NEOPLASTIC PROCESS OF B-CELL ORIGIN

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

B LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

Case #4: B Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

Clinical Vignette

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

Flow Cytometric Immunophenotyping

B Cell Tube

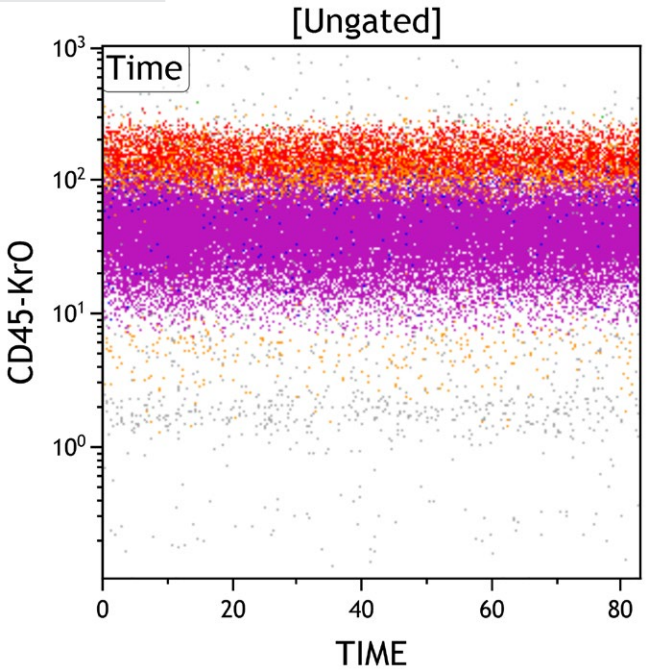

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

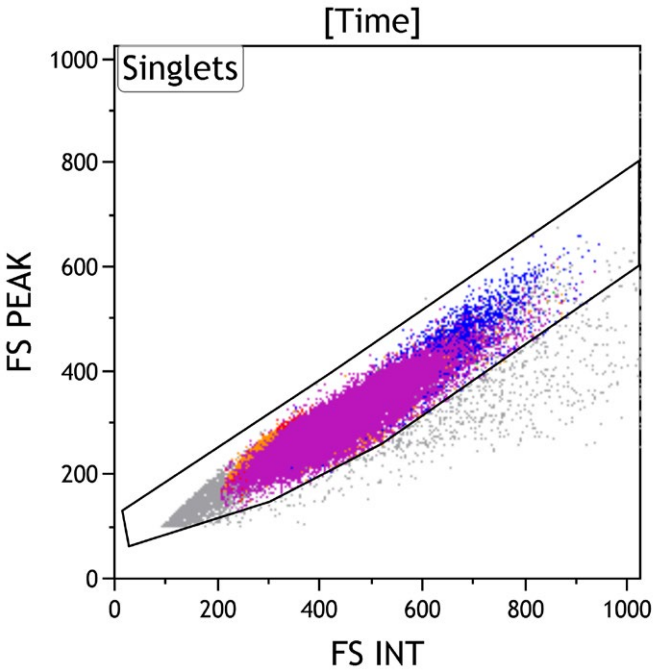

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

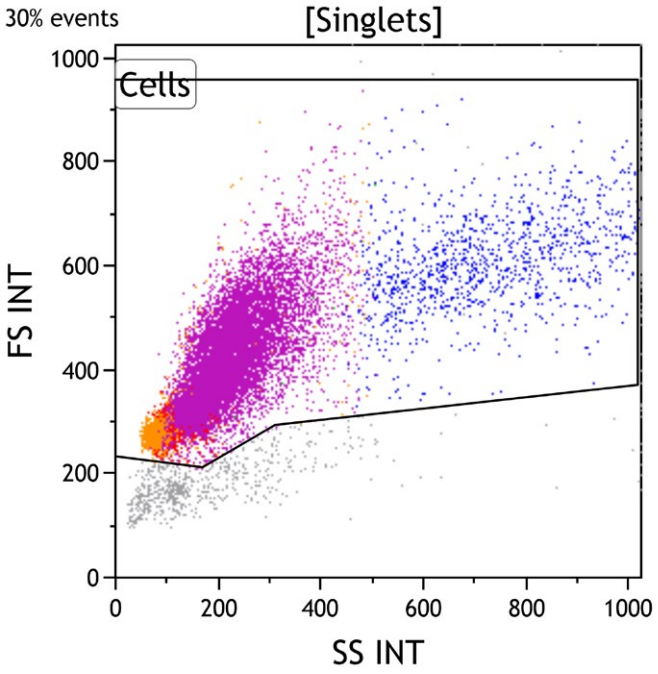

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

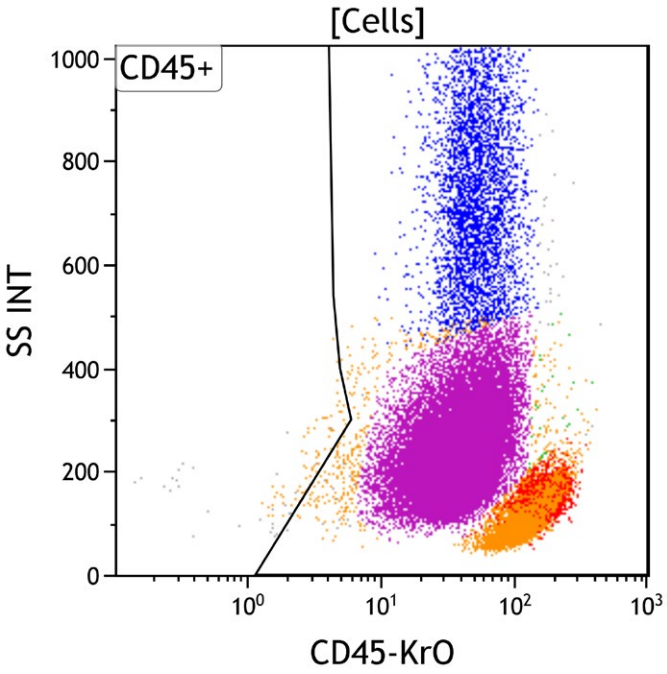

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

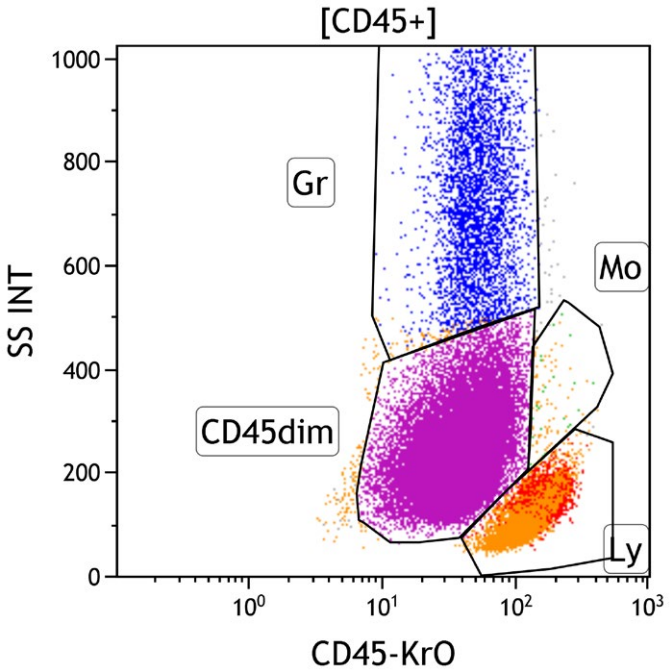

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

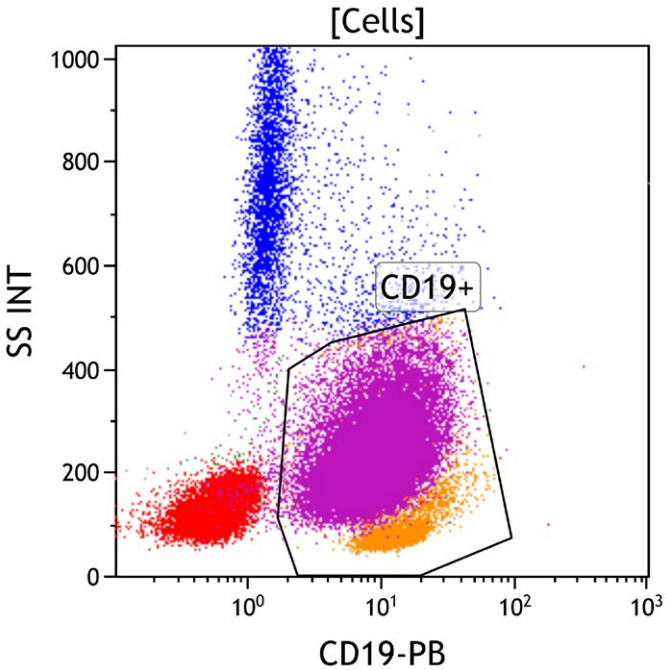

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is positive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

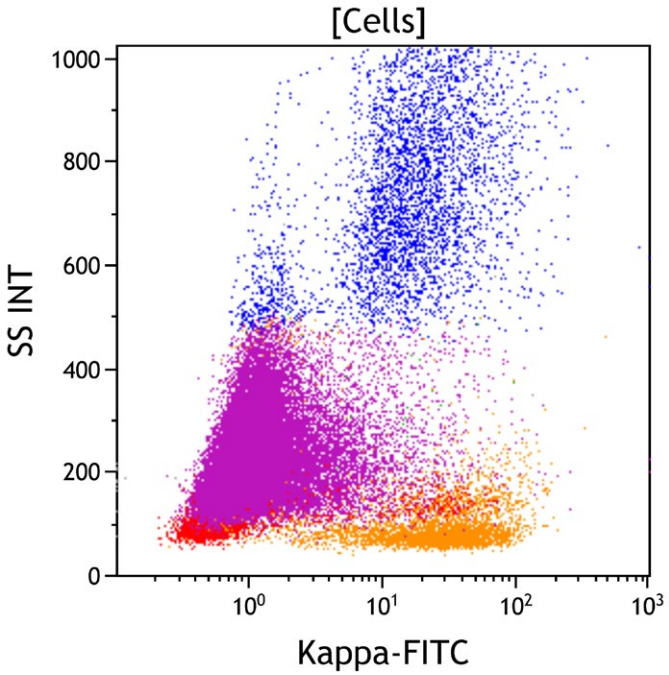

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. The Kappa light chain positive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

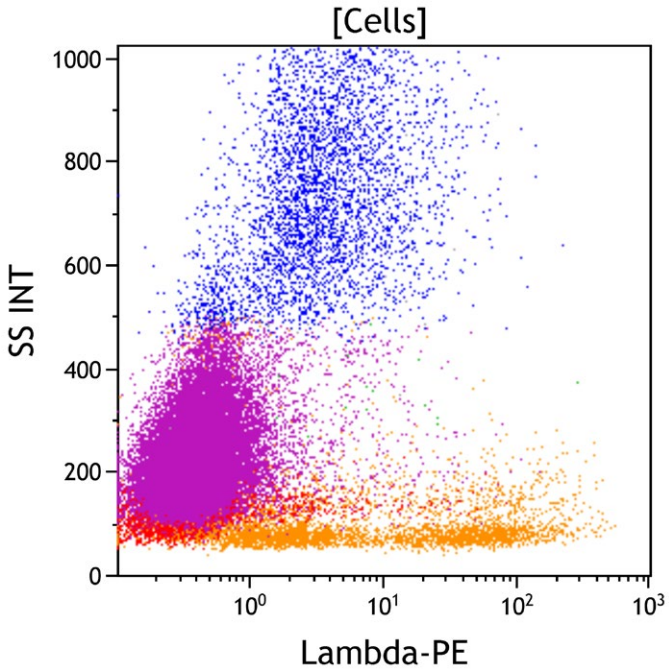

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. The lambda light chain positive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

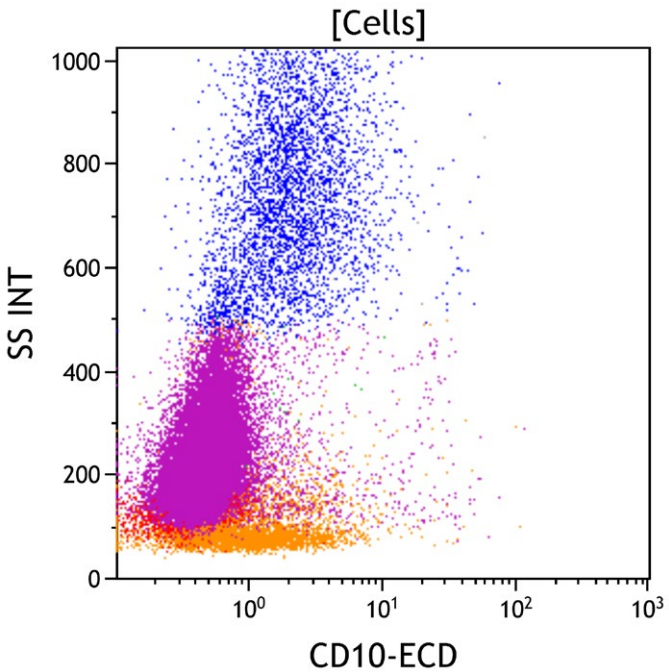

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The aberrant population (purple)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

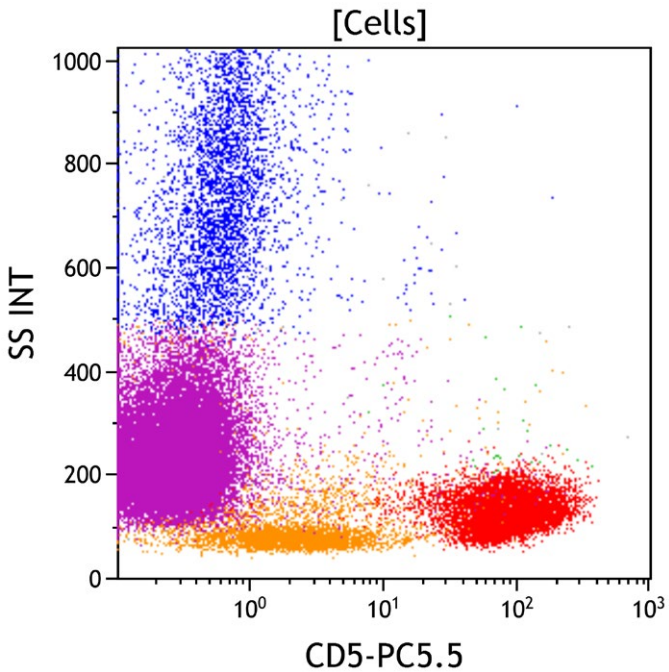

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly expressed in a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. The aberrant population (purple) is negative for CD5.

