Case #7: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Clinical Vignette

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

Flow Cytometric Immunophenotyping

B Cell Tube

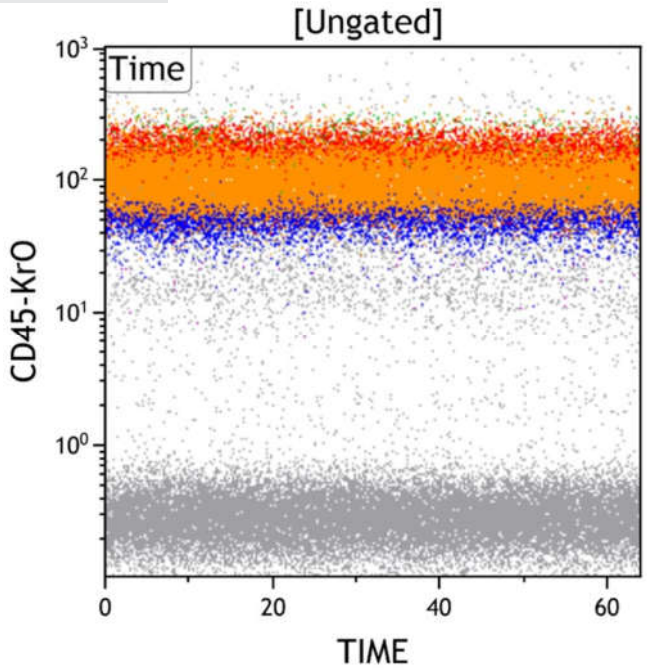

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

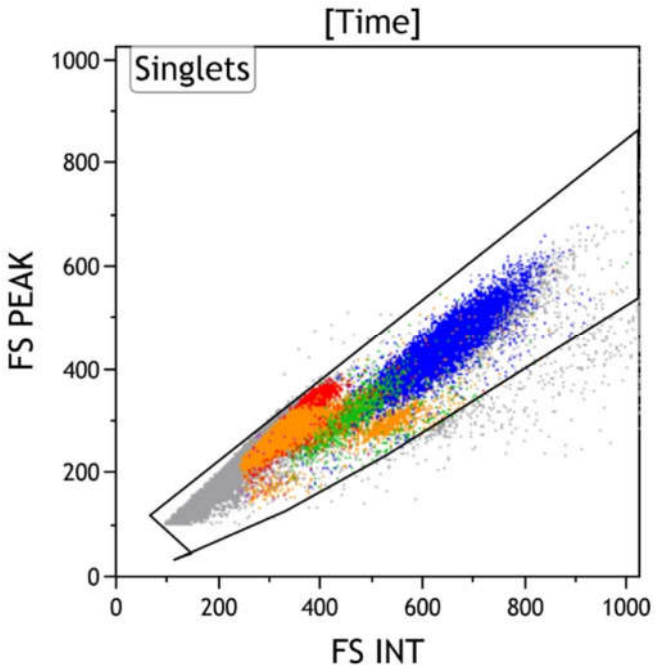

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

B Cell Tube

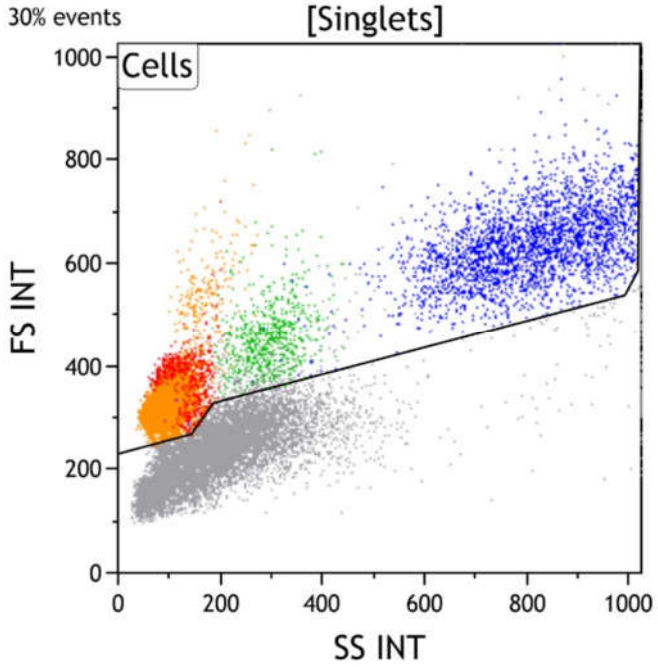

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

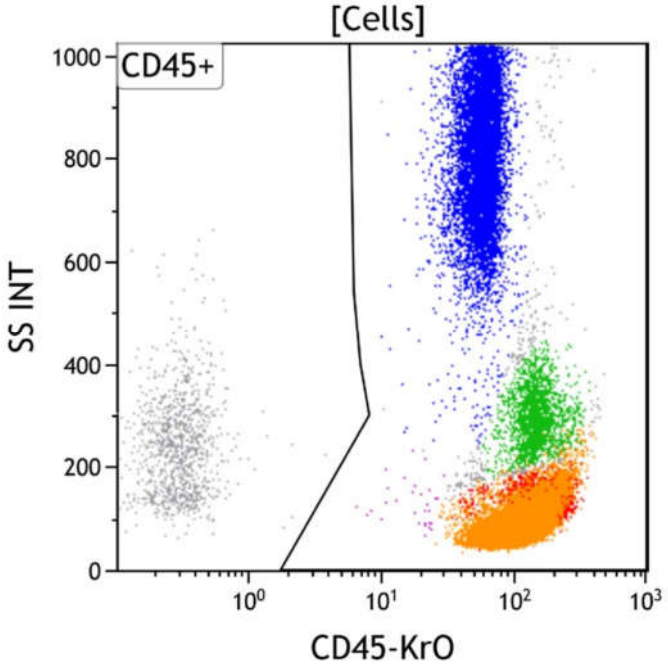

