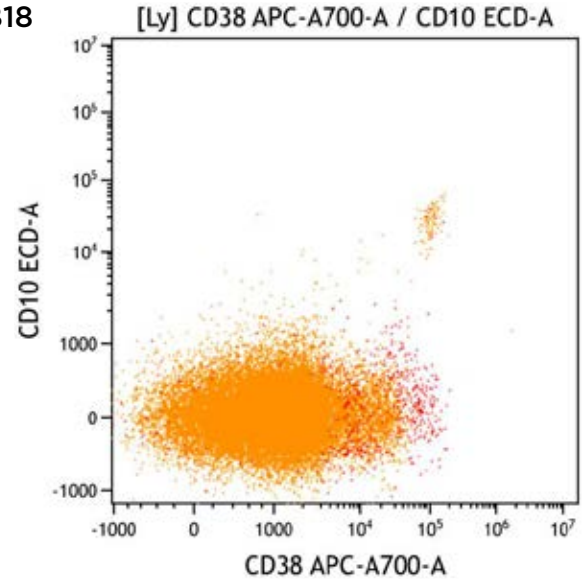

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

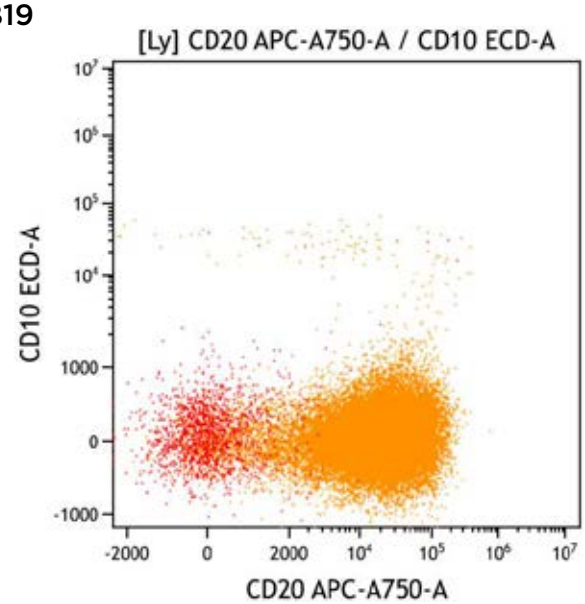

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells variably express CD20. The rare CD10 positive B cells with variably decreased CD20 represent latestage immature B cells.

B20

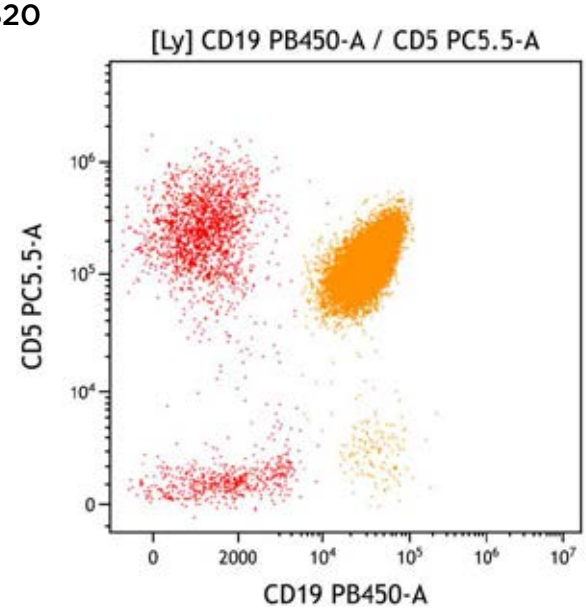

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is uniformly expressed on the expanded B cells, which is characteristic in chronic lymphocytic leukemia/ small lymphocytic lymphoma and mantle cell lymphoma. CD5 can be variably expressed on a small subset of normal mature B cells.

BACK TO CASE OVERVIEW

B21

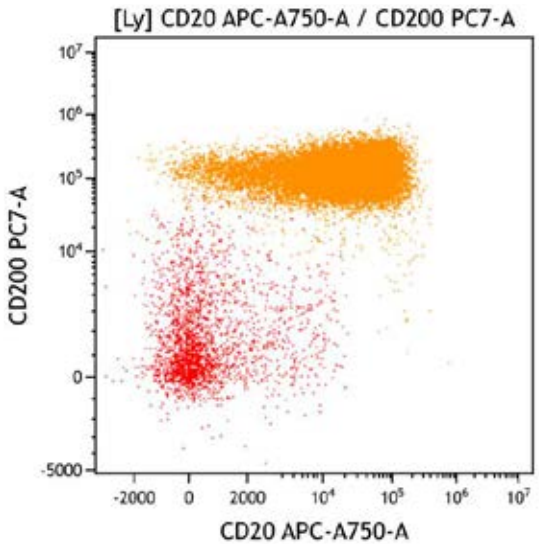

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in orange).

B22

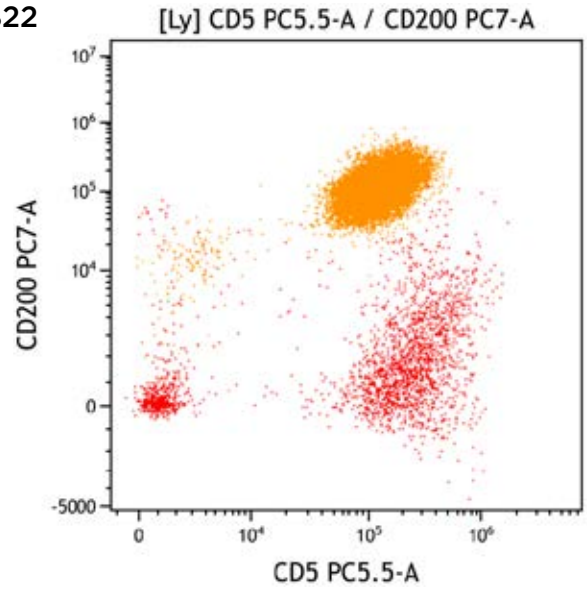

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically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

BACK TO CASE OVERVIEW

T Cell Tube

T01

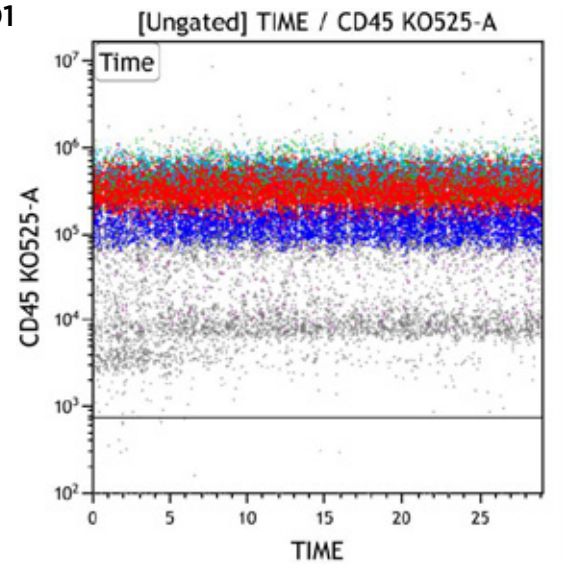

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

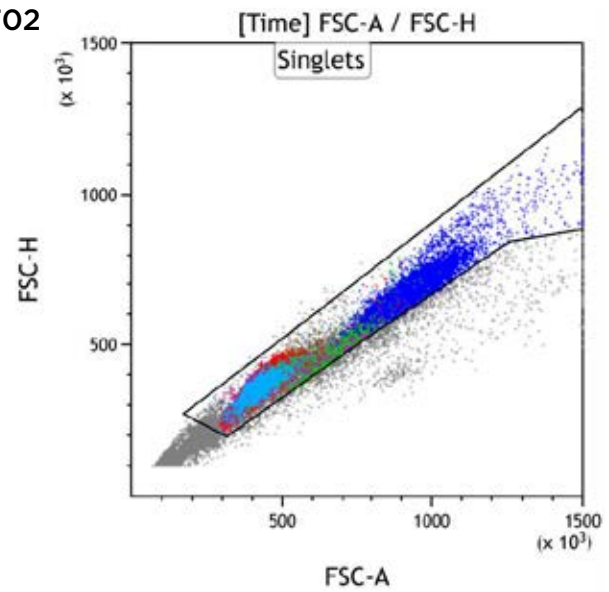

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

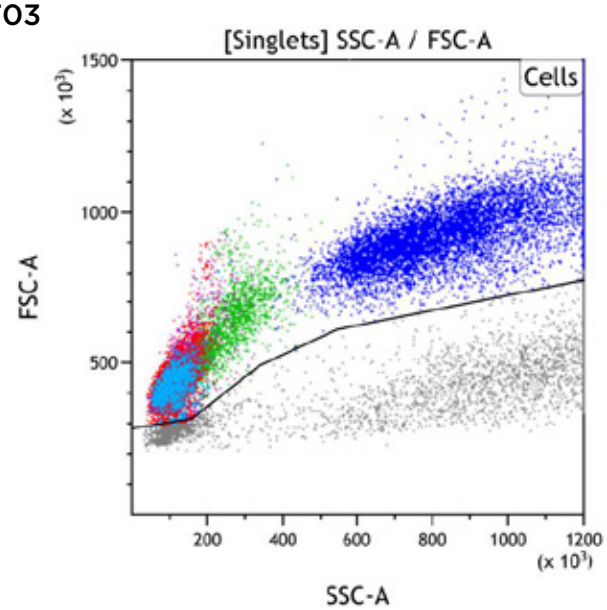

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

T04

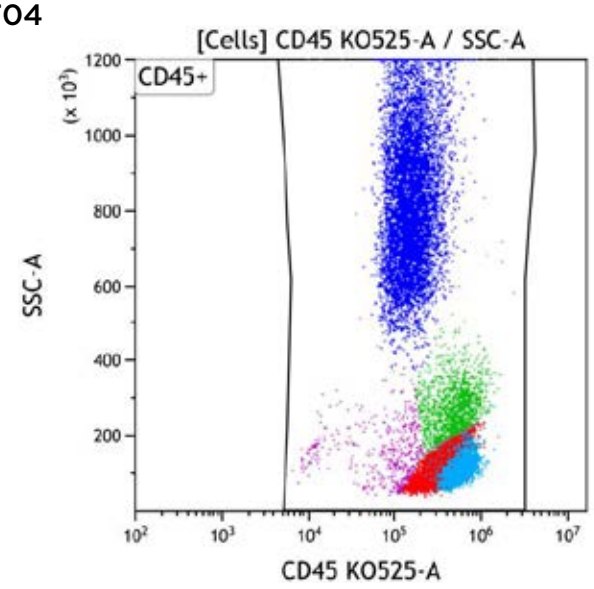

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

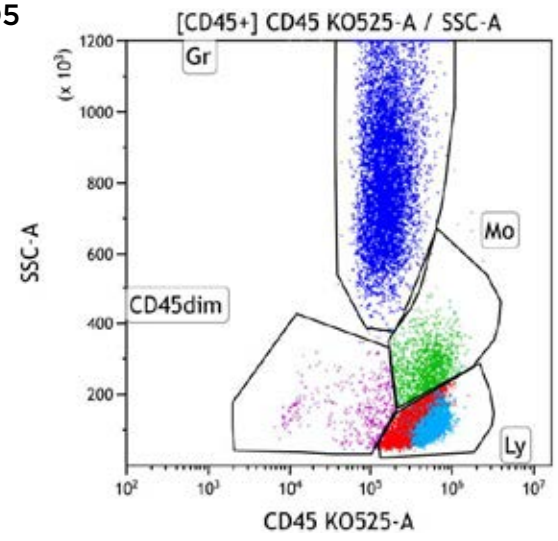

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

T06

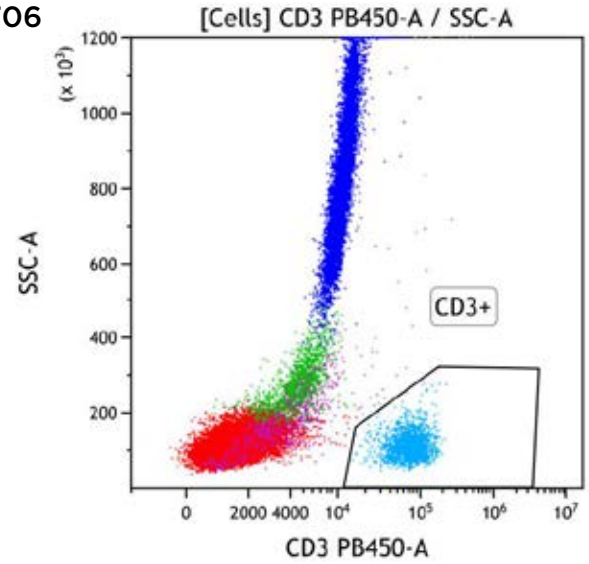

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

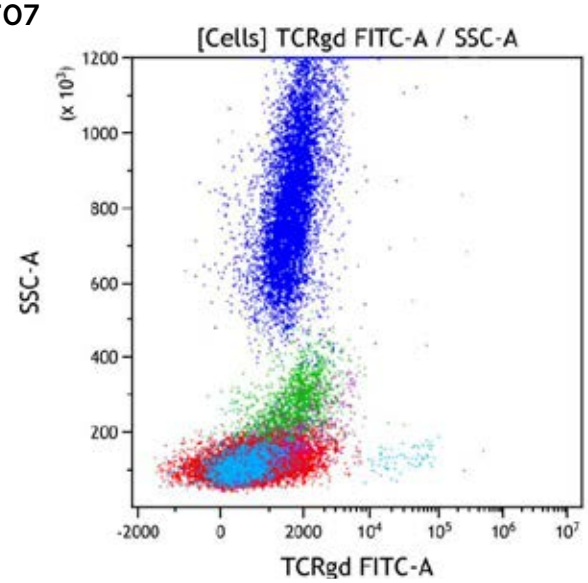

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

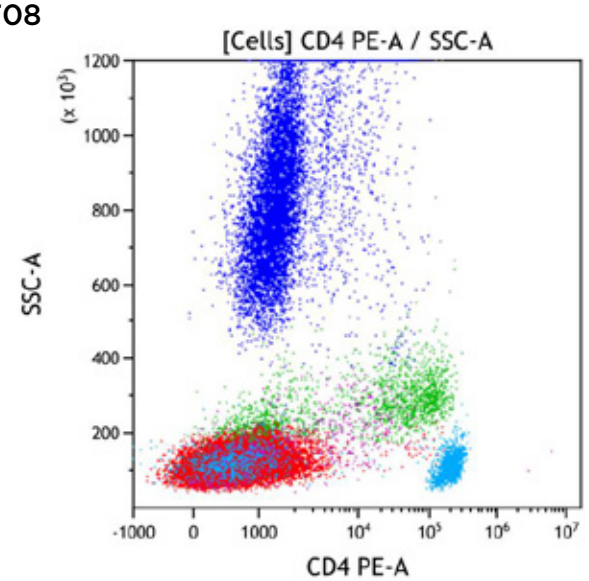

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

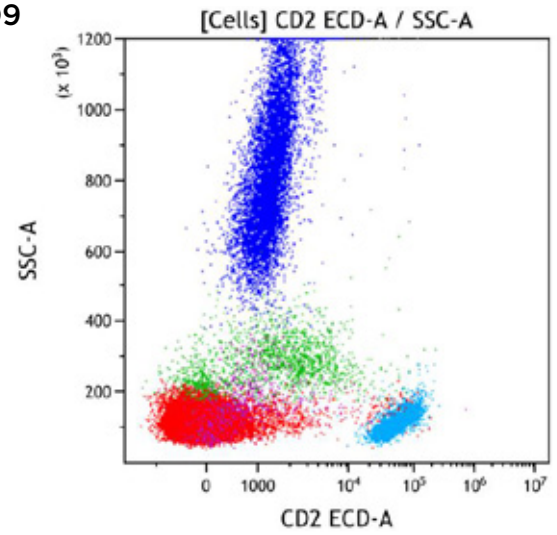

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

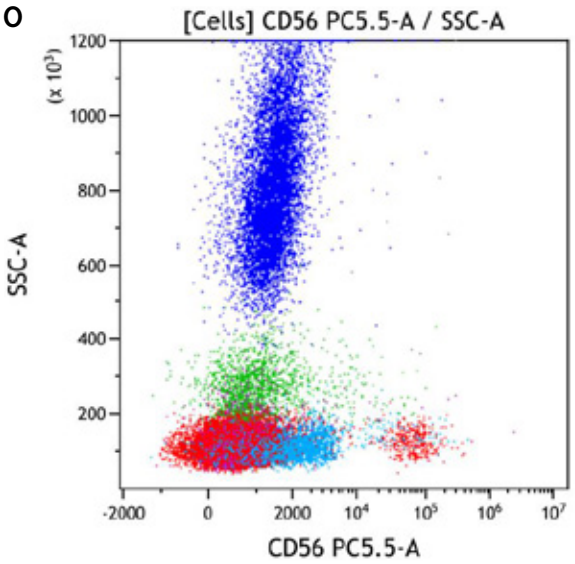

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/ delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and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

T11

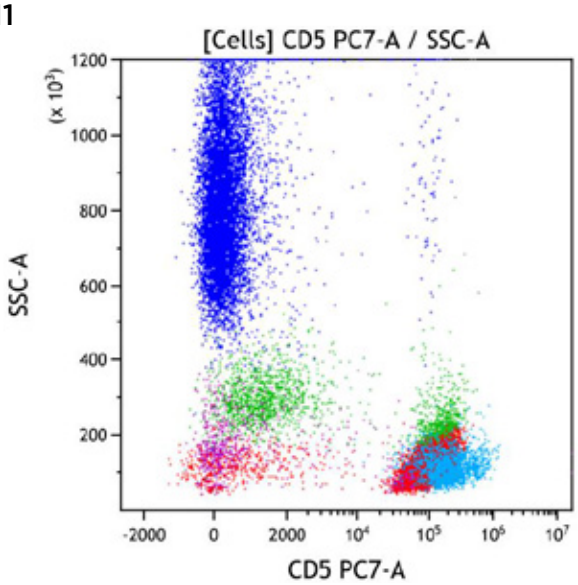

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Note a large subset of CD3-negative lymphocytes (in red) abnormally expresses CD5.

T12

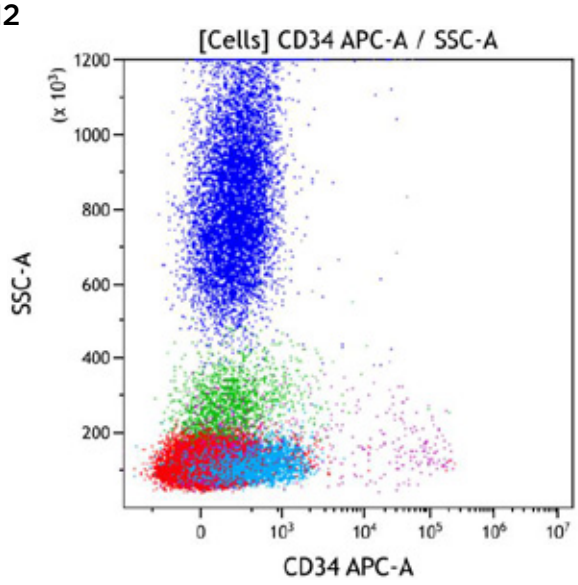

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

T13

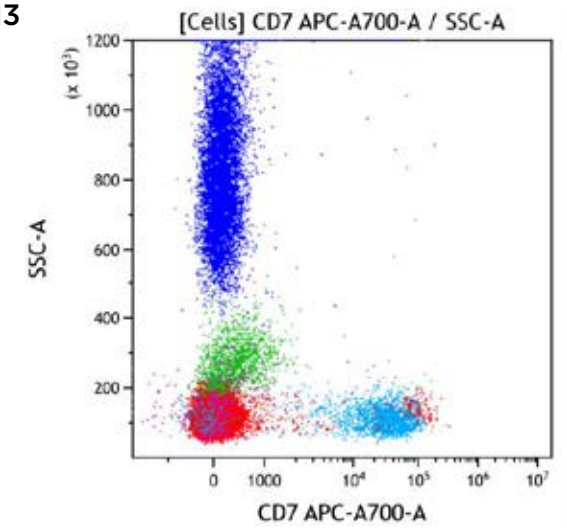

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

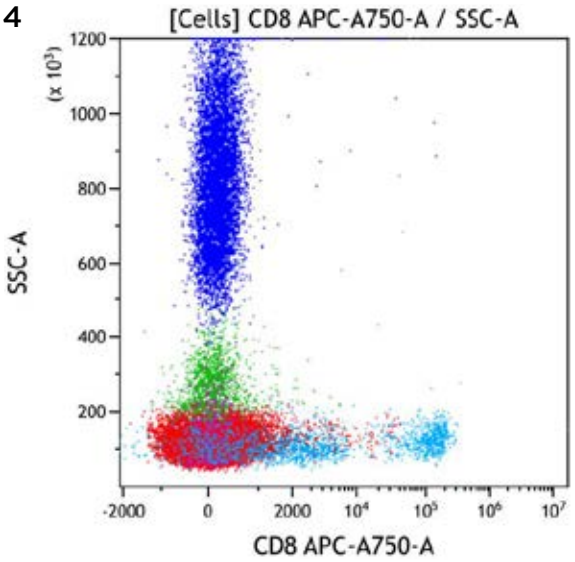

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

T15

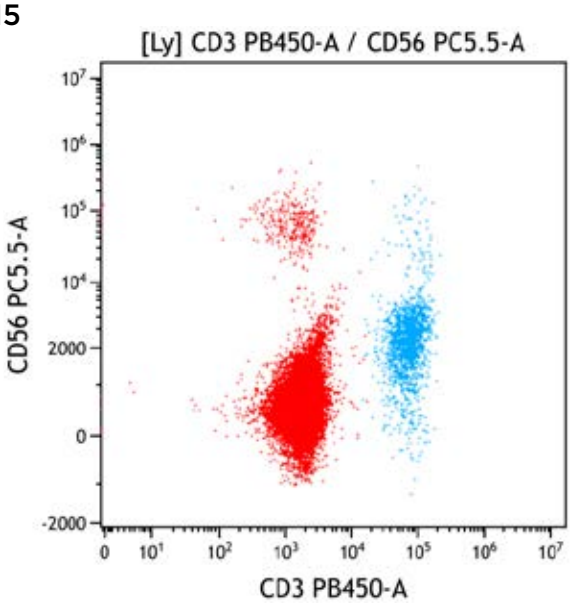

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small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

T16

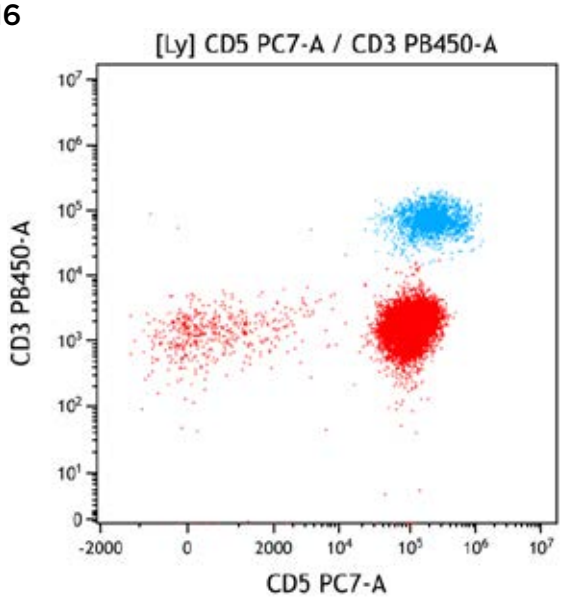

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most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

BACK TO CASE OVERVIEW

T17

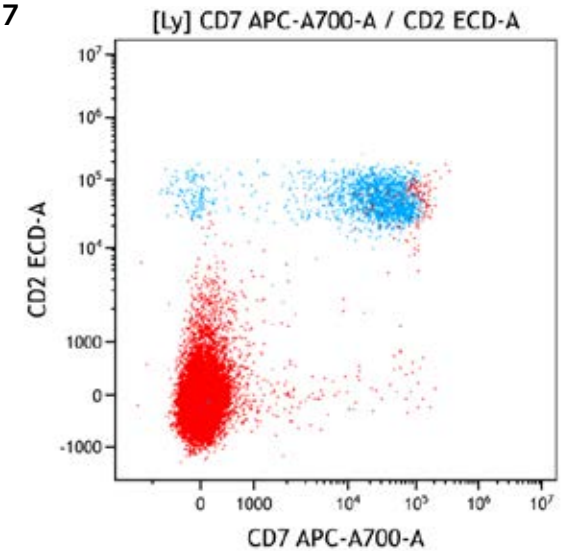

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

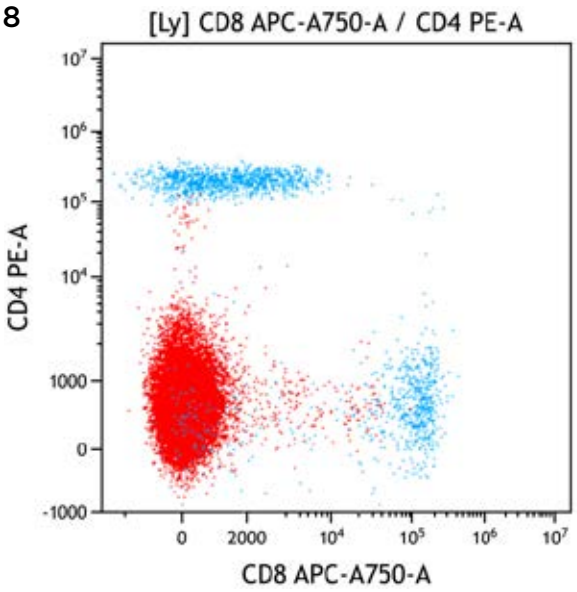

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 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 rare 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.

T19

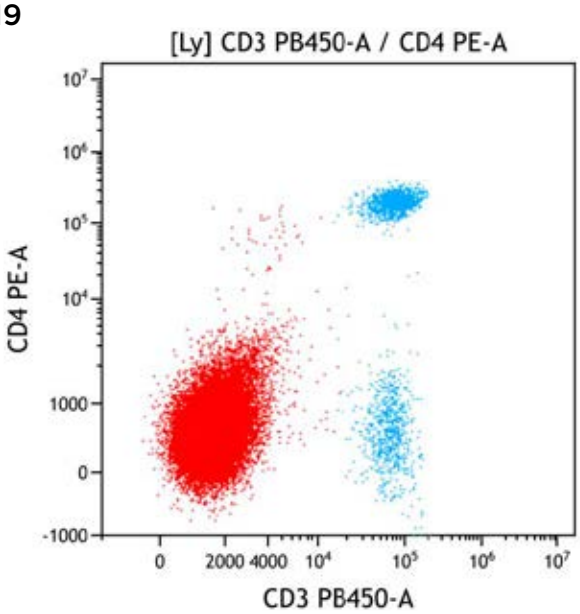

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

T20

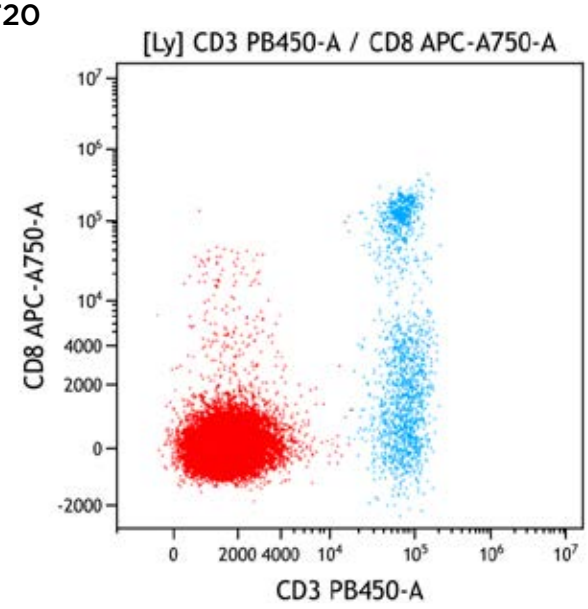

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

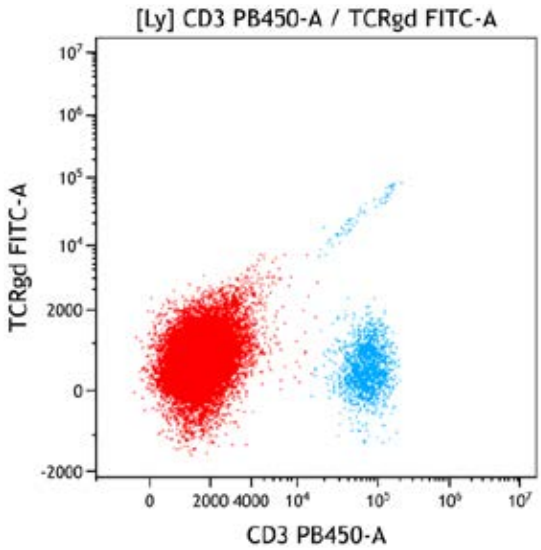

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

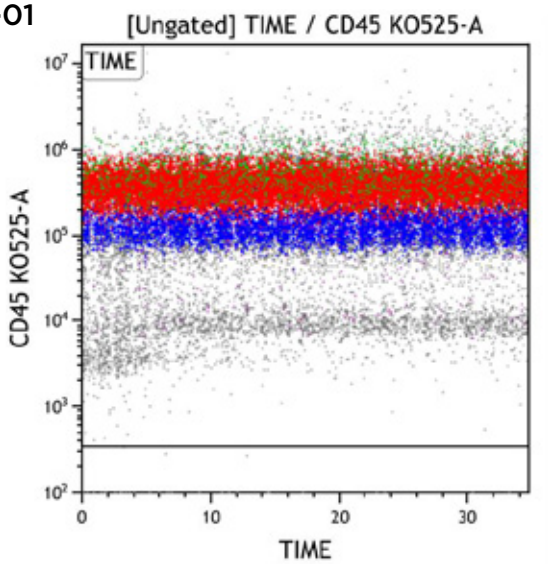

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

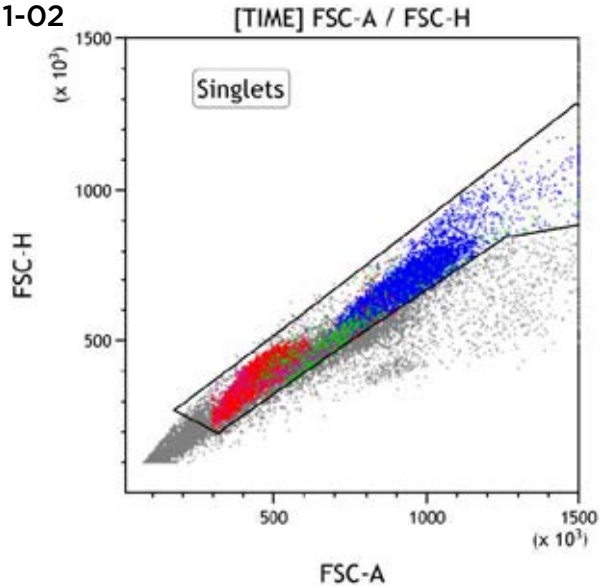

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

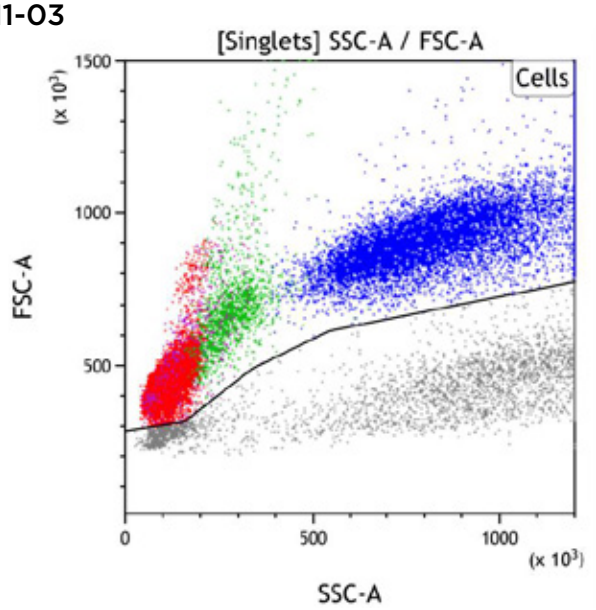

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M1-04

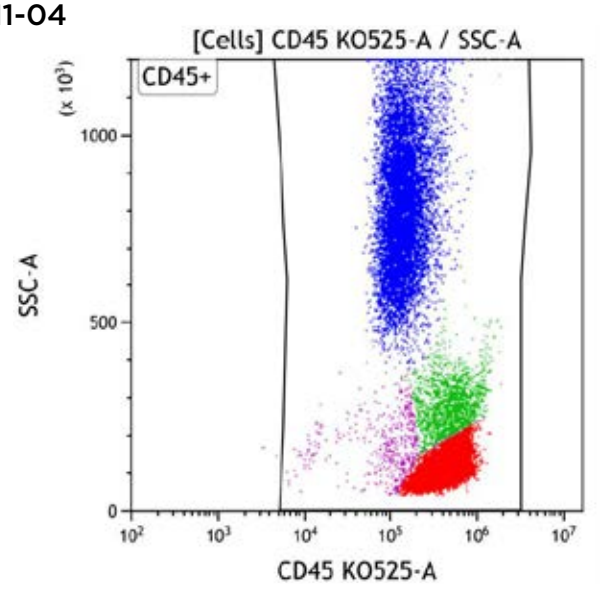

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

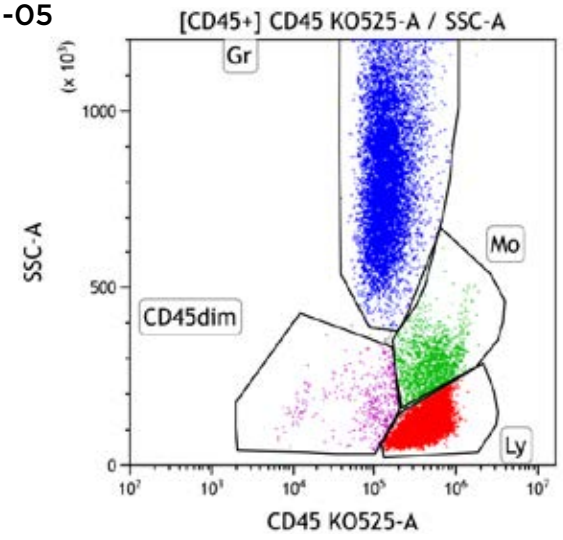

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M1-06

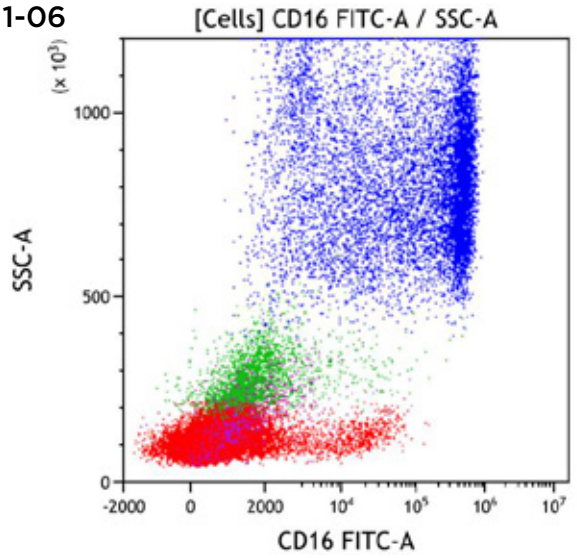

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

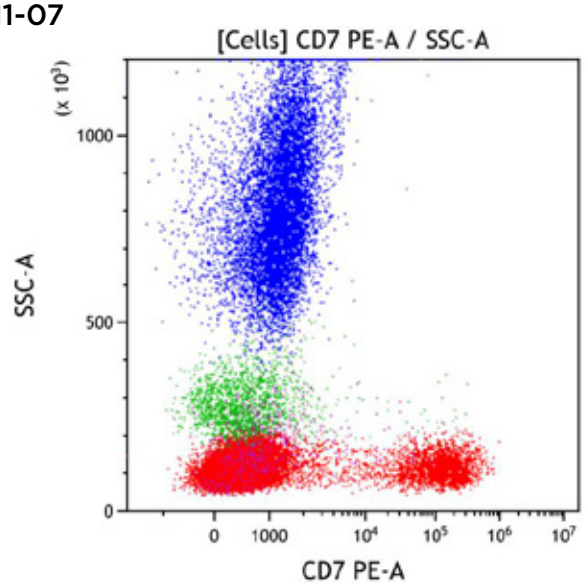

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

M1-08

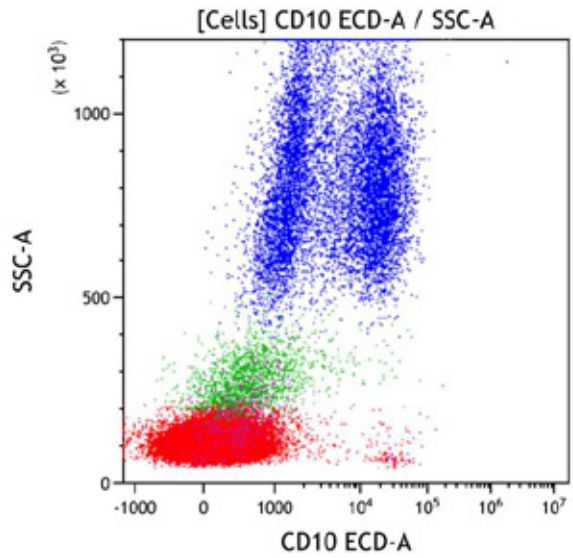

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

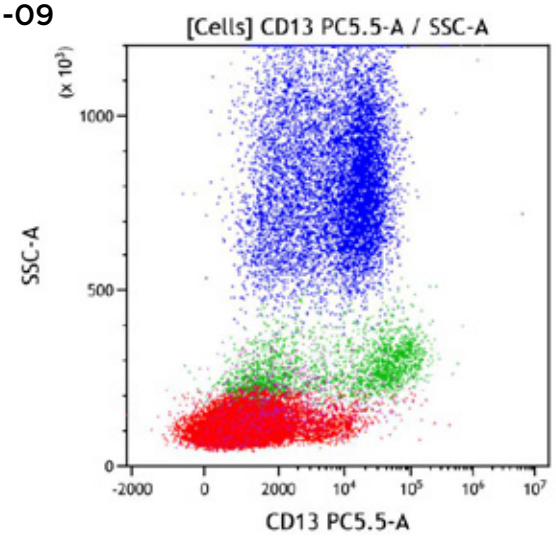

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M1-10

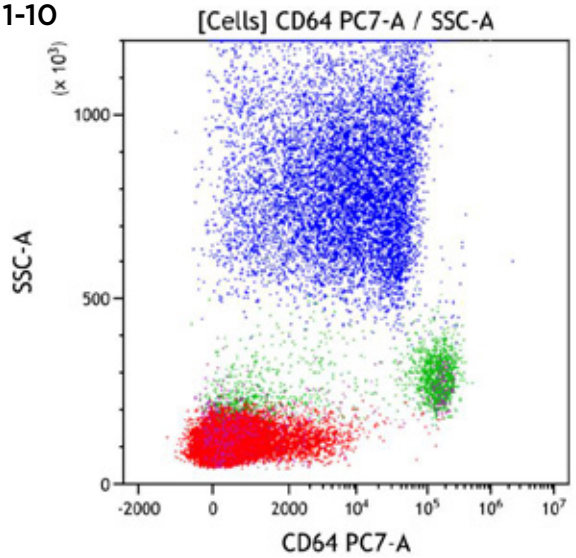

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

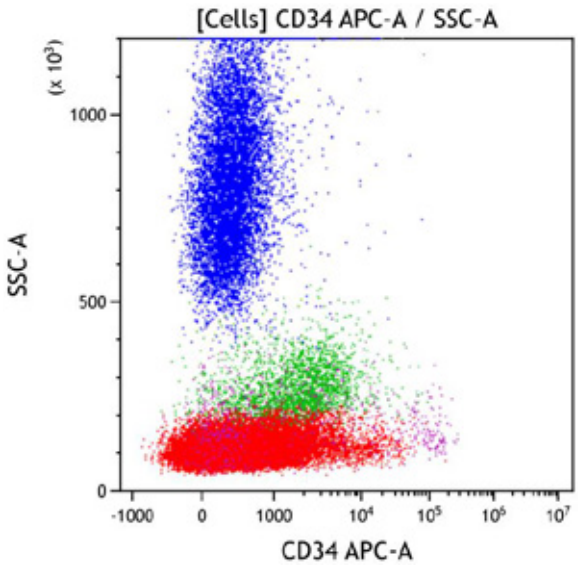

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

M1-12

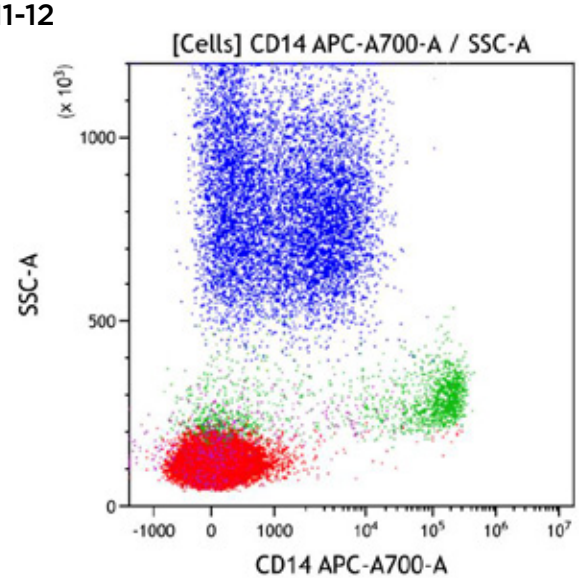

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

BACK TO CASE OVERVIEW

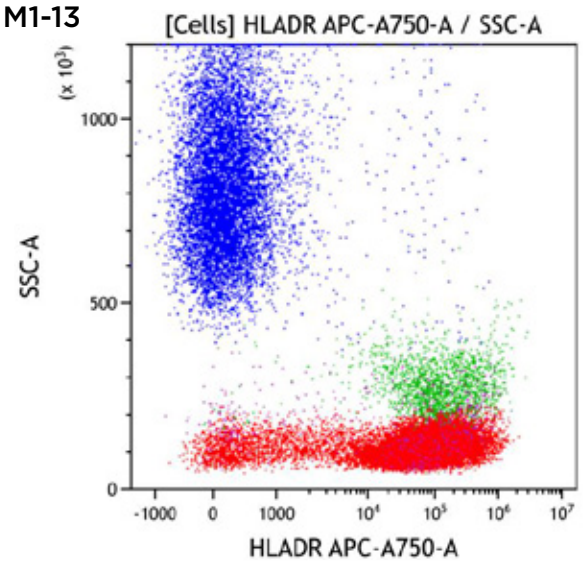

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).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

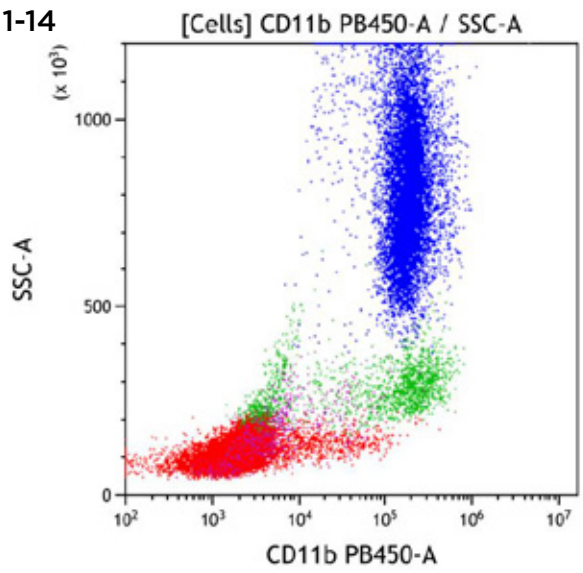

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

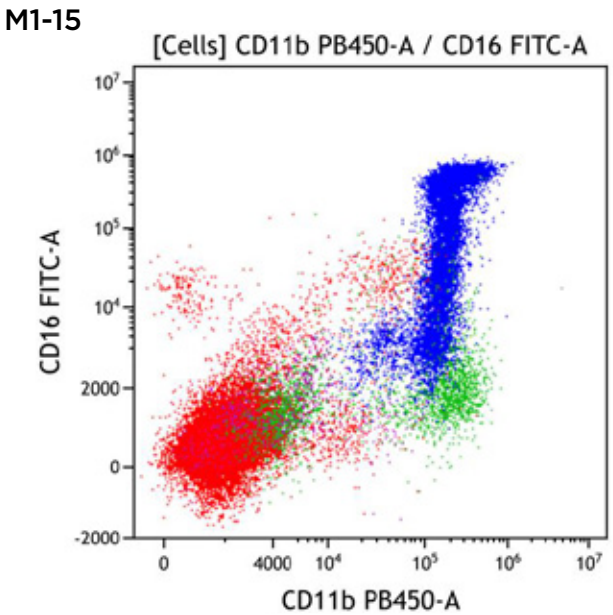

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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.

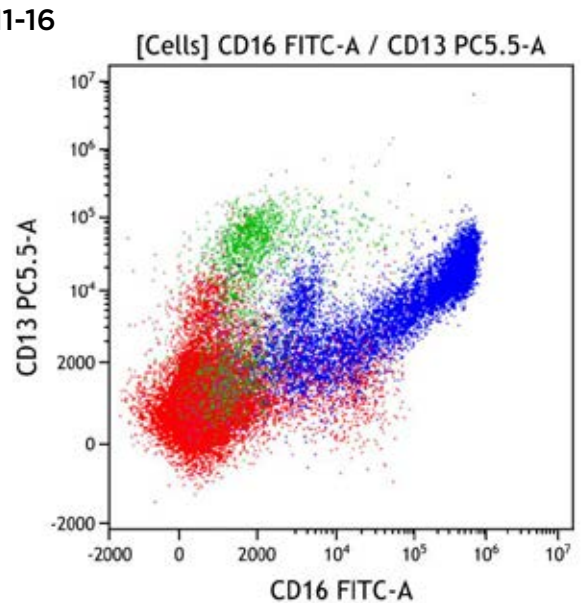

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed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

BACK TO CASE OVERVIEW

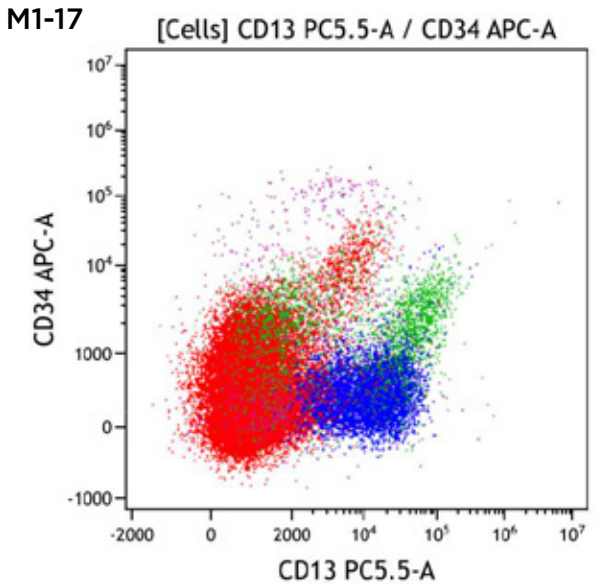

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple)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

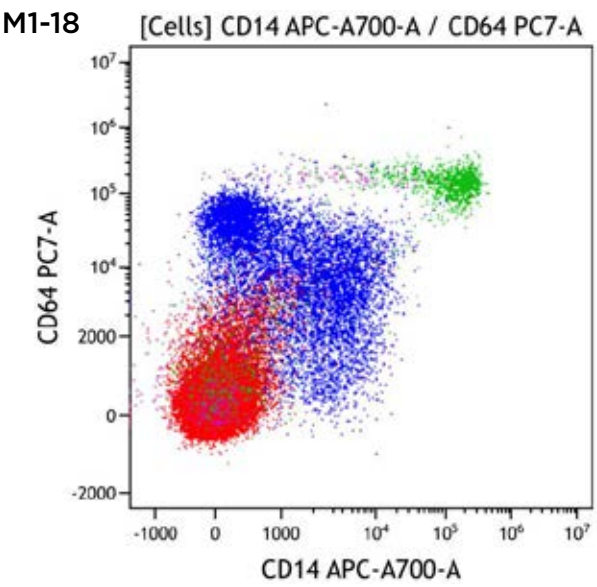

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high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

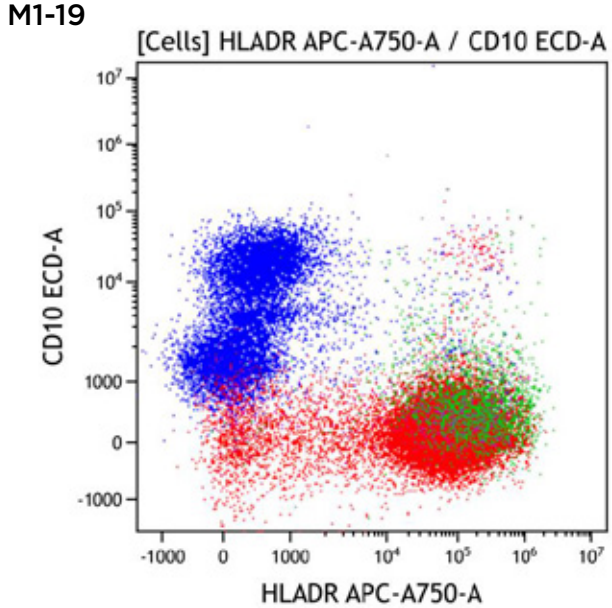

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

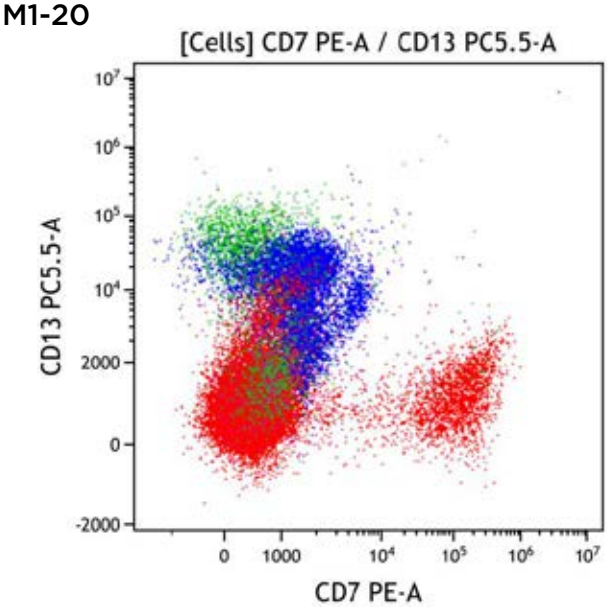

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

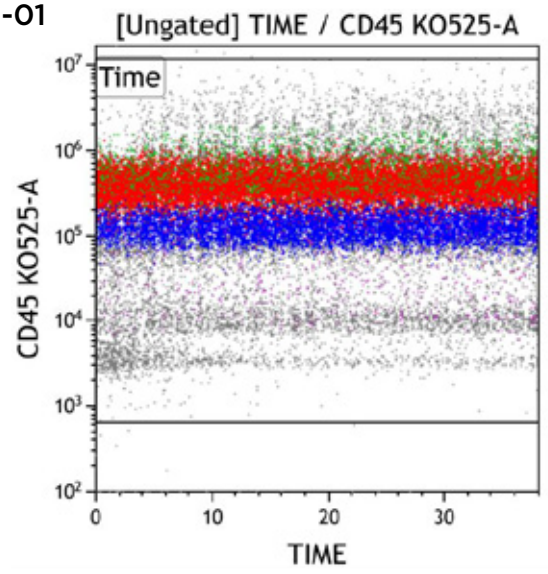

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

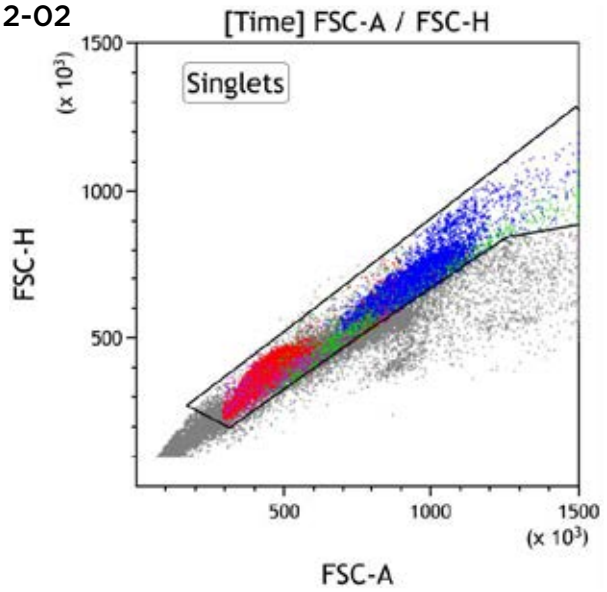

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

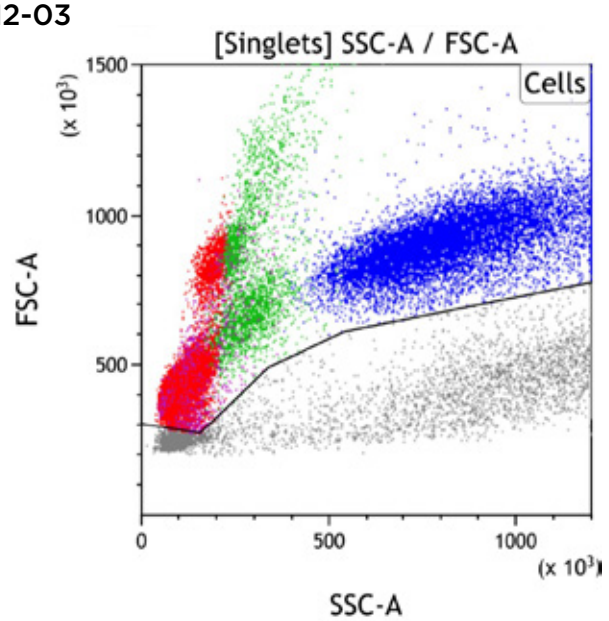

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M2-04

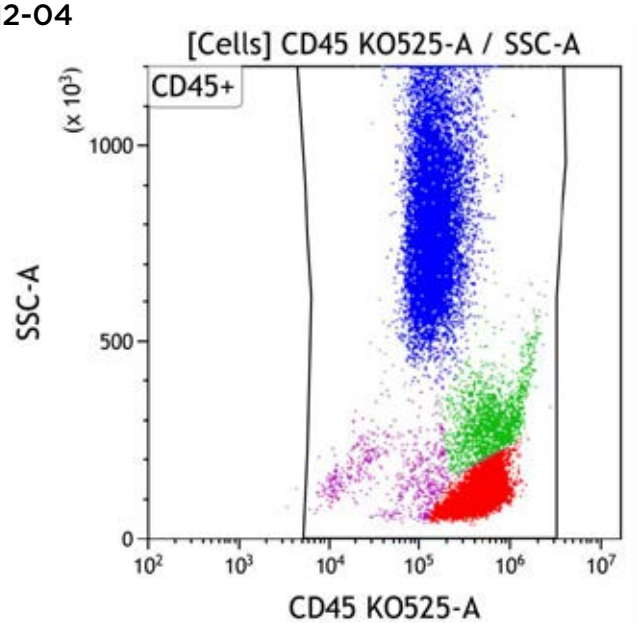

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M2-05

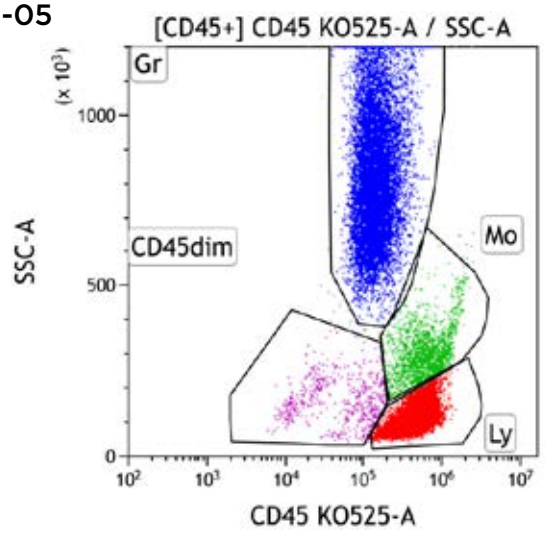

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M2-06

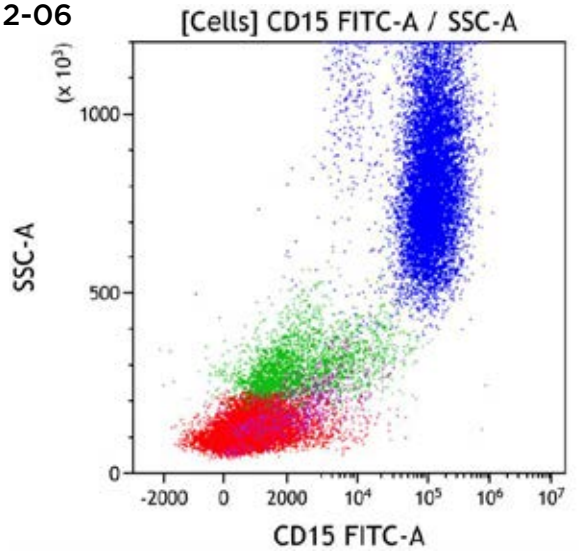

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

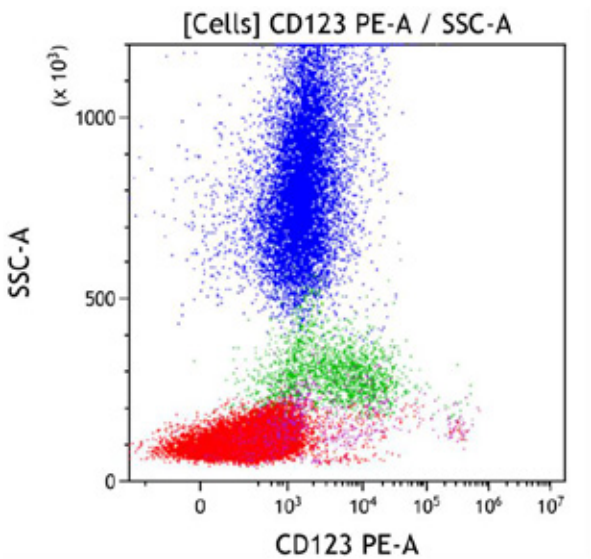

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green).

M2-08

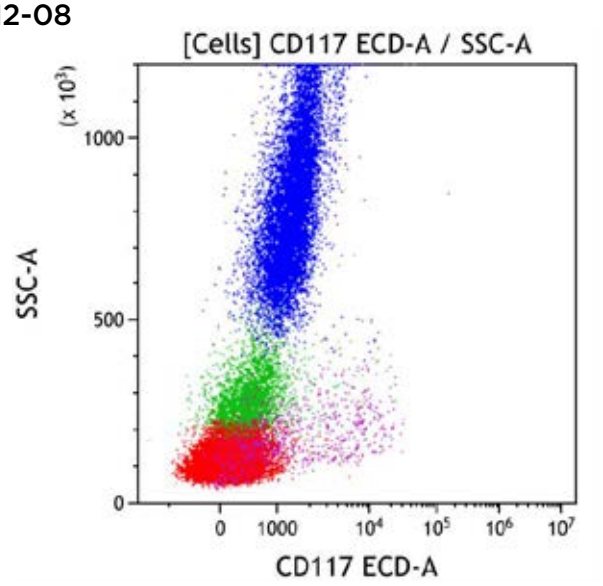

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

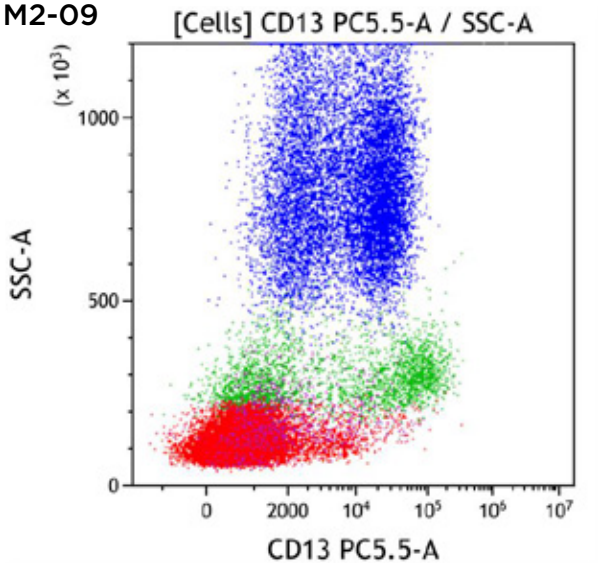

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M2-10

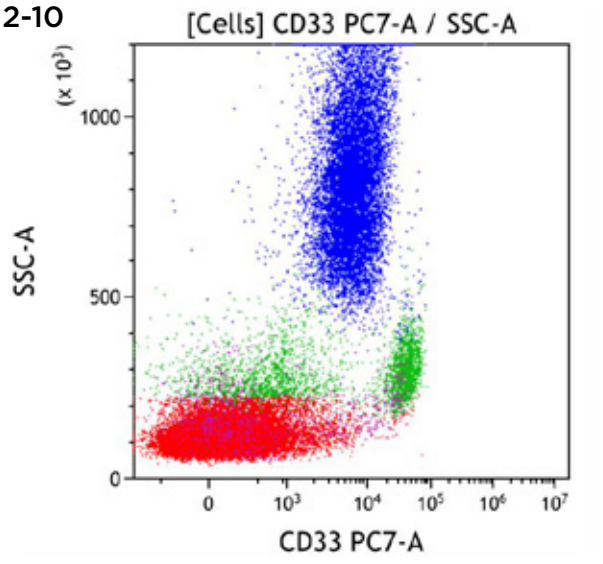

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a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

M2-11

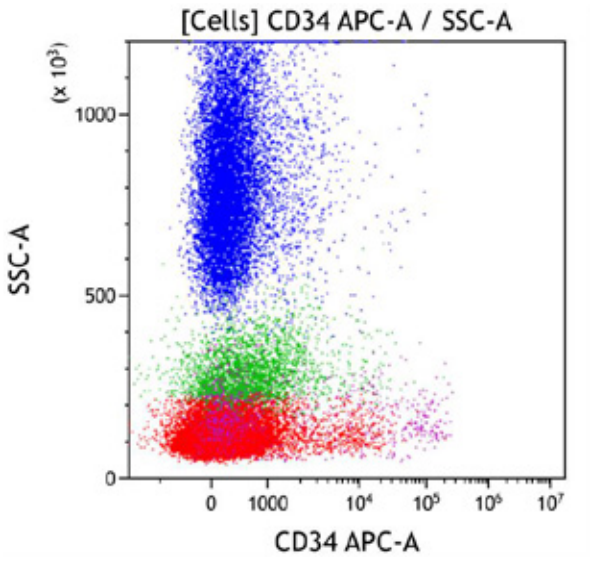

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

M2-12

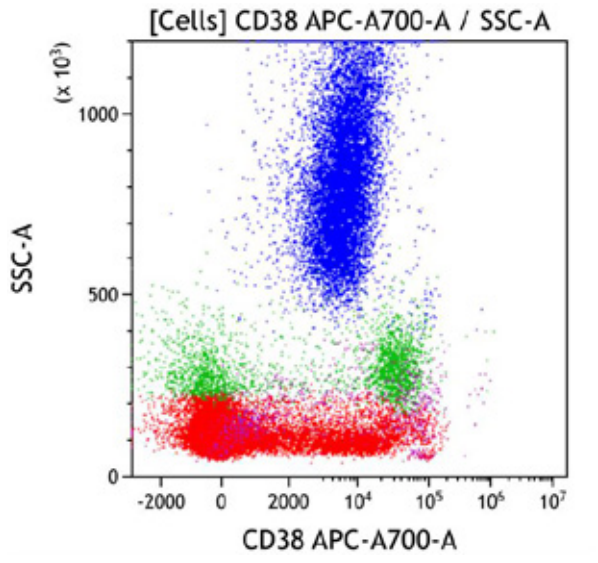

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

BACK TO CASE OVERVIEW

M2-13

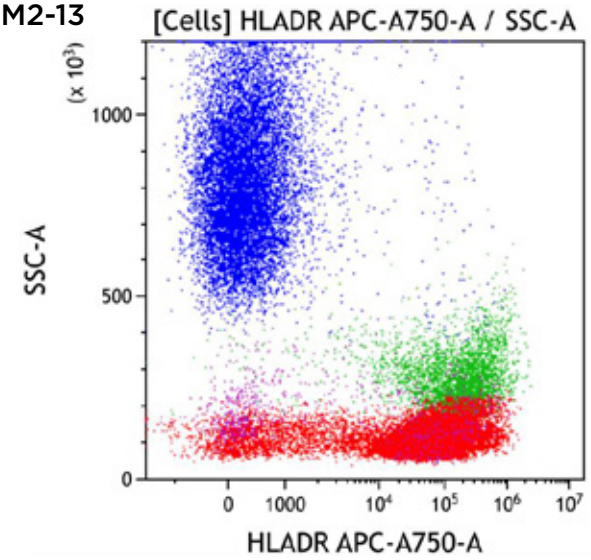

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T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

M2-14

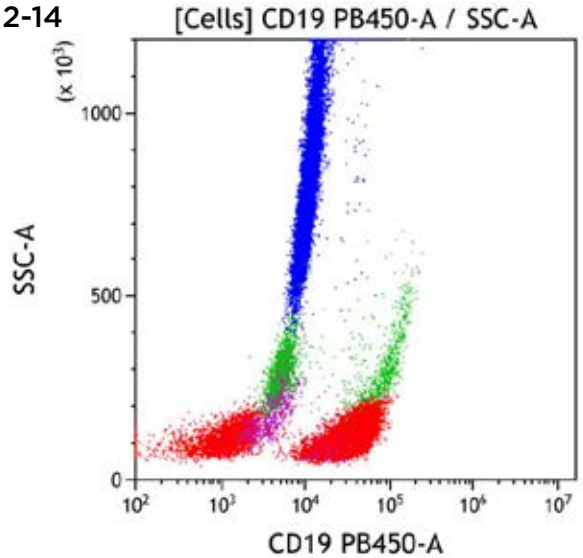

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note occasional B cells are in the monocyte gate (in green). Those are remaining doublets of neoplastic B cells.

M2-15

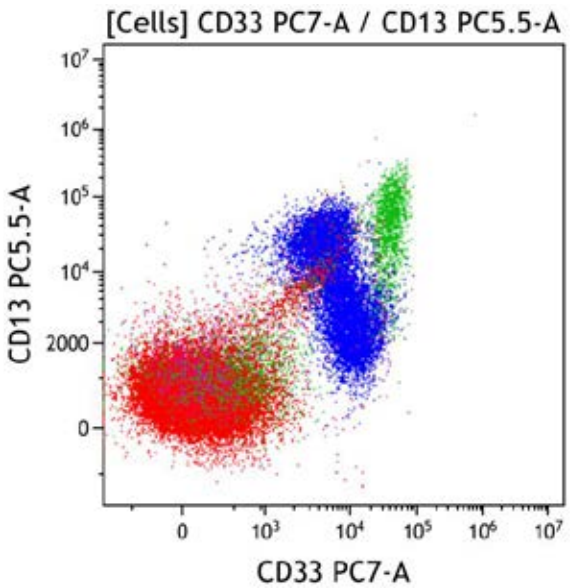

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher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

M2-16

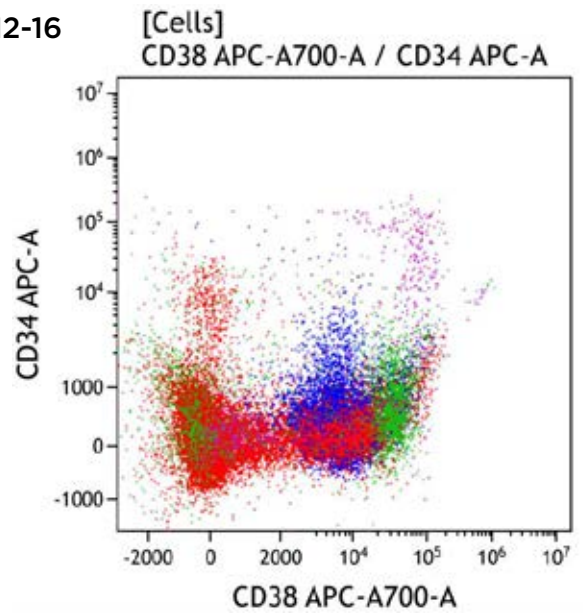

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The rare apparent CD34 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.

BACK TO CASE OVERVIEW

M2-17

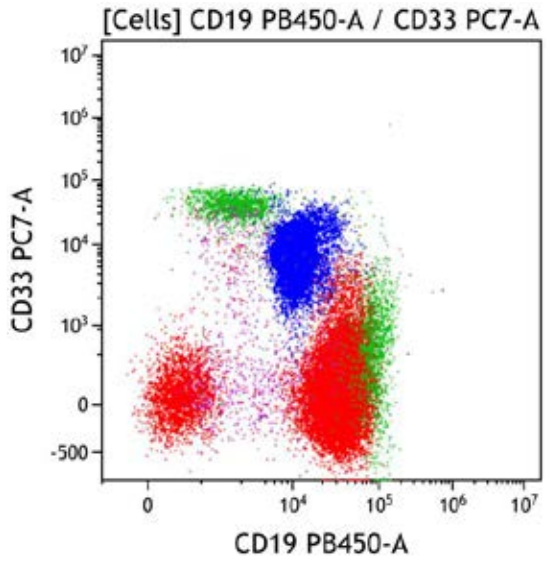

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturin granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to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

M2-18

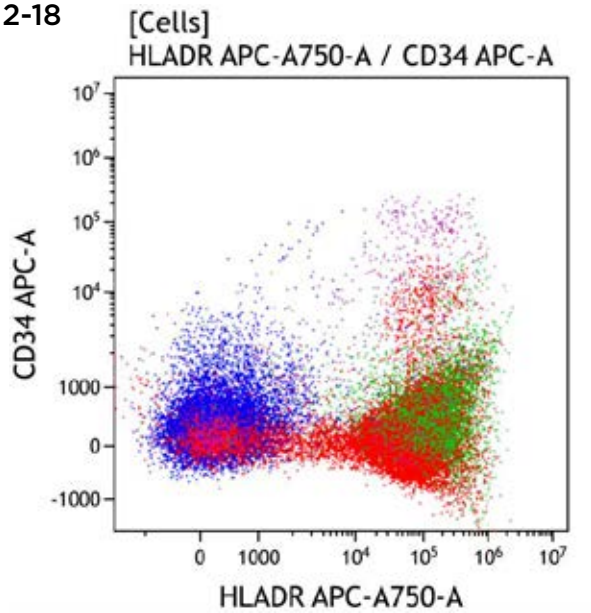

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of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

M2-19

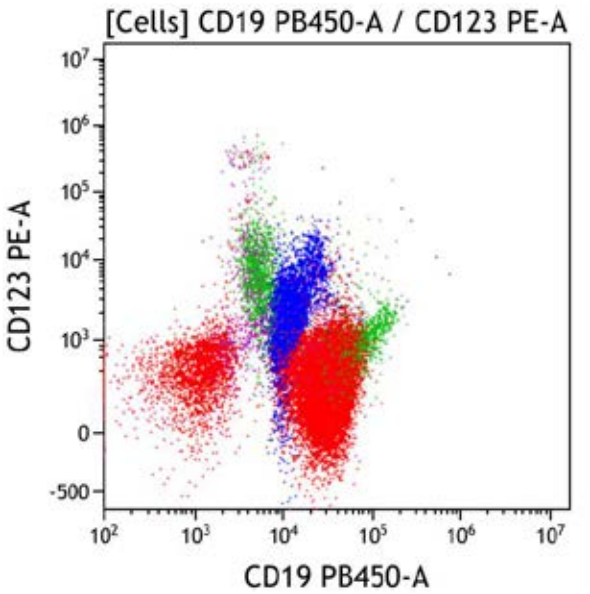

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid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

M2-20

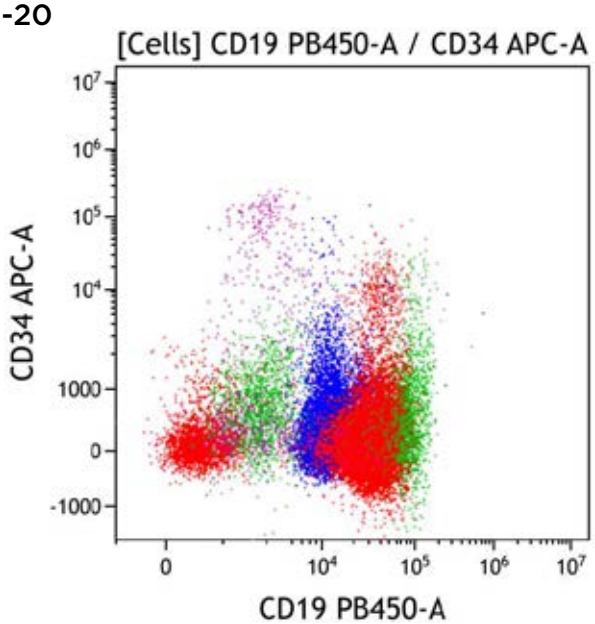

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19.

BACK TO CASE OVERVIEW

M2-21

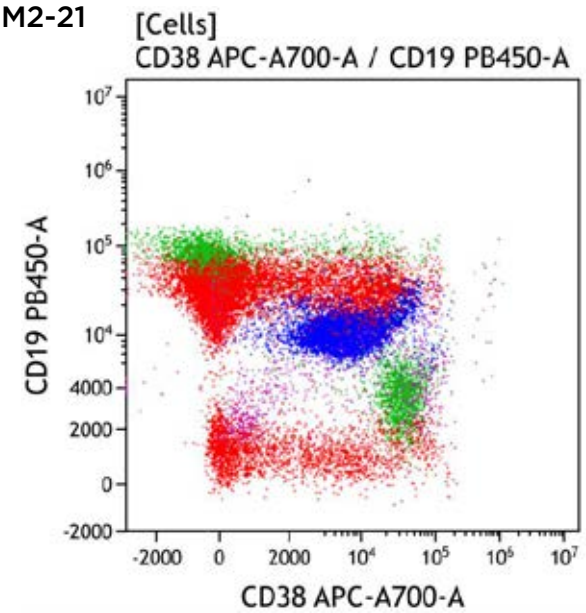

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression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

M2-22

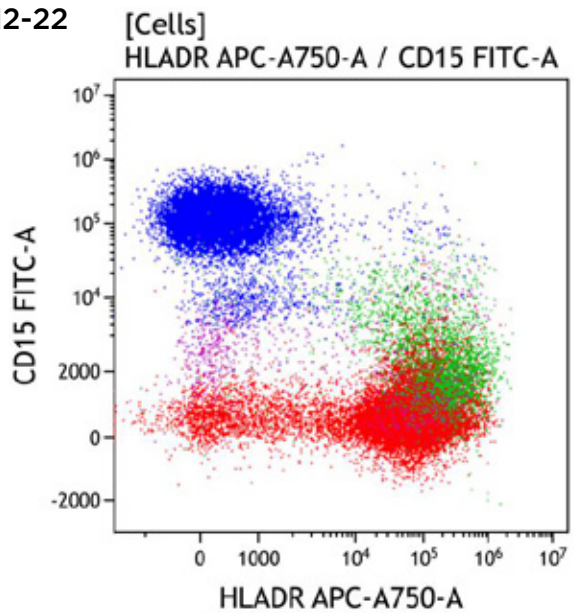

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLADR (blue),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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 9 MLL-TC4-048**
Diagnosis	**Acute myeloid leukemia with myelomonocytic maturation**
Clinical Vignette	14-year-old girl developed organomegaly. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	The flow cytometry study detected expanded myeloid blasts and mature monocytes. The blasts are CD34-positive with co-expression of myeloid markers CD13, CD33, and CD117 without lymphoid markers CD7, CD10 or CD19. In the background, complete granulocytic and monocytic maturations are present. The myeloid blasts also express CD123 and partial CD64, features that are associated with monocytic differentiation.

BACK TO CASE LISTINGS

B Cell Tube

B01

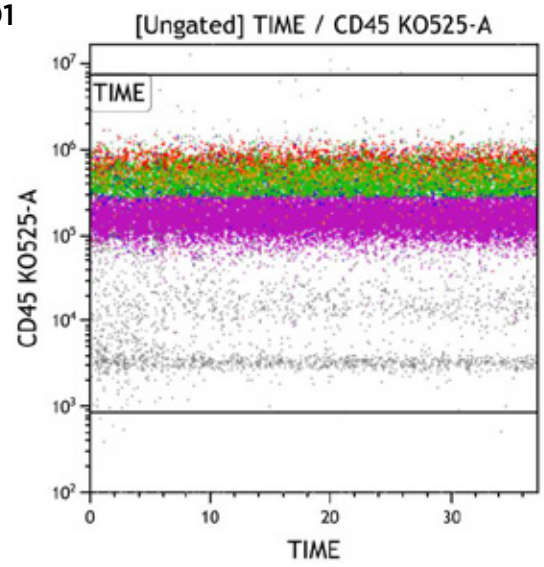

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

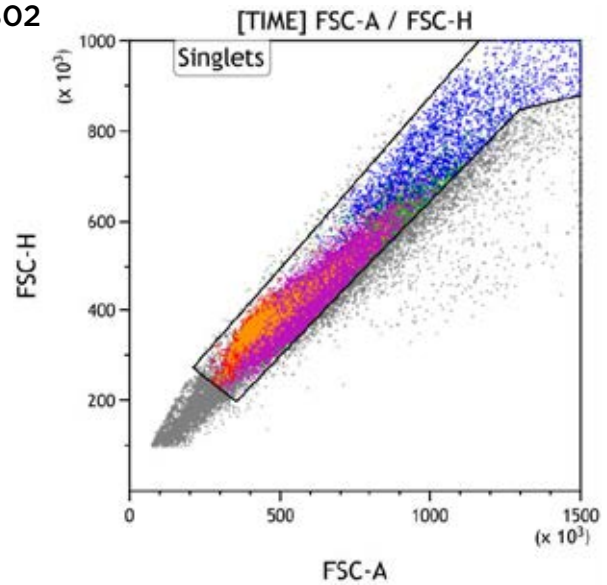

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

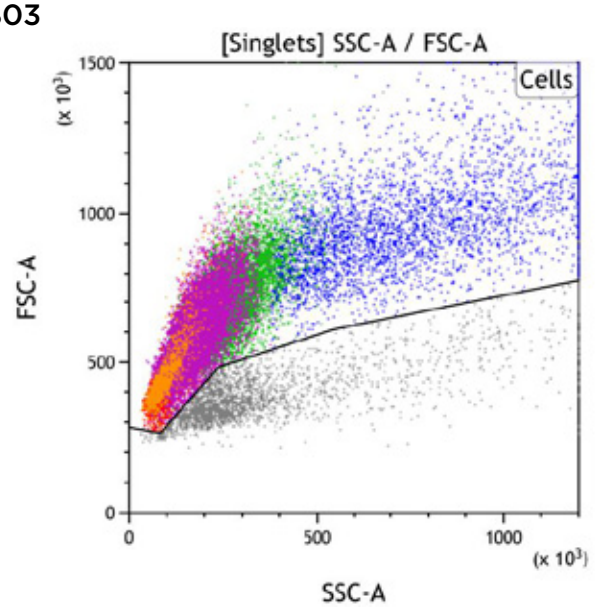

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

B04

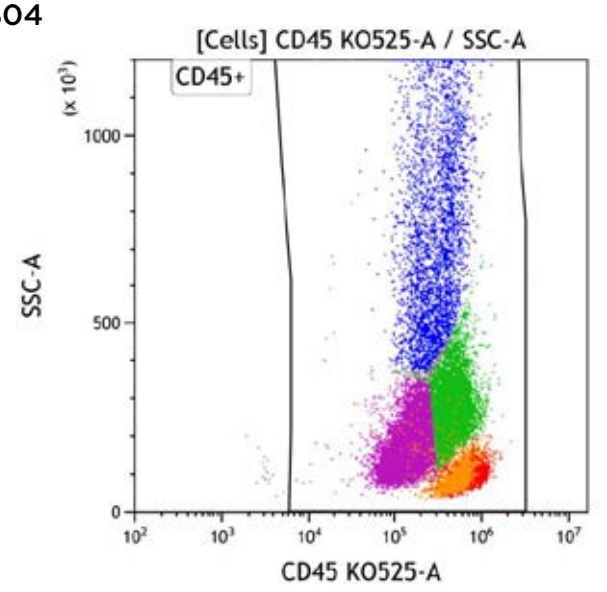

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

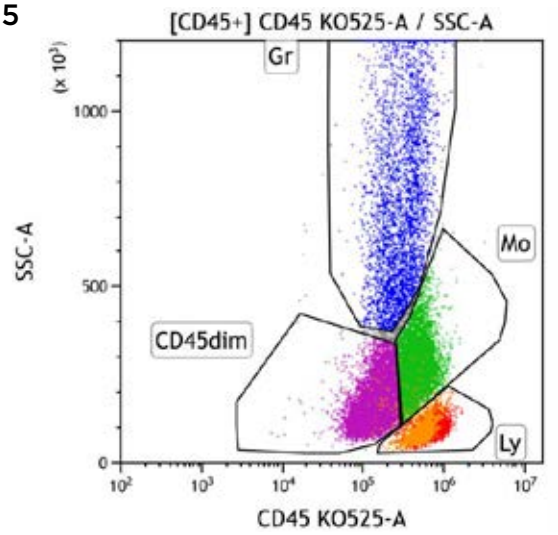

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 cells in CD45dim and Monocyte gates.

B06

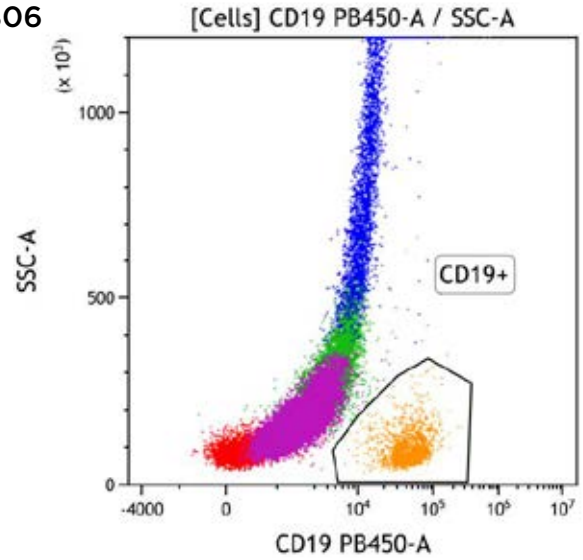

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The expanded cell populations are CD19-negative.

B07

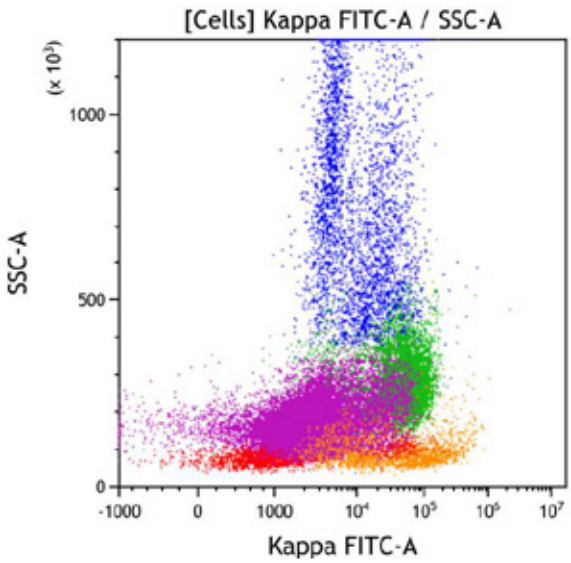

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in late stage) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent 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.

B08

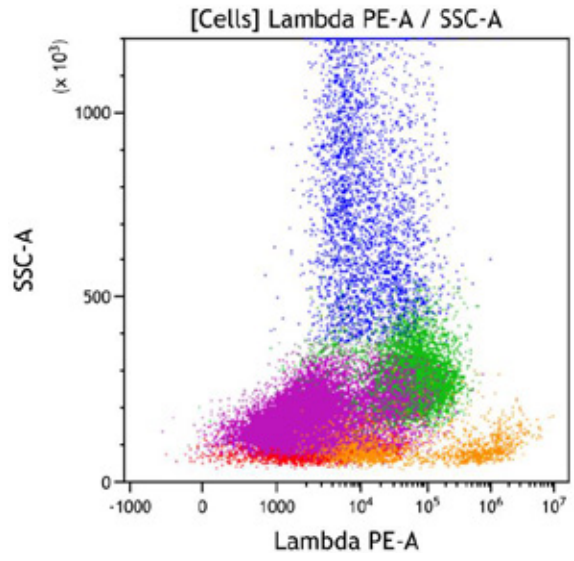

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in late stage) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent 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.

BACK TO CASE OVERVIEW

B09

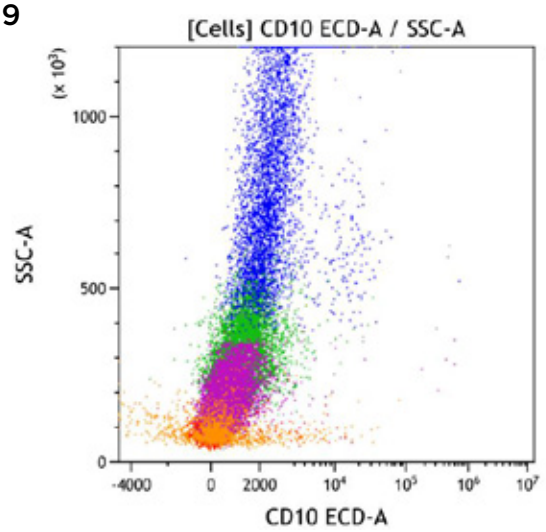

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The expanded cell populations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

B10

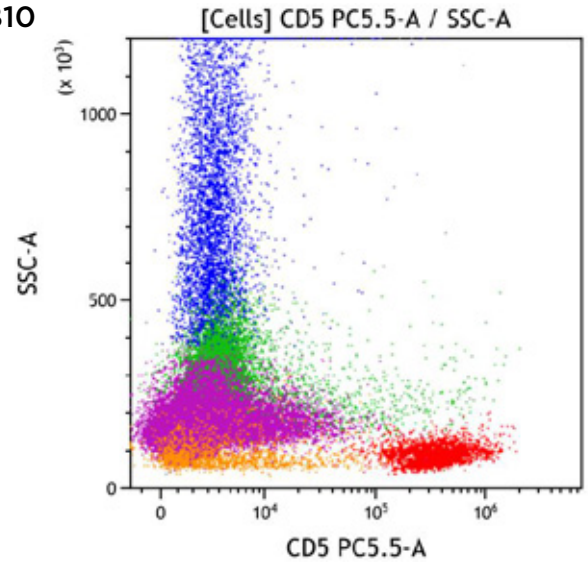

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in late stage). These lymphoid cells typically have low side scatter.