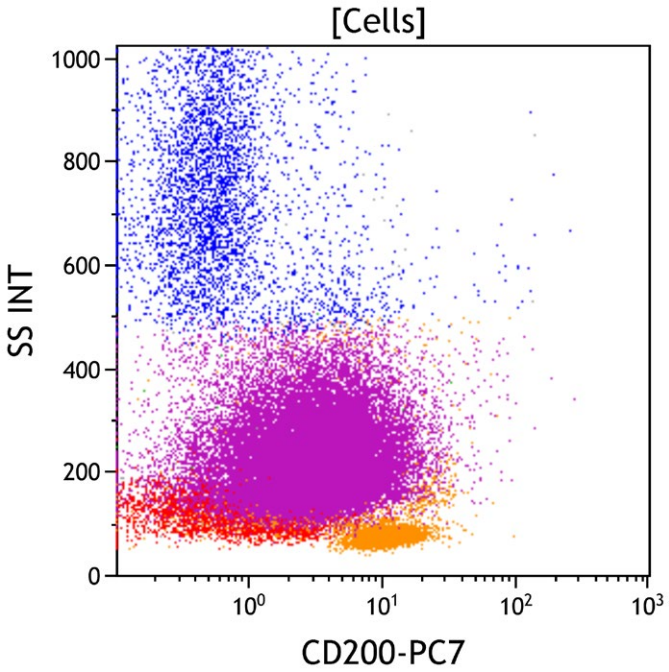

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

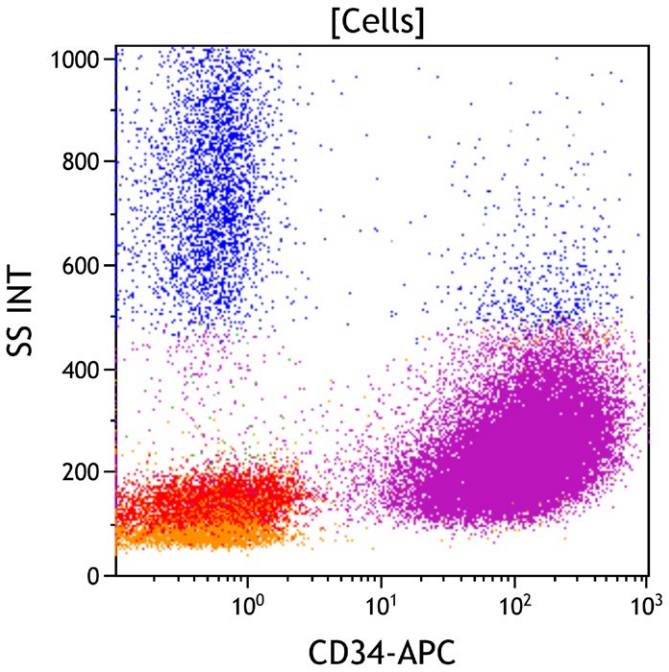

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive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

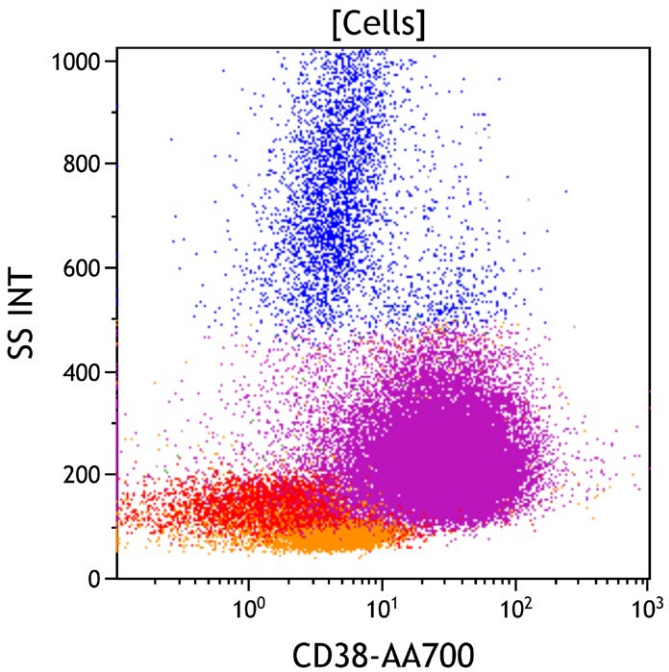

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes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

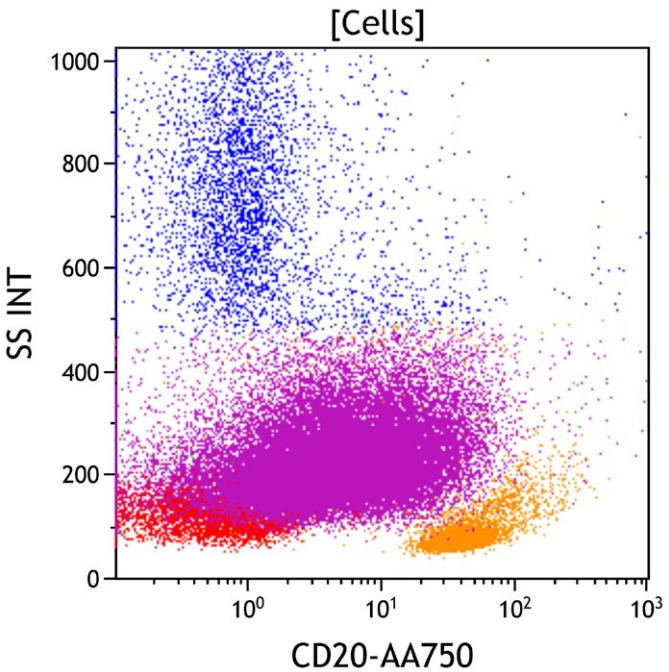

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20-positive cells are usually in the lymphocyte gate with low side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

B Cell Tube

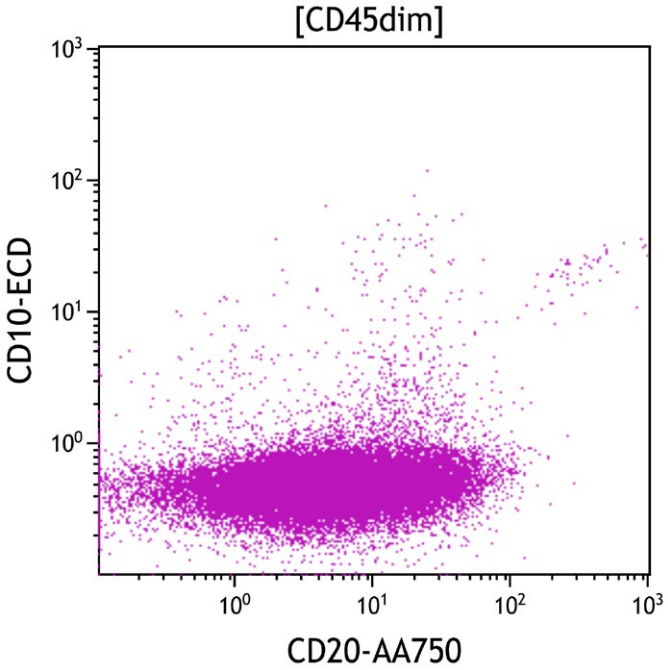

Figure 15: This CD20 vs CD10 dot plot shows all cells in the CD45dim gate. The aberrant population (purple) displays variable CD20 expression and lacks CD10 expression.

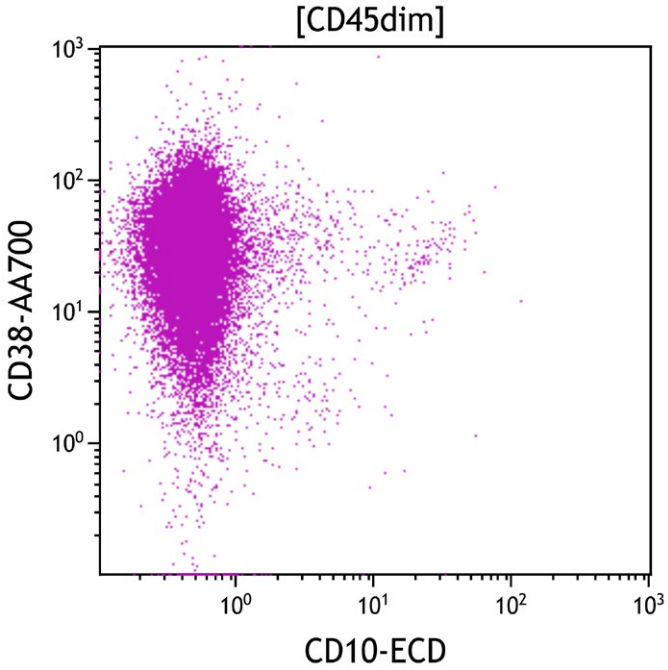

Figure 16: This CD10 vs CD38 dot plot shows all cells in the CD45dim gate. The aberrant population (purple) is positive for CD38 and negative for CD10.

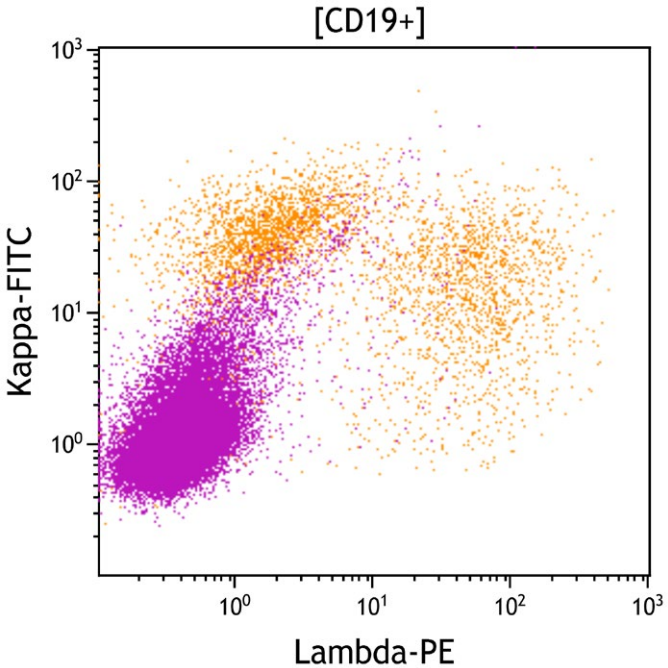

Figure 17. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells (purple) do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa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

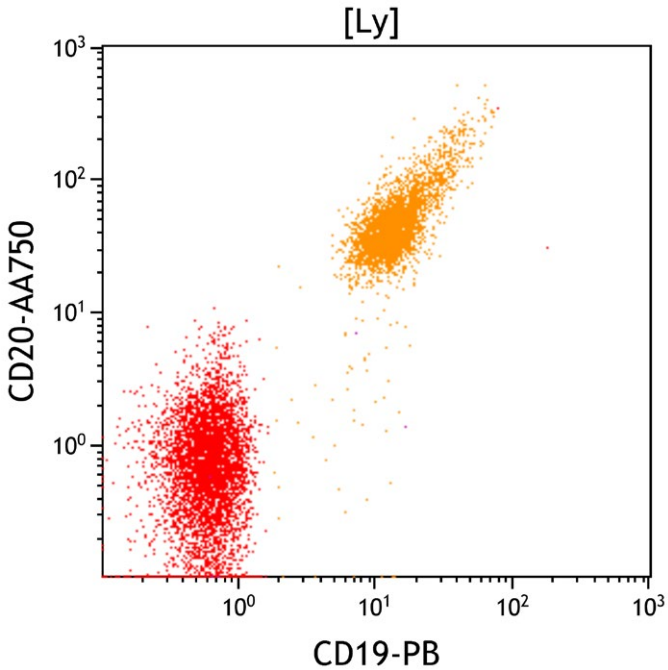

Figure 18. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression.

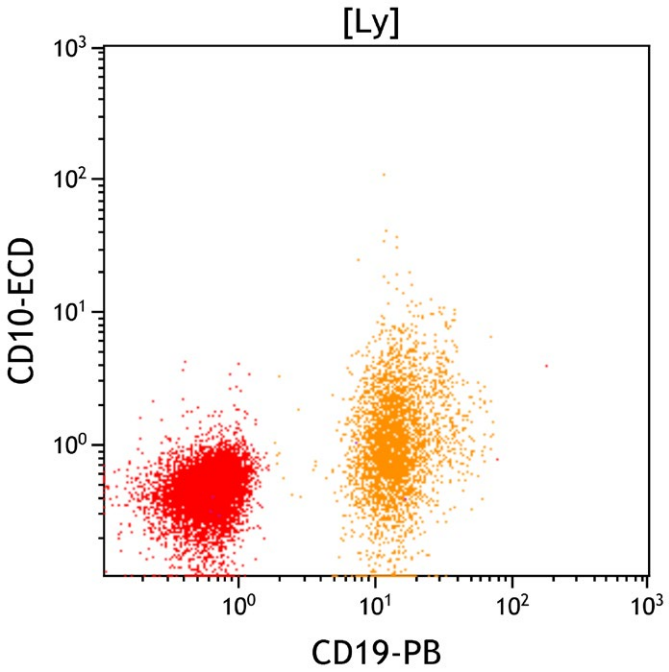

Figure 19. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells present in peripheral blood or bone marrow aspirates.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

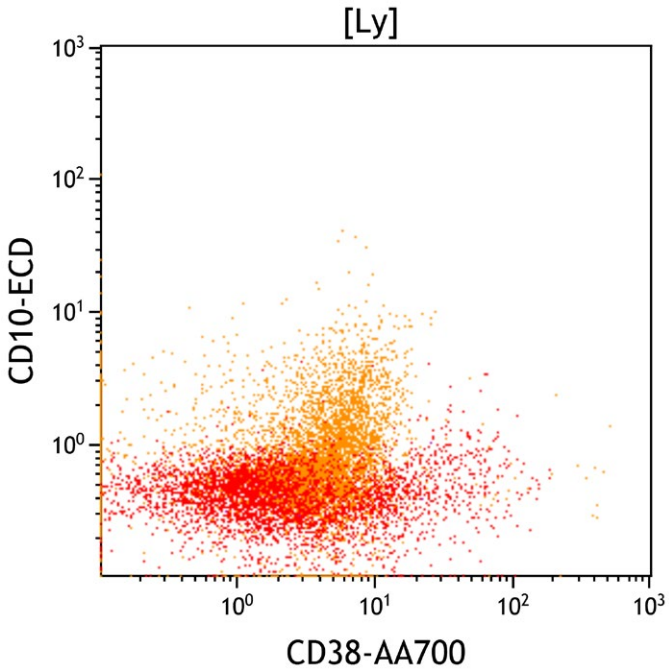

Figure 20. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable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

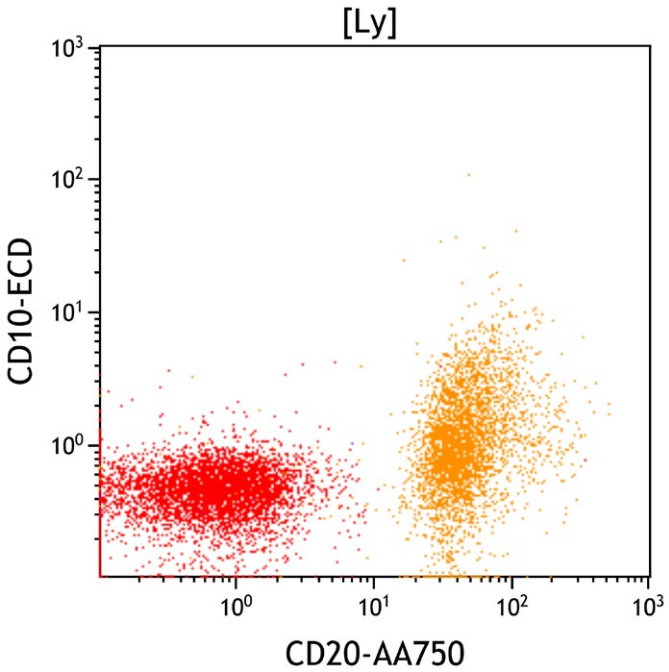

Figure 21. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20.

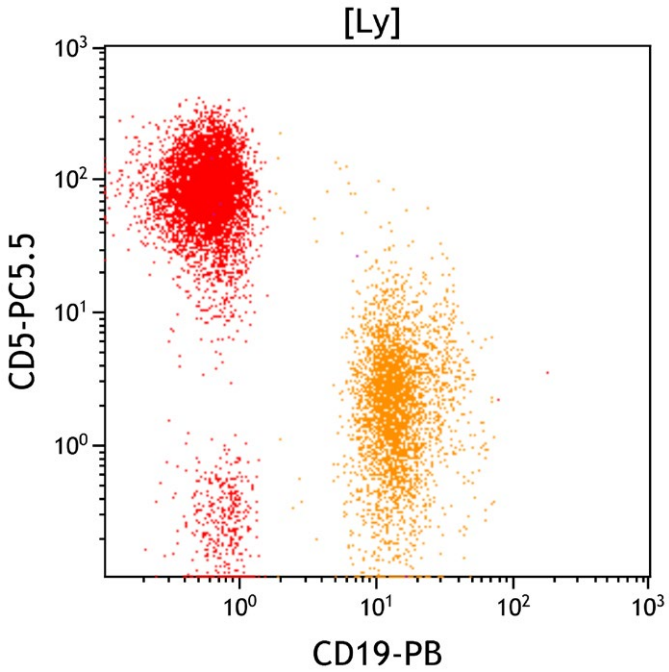

Figure 22. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells.