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

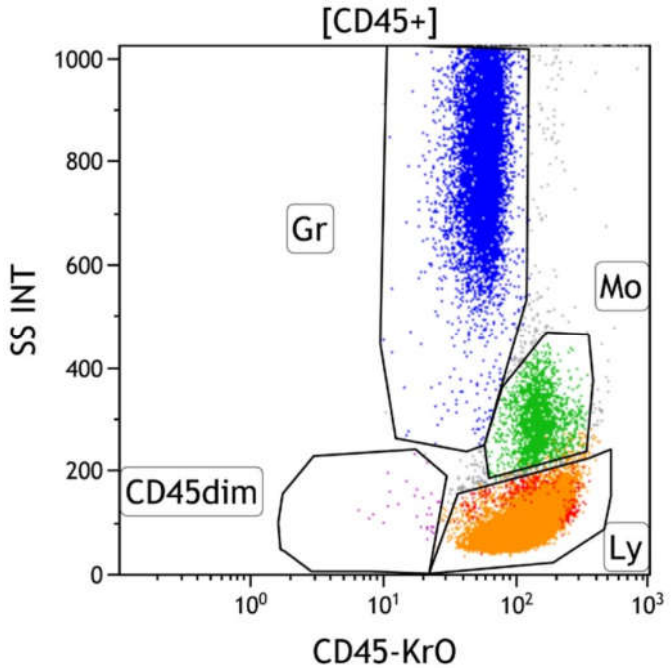

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes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

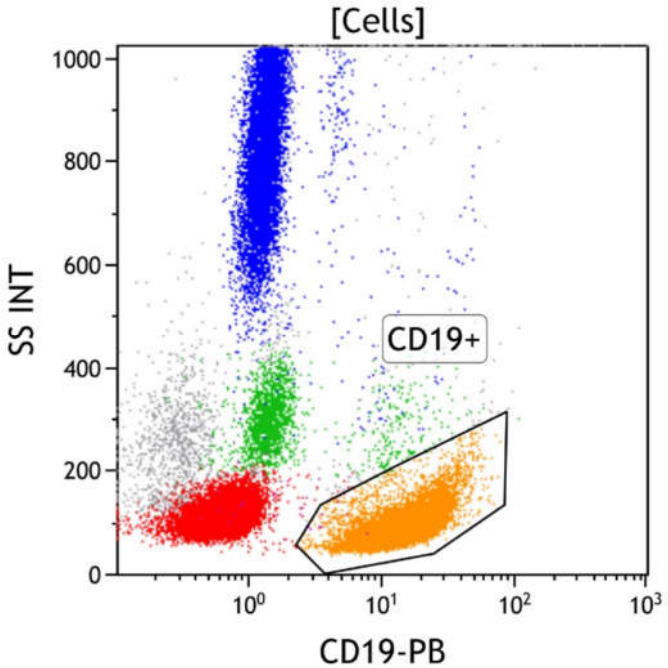

Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Note the relatively increased number of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

B Cell Tube

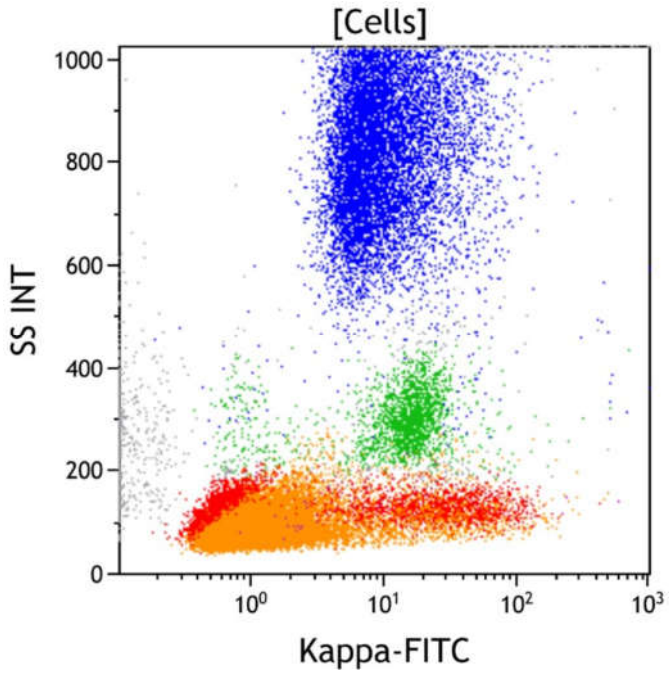

Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity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