B11

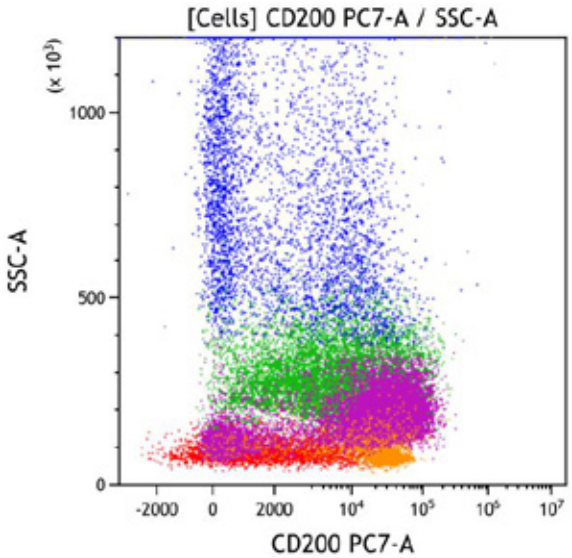

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

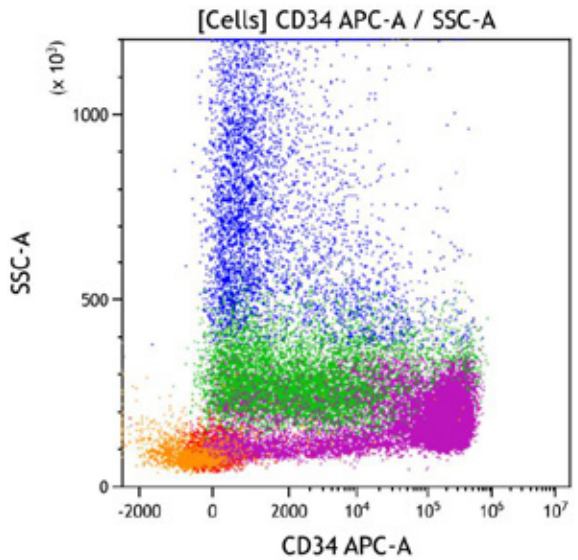

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. The expanded cell population in the CD45dim gate is CD34-positive.

BACK TO CASE OVERVIEW

B13

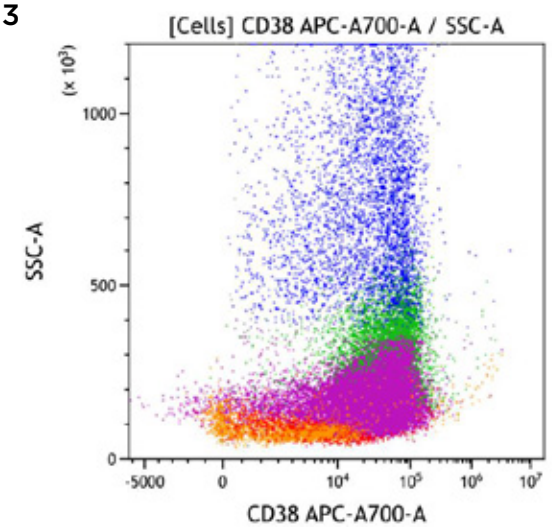

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Note the expanded cells in CD45dim and monocyte gate are CD38 positive.

B14

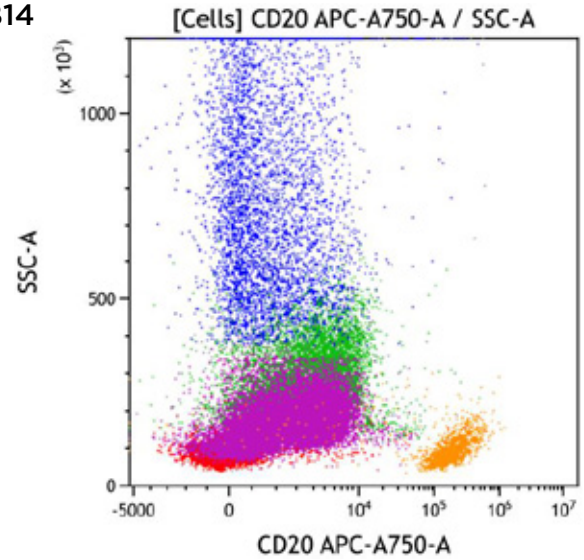

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (highlighted in late stage) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte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

B15

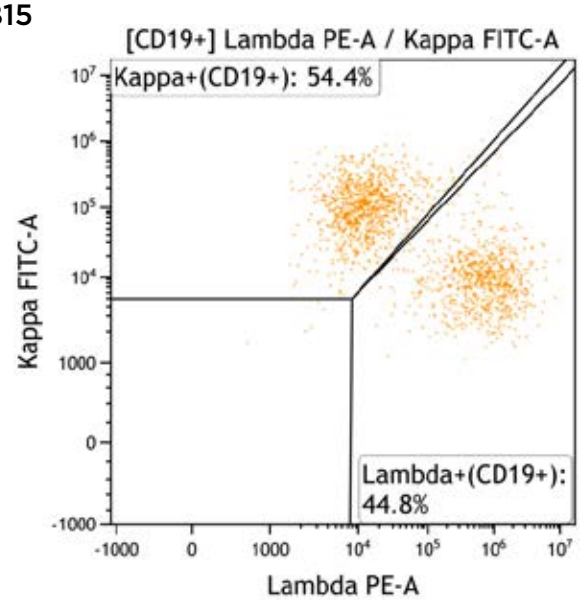

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

B16

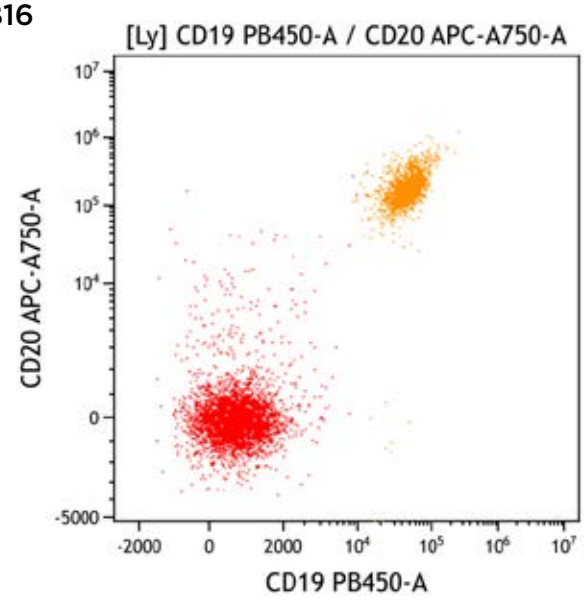

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells express both CD19 and CD20 (highlighted in late stage). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

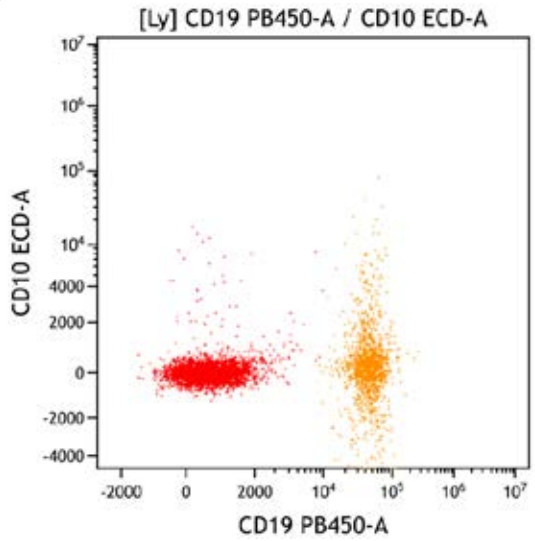

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate. B cells are CD19 positive (highlighted in late stage). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone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

B18

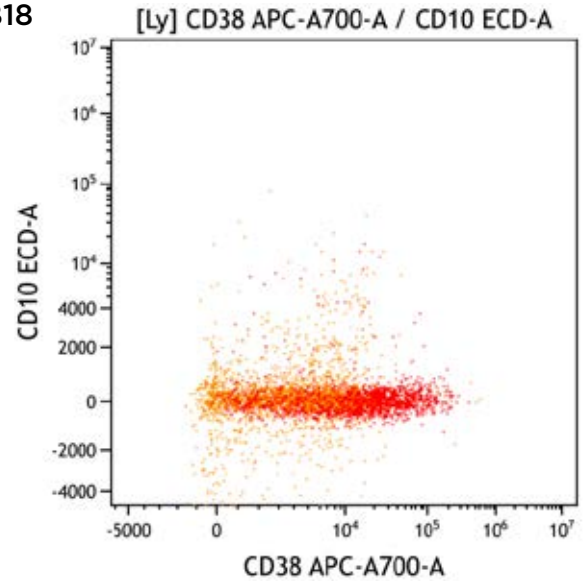

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

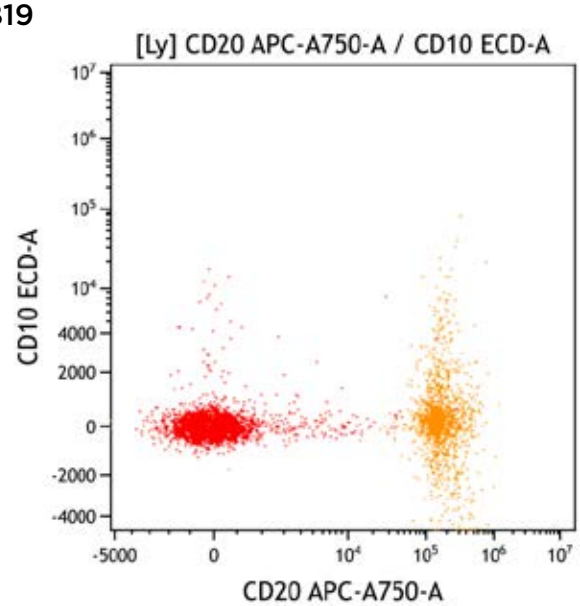

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late-stage immature B cells.

B20

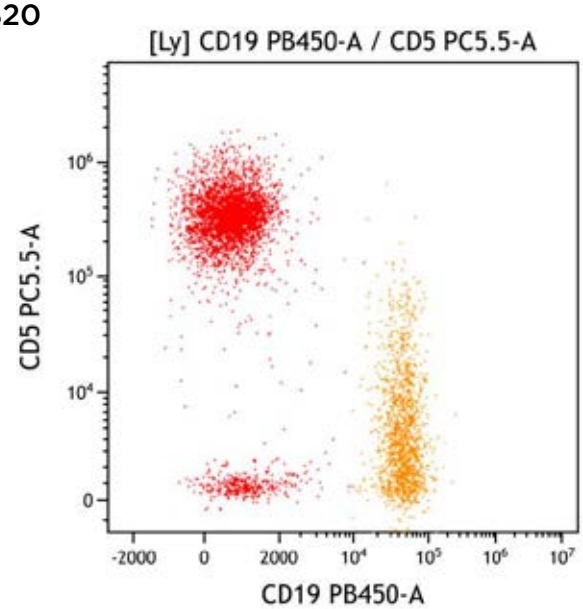

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

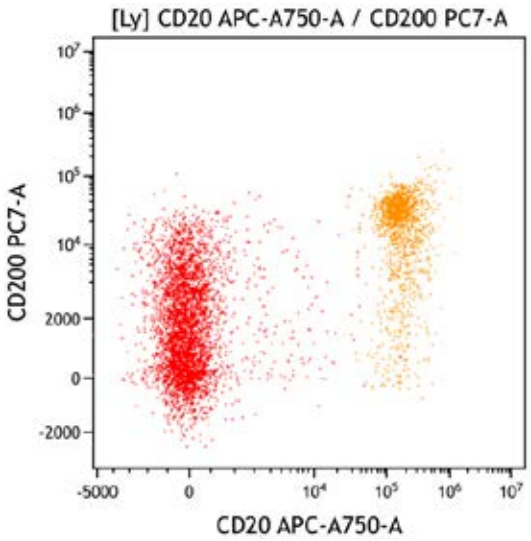

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in late stage).

B22

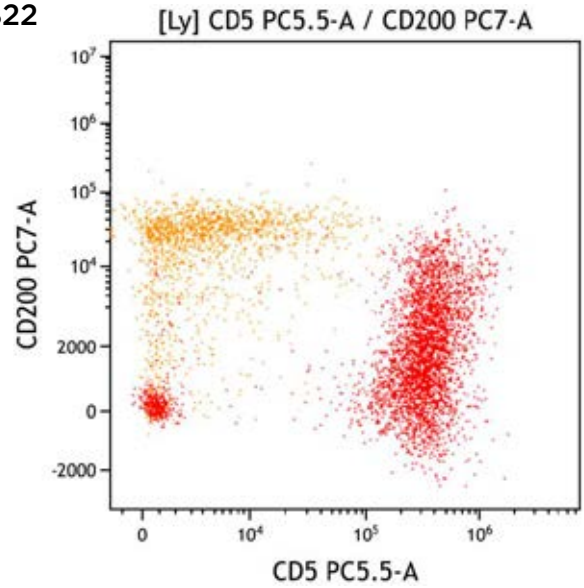

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically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

BACK TO CASE OVERVIEW

T Cell Tube

T01

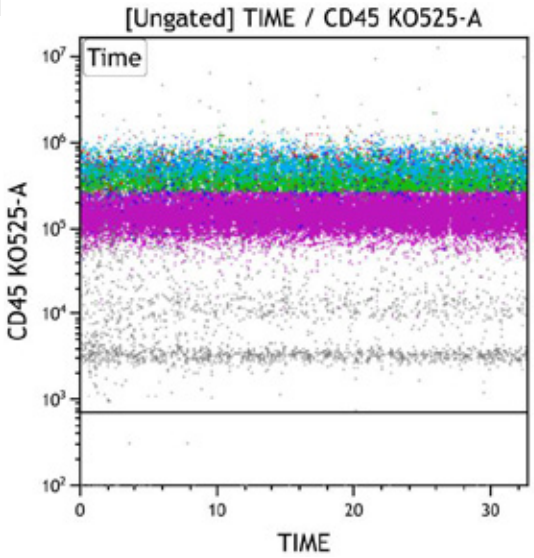

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

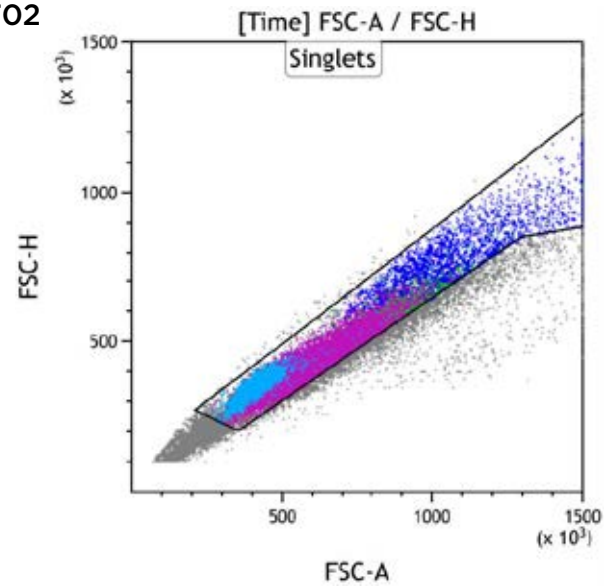

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

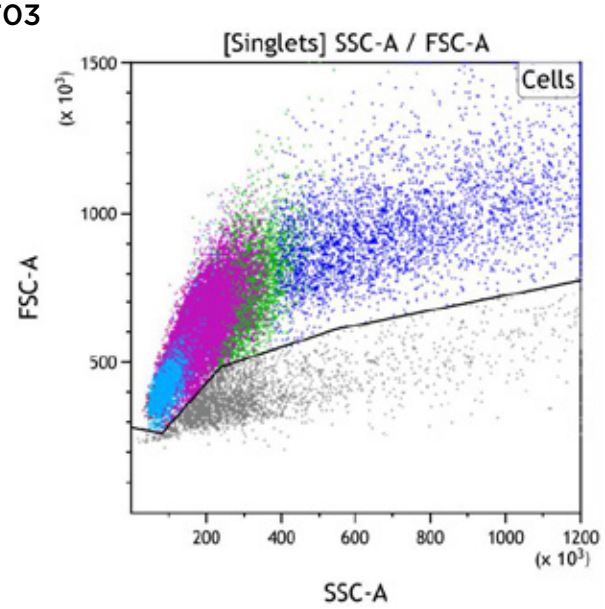

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

T04

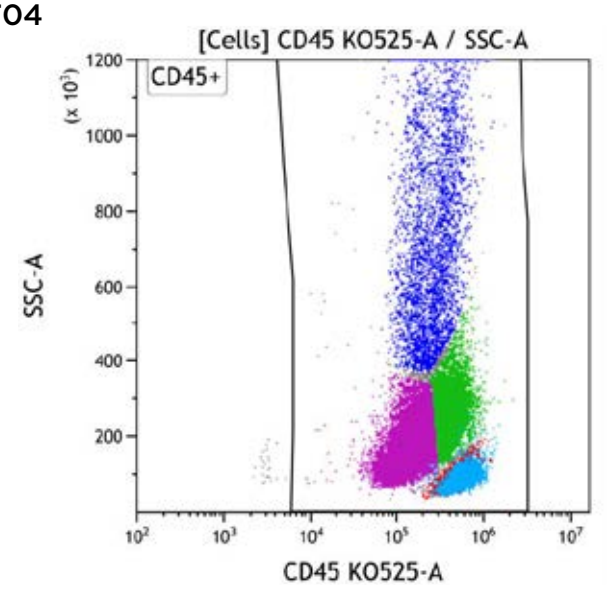

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

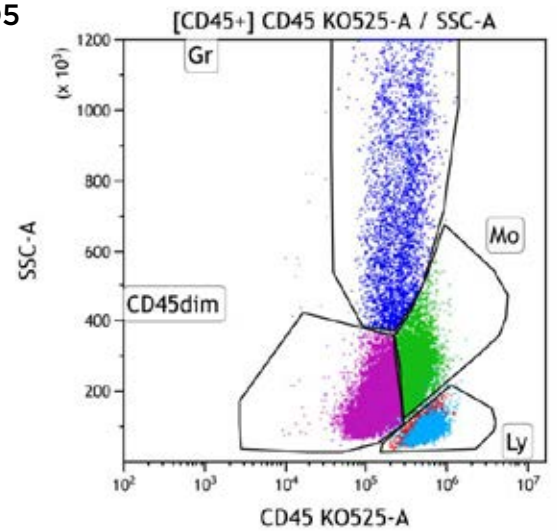

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 cell populations in the CD45dim and Monocyte gates.

T06

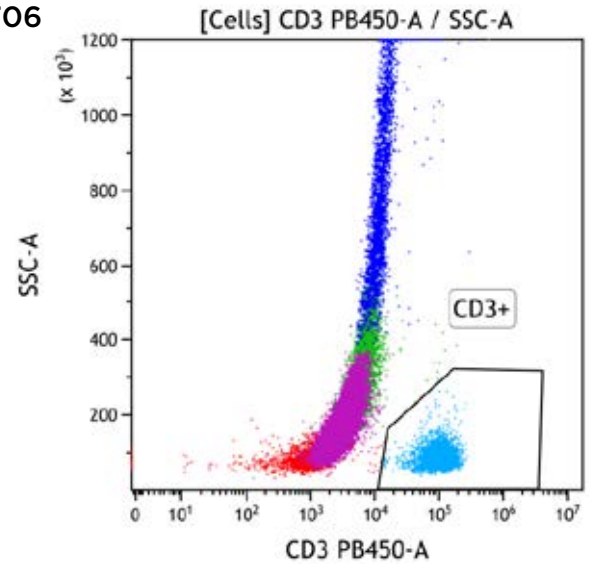

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T0

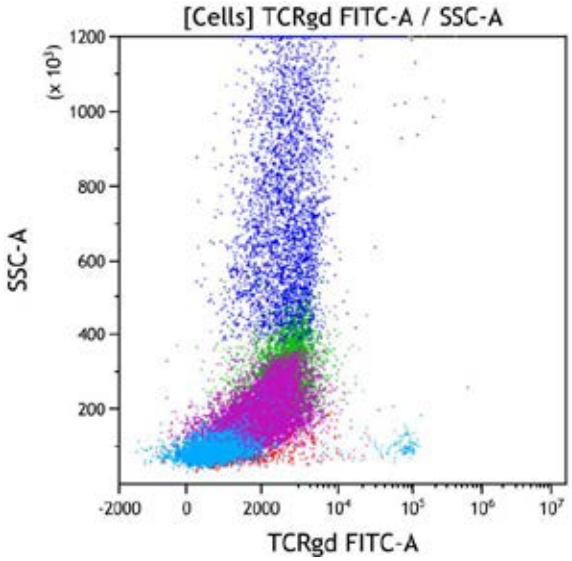

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

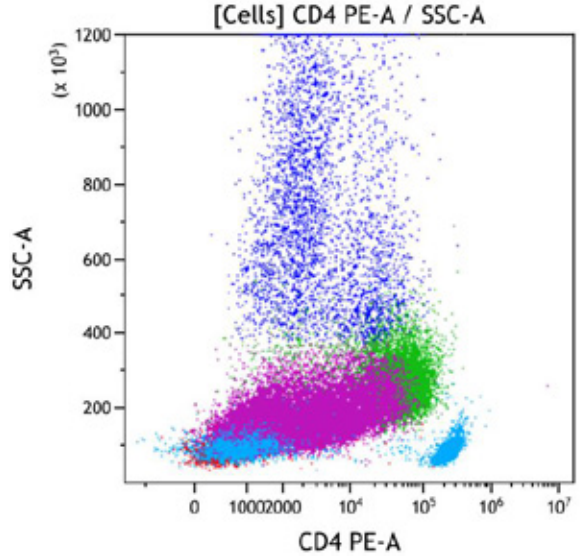

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors of multiple lineages in bone marrow. Note dim and variable CD4 expression on cells in the monocyte and CD45dim gate, respectively.

BACK TO CASE OVERVIEW

T09

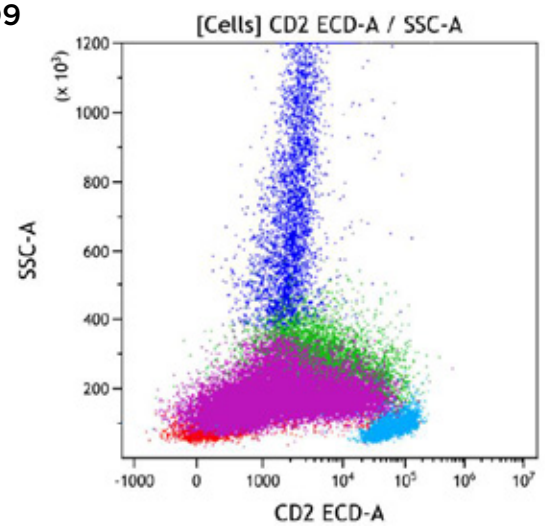

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). Note CD2 is variably expressed on a subset of cells in the CD45dim gate.

T10

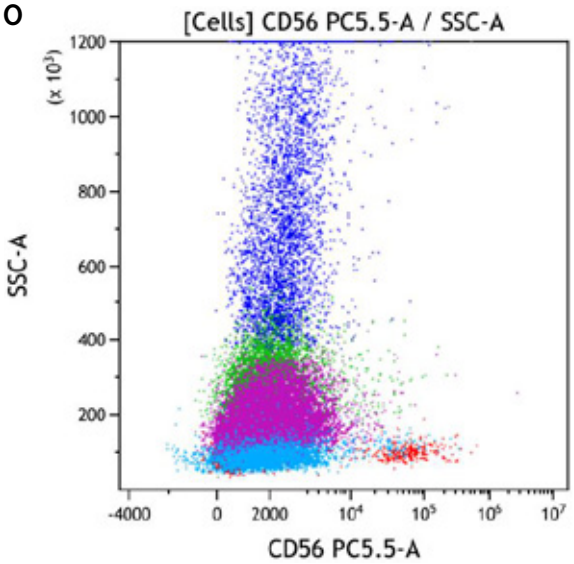

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/ delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and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

T11

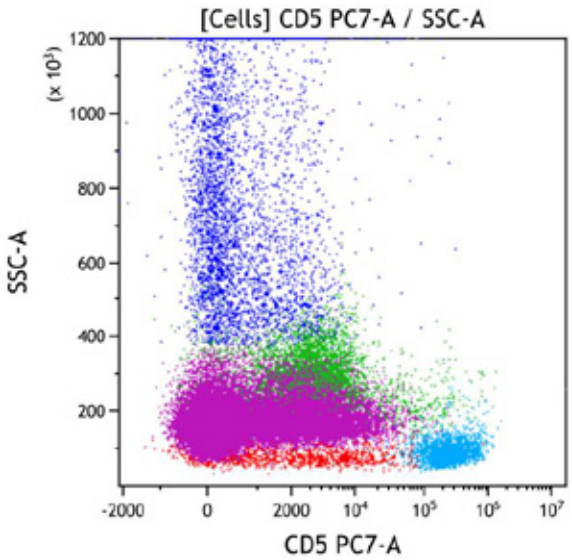

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Note variable CD5 is expressed on a subset of cells in the CD45dim gate.

T12

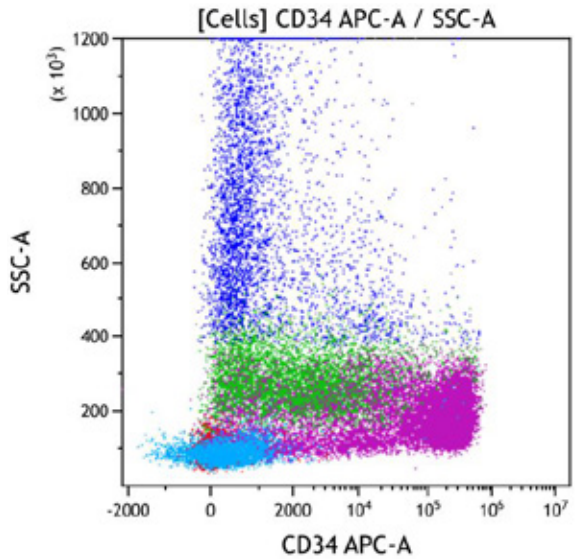

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Of note, the expanded population in the CD45dim gate is CD34-positive.

BACK TO CASE OVERVIEW

T13

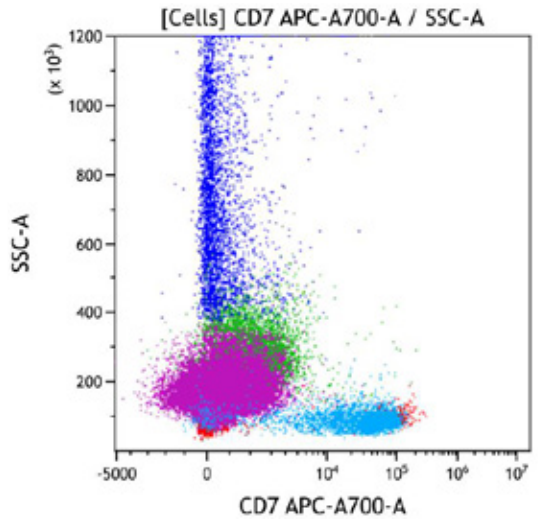

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

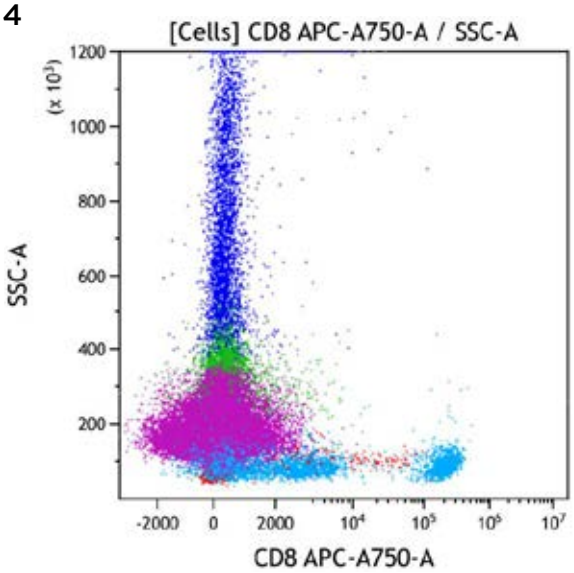

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

T15

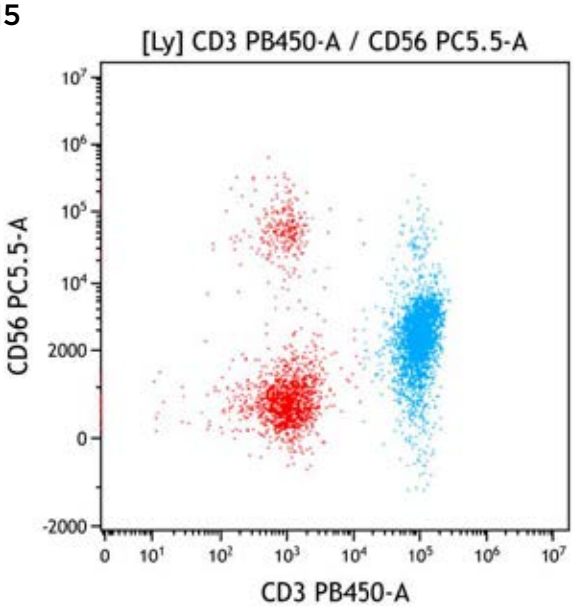

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small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

T16

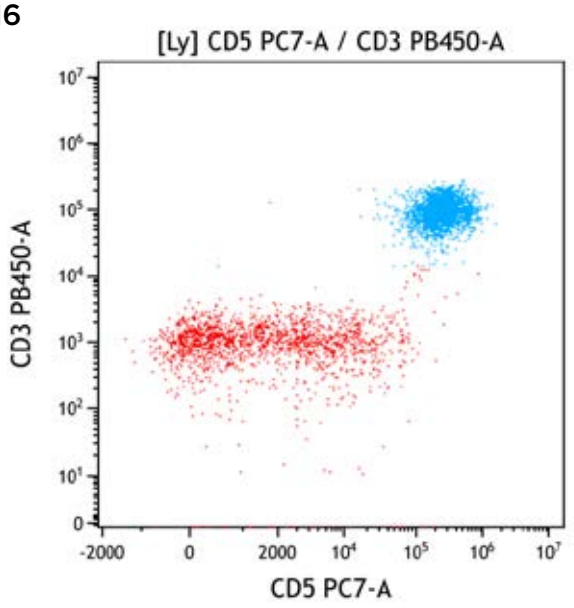

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most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

BACK TO CASE OVERVIEW

T17

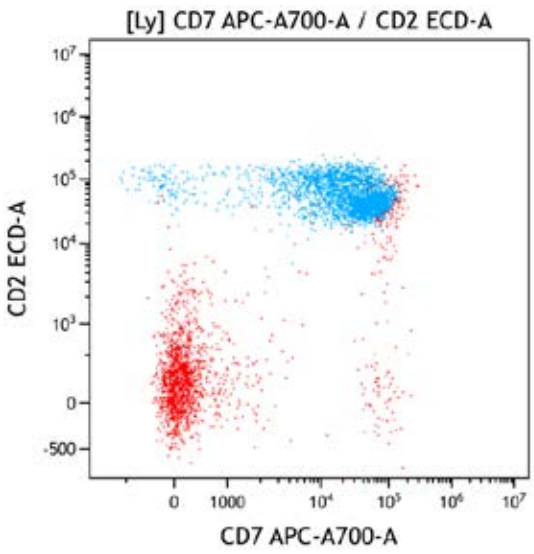

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

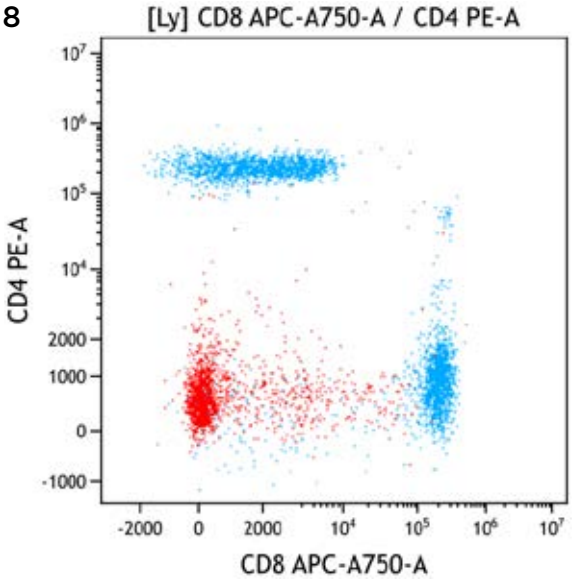

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 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.

T19

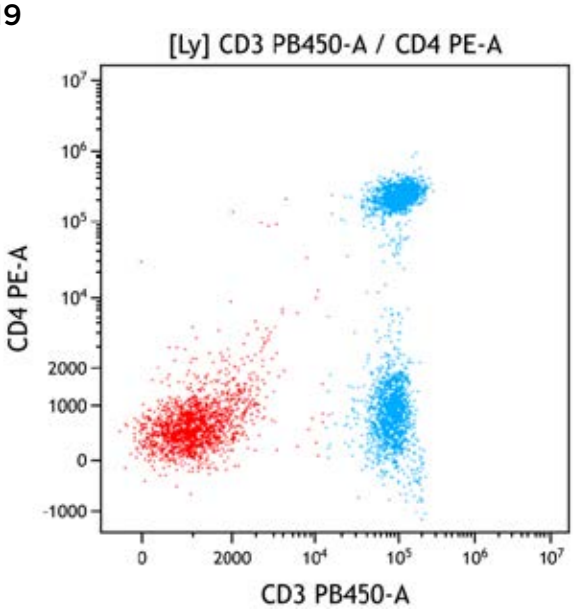

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

T20

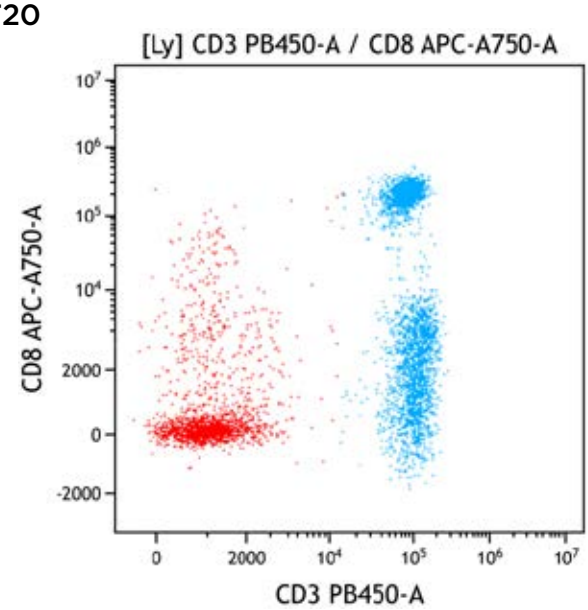

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

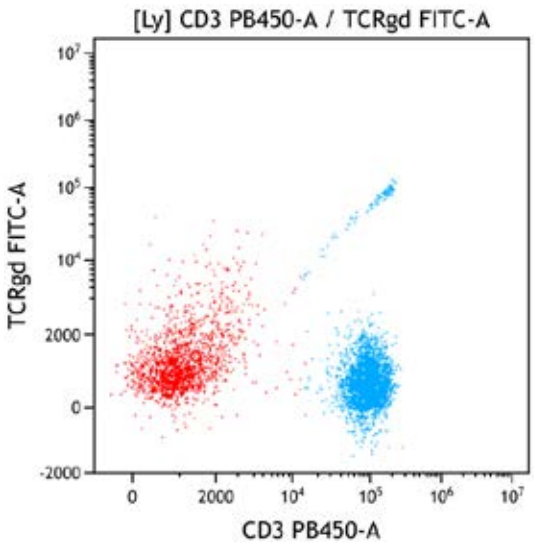

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

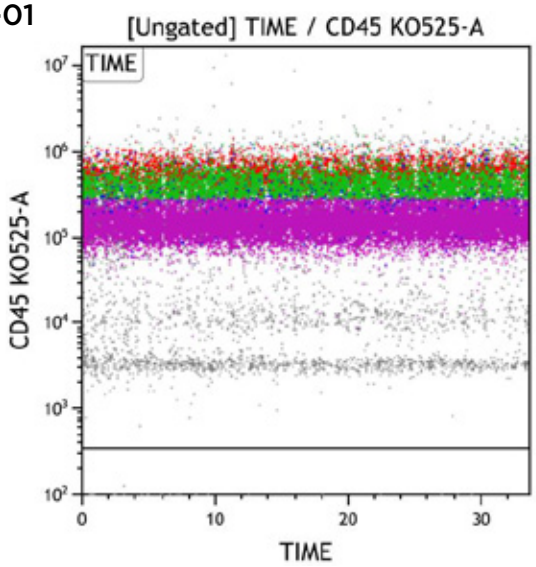

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

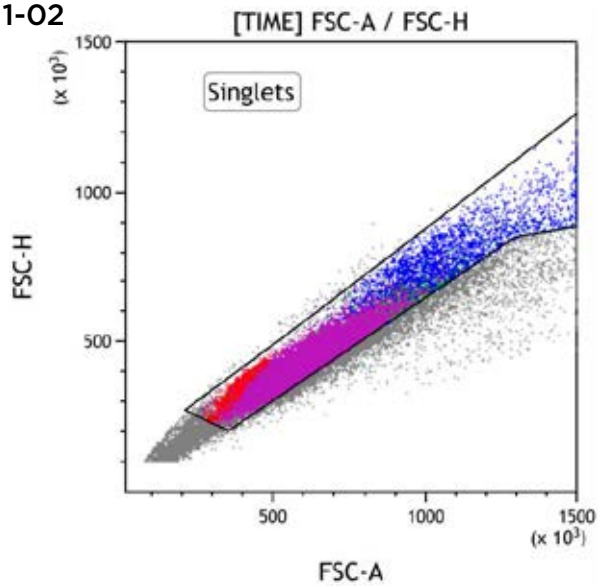

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

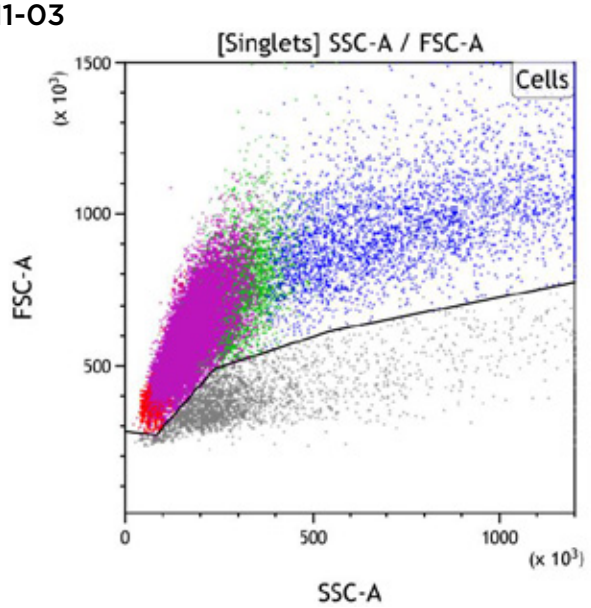

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M1-04

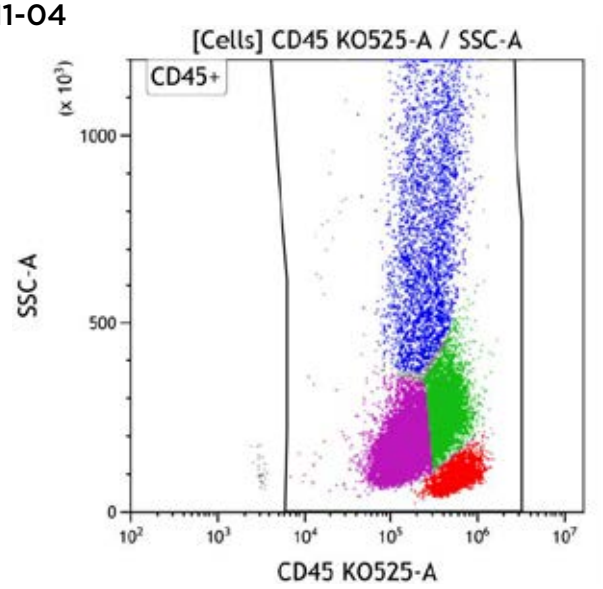

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

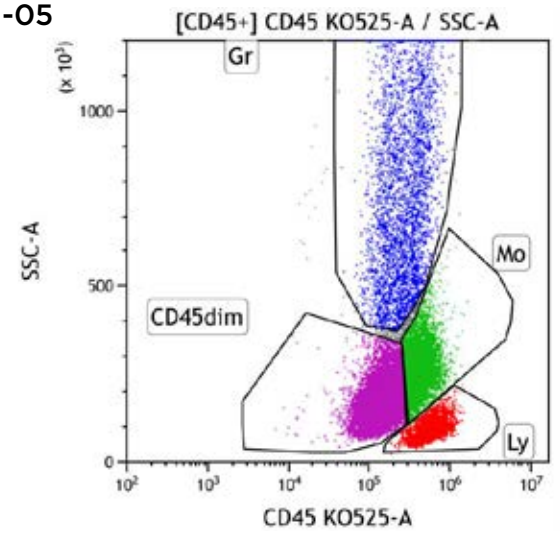

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 populations in the CD45dim and monocyte gates are expanded.

M1-06

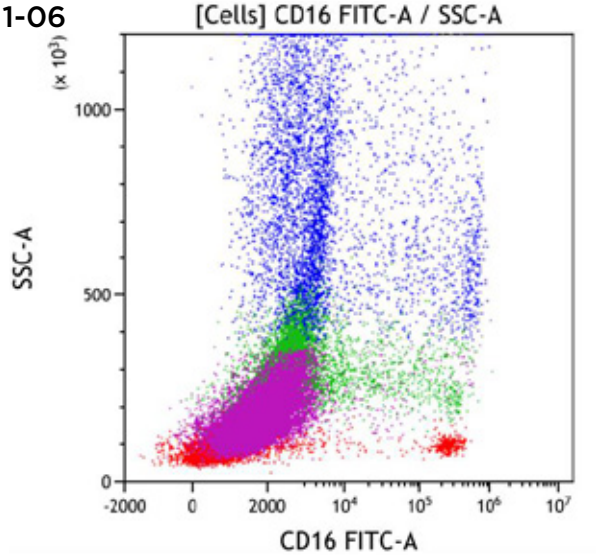

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

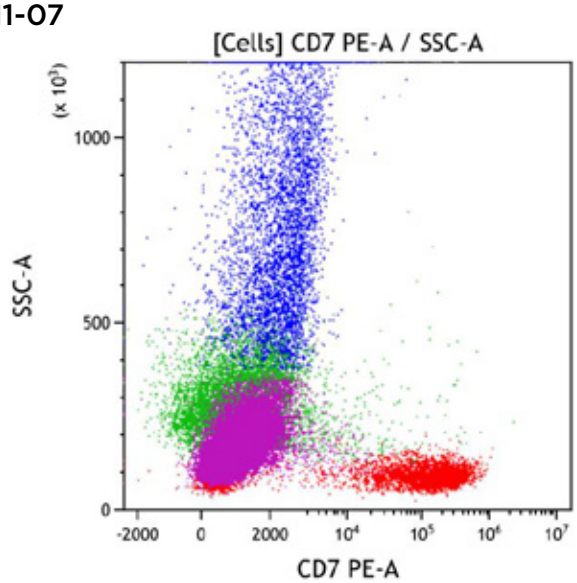

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

M1-08

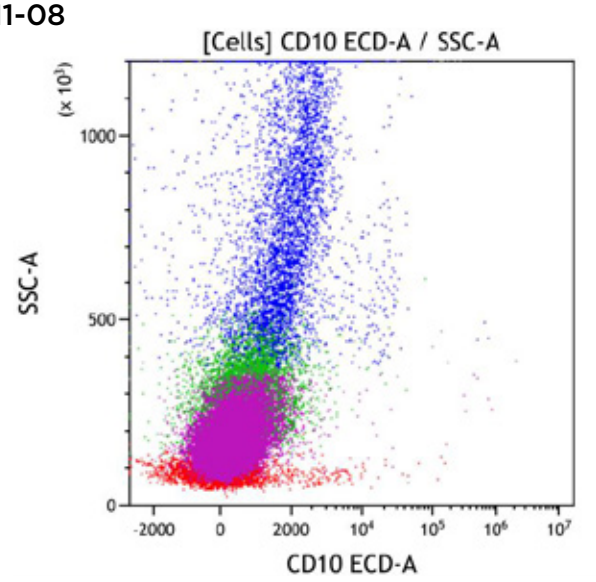

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

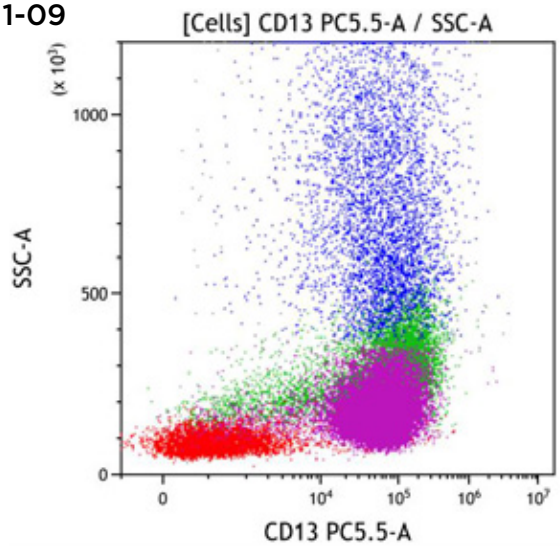

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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). Of note, the expanded population in the CD45dim gate is CD13-positive.

M1-10

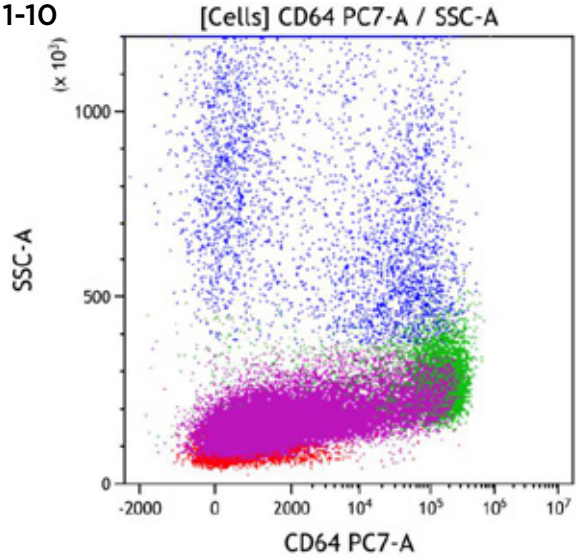

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

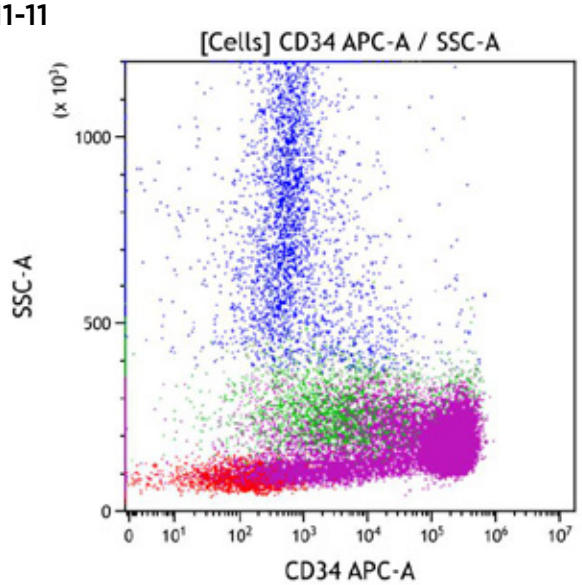

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Of note, the expanded population in the CD45dim gate is CD34-positive.

M1-12

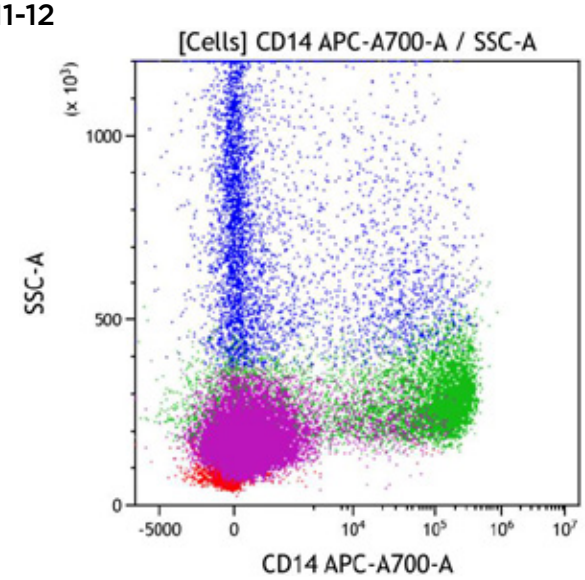

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Of note, most cells in the monocyte gate (in green) express high-level CD14, suggestive of mature monocytes.

BACK TO CASE OVERVIEW

M1-13

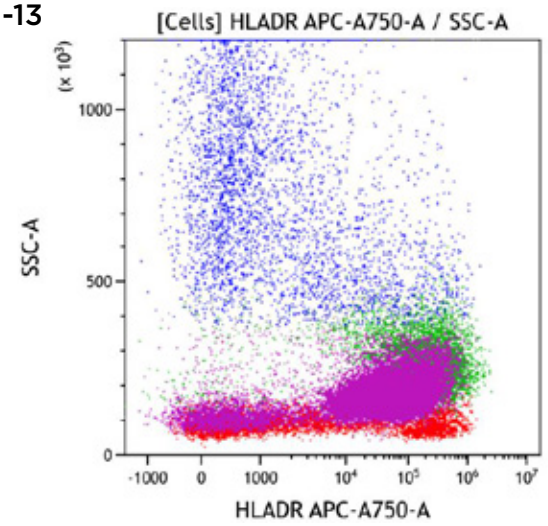

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

M1-14

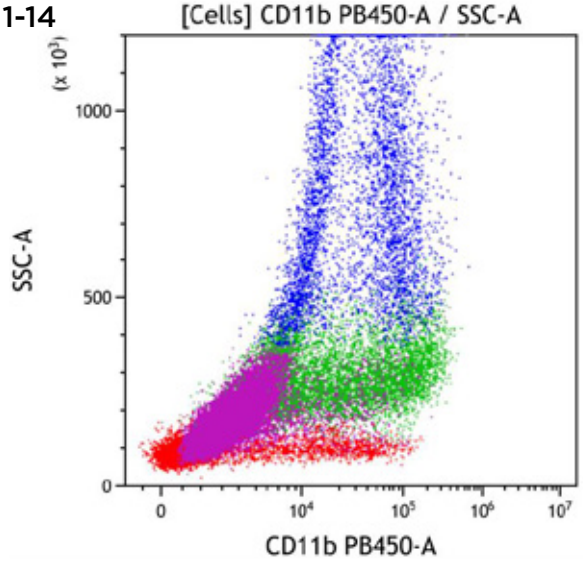

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

M1-15

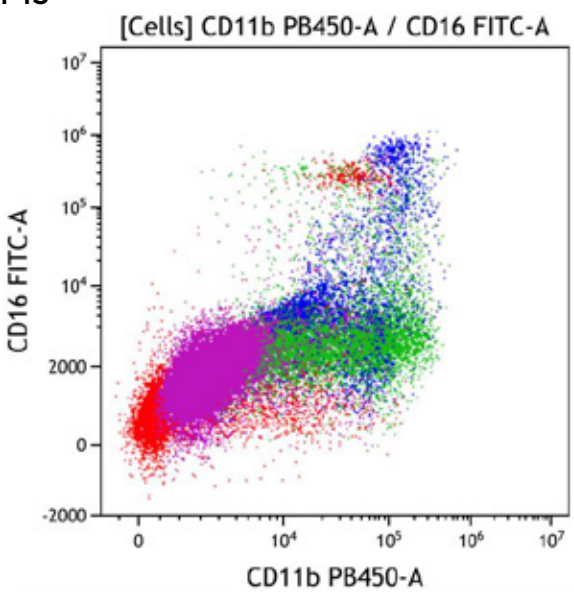

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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.

M1-16

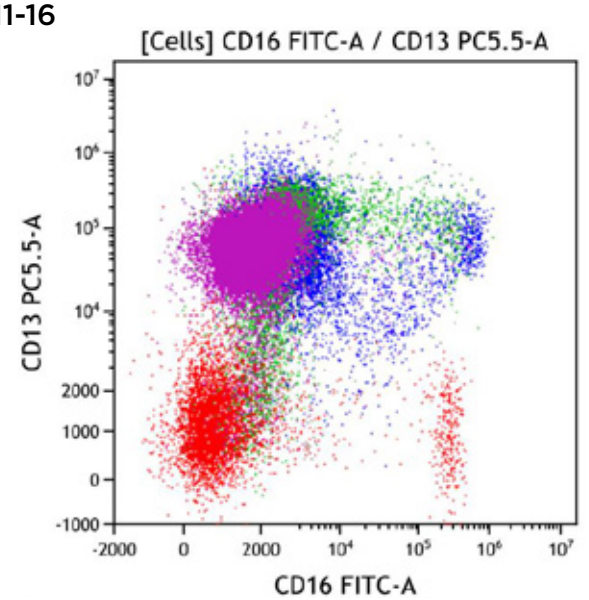

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed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

BACK TO CASE OVERVIEW

M1-17

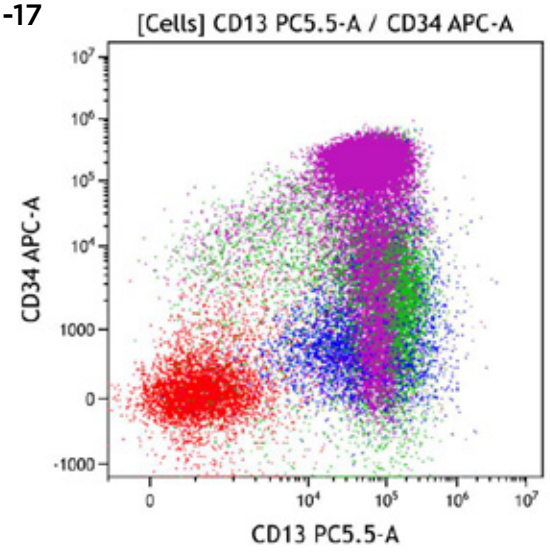

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphoid cells (red). Of note, most of cells in the CD45dim gate have coexpression of CD13 and CD34.

M1-18

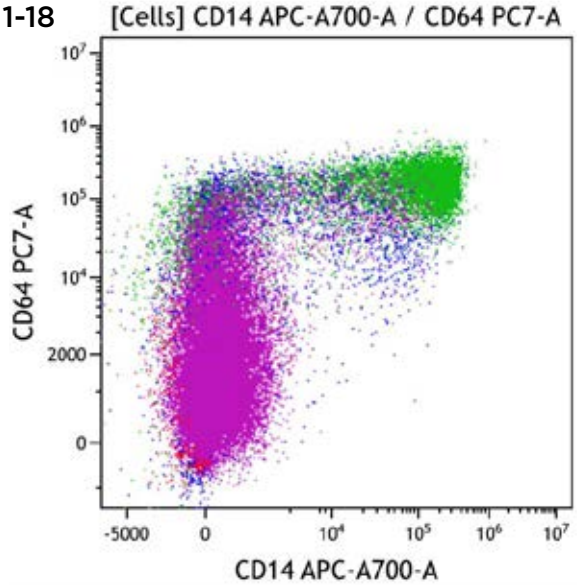

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high expression of CD64 without CD14 (blue top left) and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64 (green). Of note, most cells in the expanded monocyte gate express high-level CD64 and CD14, suggestive of mature monocytes.

M1-19

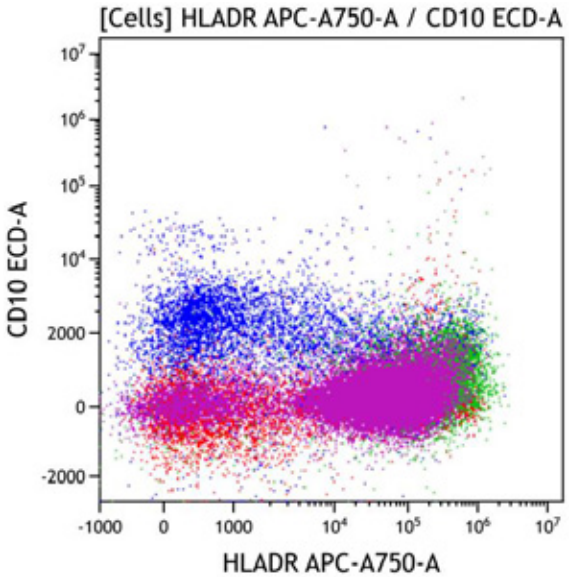

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

M1-20

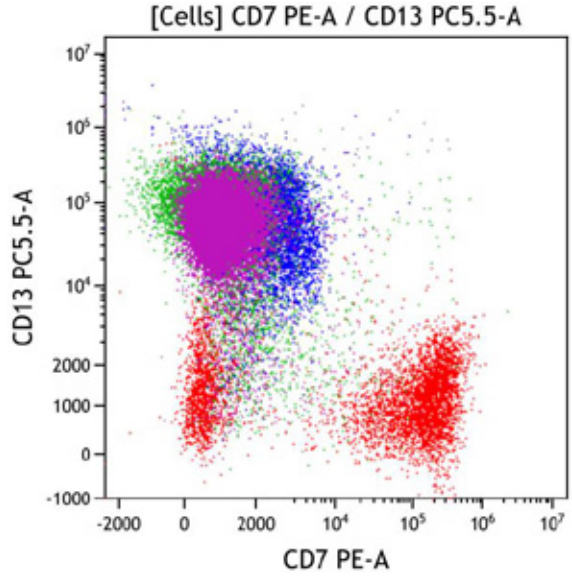

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

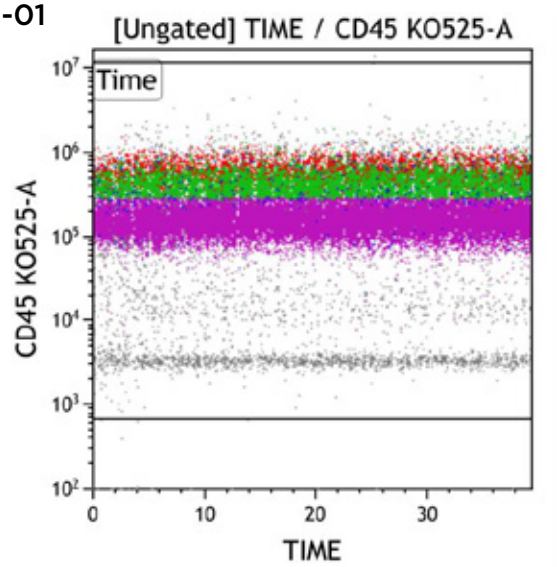

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

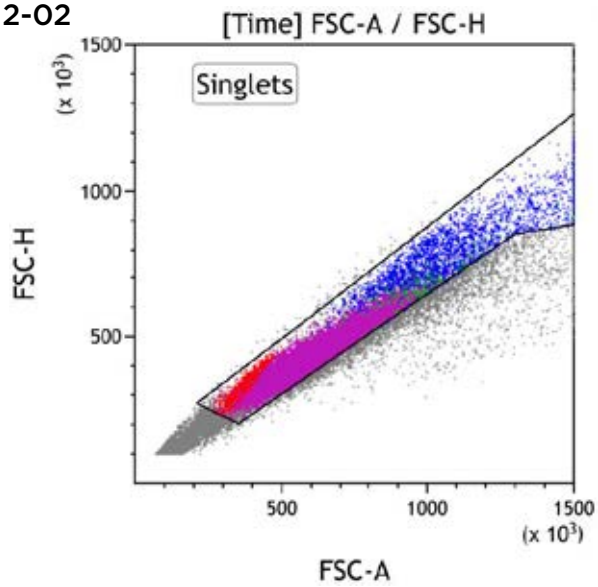

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

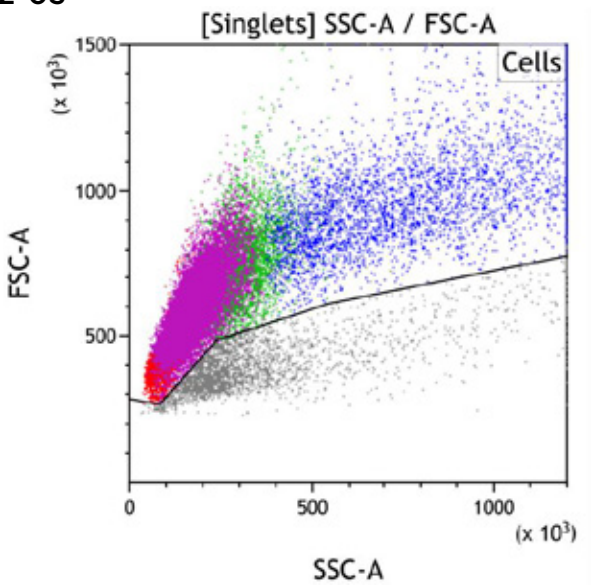

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M2-04

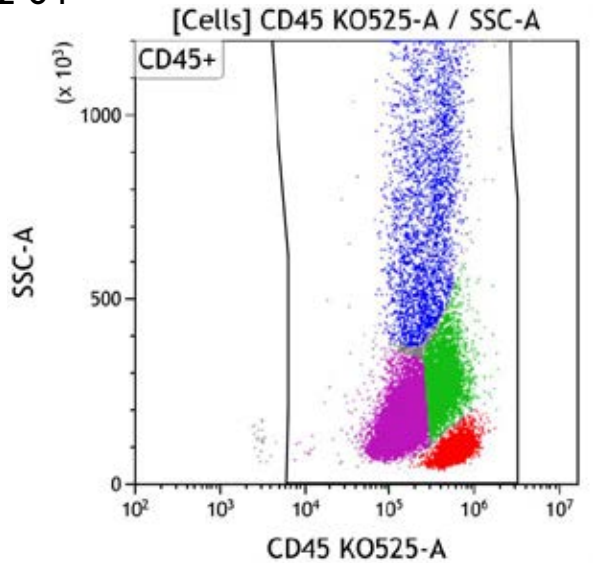

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M2-05

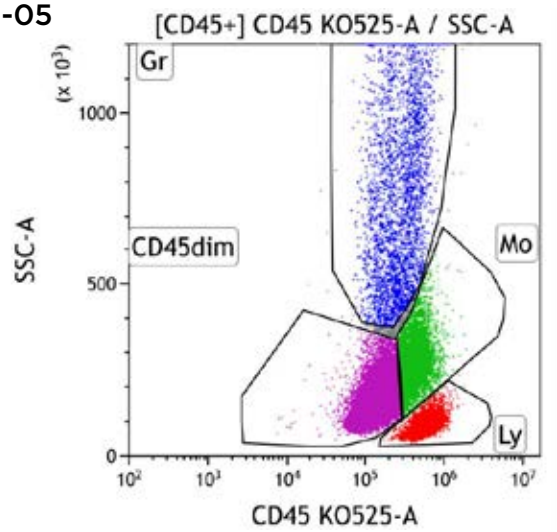

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (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. Note the expanded populations in the CD45dim and monocyte gates.

M2-06

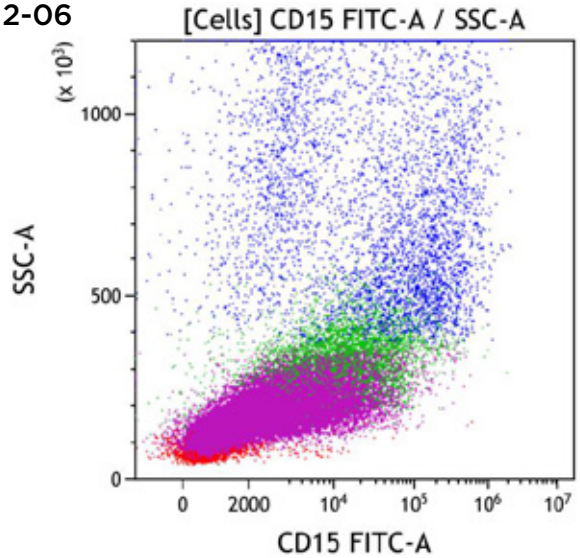

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

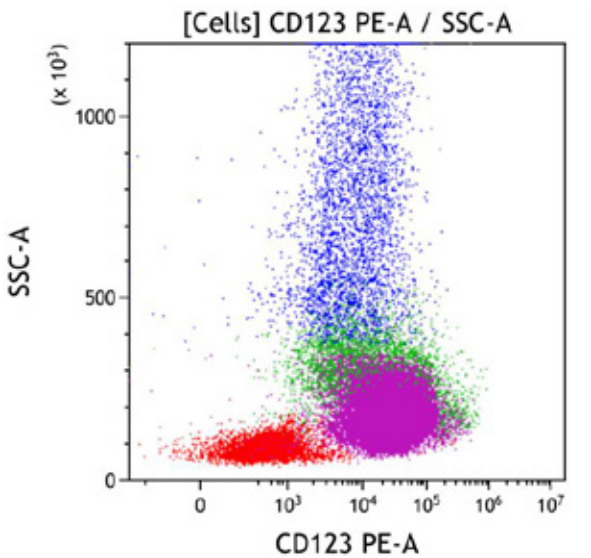

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green). Of note, the blasts are CD123 positive, but not as bright as basophils or plasmacytoid dendritic cells.

M2-08

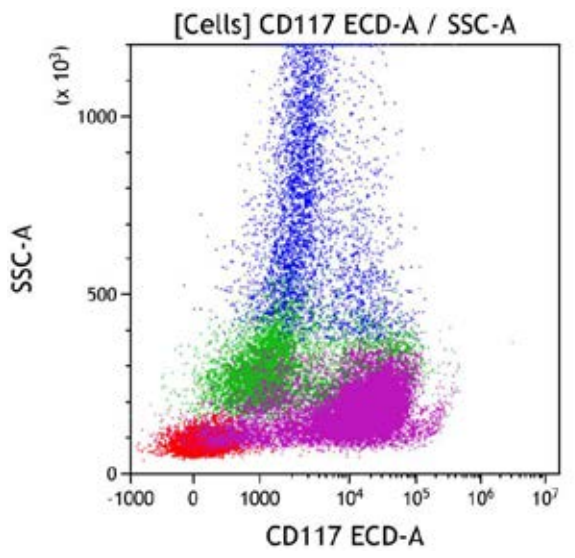

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Of note, the expanded population in the CD45dim gate is CD117-positive.

BACK TO CASE OVERVIEW

M2-09

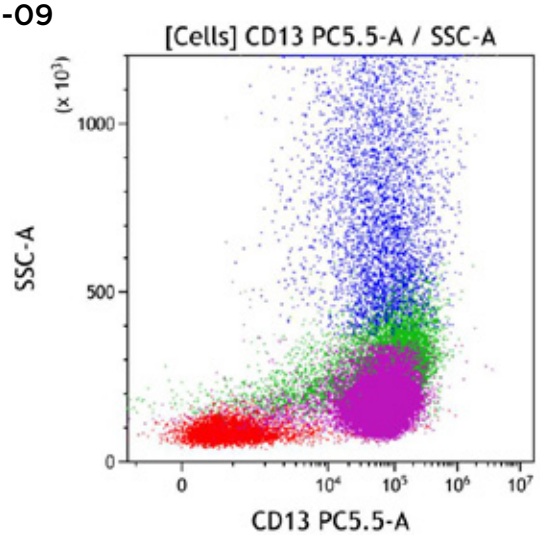

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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). Of note, the expanded population in the CD45dim gate is CD13-positive.

M2-10

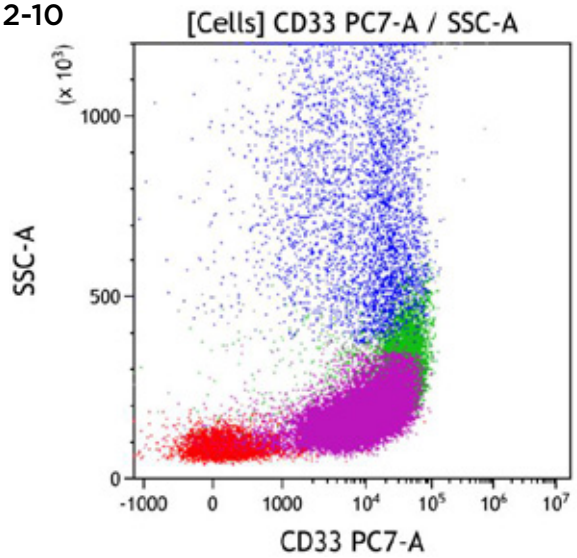

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34+ myeloid progenitors (purple). Of note, the expanded population in the CD45dim gate is CD33-positive.

M2-11

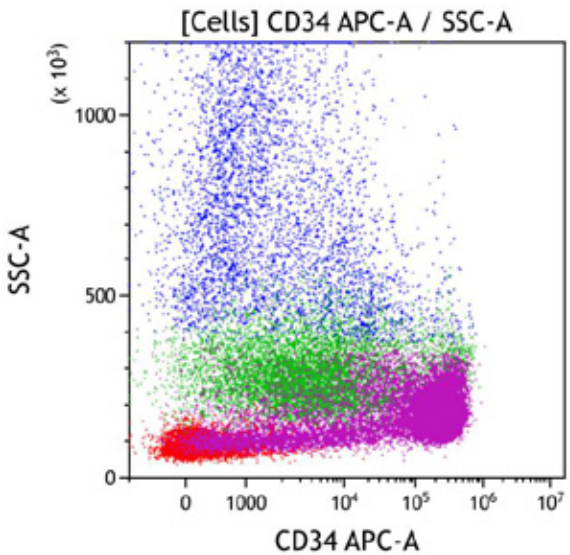

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Of note, the expanded population in the CD45dim gate is CD34-positive.

M2-12

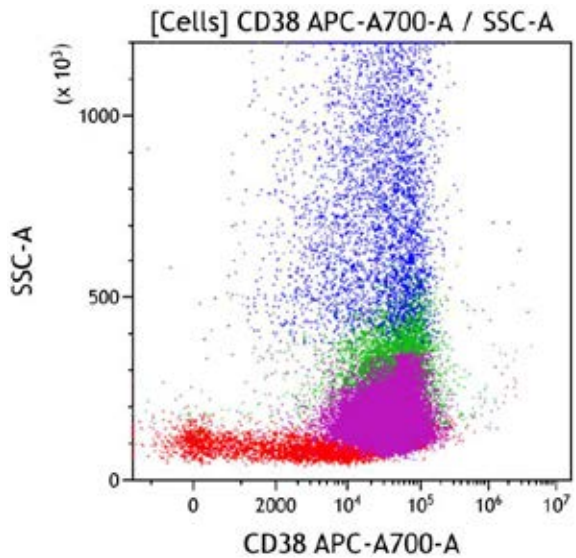

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

BACK TO CASE OVERVIEW

M2-13

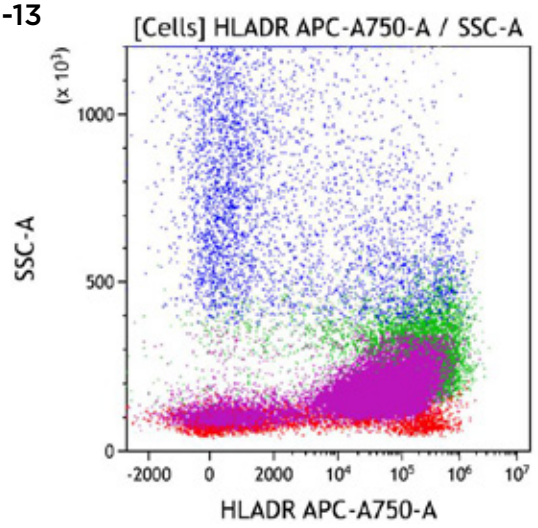

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T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

M2-14

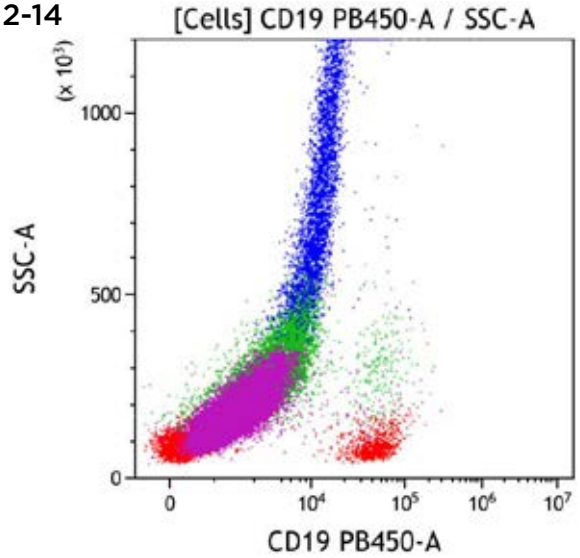

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Of note, the expanded population in the CD45dim gate is CD19-negative.

M2-15

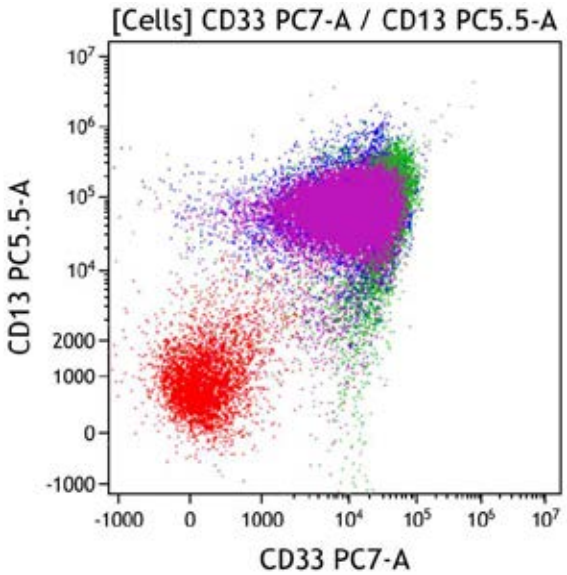

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher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

M2-16

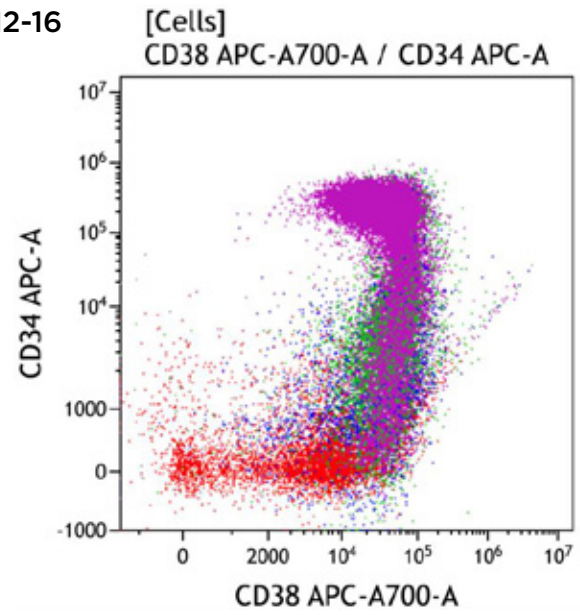

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable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

BACK TO CASE OVERVIEW

M2-17

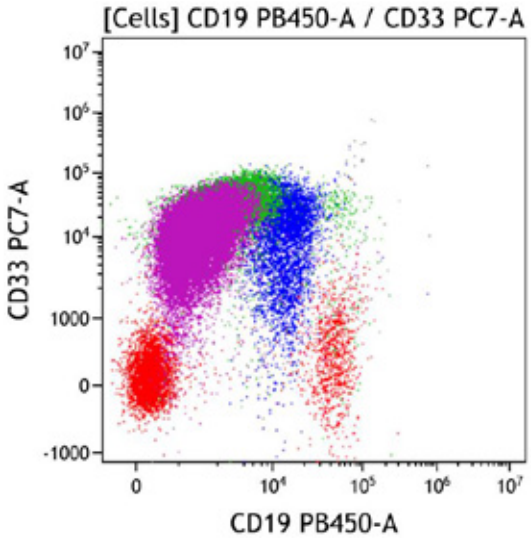

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Of note, the expanded population in the CD45dim gate is CD33-positive and CD19-negative.

M2-18

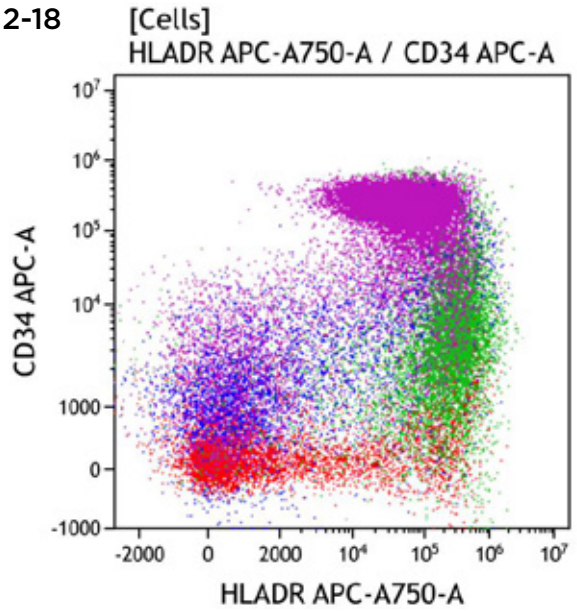

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of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

M2-19

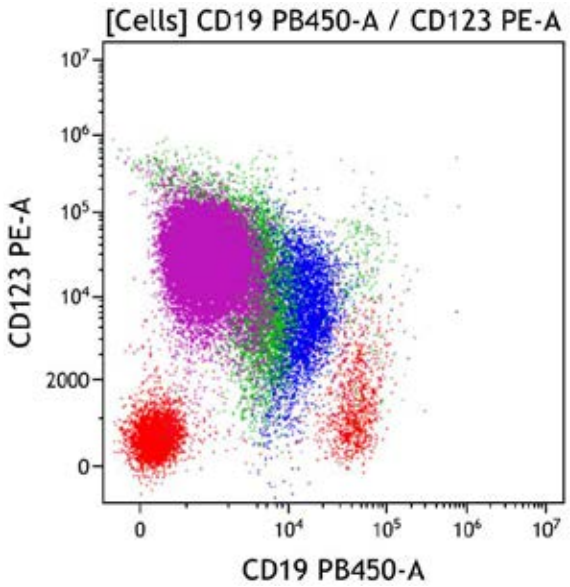

Figure 19. This CD19 vs CD123 ot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not expression CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Of note, the expanded population in the CD45dim gate is CD123-positive.

M2-20

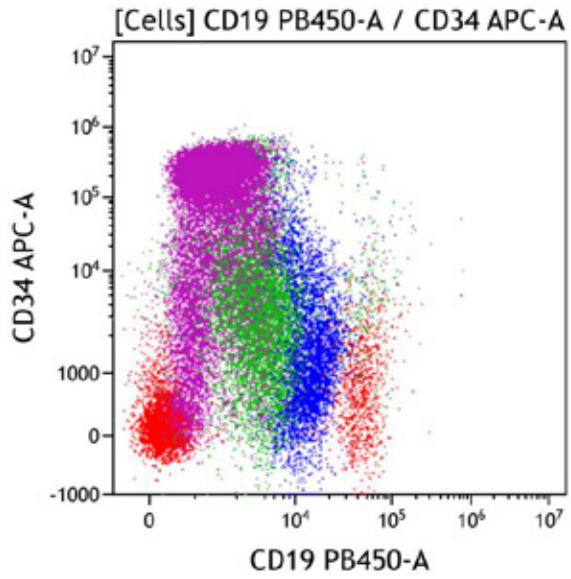

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19.

BACK TO CASE OVERVIEW

M2-21

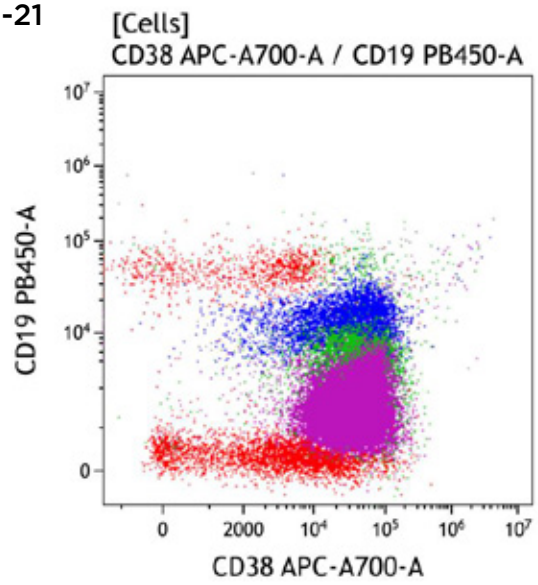

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression 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.

M2-22

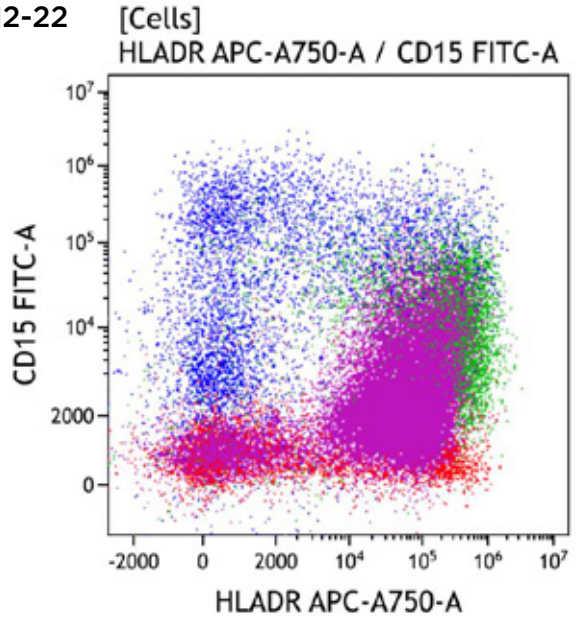

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR (blue),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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 10 QWA-TC4-040**
Diagnosis	**Plasma cell neoplasm**
Clinical Vignette	60-year-old female with history of anemia and thrombocytopenia. A bone marrow sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	The flow cytometry study detected a population of abnormal CD45-dim population with increased SSC. This population is uniformly positive for CD38, CD56, CD117, and CD200 without CD10, CD14, CD16, CD19, CD20, CD33, CD64 or T cell markers. This population does not have a normal counterpart, and most consistent with neoplastic plasma cells. Neoplastic plasma cells can have dim to negative CD45, moderate to high SSC, and uniform expression of CD38. Some plasma cell neoplasm can be uniformly positive for CD56, CD117 or CD200, such as in this case. The plasma cell neoplasm was further confirmed using a plasma cell panel, which is not included in the ClearLLab 10C panels. It is important to recognize unusual population in panels that are not specifically designed to detect.

BACK TO CASE LISTINGS

B Cell Tube

B01

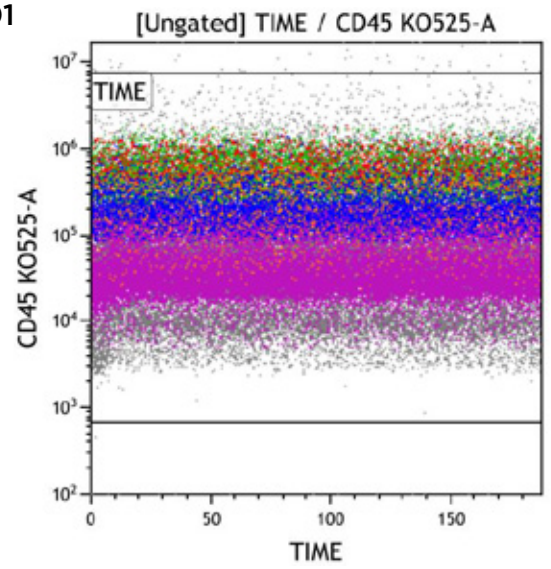

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

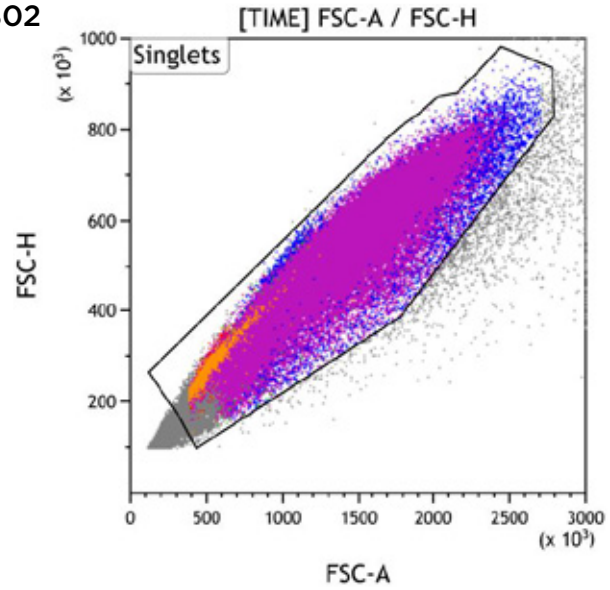

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

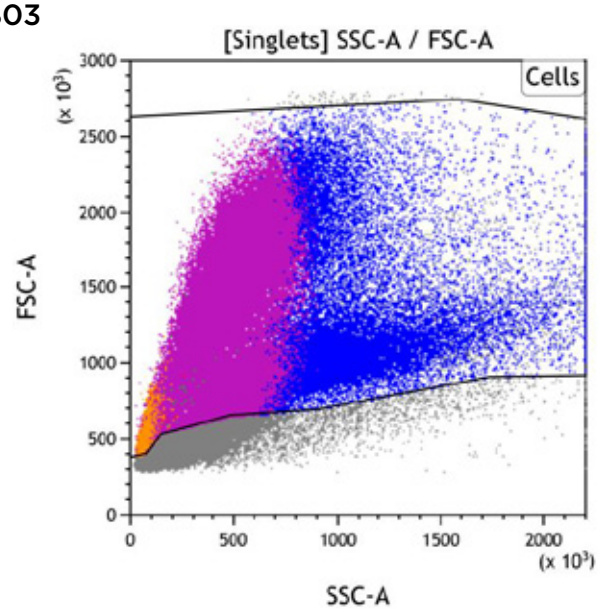

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. Of note, a subset of cells in the CD45dim gate (in purple) shows decreased FSC and increased SSC, a feature that is typically associated with cell degeneration.