B Cell Tube

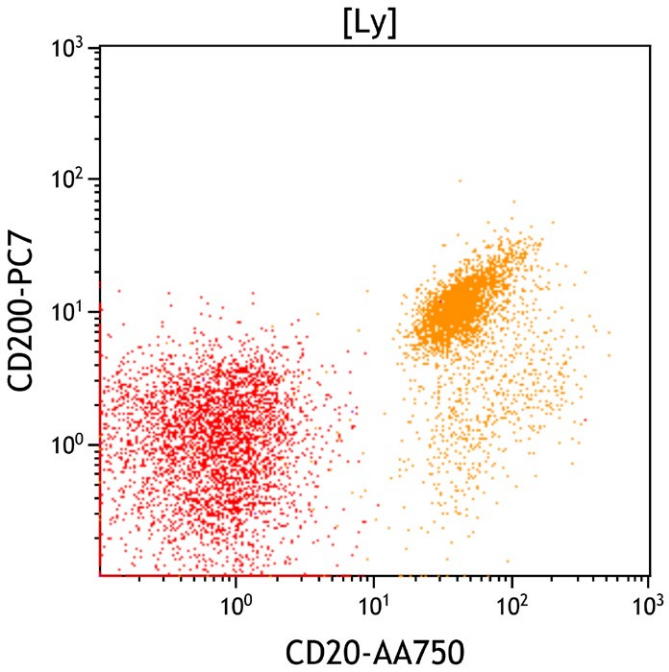

Figure 23. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

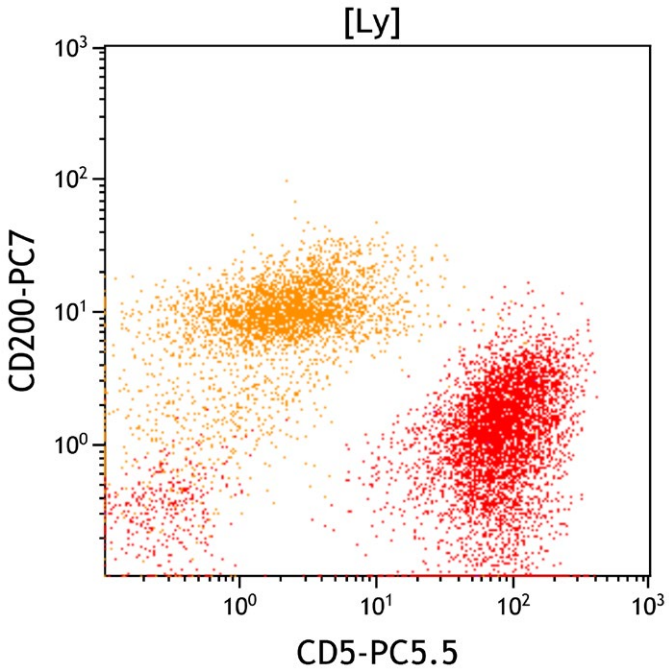

Figure 24. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma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

T Cell Tube

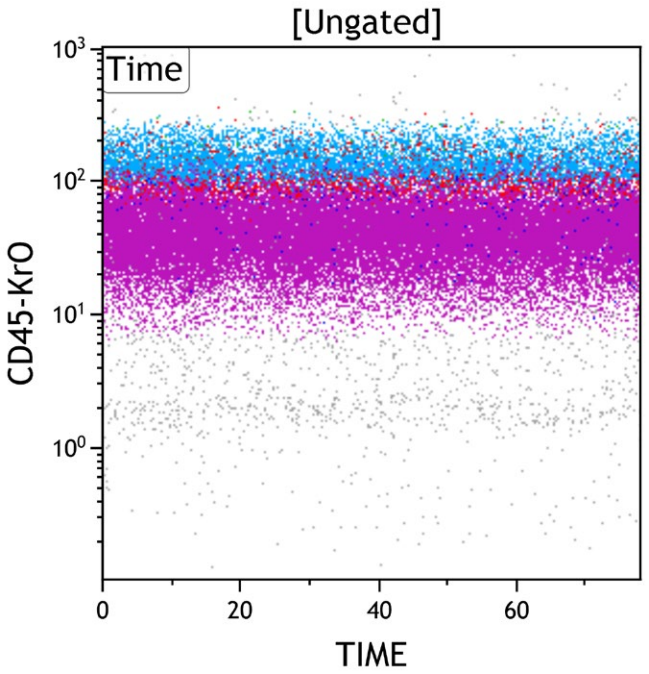

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

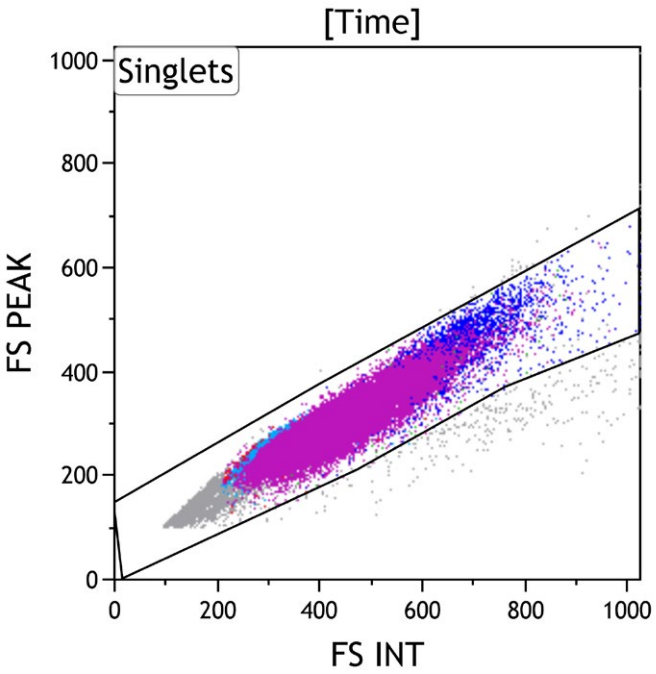

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

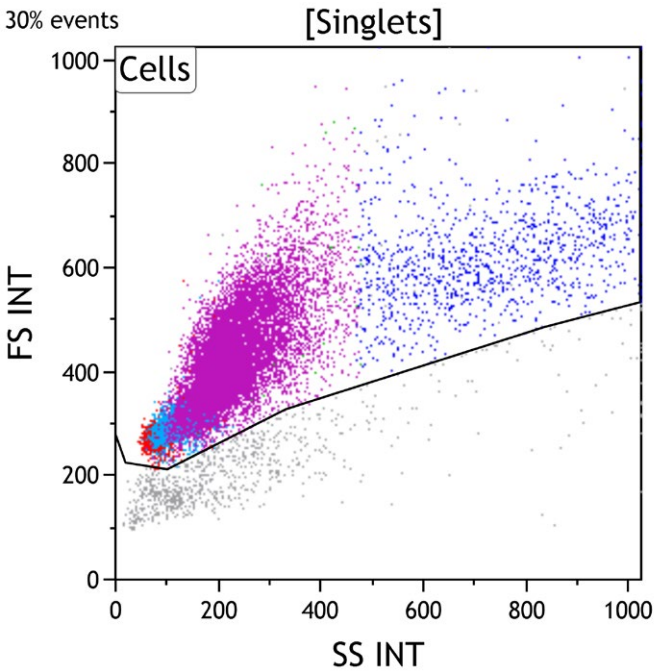

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

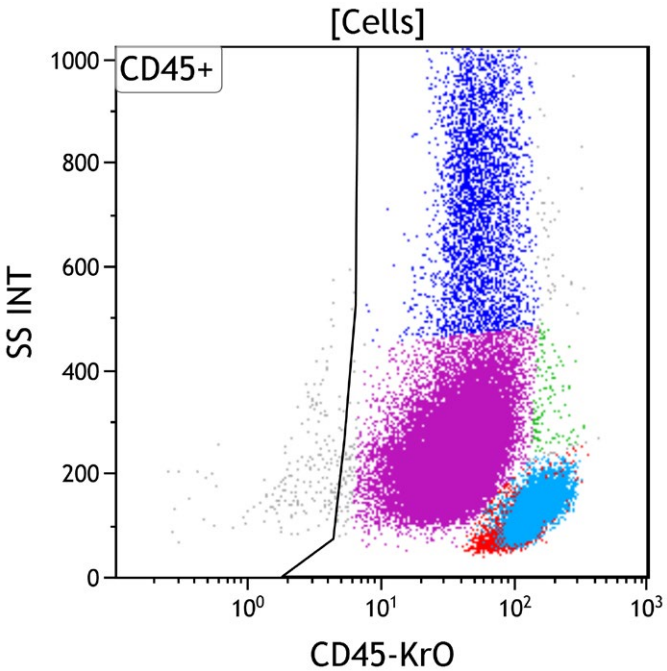

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

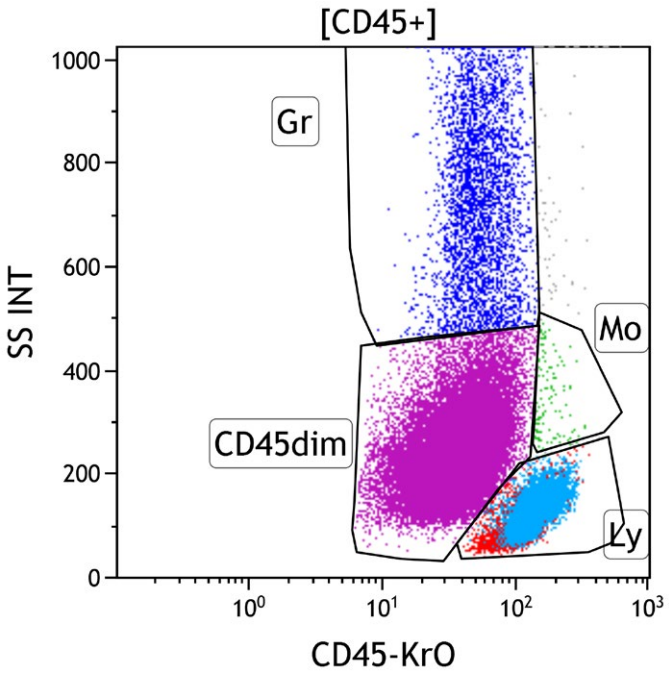

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/aqua), monocytes (Gate Mo, green), and granulocytes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

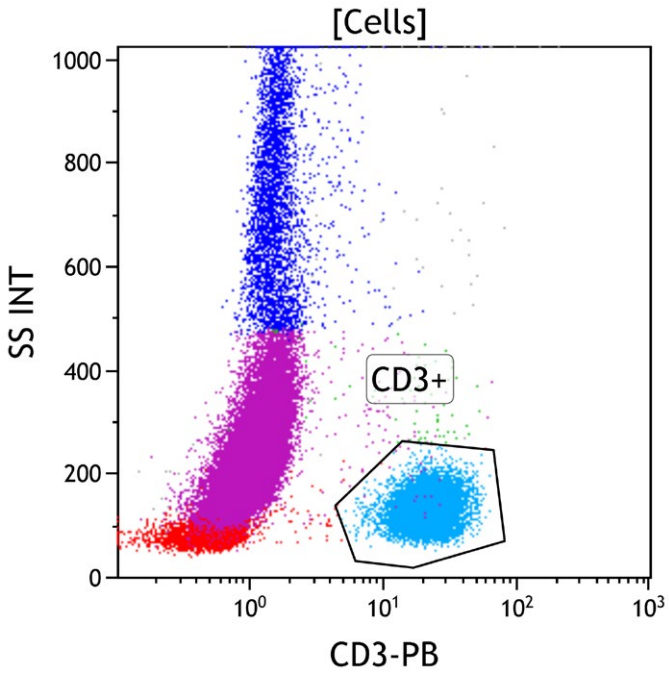

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies surface CD3 positive cells (in aqua). Surface CD3 typically expressed on mature T cell. The aberrant population (purple) is negative for CD3.

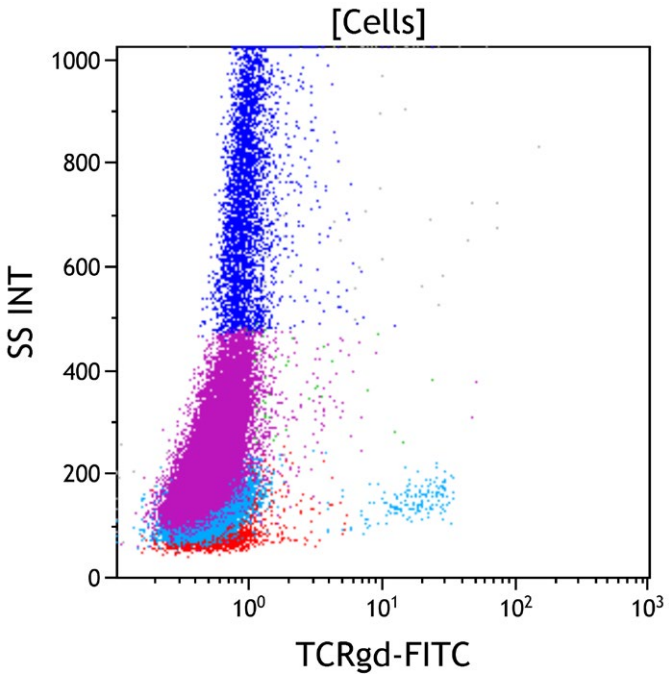

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (in aqua). The aberrant population (purple) is negative for TCRγδ.

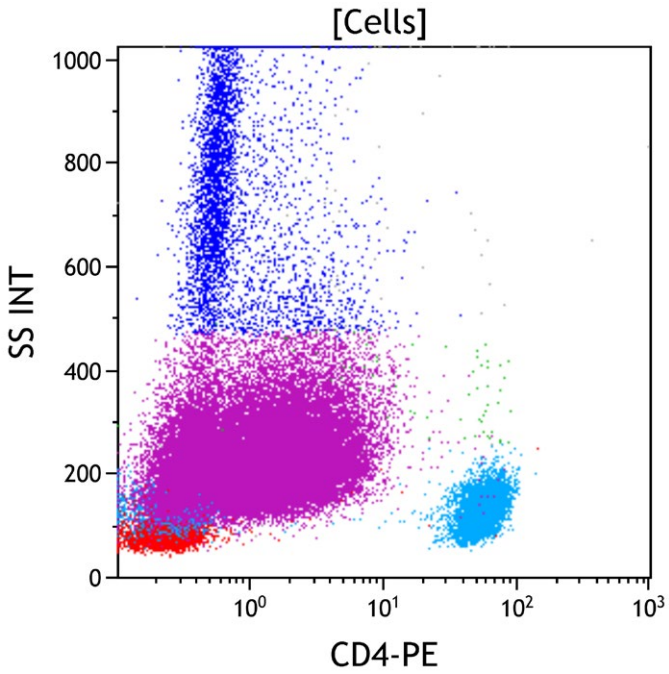

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). The aberrant population (purple) is dimly positive for CD4.

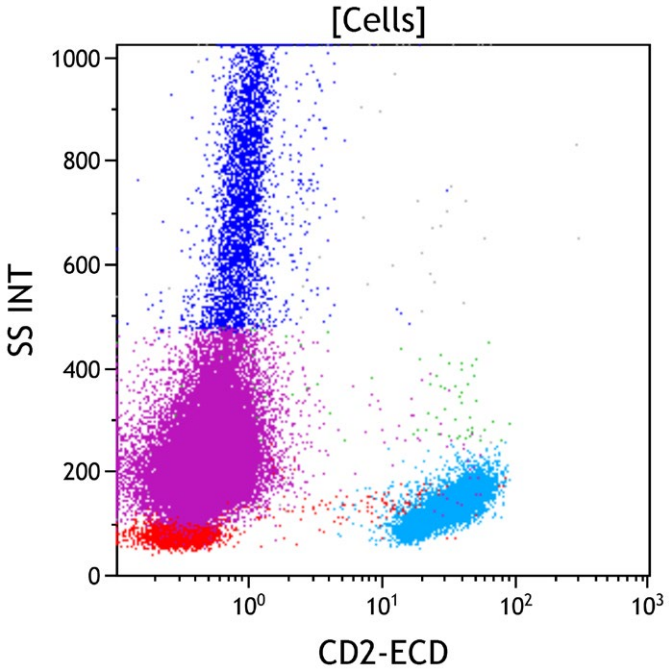

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells. The aberrant population (purple) is negative for CD2.

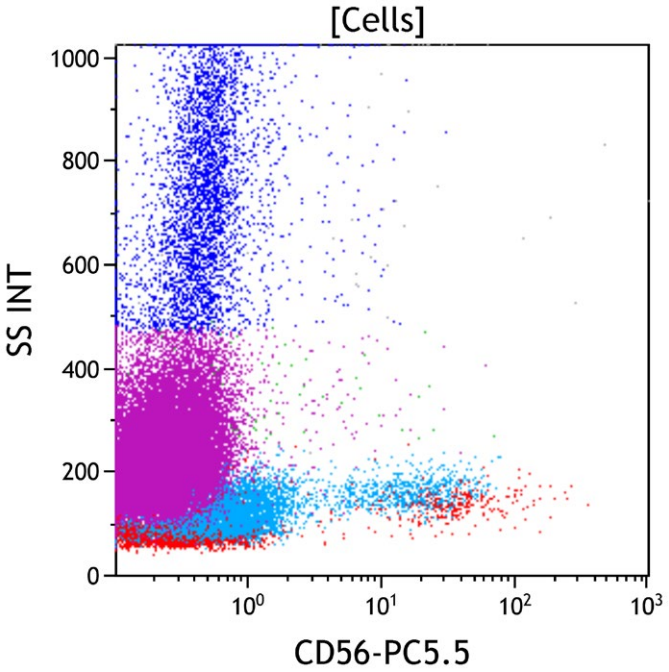

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). The aberrant population (purple) is negative for CD56.

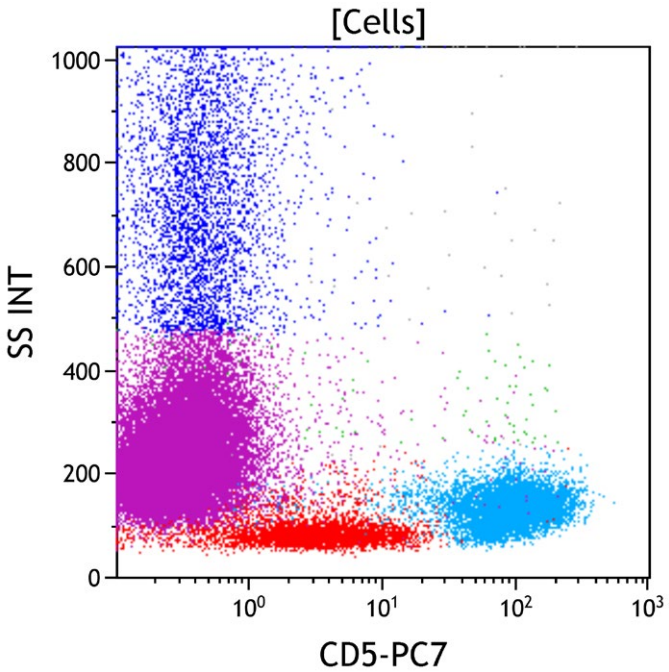

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. The aberrant population (purple)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

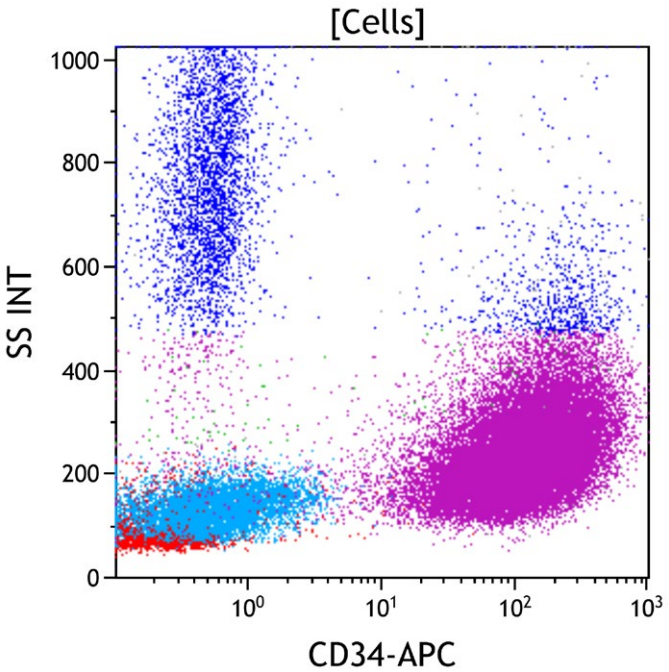

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with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

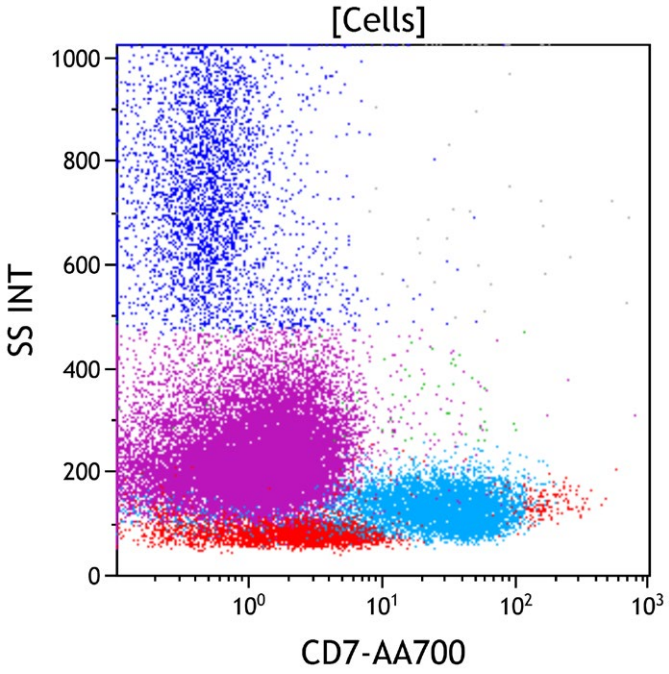

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

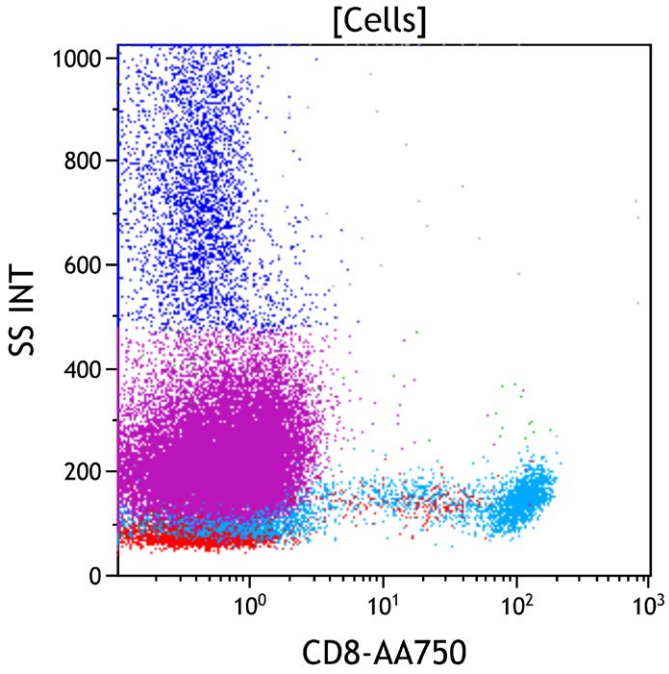

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. The aberrant population (purple) is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

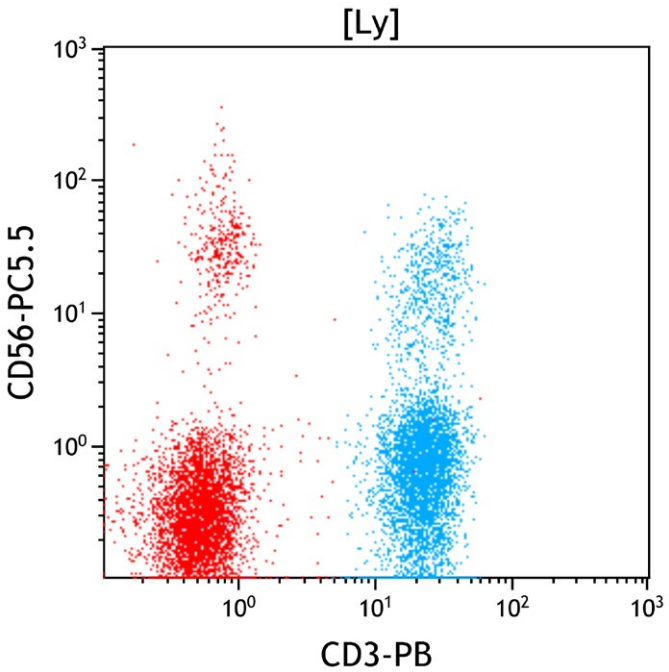

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset (red,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

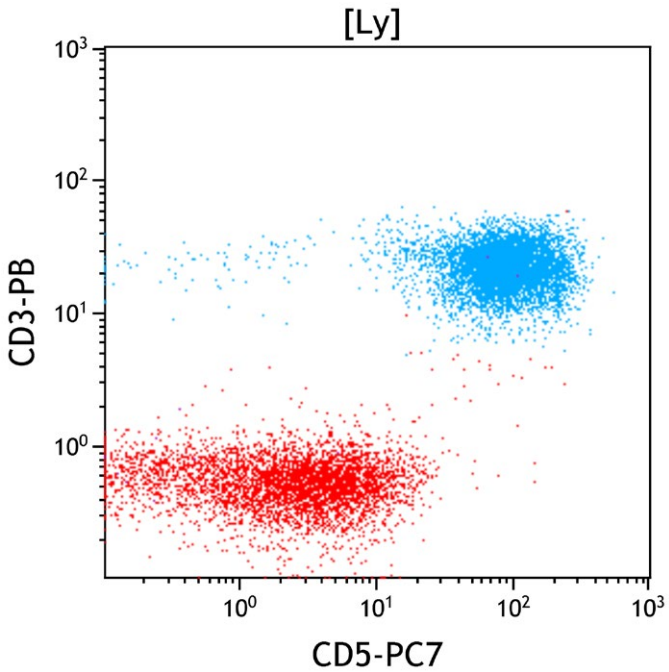

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

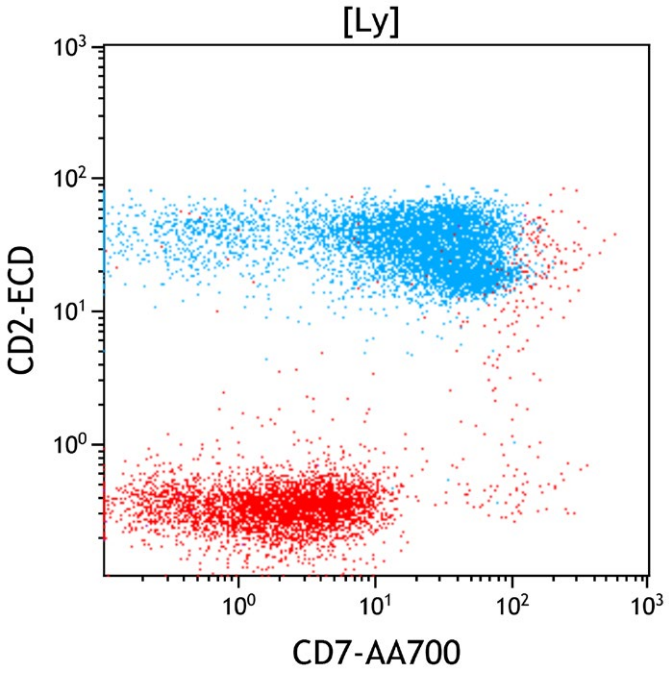

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

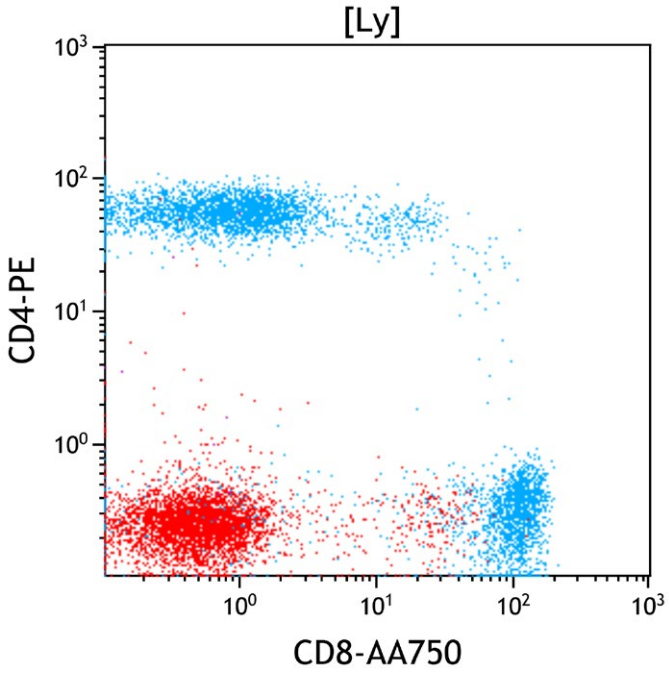

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional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

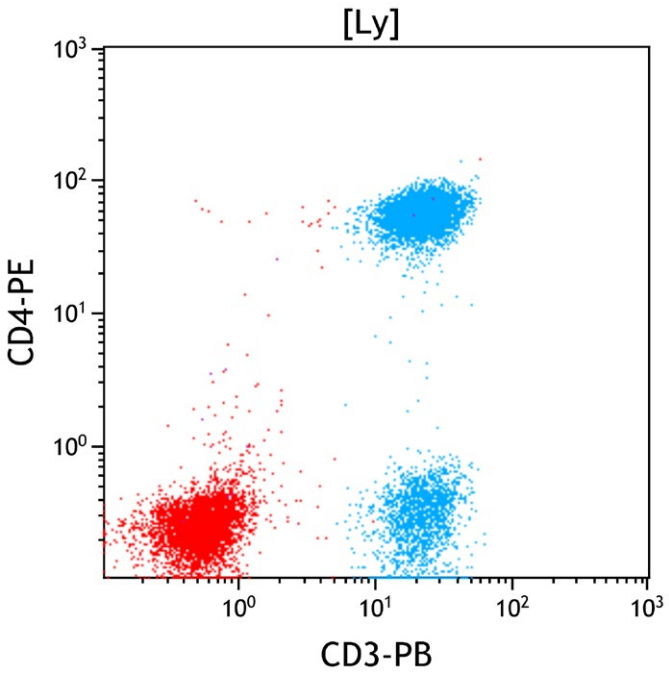

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

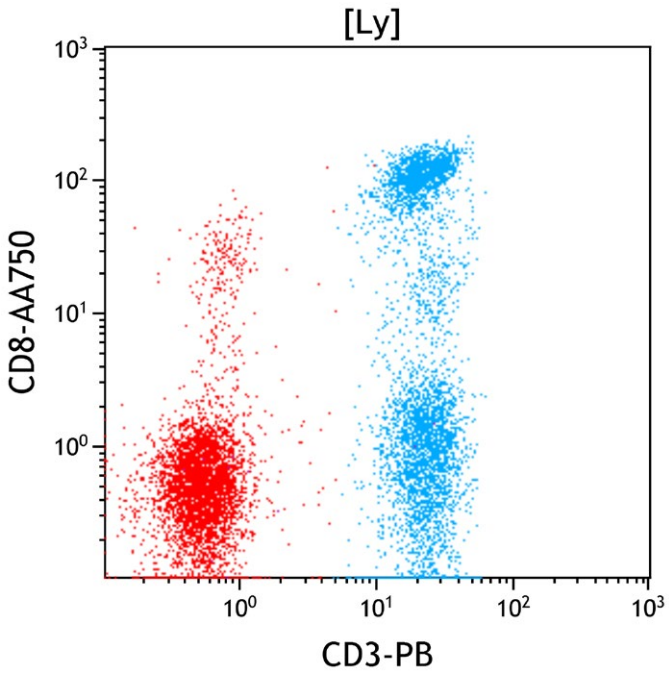

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 (red, upper left) and lacks CD3 expression.