B04

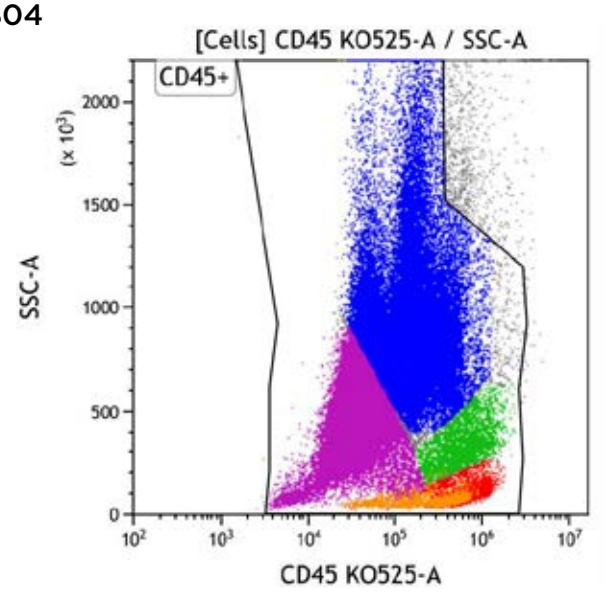

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

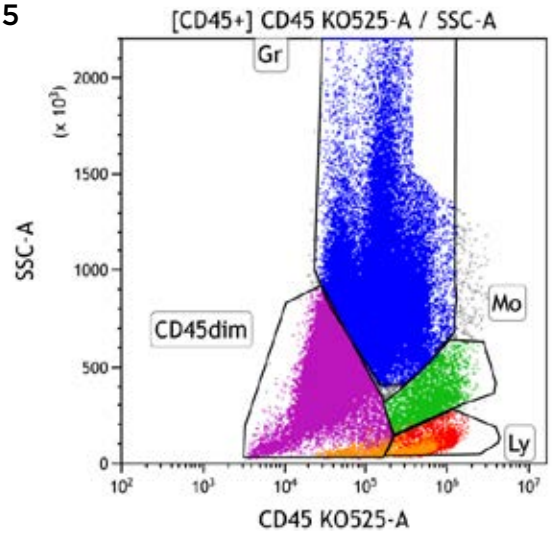

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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 populations in the CD45dim with increased SSC.

B06

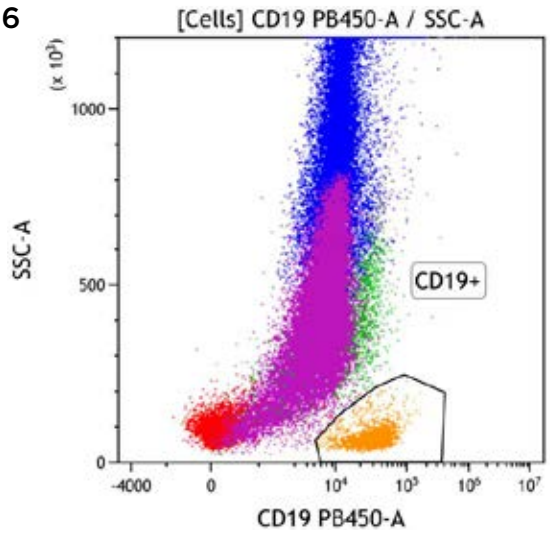

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Of note, the expanded population in the CD45dim gate is CD19-negative.

B07

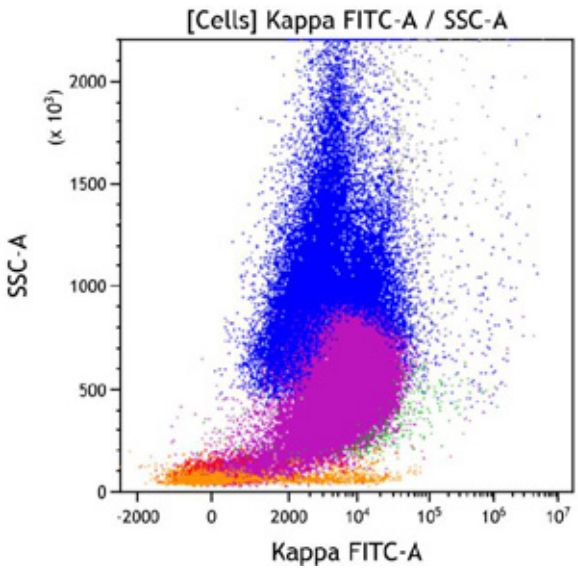

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in orange) and latestage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa 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. .

B08

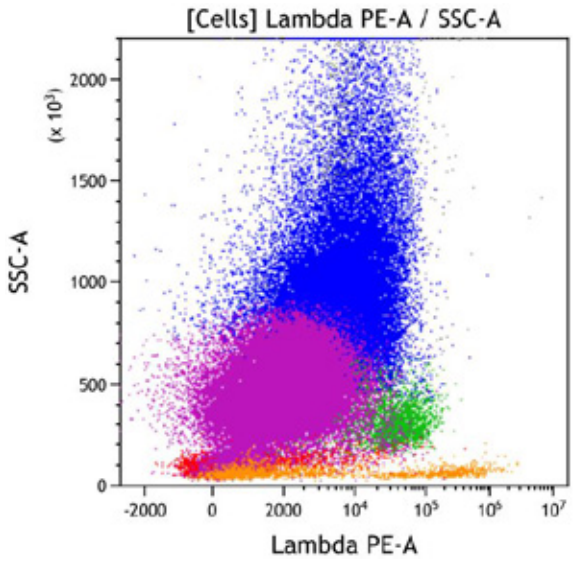

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent 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.

BACK TO CASE OVERVIEW

B09

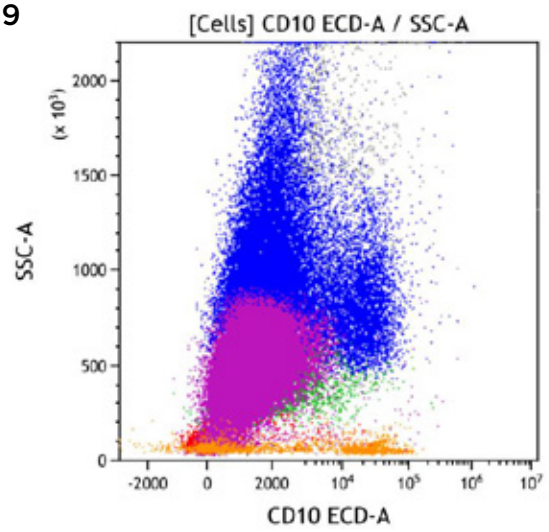

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

B10

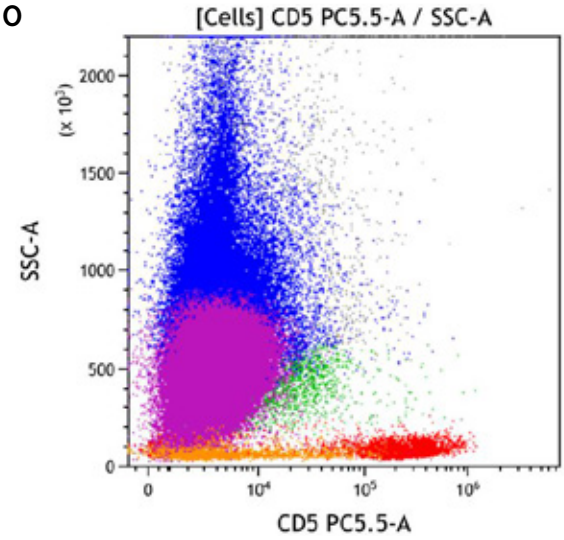

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in orange). These lymphoid cells typically have low side scatter.

B11

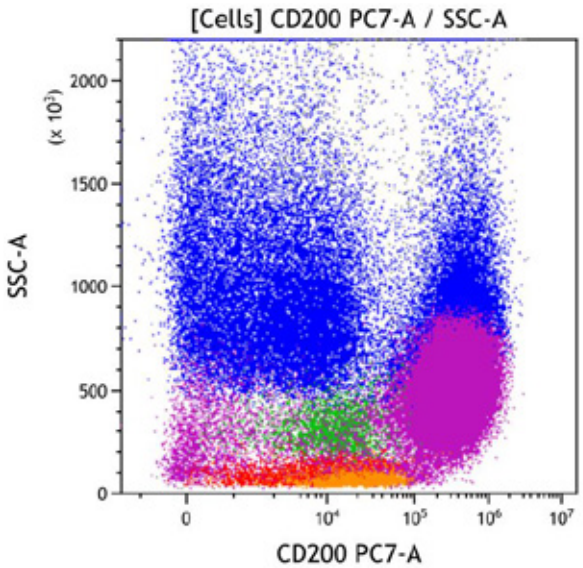

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). Of note, the expanded population in the CD45dim gate expresses bright CD200.

B12

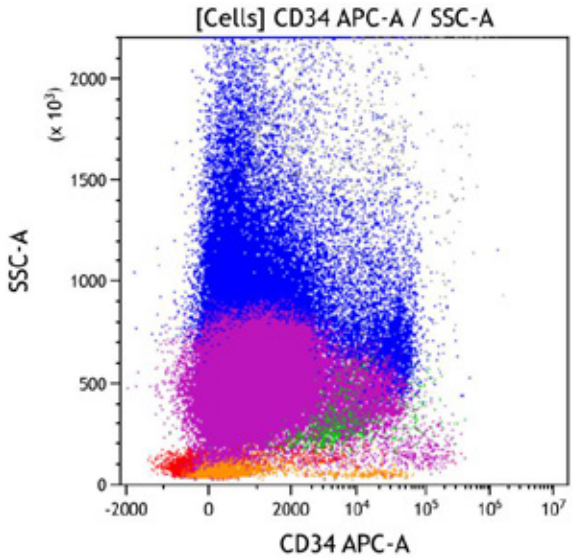

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

B13

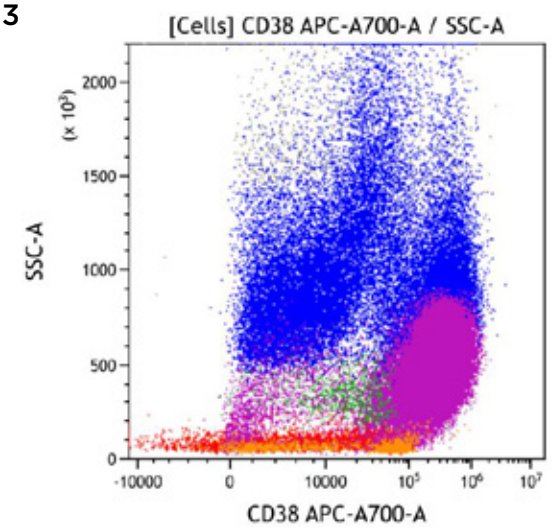

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34-positive hematopoietic stem cells express CD38 at variably low to absent level. Of note, the expanded population in the CD45dim gate expresses bright CD38.

B14

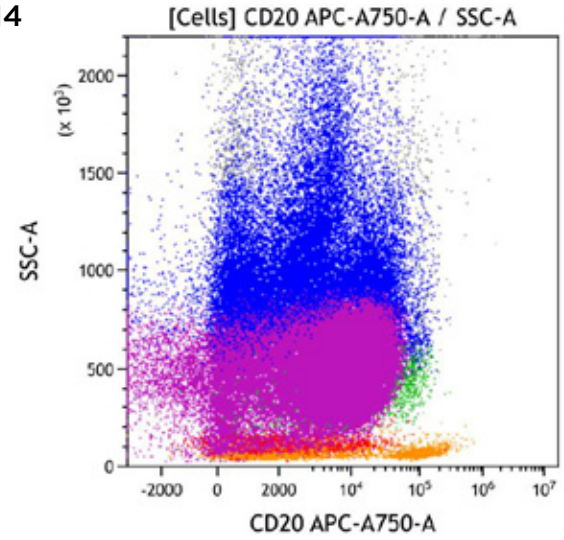

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (highlighted in orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate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

B15

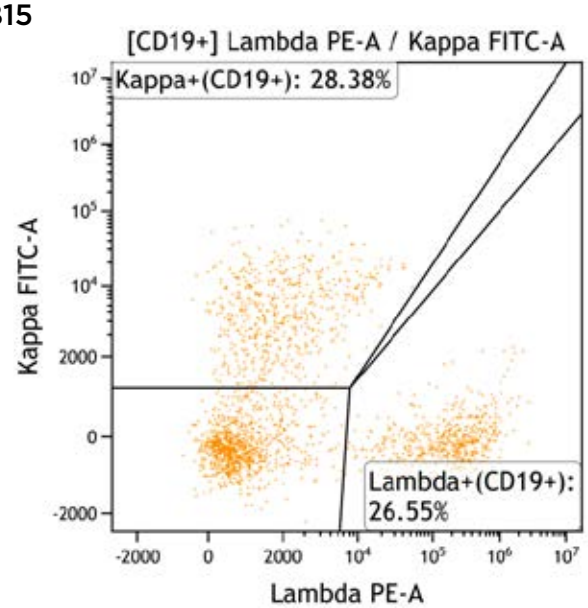

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

B16

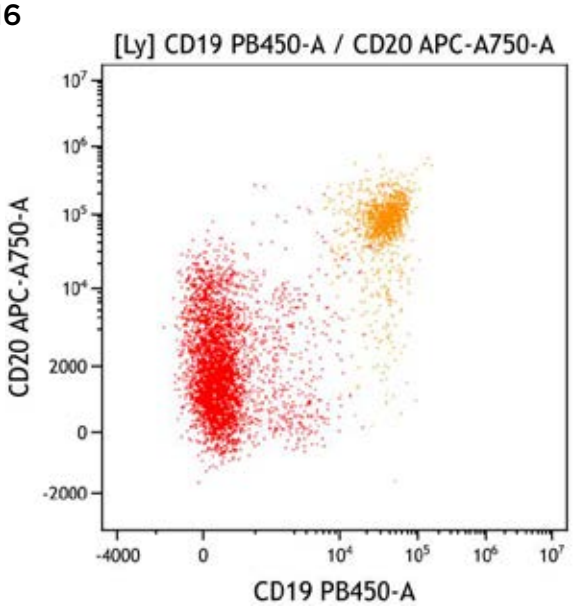

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells express both CD19 and CD20 (highlighted in orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

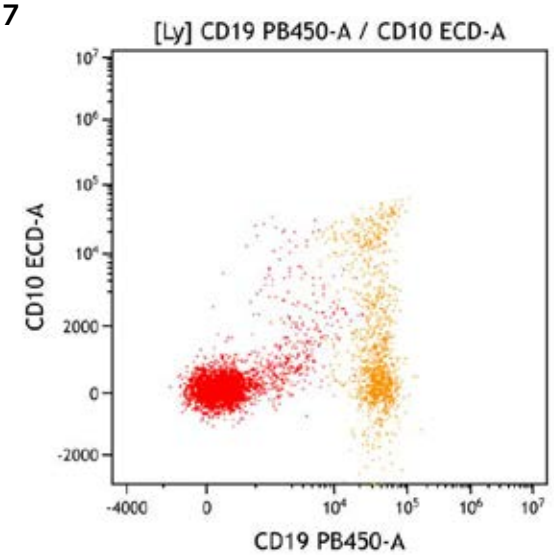

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate. B cells are CD19 positive (highlighted in orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow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

B18

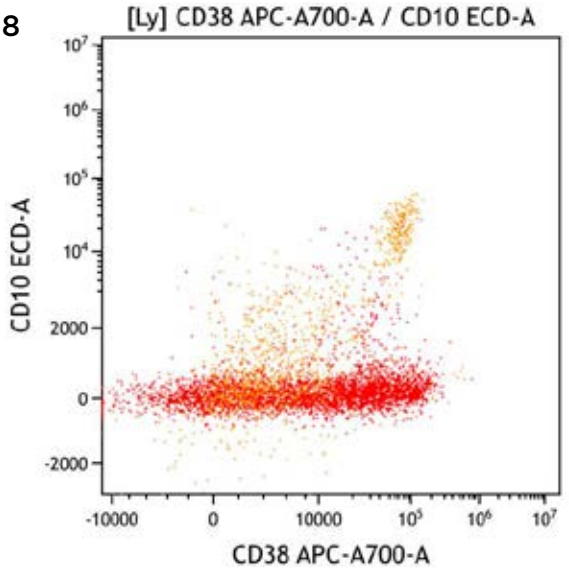

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

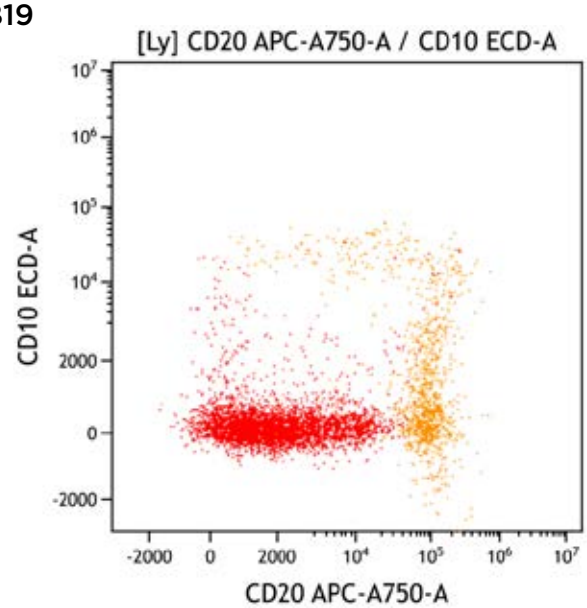

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late-stage immature B cells.

B20

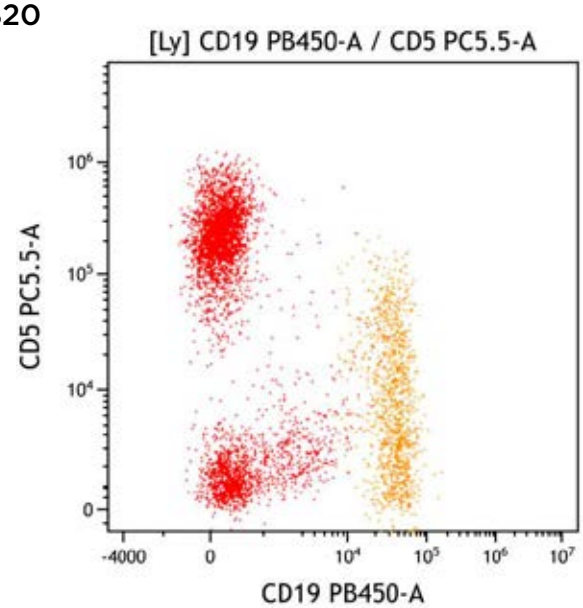

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is variably expressed at a low level on a subset of normal mature B cells and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

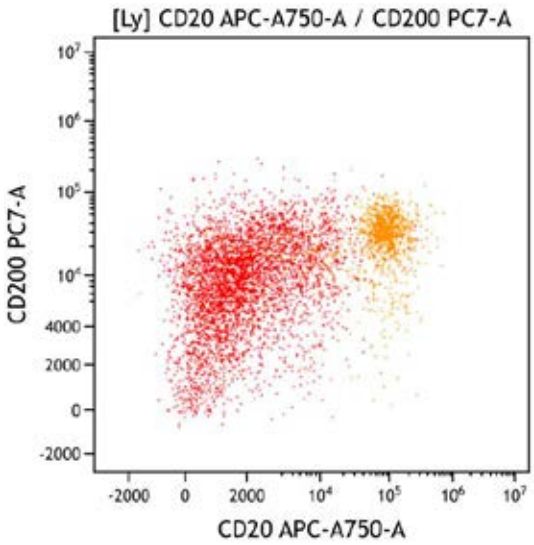

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in orange).

B22

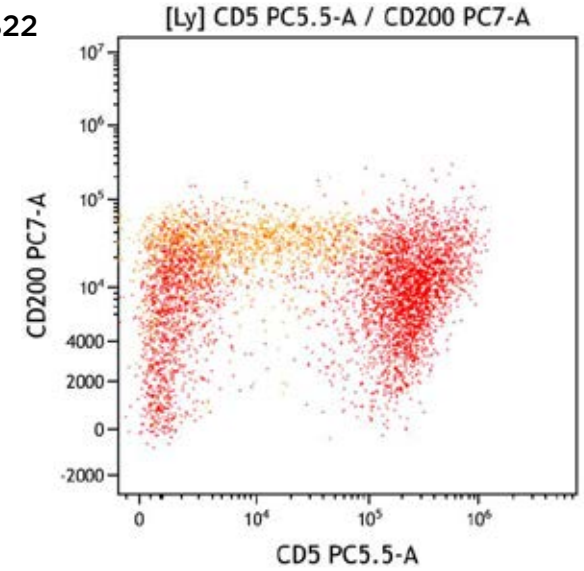

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically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

BACK TO CASE OVERVIEW

T Cell Tube

T01

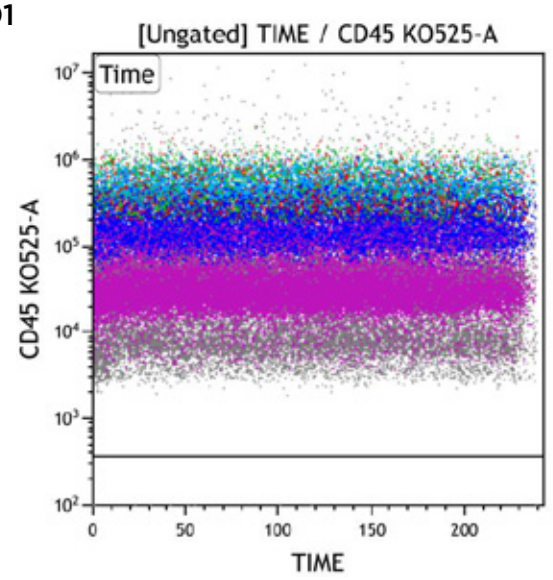

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

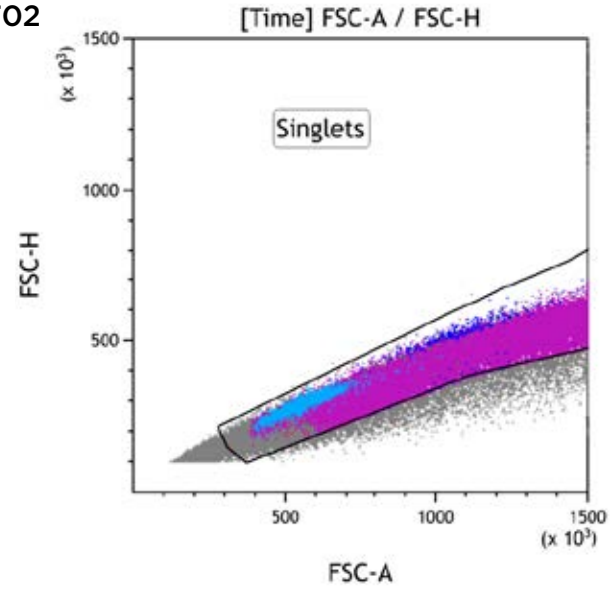

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

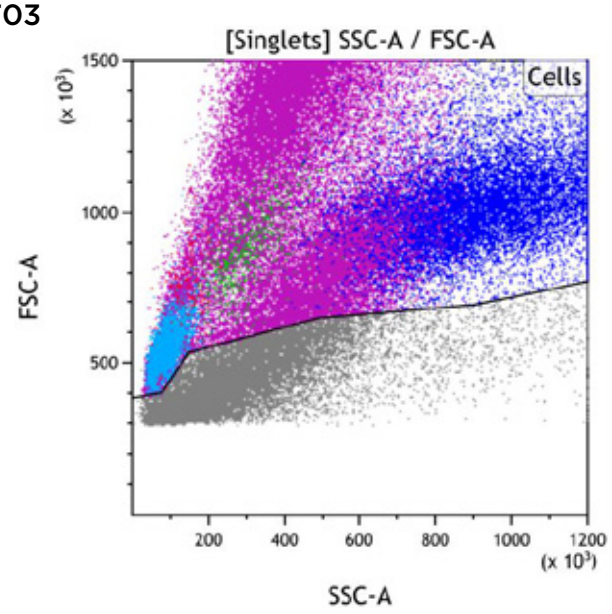

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. Of note, a subset of cells in the CD45dim gate (in purple) shows decreased FSC and increased SSC, a feature that is typically associated with cell degeneration.

T04

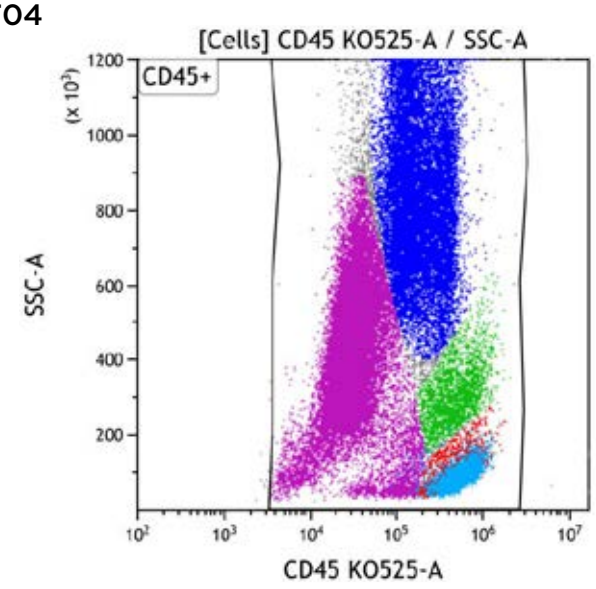

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

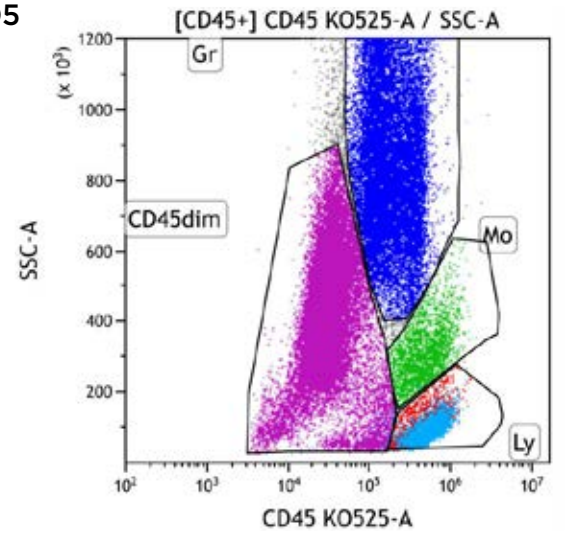

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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. Of note, cells in the CD45dim gate have abnormally increased SSC.

T06

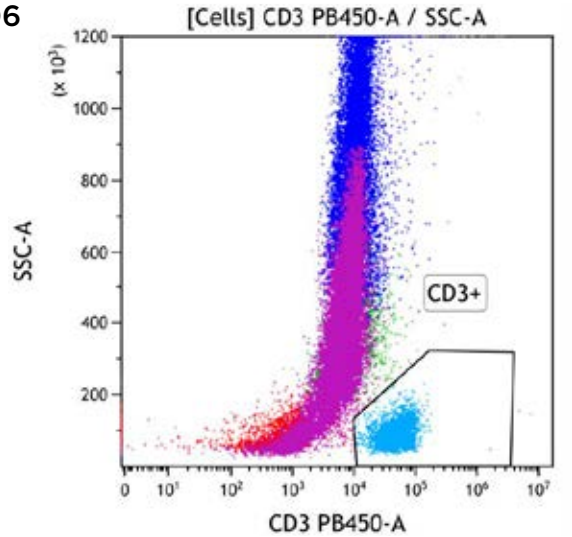

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

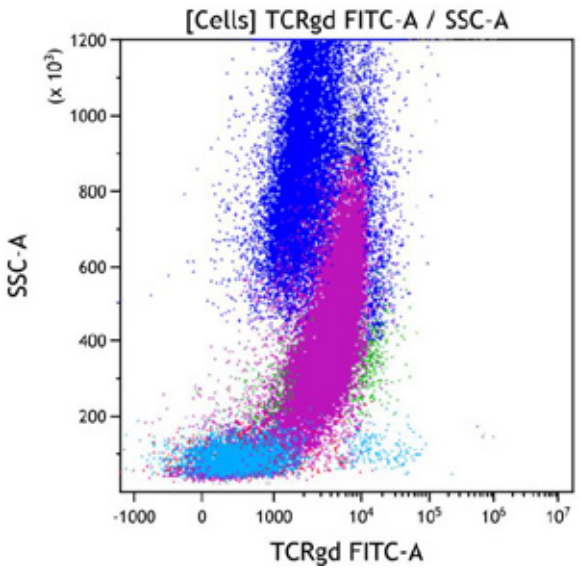

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

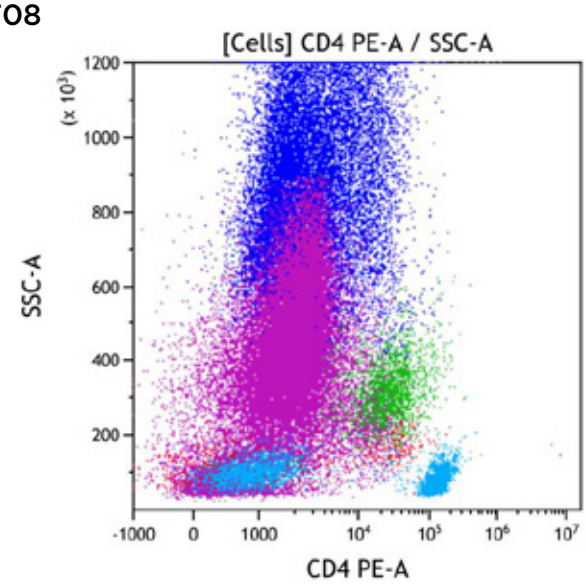

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

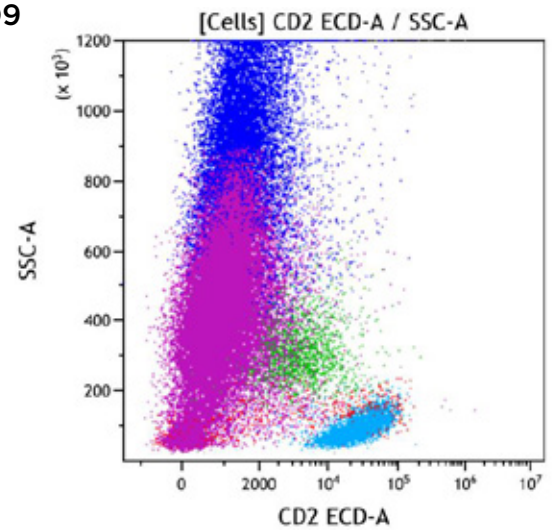

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

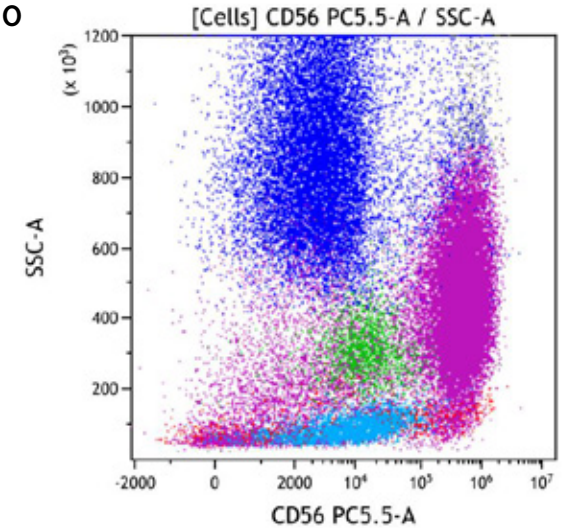

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/ delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. Of note, the expanded population in the CD45dim gate expresses uniform CD56.

T11

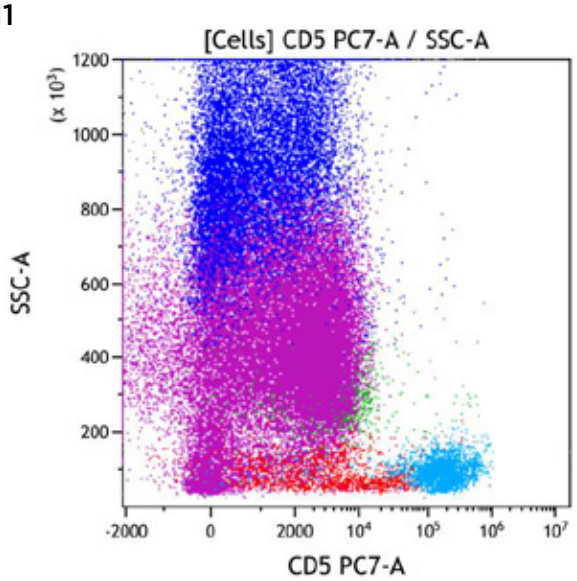

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

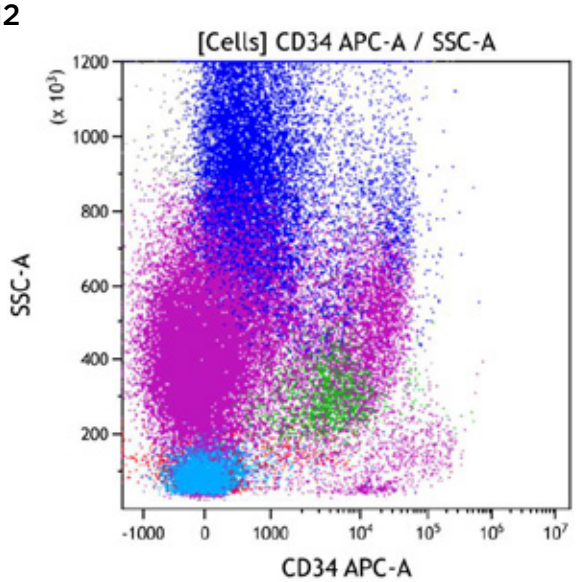

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

T13

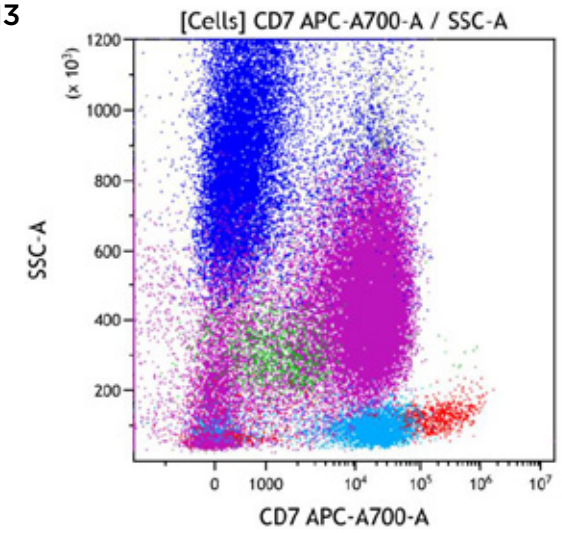

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

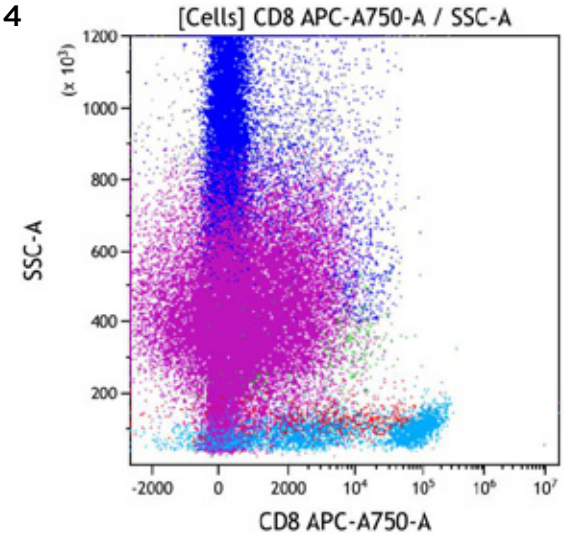

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

T15

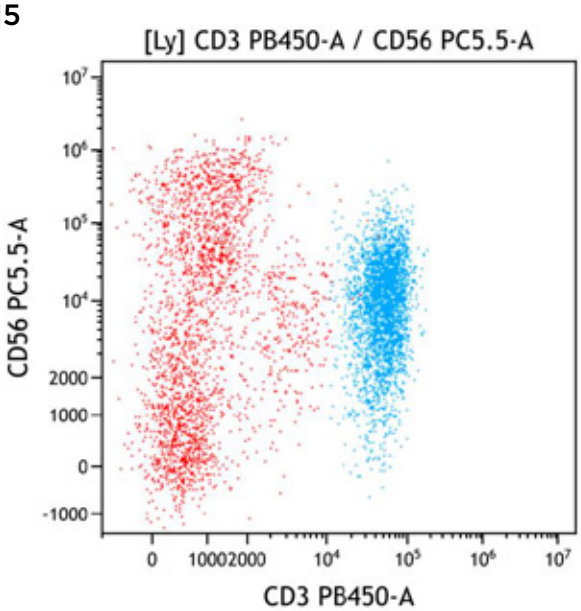

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small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

T16

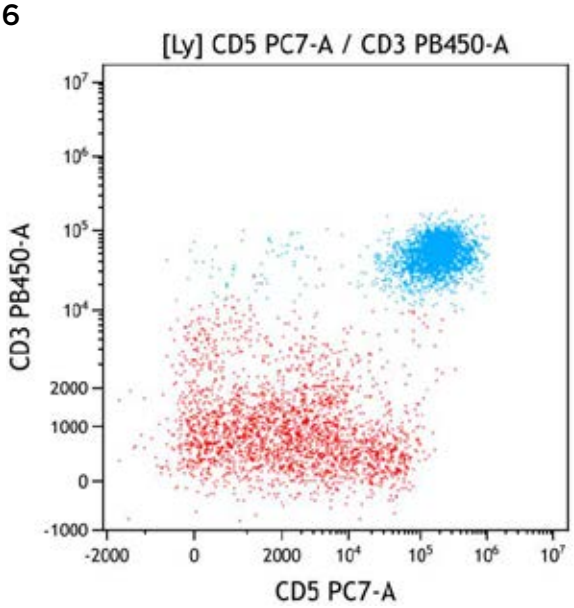

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most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

BACK TO CASE OVERVIEW

T17

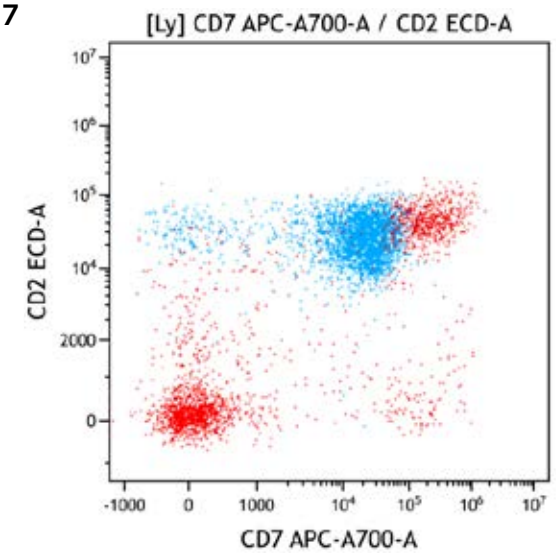

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

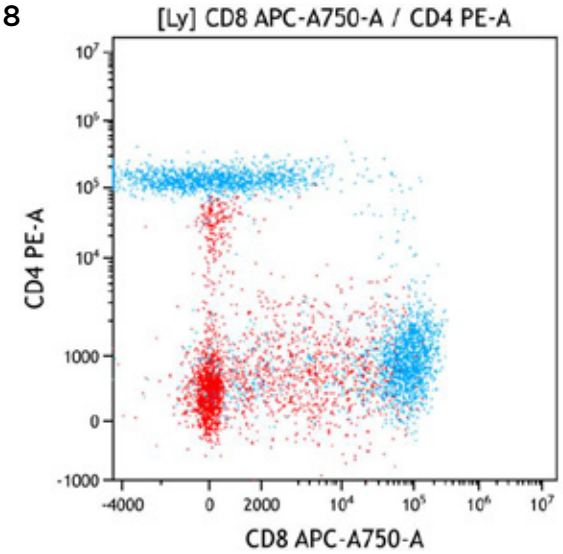

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 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.

T19

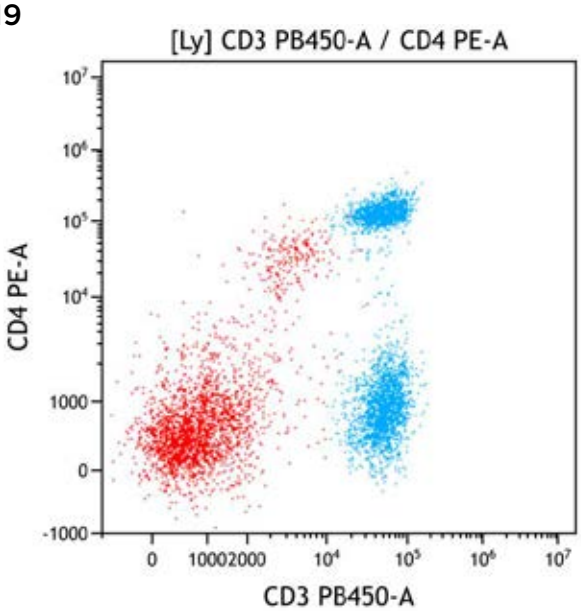

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

T20

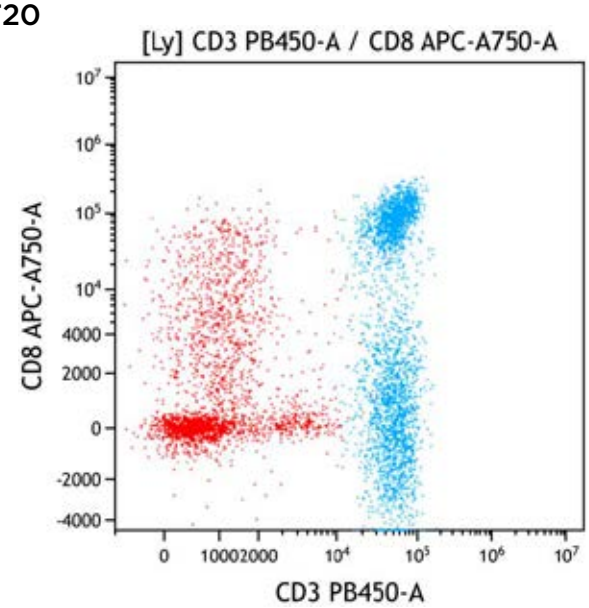

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

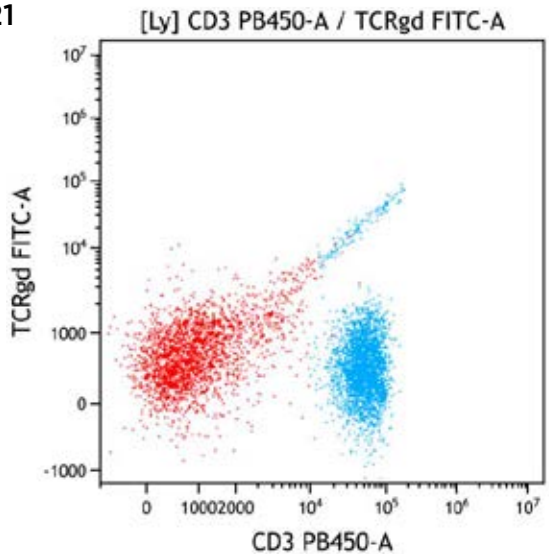

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

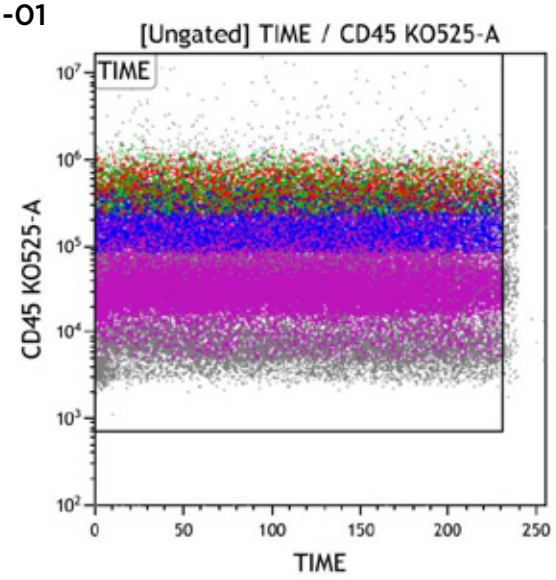

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

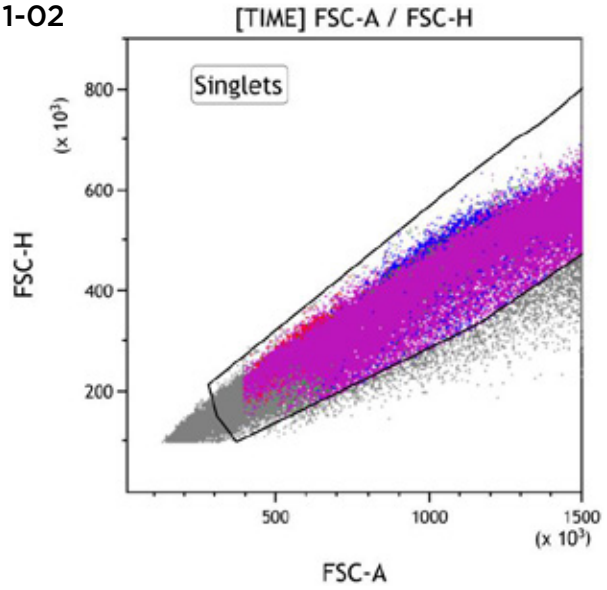

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

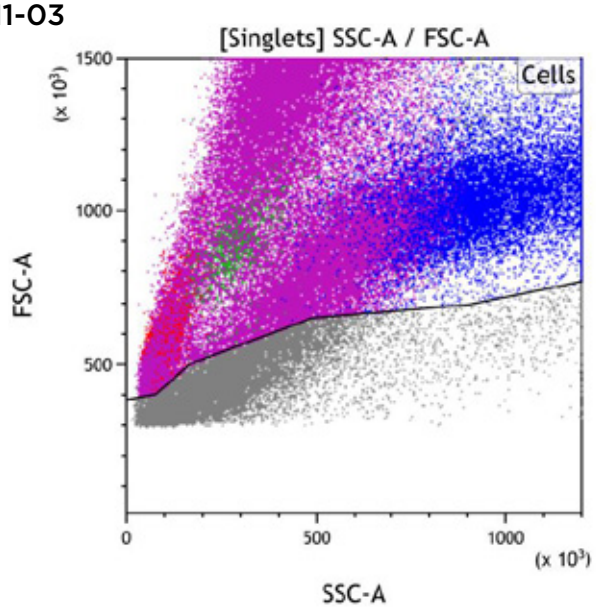

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. Of note, cells in the CD45dim gate have increased SSC.

M1-04

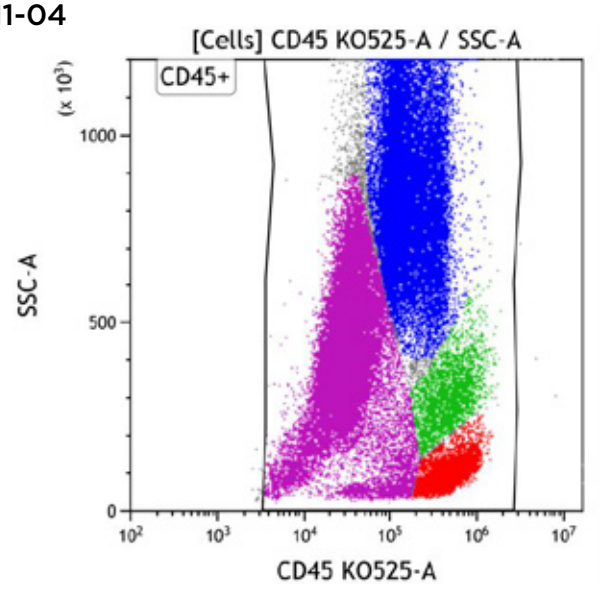

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

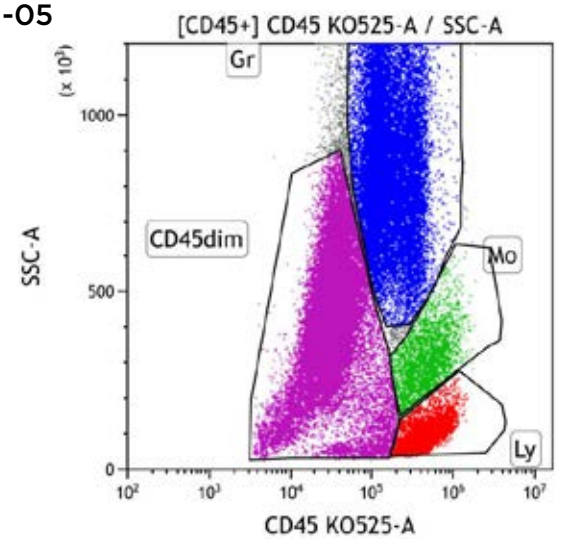

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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. Of note, cells in the CD45dim gate have abnormally increased SSC.

M1-06

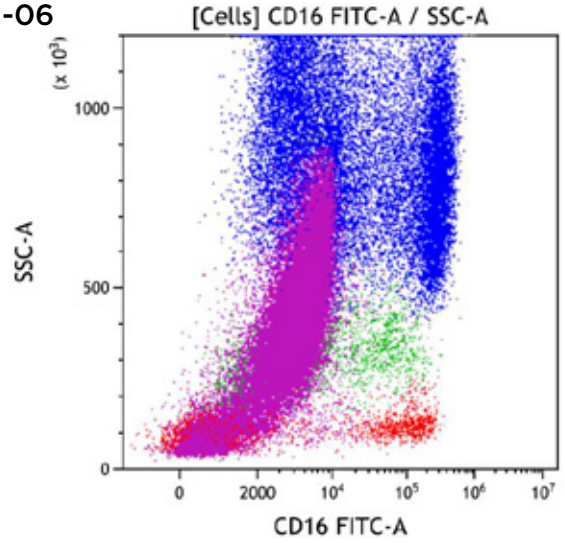

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

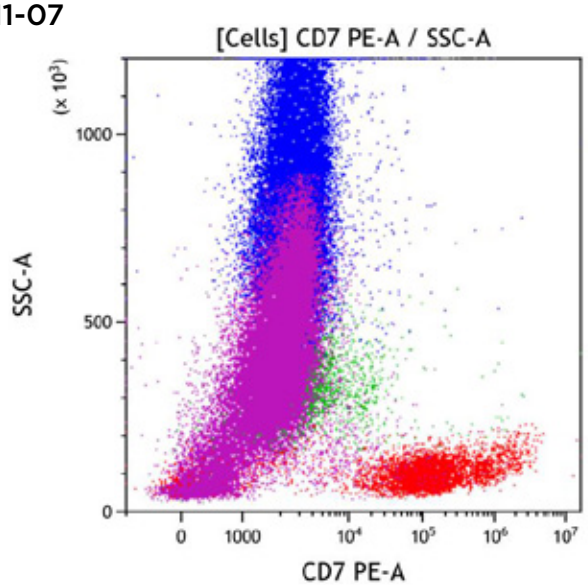

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

M1-08

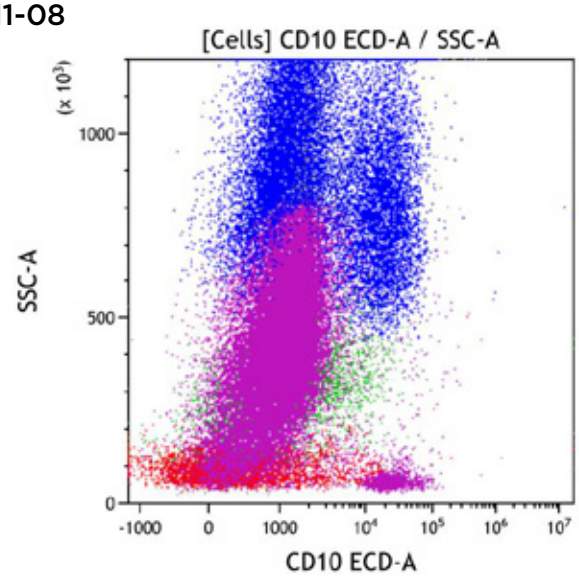

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

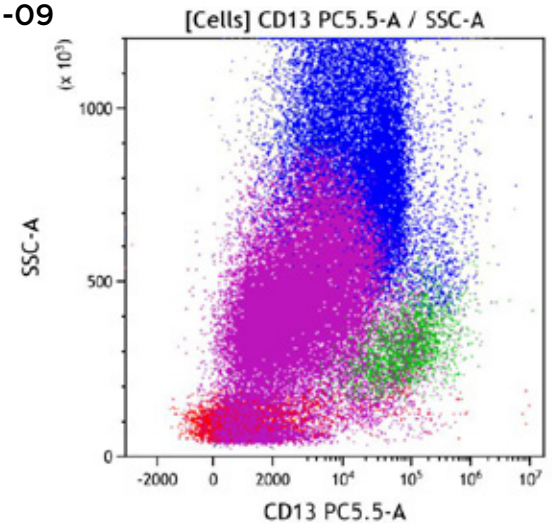

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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). Of note, most cells in the CD45dim gate (purple) are CD13-negative.

M1-10

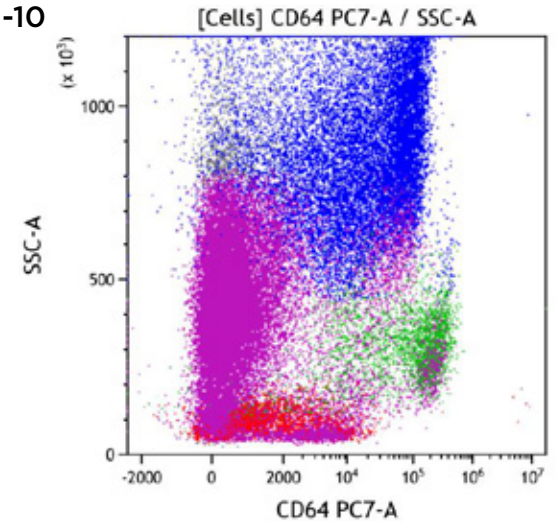

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34+ progenitors. Of note, most cells in the CD45dim gate (purple) are CD64-negative.

M1-11

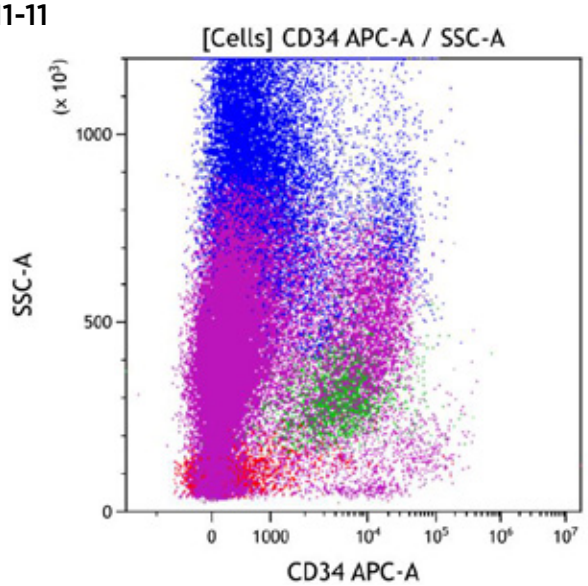

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

M1-12

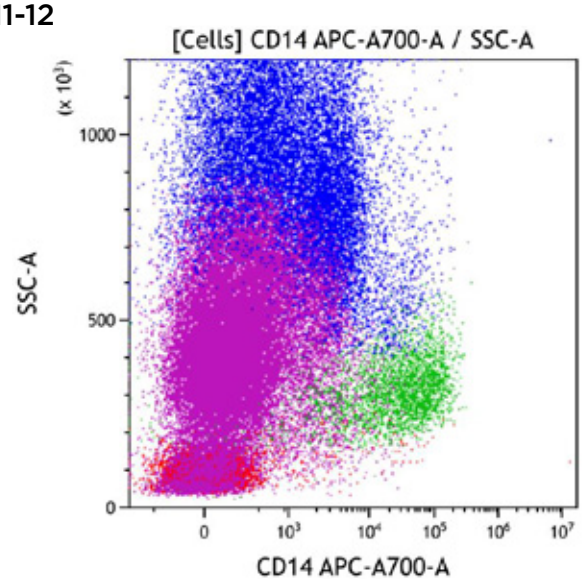

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Of note, most cells in the CD45dim gate (purple) are CD14-negative.

BACK TO CASE OVERVIEW

M1-13

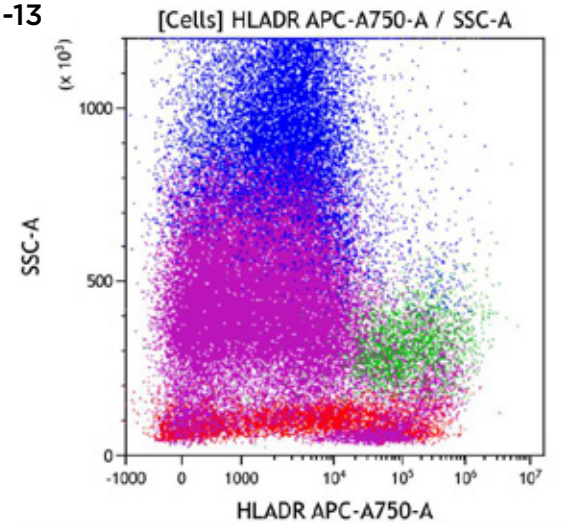

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

M1-14

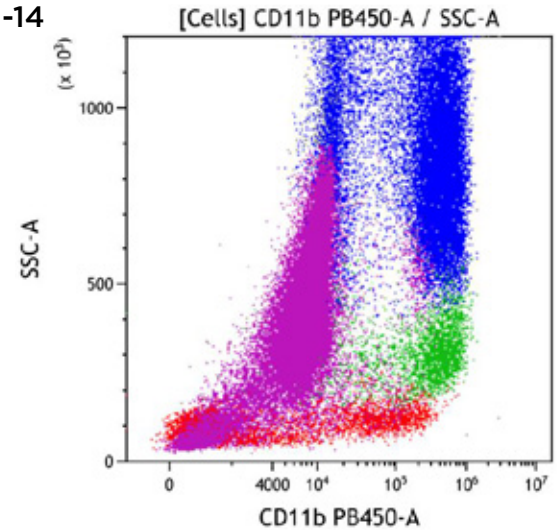

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

M1-15

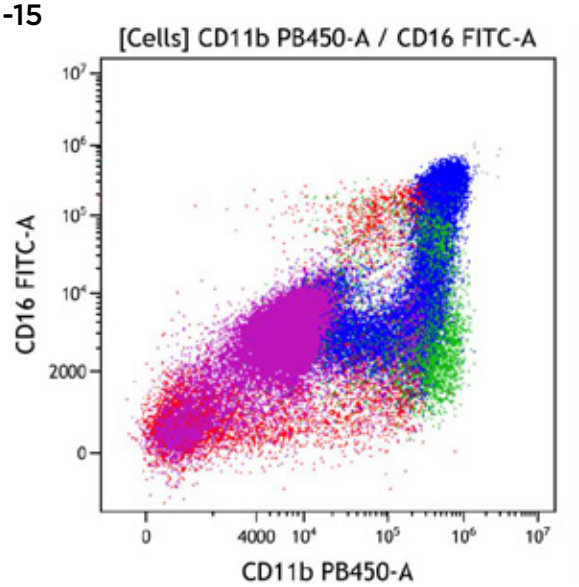

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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.

M1-16

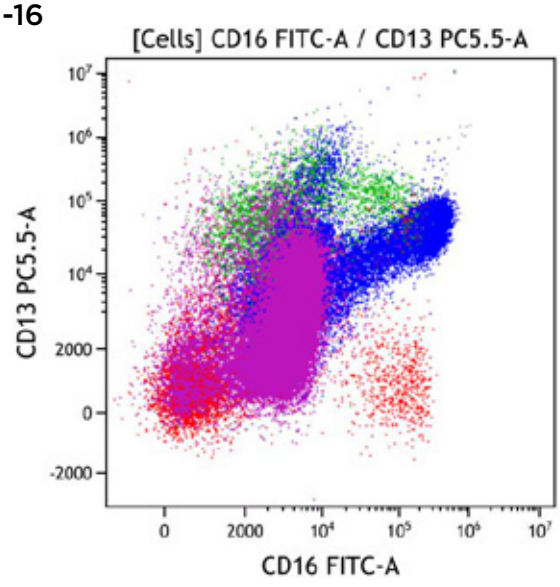

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed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

BACK TO CASE OVERVIEW

M1-17

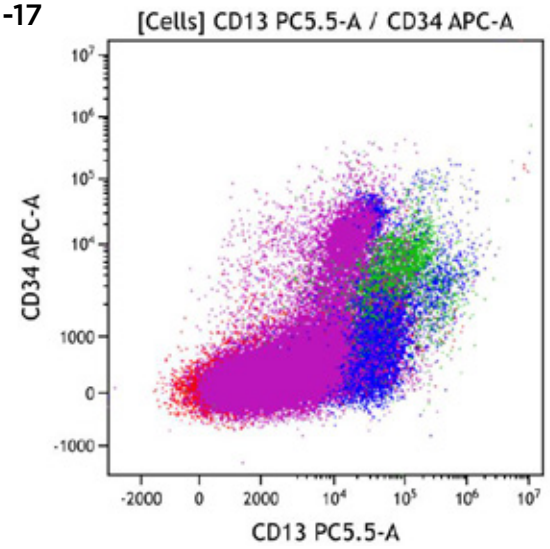

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple)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

M1-18

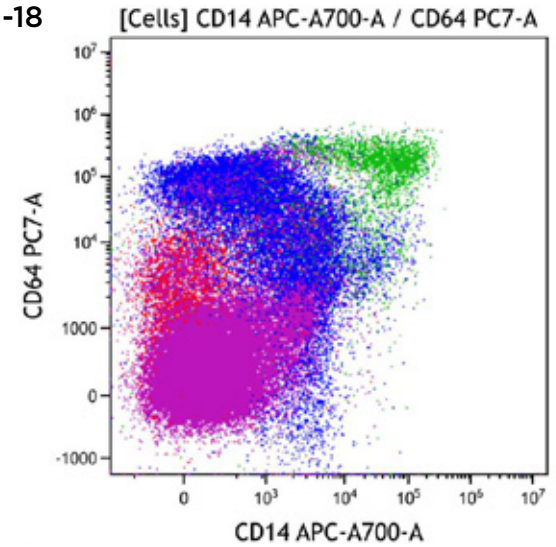

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high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

M1-19

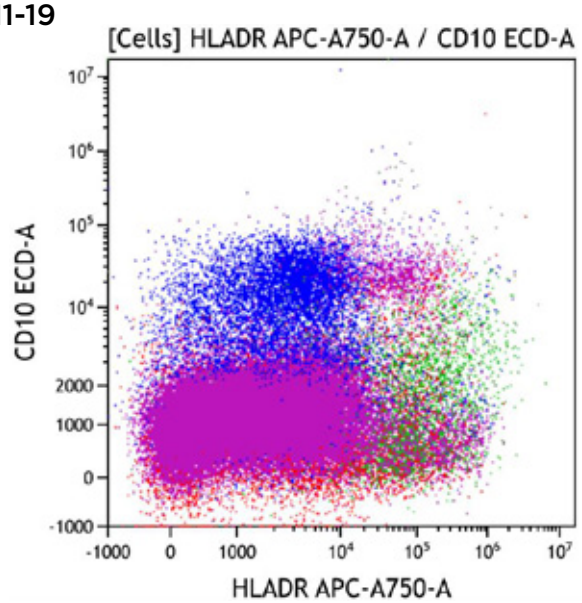

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

M1-20

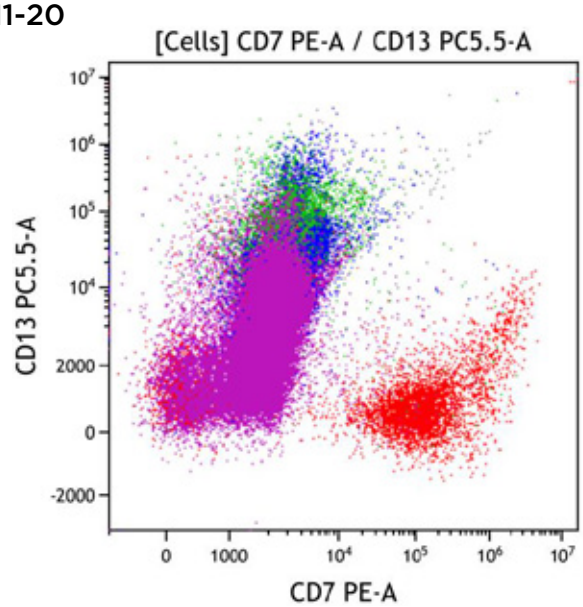

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

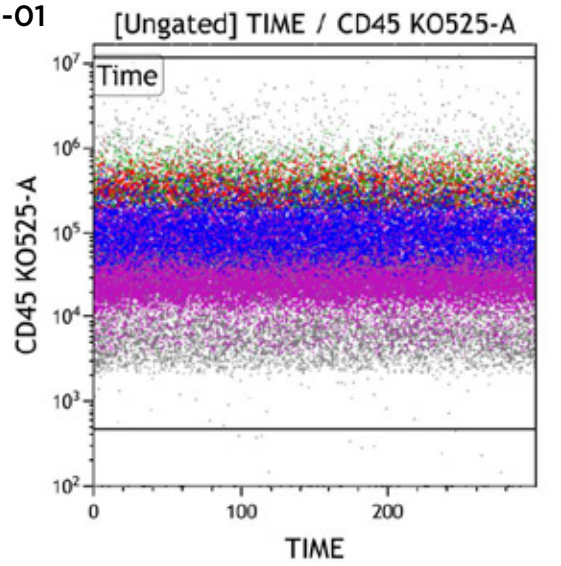

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

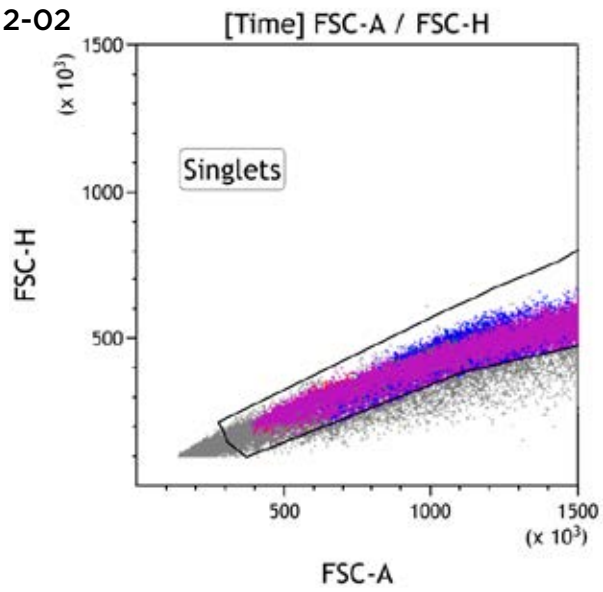

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

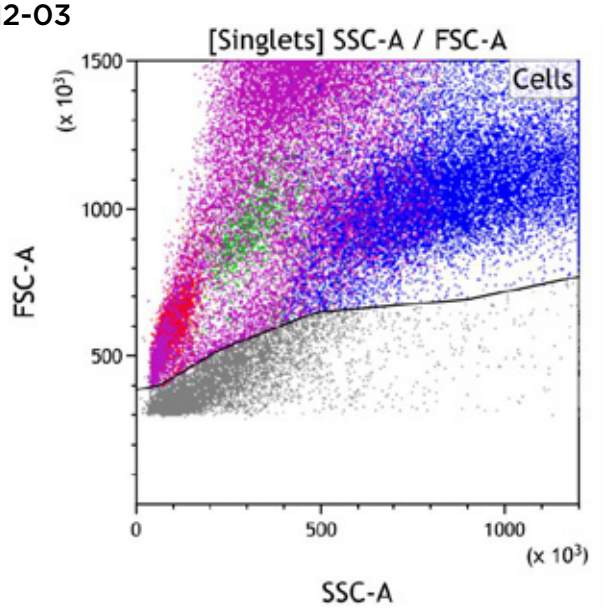

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. Of note, a subset of cells in the CD45dim gate (in purple) shows decreased FSC and increased SSC, a feature that is typically associated with cell degeneration.

M2-04

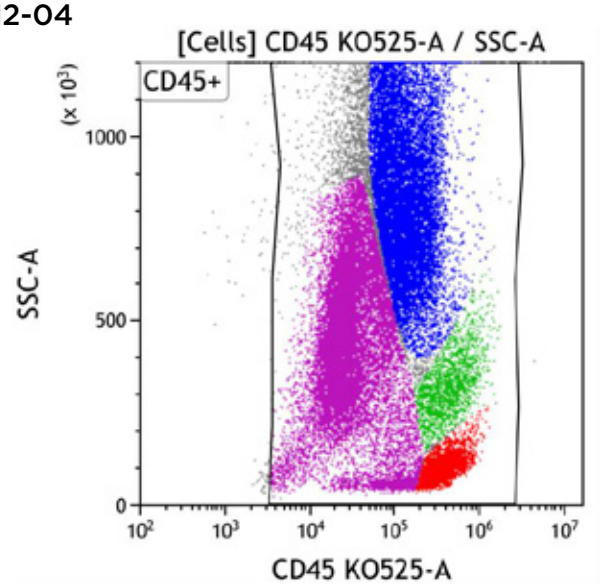

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M2-05

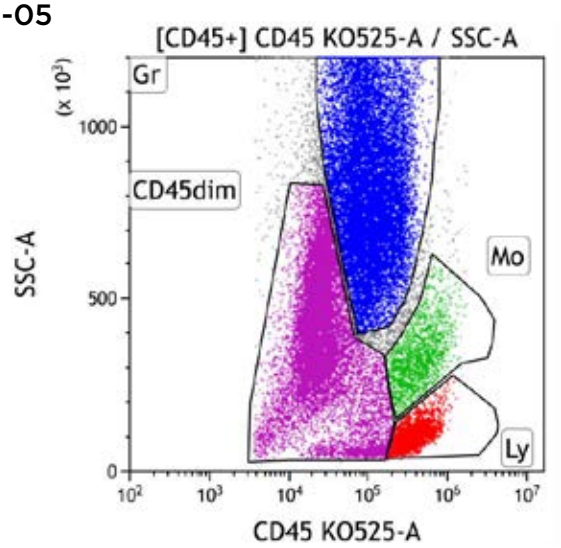

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors, (e.g., 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. Of note, cells in the CD45dim gate have abnormally increased SSC.

M2-06

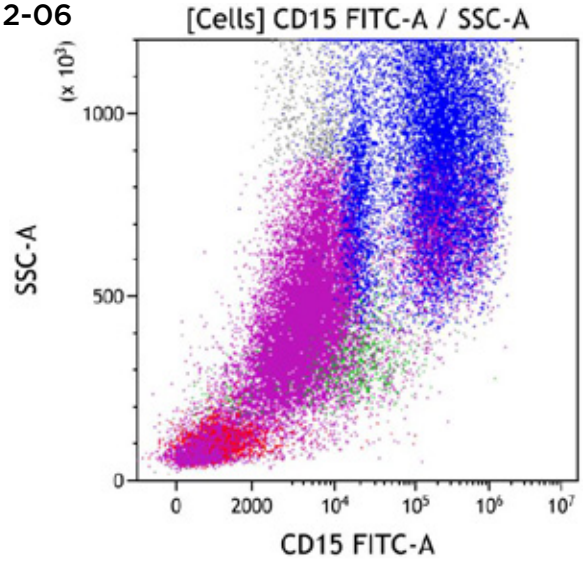

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

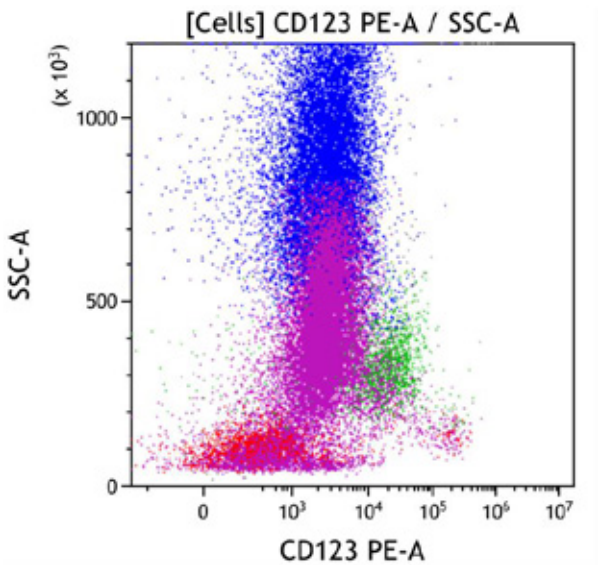

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green).

M2-08

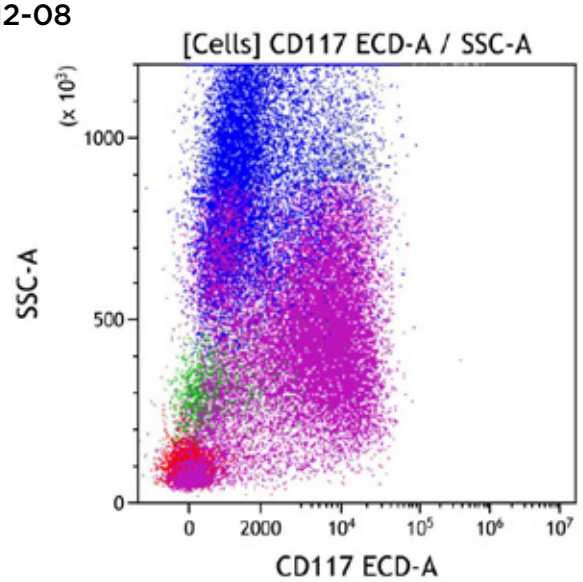

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Of note, most cells in the CD45dim gate (purple) are CD117-positive.

BACK TO CASE OVERVIEW

M2-09

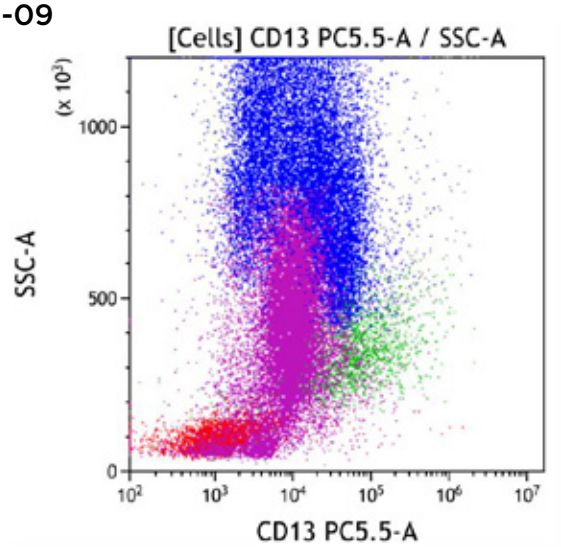

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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). Of note, most cells in the CD45dim gate (purple) are dimly positive for CD13.

M2-10

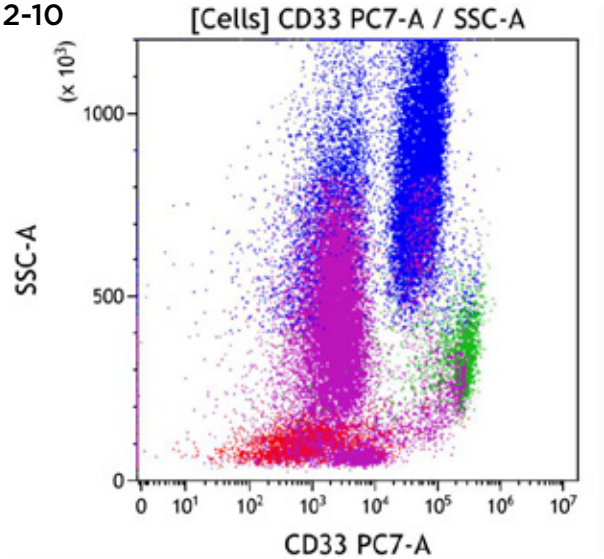

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a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

M2-11

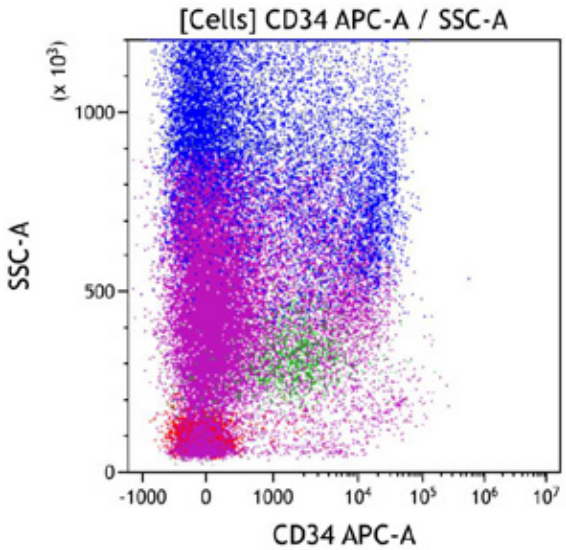

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

M2-12

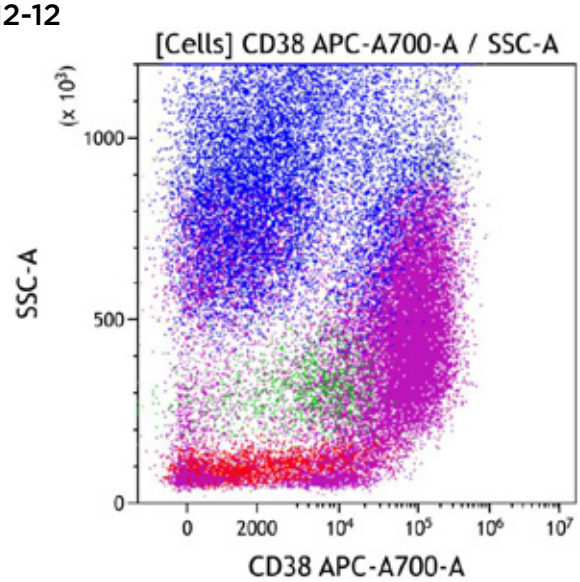

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level. Of note, most cells in the CD45dim gate (purple) are CD38-positive.

BACK TO CASE OVERVIEW

M2-13

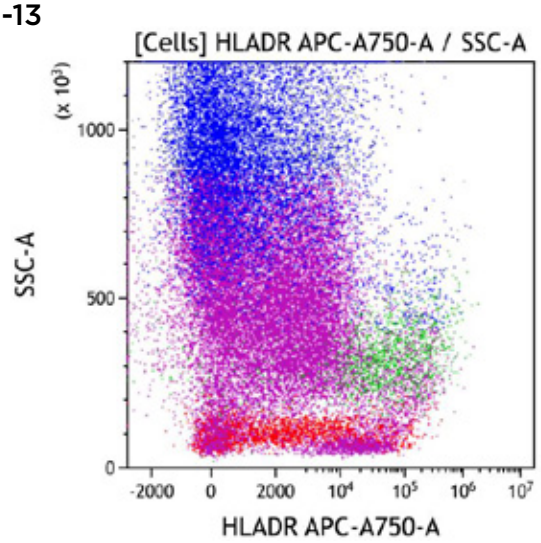

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T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

M2-14

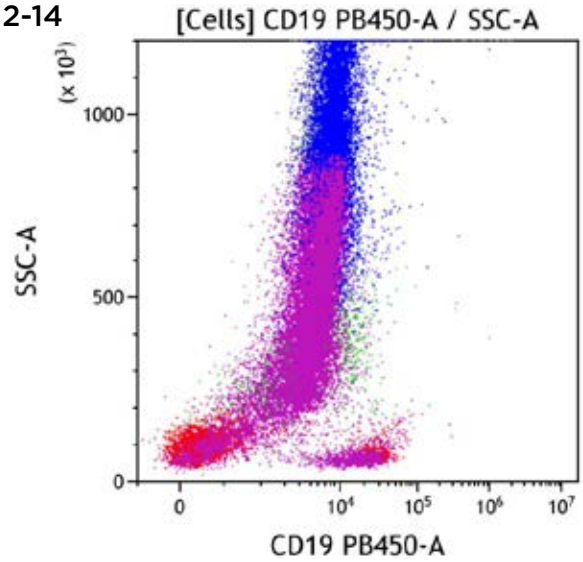

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Of note, most cells in the CD45dim gate (purple) are CD19-negative.

M2-15

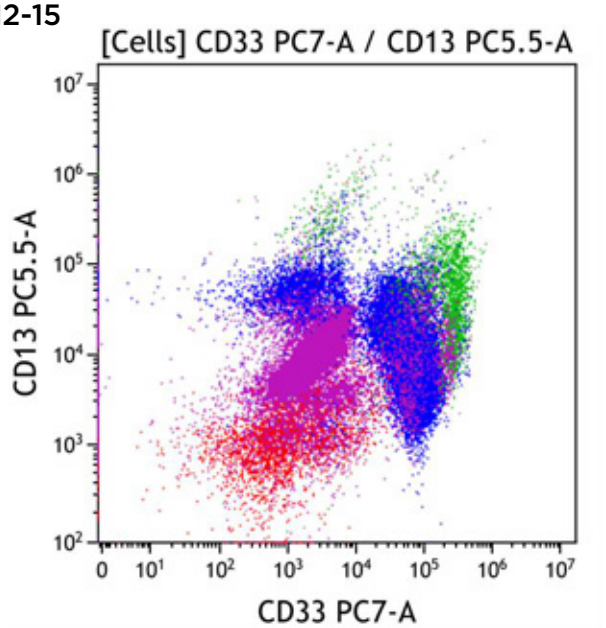

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher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

M2-16

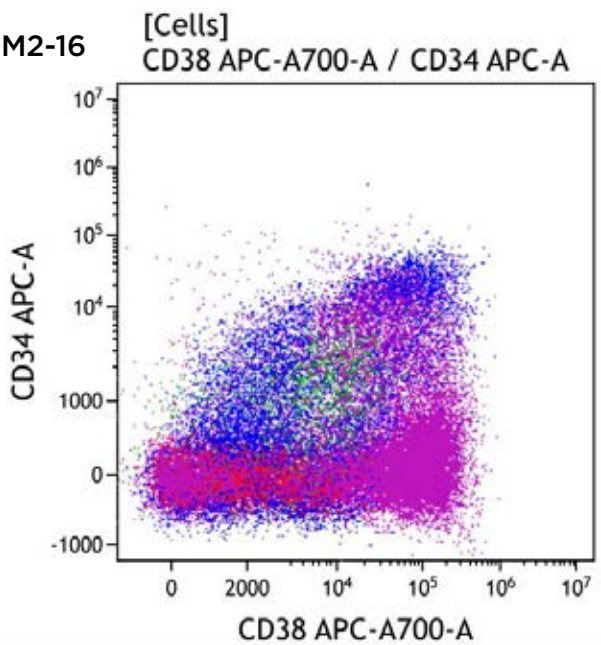

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable 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.

BACK TO CASE OVERVIEW

M2-17

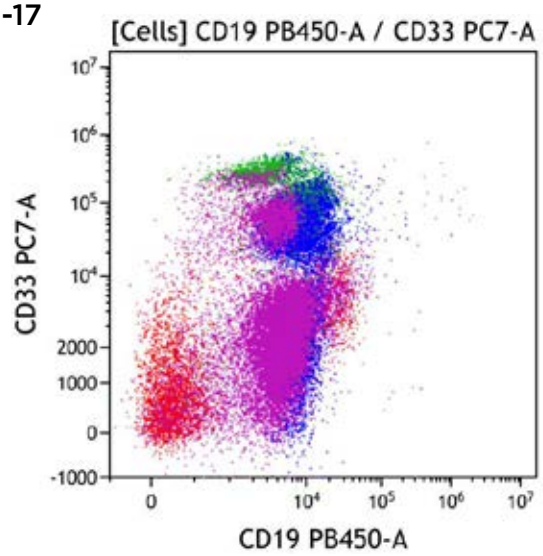

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to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

M2-18

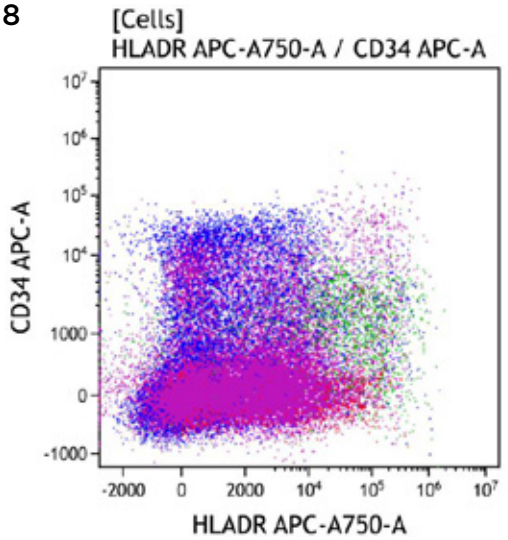

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of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

M2-19

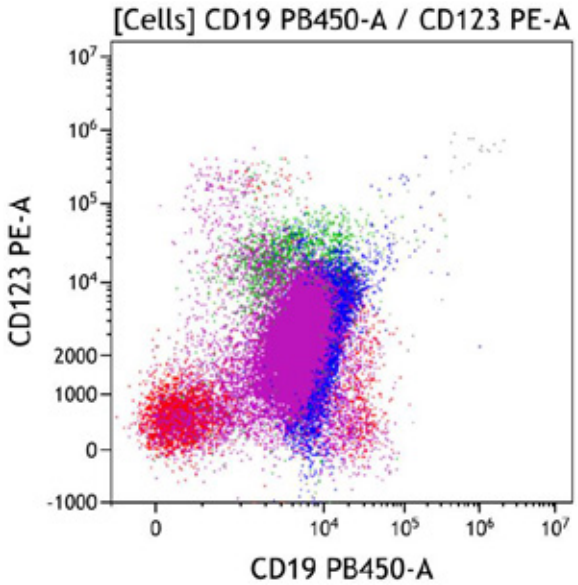

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid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

M2-20

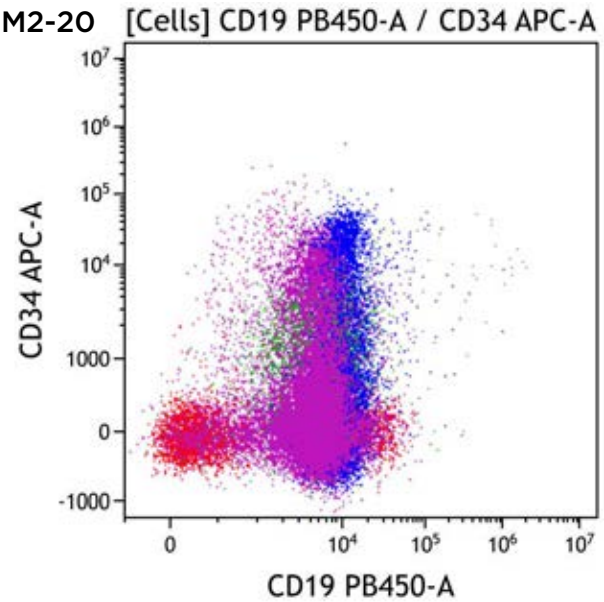

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19.