T Cell Tube

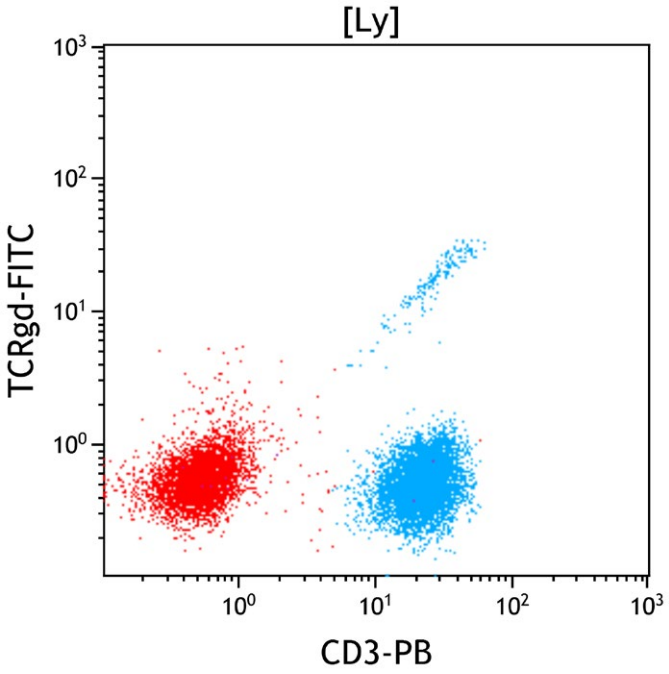

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCRγδ, which is co-expressed with CD3. The highly linear relationship between CD3 and TCRγδ is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased expression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

M1 Cell Tube

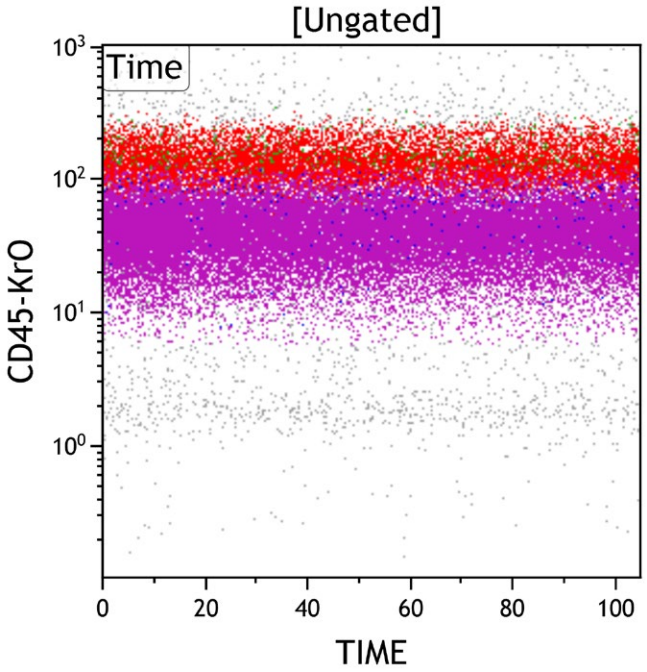

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

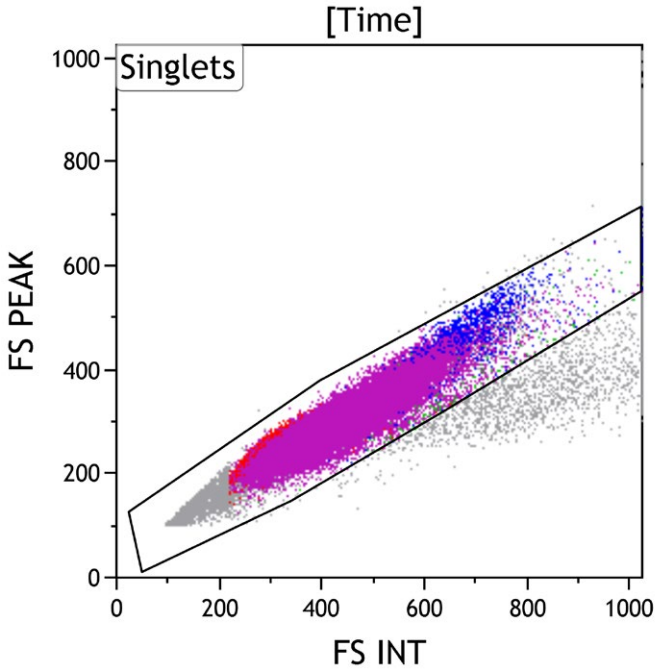

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

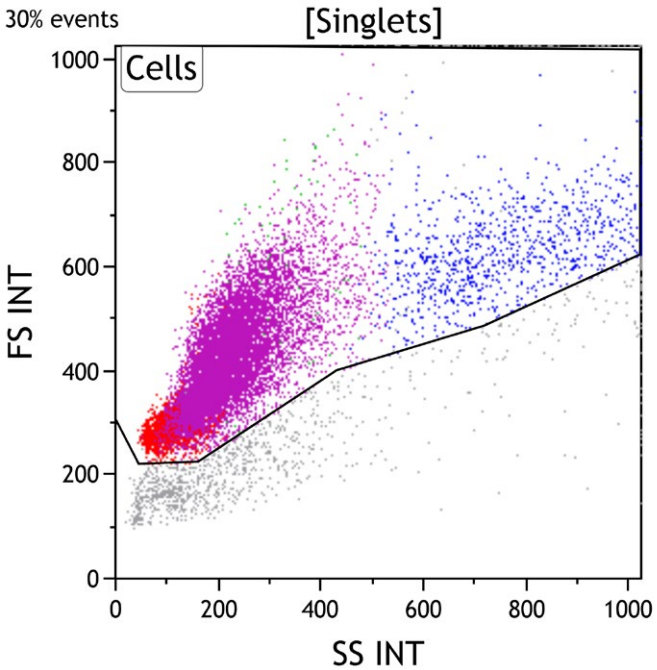

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

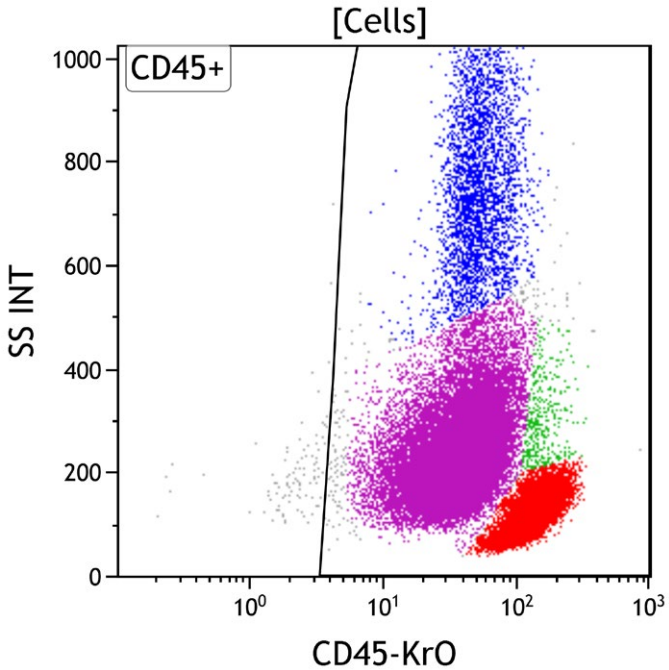

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

M1 Cell Tube

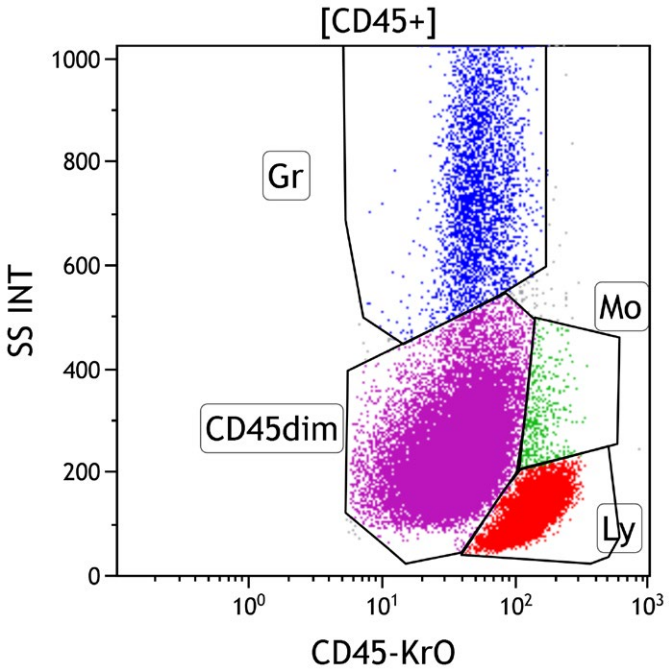

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

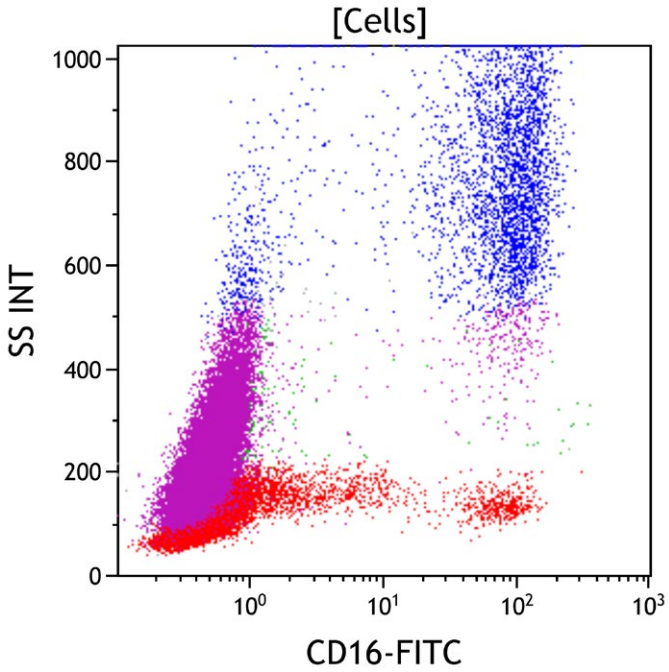

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on granulocytes (blue). Most NK cells express CD16 (red). The aberrant population (purple) is negative for CD16.

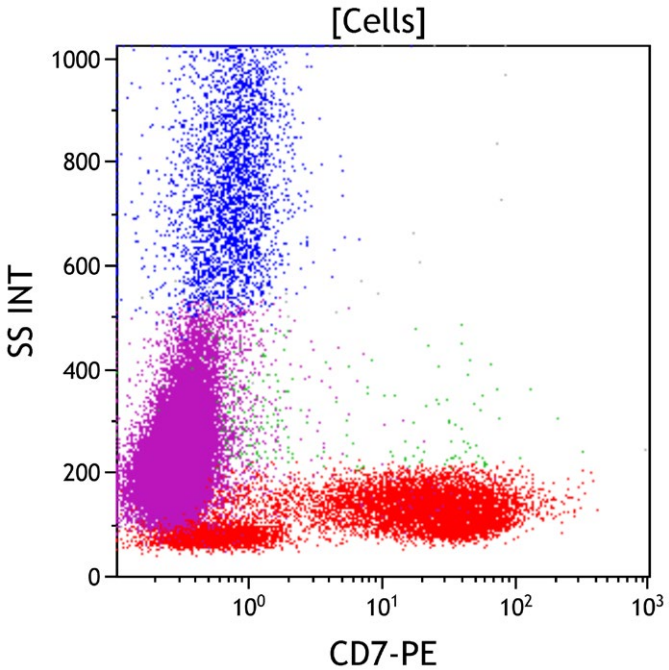

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

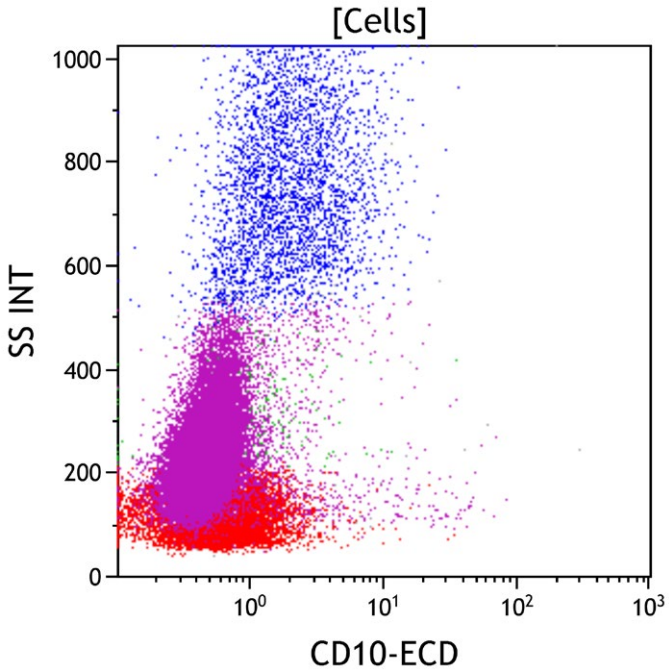

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show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

M1 Cell Tube

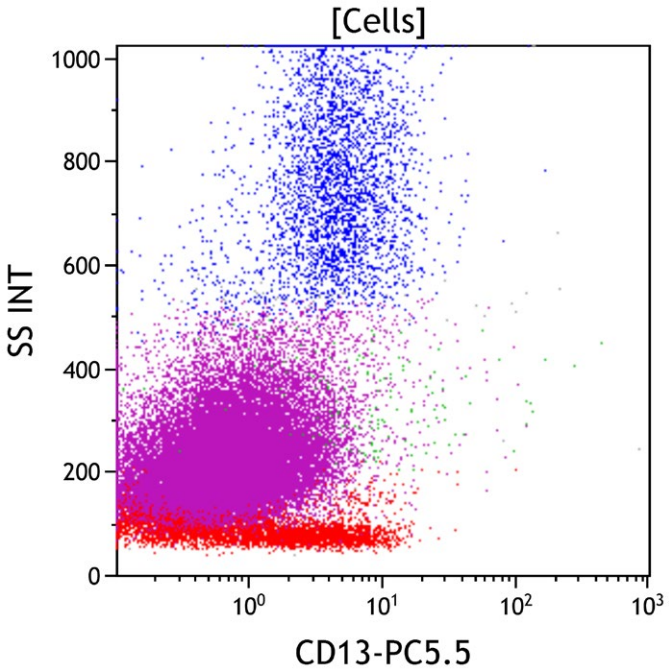

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on mature and a subset of immature granulocytes (in blue, with high side scatter). The aberrant population (purple) is dimly positive for CD13.

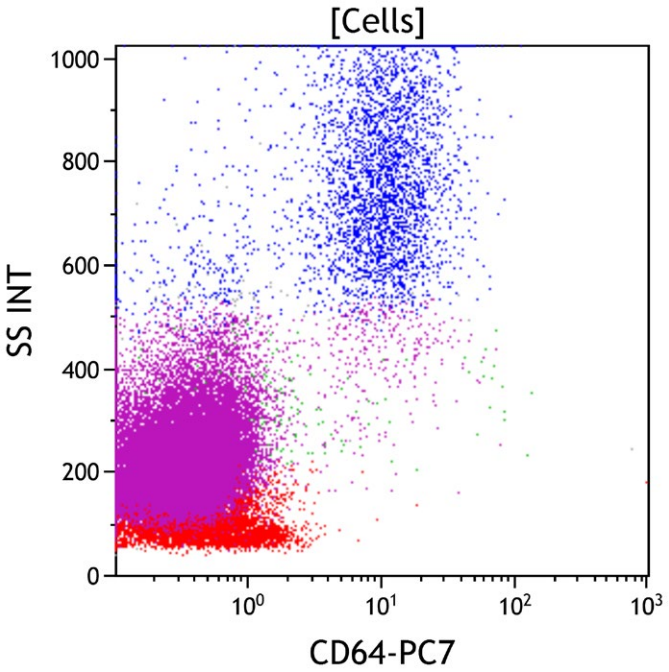

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid cells or most CD34 positive progenitors. The aberrant population (purple)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

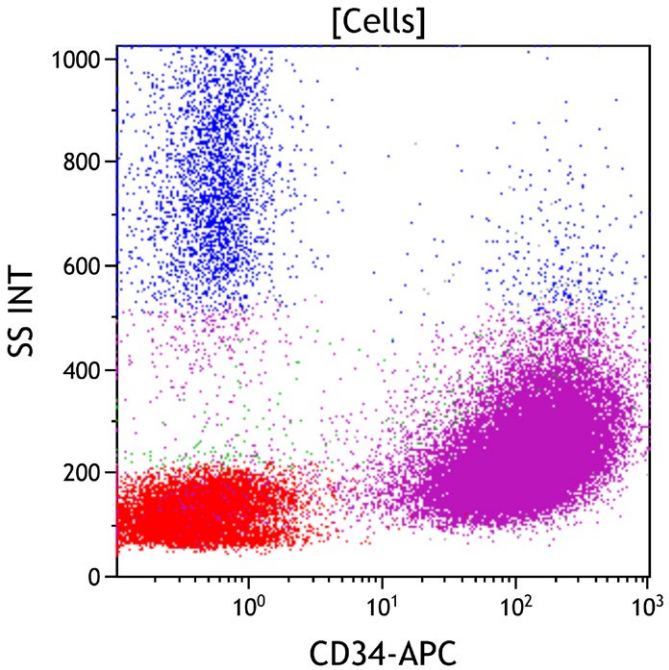

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive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

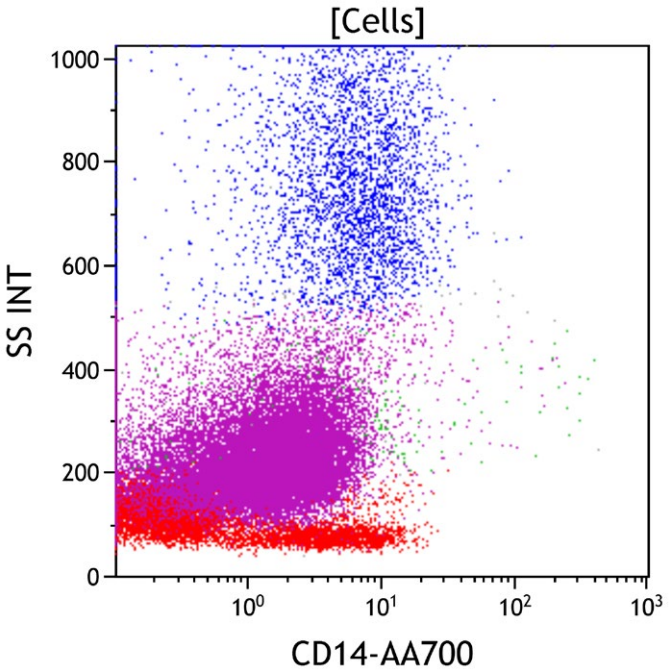

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed on granulocytes (blue) at a low level. The aberrant population (purple) is negative for CD14.

M1 Cell Tube

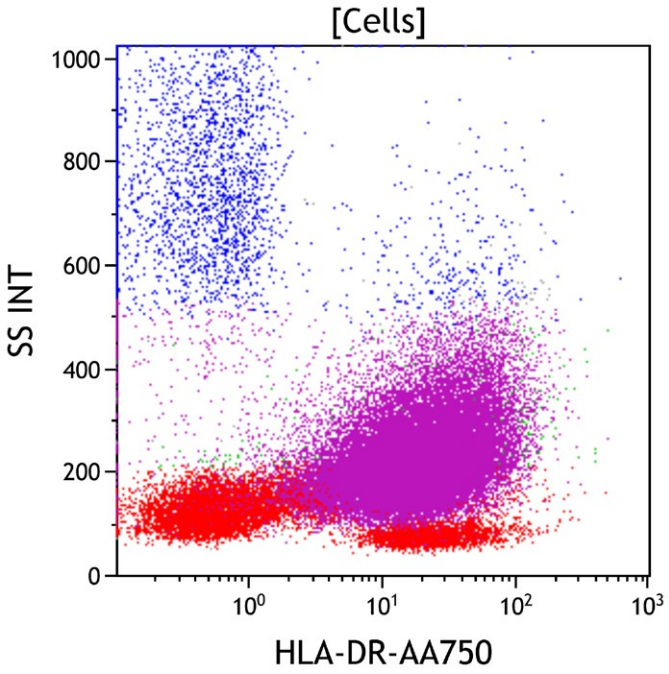

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. The aberrant population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

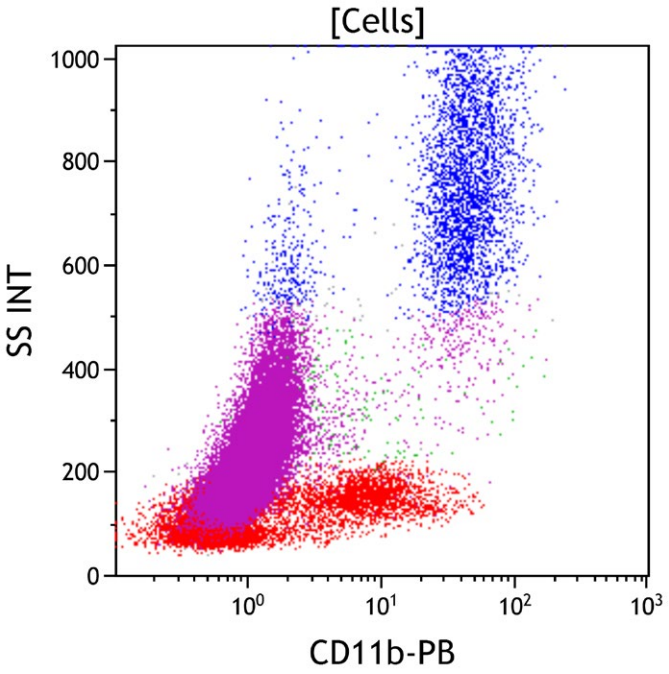

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (in blue), monocytes, basophils, and NK cells . The aberrant population (purple) is negative for CD11b.

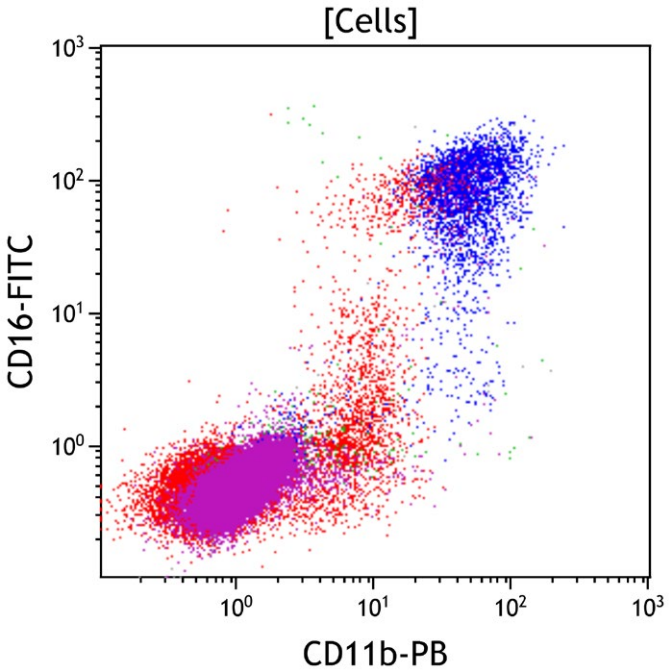

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, granulocytes (blue), and NK cells. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is negative for CD11b and CD16.

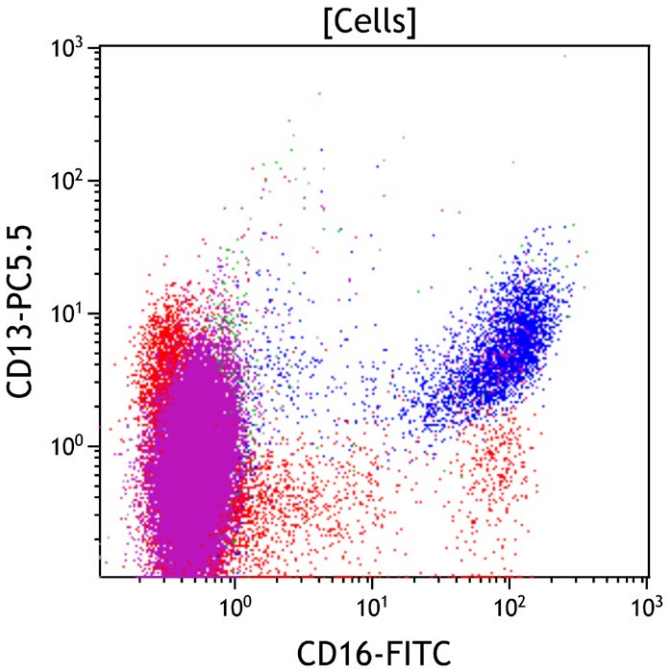

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red). The aberrant population (purple) is dimly positive for CD13 and negative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

M1 Cell Tube

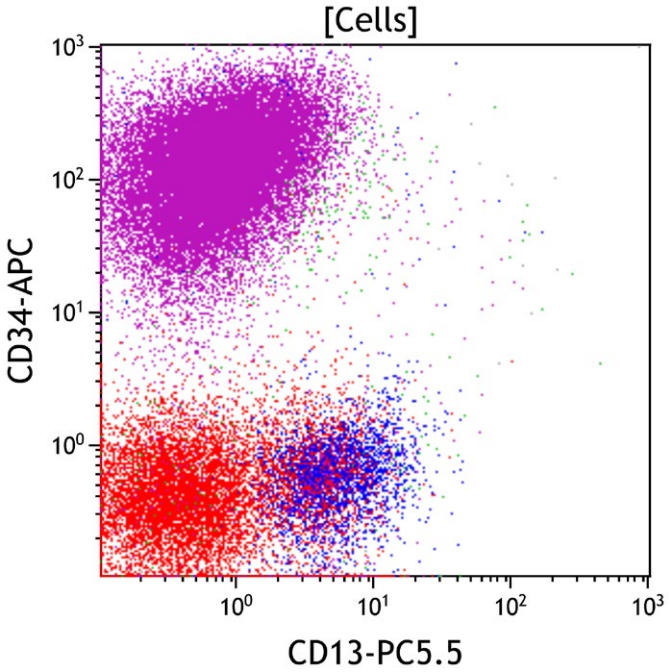

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. The aberrant population (purple) is strongly positive for CD34 and dimly positive for CD13.

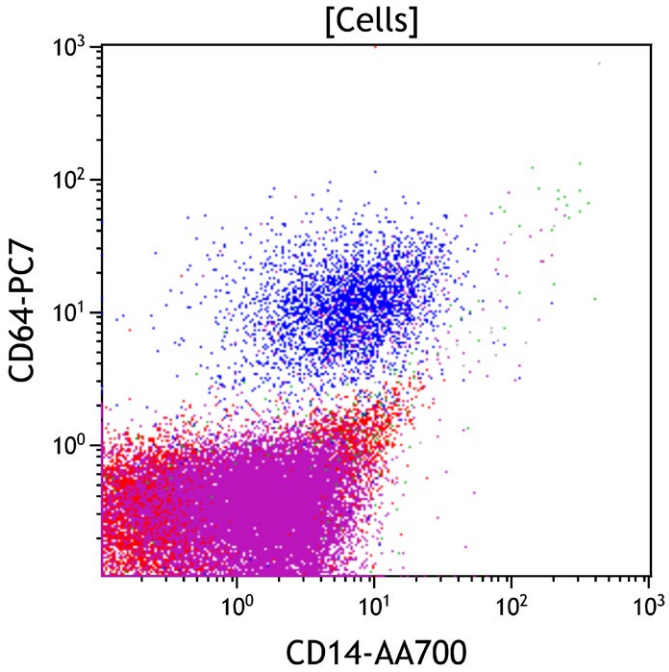

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes (blue). The aberrant population (purple) is negative for CD14 and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

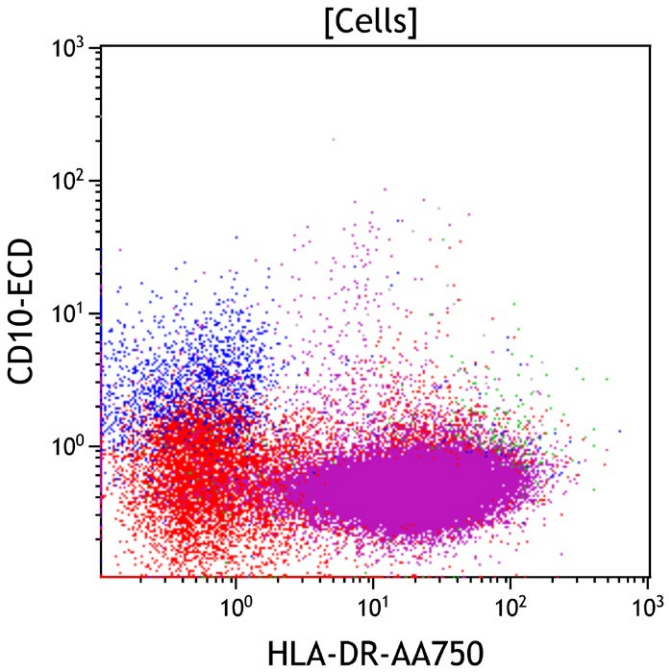

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells and CD34 positive progenitors. CD10 is expressed on granulocytes (blue). The aberrant population (purple) is positive for HLA-DR and negative for CD10.

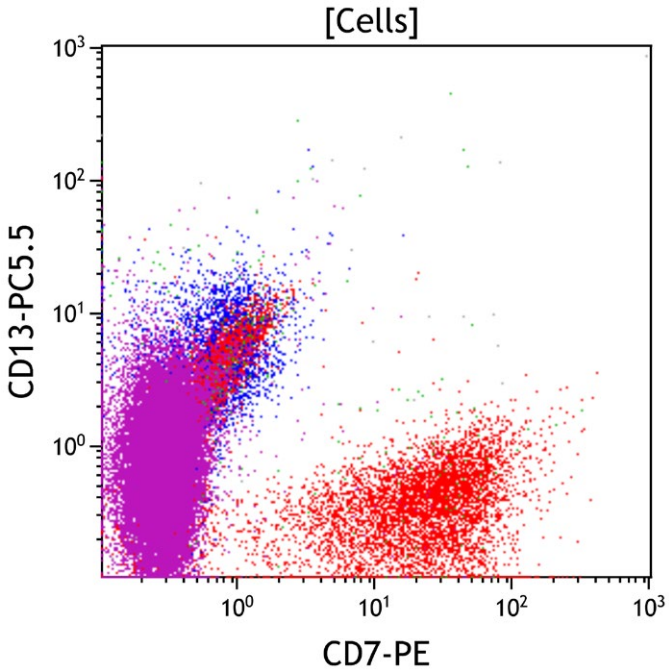

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on granulocytes (blue), monocytes, and basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The aberrant population (purple) is dimly positive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