BACK TO CASE OVERVIEW

M2-21

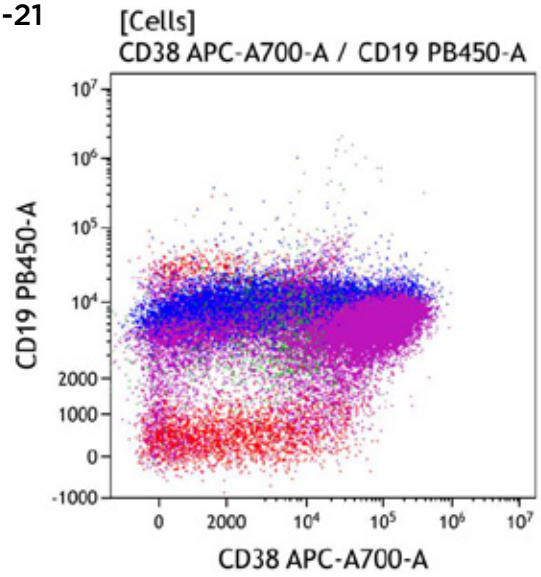

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression 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.

M2-22

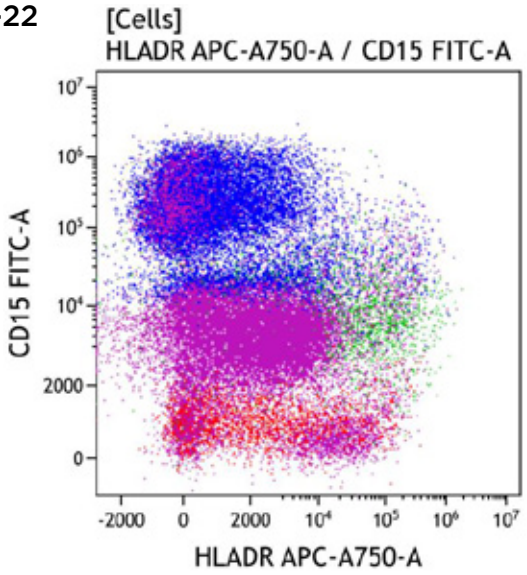

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLA-DR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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 11 QWA-TC4-071**
Diagnosis	**Mantle Cell Lymphoma**
Clinical Vignette	79-year-old female was recently diagnosed with non-Hodgkin B cell lymphoma. This biopsy was performed for lymphoma staging.
Flow Cytometry Result Interpretation	The flow cytometry study detected a kappa light chain restricted B cell population with abnormal expression of CD5 and absence of CD200. The findings are consistent with mantle cell lymphoma. Mantle cell lymphoma typically express CD5 without CD200 with normal CD20. In contrast, chronic lymphocytic leukemia/small lymphocytic lymphoma is typically express CD5 and CD200 with variably decreased CD20.

BACK TO CASE LISTINGS

B Cell Tube

B01

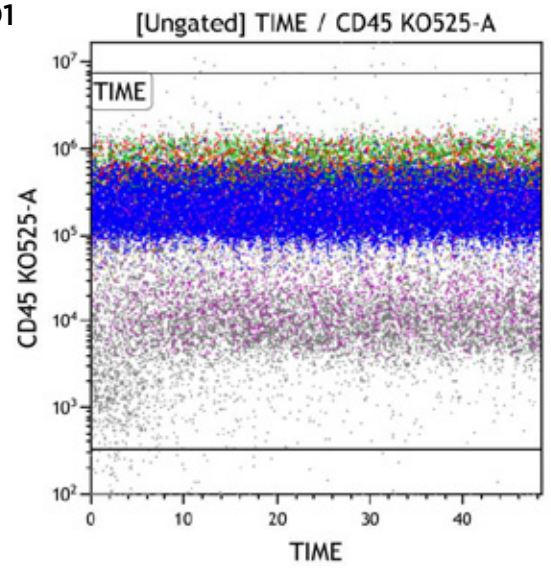

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

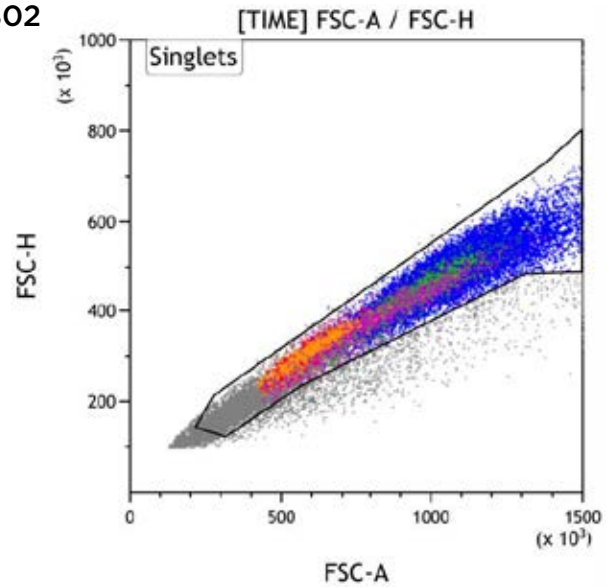

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

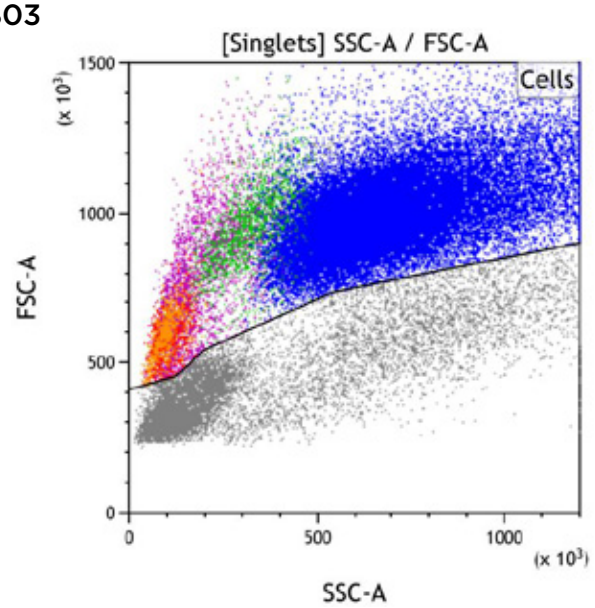

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

B04

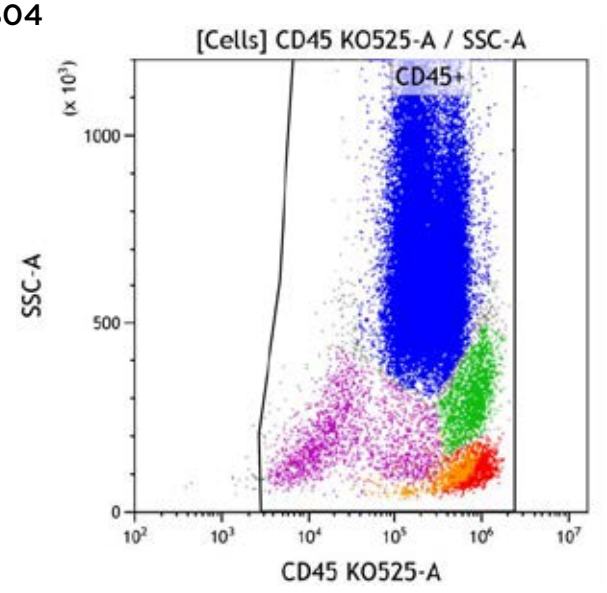

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

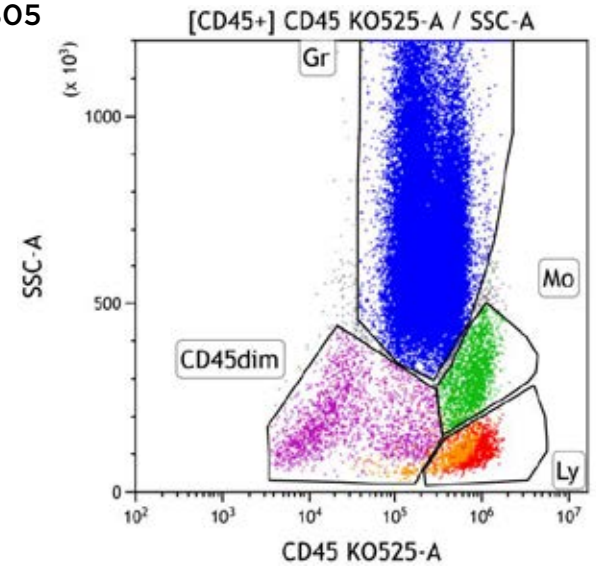

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., myeloblasts and immature B cells). Basophils, plas-macytoid 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.

B06

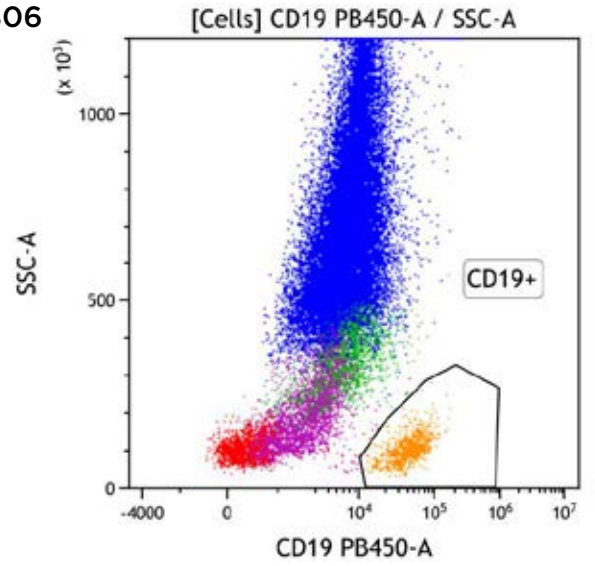

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter

B07

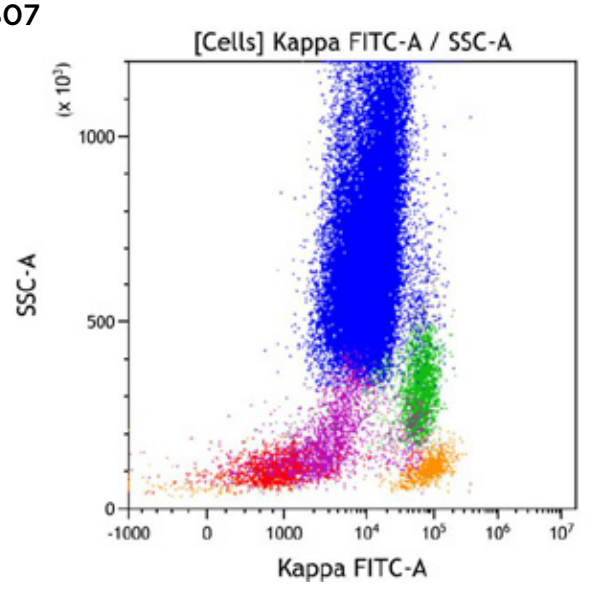

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in orange) and latestage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa 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.

B08

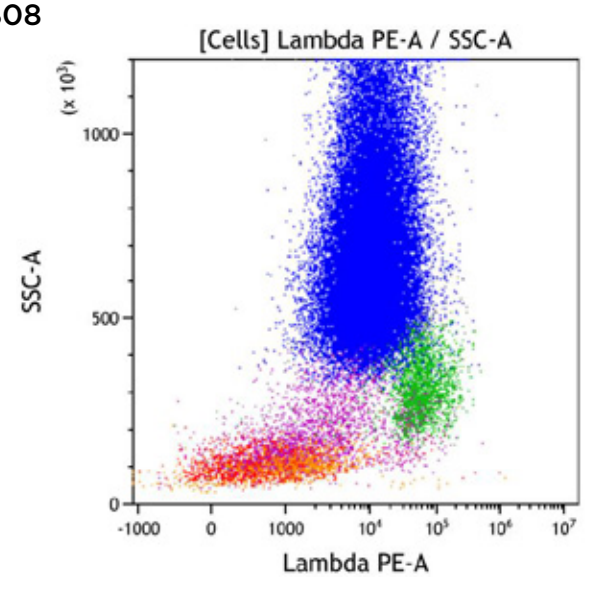

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent 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.

BACK TO CASE OVERVIEW

B09

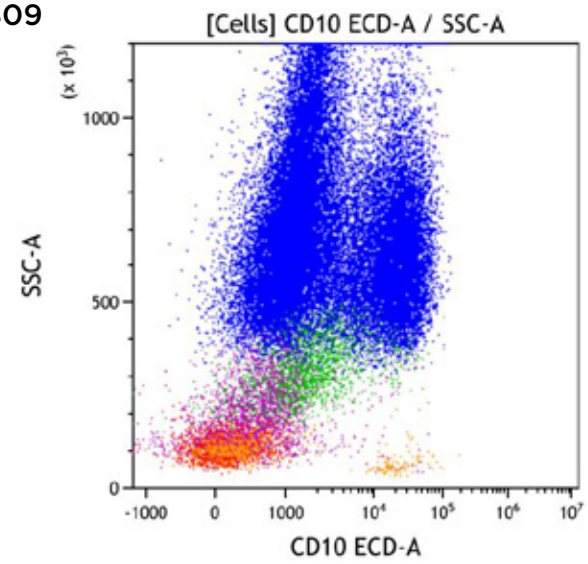

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

B10

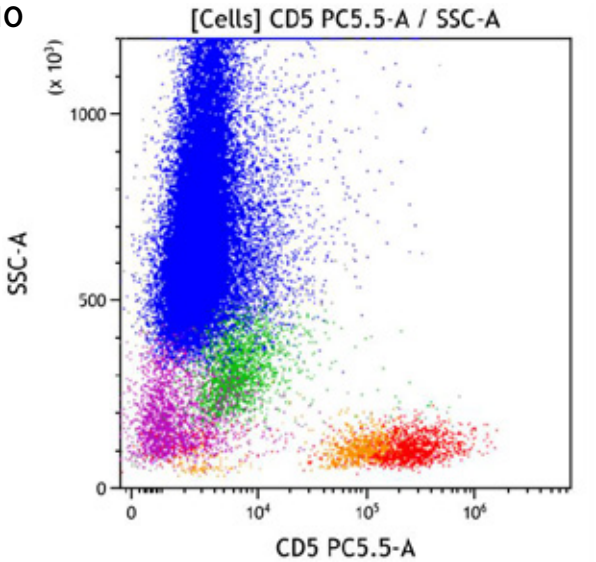

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in orange). These lymphoid cells typically have low side scatter. Of note, most of B cells (in orange) are uniformly positive for CD5.

B11

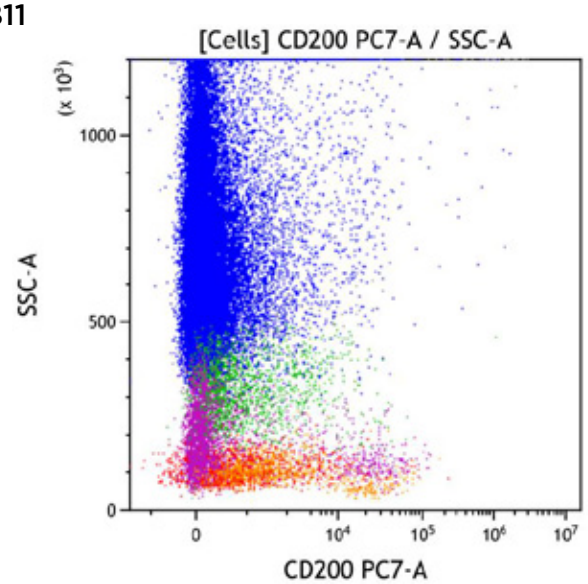

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

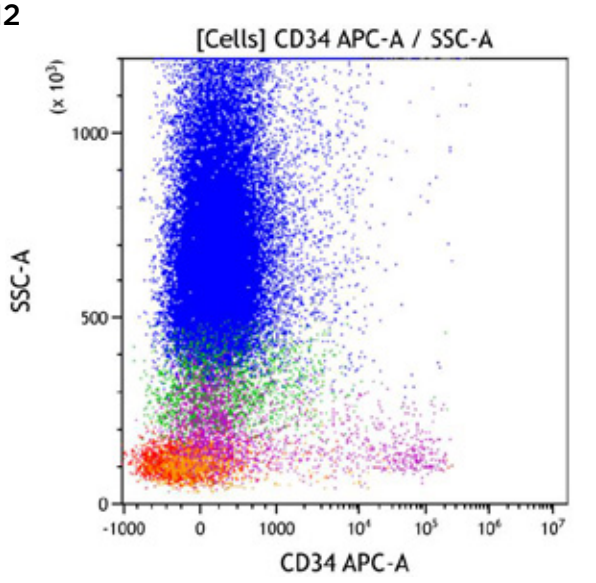

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts).CD34-positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

B13

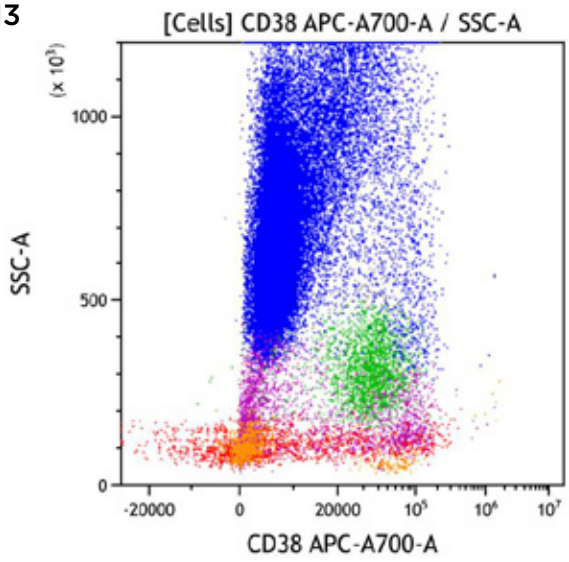

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

B14

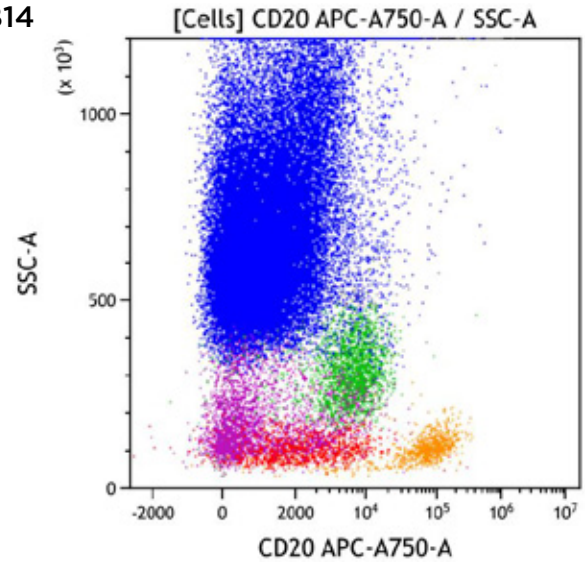

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (highlighted in orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate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

B15

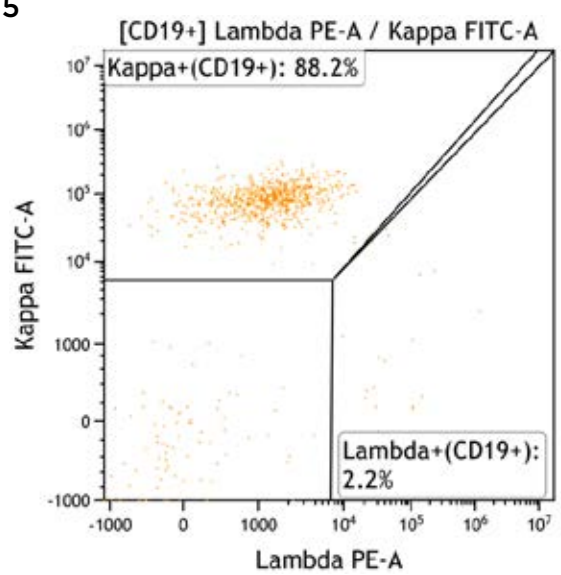

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lamb-da light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

B16

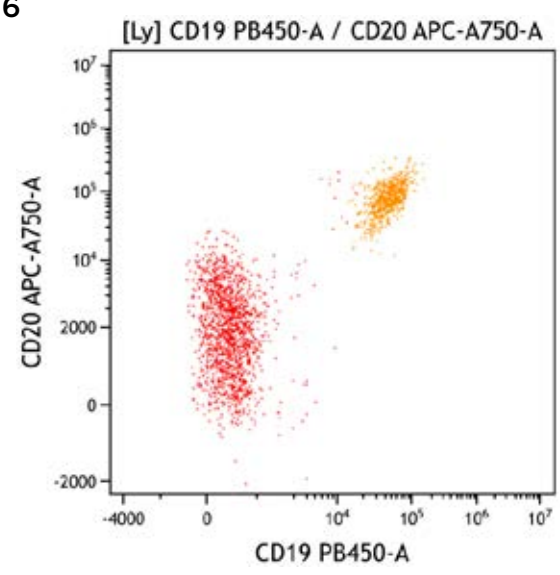

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells express both CD19 and CD20 (highlighted in orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression. Of note, B cells uniformly express CD19 and CD20, which is a normal pattern for mature B cells.

BACK TO CASE OVERVIEW

B17

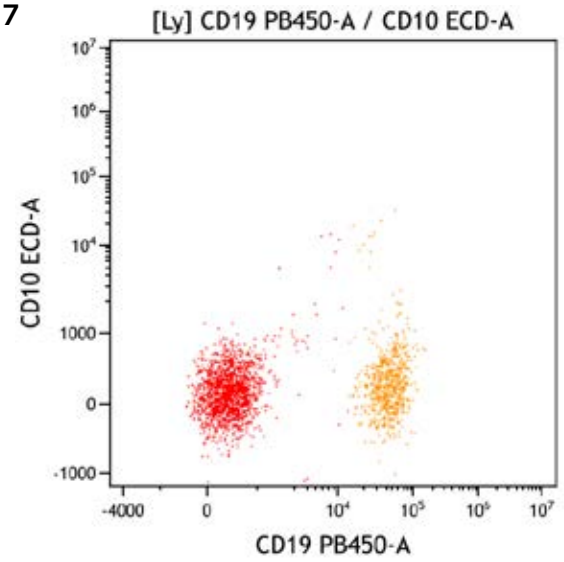

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate. B cells are CD19 positive (highlighted in orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow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

B18

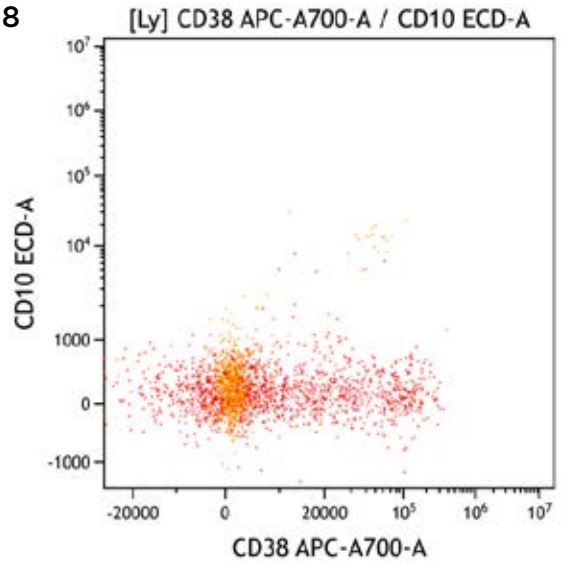

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

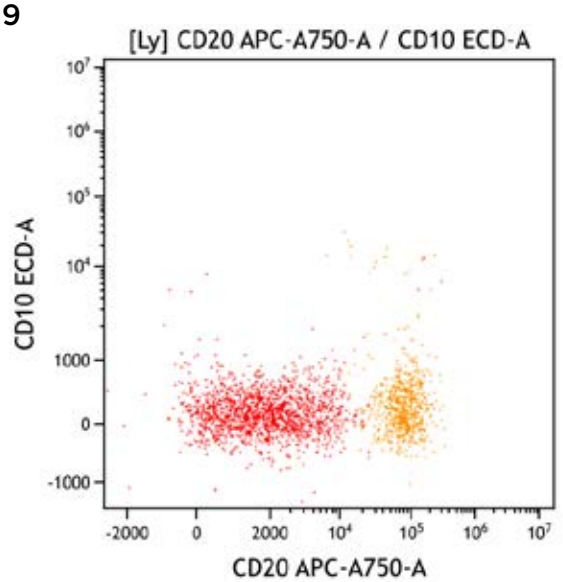

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late-stage immature B cells.

B20

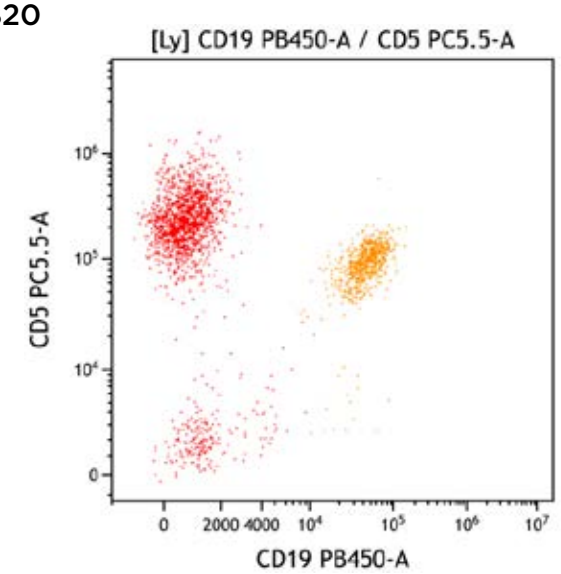

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is variably expressed at a low level on a subset of normal mature B cells and expressed on some subtypes of neoplastic B cells. Of note, most of B cells express CD5 at a level slightly dimmer than T cells.

BACK TO CASE OVERVIEW

B21

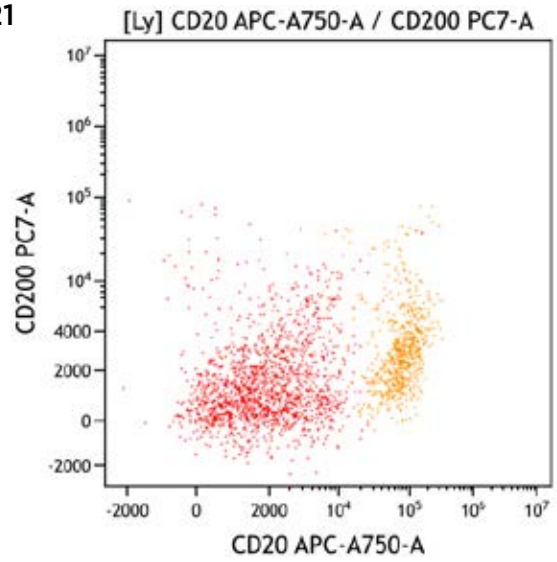

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in orange). Of note, most of B cells are CD200 negative.

B22

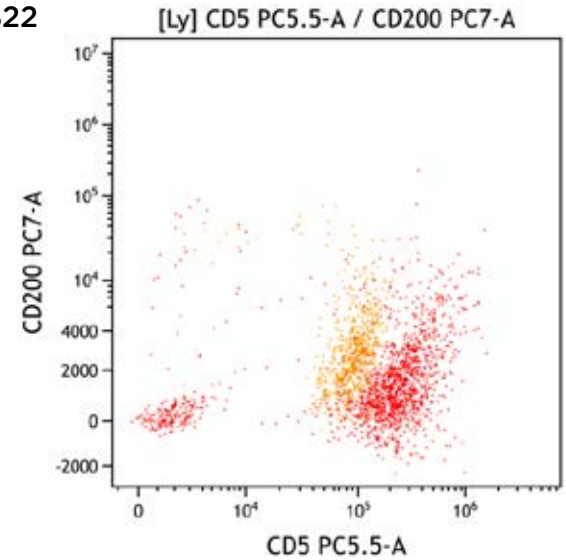

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. Of note, most of B cells are CD5-positive and CD200-negative.

BACK TO CASE OVERVIEW

T Cell Tube

T01

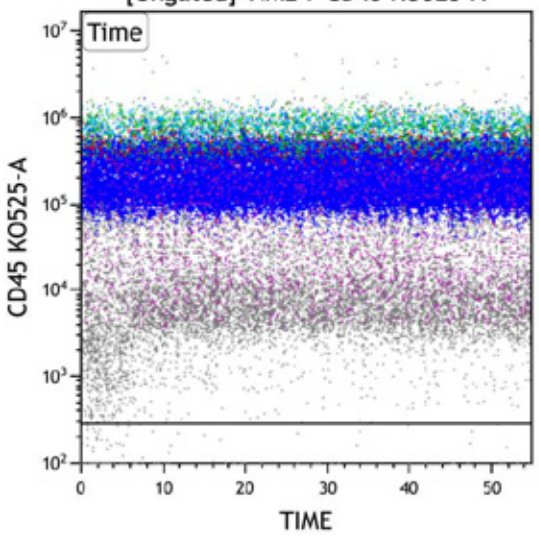

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

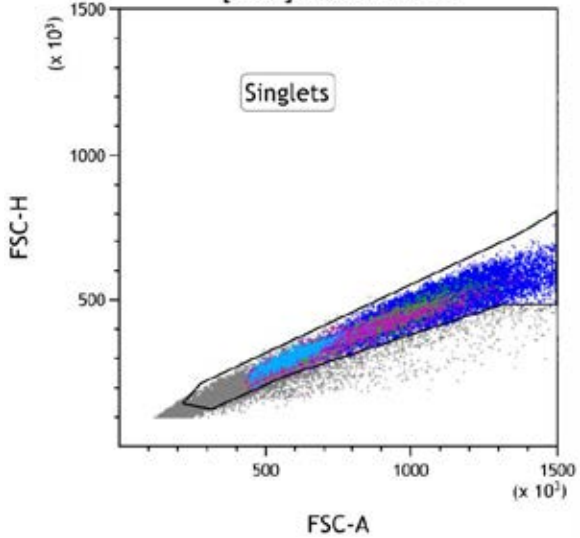

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

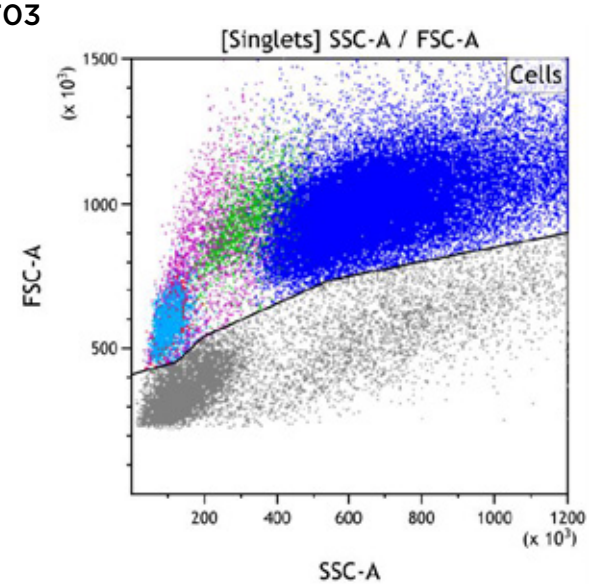

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

T04

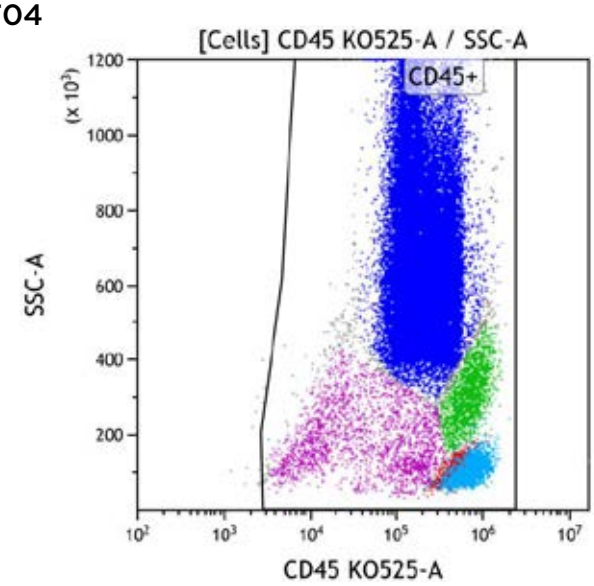

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

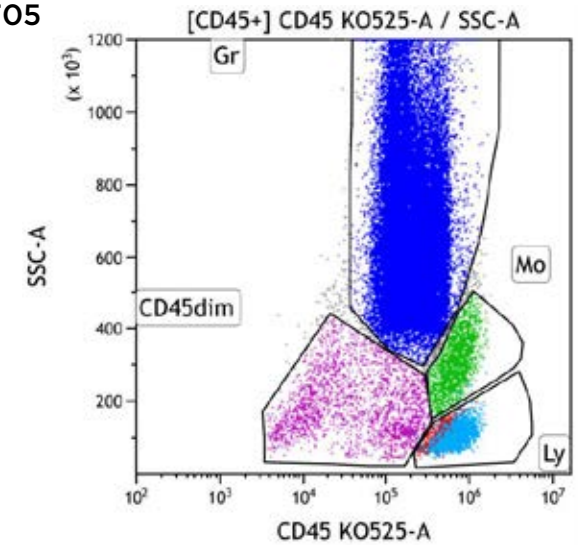

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

T06

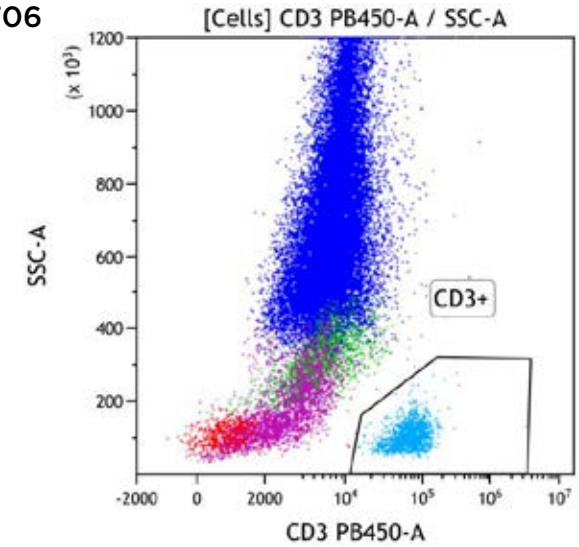

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

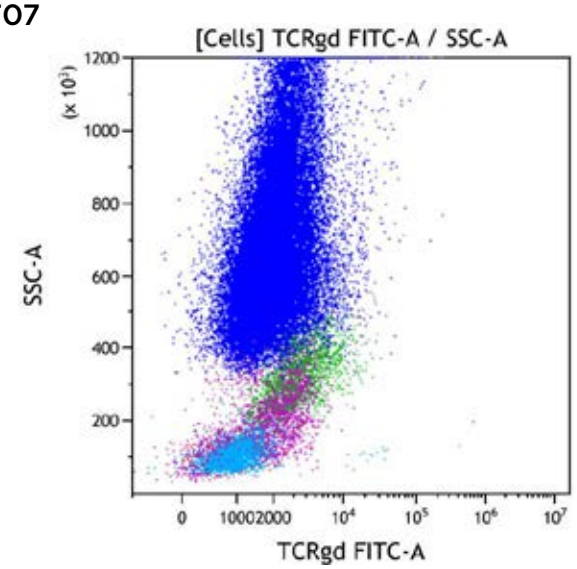

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

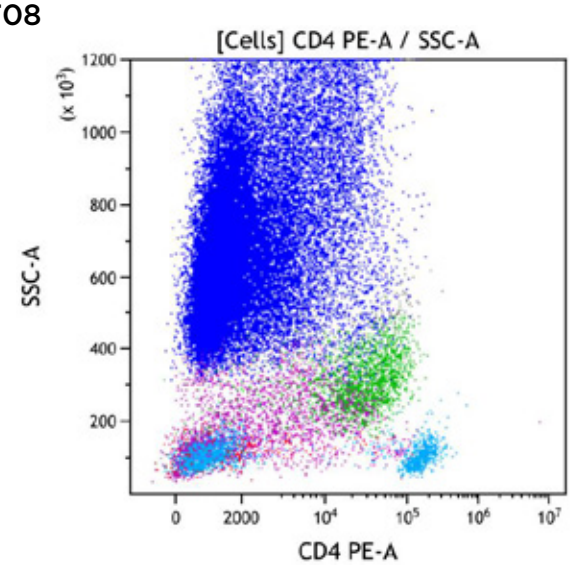

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

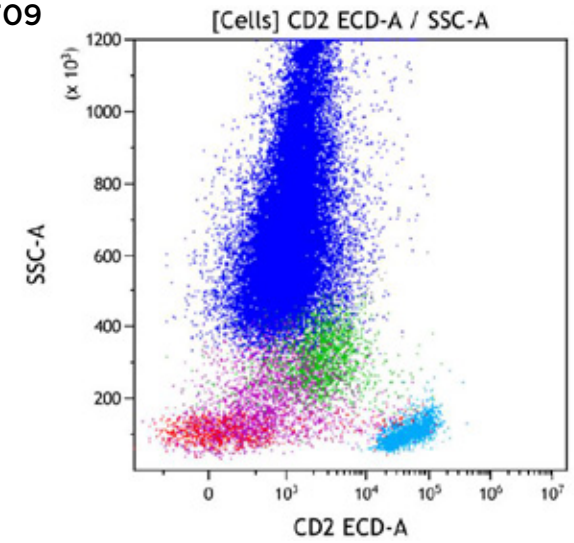

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

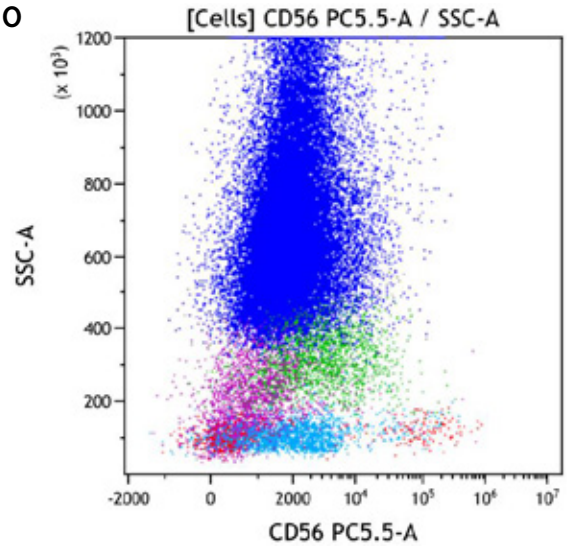

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and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

T11

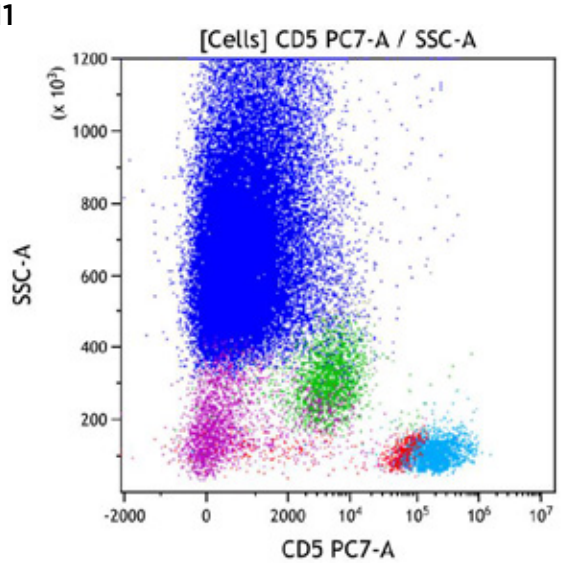

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Of note, most of CD3-negative mature lymphocytes (in red) express CD5.

T12

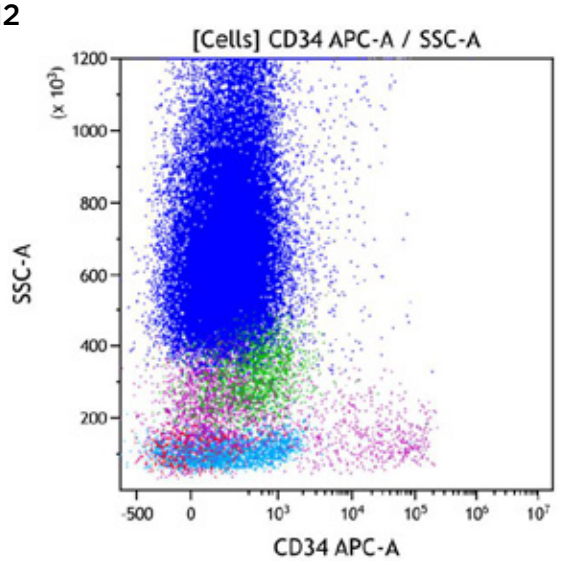

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

BACK TO CASE OVERVIEW

T13

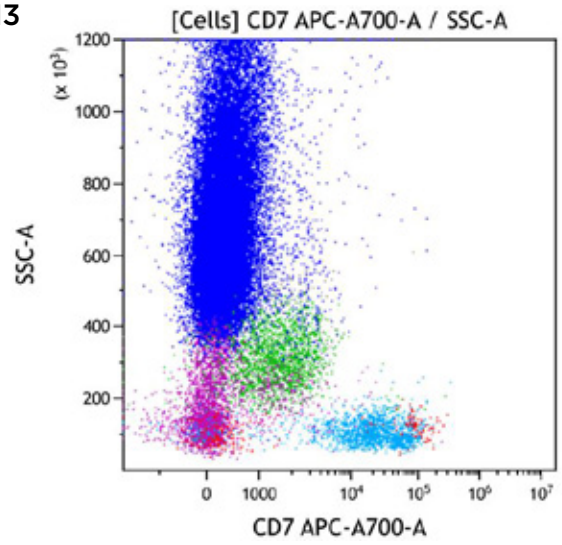

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

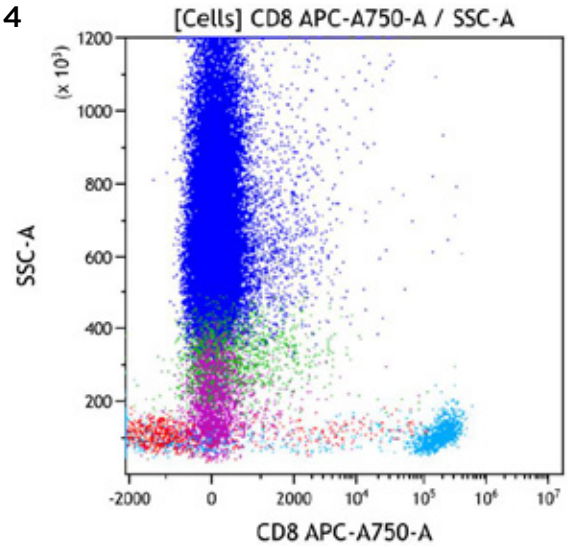

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

T15

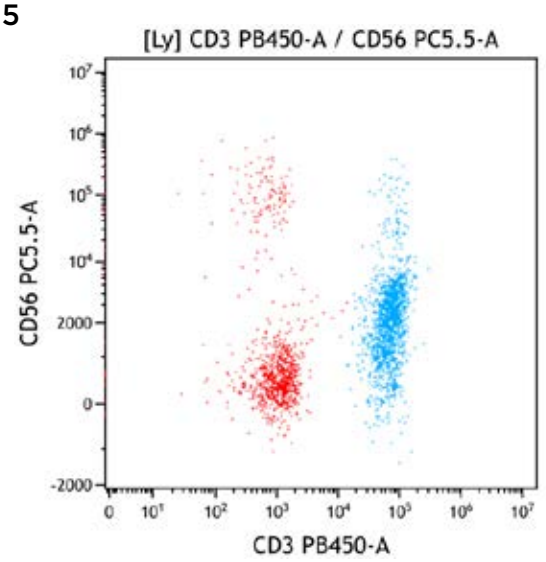

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small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

T16

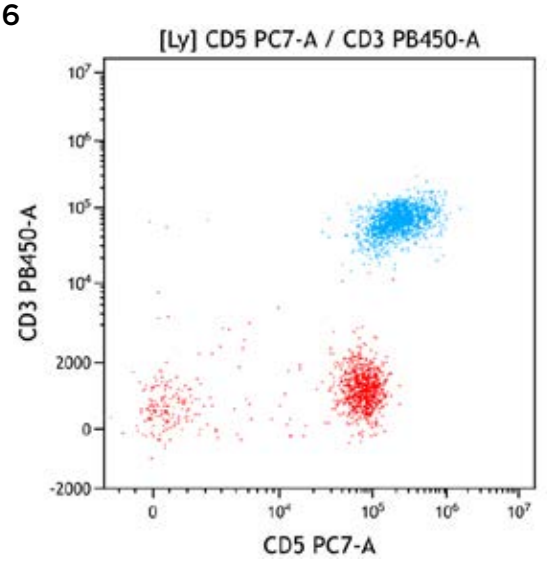

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most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

BACK TO CASE OVERVIEW

T17

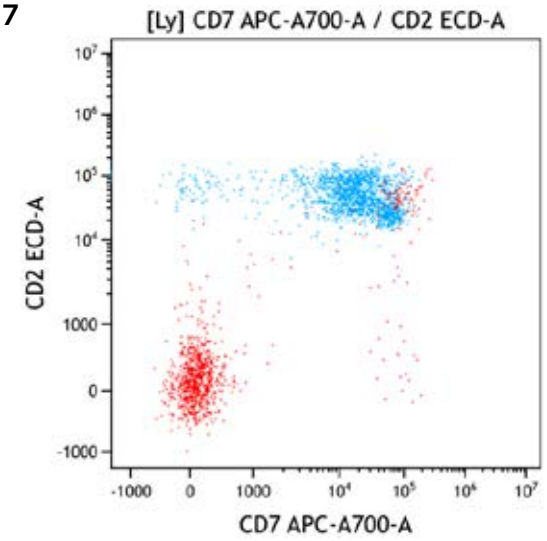

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

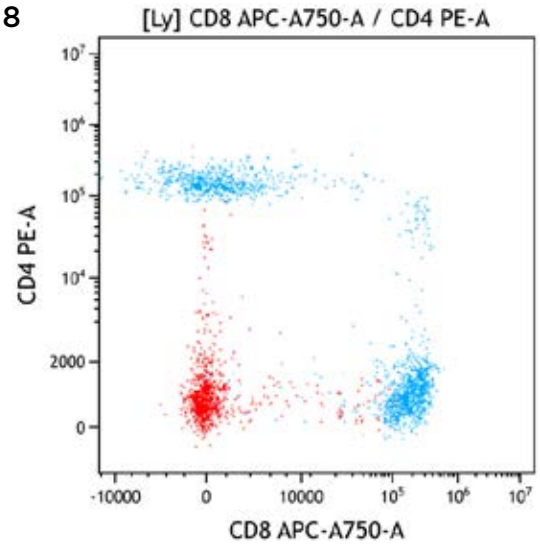

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 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.

T19

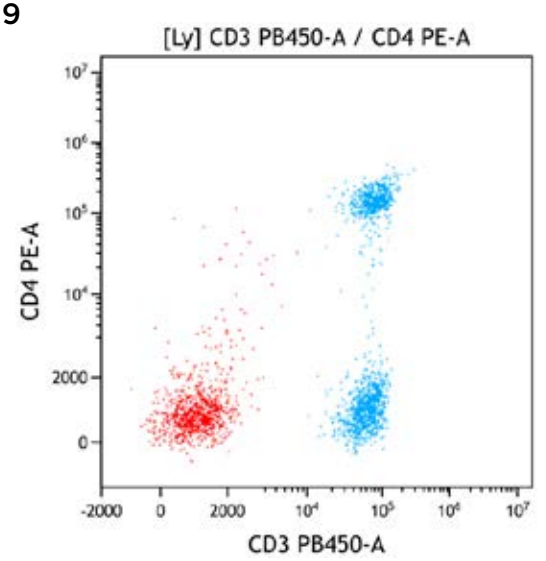

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

T20

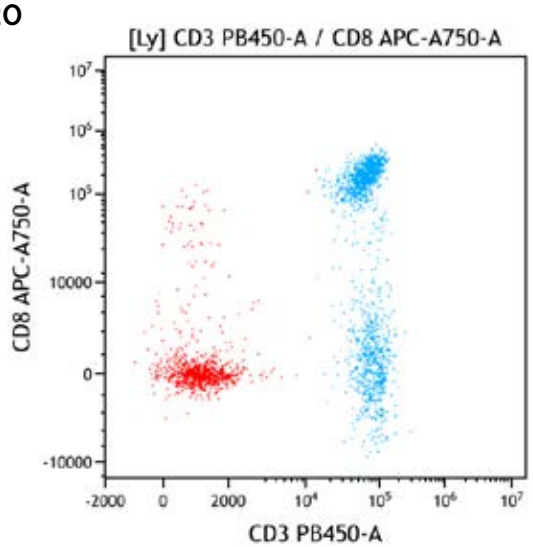

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

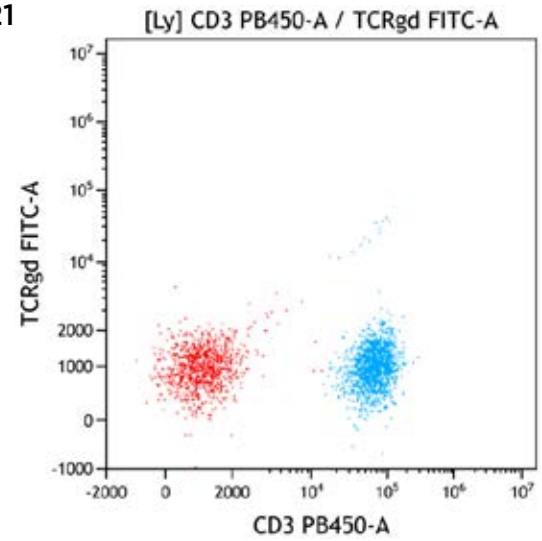

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

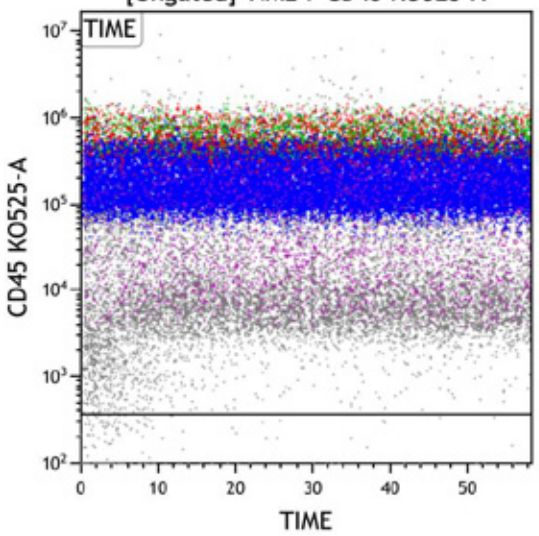

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

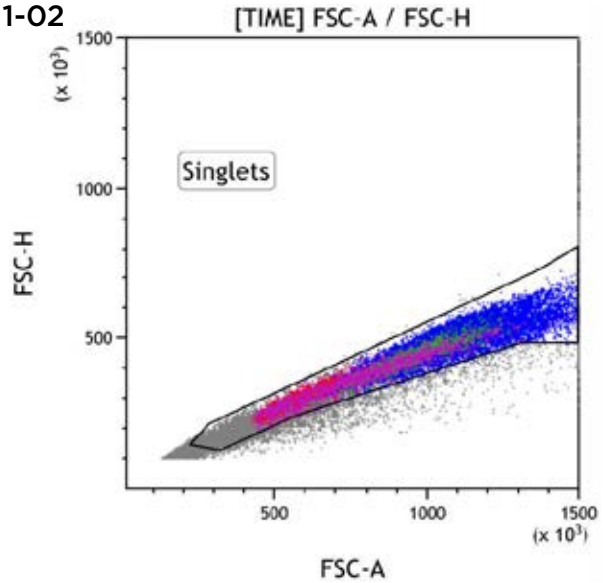

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

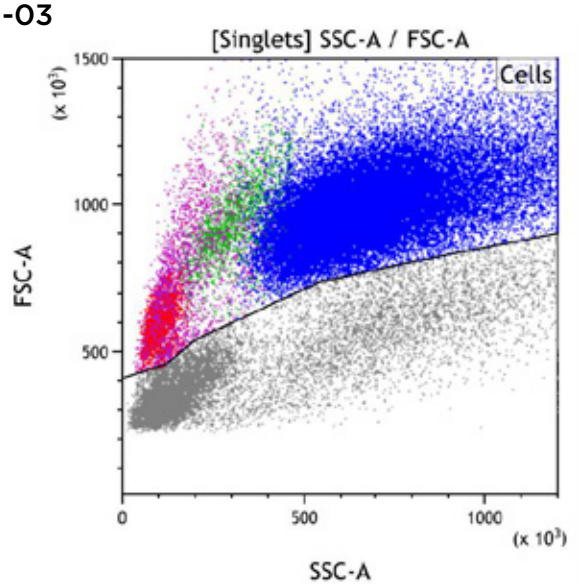

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M1-04

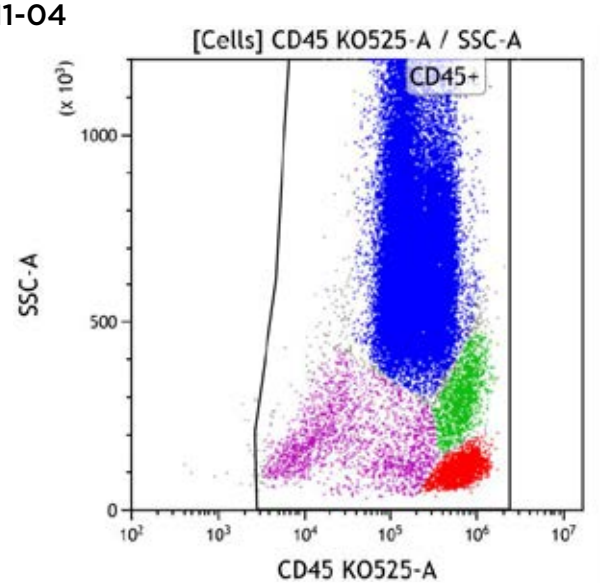

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

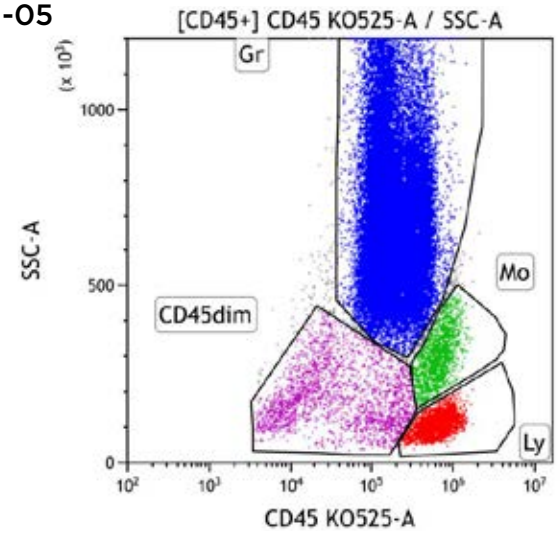

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M1-06

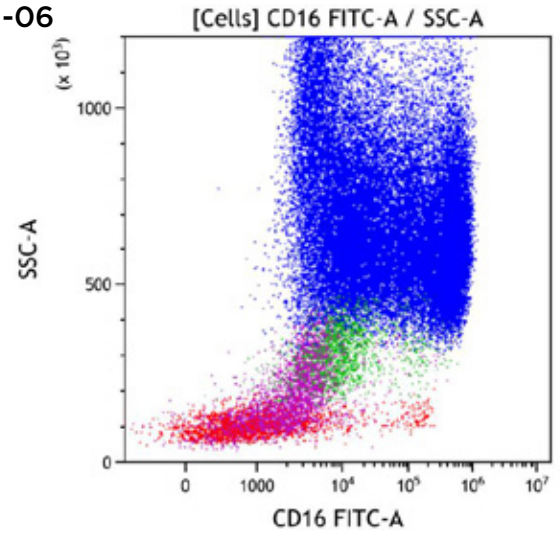

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

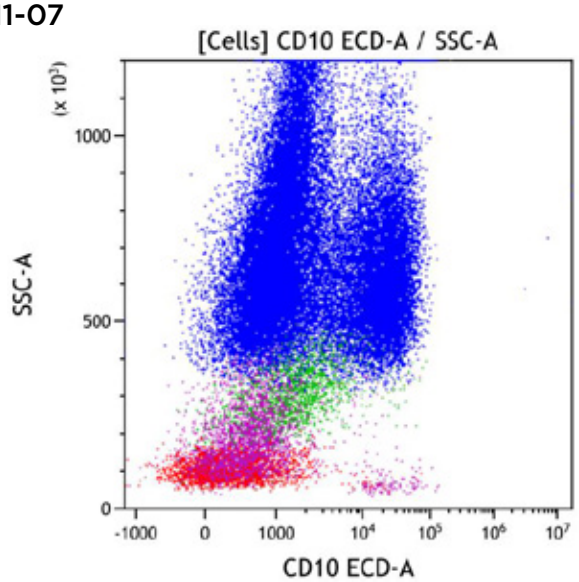

Figure 7: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

M1-08

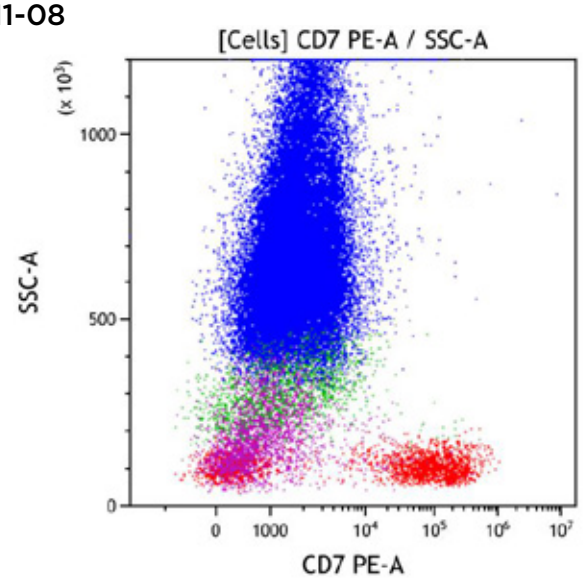

Figure 8: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage-committed progenitors (purple).

BACK TO CASE OVERVIEW

M1-09

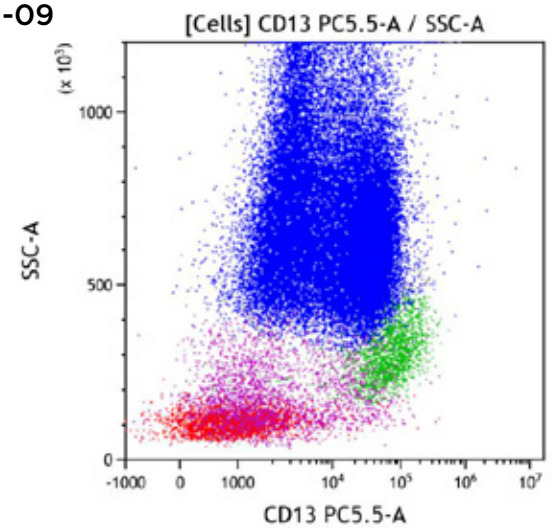

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M1-10

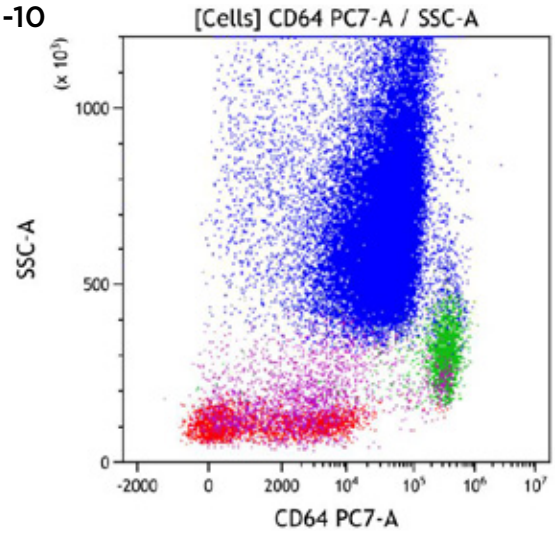

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

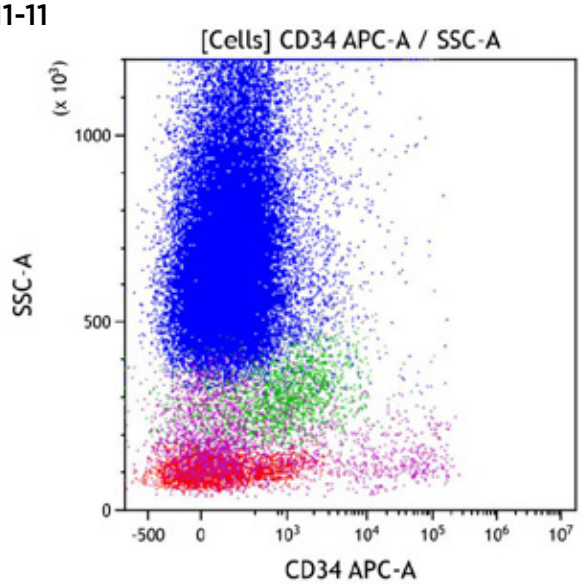

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

M1-12

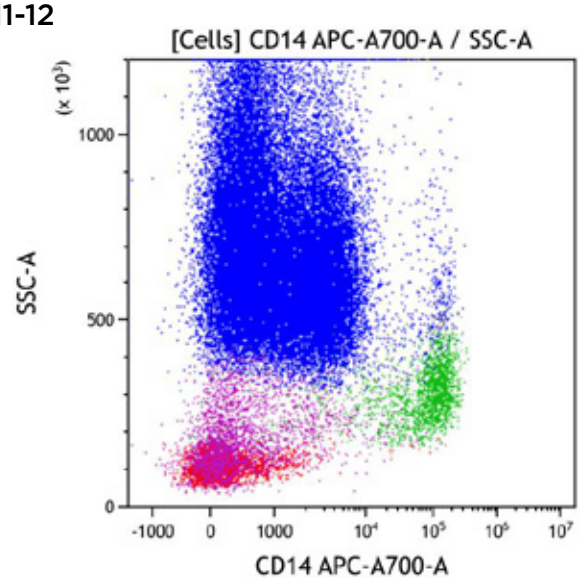

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

BACK TO CASE OVERVIEW

M1-13

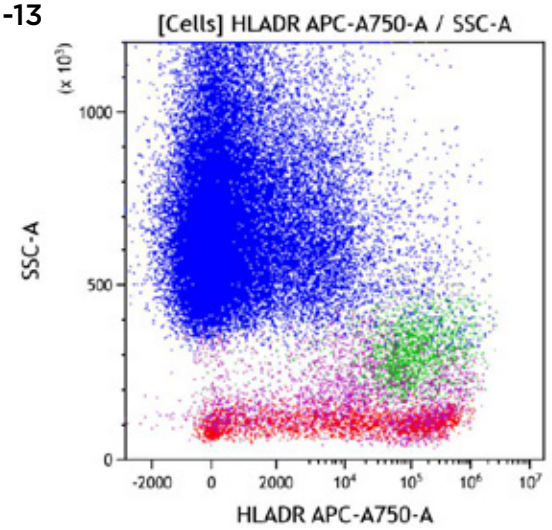

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

M1-14

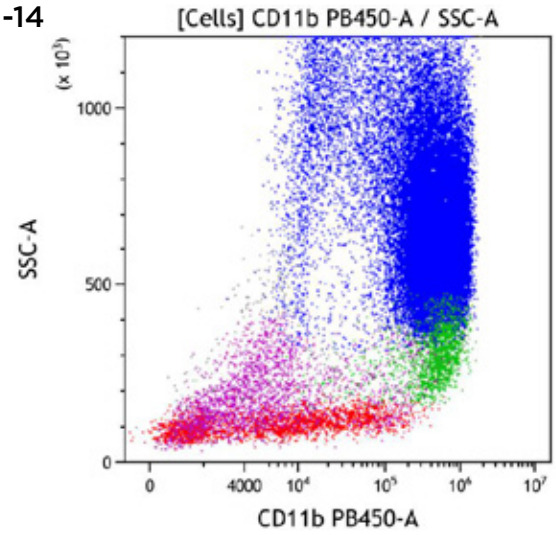

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

M1-15

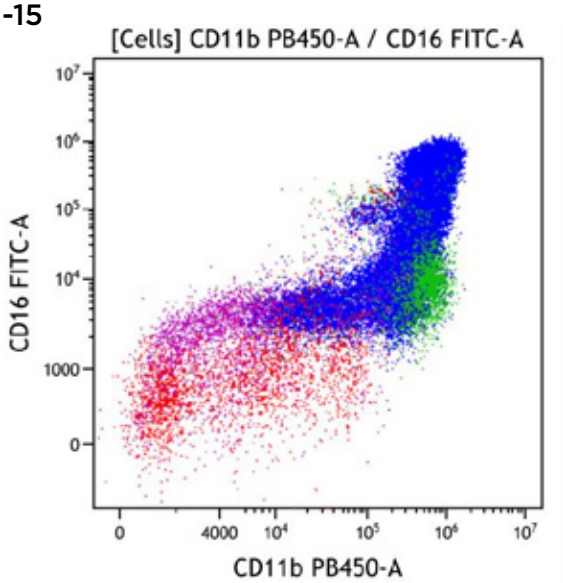

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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.

M1-16

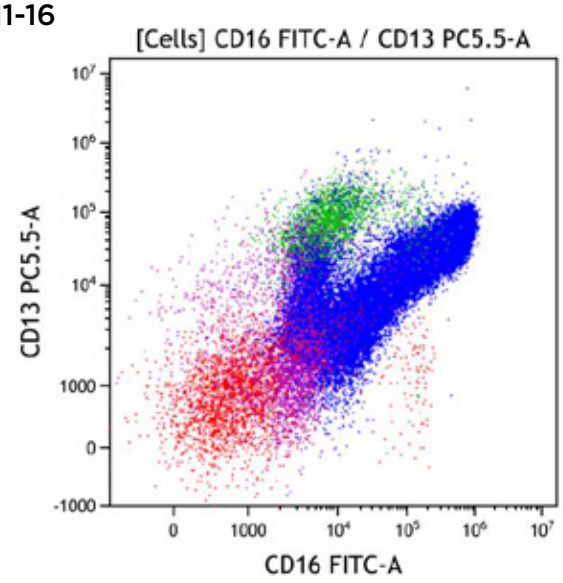

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed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

BACK TO CASE OVERVIEW

M1-17

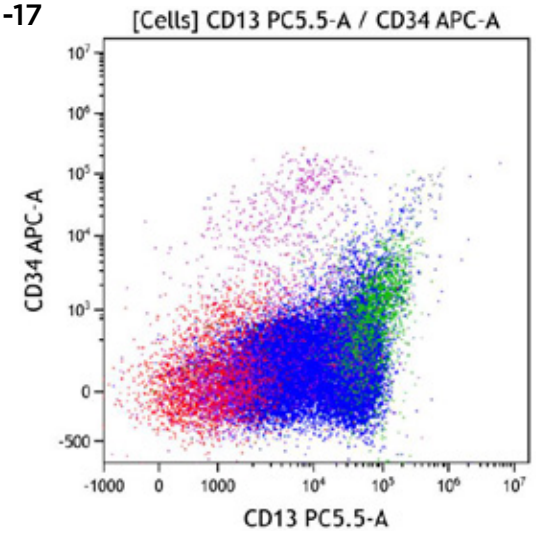

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple)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

M1-18

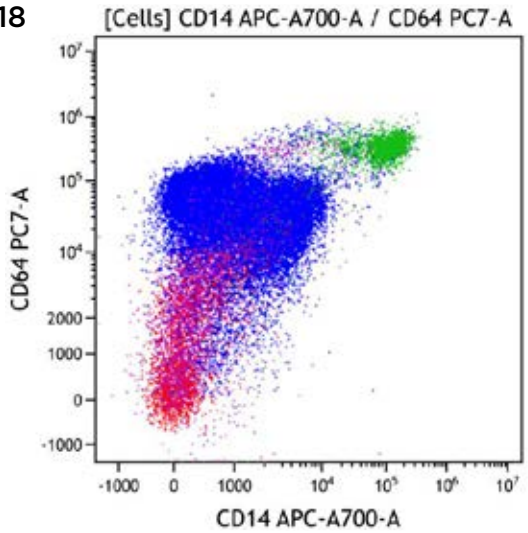

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high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

M1-19

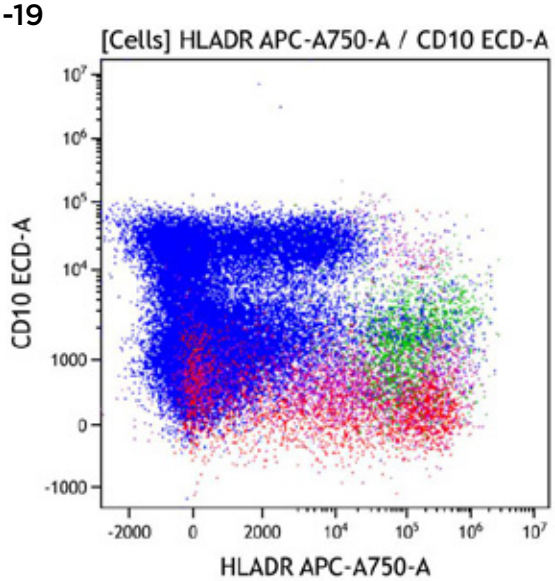

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

M1-20

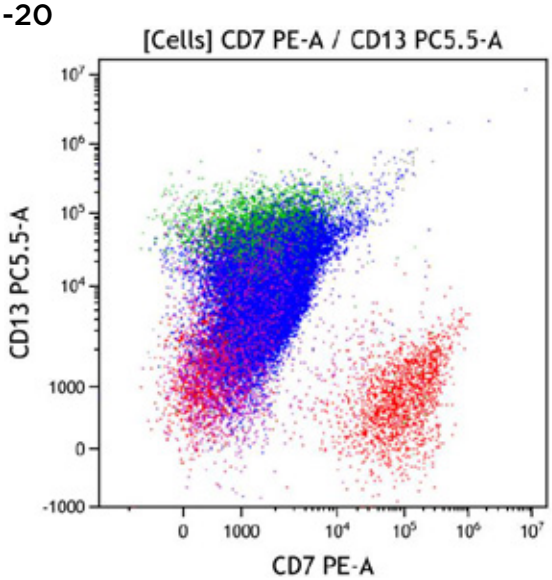

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

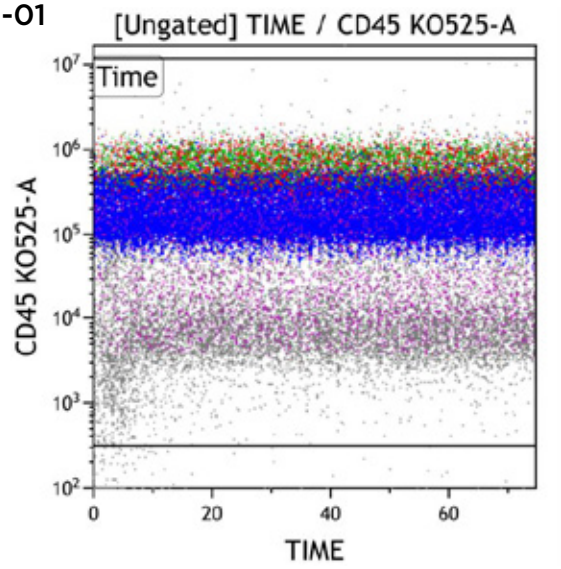

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

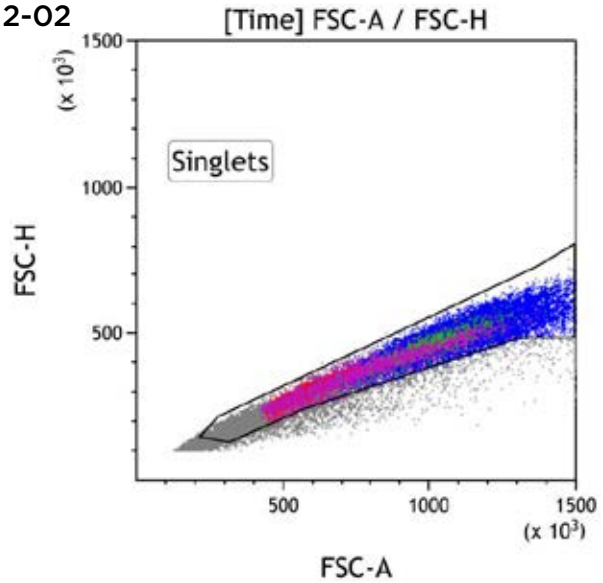

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

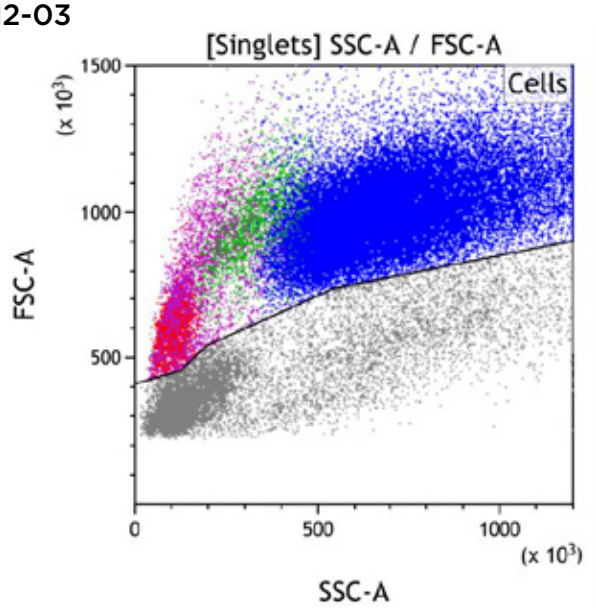

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M2-04

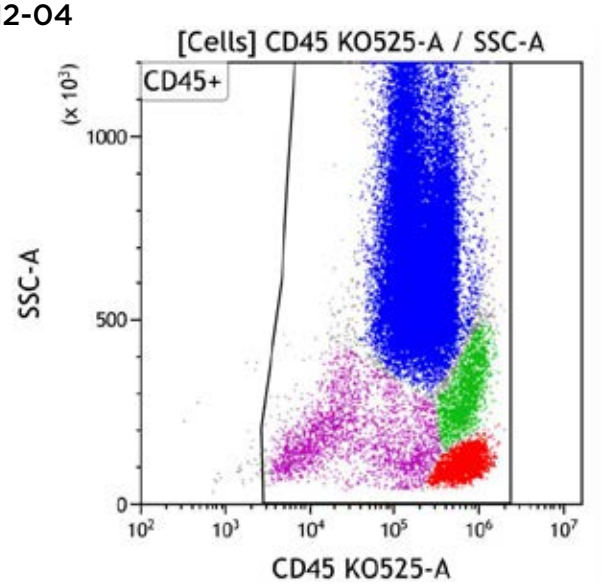

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M2-05

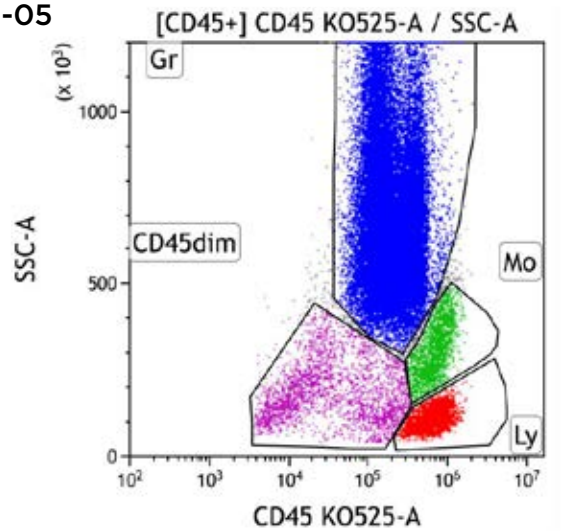

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (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.

M2-06

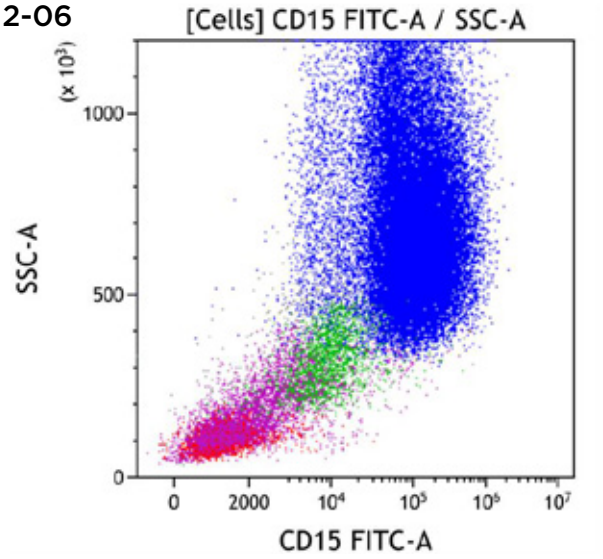

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

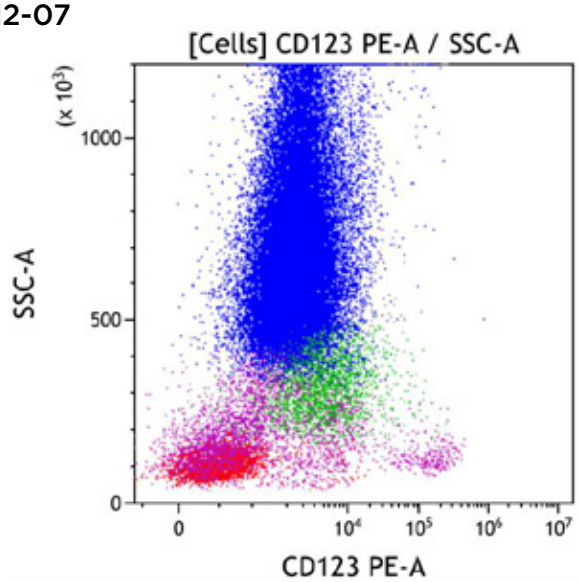

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green).