M2 Cell Tube

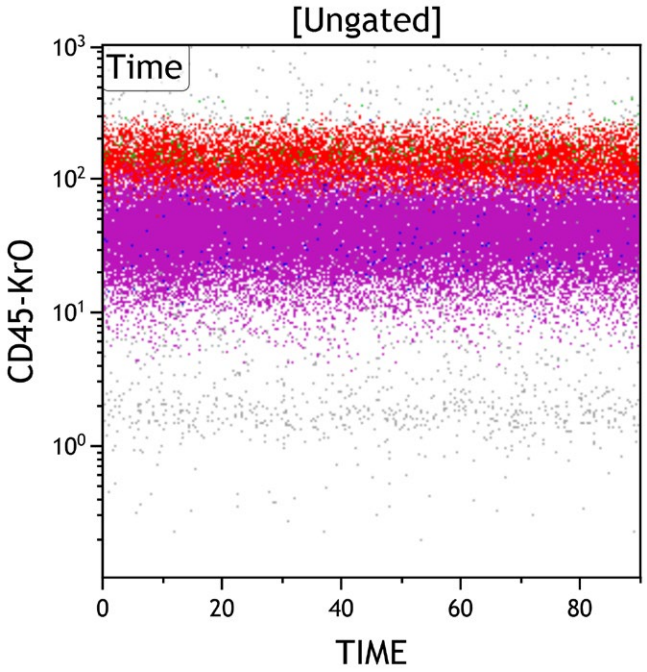

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

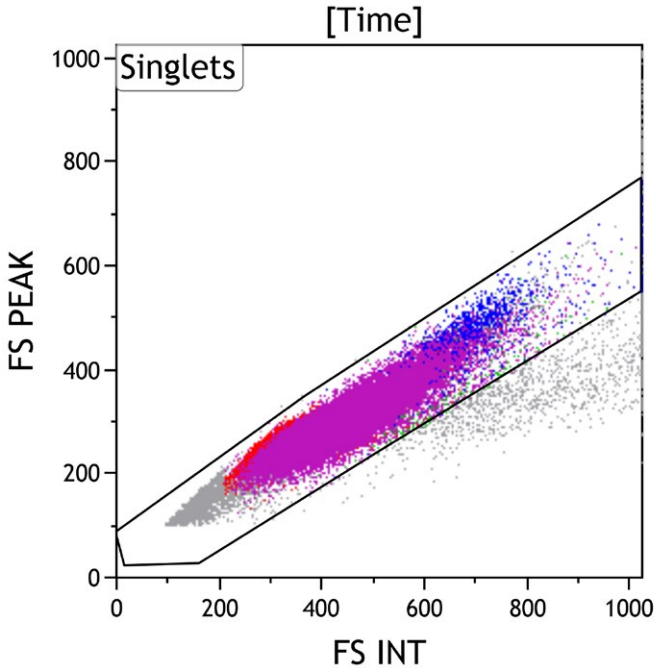

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

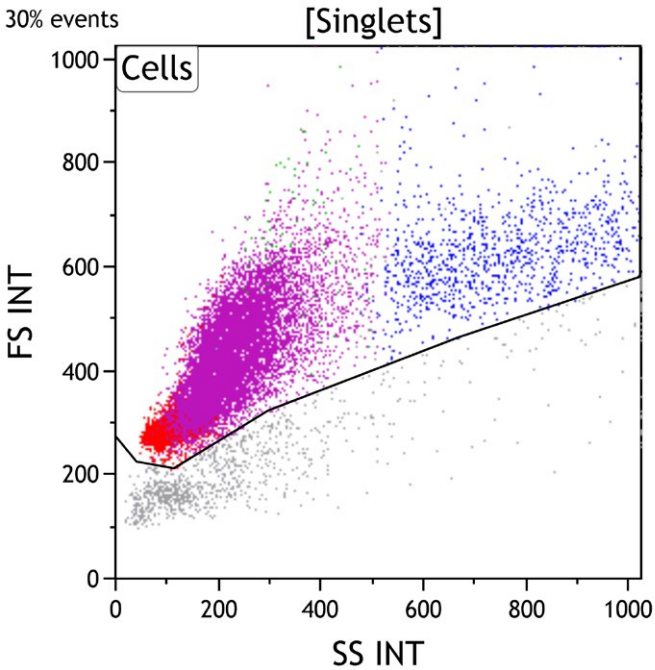

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

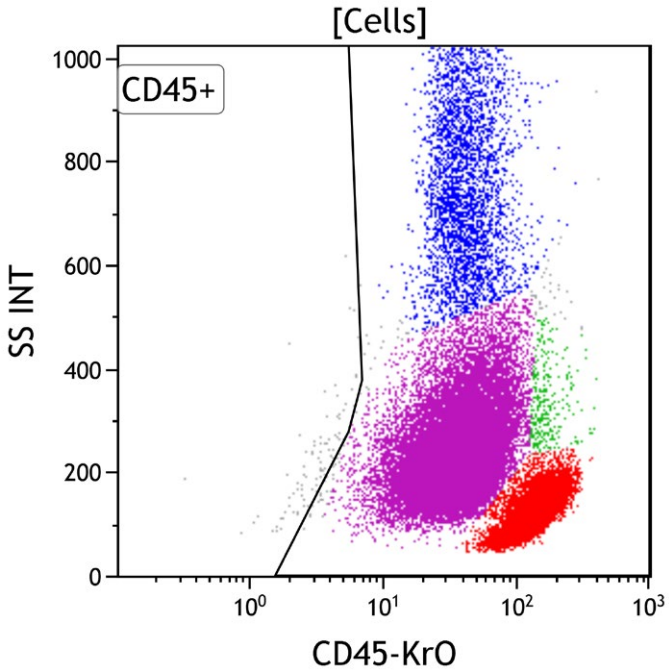

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

M2 Cell Tube

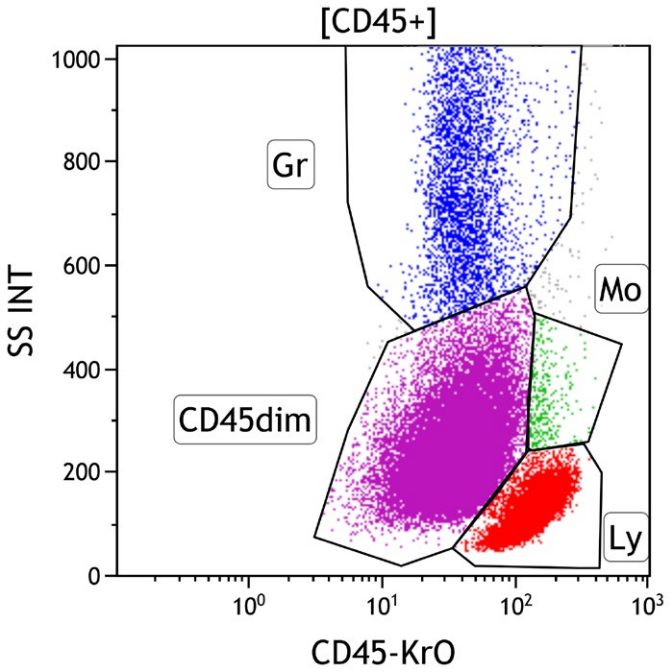

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

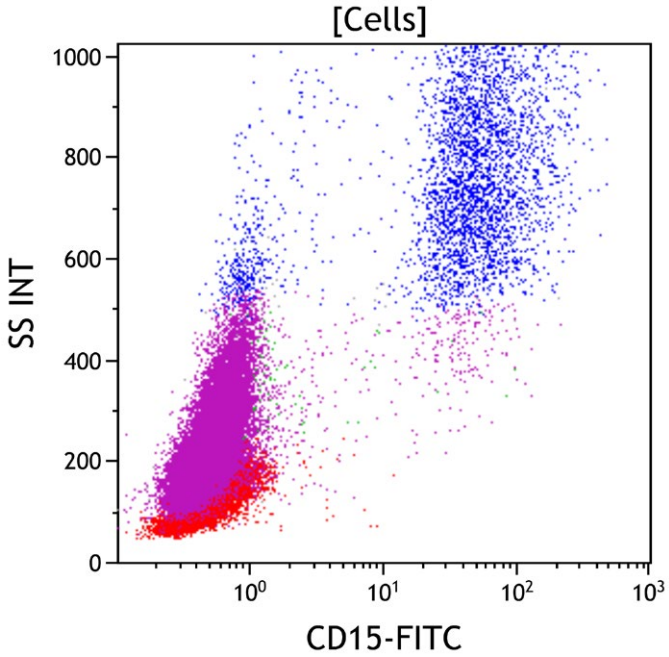

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on mature granulocytes (in blue). The aberrant population (purple) is negative for CD15.

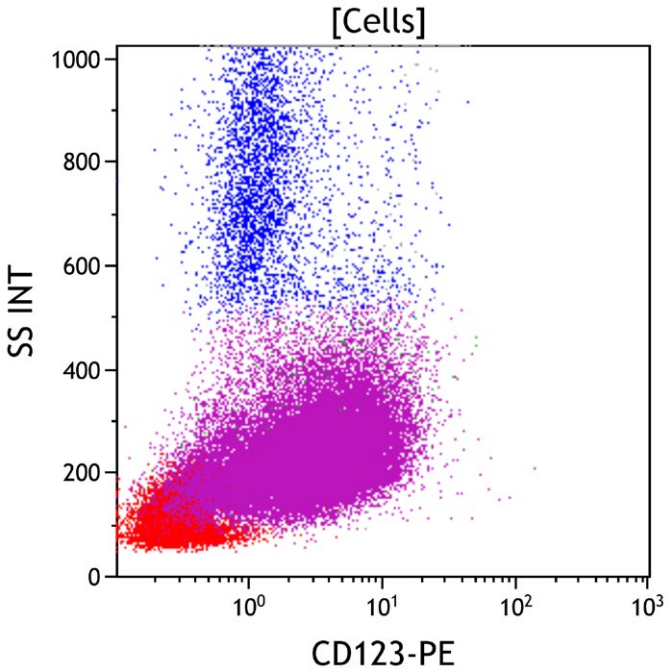

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. The aberrant population (purple) displays dim to intermediate CD123 expression.

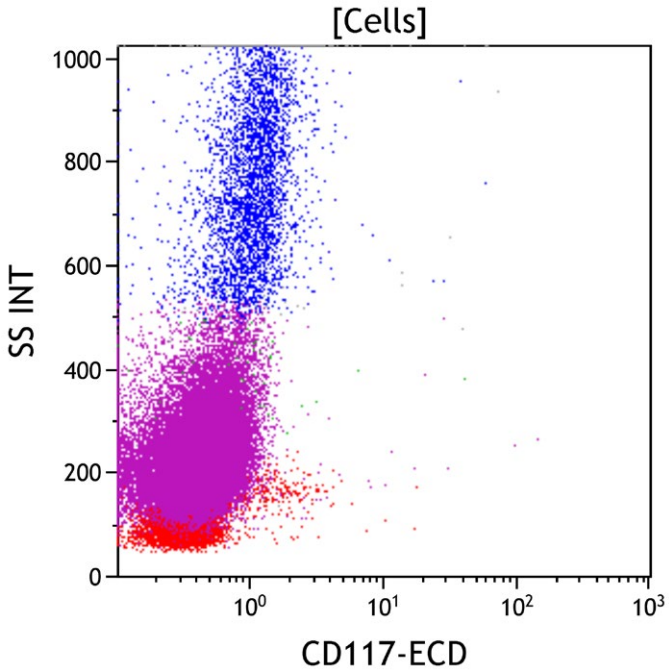

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

M2 Cell Tube

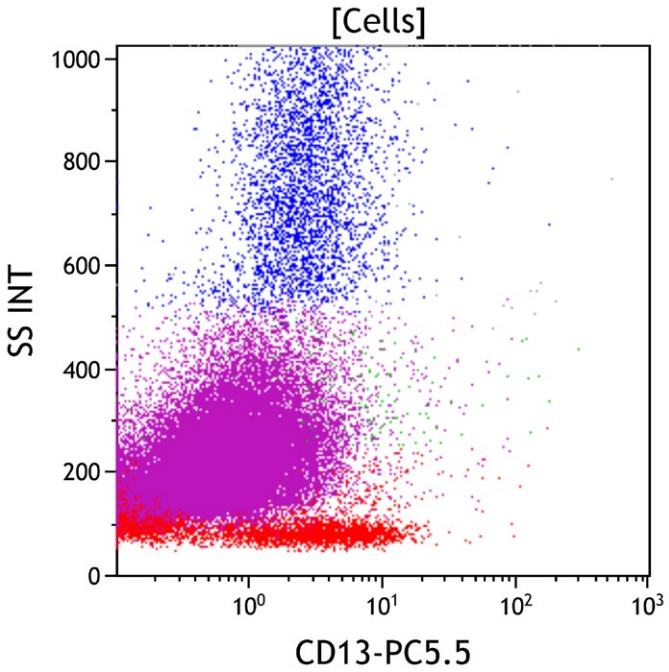

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (in blue, with high side scatter) and on mature monocytes. The aberrant population (purple) is dimly positive for CD13.

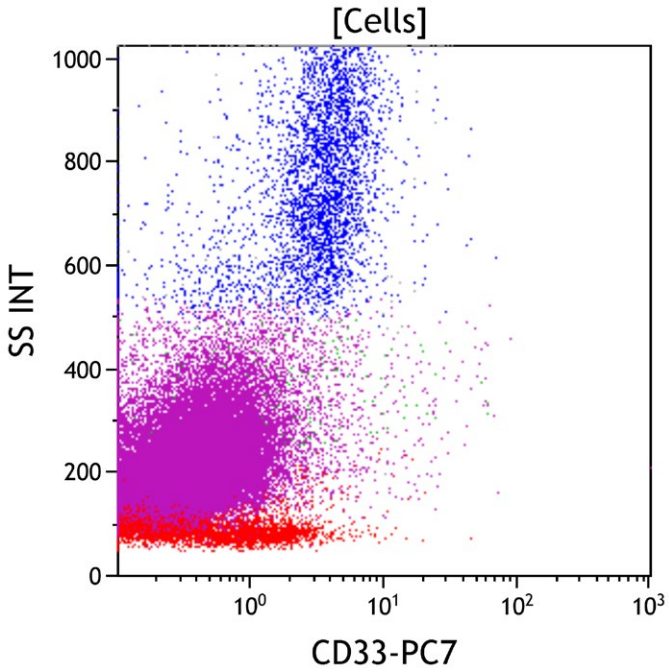

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at a high level on monocytes and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells (red), and a subset of CD34 positive myeloid progenitors. The aberrant population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

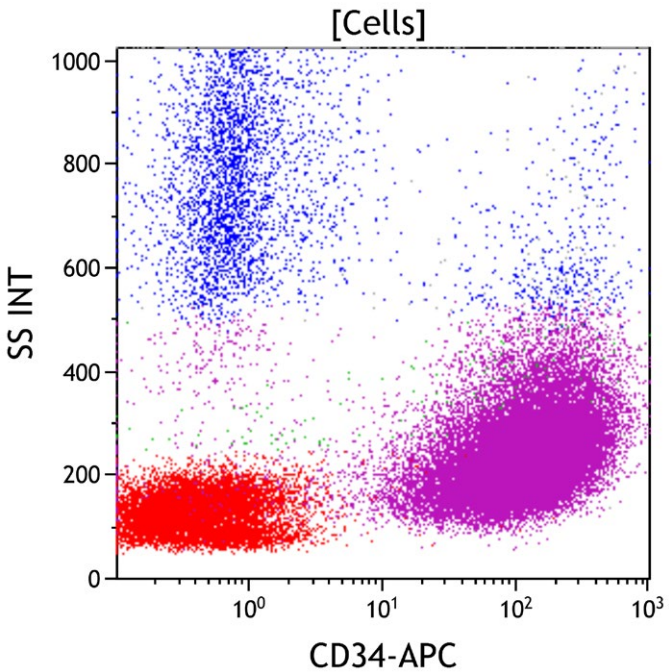

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The aberrant population (purple) is strongly positive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

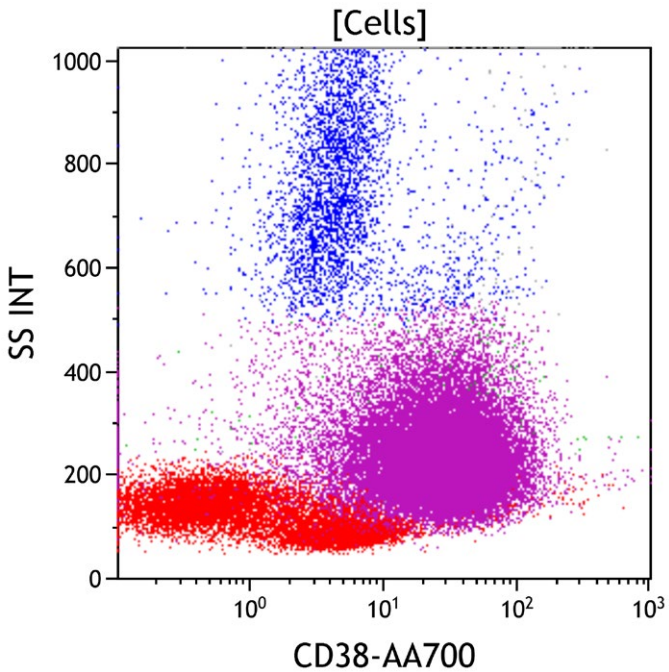

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variable level on activated lymphocytes (red).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