M2-08

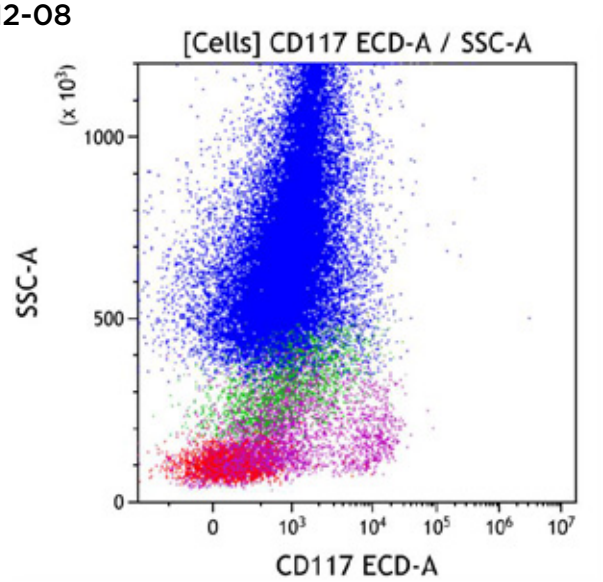

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

BACK TO CASE OVERVIEW

M2-09

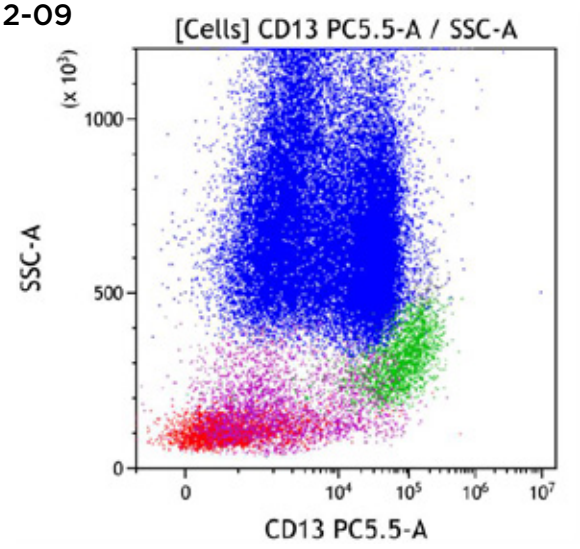

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M2-10

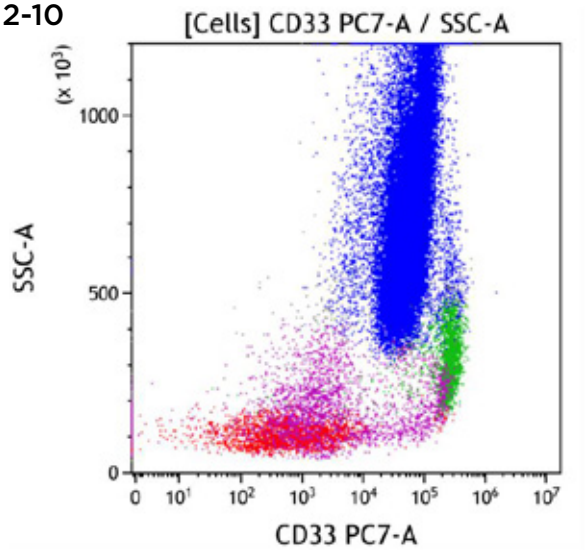

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a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

M2-11

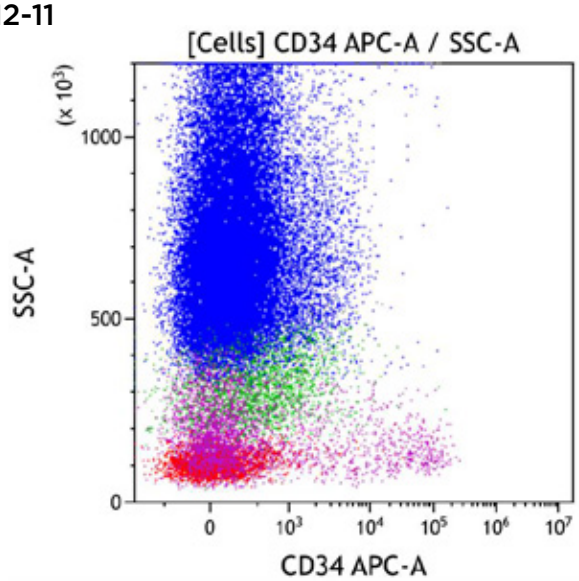

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

M2-12

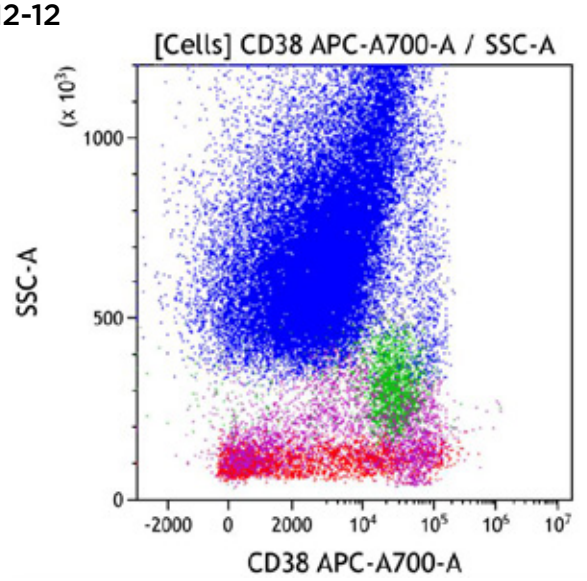

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

BACK TO CASE OVERVIEW

M2-13

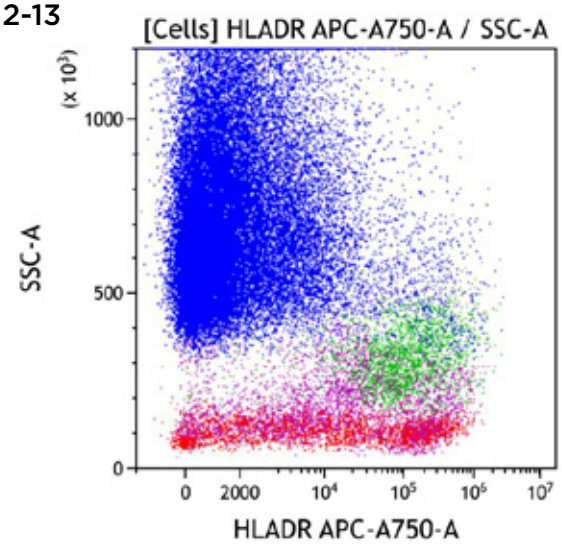

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T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

M2-14

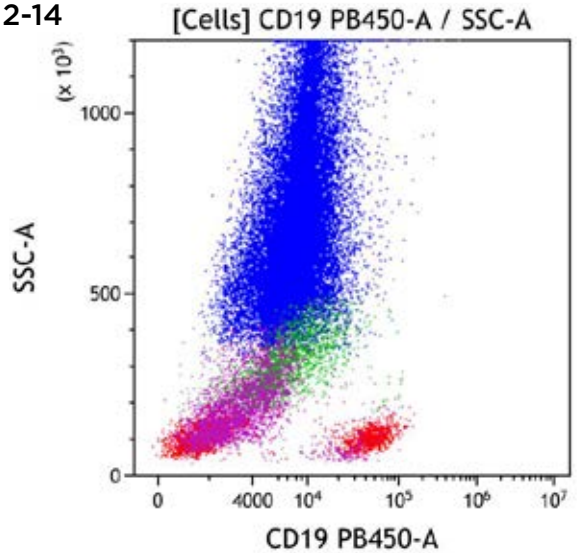

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

M2-15

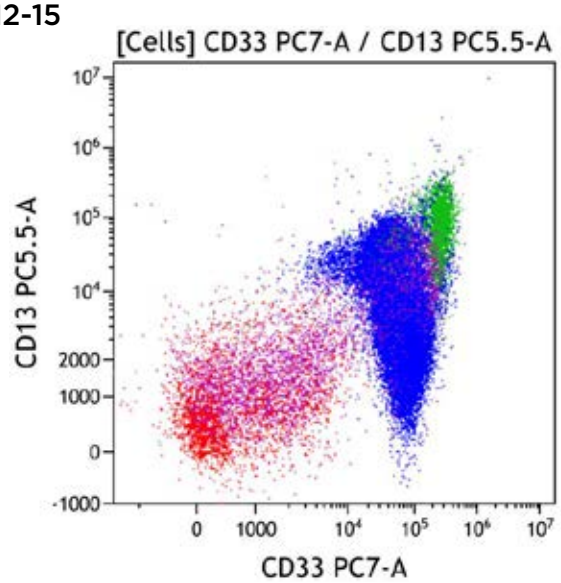

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher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

M2-16

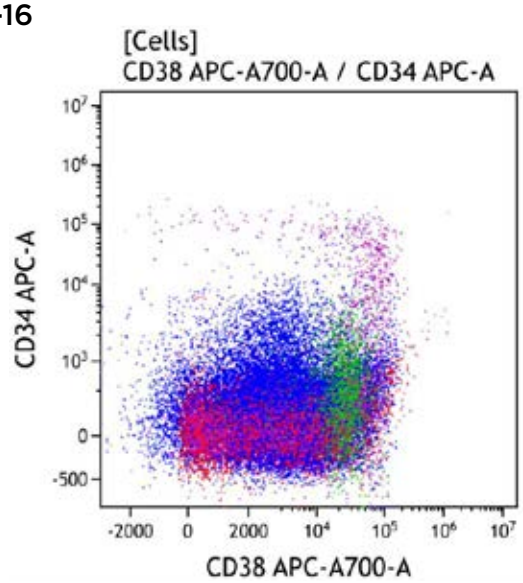

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple,not many in this sample). The apparent variable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

BACK TO CASE OVERVIEW

M2-17

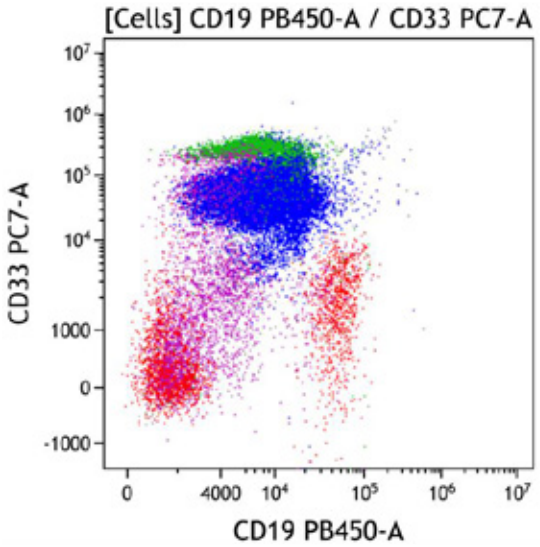

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to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

M2-18

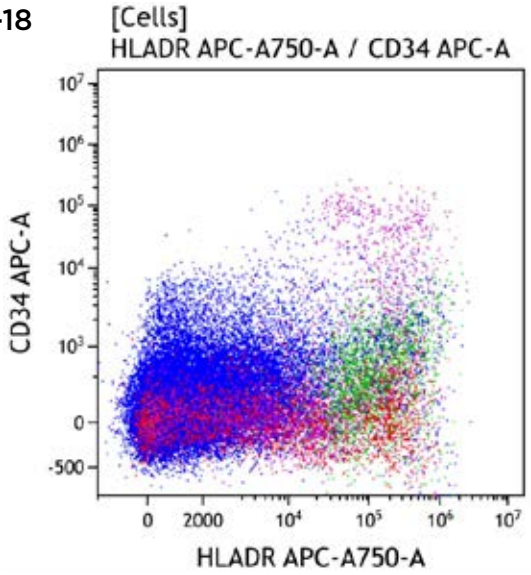

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of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

M2-19

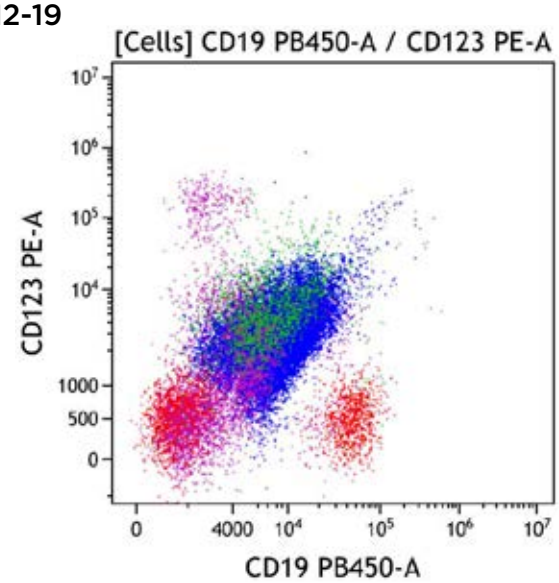

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid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

M2-20

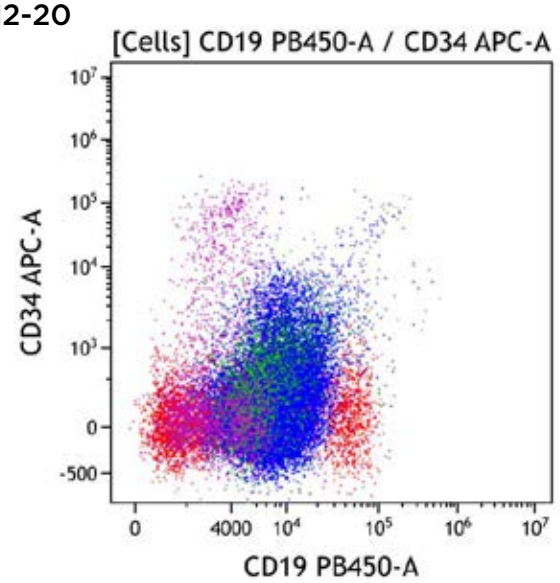

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19.

BACK TO CASE OVERVIEW

M2-21

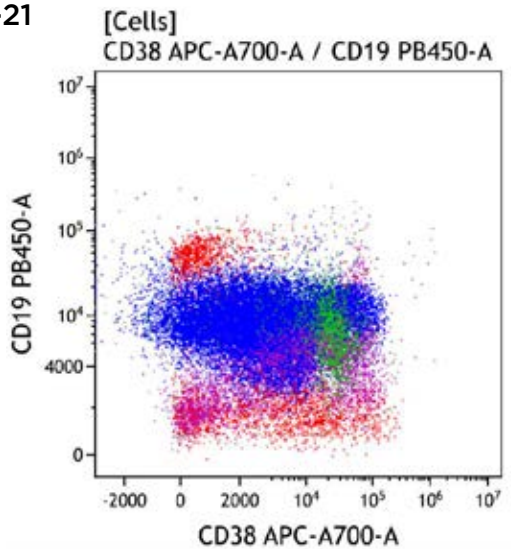

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression 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.

M2-22

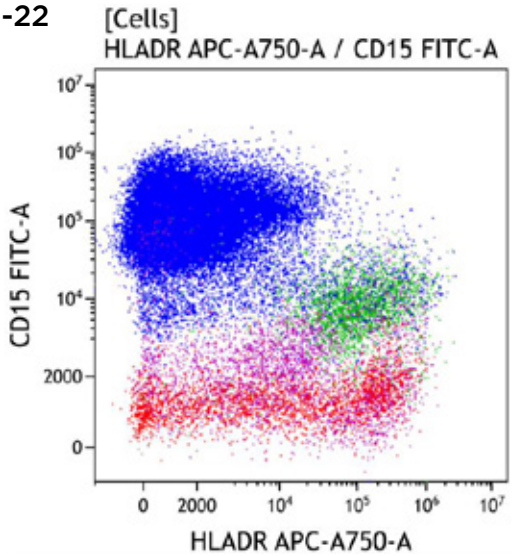

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLADR (blue),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

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 12 UMF-TC4-115**
Diagnosis	**Acute Myeloid Leukemia**
Clinical Vignette	80-year-old female developed anemia and thrombocytopenia. Bone marrow biopsy was performed for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	The flow cytometry study detected an expanded population of CD34-positive blasts, which is partially positive for CD13, CD33, CD117 and CD123 without lymphoid marker CD7, CD10 or CD19 or mature myeloid marker CD14 or CD16. The population is consistent with myeloid blasts with minimal differentiation. The finding supports acute myeloid leukemia.

BACK TO CASE LISTINGS

B Cell Tube

B01

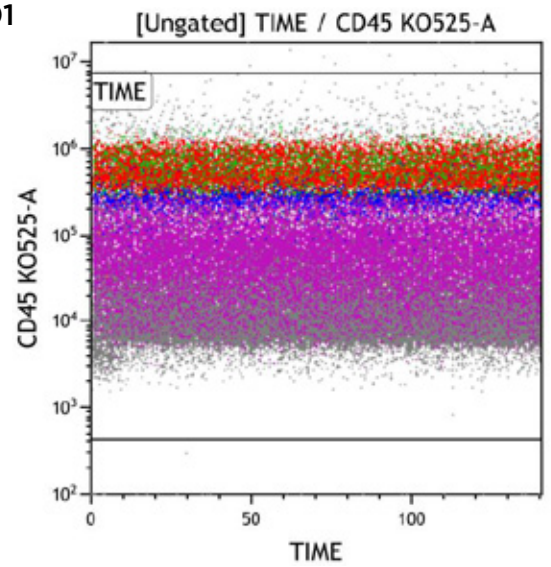

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

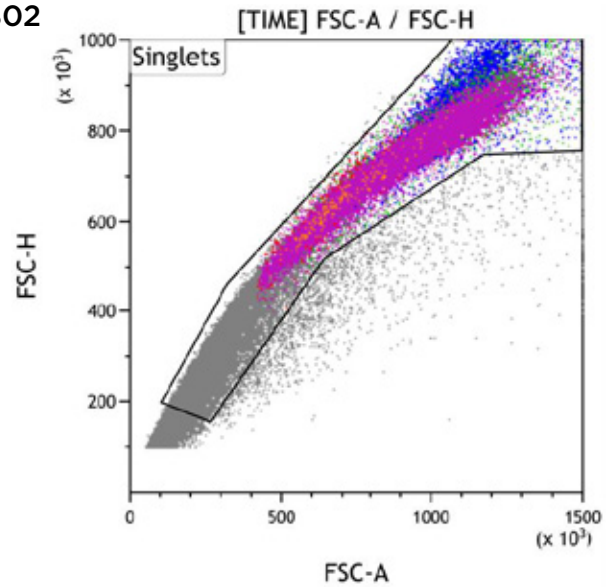

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

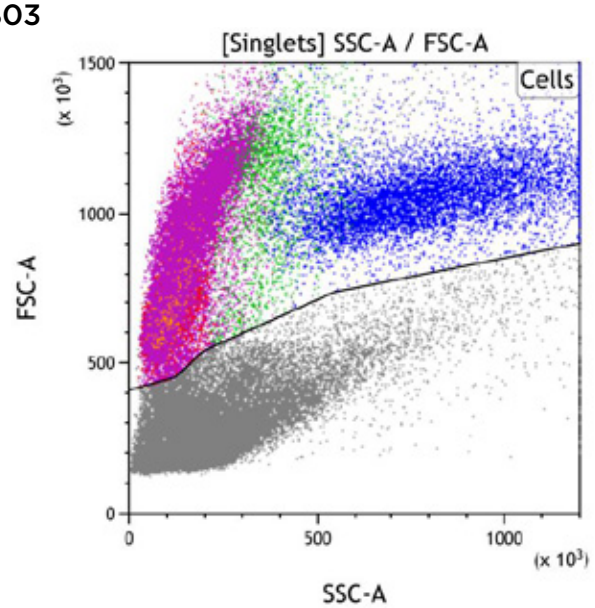

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

B04

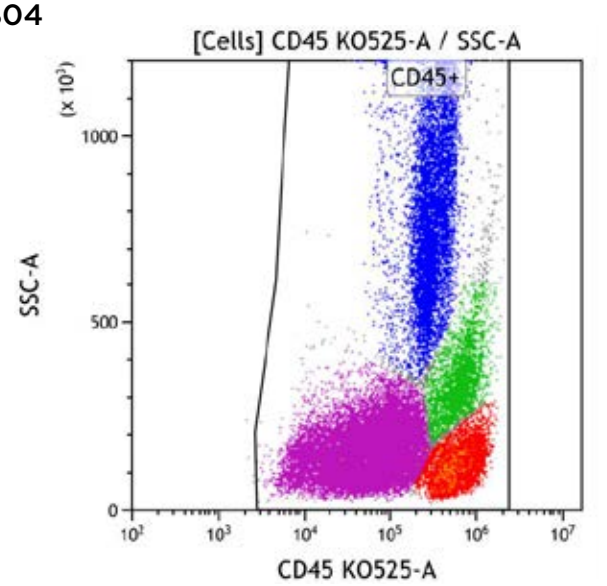

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

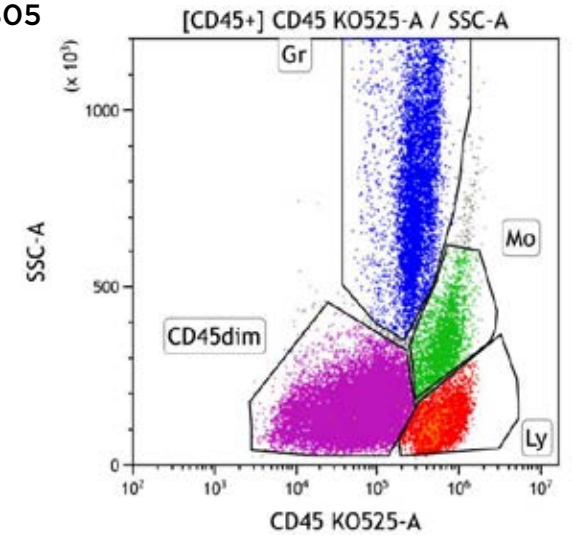

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

B06

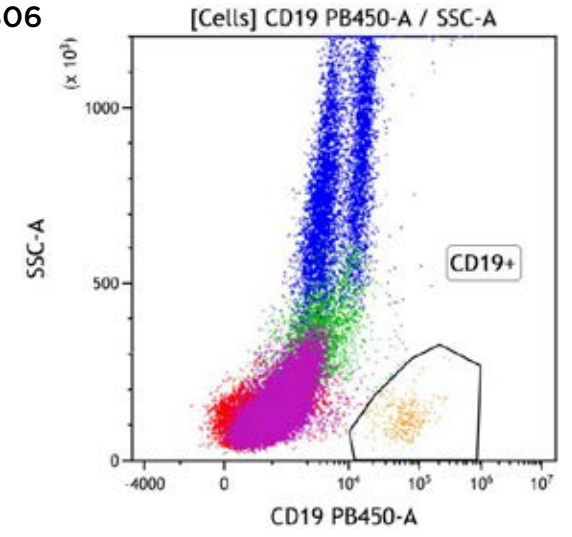

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B07

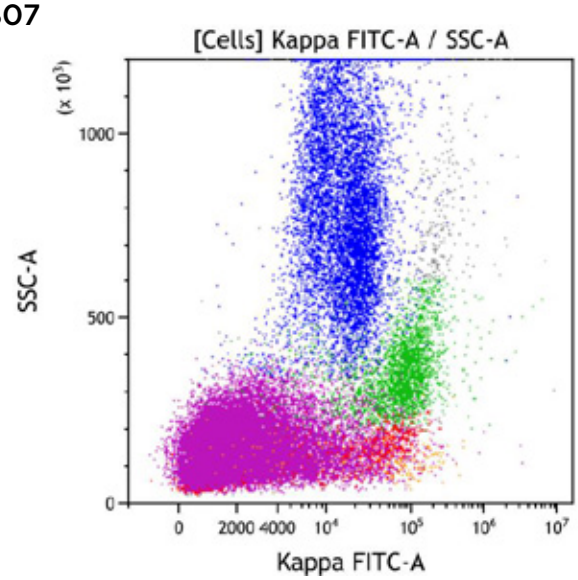

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent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

B08

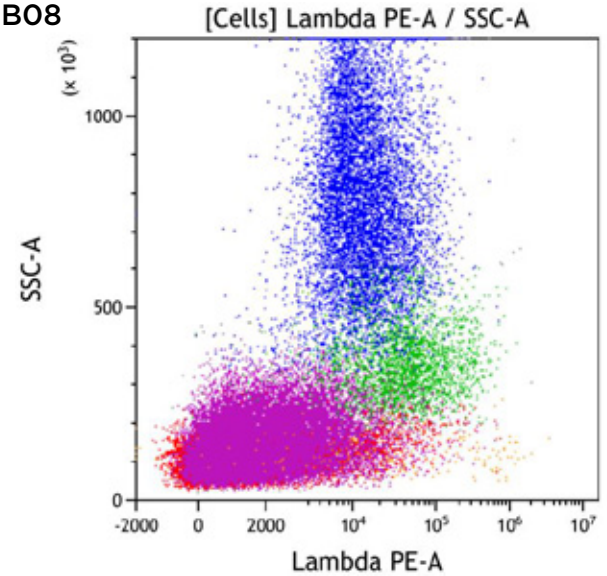

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent 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.

BACK TO CASE OVERVIEW

B09

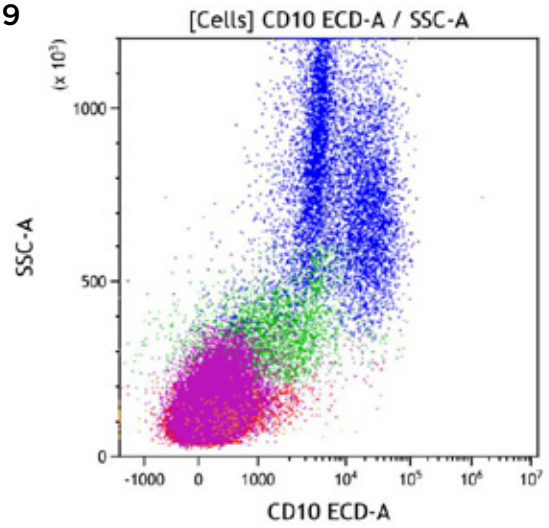

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Of note, the expanded population in the CD45dim gate is CD10-negative.

B10

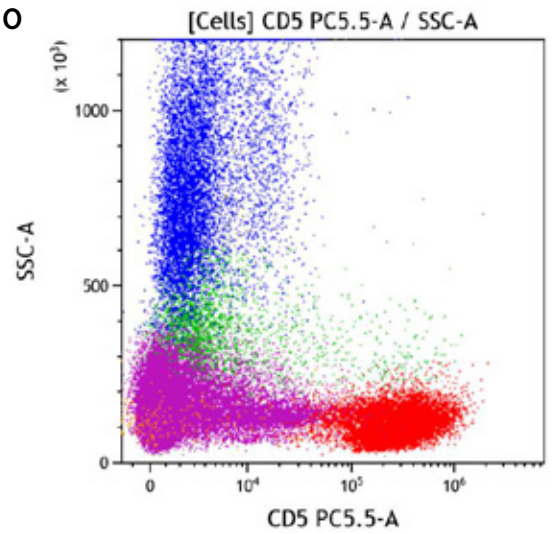

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in orange). These lymphoid cells typically have low side scatter.

B11

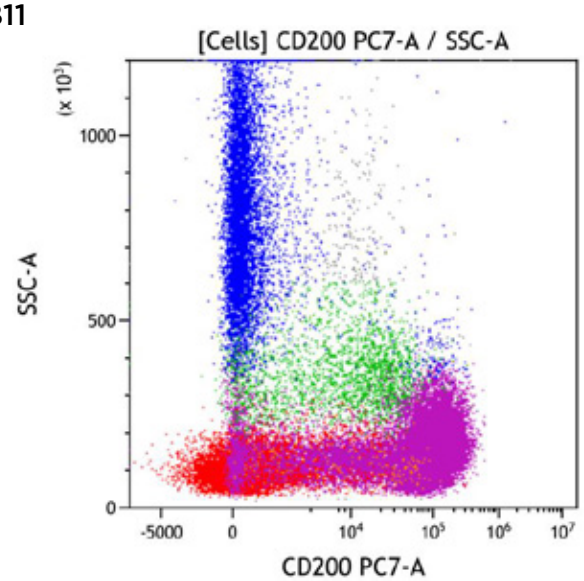

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

B12

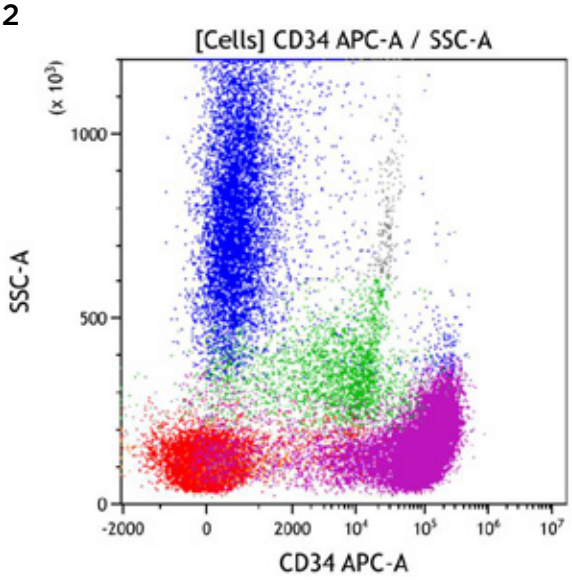

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Of note, the expanded population in the CD45dim gate is CD34-positive.

BACK TO CASE OVERVIEW

B13

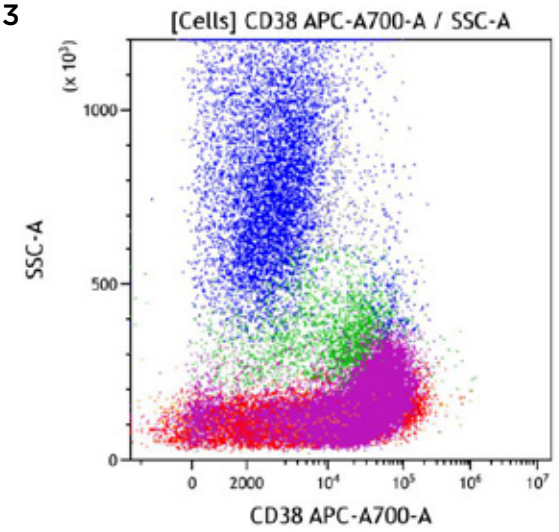

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

B14

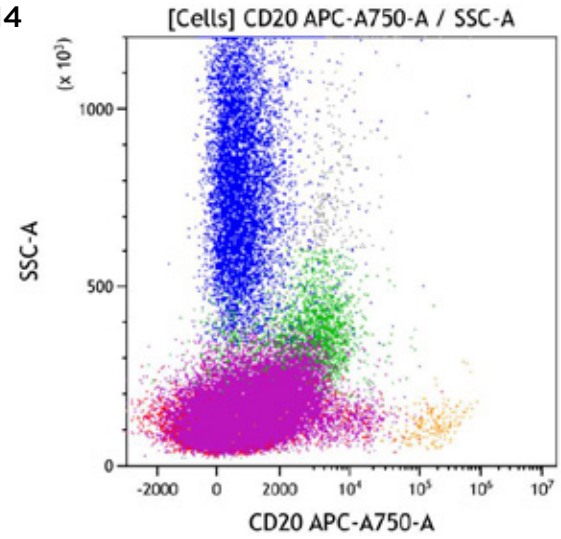

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (highlighted in orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate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

B15

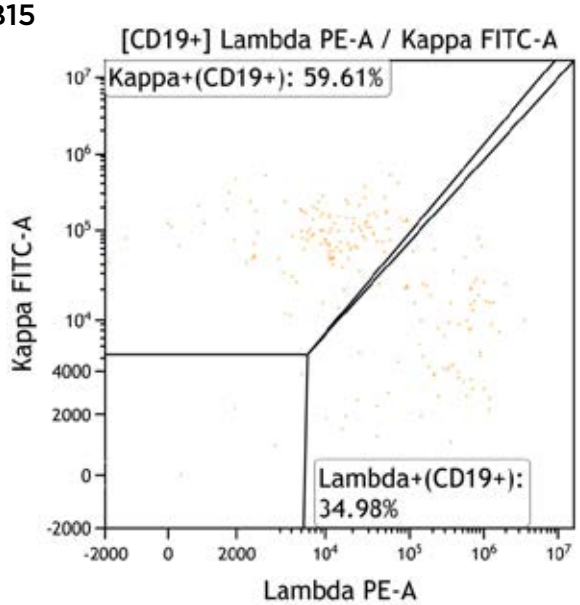

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

B16

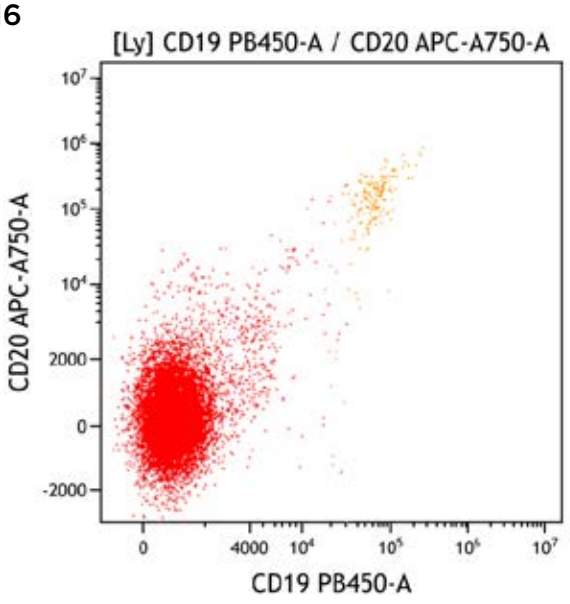

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells express both CD19 and CD20 (highlighted in orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

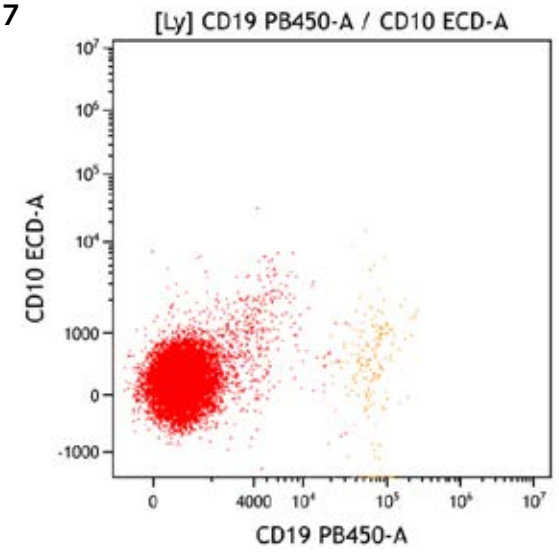

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate. B cells are CD19 positive (highlighted in orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow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

B18

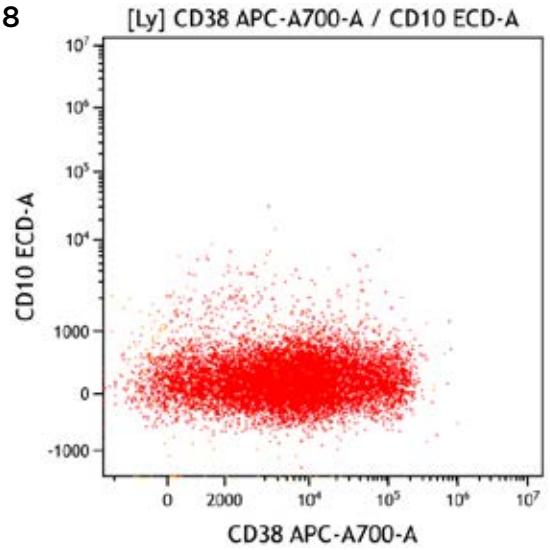

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

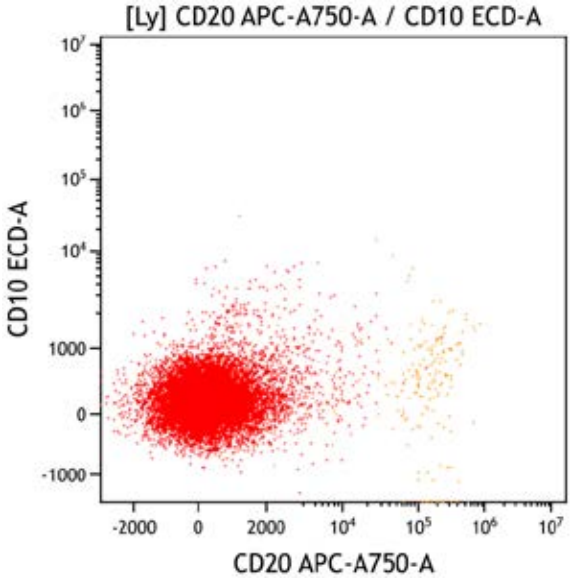

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late-stage immature B cells.

B20

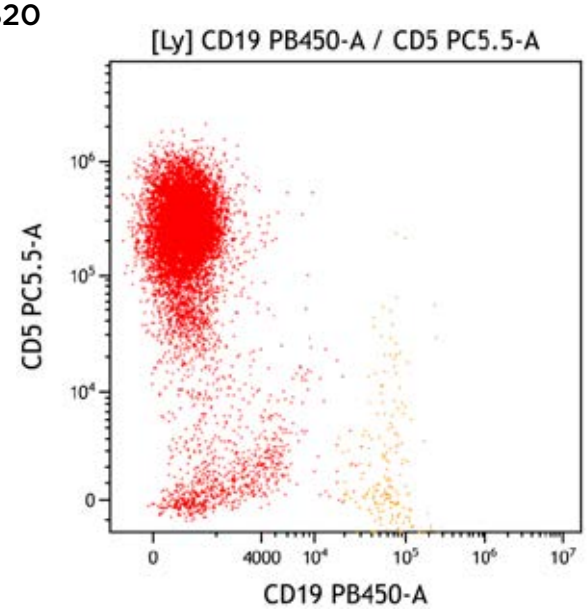

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

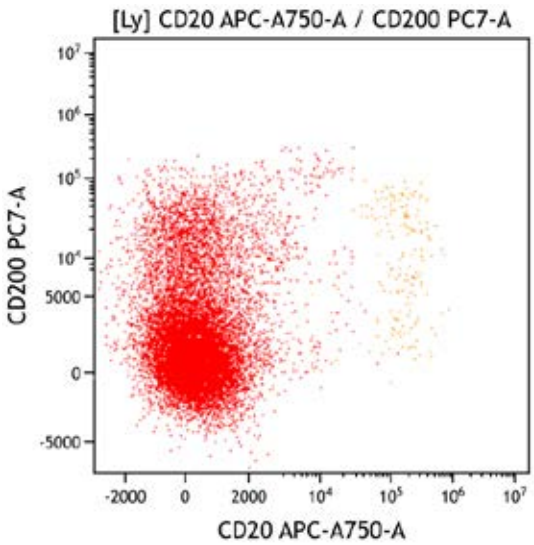

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in orange).

B22

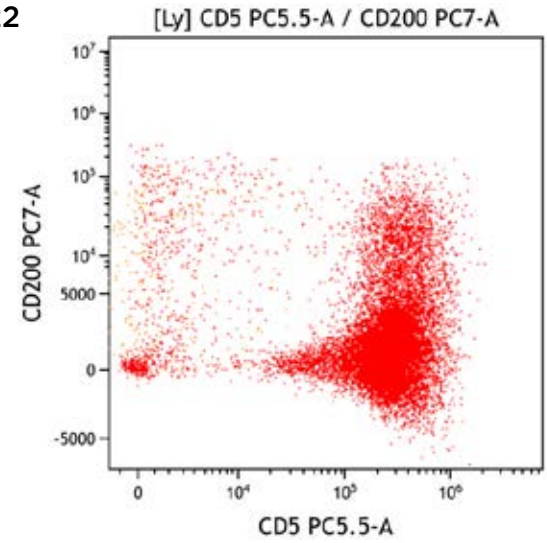

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically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

BACK TO CASE OVERVIEW

T Cell Tube

T01

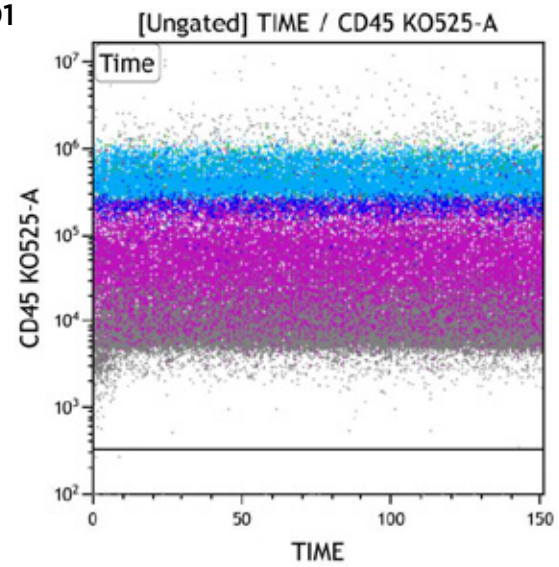

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

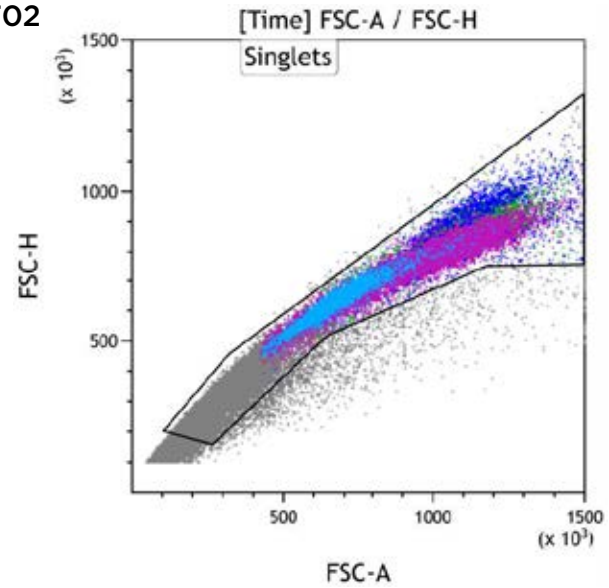

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

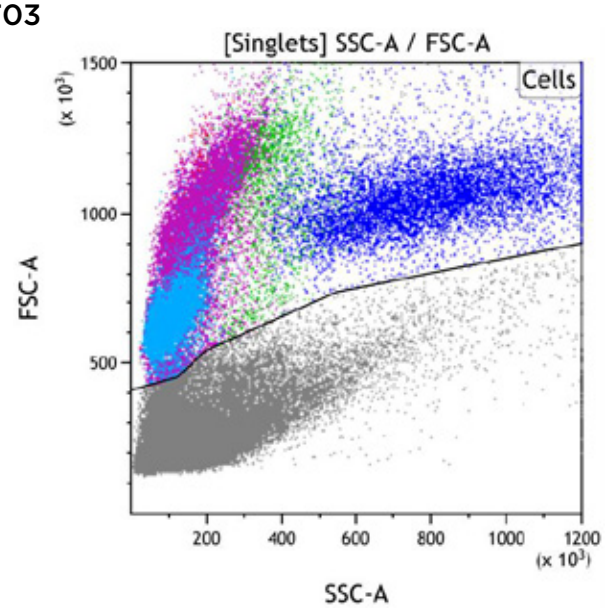

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

T04

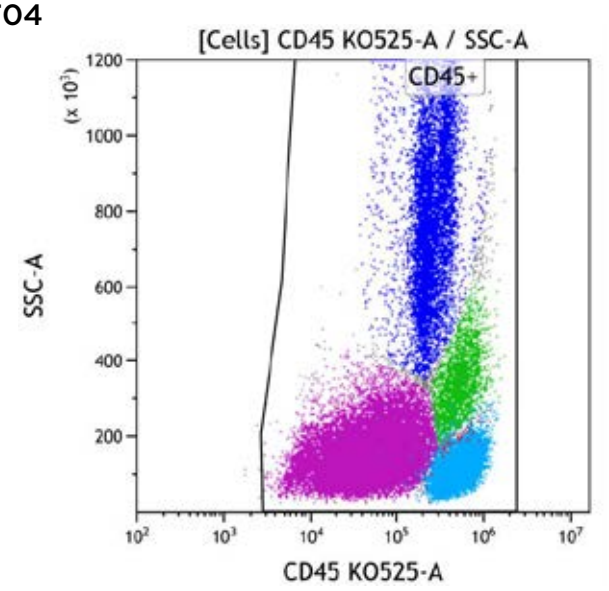

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

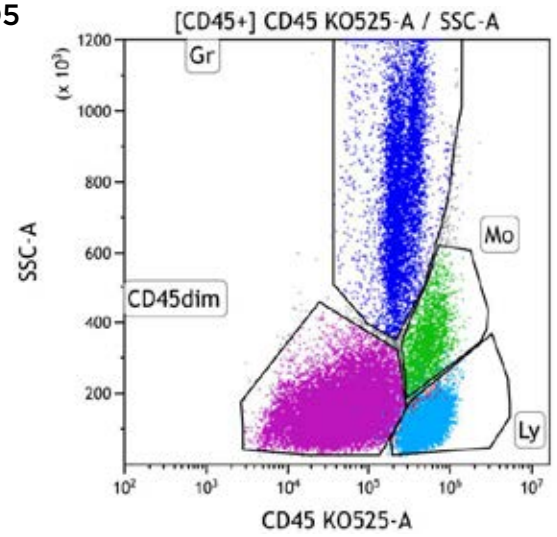

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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. Of note, cells in the CD45dim gate are expanded (in purple).

T06

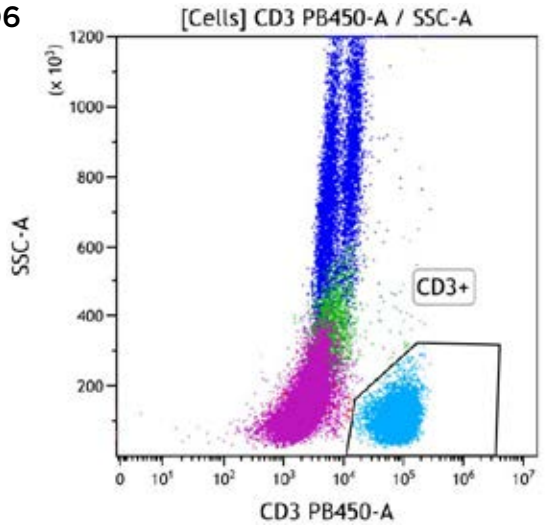

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

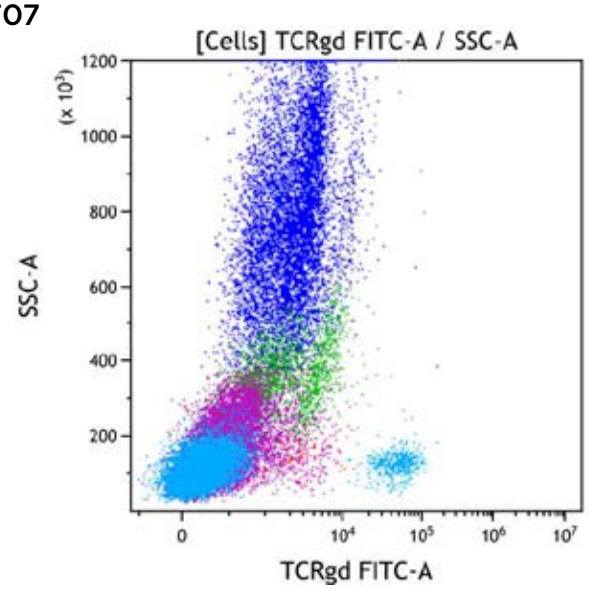

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

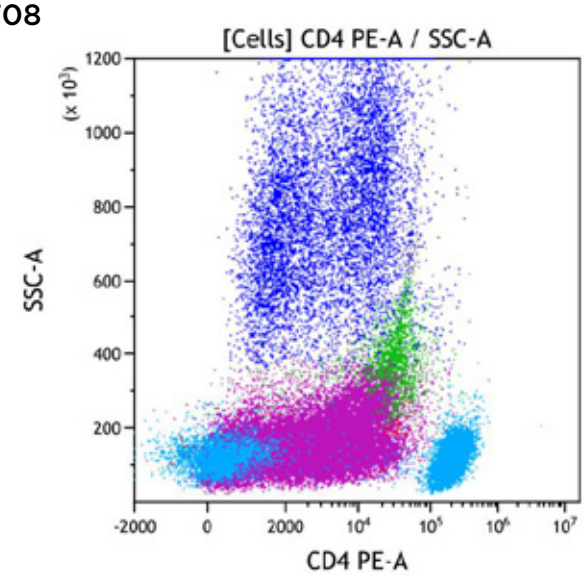

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

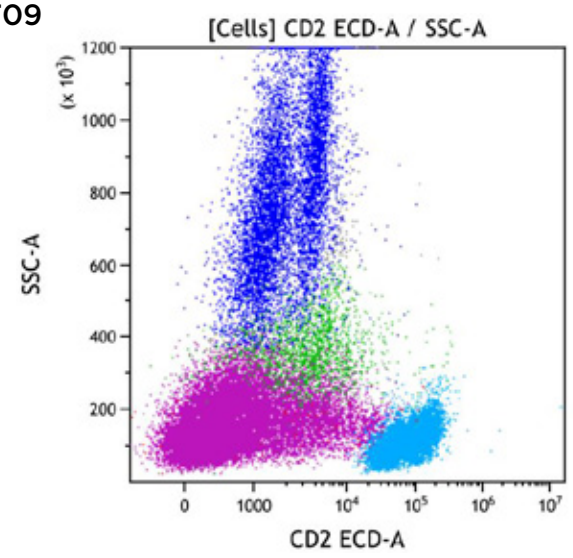

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

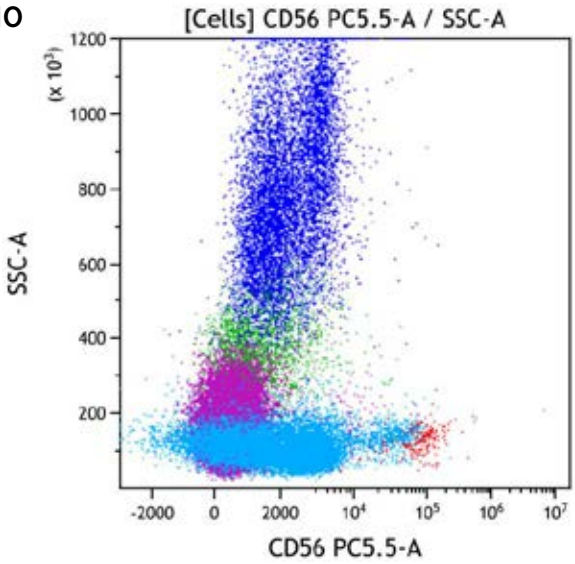

Figure 10. This CD56 vs Side Scatter dot plot shows all viable Cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and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

T11

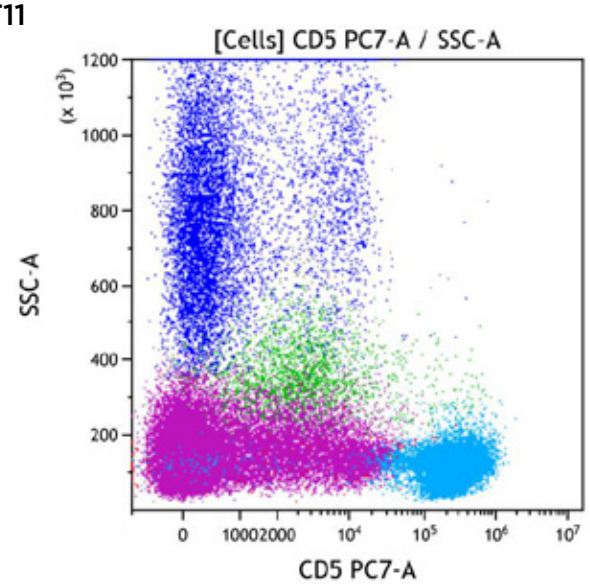

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5. Note the expanded populations in the CD45dim gate (in purple) is partiall

T12

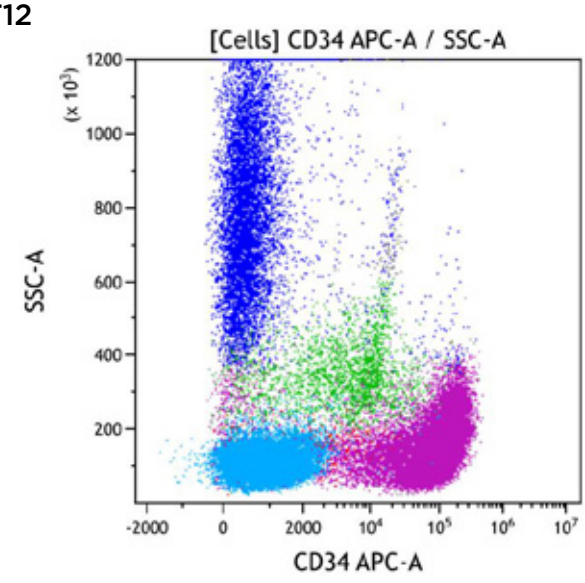

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Of note, the expanded population in the CD45dim gate is CD34-positive. w.

BACK TO CASE OVERVIEW

T13

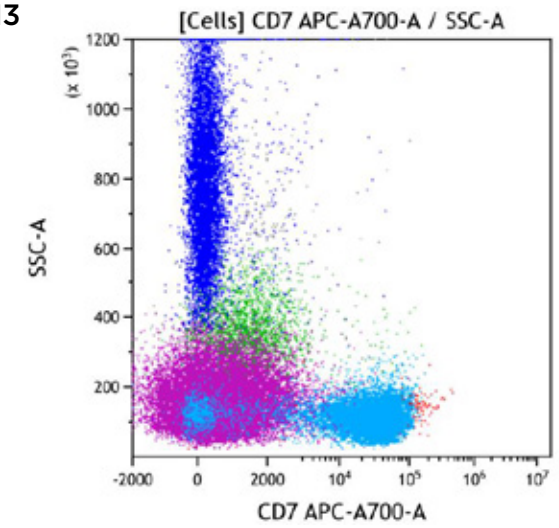

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

T14

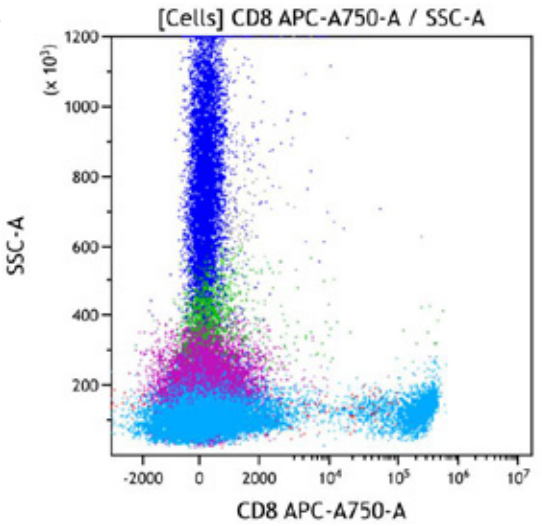

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells M(aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. .

T15

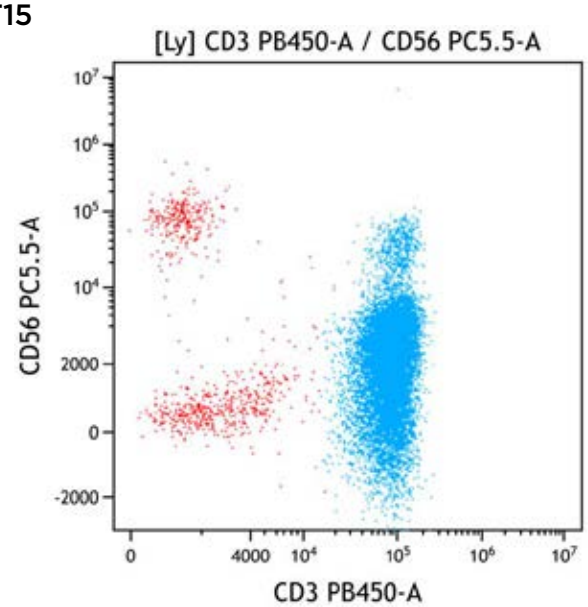

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small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

T16

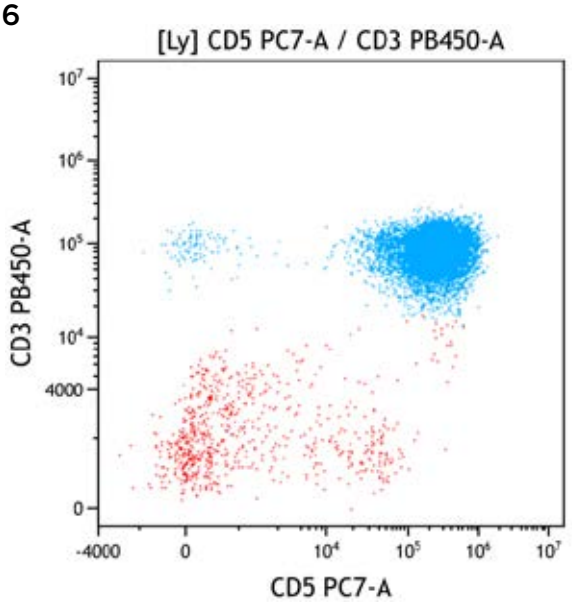

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most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

BACK TO CASE OVERVIEW

T17

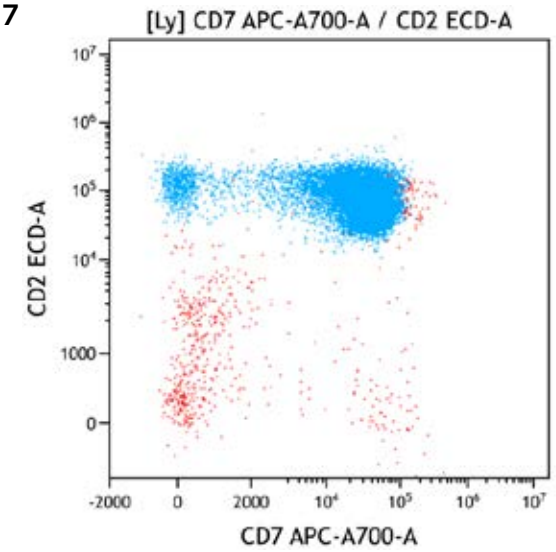

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red)

T18

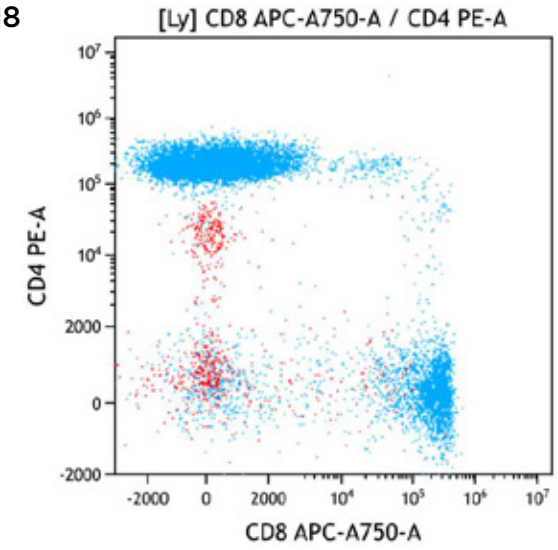

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 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.

T19

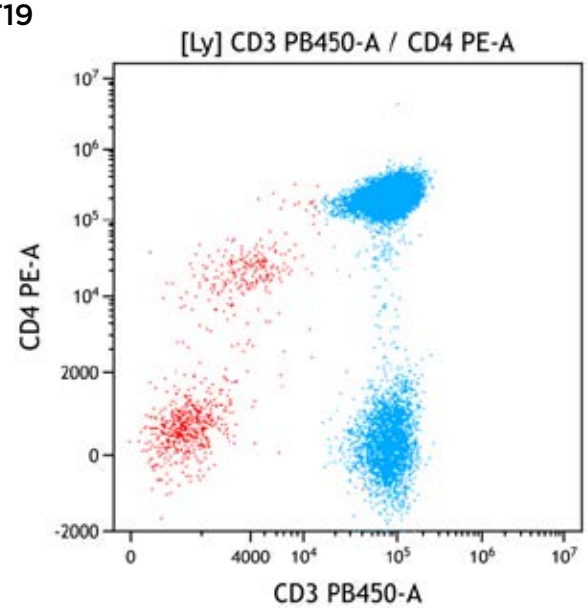

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. .

T20

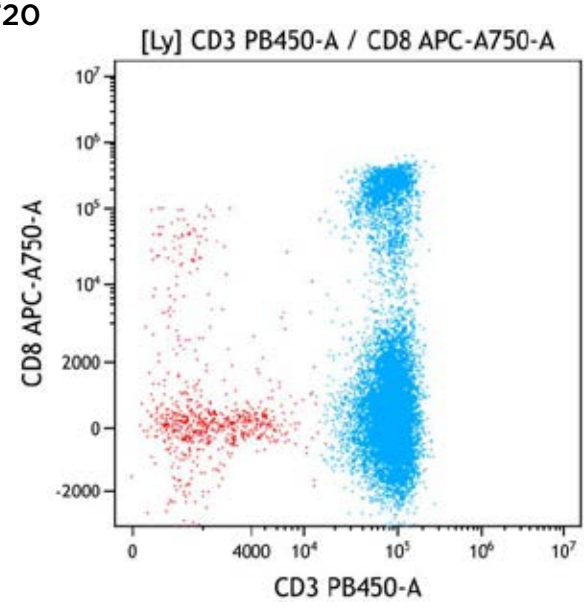

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3

BACK TO CASE OVERVIEW

T21

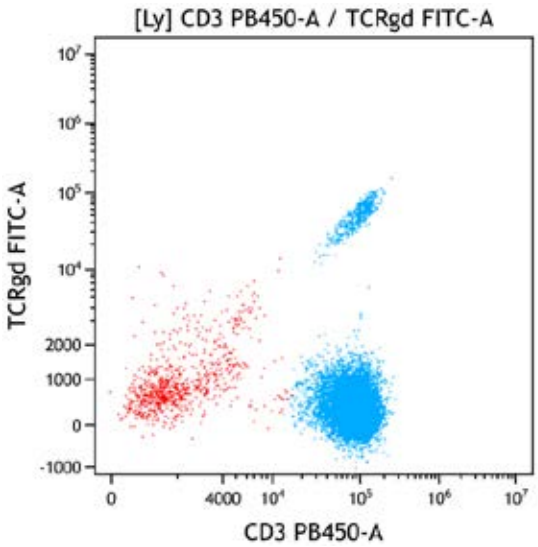

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

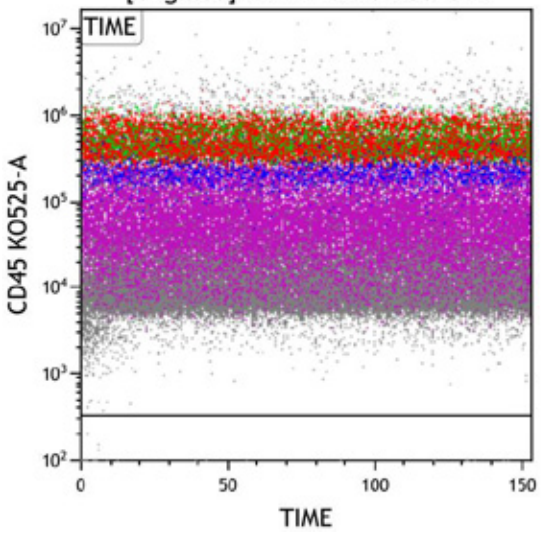

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M1-02

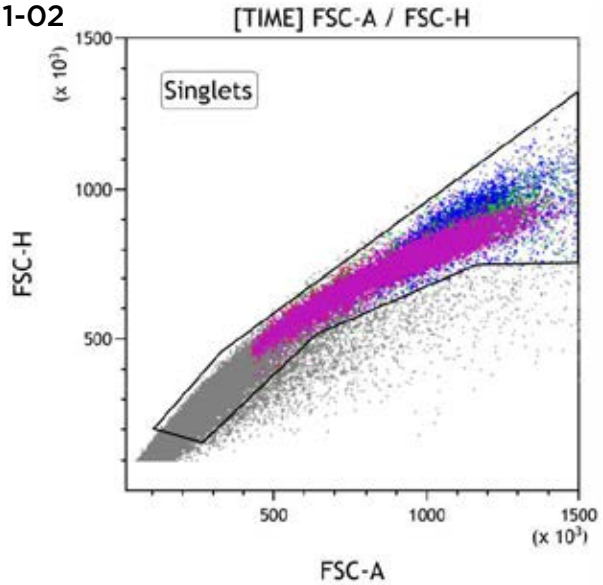

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

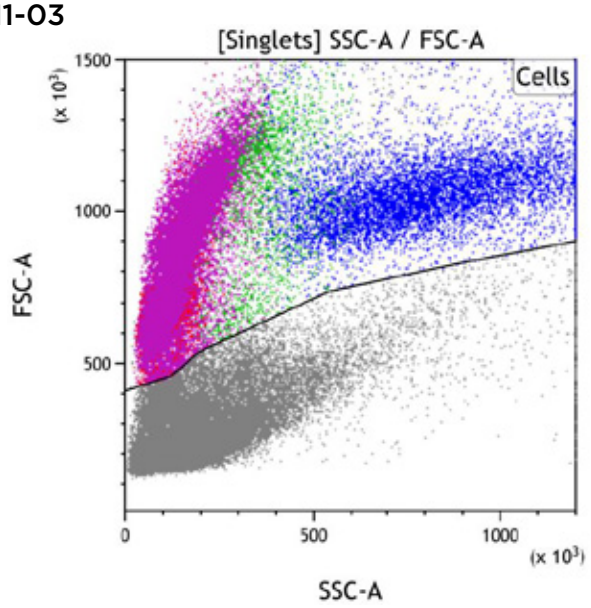

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M1-04

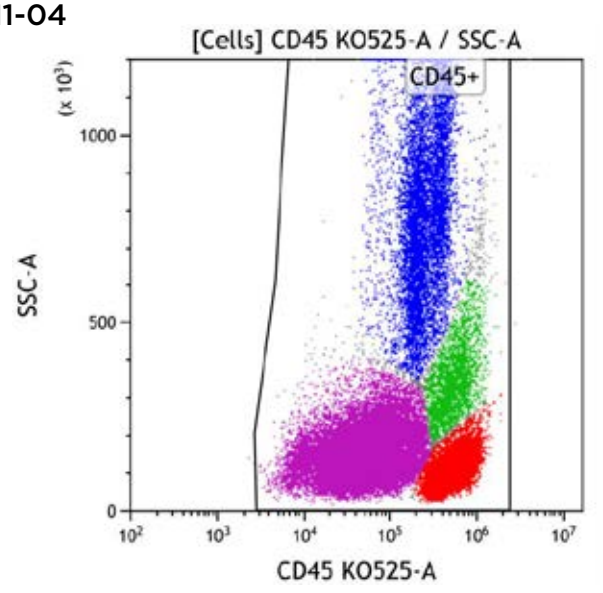

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M1-05

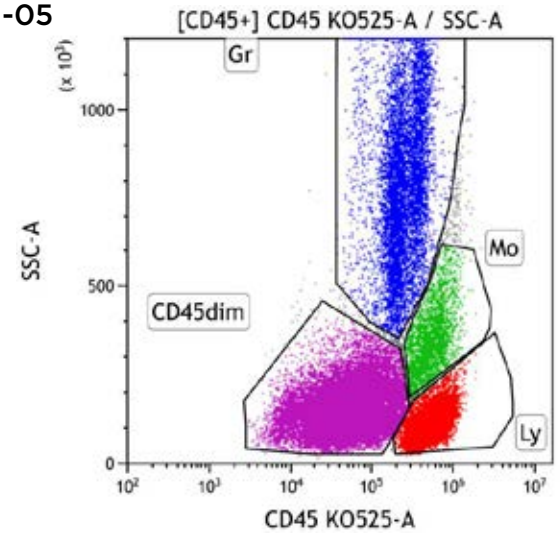

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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. Of note, cells in the CD45dim gate are expanded (in purple).

M1-06

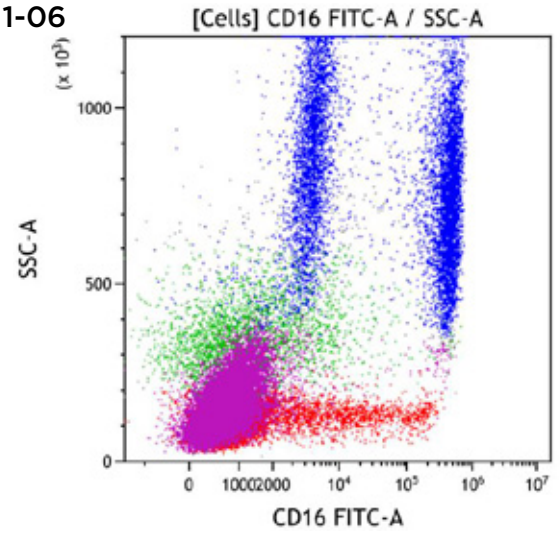

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

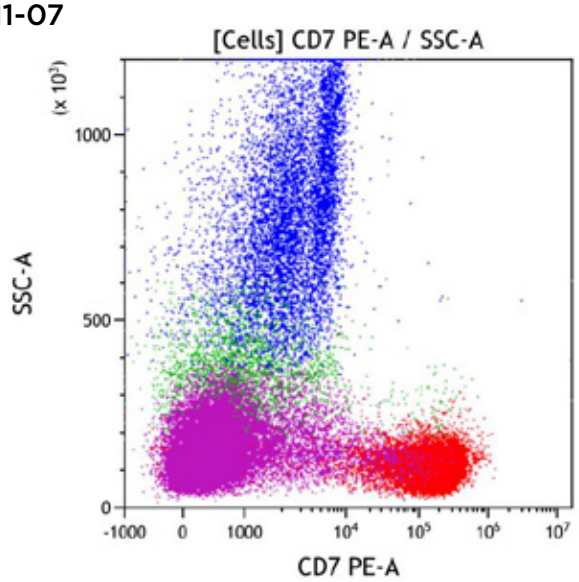

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

M1-08

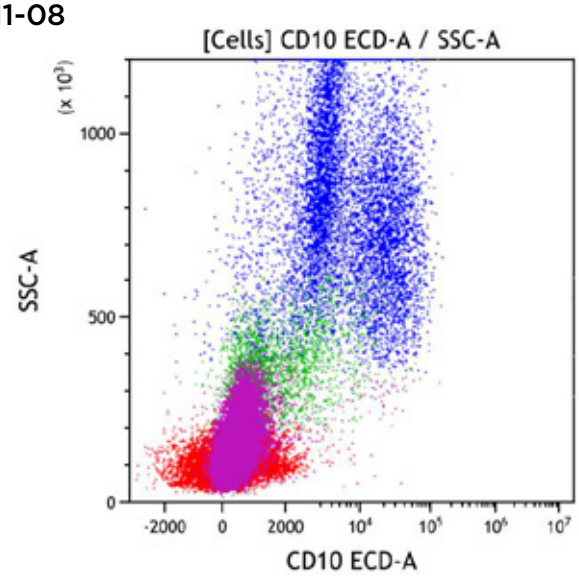

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

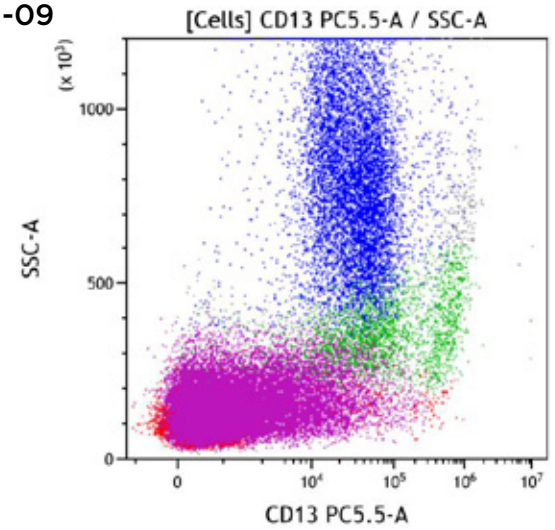

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M1-10

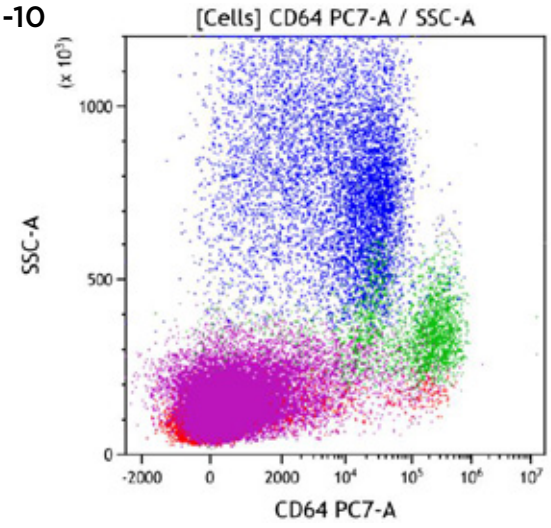

Figure 10. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

M1-11

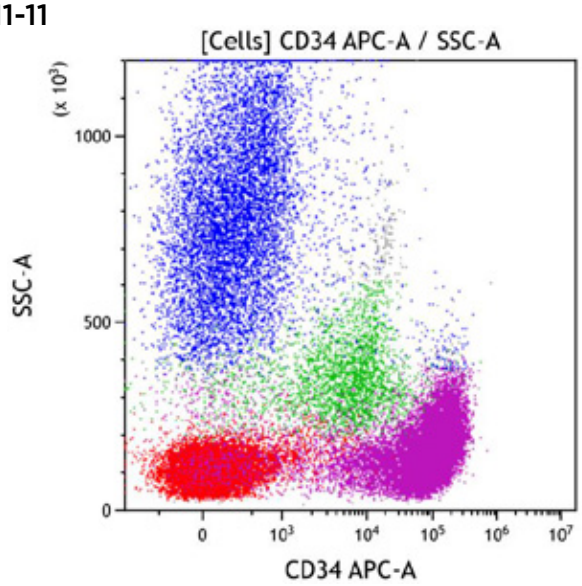

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate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

M1-12

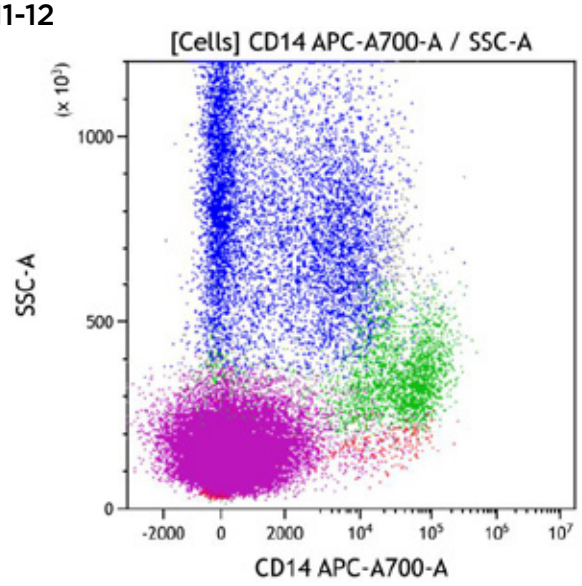

Figure 12. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Of Nnote, most cells in the CD45dim gate (purple) are CD14-negative.

BACK TO CASE OVERVIEW

M1-13

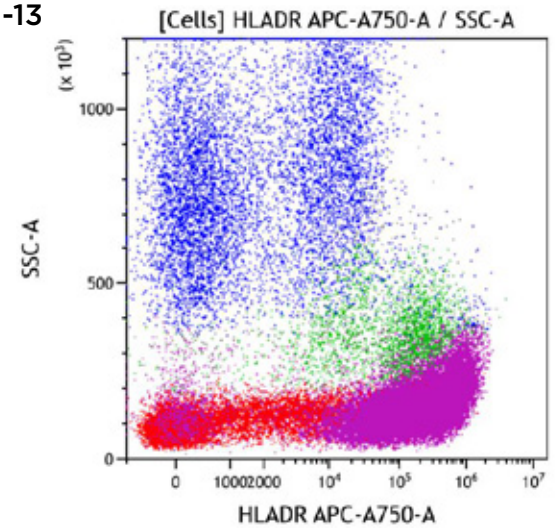

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red). Of note, most cells in the CD45dim gate (purple) are CD34-positive.

M1-14

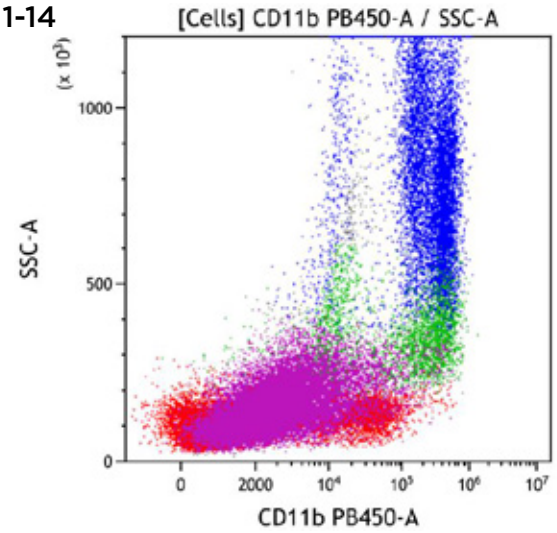

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

M1-15

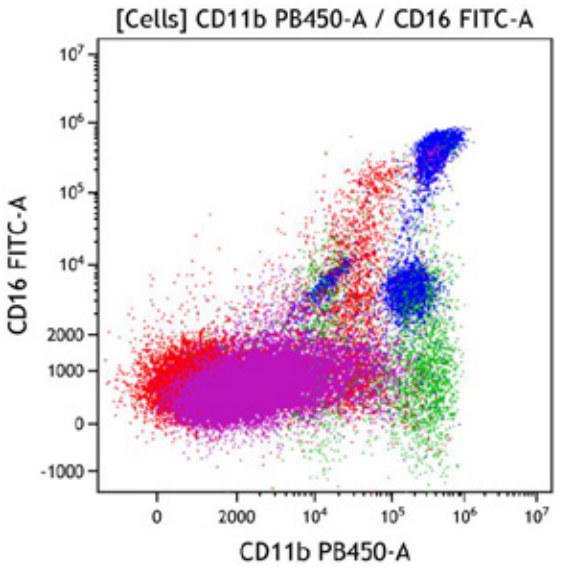

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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.

M1-16

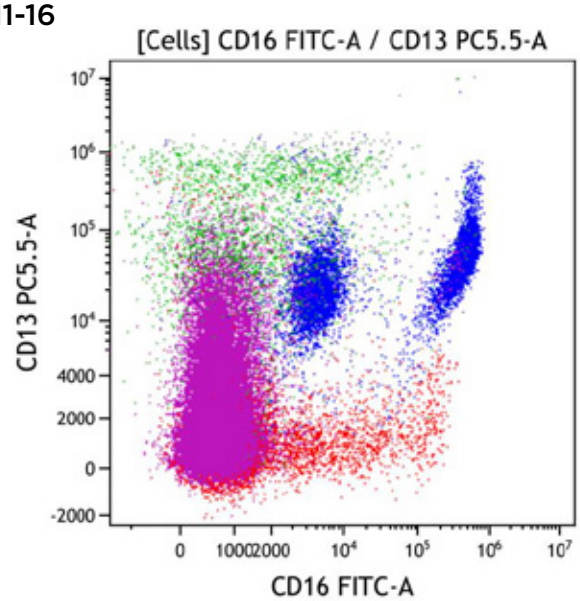

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed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

BACK TO CASE OVERVIEW

M1-17

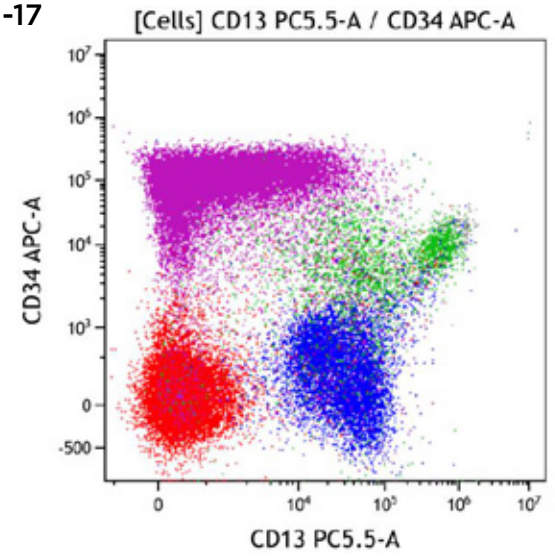

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple)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

M1-18

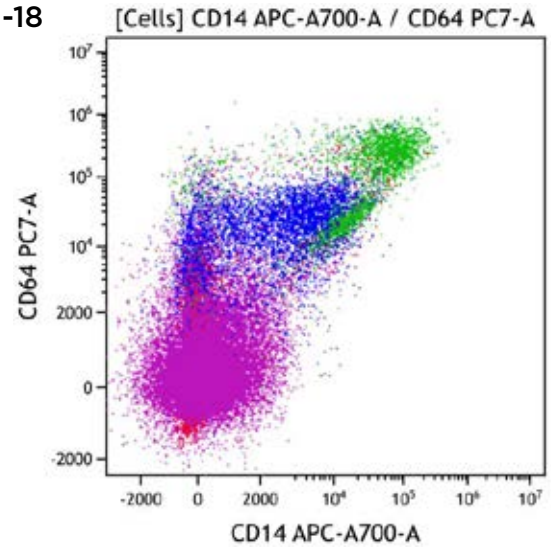

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high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

M1-19

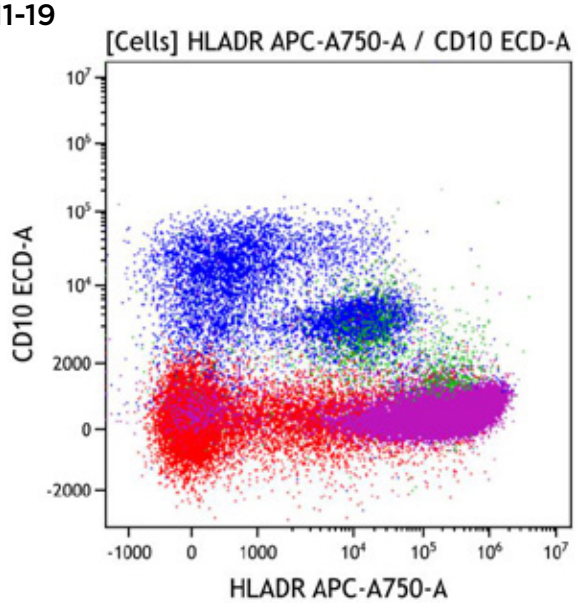

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple). Of note, most cells in the CD45dim gate (purple) are HLA-DR positive.

M1-20

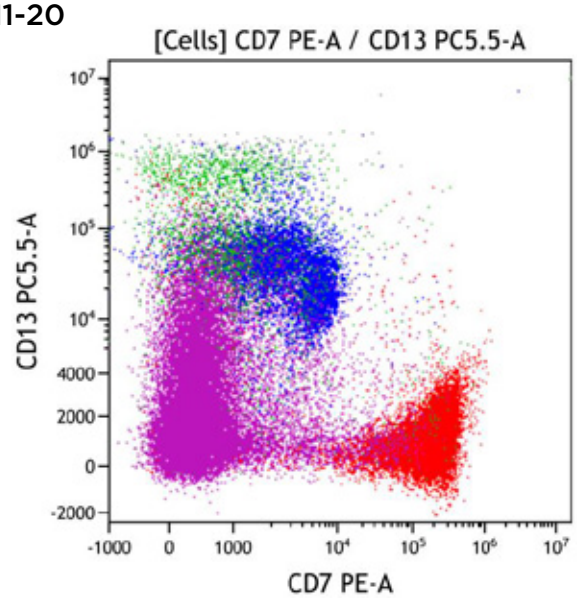

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

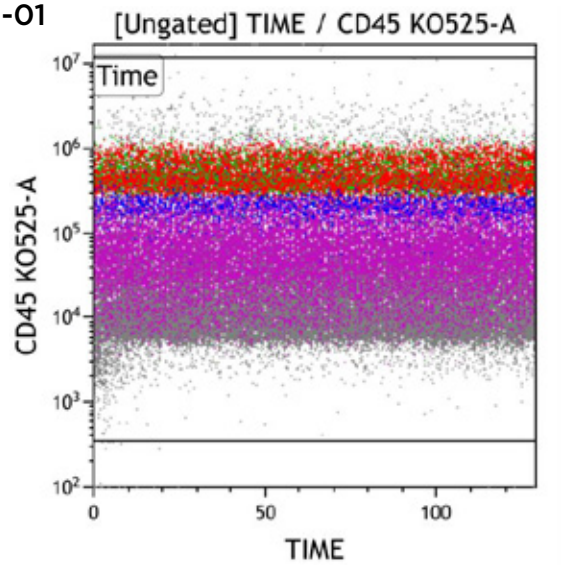

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

M2-02

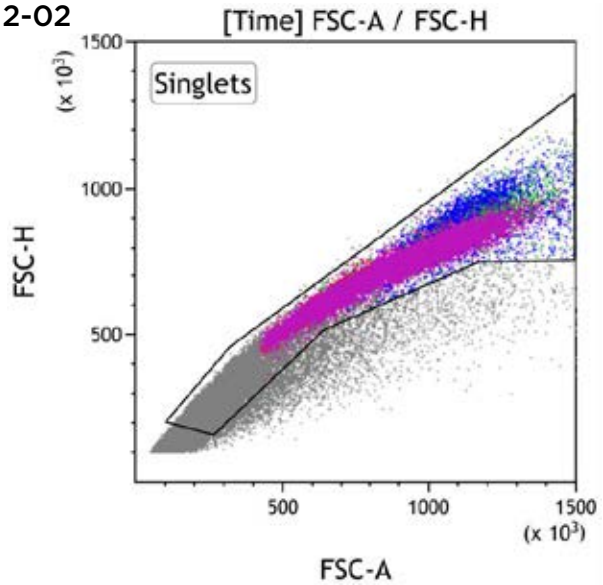

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

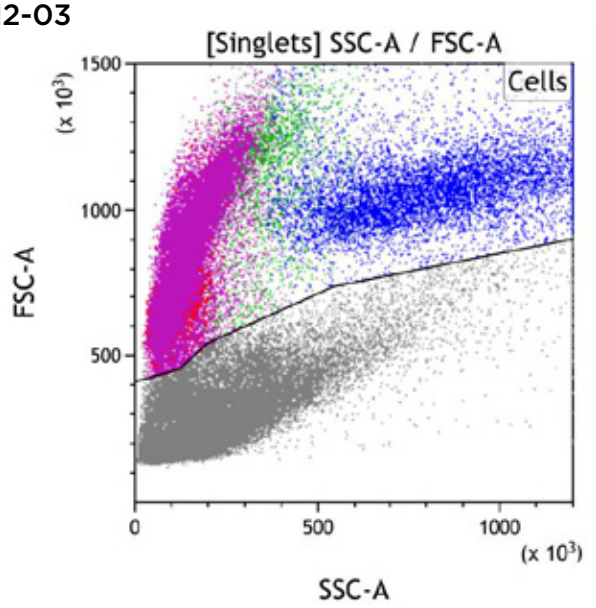

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

M2-04

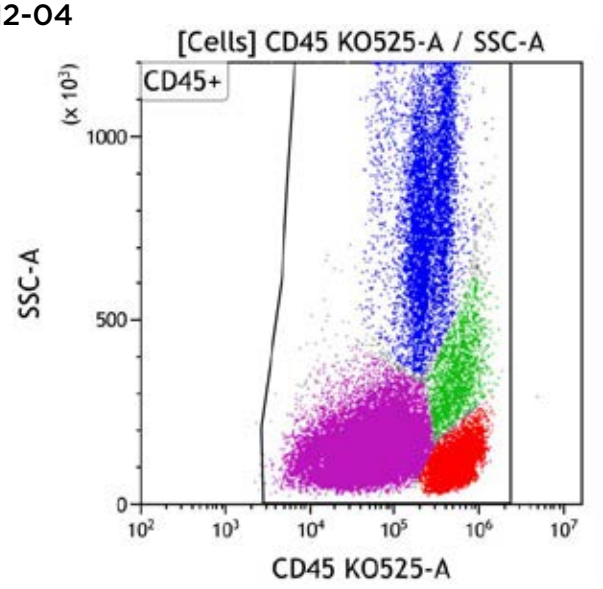

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

M2-05

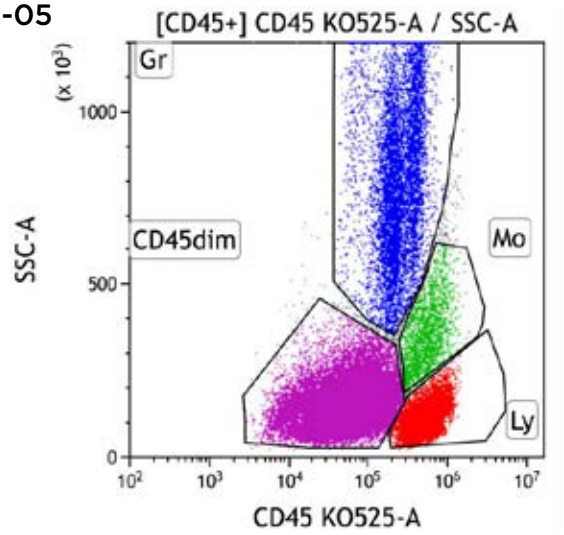

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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. Of note, cells in the CD45dim gate are expanded (in purple).

M2-06

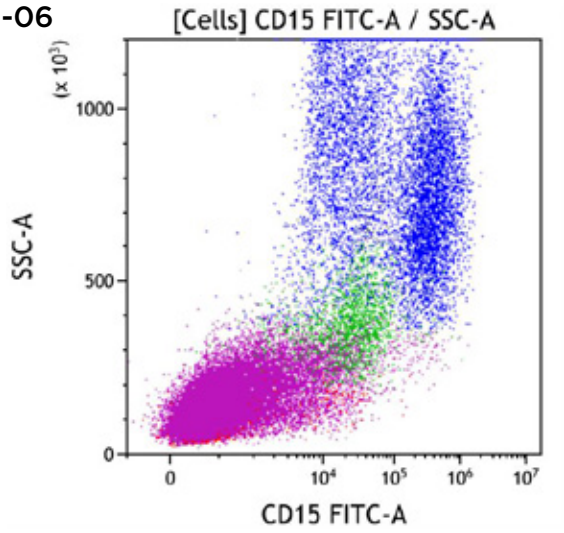

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green). Of note, most cells in the CD45dim gate (purple) are CD15-negative.

M2-07

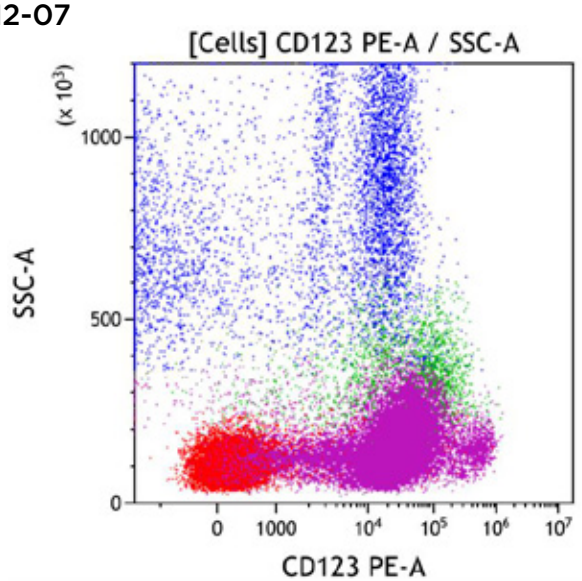

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green). Of note, most cells in the CD45dim gate (purple) are CD123-positive.

M2-08

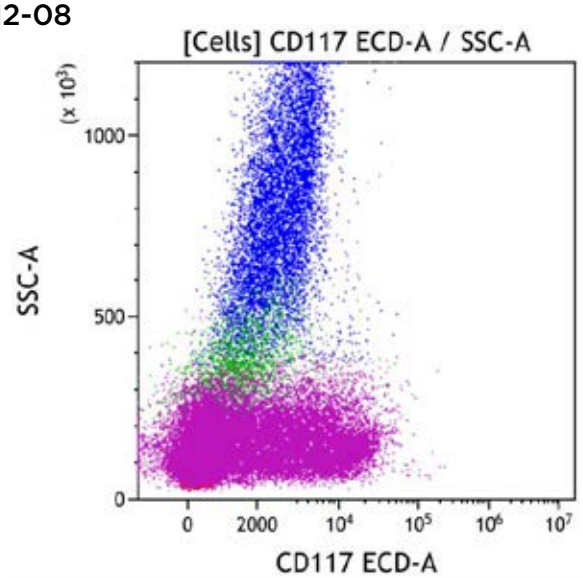

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Of note, most cells in the CD45dim gate (purple) are partially positive for CD117.

BACK TO CASE OVERVIEW

M2-09

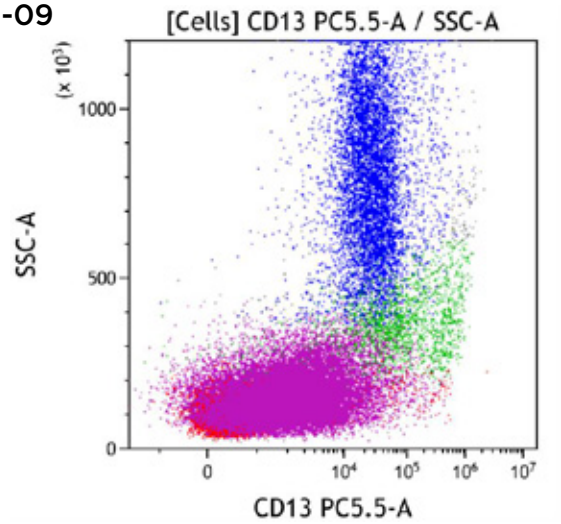

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M2-10

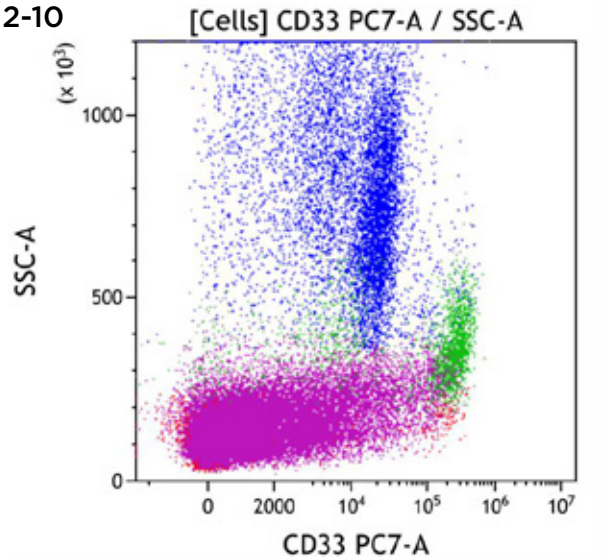

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granu locytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34+ myeloid progenitors (purple). Of note, most cells in the CD45dim gate (purple) are partially positive for CD33.

M2-11

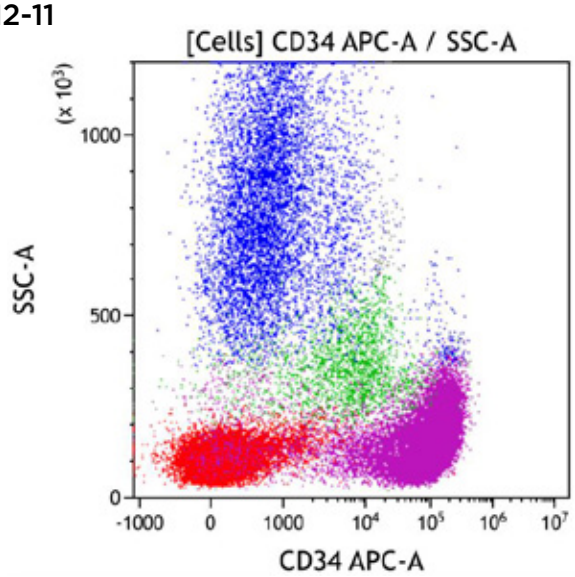

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Of note, the expanded population in the CD45dim gate is CD34-positive.

M2-12

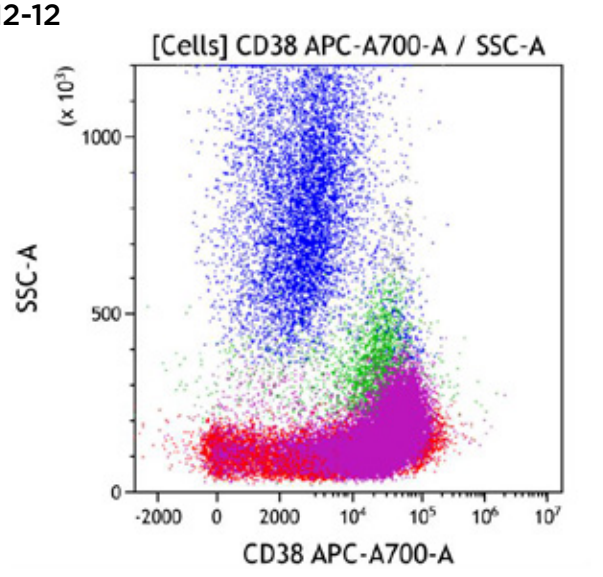

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

BACK TO CASE OVERVIEW

M2-13

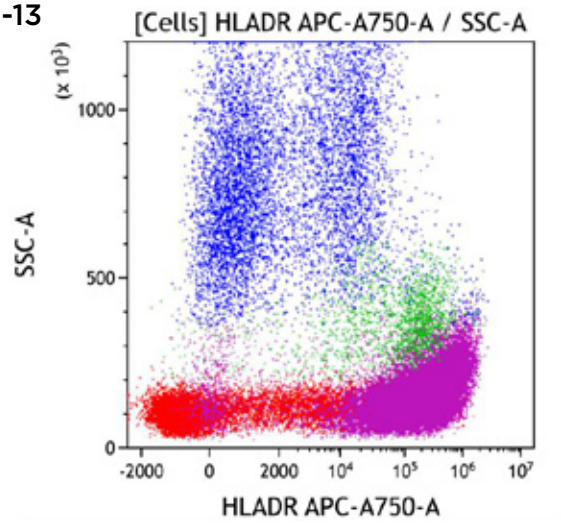

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red). Of note, the expanded population in the CD45dim gate is HLA-DR-positive.

M2-14

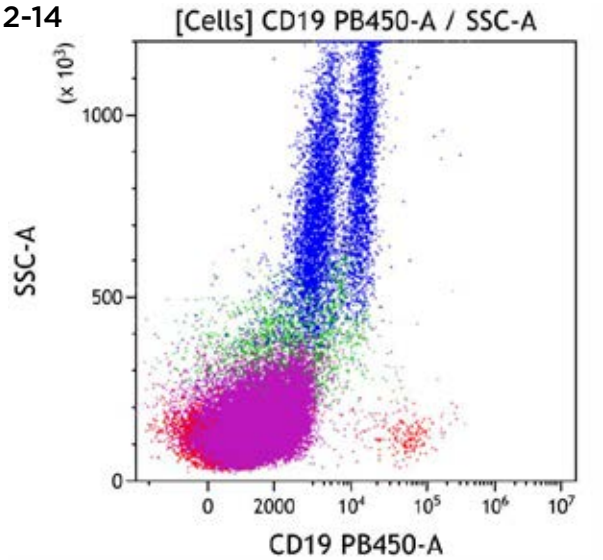

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Of note, the expanded population in the CD45dim gate is CD19-negative.

M2-15

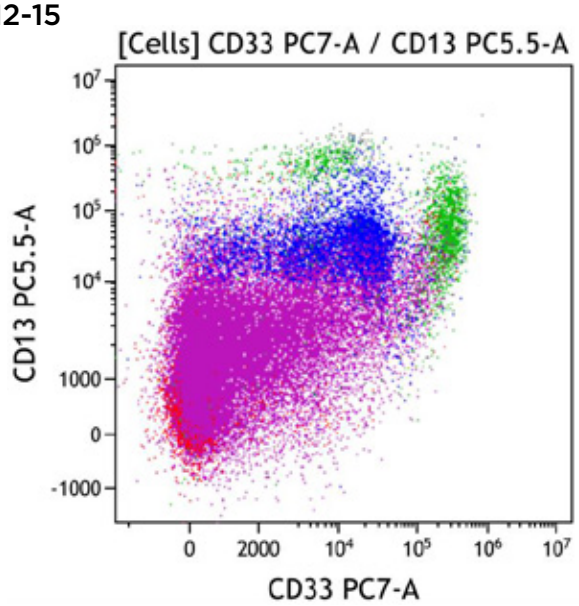

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher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

M2-16

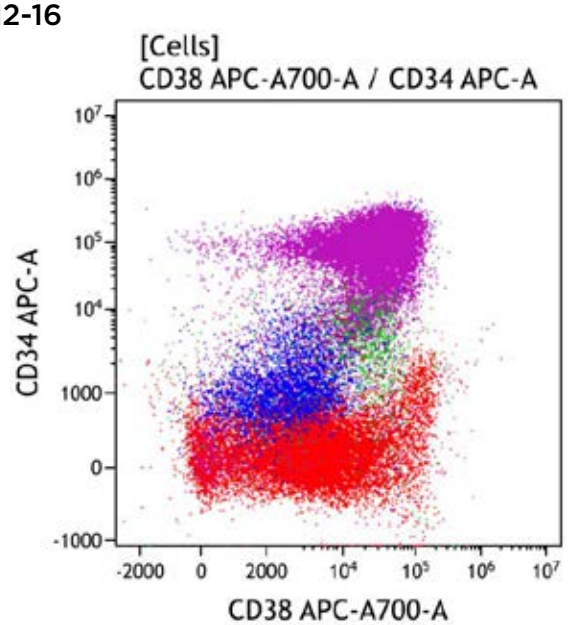

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable 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.

BACK TO CASE OVERVIEW

M2-17

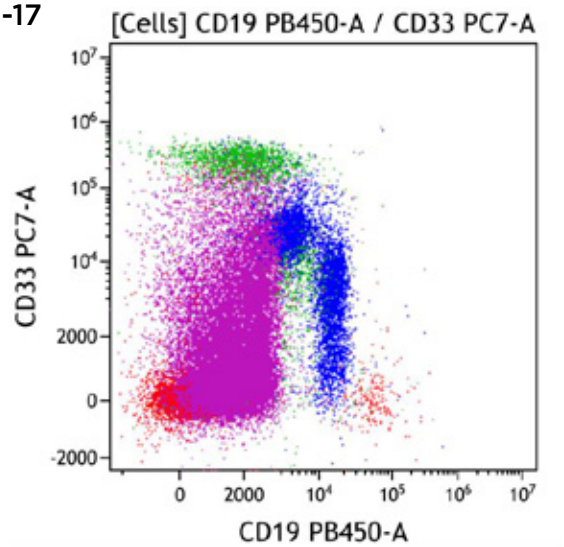

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to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

M2-18

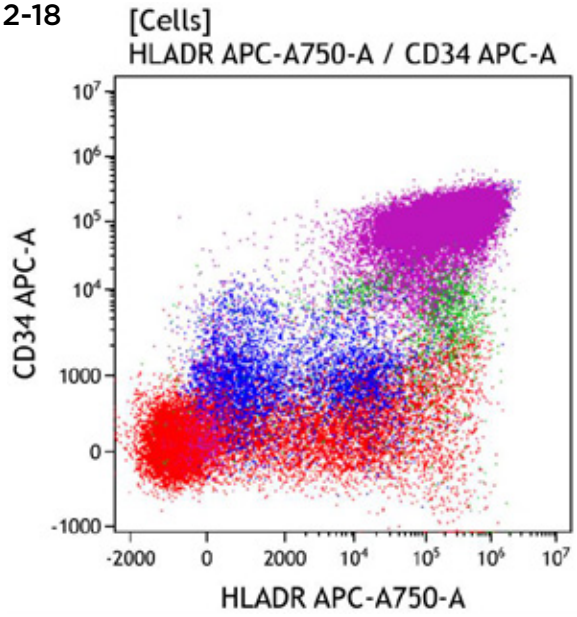

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of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

M2-19

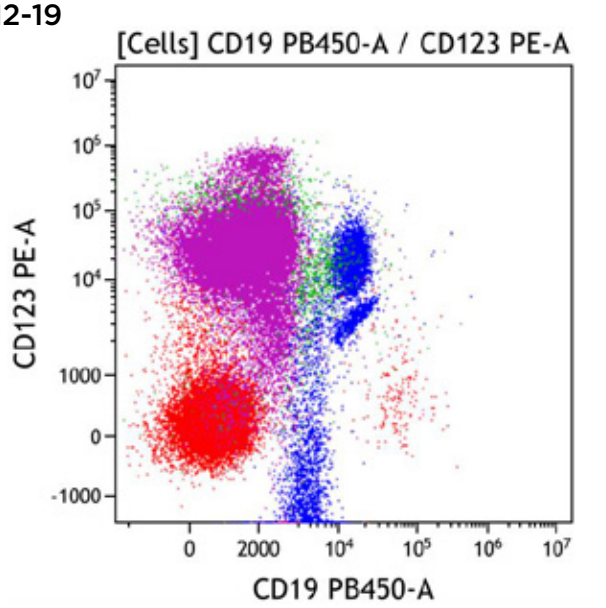

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid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

M2-20

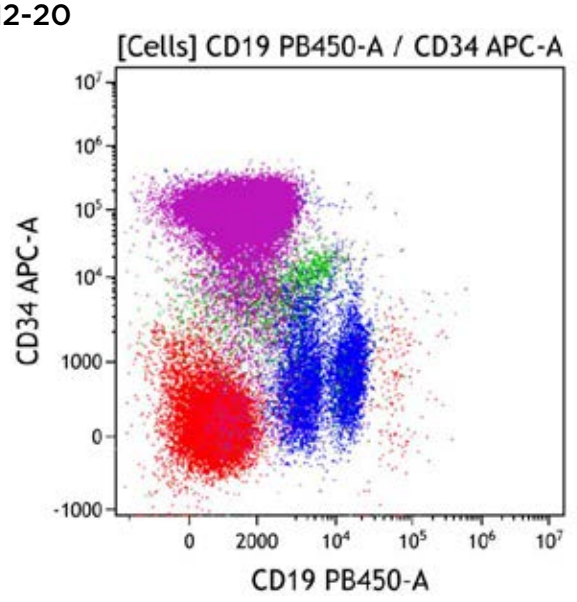

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19

BACK TO CASE OVERVIEW

M2-21

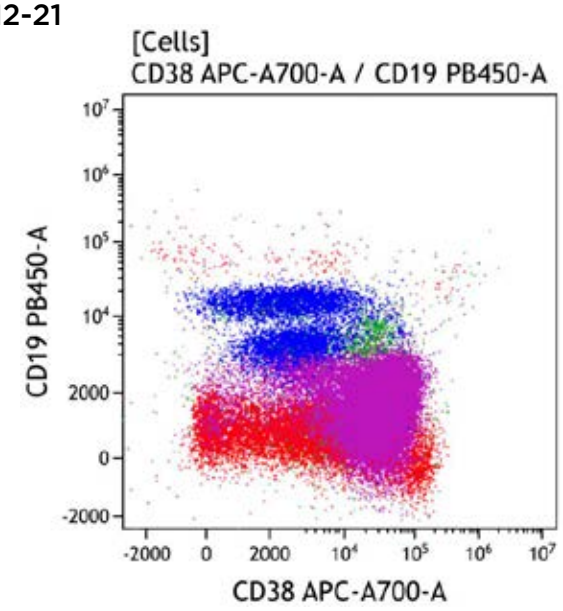

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression 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.

M2-22

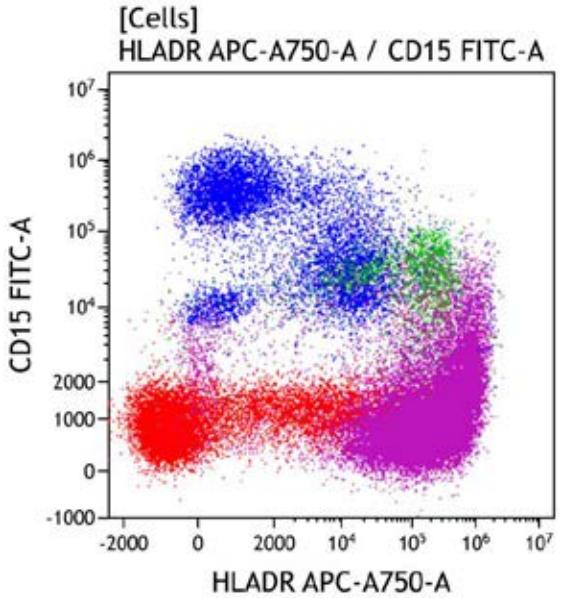

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLADR (blue), except the earliest forms where CD15 is being acquired. CD34+ myeloid progenitors (purple) express HLA-DR but only transiently express CD15. Of note, a small subset of cells in the CD45dim gate is partially positive for CD15.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 13 MLL-TC4-038**
Diagnosis	**Normal Bone Marrow**
Clinical Vignette	64-year-old male with a recent diagnosis of non-Hodgkin B cell lymphoma. This specimen was collected for lymphoma staging.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations. 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.

BACK TO CASE LISTINGS

B Cell Tube

B01

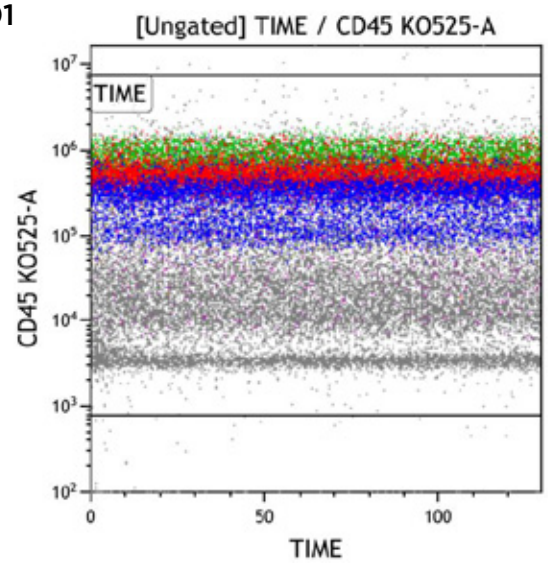

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

B02

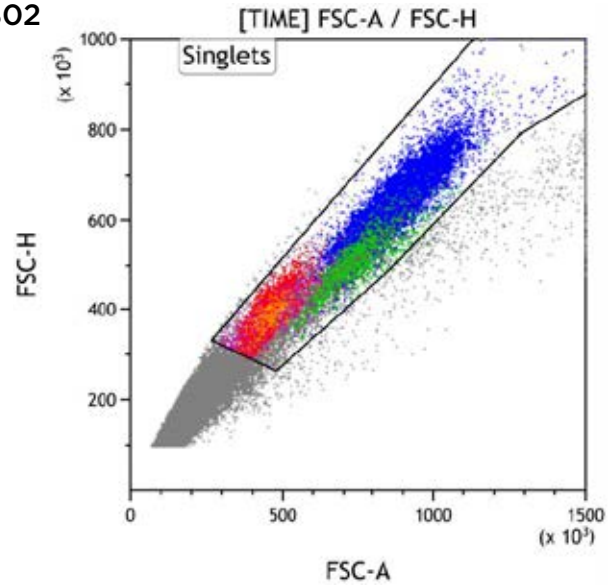

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

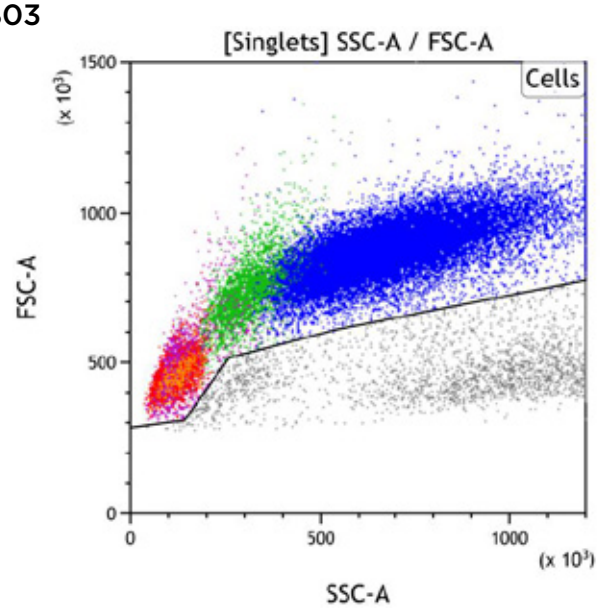

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

B04

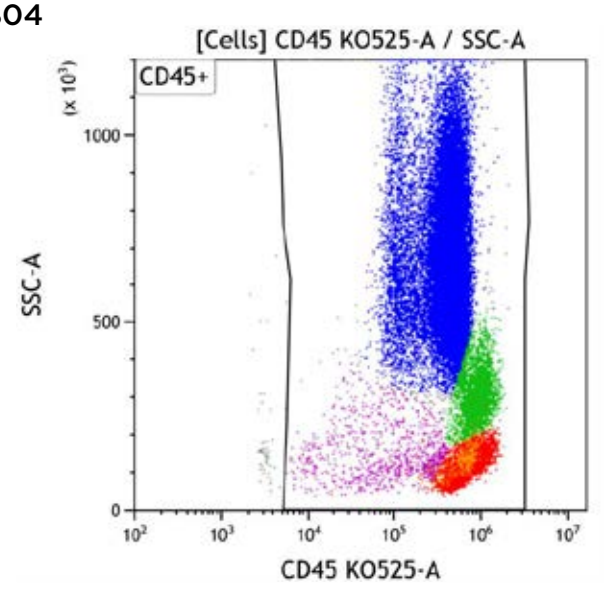

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

B05

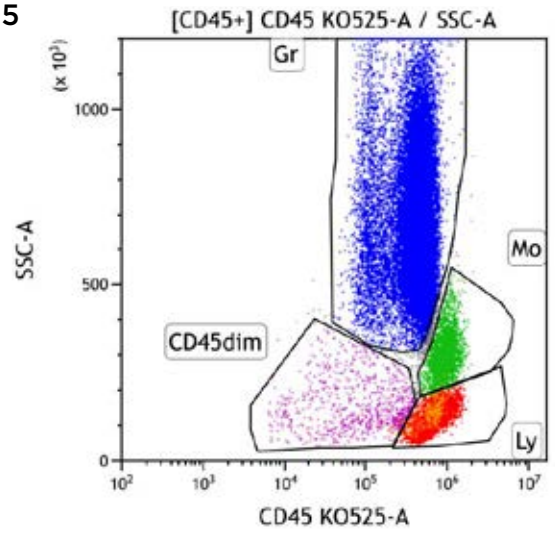

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

B06

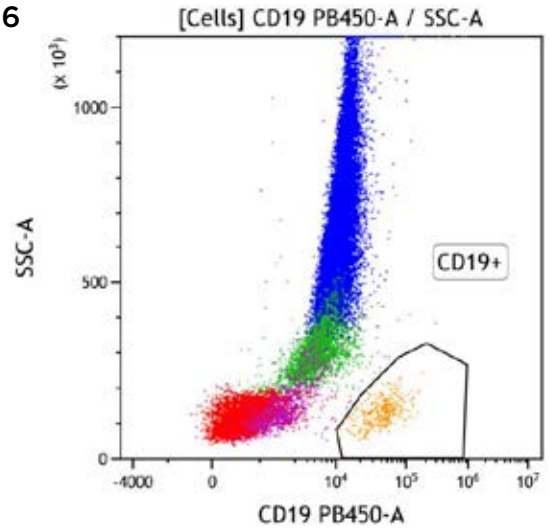

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B07

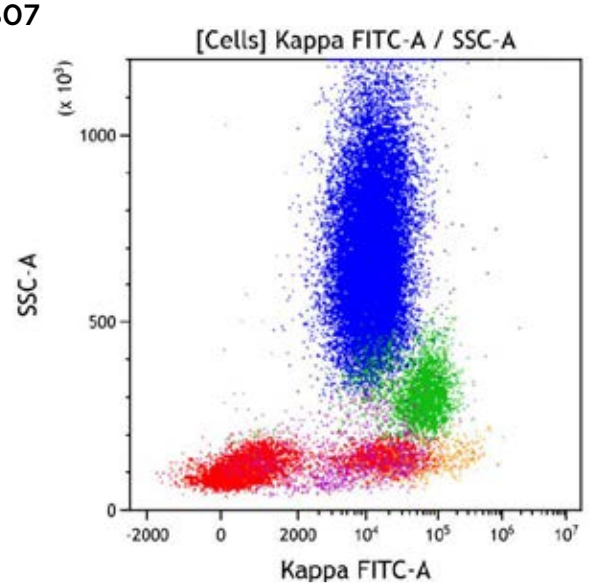

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (highlighted in yellow) and latestage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa 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.

B08

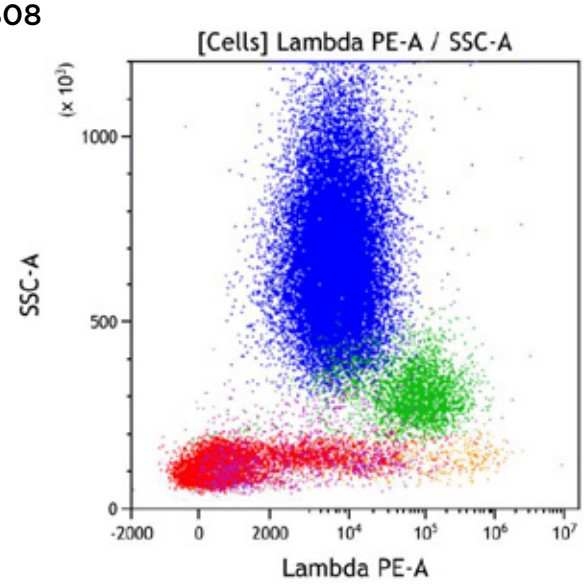

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (highlighted in yellow) and latestage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda 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.

BACK TO CASE OVERVIEW

B09

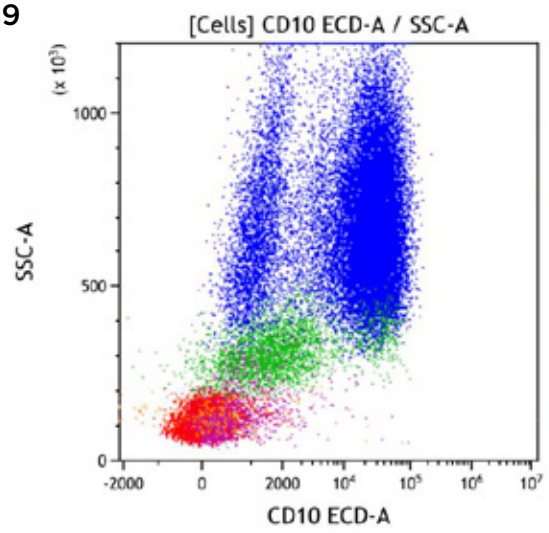

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

B10

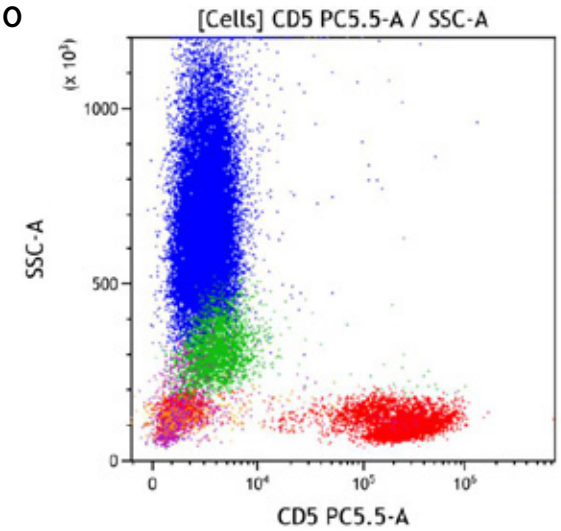

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells, as well as dimly a subset of mature B cells (highlighted in yellow). These lymphoid cells typically have low side scatter.

B11

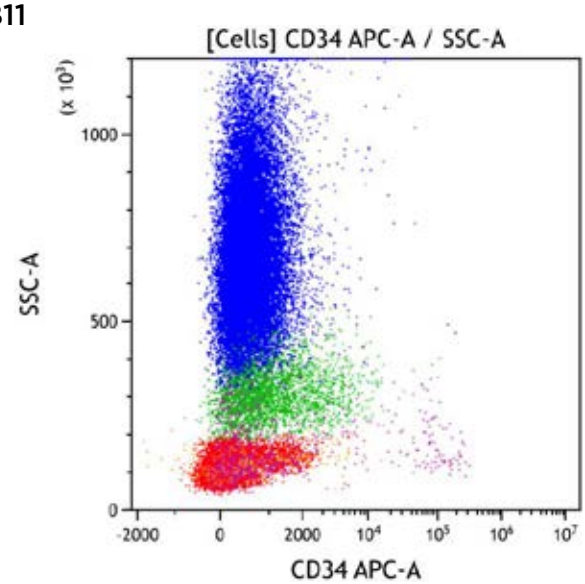

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate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

B12

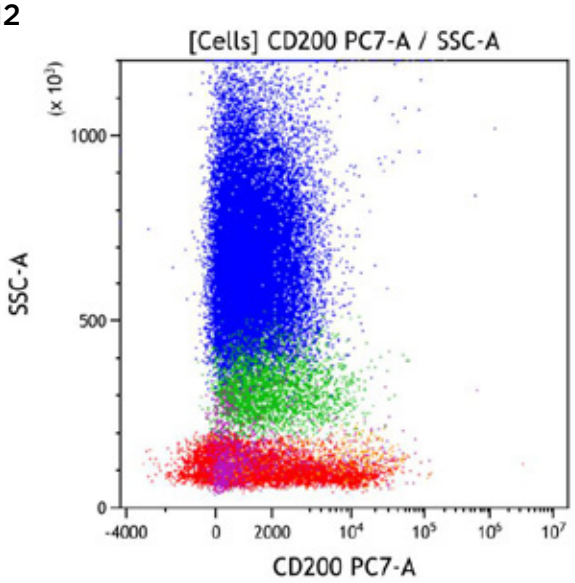

Figure 12. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

BACK TO CASE OVERVIEW

B13

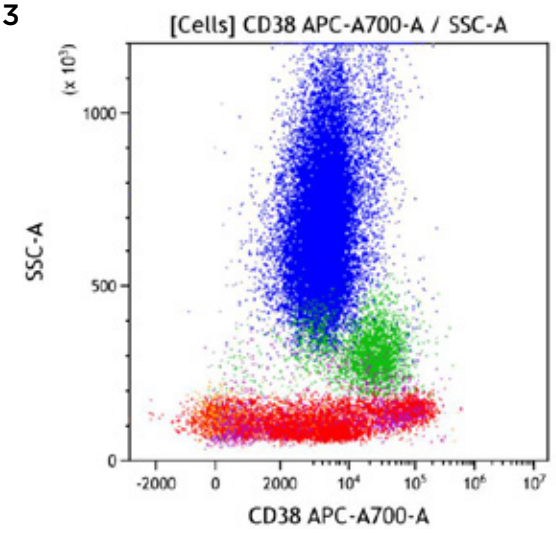

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated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

B14

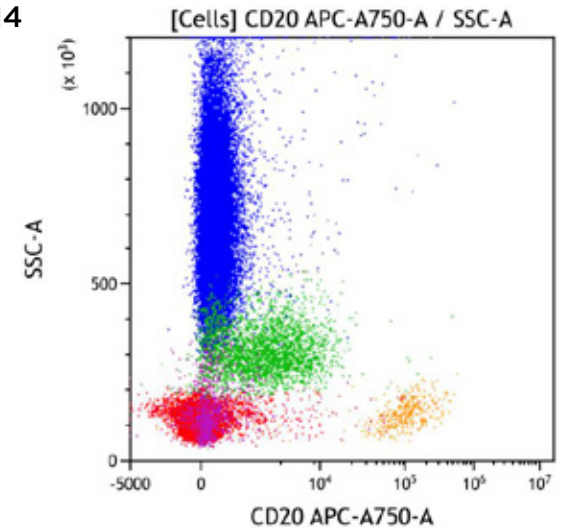

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (highlighted in orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate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

B15

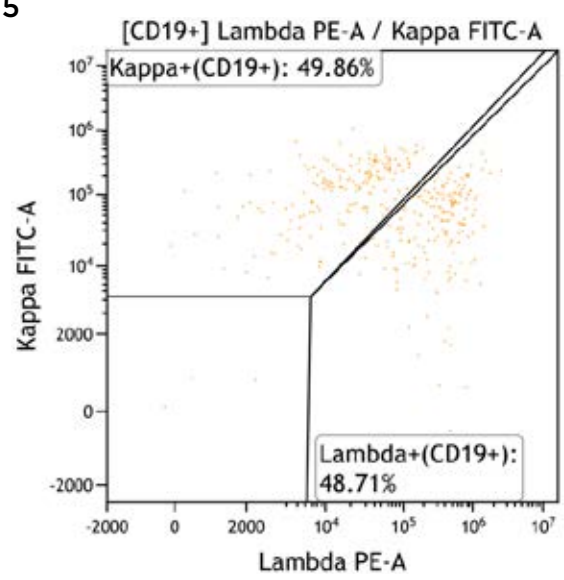

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

B16

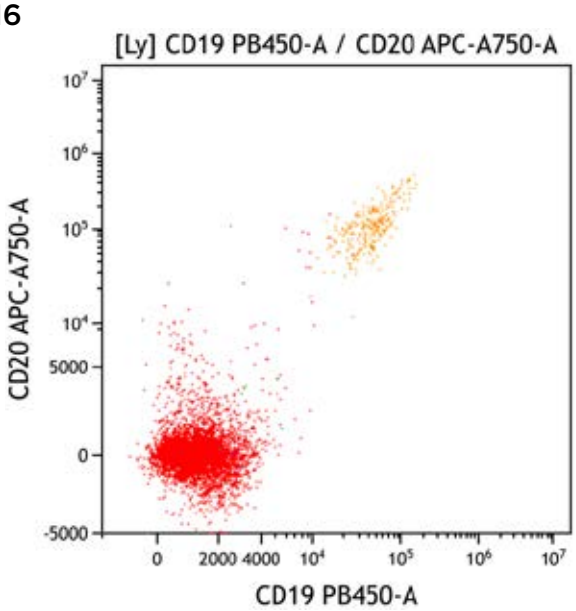

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate. Mature B cells express both CD19 and CD20 (highlighted in orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

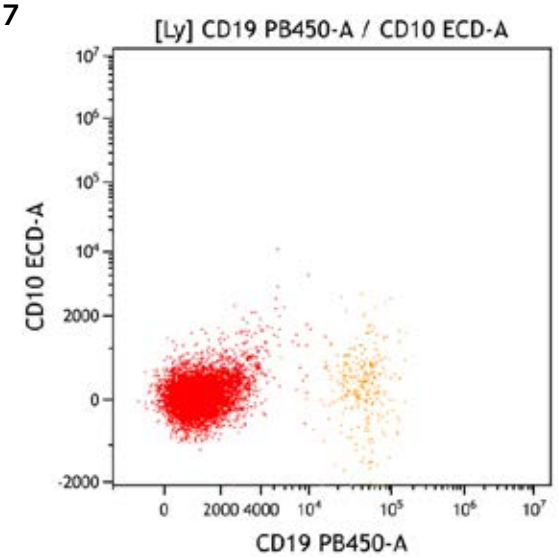

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate. B cells are CD19 positive (highlighted in yellow). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late-stage immature B cells is present in peripheral blood and bone marrow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

B18

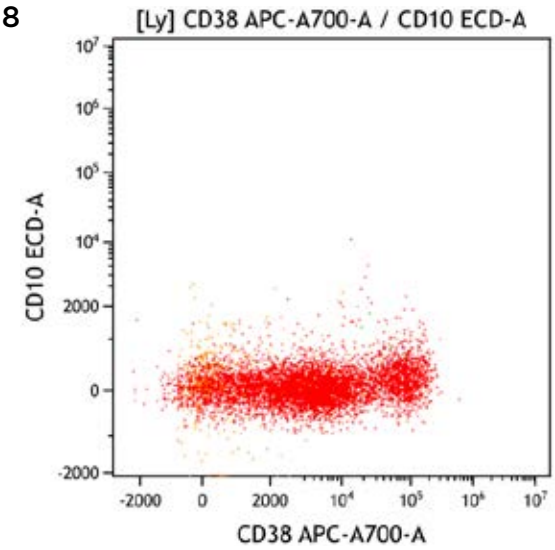

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate. CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable CD38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

B19

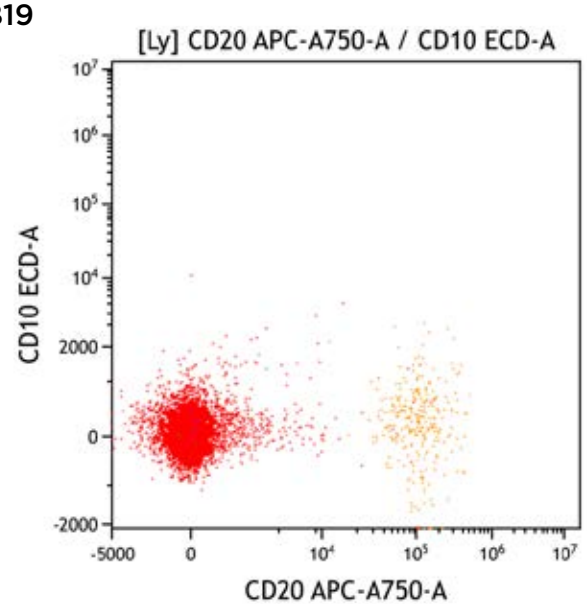

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate. Most mature B cells uniformly express high-level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late-stage immature B cells.

B20

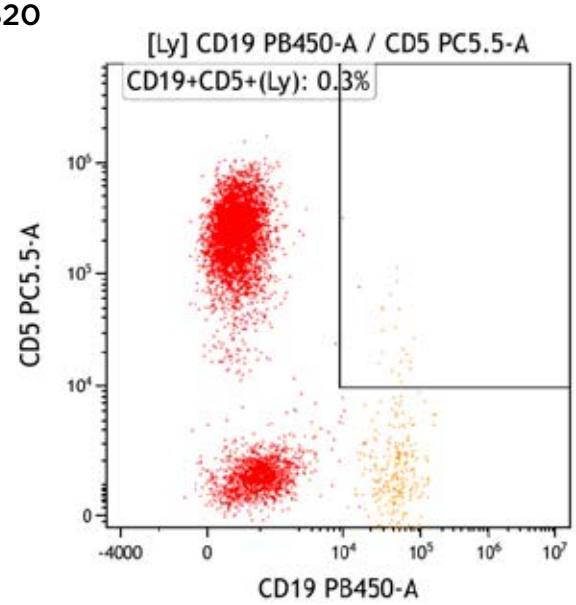

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate. CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

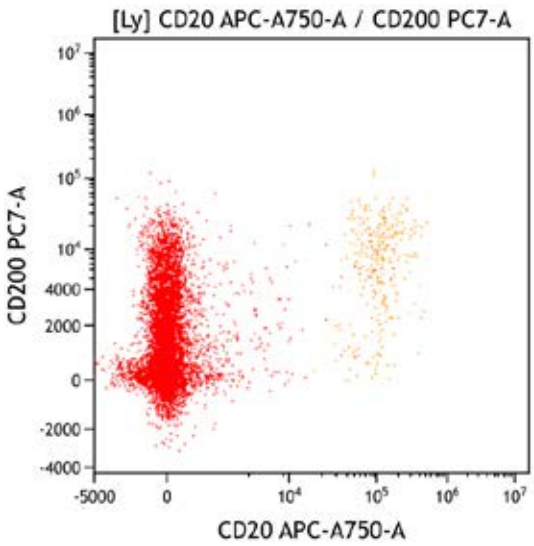

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells express CD200 at a low to moderate level (highlighted in orange).

B22

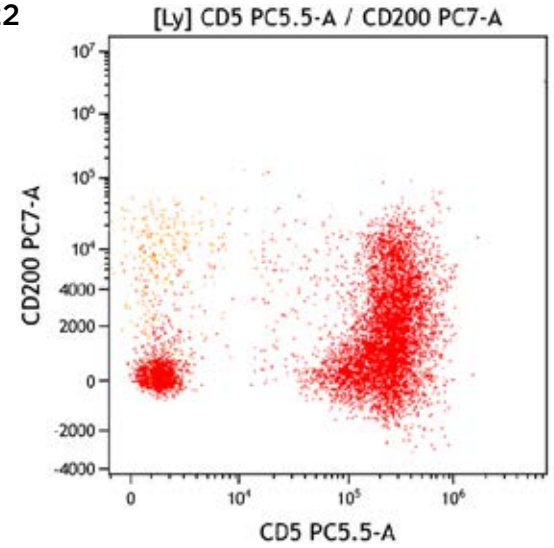

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate. Most mature B cells normally express CD200 and a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically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

BACK TO CASE OVERVIEW

T Cell Tube

T01

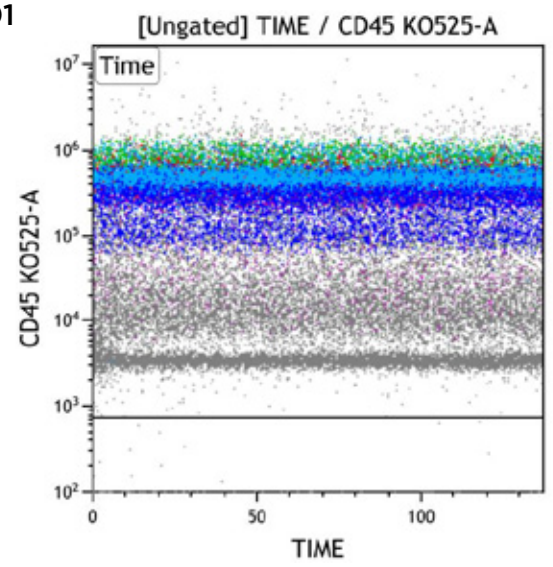

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

T02

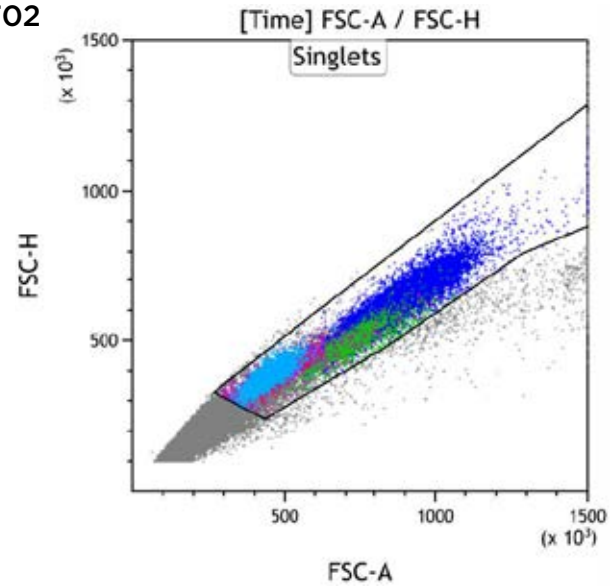

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

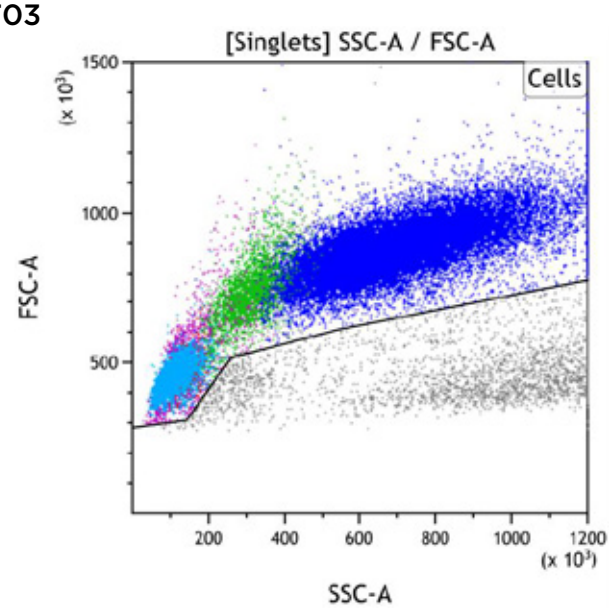

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

T04

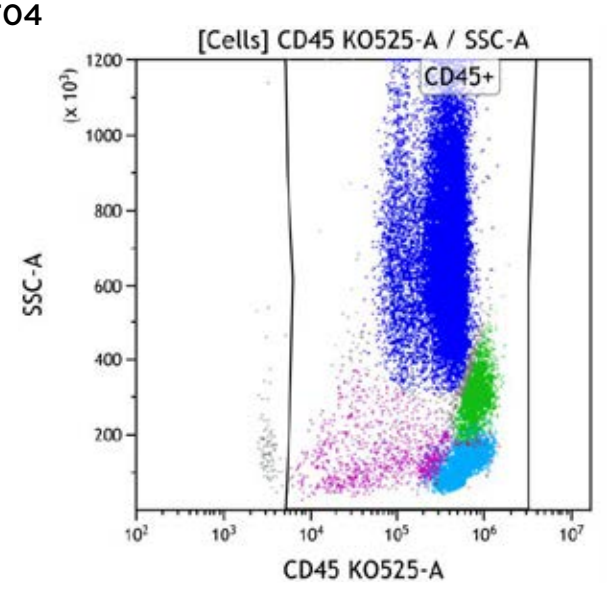

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris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

BACK TO CASE OVERVIEW

T05

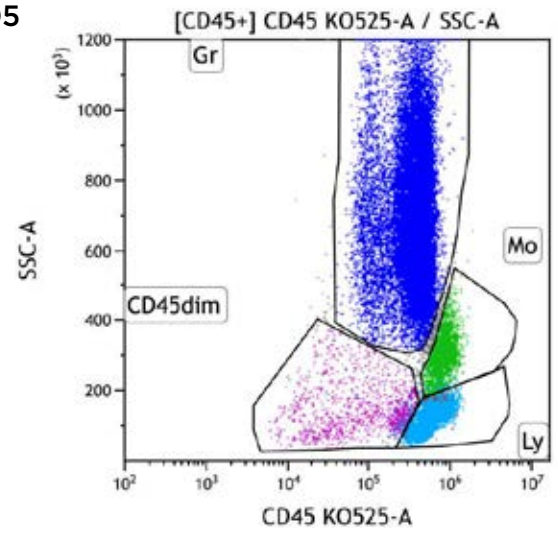

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

T06

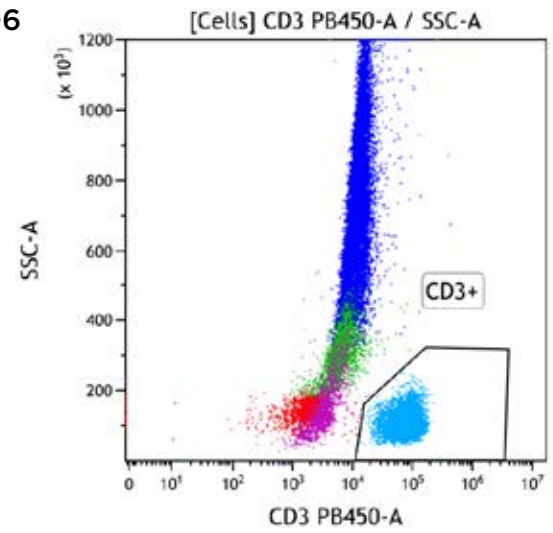

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate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

T07

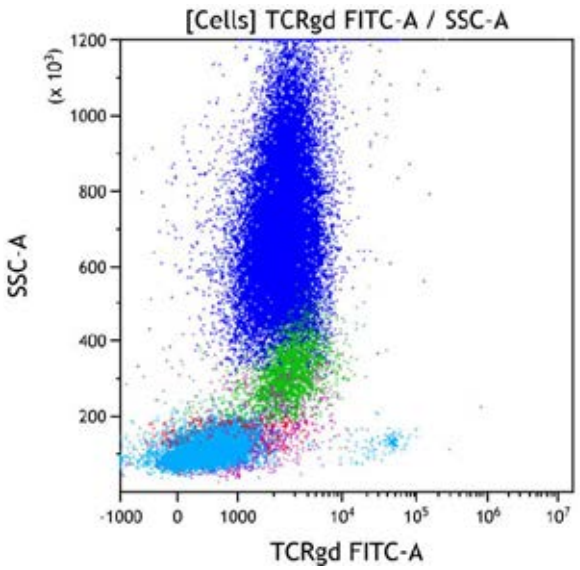

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of the T cell receptor and expressed on a small subset of cytotoxic T cells (aqua).

T08

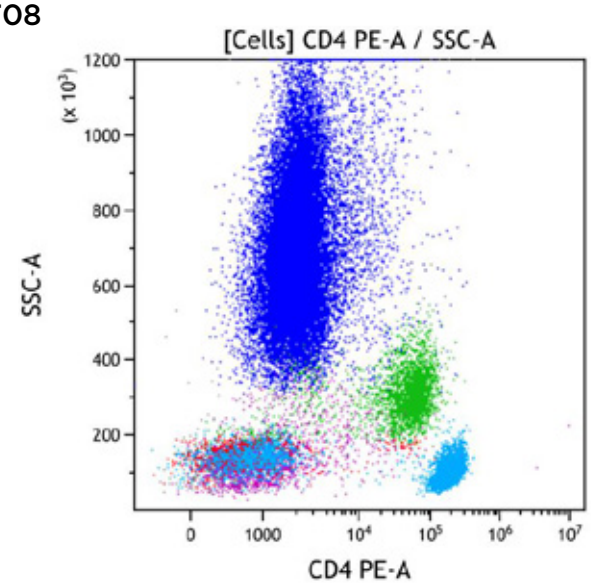

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4+ T cells. It is also expressed at a low level on immature progenitors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

BACK TO CASE OVERVIEW

T09

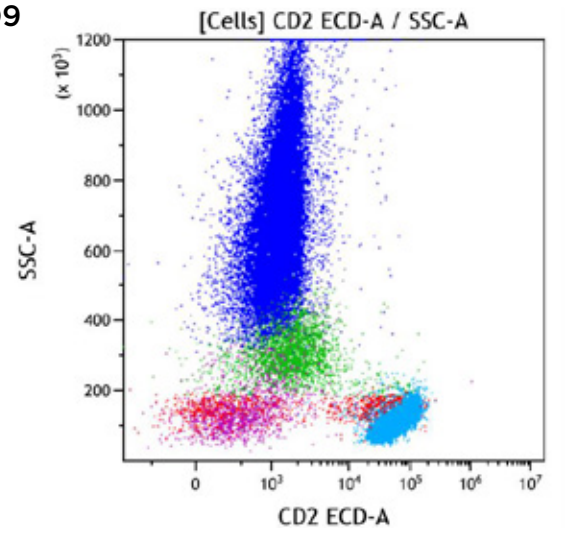

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

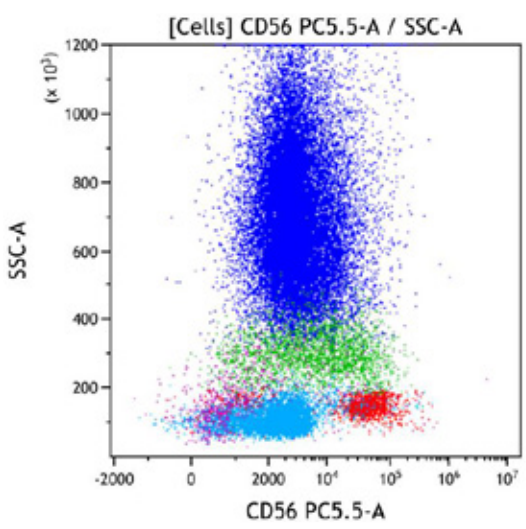

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/ delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and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

T11

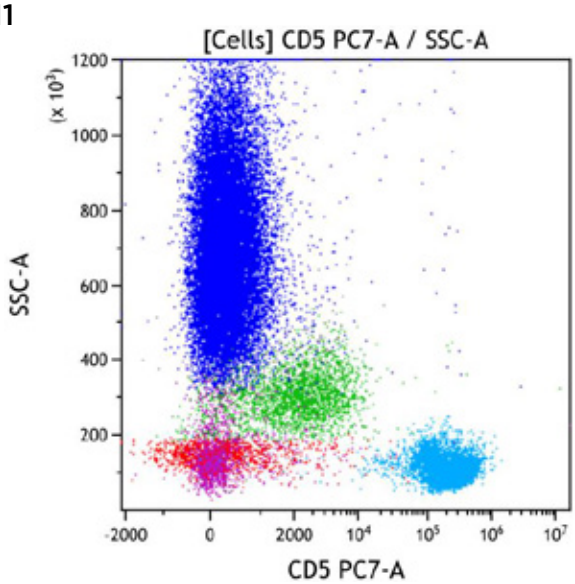

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

T12

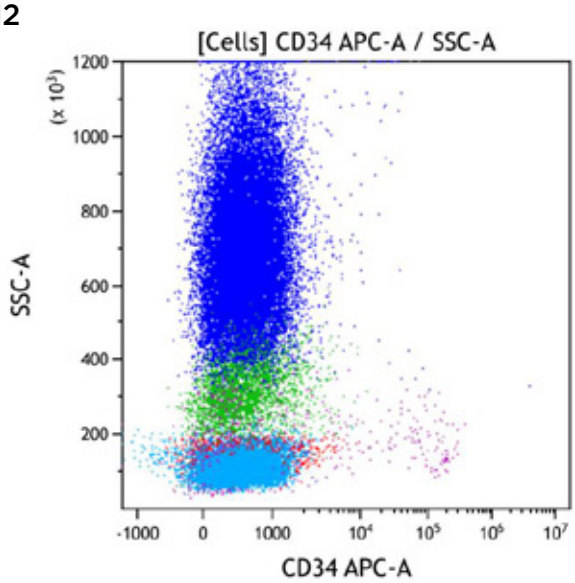

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

BACK TO CASE OVERVIEW

T13

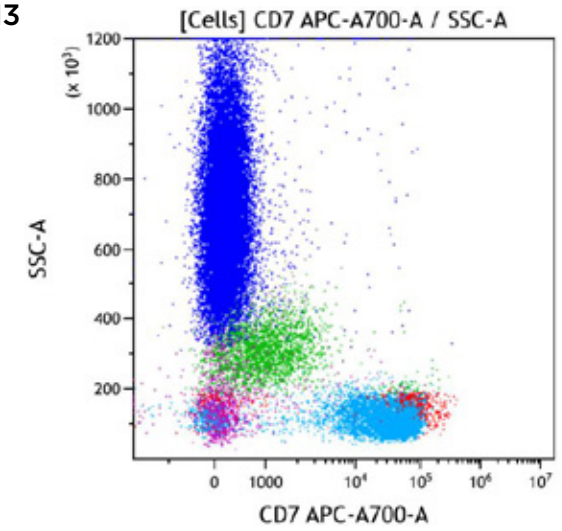

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage committed CD34 positive progenitors.

T14

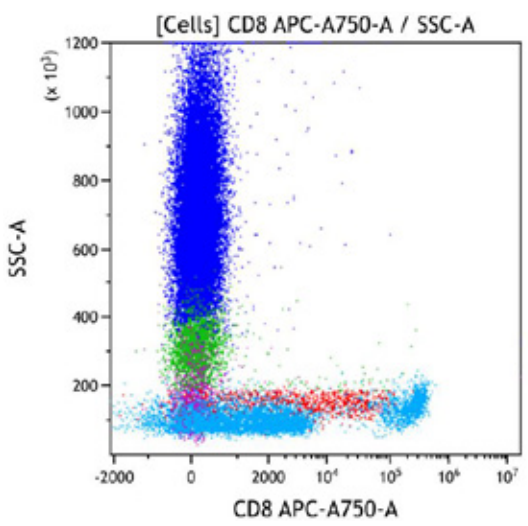

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

T15

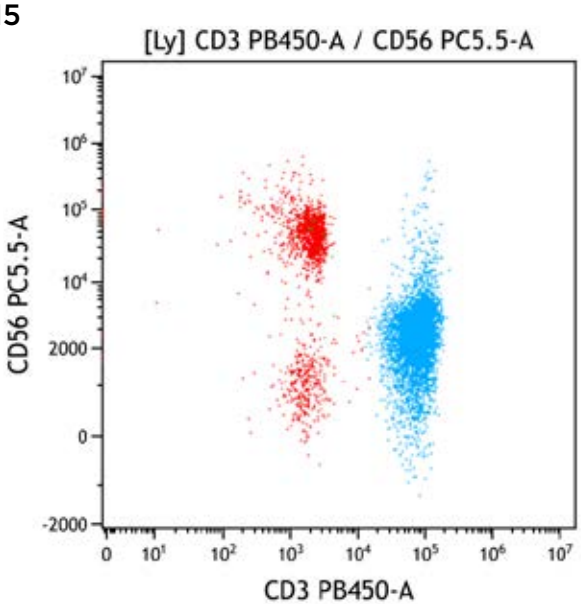

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate. T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red). Small subsets of mature T cells also express CD56, in particular T cells with natural killer activity (NK/T cells) and gamma-delta T cells.

T16

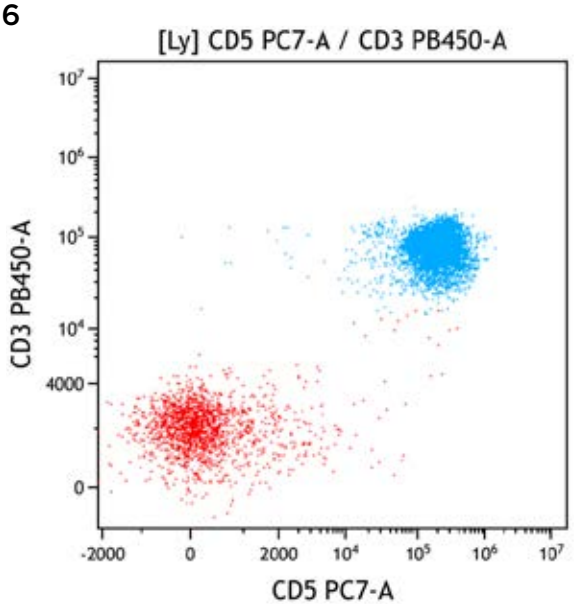

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate. CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells (red).

BACK TO CASE OVERVIEW

T17

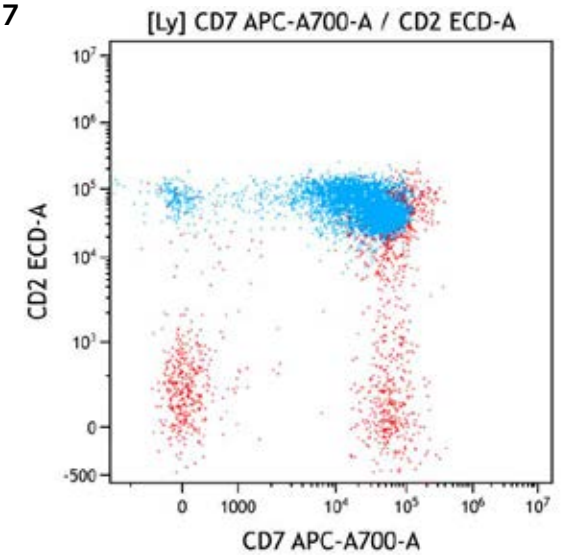

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate. CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

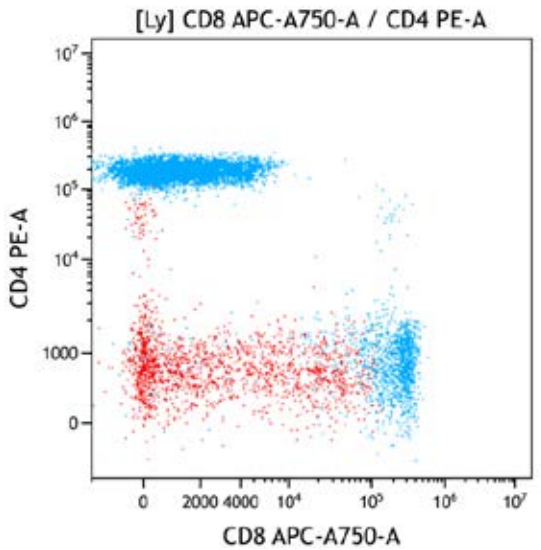

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate. CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional CD4 and CD8 double negative or double positive T cells are also present. The double negative T cells typically consist mostly of gamma/deltaT 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.

T19

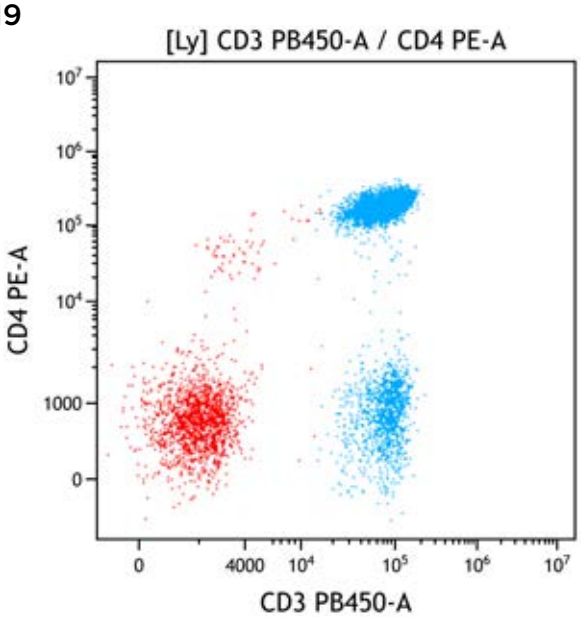

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate. All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

T20

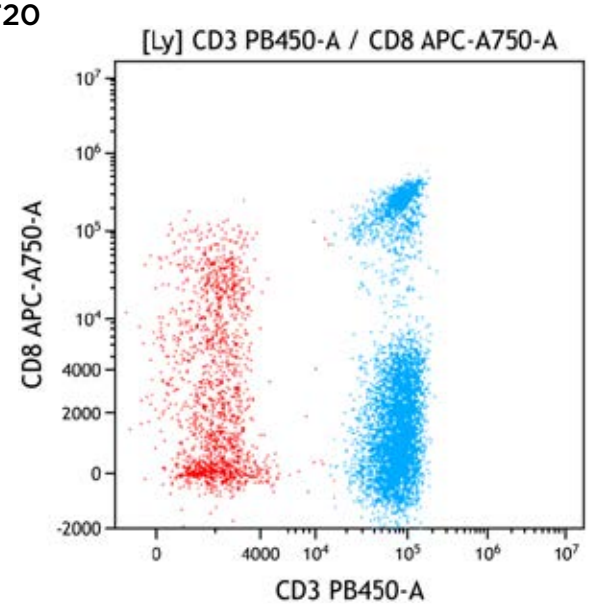

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate. All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

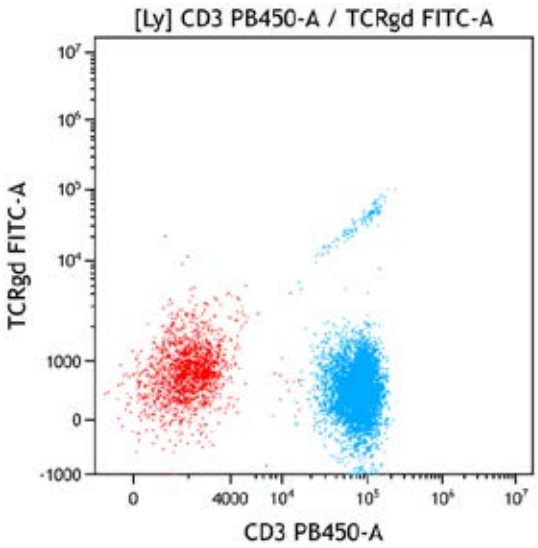

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate. A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

BACK TO CASE OVERVIEW

M1 Cell Tube

M1-01

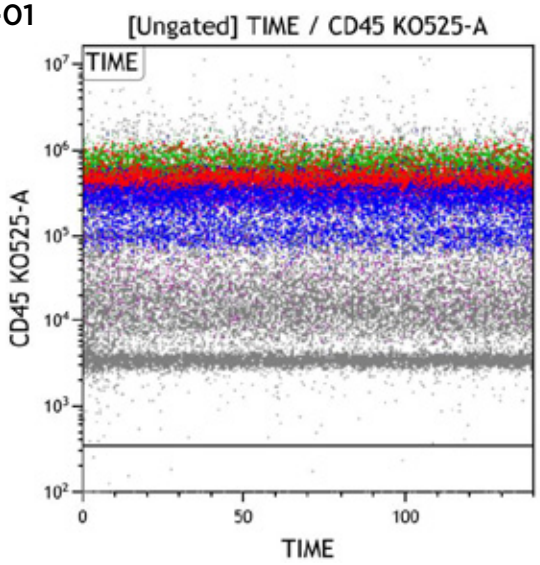

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be included in the Time gate.

M1-02

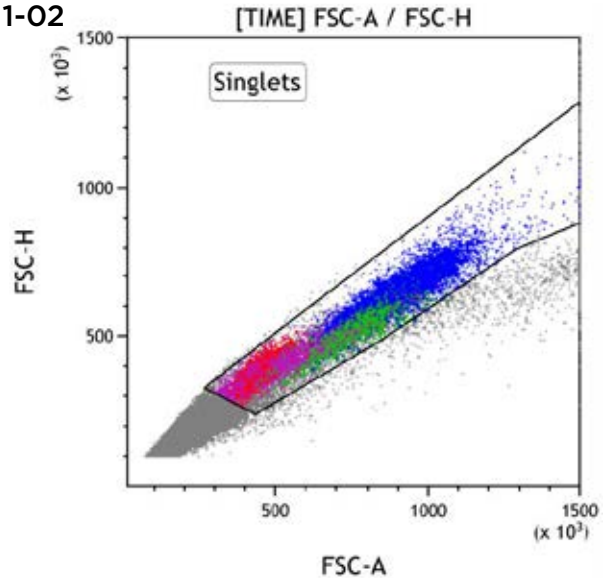

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

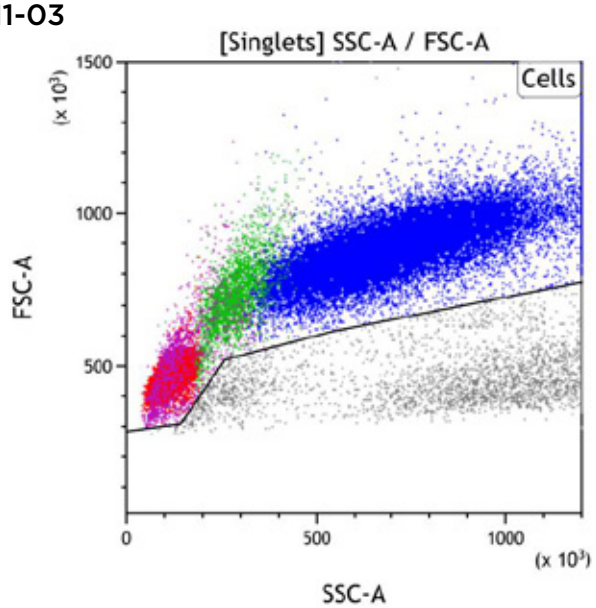

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

M1-04

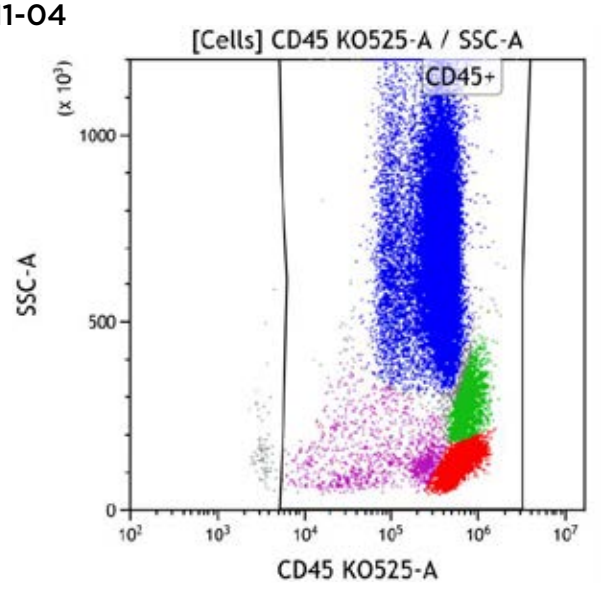

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M1-05

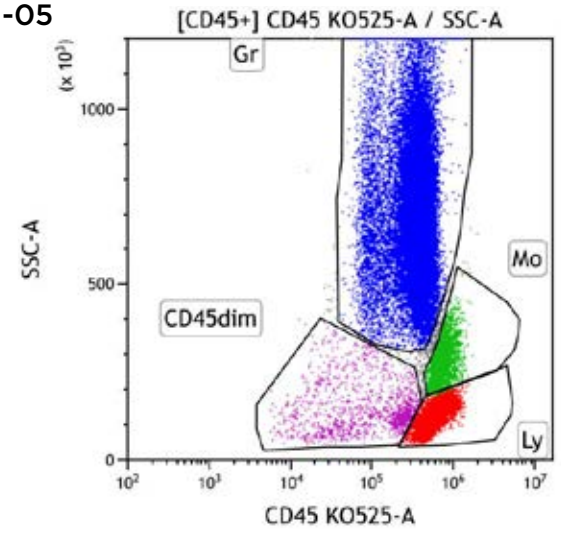

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M1-06

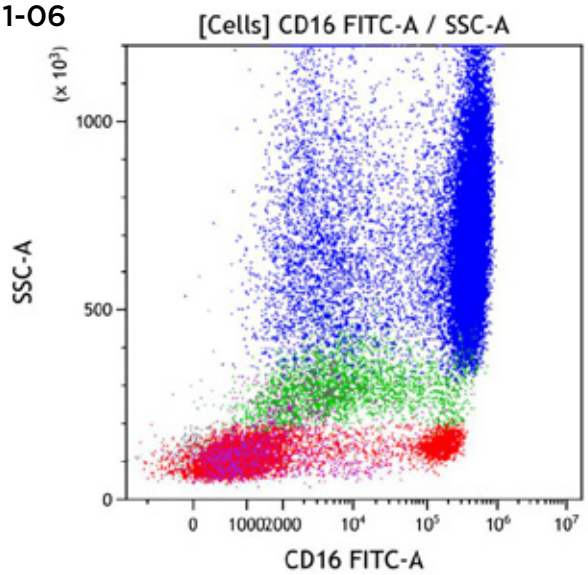

Figure 6. This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

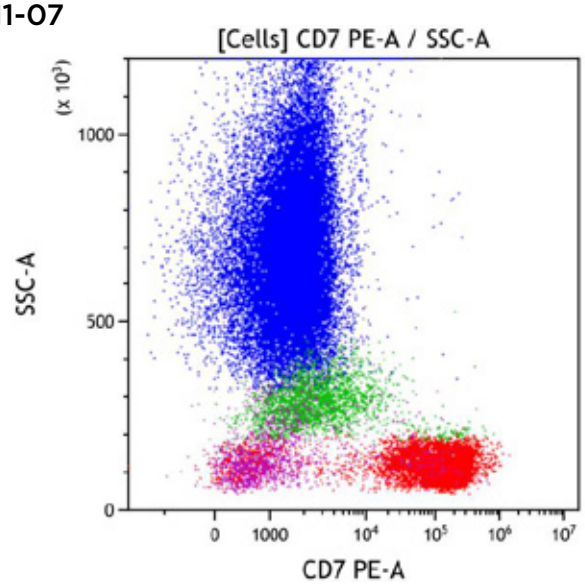

Figure 7. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of the lineage-committed progenitors (purple).

M1-08

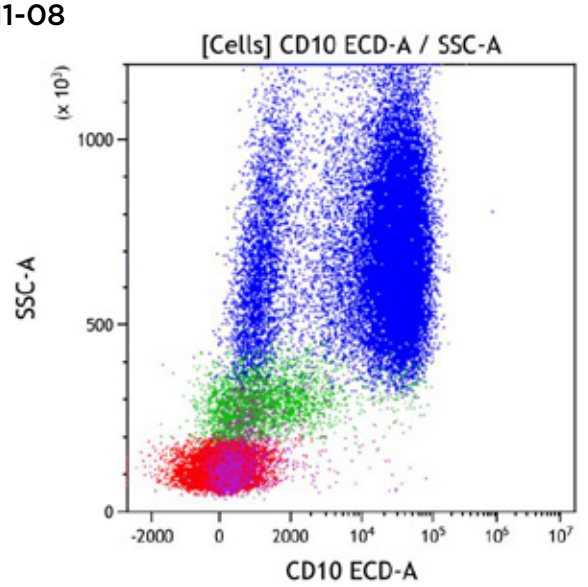

Figure 8. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells (purple), mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

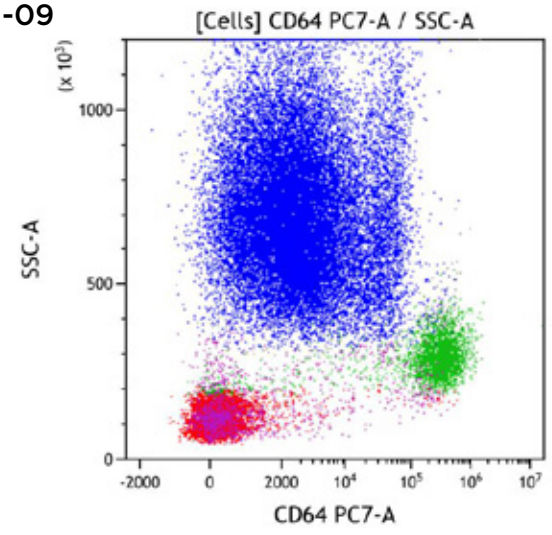

Figure 9. This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34+ progenitors.

M1-10

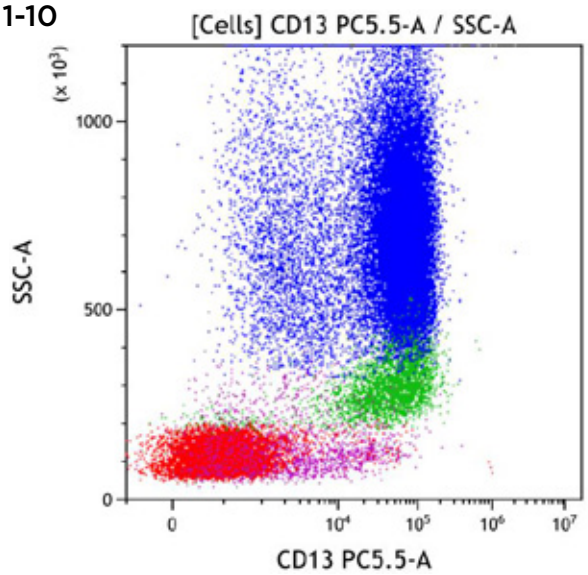

Figure 10. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M1-11

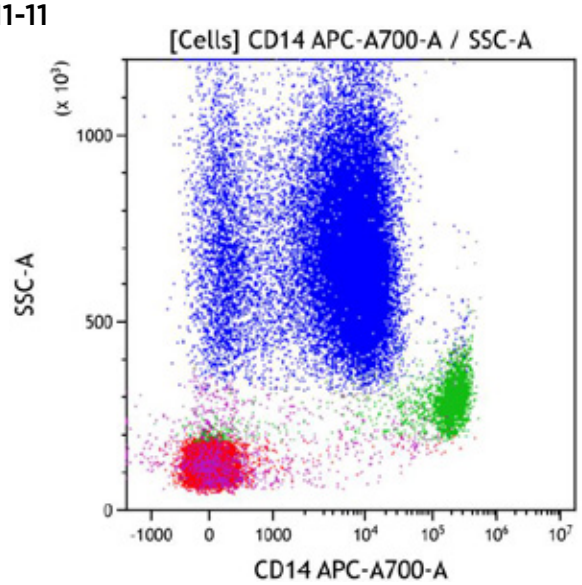

Figure 11. This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level.

M1-12

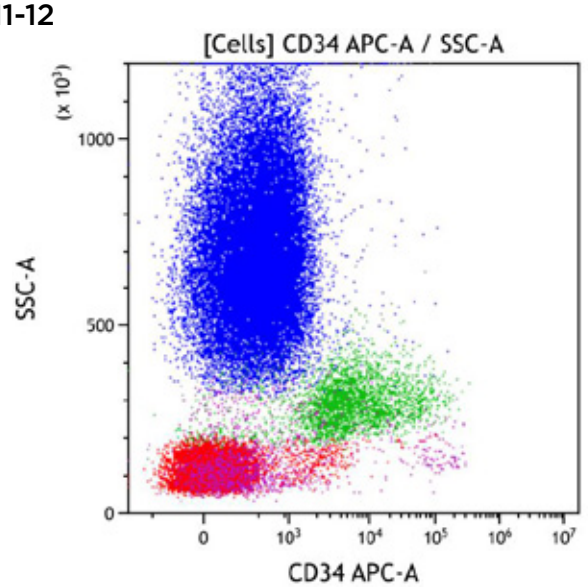

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

BACK TO CASE OVERVIEW

M1-13

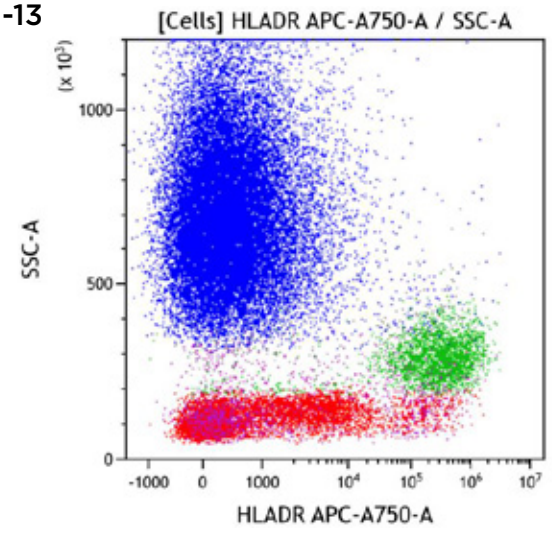

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34+ progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

M1-14

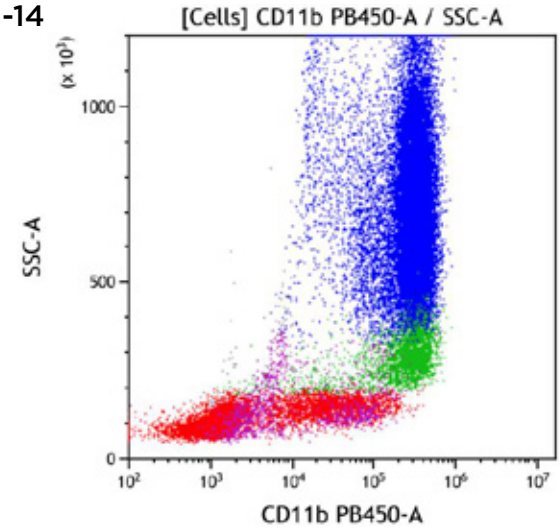

Figure 14. This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils (purple).

M1-15

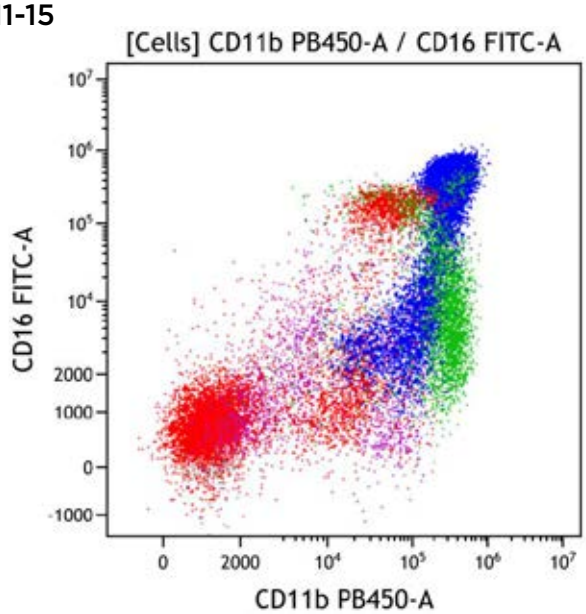

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), maturing granulocytes (blue), NK cells (red) and basophils (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, most promyelocytes 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.

M1-16

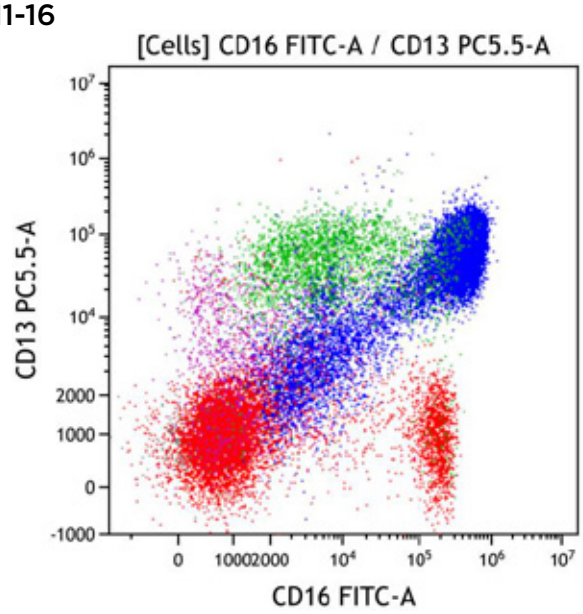

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34+ progenitors (purple). CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red). During granulocytic maturation, CD13 is expressed 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).

BACK TO CASE OVERVIEW

M1-17

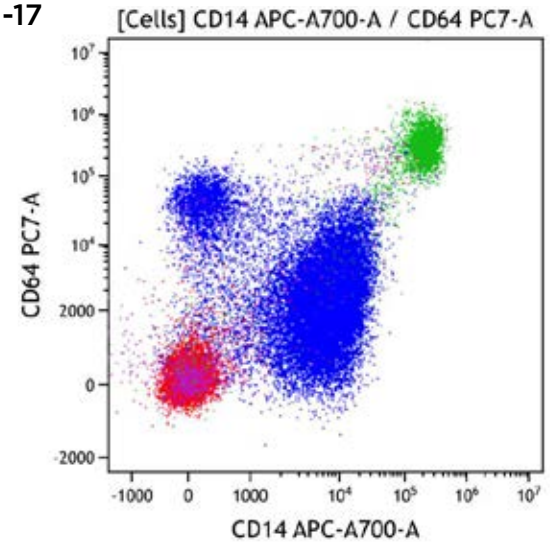

Figure 17. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on maturing granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes (blue). Immature monocytes show high 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).

M1-18

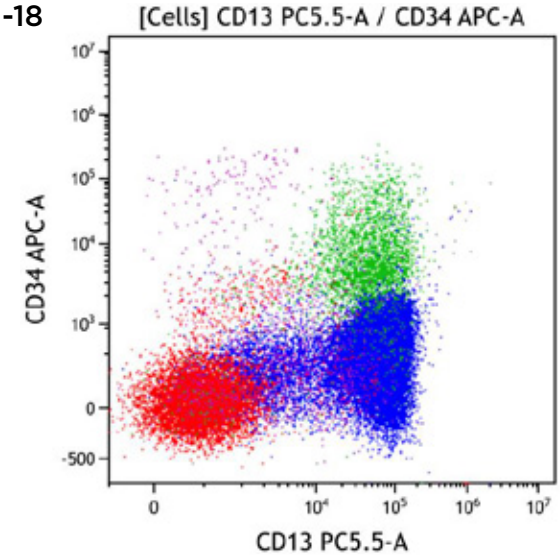

Figure 18. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors (purple) or mature lymphoid cells (red).

M1-19

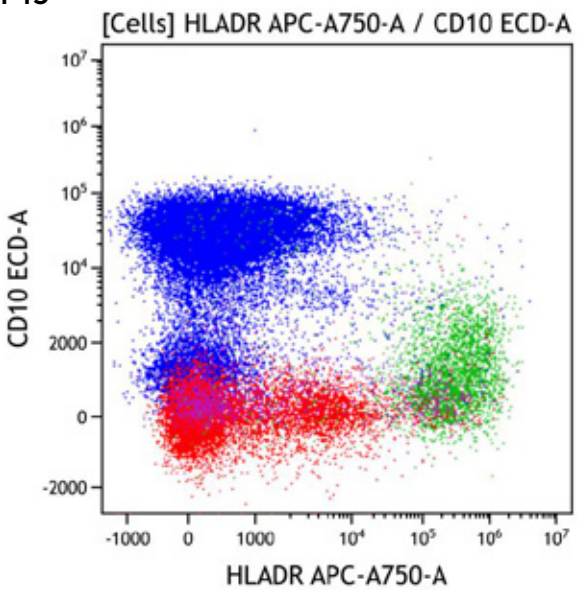

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34+ progenitors (purple). CD10 is expressed by mature granulocytes (blue) and immature B cells (purple). Immature B cells express both CD10 and HLA-DR (purple).

M1-20

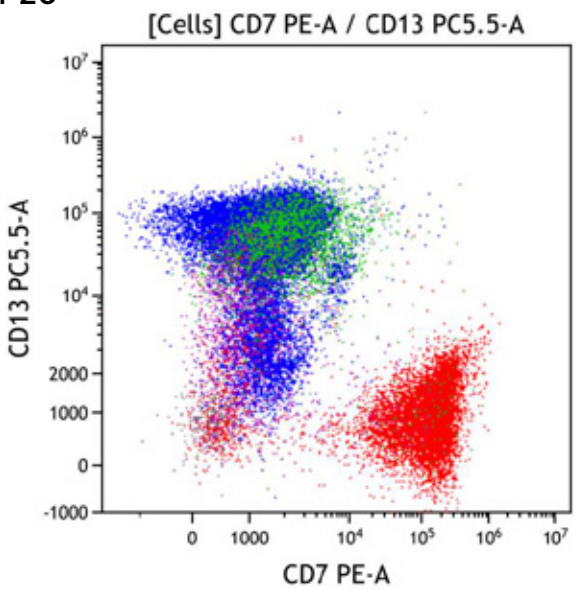

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on maturing granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors (purple). Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

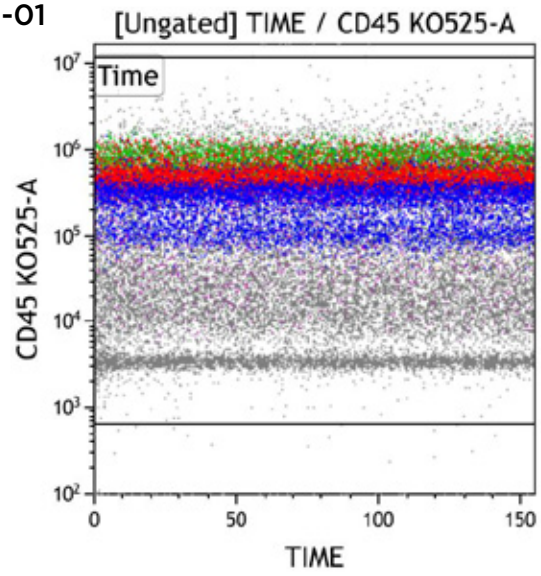

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be included in the Time gate.