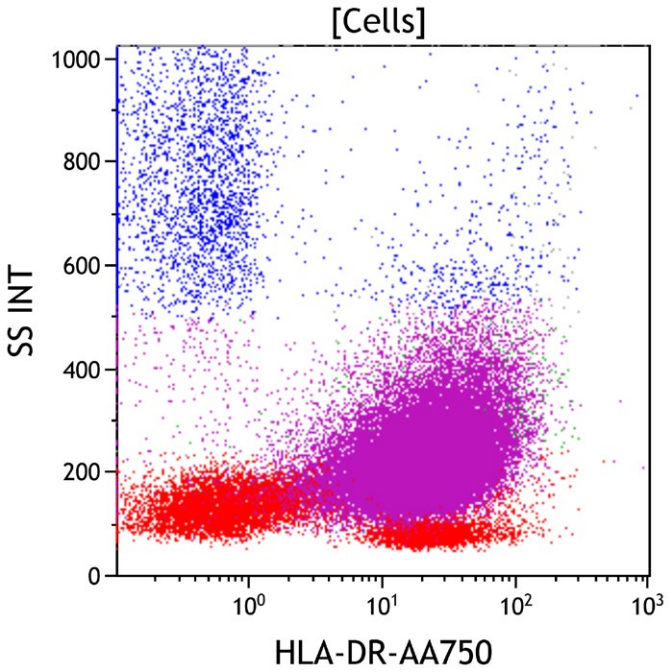

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red). The aberrant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

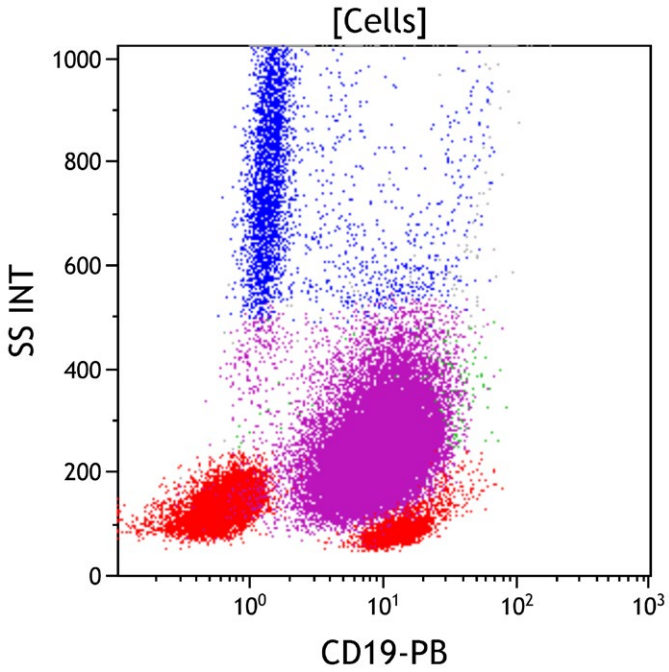

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The aberrant population (purple) is positive for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

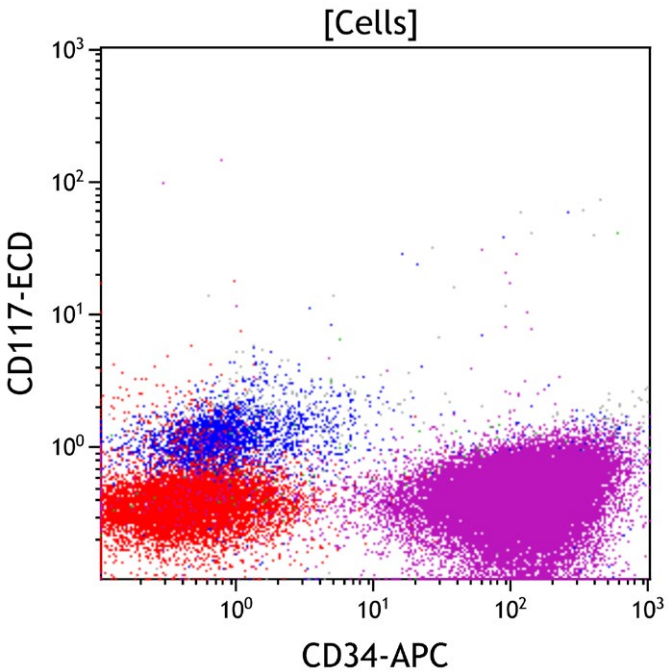

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes and early erythroid precursors, but negative on early B cell precursors. The aberrant population (purple) is strongly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

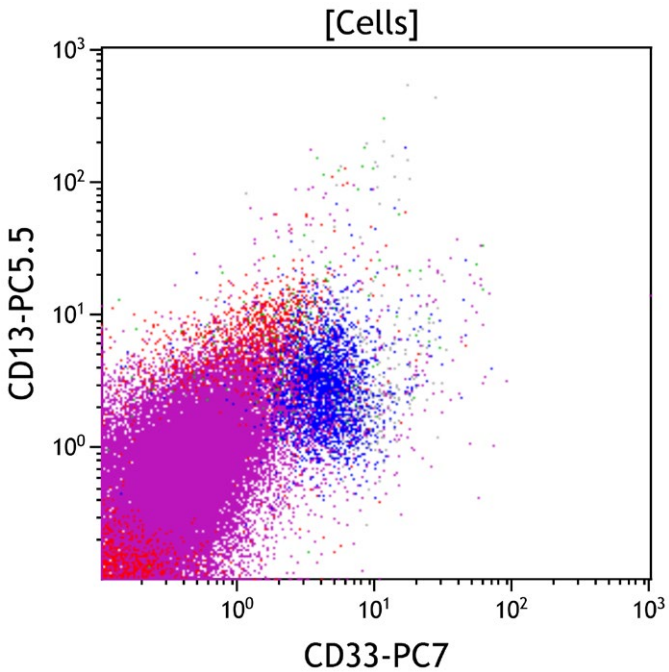

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, granulocytes (blue), basophils, and CD34 positive progenitors. Lymphocytes largely do not express either CD13 or CD33 (red). The aberrant population (purple) displays dim CD13 expression and is negative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

M2 Cell Tube

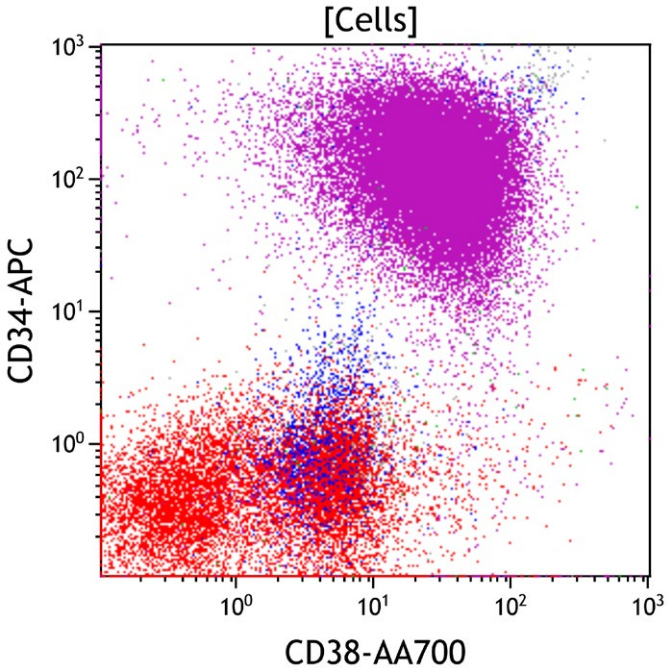

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. The aberrant population (purple) is positive for CD34 and CD38. The level of CD38 expression is slightly and variably lower than that seen on normal immature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

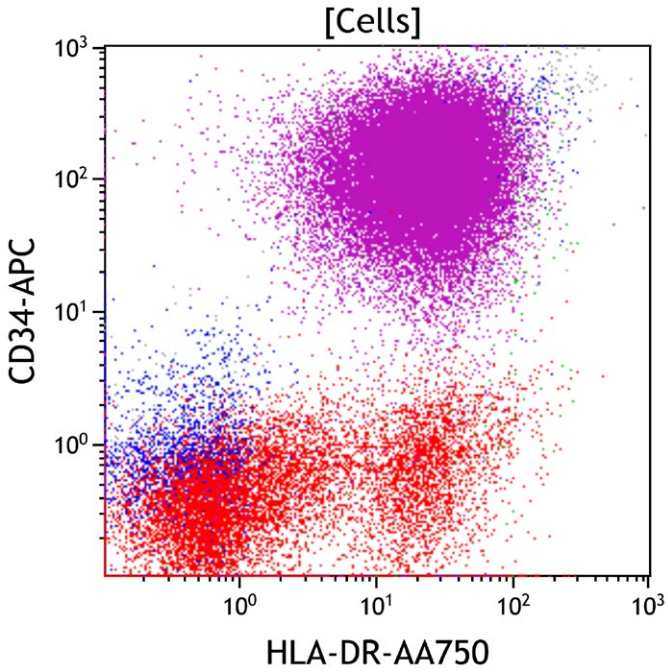

Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes (green), plasmacytoid dendritic cells and CD34 positive progenitors. CD34 is expressed on early progenitors. The aberrant population (purple) is strongly positive for CD34 and HLA-DR.

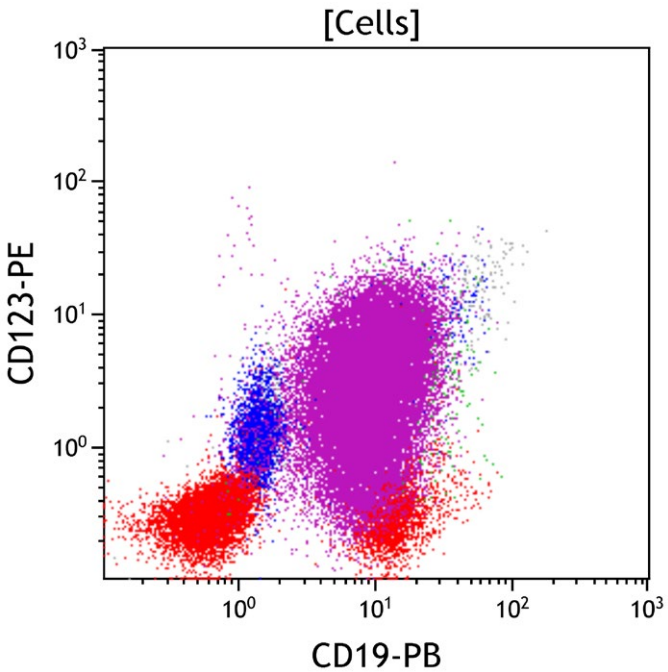

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 positive B cells normally do not express significant CD123. The aberrant population (purple) is positive for CD19 and CD123.

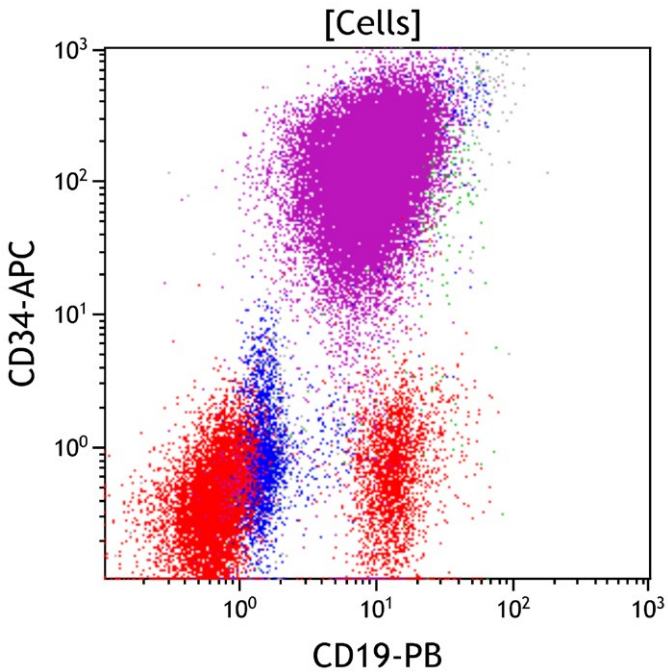

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red) do not express CD34. The aberrant population (purple) is positive for CD19 and CD34.

M2 Cell Tube

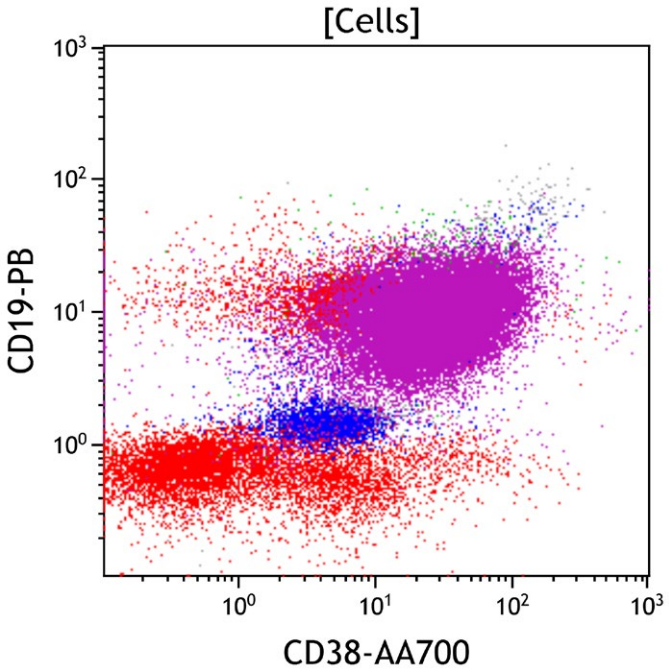

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Mature CD19 positive B cells (red) show intermediate CD38. The aberrant population (purple) is positive for CD19 and CD38. The level of CD38 expression is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

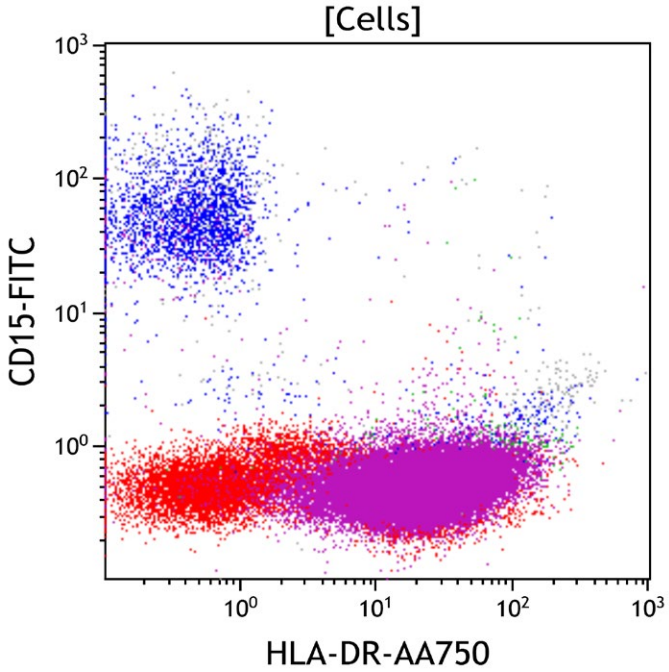

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed by granulocytes (blue) and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes (blue) do not express HLA-DR. The aberrant population (purple) is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

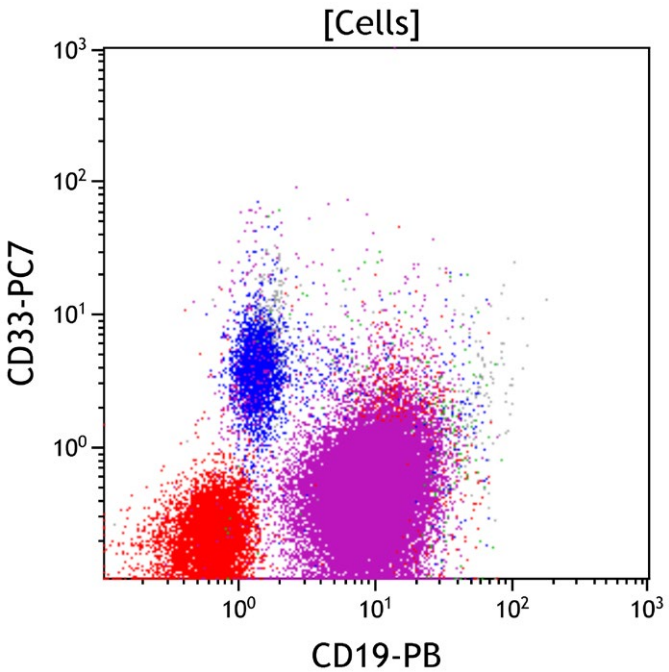

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes and granulocytes (blue). Mature CD19 positive B cells (red) do not normally express significant CD33. The aberrant population (purple) is positive for CD19 and negative for CD33.

Results of Flow Cytometric Immunophenotyping

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

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