M2-02

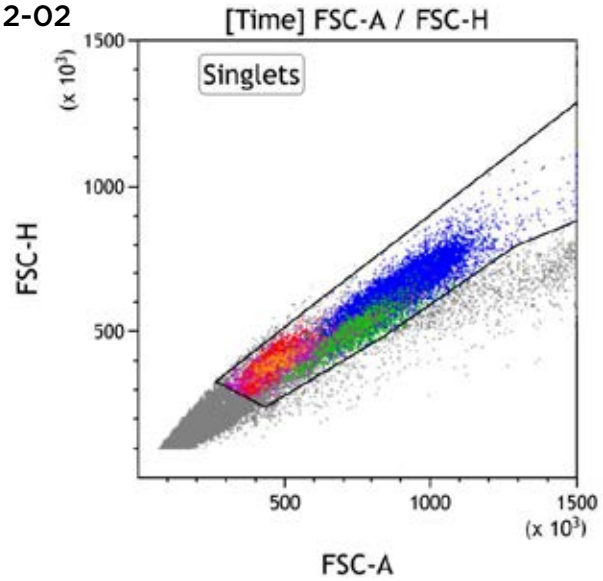

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

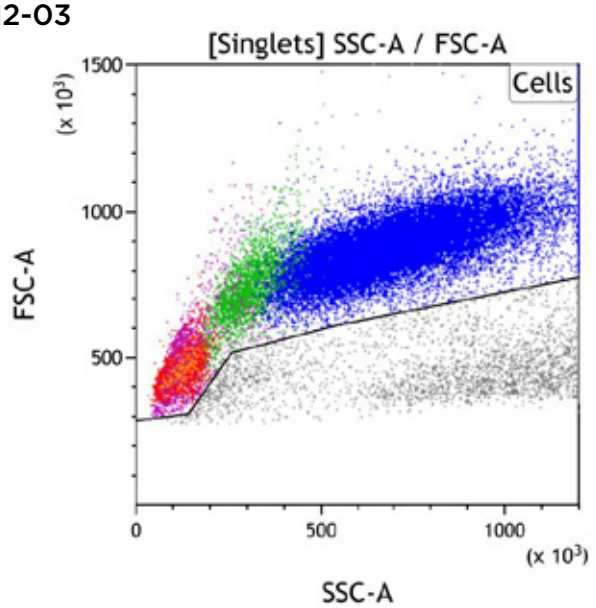

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

M2-04

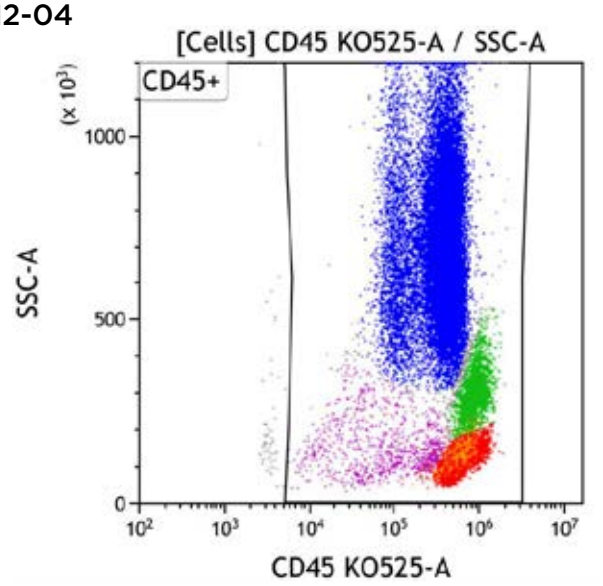

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (gray colored events) usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M2-05

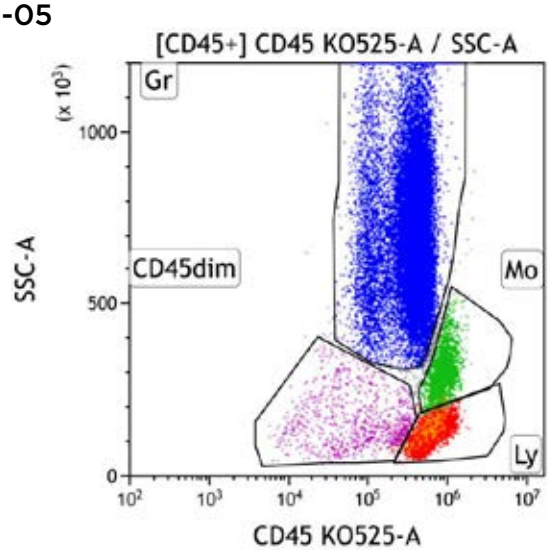

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45 dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M2-06

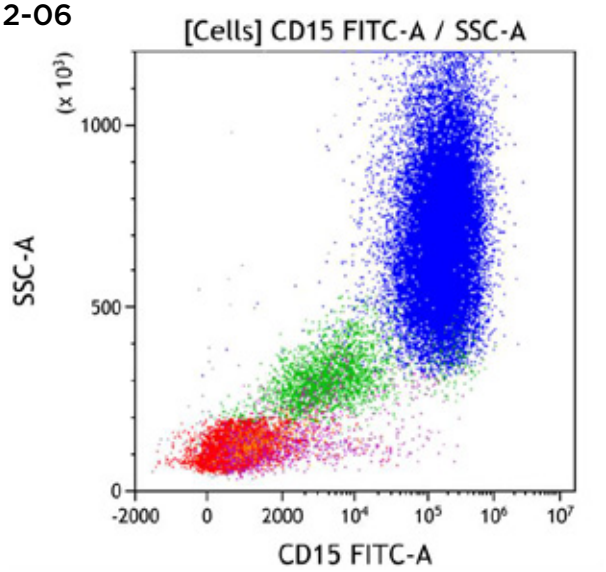

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes (green).

M2-07

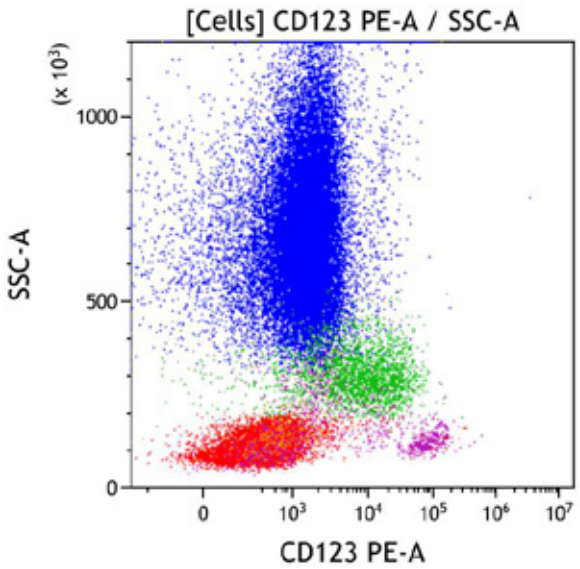

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells (purple) and at a lower level on CD34 positive myeloid progenitors (purple) and monocytes (green).

M2-08

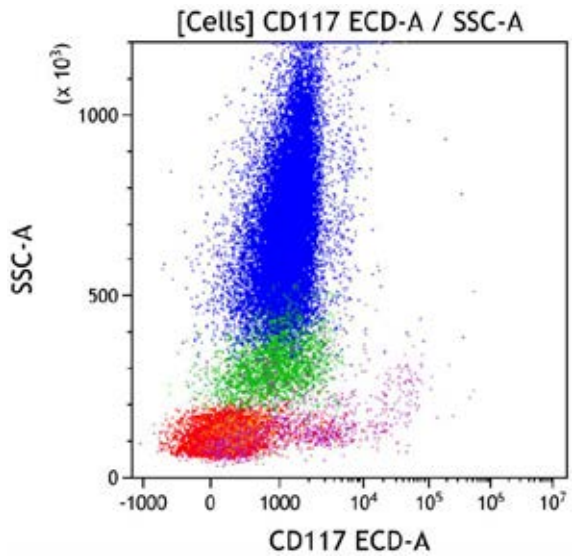

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34+ myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells.

BACK TO CASE OVERVIEW

M2-09

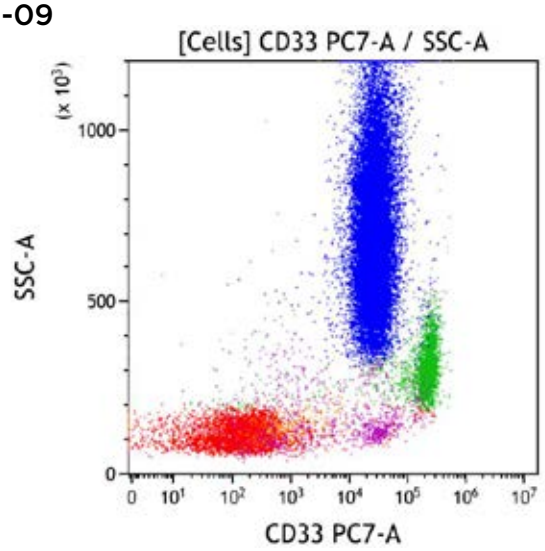

Figure 9. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its high level on immature and mature monocytes (green), at a slightly lower level on immature granulocytes (blue), and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34+ myeloid progenitors (purple).

M2-10

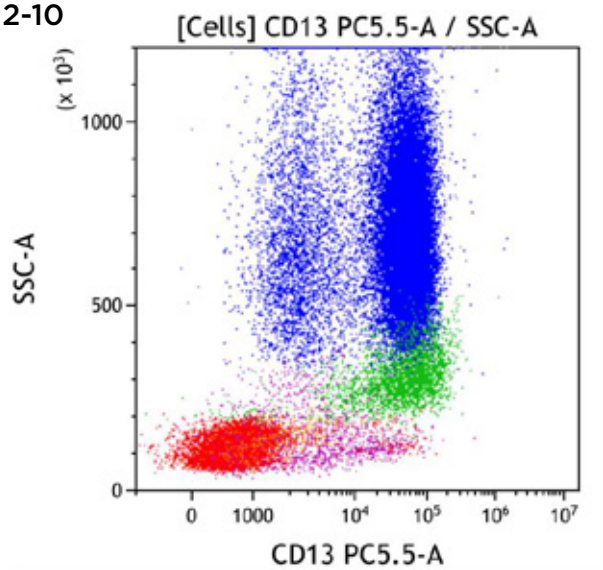

Figure 10. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature 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).

M2-11

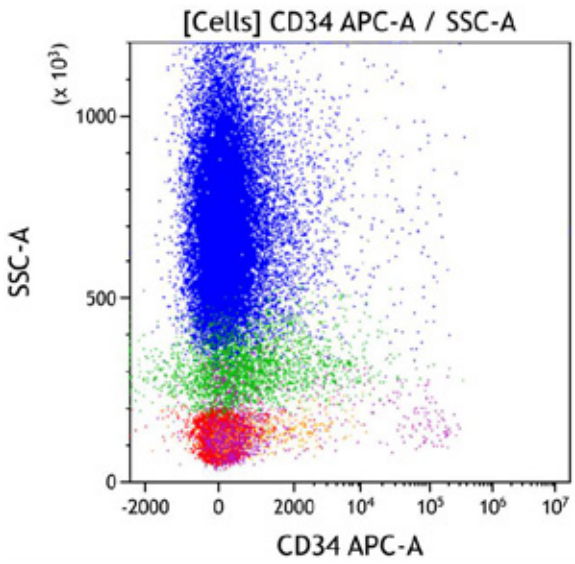

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34-positive progenitors typically have low to intermediate side scatter in the CD45 dim gate (purple) with immature B cell progenitors having lower side scatter than immature myeloid progenitors.

M2-12

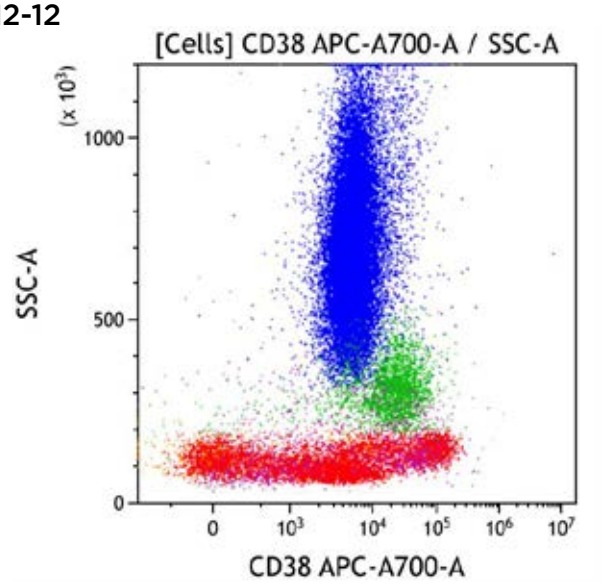

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors (purple), at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red). CD34 positive hematopoietic stem cells express CD38 at variably low to absent level.

BACK TO CASE OVERVIEW

M2-13

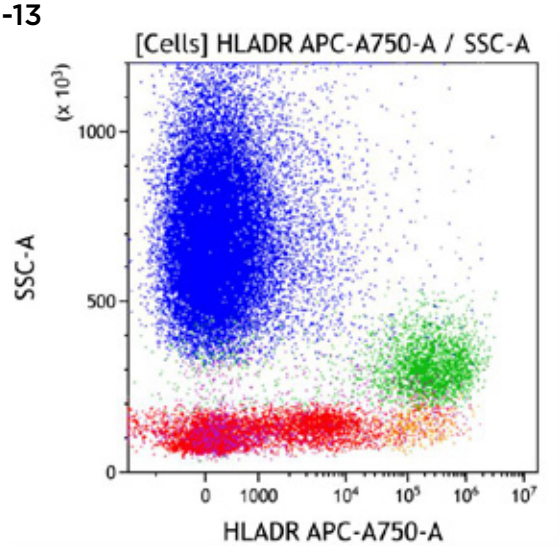

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors (purple), mature (red) and immature (purple) B cells, and activated T cells (red).

M2-14

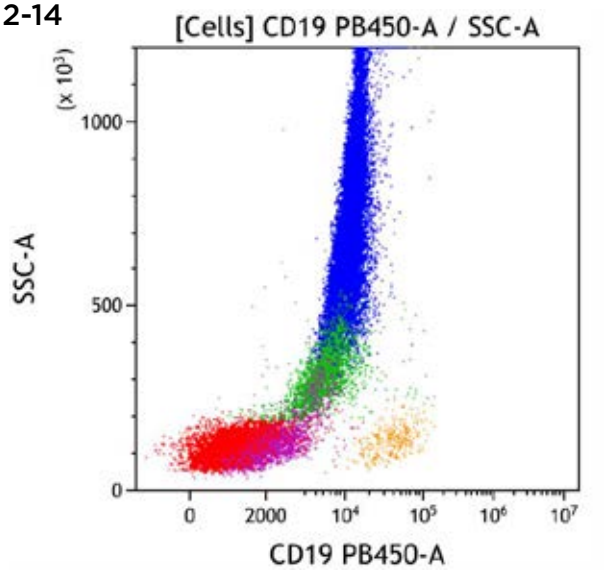

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

M2-15

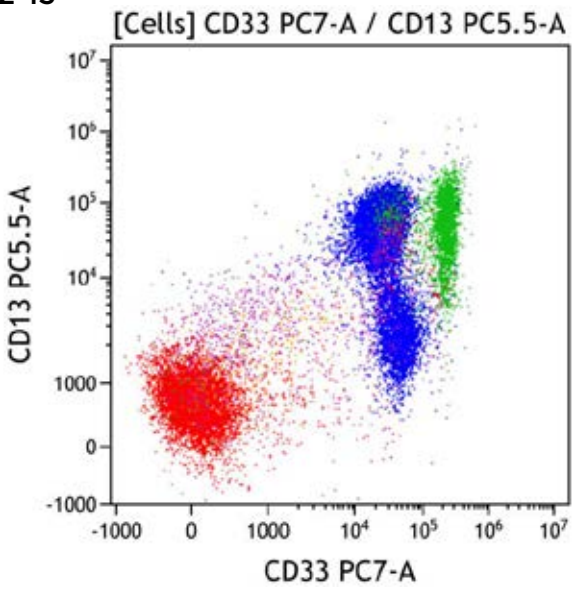

Figure 15. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), maturing granulocytes (blue), basophils, and CD34 positive progenitors (purple). Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 (blue bottom) than more mature granulocytes (blue left). Lymphocytes largely do not express either CD13 or CD33 (red).

M2-16

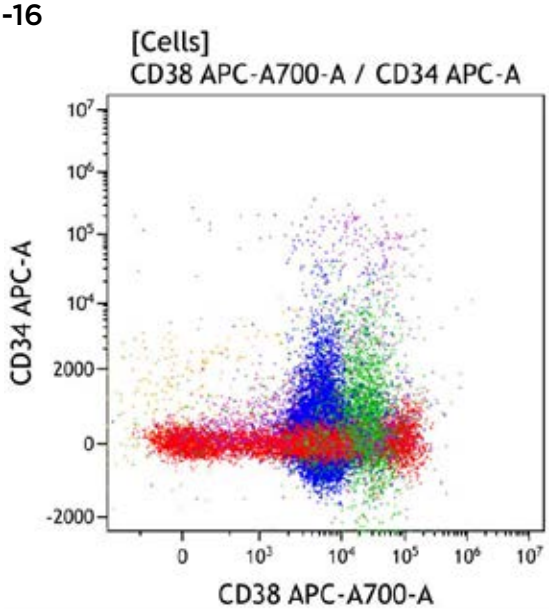

Figure 16. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34 (purple). Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38 (purple, not many in this sample). The apparent variable 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.

BACK TO CASE OVERVIEW

M2-17

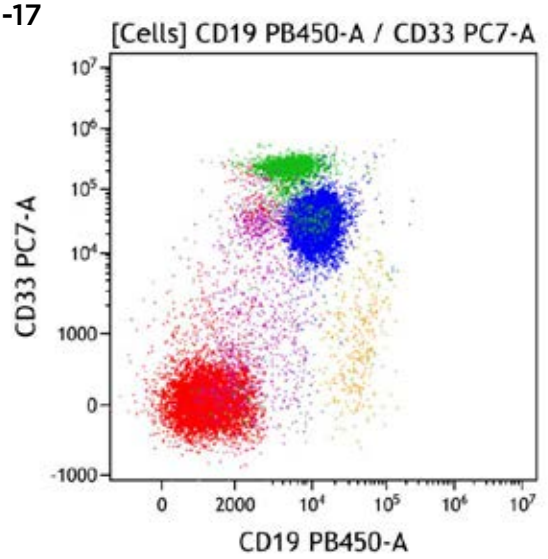

Figure 17. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). B cells do not normally express significant CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

M2-18

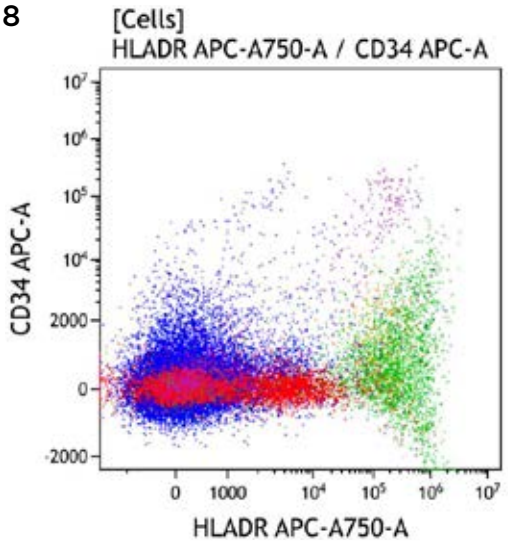

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLADR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34+ progenitors (purple). CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR (purple) with the highest level of HLA-DR seen on early monocytes (purple right).

M2-19

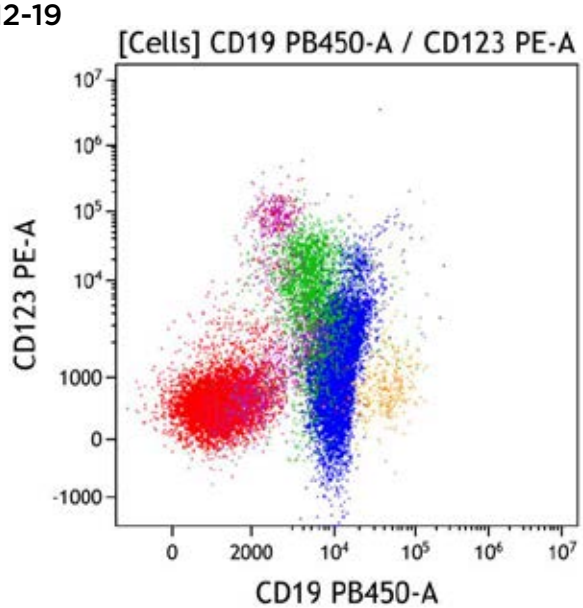

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed by B cells. CD123 is expressed by basophils, plasmacytoid dendritic cells, monocytes (green) and CD34+ progenitors (purple). CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid dendritic cells do not expression CD19. The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

M2-20

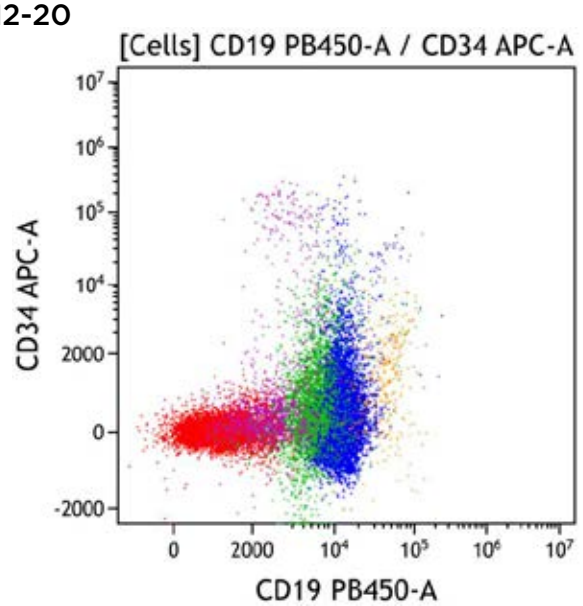

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34+ myeloid progenitors do not express CD19.

BACK TO CASE OVERVIEW

M2-21

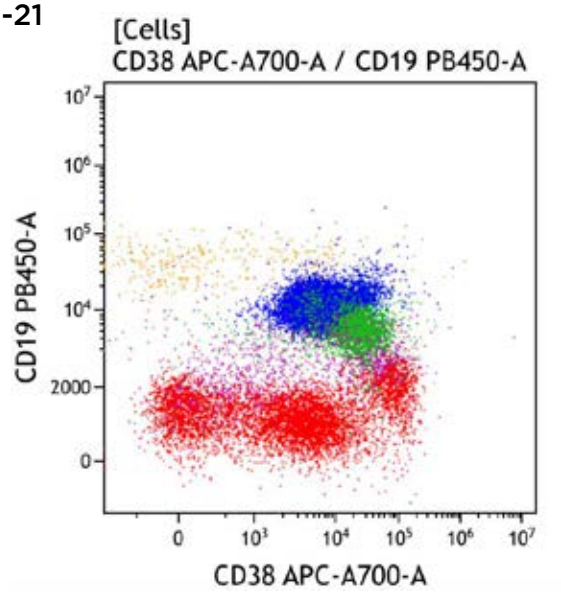

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors (purple). Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38 (purple). Mature CD19+ B cells show lower expression 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.

M2-22

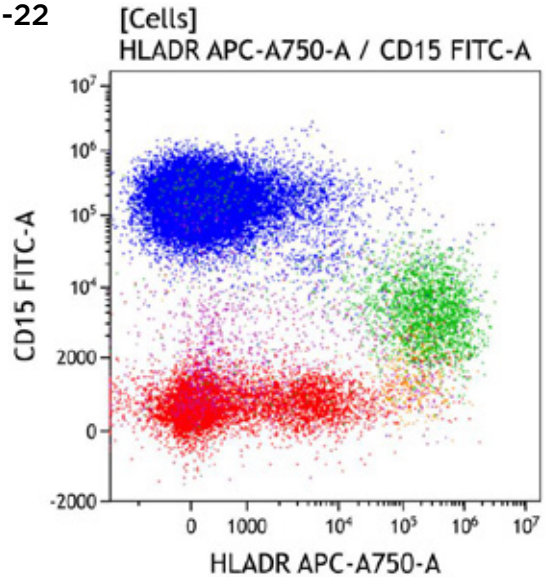

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by maturing granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors (purple). Maturing granulocytes do not express HLADR (blue), except the earliest forms where CD15 is being acquired. CD34+ myeloid progenitors (purple) express HLA-DR but only transiently express CD15.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 14 LHS-TC4-039**
Diagnosis	**Normal peripheral blood**
Clinical Vignette	This is a 3-year-old female. A peripheral blood sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations in this case. Not that correlation with clinical and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay.

BACK TO CASE LISTINGS

B Cell Tube

B01

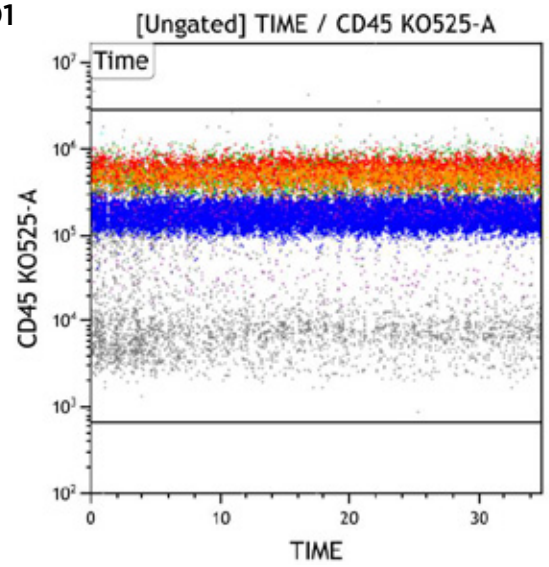

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

B02

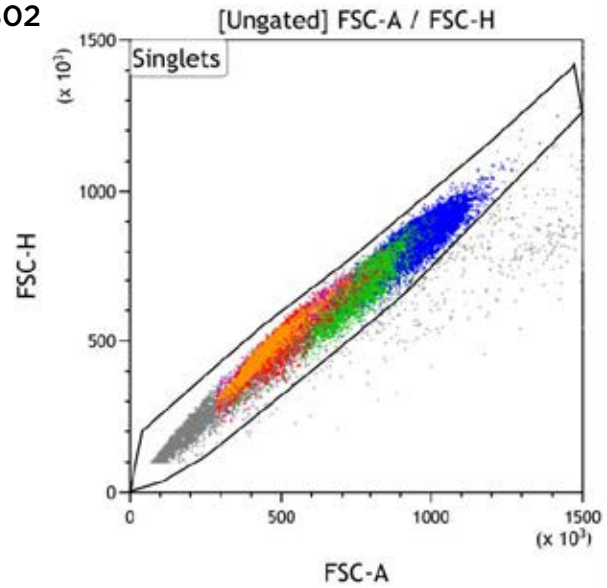

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

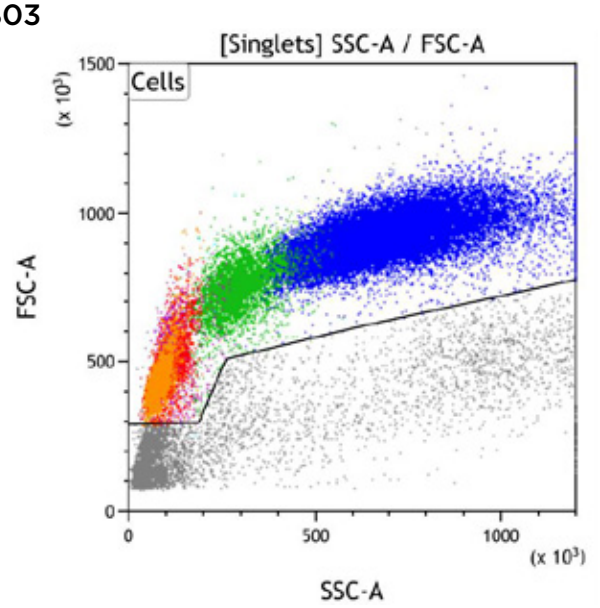

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

B04

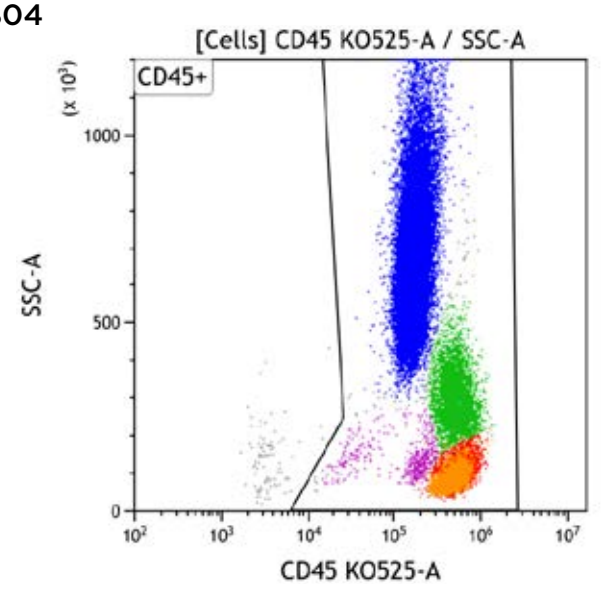

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population (Gray colored events) usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

B05

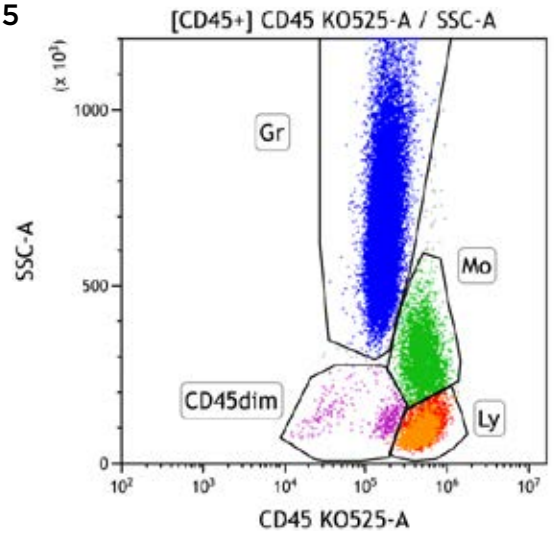

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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 followed throughout the analysis.

B06

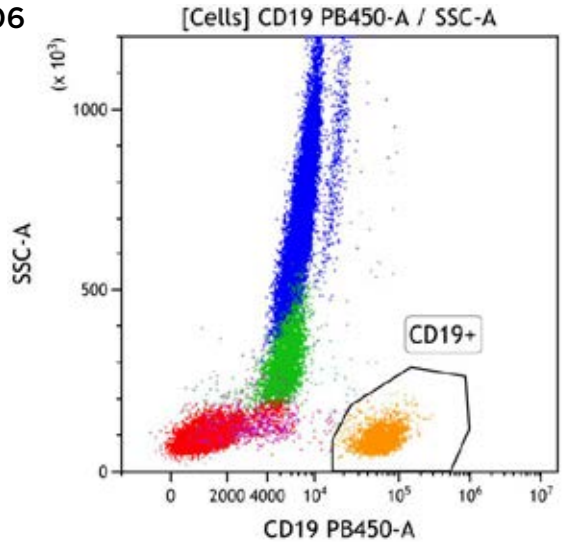

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B07

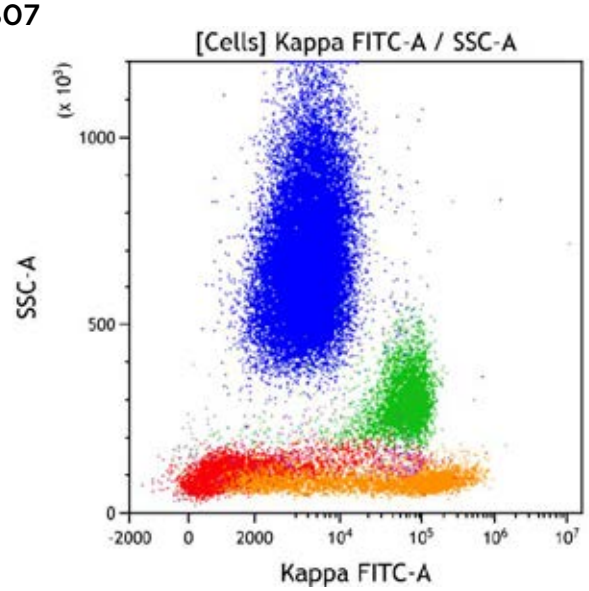

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity 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.

B08

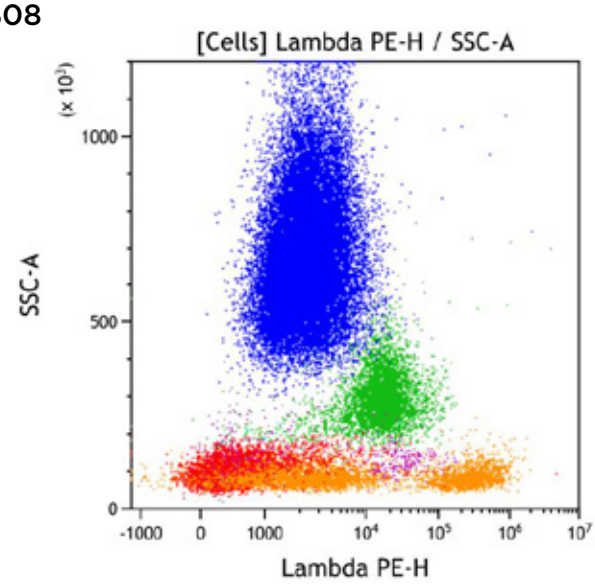

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity 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.

BACK TO CASE OVERVIEW

B09

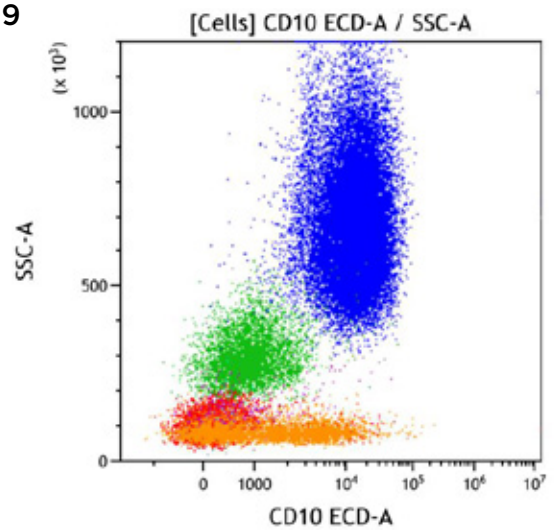

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

B10

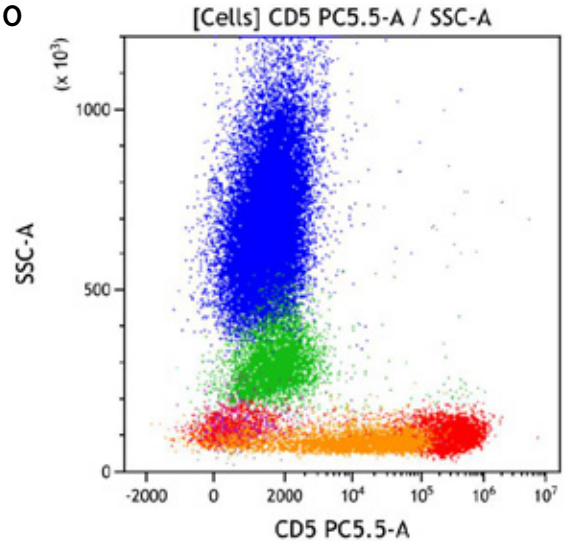

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on immature and mature T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter.

B11

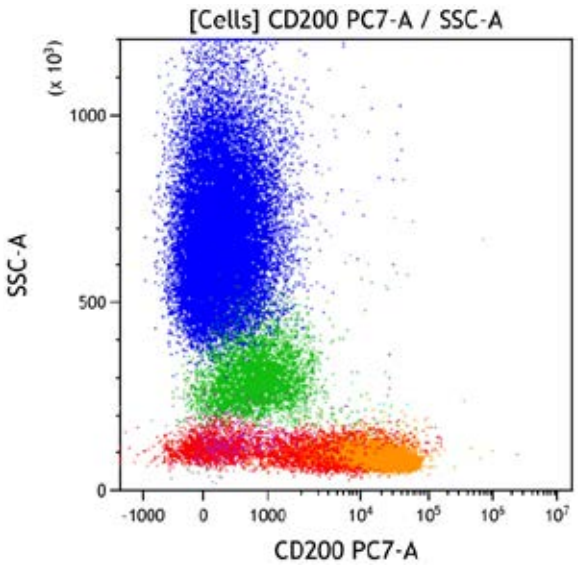

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

B12

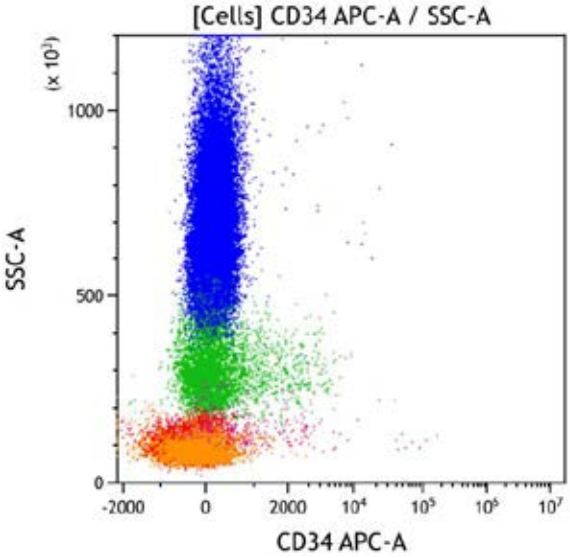

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

BACK TO CASE OVERVIEW

B13

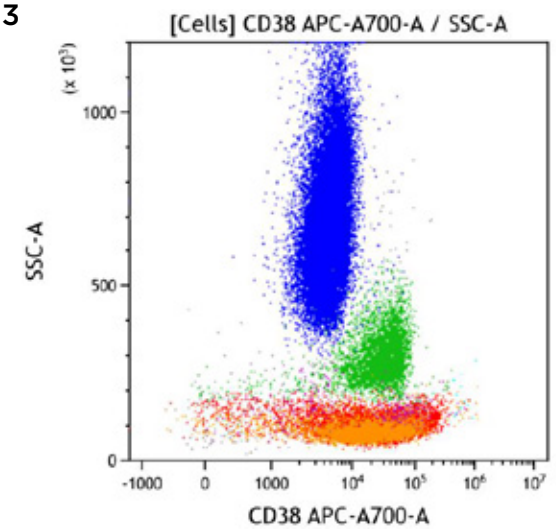

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably level on activated mature lymphocytes (red/orange).

B14

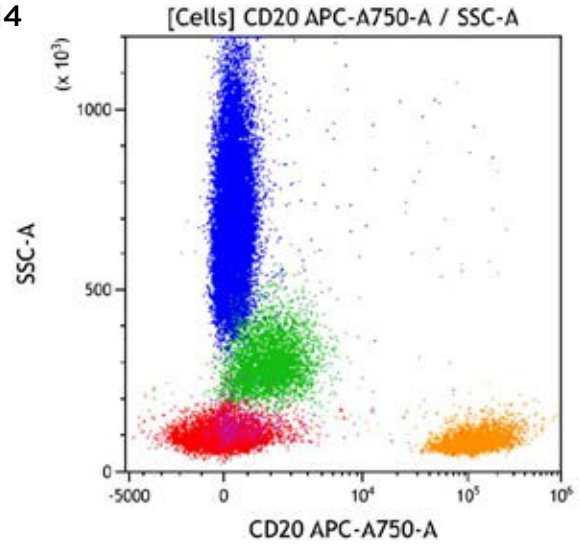

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.

B15

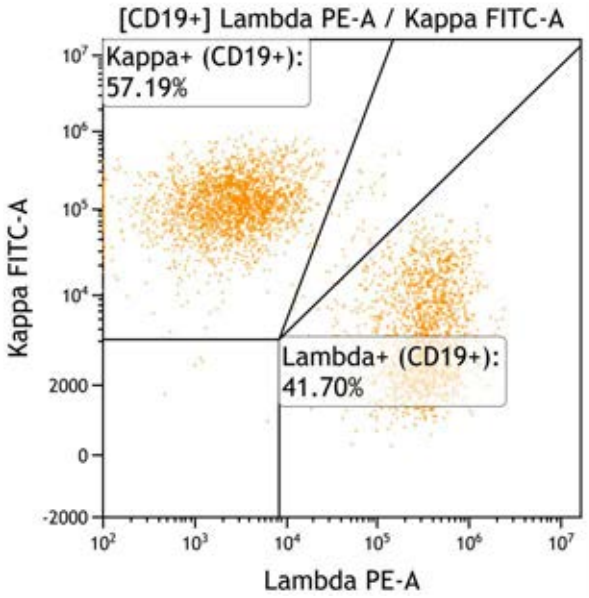

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

B16

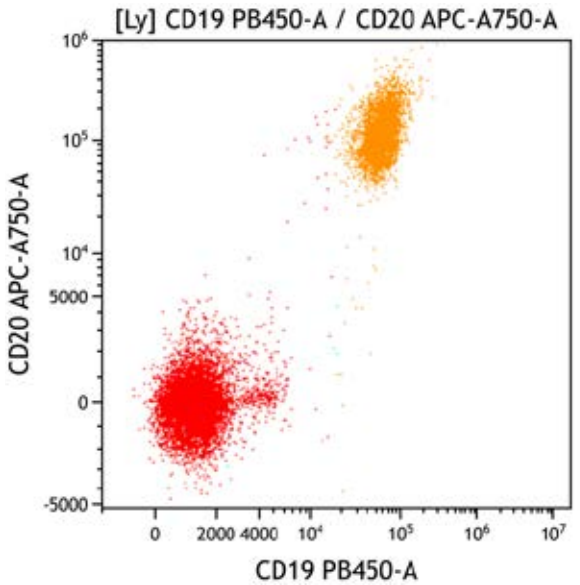

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

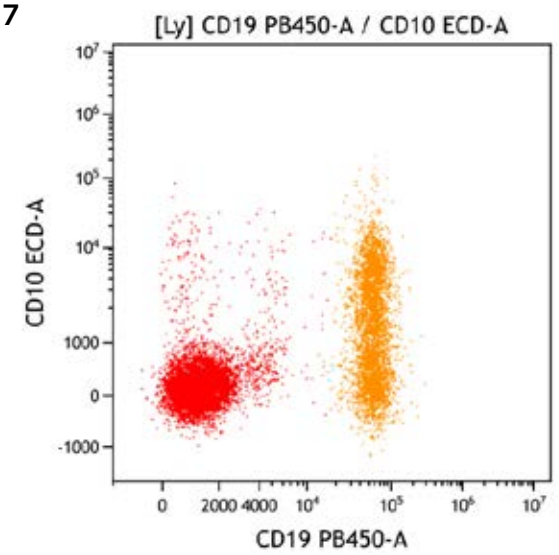

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of latestage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45dim gate.

B18

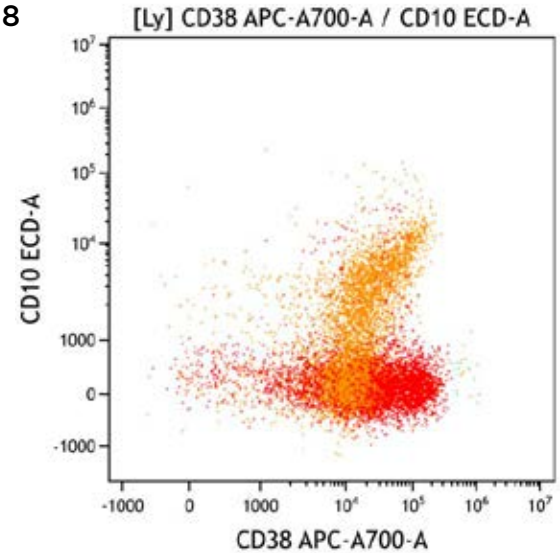

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low level expression of CD38. T cells (red) show variable CD38 expression dependent on activation state. The 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.

B19

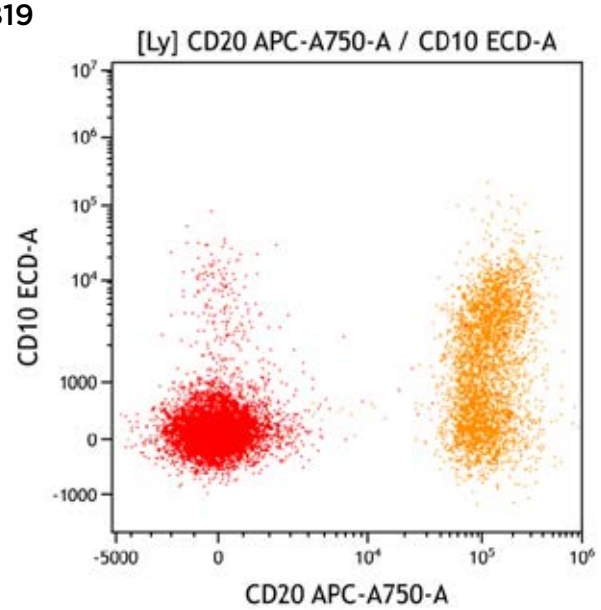

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20 without significant CD10. The few CD10 positive B cells represent late stage immature B cells.

B20

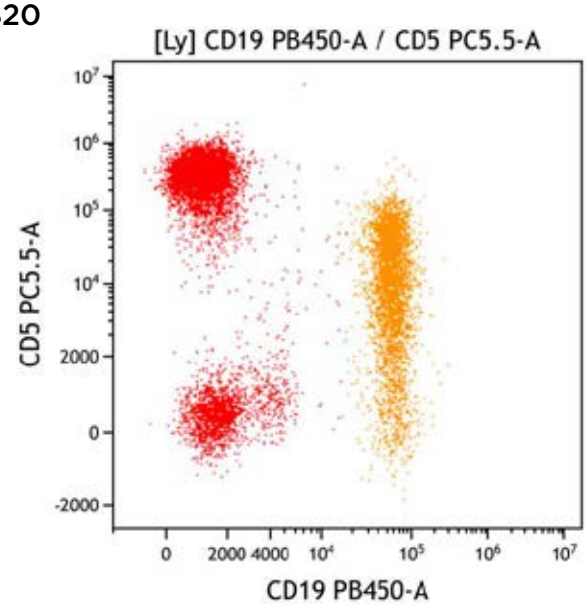

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

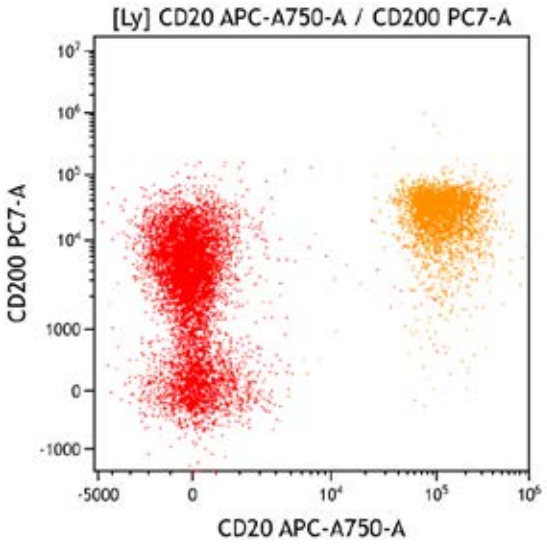

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

B22

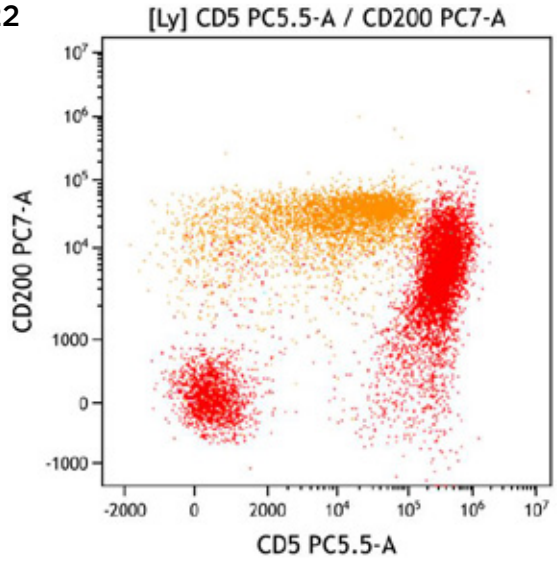

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

BACK TO CASE OVERVIEW

T Cell Tube

T01

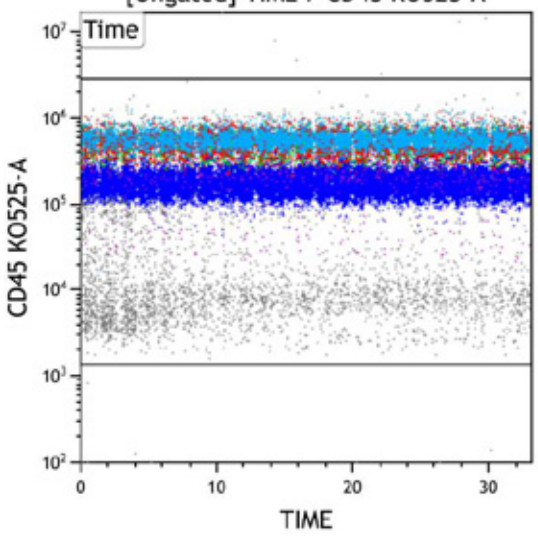

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

T02

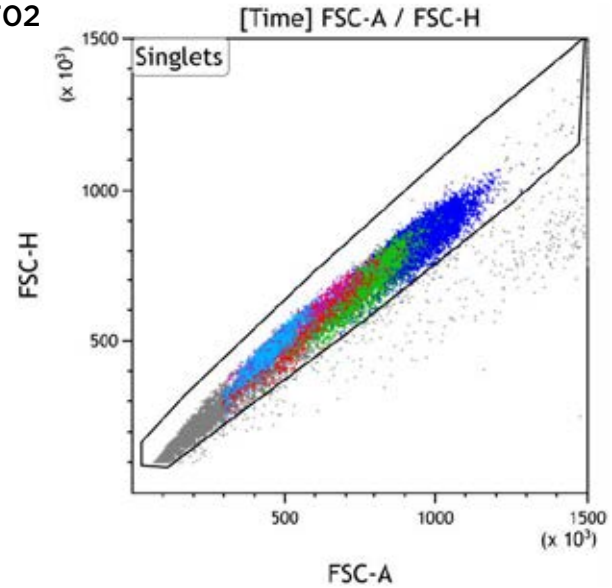

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

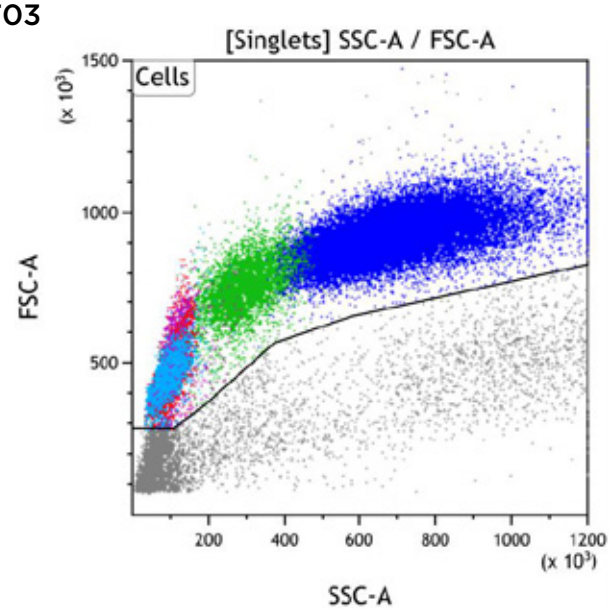

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

T04

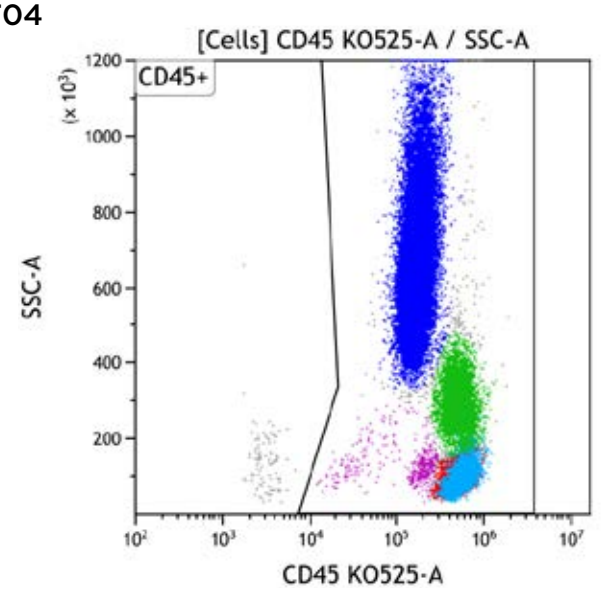

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

T05

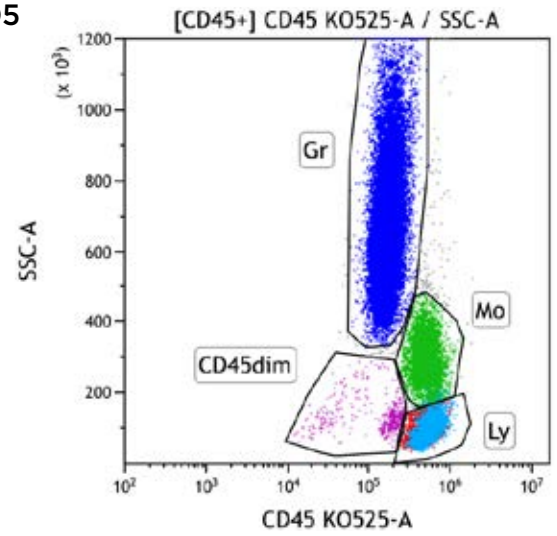

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

T06

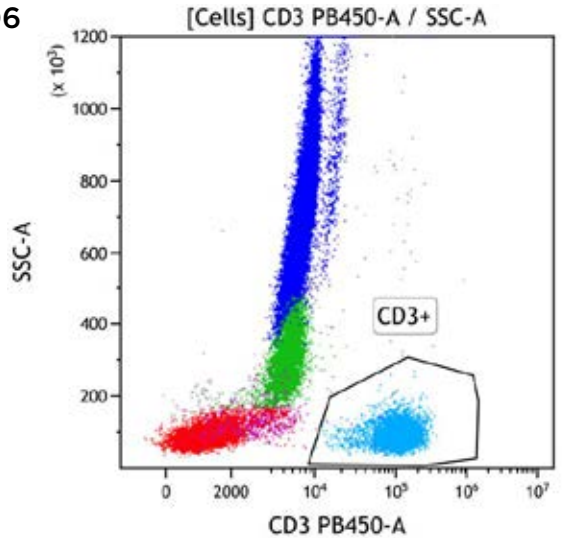

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

T07

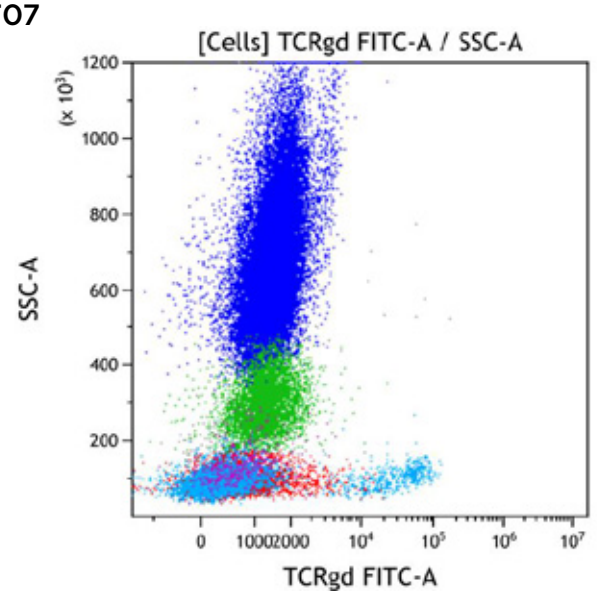

Figure 7. This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

T08

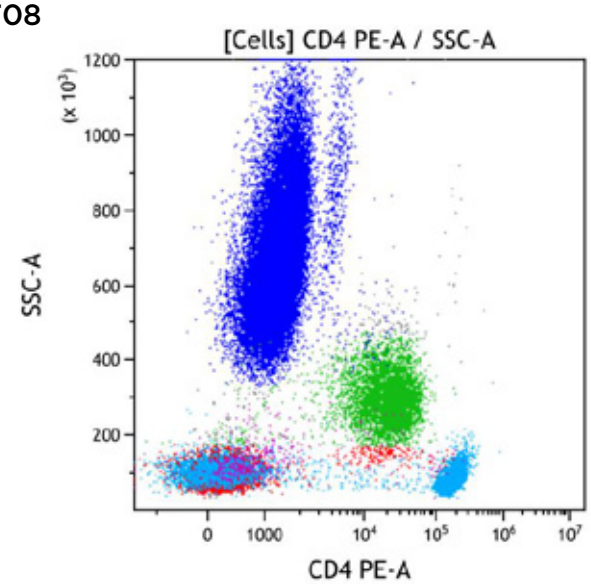

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells.

BACK TO CASE OVERVIEW

T09

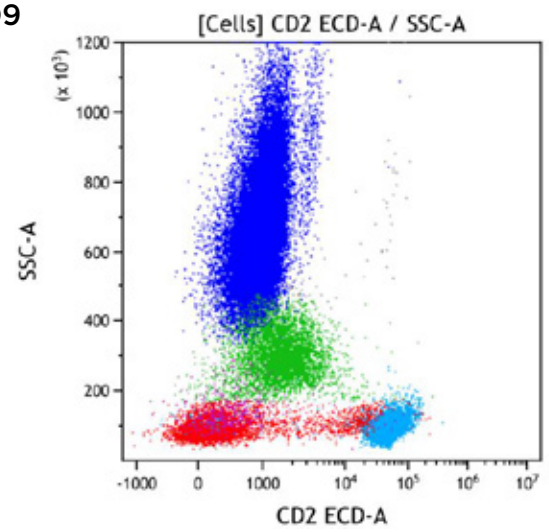

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

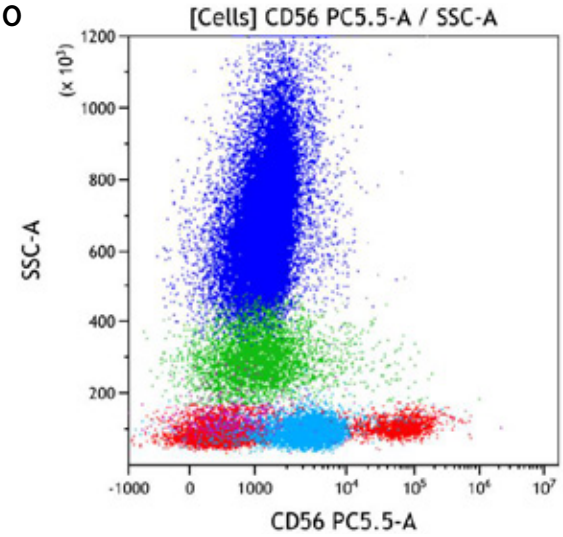

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD5 is also partially expressed on monocytes (green) in both reactive and neoplastic conditions.

T11

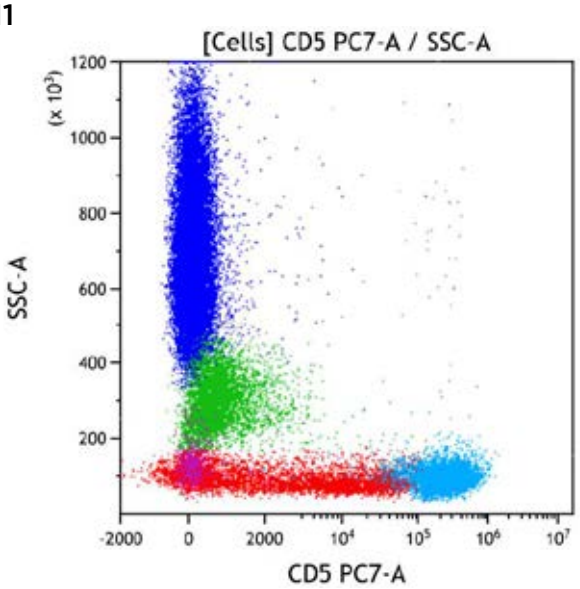

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

T12

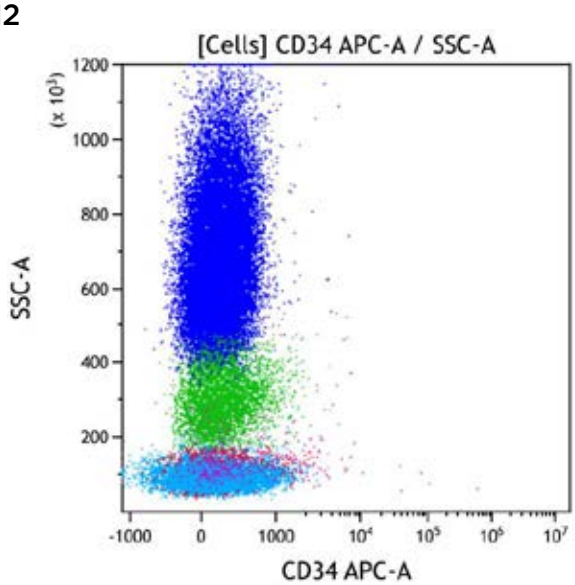

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

BACK TO CASE OVERVIEW

T13

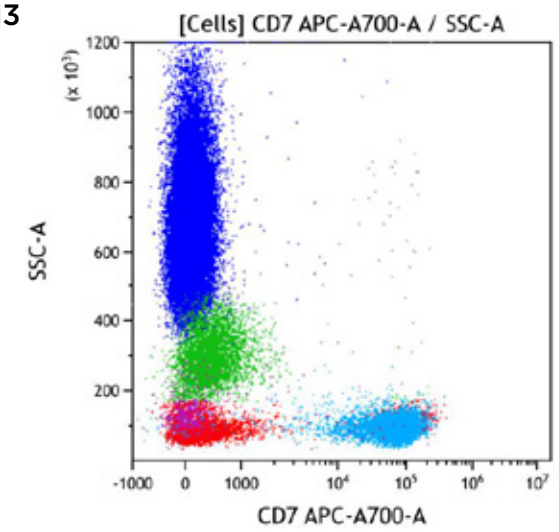

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of lineage committed CD34 positive progenitors.

T14

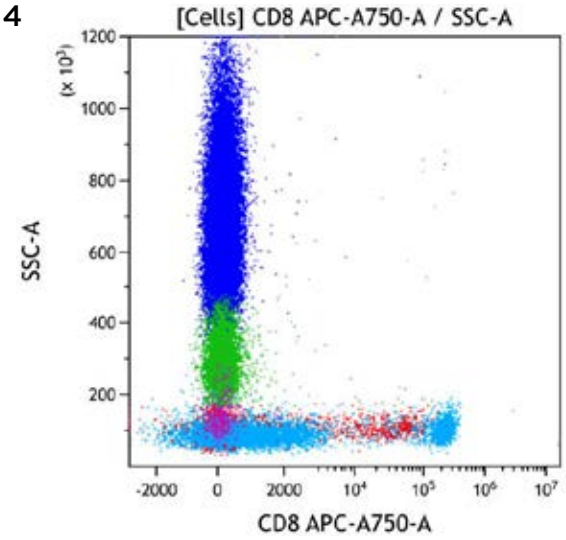

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells.

T15

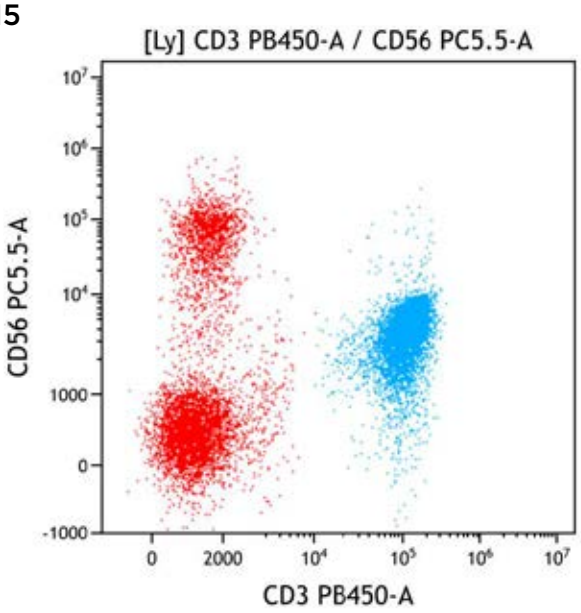

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

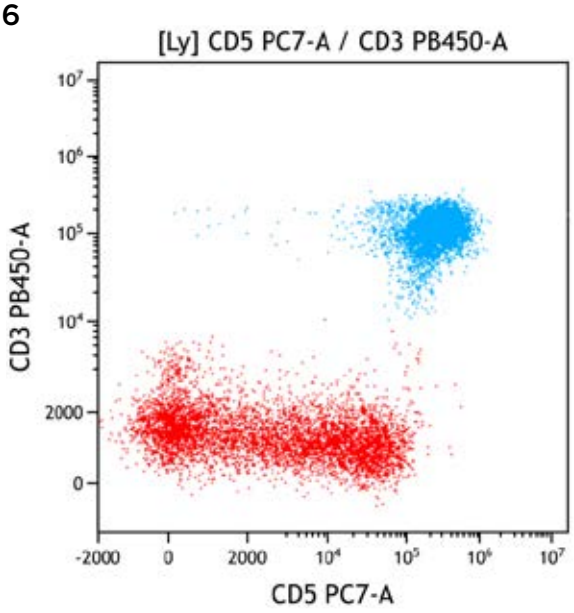

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells and B cells.

BACK TO CASE OVERVIEW

T17

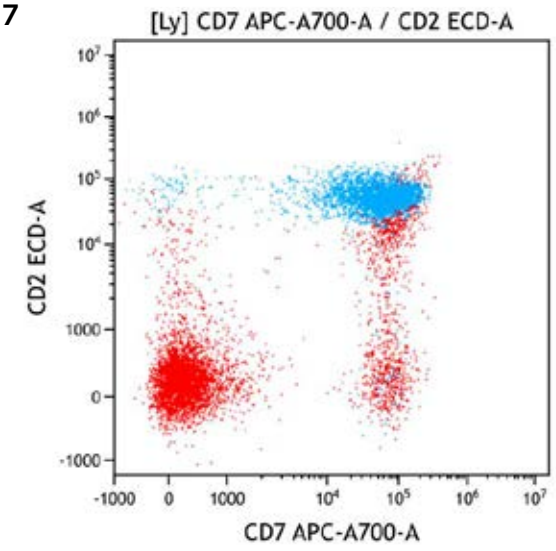

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

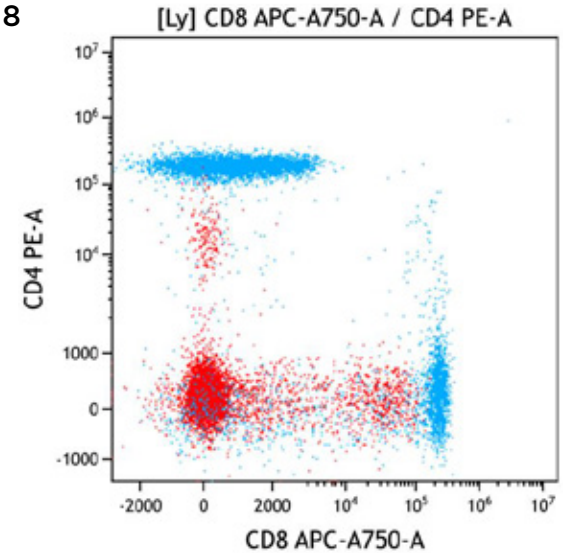

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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.

T19

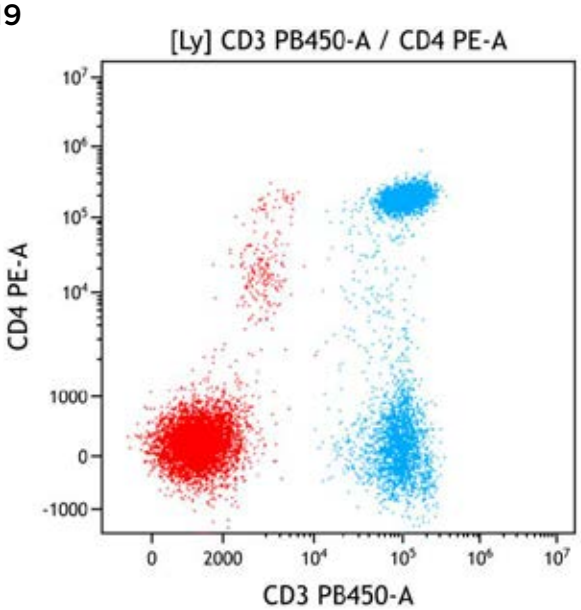

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells (red, lower left) lack expression of both CD3 and CD4. Of note, the few 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.

T20

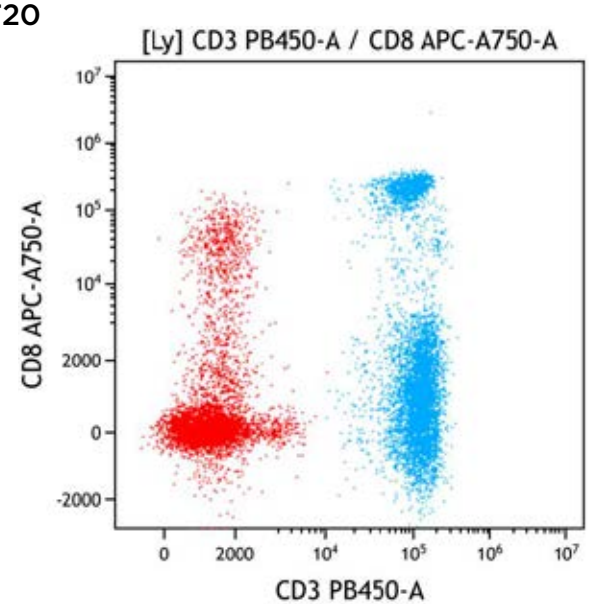

Figure 20. This CD3 vs CD8 dot plotshows all cells in the lymphocyte gate (Ly). All CD8 positive T cells (aqua) express CD3. A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

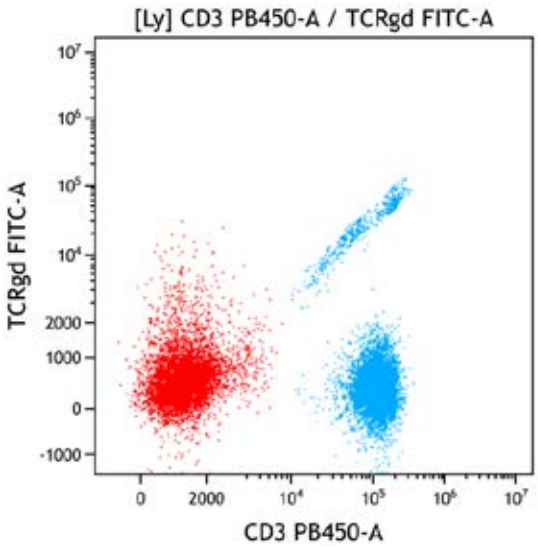

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/ delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

BACK TO CASE OVERVIEW

M1Cell Tube

M1-01

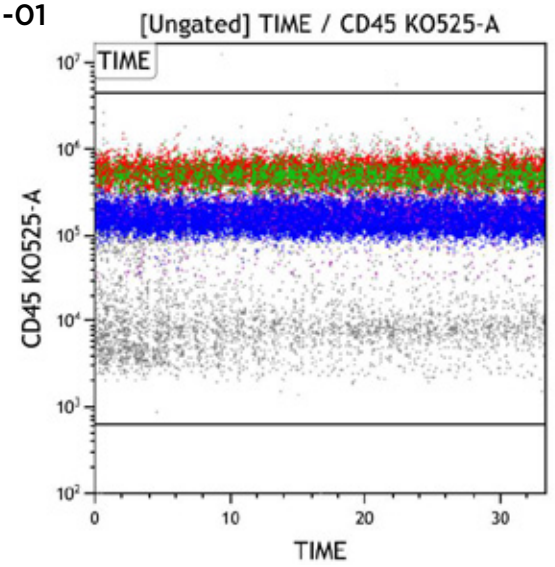

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

M1-02

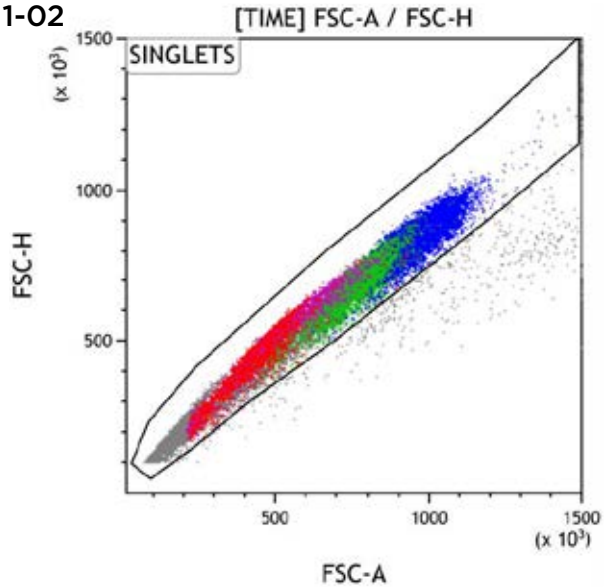

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M1-03

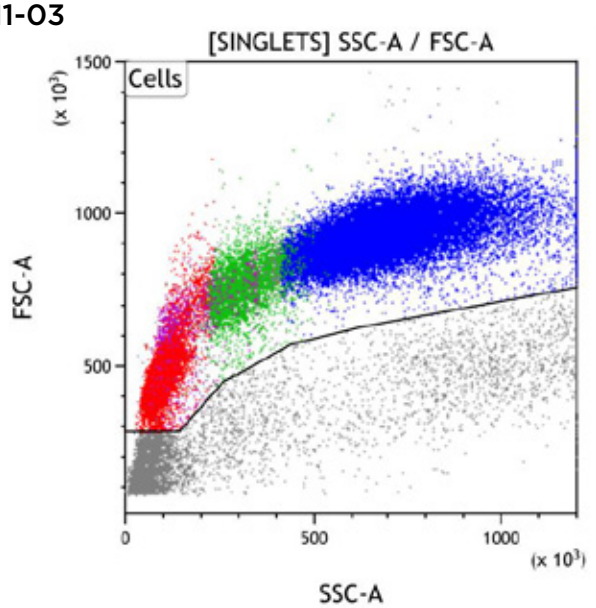

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

M1-04

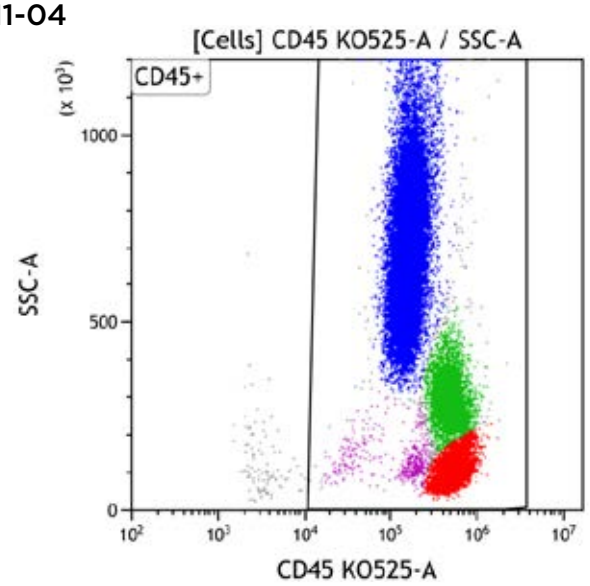

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M1-05

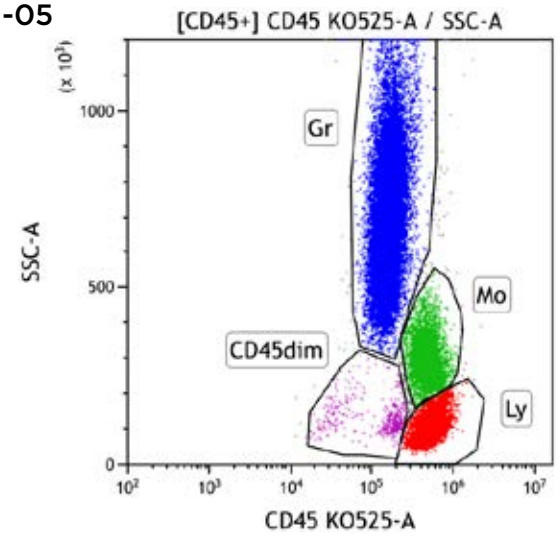

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M1-06

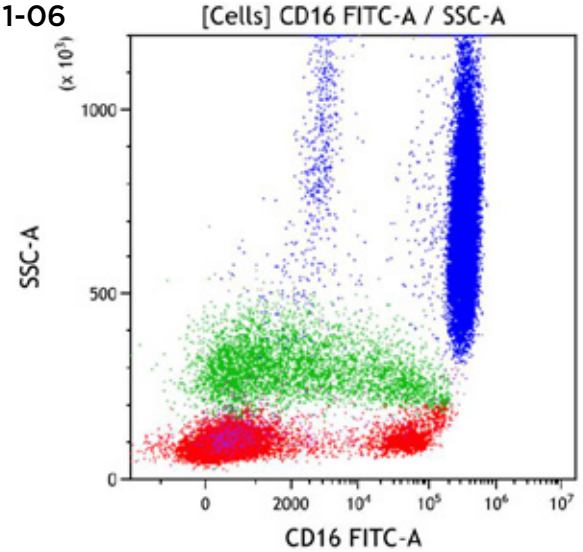

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green).

M1-07

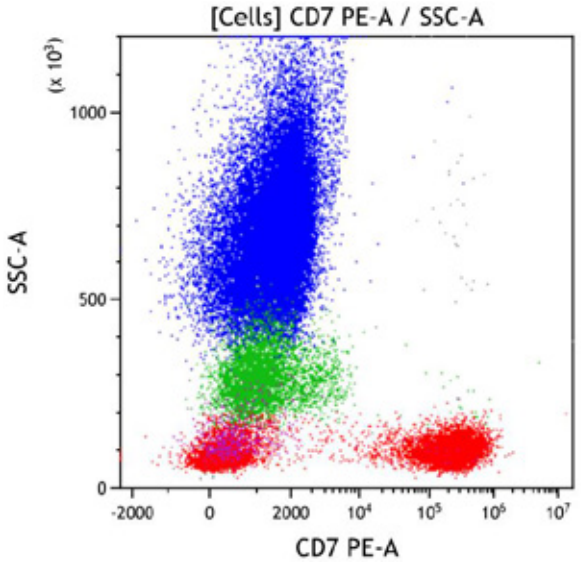

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells, and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of lineage committed progenitors.

M1-08

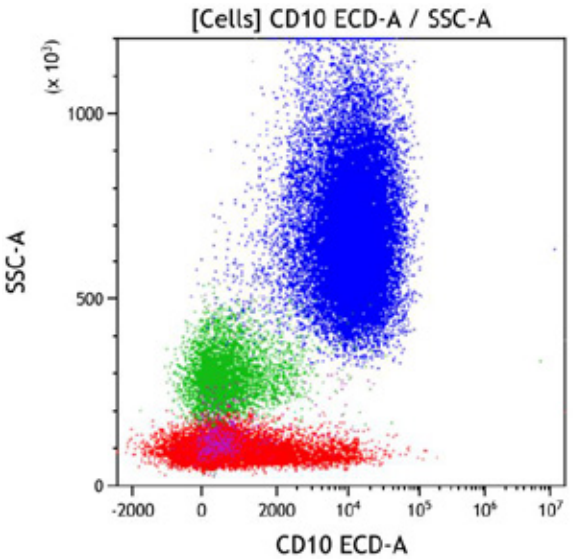

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter.

BACK TO CASE OVERVIEW

M1-09

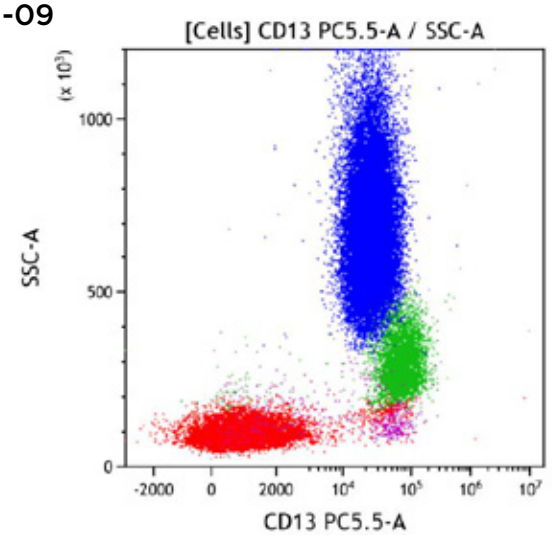

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature granulocytes (blue), mature monocytes (green), and myeloid progenitors (purple).

M1-10

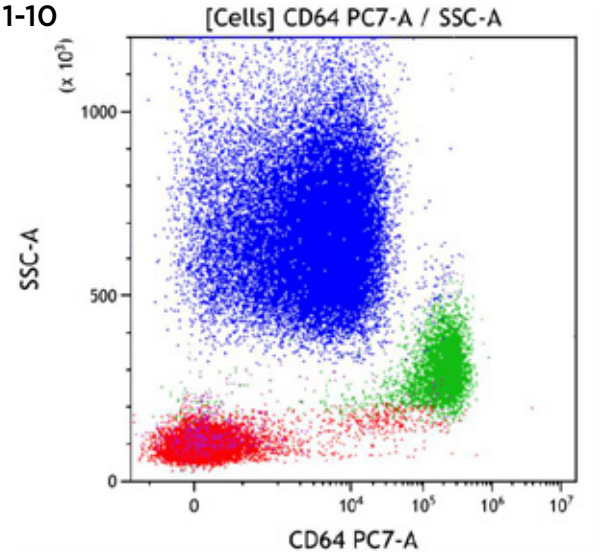

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on monocytes (green). Activated mature monocytes express CD64 at lower level and have lower side scatter. CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphocytes (red) or most CD34 positive progenitors.

M1-11

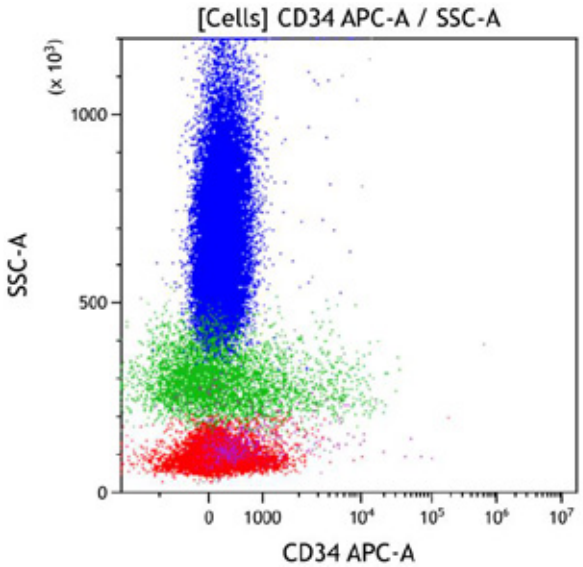

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

M1-12

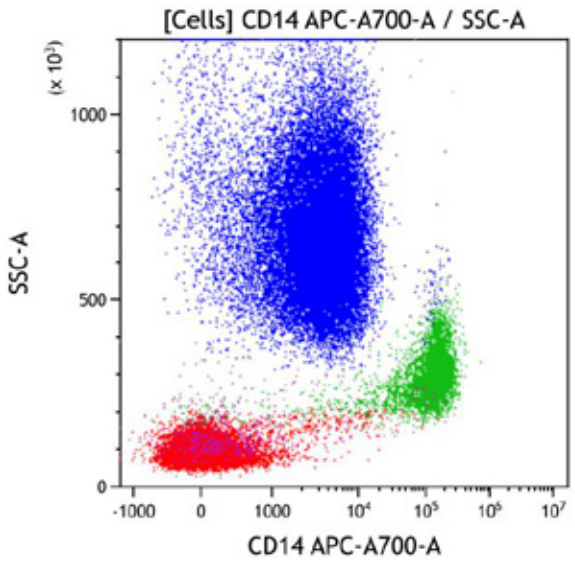

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a low level on mature granulocytes (blue). Activated mature monocytes express CD14 at a lower level and have lower side scatter.

BACK TO CASE OVERVIEW

M1-13

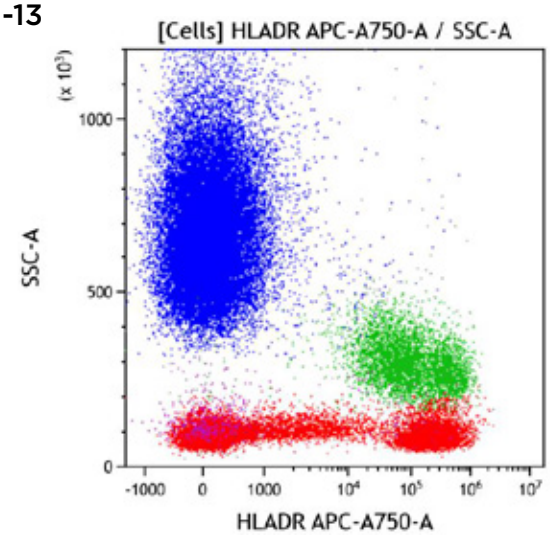

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 progenitors, immature and mature B cells (red), and activated T cells (red).

M1-14

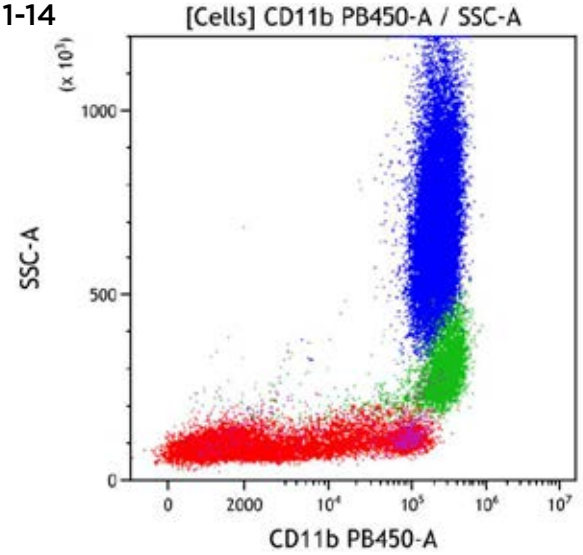

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes (blue) and monocytes (green). CD11b is also expressed on NK cells (red) and basophils.

M1-15

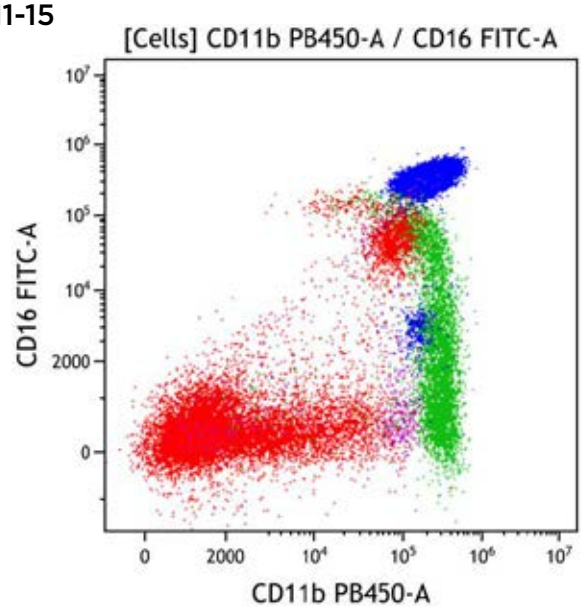

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue) and a subset of NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). Activated mature monocytes express CD16 at a variable level and are CD11b positive.

M1-16

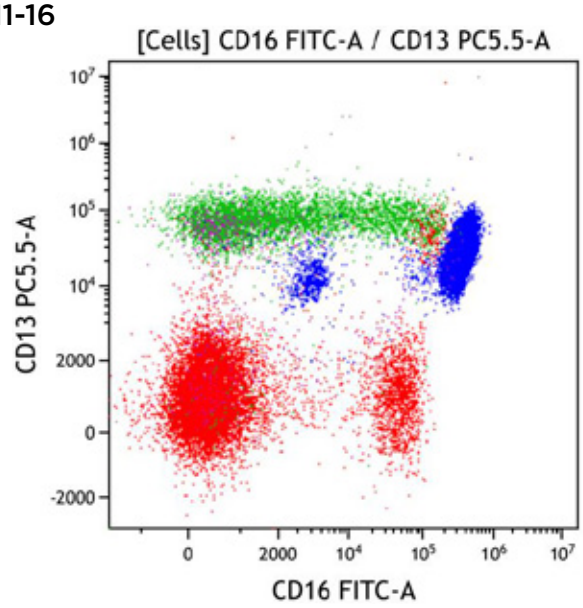

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes (blue) and a subset of NK cells (red).

BACK TO CASE OVERVIEW

M1-17

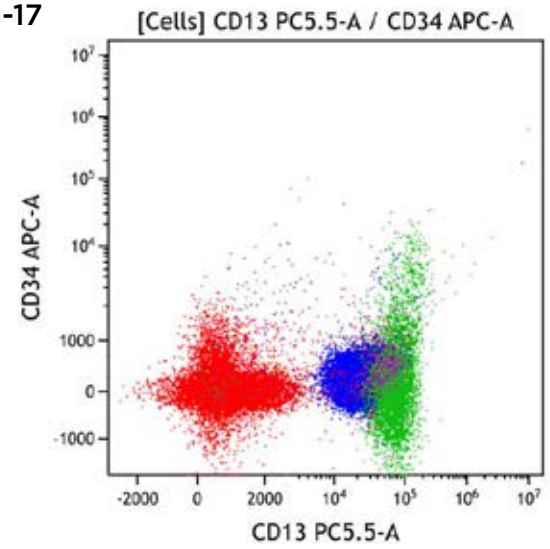

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphocytes.

M1-18

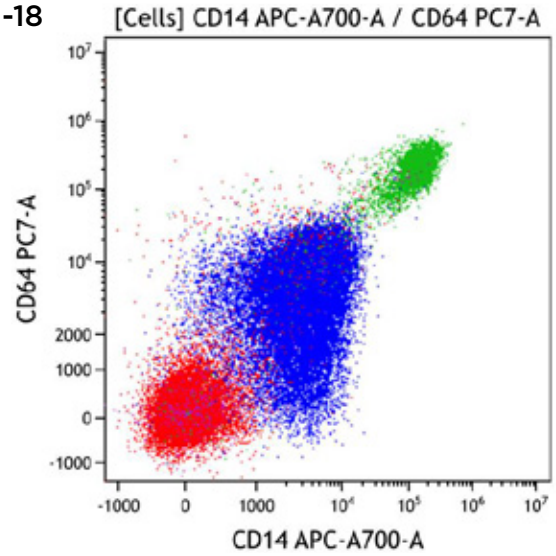

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on mature monocytes and a lower level on granulocytes (blue). Activated mature monocytes express CD14 and CD64 at a variably low level.

M1-19

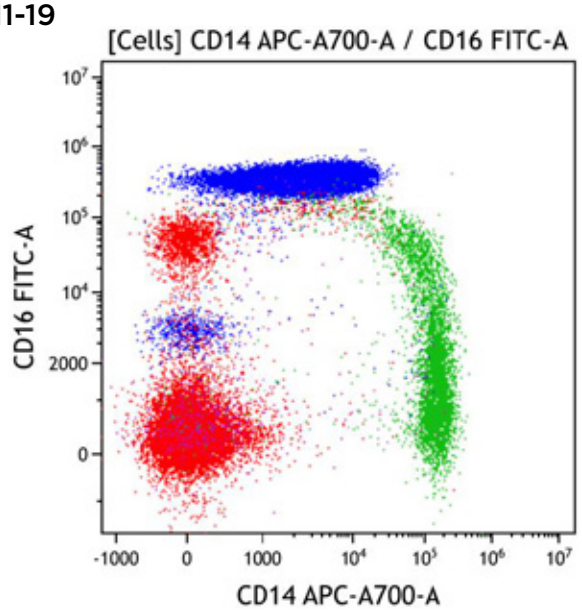

Figure 19. This CD14 vs CD16 dot plot shows all viable cells. CD14 is expressed at a high level on monocytes (green) and a lower level on granulocytes (blue). CD16 is expressed on granulocytes and a subset of NK cells (red). Activated mature monocytes express CD14 and CD16 at a variably low level.

M1-20

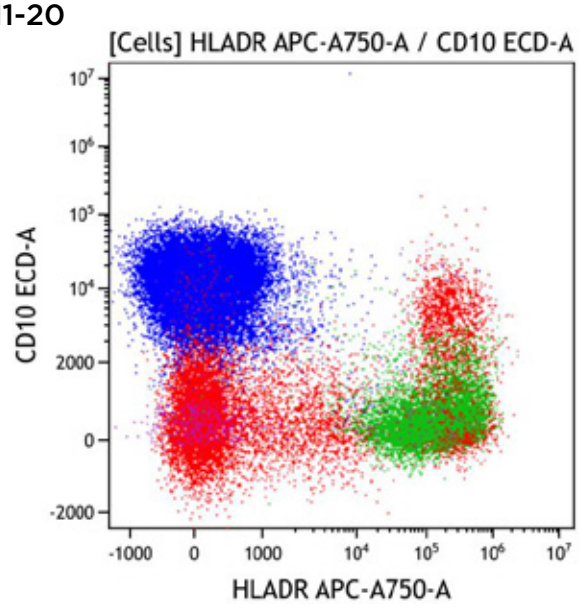

Figure 20. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on mature granulocytes (blue).

BACK TO CASE OVERVIEW

M1-21

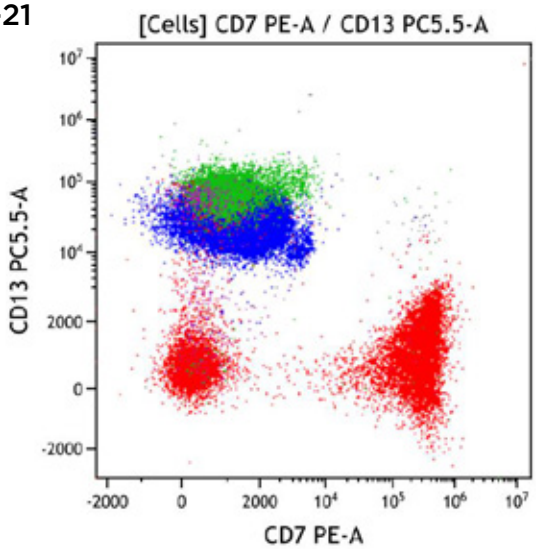

Figure 21. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

BACK TO CASE OVERVIEW

M2 Cell Tube

M2-01

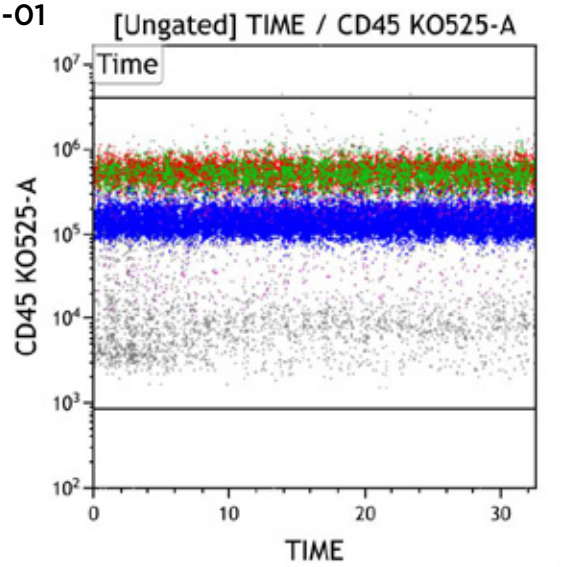

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

M2-02

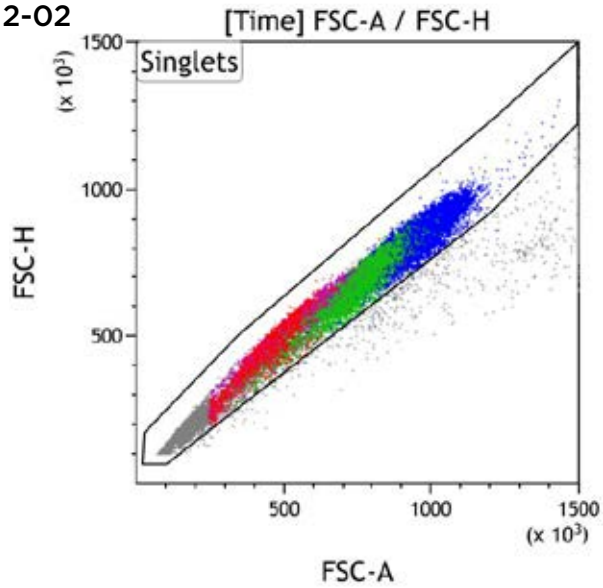

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

M2-03

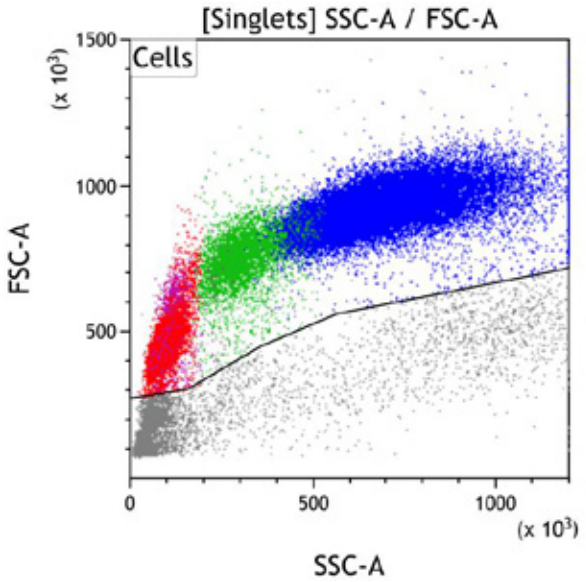

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

M2-04

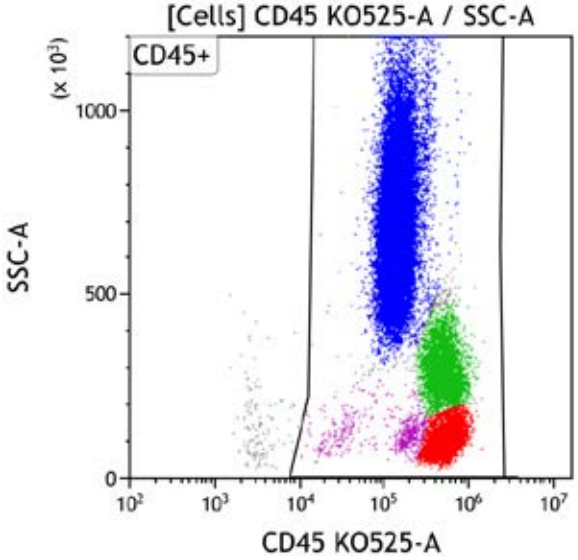

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, or non-hematopoietic cells.

BACK TO CASE OVERVIEW

M2-05

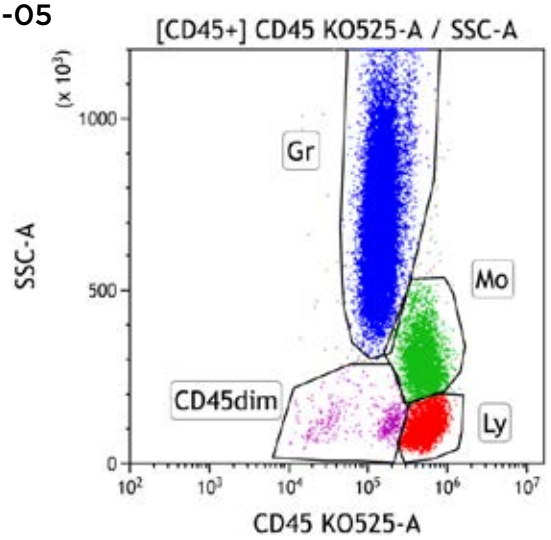

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., 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.

M2-06

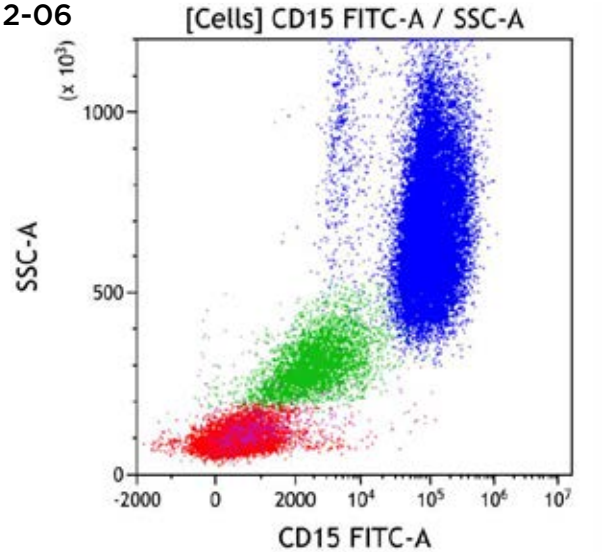

Figure 6. This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and at a lower level on monocytes (green).

M2-07

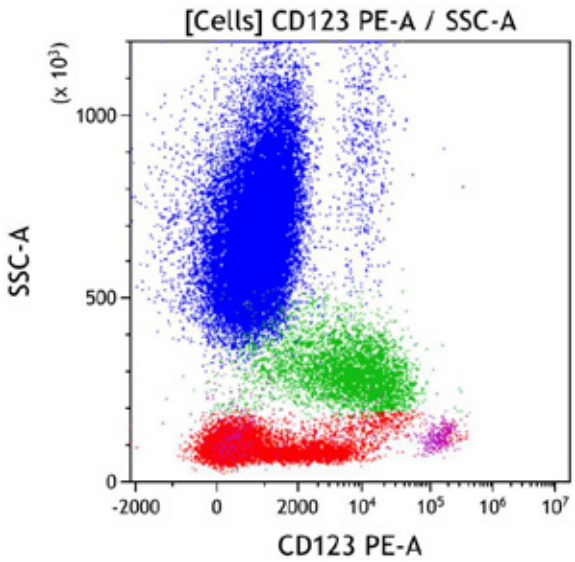

Figure 7. This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

M2-08

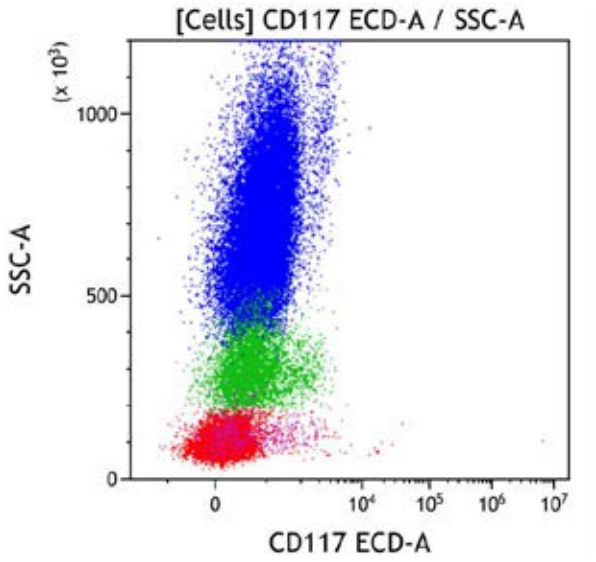

Figure 8. This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD117.

BACK TO CASE OVERVIEW

M2-09

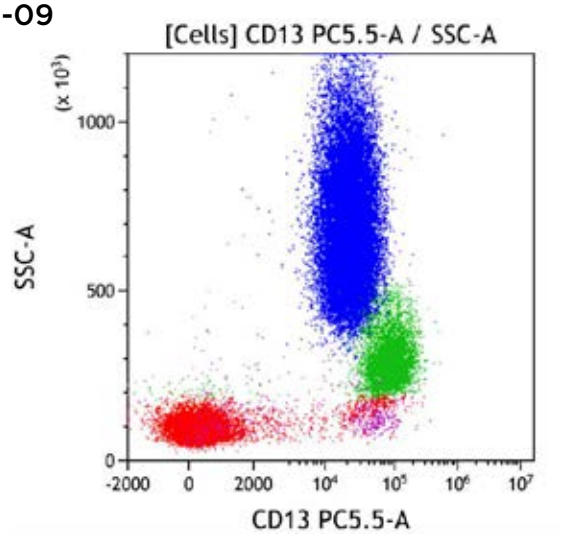

Figure 9. This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and variably on myeloid progenitors.

M2-10

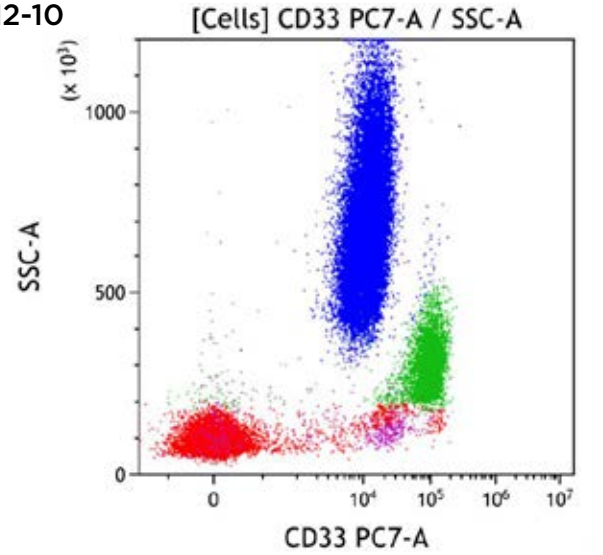

Figure 10. This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors.

M2-11

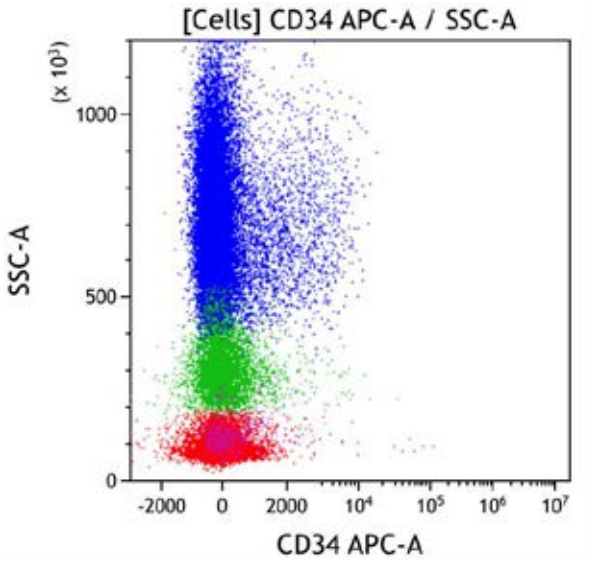

Figure 11. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

M2-12

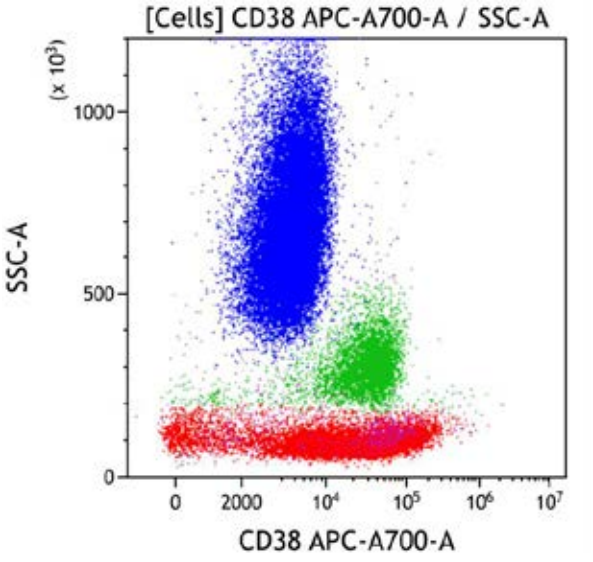

Figure 12. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature lymphocytes (red).

BACK TO CASE OVERVIEW

M2-13

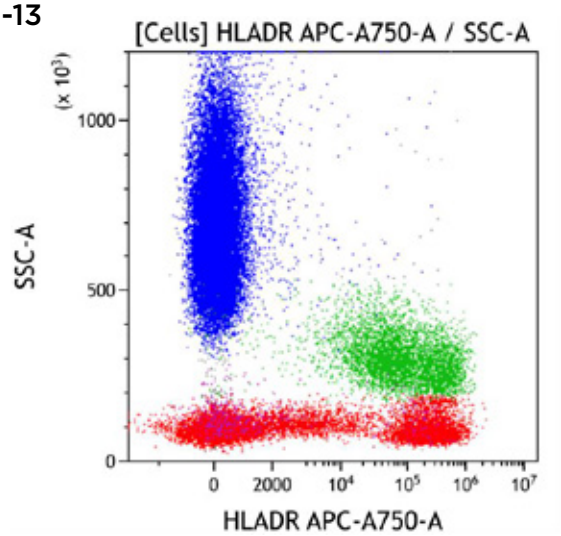

Figure 13. This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red, lower right), and activated T cells.

M2-14

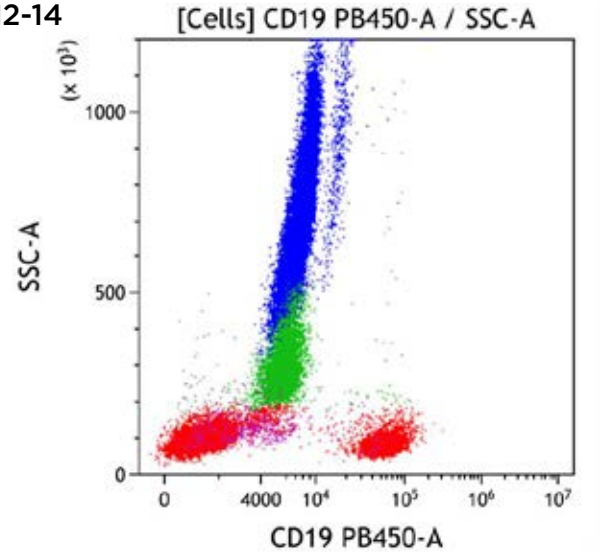

Figure 14. This CD19 vs Side Scatter dot plot shows events in the Cells gate. CD19 is expressed on immature and mature B cells (red, lower middle), as well as most plasma cells. These cells typically have low to moderate side scatter.

M2-15

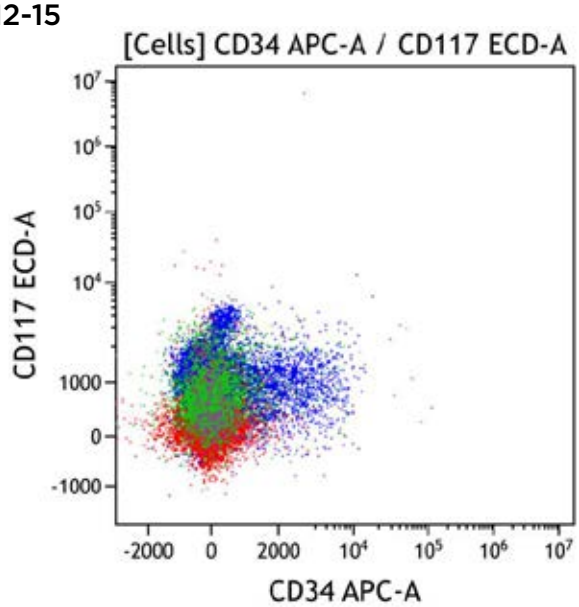

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34 and CD117. CD34 and CD117 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

M2-16

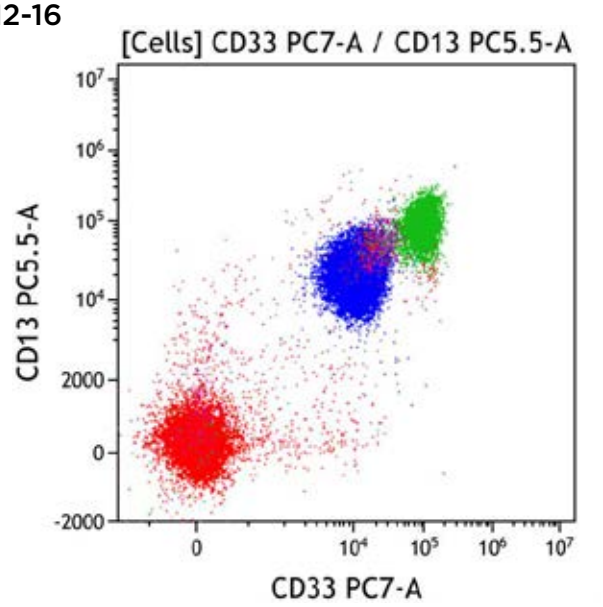

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes (red) do not express either CD13 or CD33.

BACK TO CASE OVERVIEW

M2-17

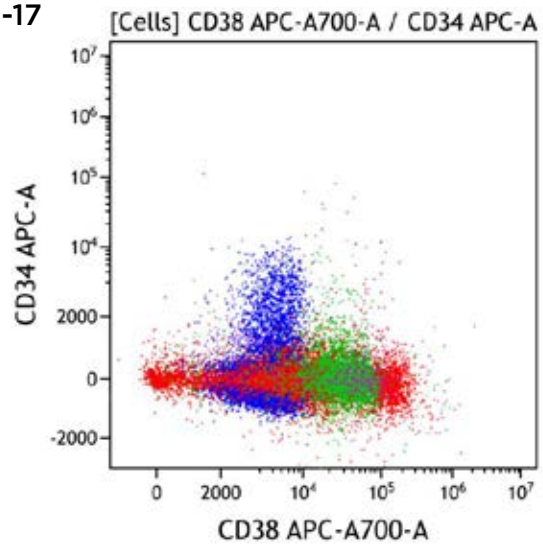

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34 with variable CD38. CD34 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

M2-18

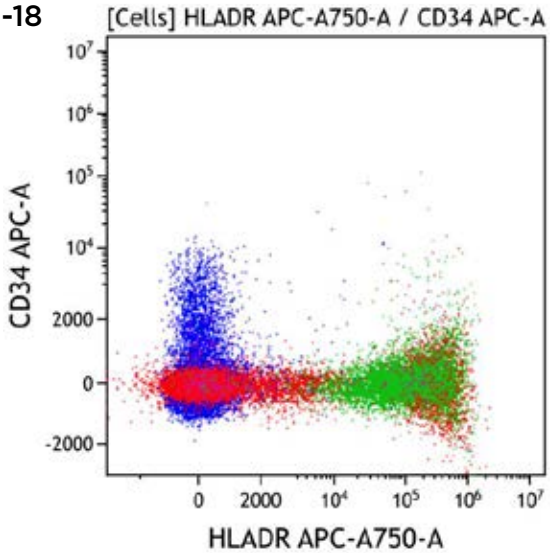

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34. CD34 positive progenitors normally represent less than 0.01% of the white blood cells in peripheral blood.

M2-19

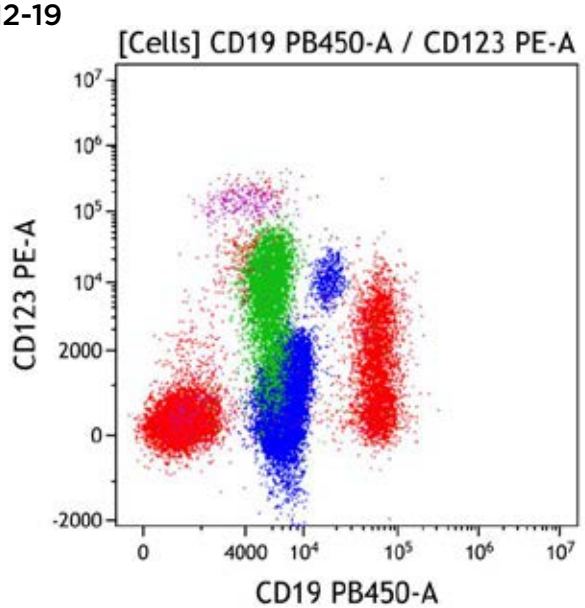

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red) normally do not express significant CD123. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence, largely from eosinophils.

M2-20

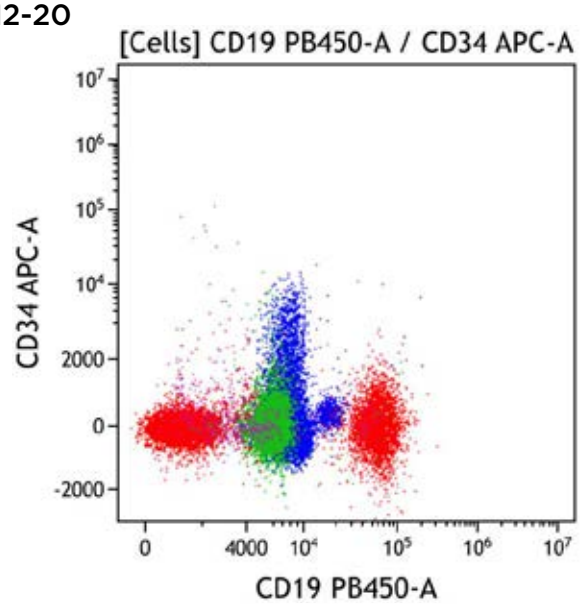

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed by B cells. CD34 is expressed on early progenitors. A subset of lymphocytes (red) are CD19 positive B cells without expression of CD34.

BACK TO CASE OVERVIEW

M2-21

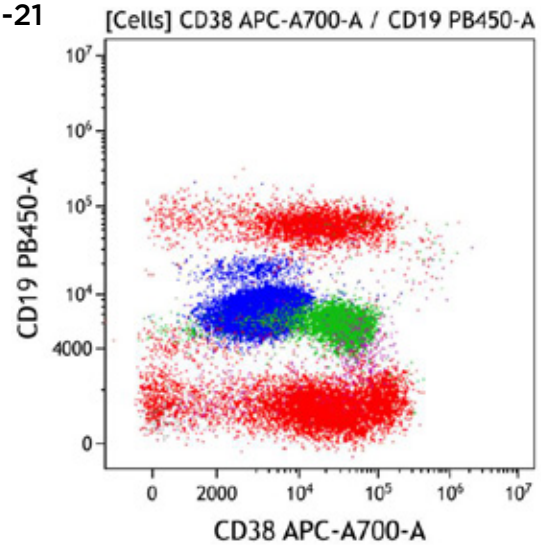

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells show intermediate expression of CD38 (red). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils.

M2-22

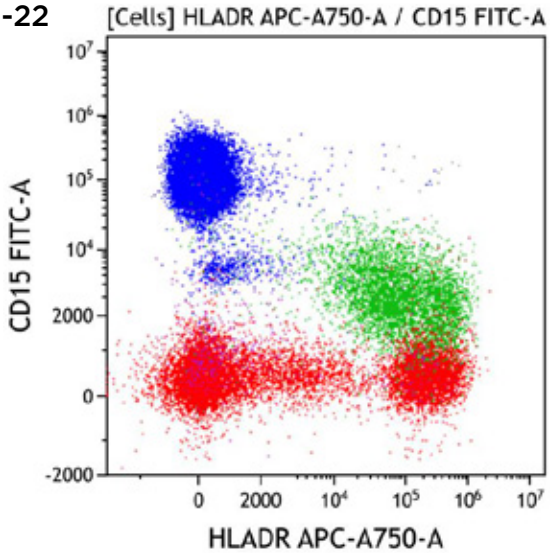

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR.

M2-23

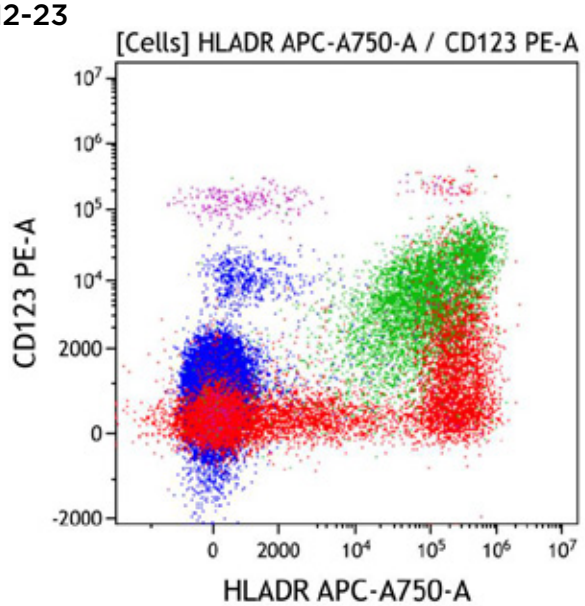

Figure 23. This HLA-DR vs CD123 dot plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Mature granulocytes (blue) do not express HLA-DR. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes, and CD34 positive progenitors.

M2-24

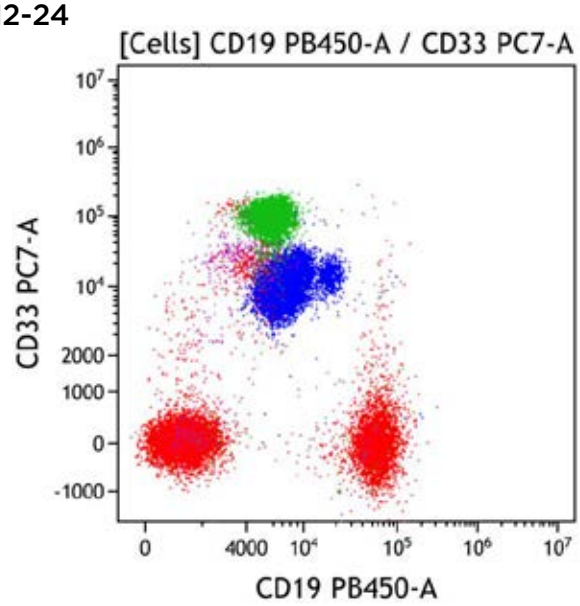

Figure 24. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed by B cells (red). CD33 is expressed by monocytes (green) and granulocytes (blue). CD19 positive B cells normally do not normally express significant CD33. The apparent CD19 positivity on granulocytes (blue) is due to autofluorescence, largely from eosinophils. A subset of lymphocytes (red) are CD19 positive B cells without expression of CD33.

BACK TO CASE OVERVIEW

CASE OVERVIEW

Case #	**Case 15 QTX-TC4-297**
Diagnosis	**Normal lymph node**
Clinical Vignette	This is a 63-year-old female. A lymph node biopsy sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.
Flow Cytometry Result Interpretation	Flow cytometric immunophenotyping identified no immunophenotypically aberrant populations in this case. Not that correlation with clinical and laboratory data is recommended, and that a malignant process cannot be ruled solely on the basis of this assay.

BACK TO CASE LISTINGS

B Cell Tube

B01

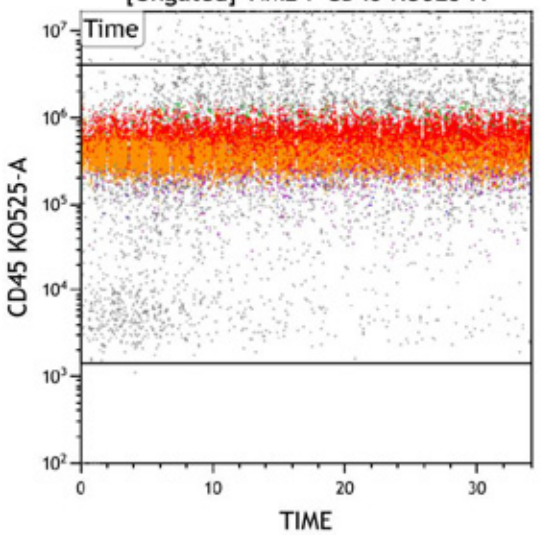

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Times gate.

B02

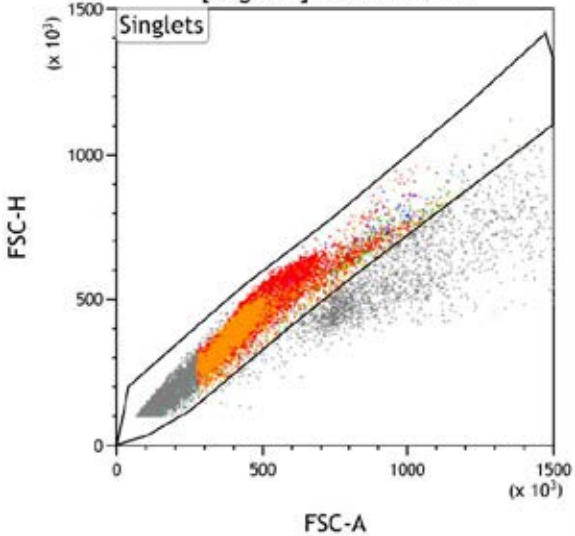

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

B03

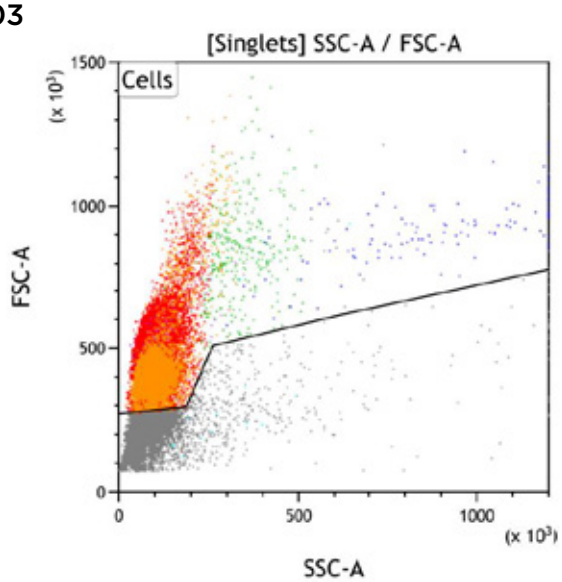

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate. Exclusion of non-viable cells can be particularly important for tissue specimens to minimize artifacts.

B04

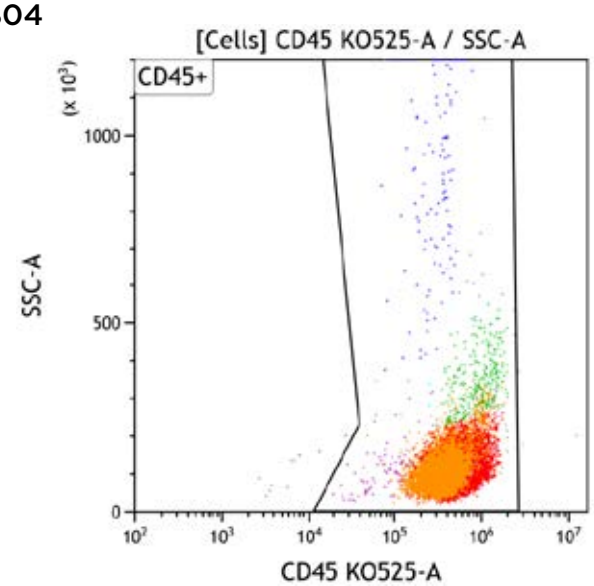

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

BACK TO CASE OVERVIEW

B05

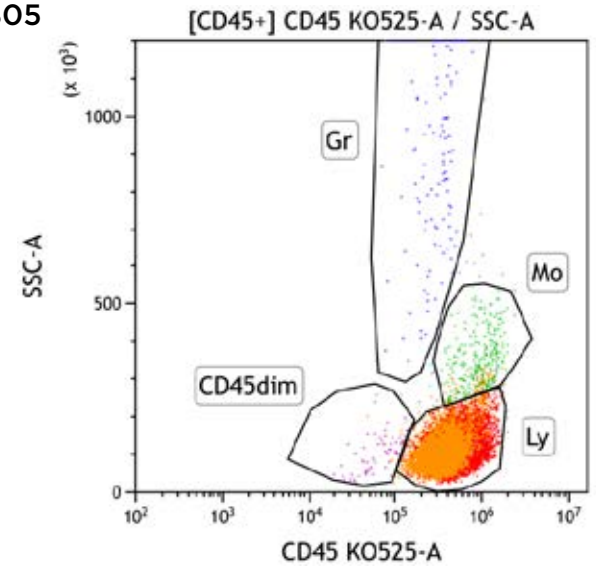

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) is occupied by early progenitors (e.g., 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 followed throughout the analysis. In this case, the white blood cells are composed predominantly of lymphocytes, as is typical for lymph nodes.

B06

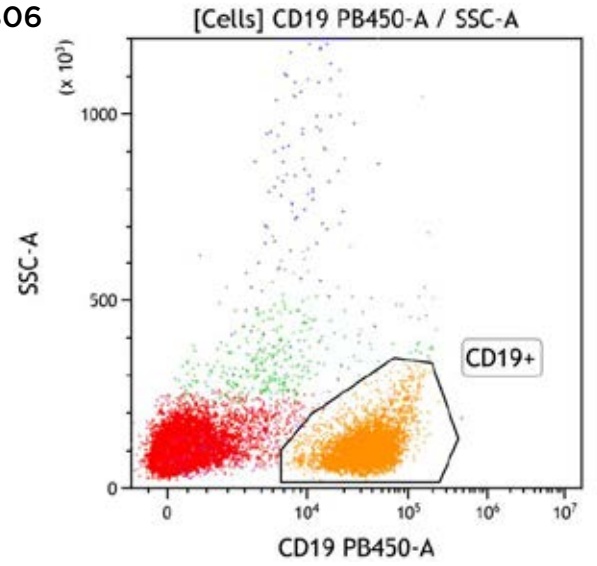

Figure 6. This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells (orange). CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter.

B07

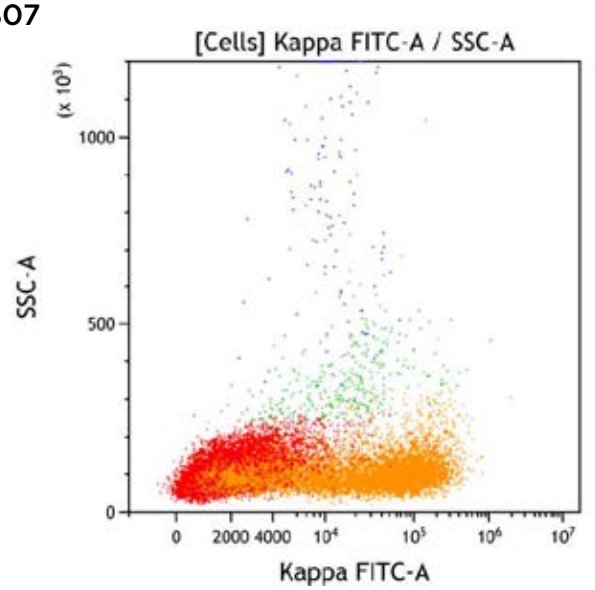

Figure 7. This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The Kappa light chain positive cells are shown on the right side of the plot.

B08

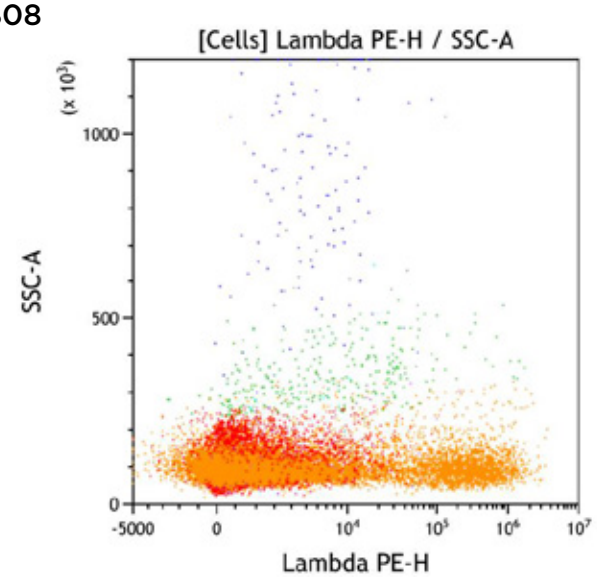

Figure 8. This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late-stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The lambda light chain positive cells are shown on the right side of the plot.

BACK TO CASE OVERVIEW

B09

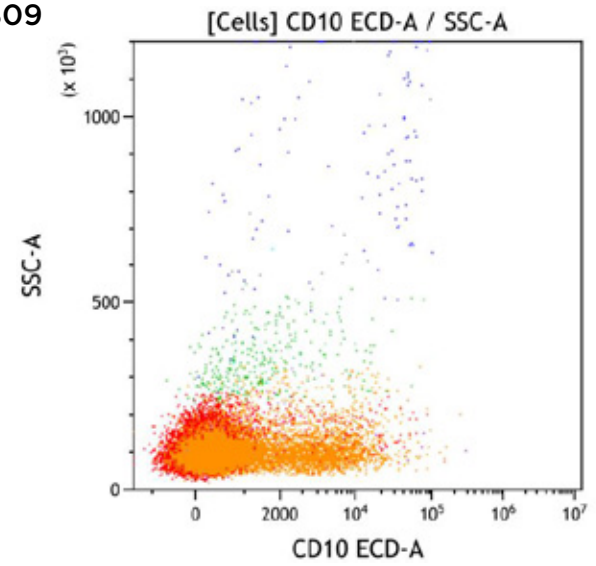

Figure 9. This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). A subset of CD19 positive B cells (orange) express CD10 and likely represent germinal center B cells.

B10

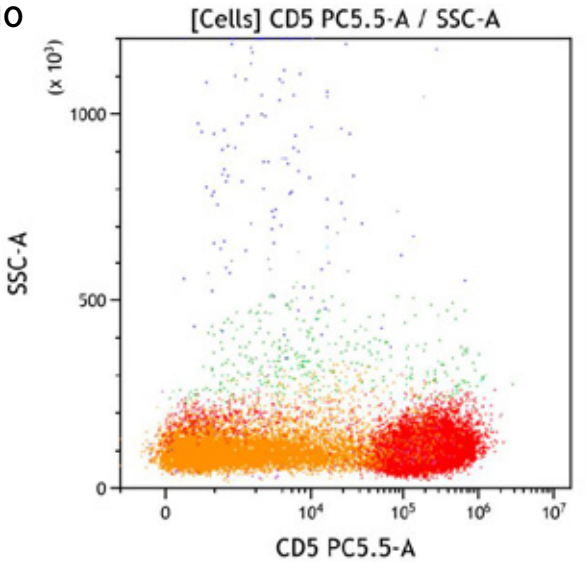

Figure 10. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter.

B11

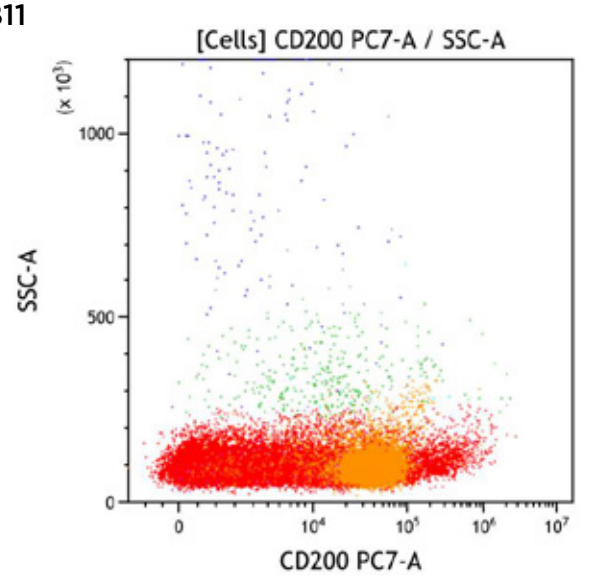

Figure 11. This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive).

B12

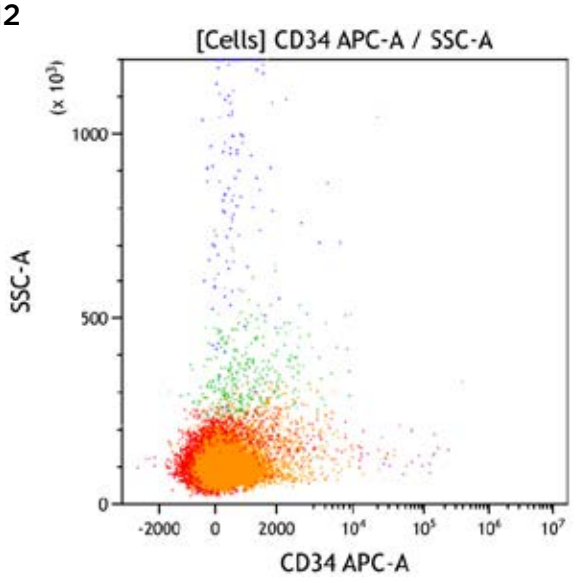

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature lymphocytes are negative for CD34.

BACK TO CASE OVERVIEW

B13

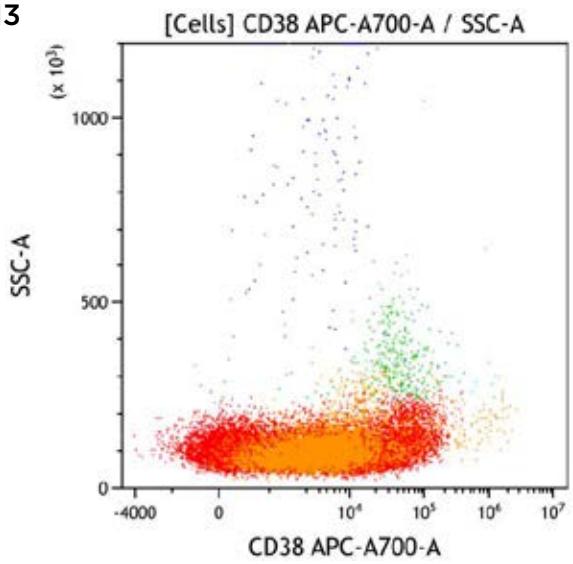

Figure 13. This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably level on activated mature lymphocytes (red/orange).

B14

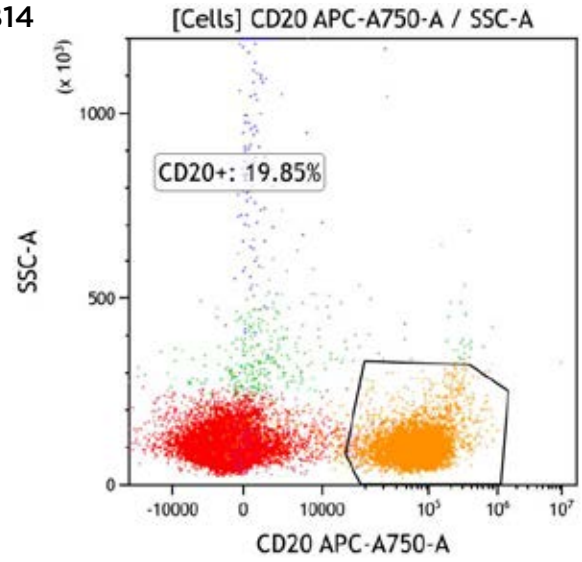

Figure 14. This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter.

B15

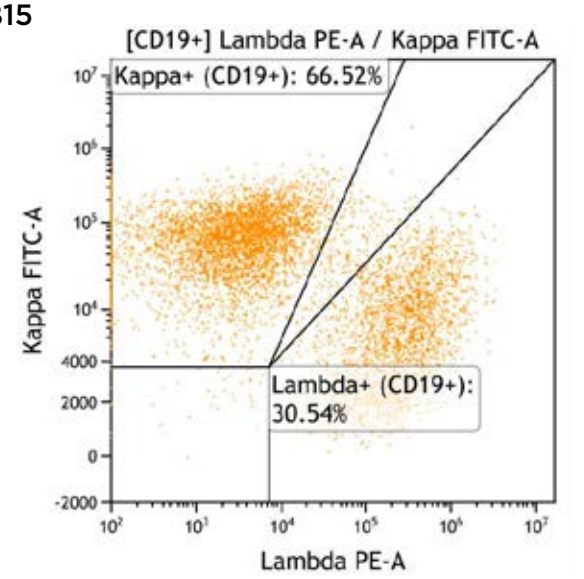

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda ratio is ~1.4 with a range between 1 to 2. Increased background due to adherent plasma immunoglobulin is common.

B16

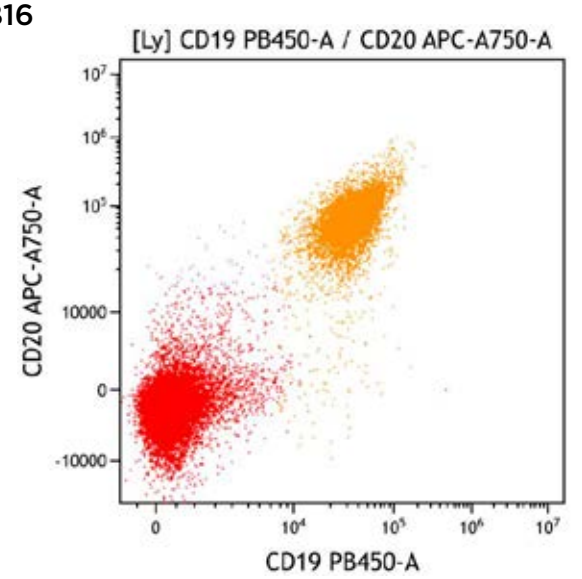

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells (orange) express both CD19 and CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

BACK TO CASE OVERVIEW

B17

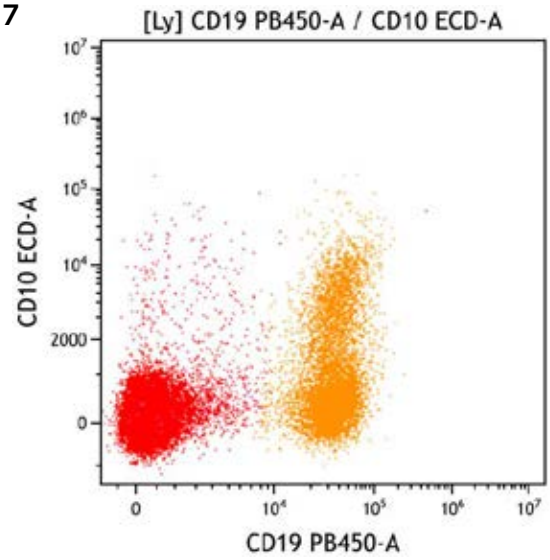

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells (orange) are CD19 positive. CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of latestage immature B cells is present in peripheral blood and bone marrow aspirates. The dim CD10 positivity seen here is typical of germinal center B cells.

B18

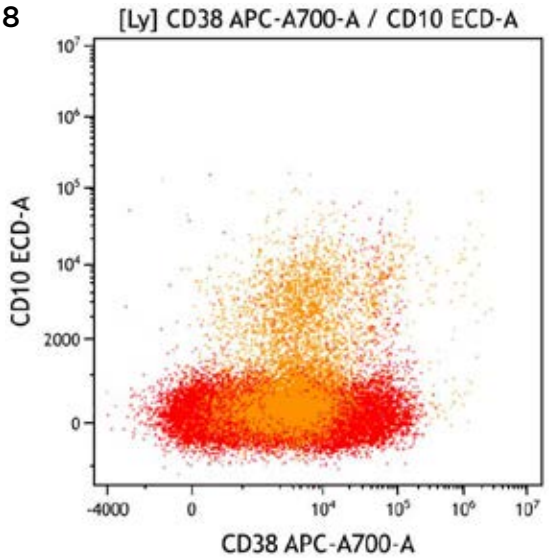

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low level expression of CD38. Germinal 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. 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.

B19

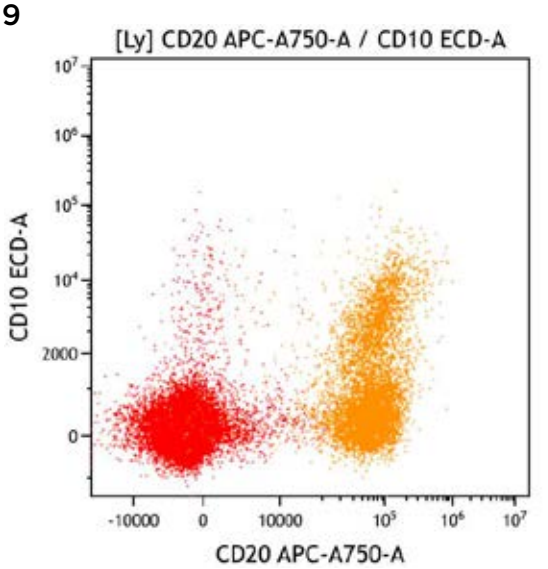

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) uniformly express high level CD20. Germinal center B cells in the lymph node express CD10 at a low level. The few CD10 positive B cells represent late stage immature B cells.

B20

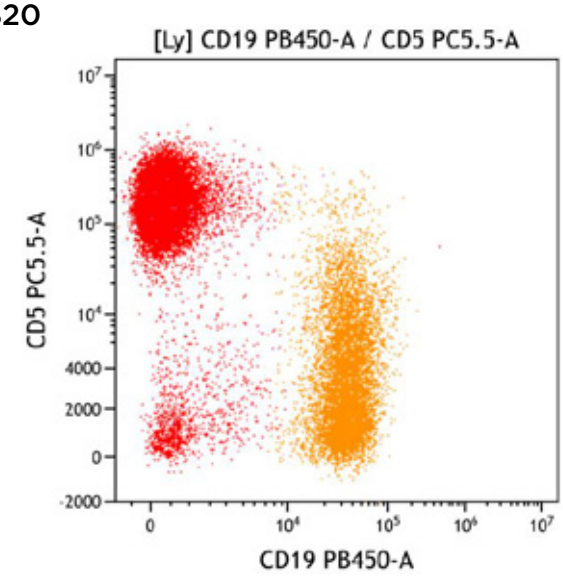

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells.

BACK TO CASE OVERVIEW

B21

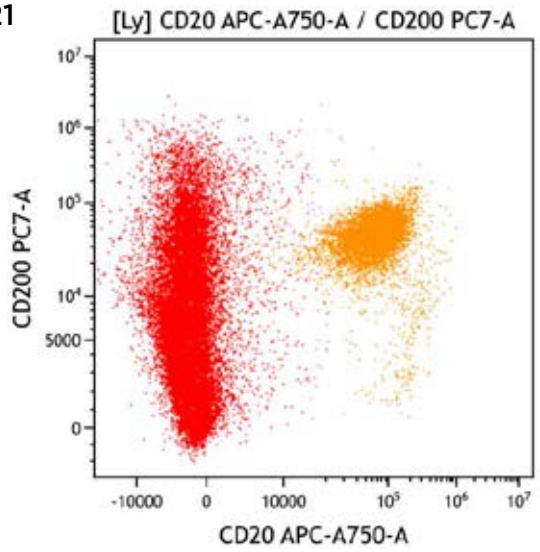

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) express CD200 at a low to moderate level.

B22

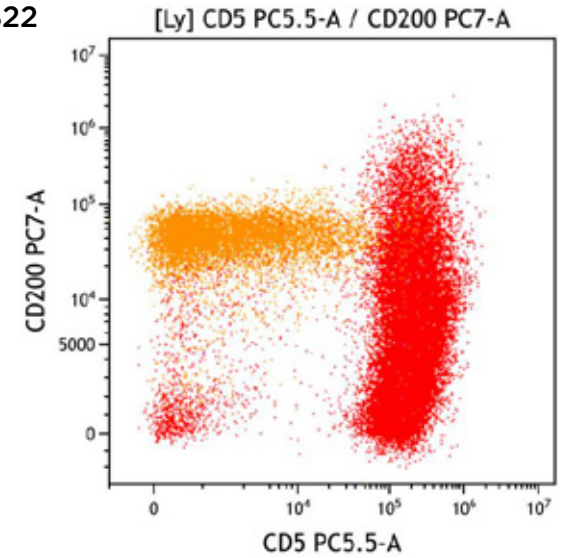

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells (orange) normally express CD200 with a subset variably express CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200.

BACK TO CASE OVERVIEW

T Cell Tube

T01

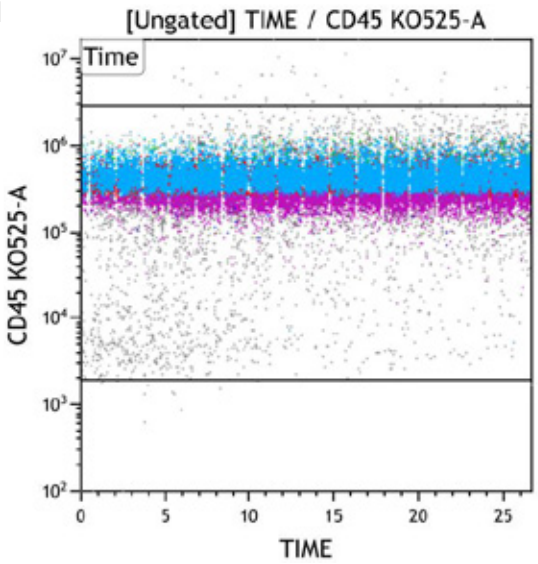

Figure 1. This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can be excluded in the Time gate.

T02

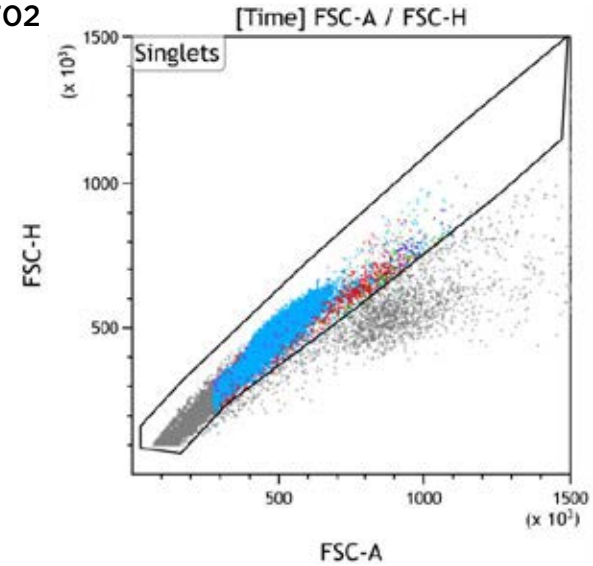

Figure 2. This FSC-A vs FSC-H dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship FSC-A vs FSC-H and are included in the Singlets gate, while doublets lie outside the linear relationship.

T03

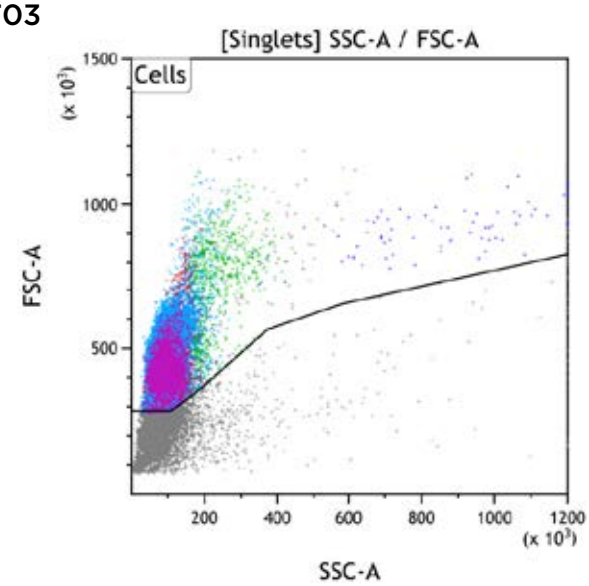

Figure 3. This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased side scatter. Viable cells are included in the Cells gate.

T04

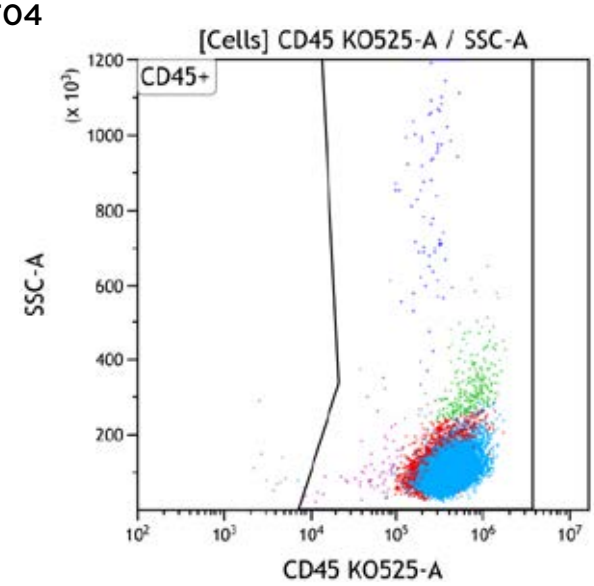

Figure 4. This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic cells.

BACK TO CASE OVERVIEW

T05

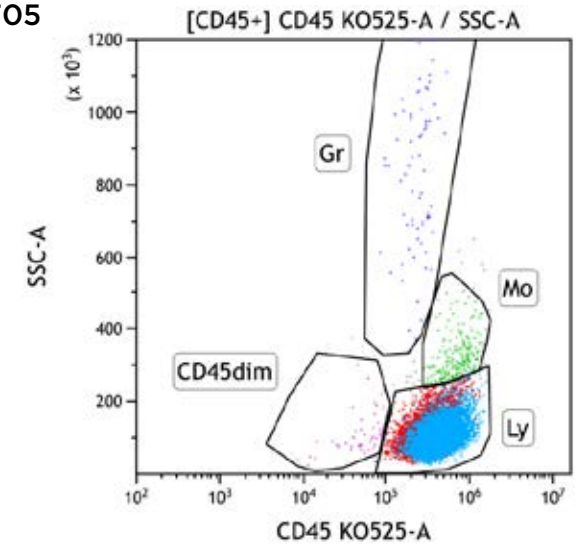

Figure 5. This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white blood cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes (Gate Gr, blue). The CD45dim gate (purple) covers the area typically occupied by early progenitors (e.g., myeloblasts andimmature 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, as is typical for lymph node.

T06

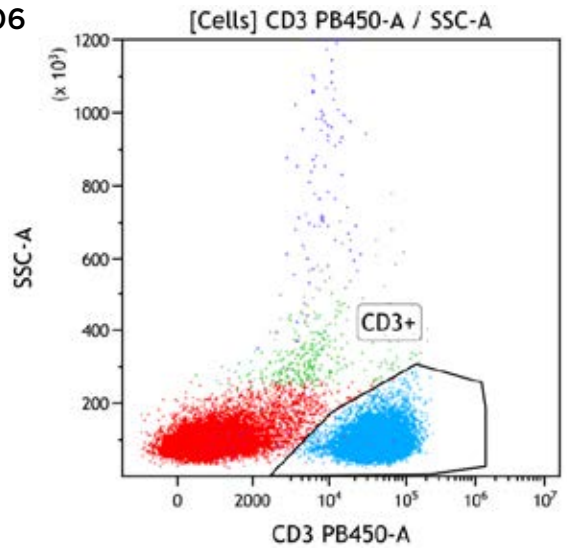

Figure 6. This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter.

T07

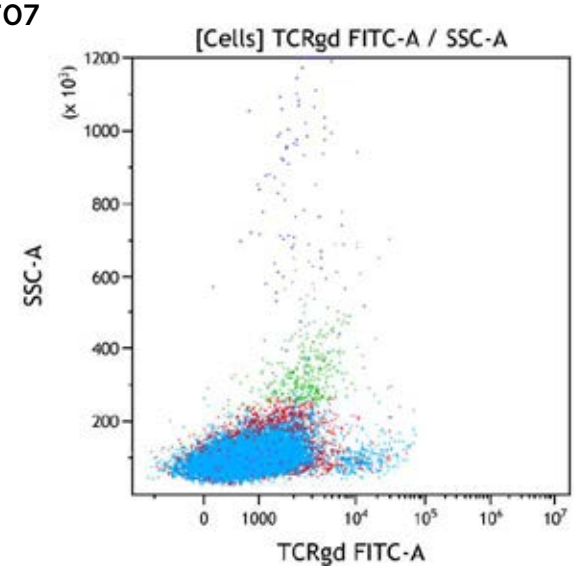

Figure 7. This TCR**γδ** vs Side Scatter dot plot shows all viable cells. TCR**γδ** is a subtype of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

T08

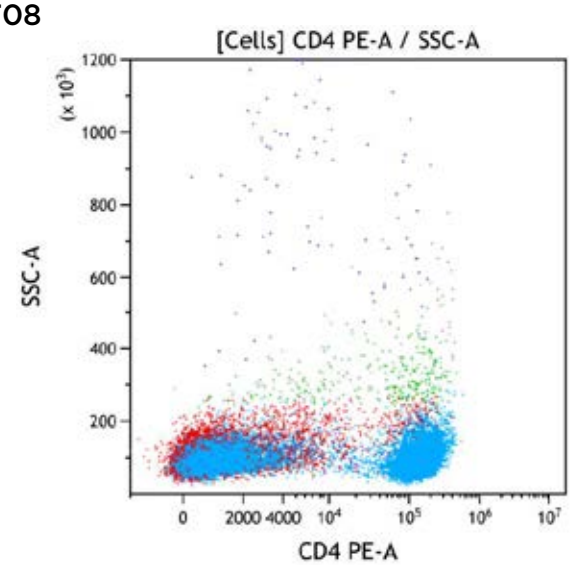

Figure 8. This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells (aqua) at a high level. CD4 is also expressed on monocytes (green) at a level lower than that of CD4 positive T cells, but are essentially absent in this case.

BACK TO CASE OVERVIEW

T09

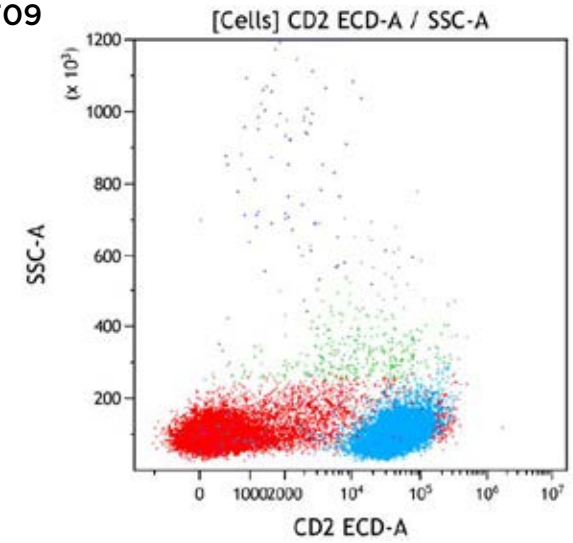

Figure 9. This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expression by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

T10

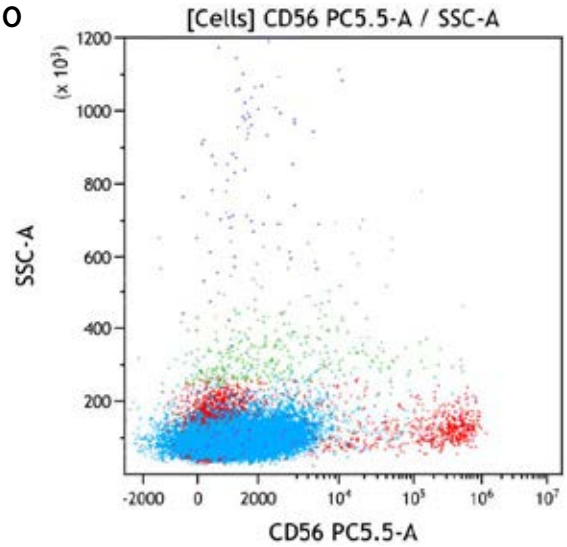

Figure 10. This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytes (green) in both reactive and neoplastic conditions. NK cells are generally infrequent in normal tissues.

T11

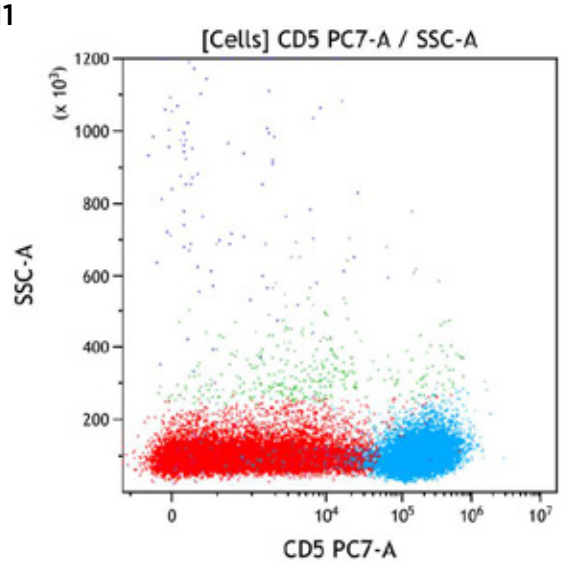

Figure 11. This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells expresses CD5.

T12

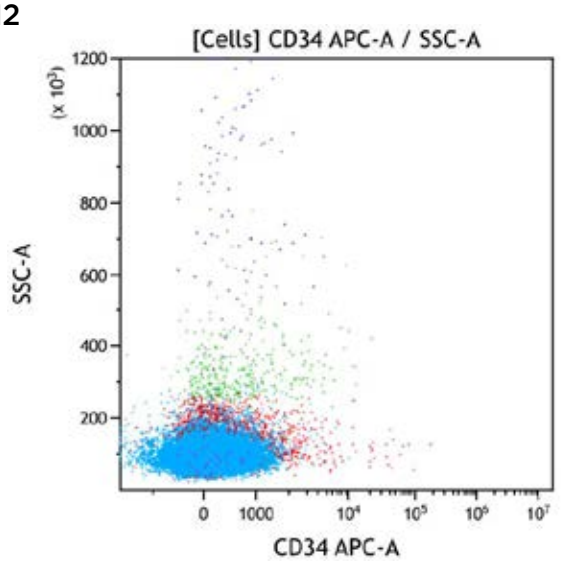

Figure 12. This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature lymphocytes are negative for CD34. Tissues rarely contain CD34 positive progenitor populations.

BACK TO CASE OVERVIEW

T13

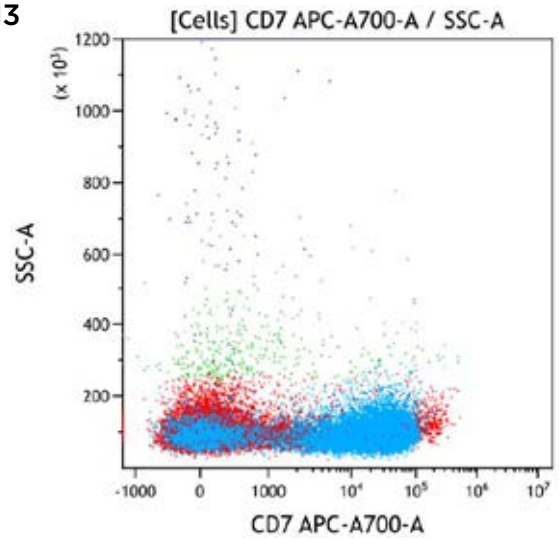

Figure 13. This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly expressed on a subset of plasmacytoid dendritic cells and a subset of lineage committed CD34 positive progenitors.

T14

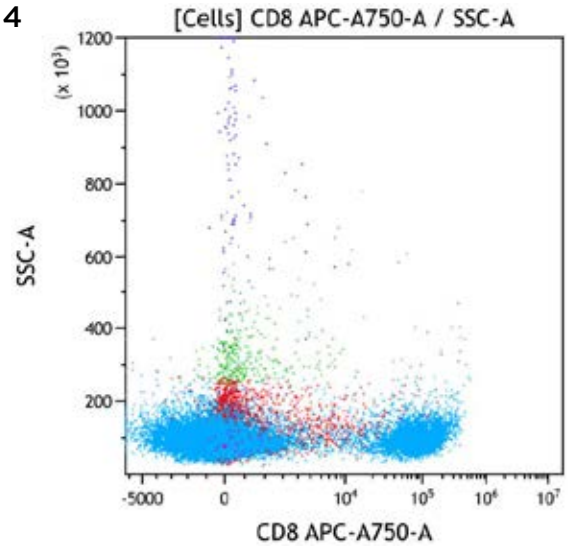

Figure 14. This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma/delta T cells.

T15

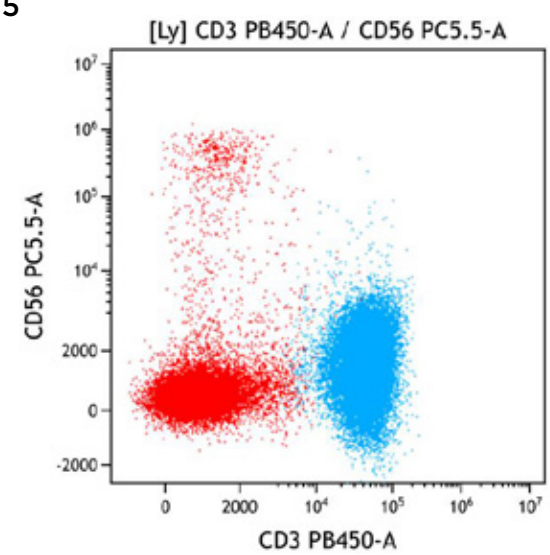

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red). 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.

T16

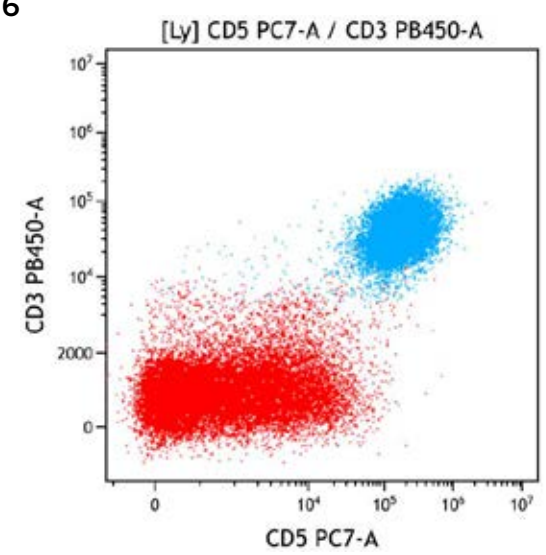

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are co-expressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed on most NK cells and B cells.

BACK TO CASE OVERVIEW

T17

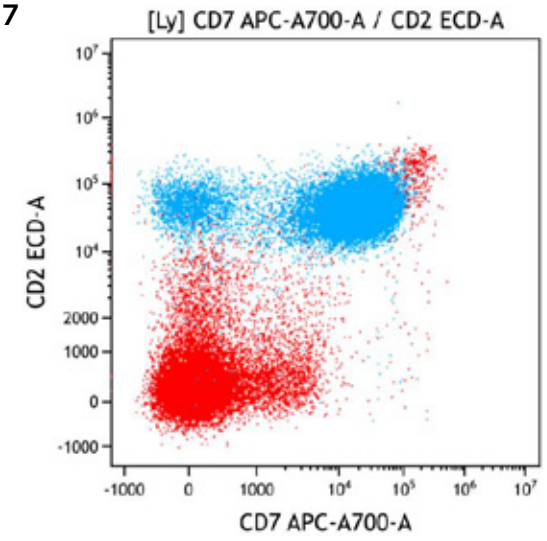

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are co-expressed on the large majority of mature T cells (aqua) and NK cells (red).

T18

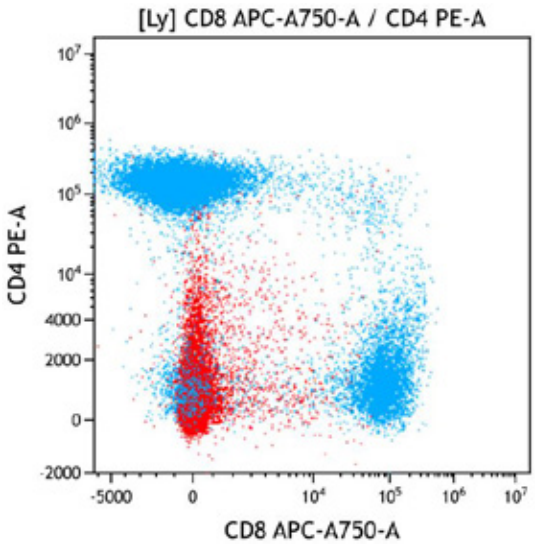

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional 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.

T19

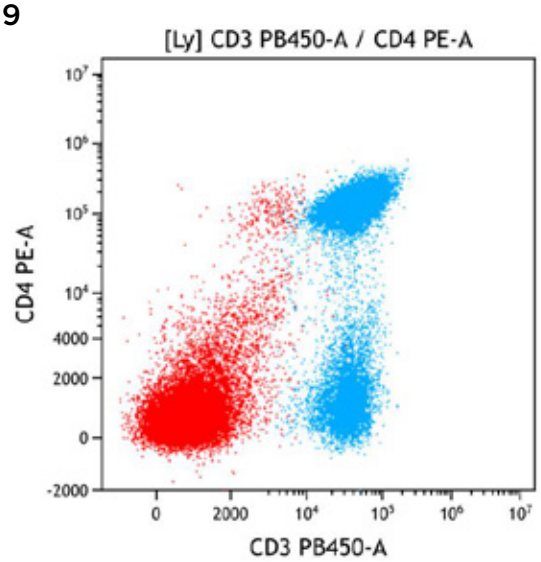

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. Of note, the few CD4 positive but CD3 negative cells (red, upper left) are monocytes included in the lymphocyte gate. It demonstrates that CD45 vs Side scatter gating alone does not allow pure lymphocyte identification.

T20

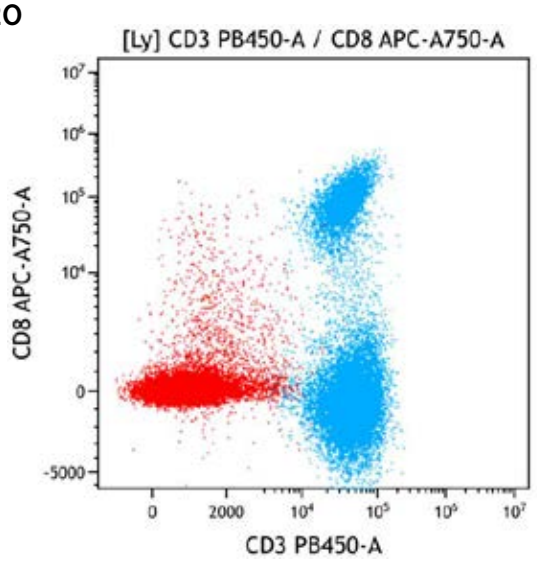

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red) without CD3.

BACK TO CASE OVERVIEW

T21

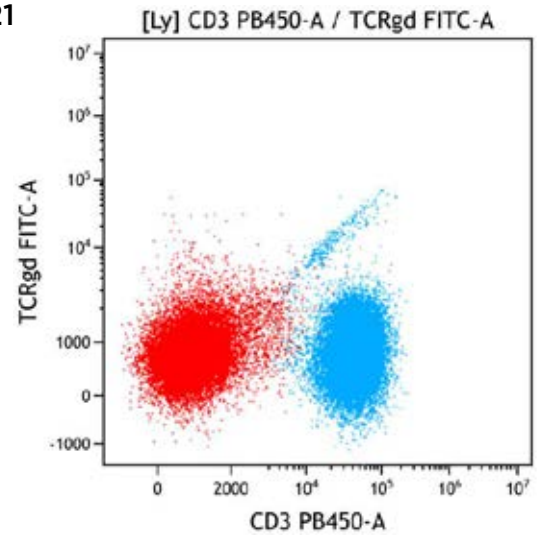

Figure 21. This CD3 vs TCR**γδ** dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells expresses TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression of one shows increased expression of the other.

BACK TO CASE OVERVIEW

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