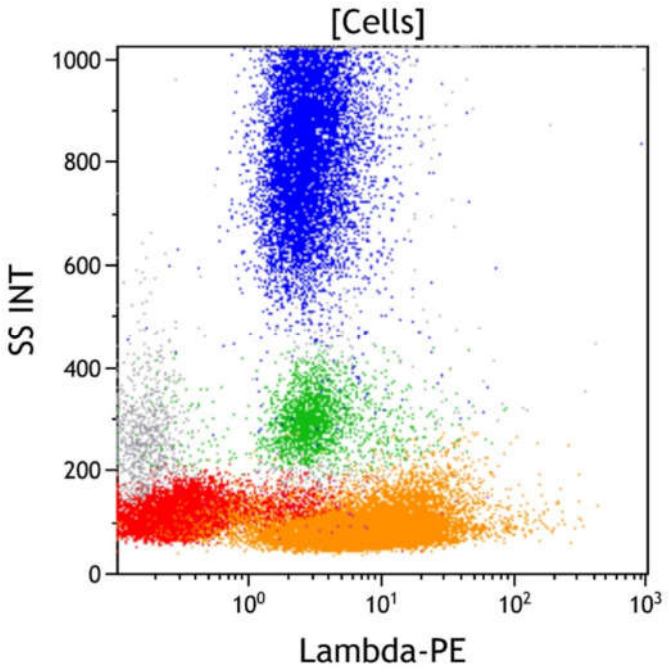

Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity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

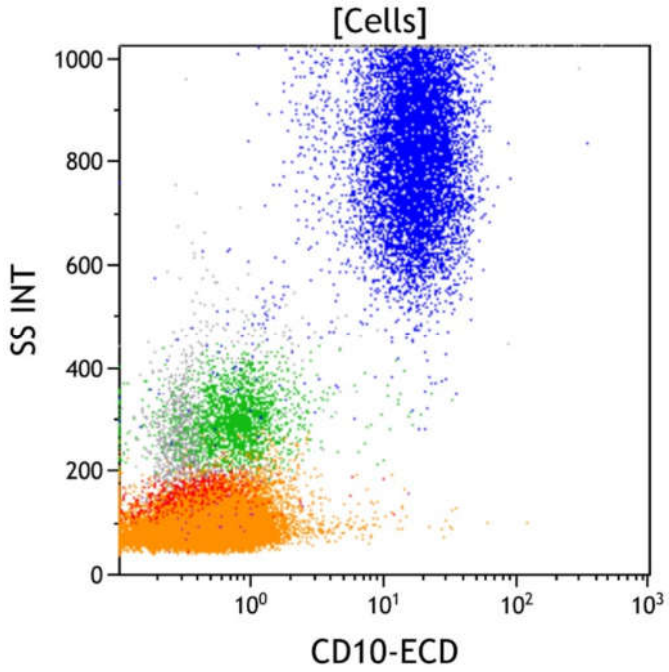

Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The CD19 positive population (orange)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

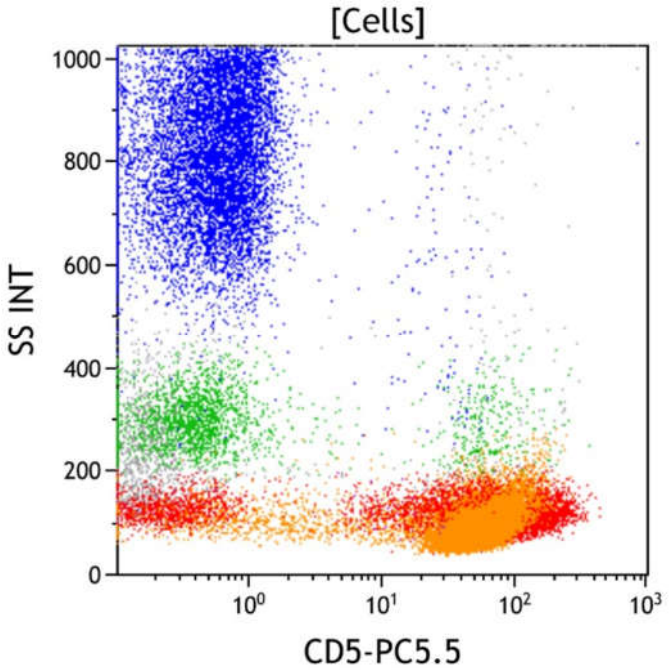

Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature T cells (red), as well as dimly expressed in a subset of mature B cells. These lymphoid cells typically have low side scatter. The CD19 positive population (orange) expresses CD5 at a level similar to T cells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

B Cell Tube

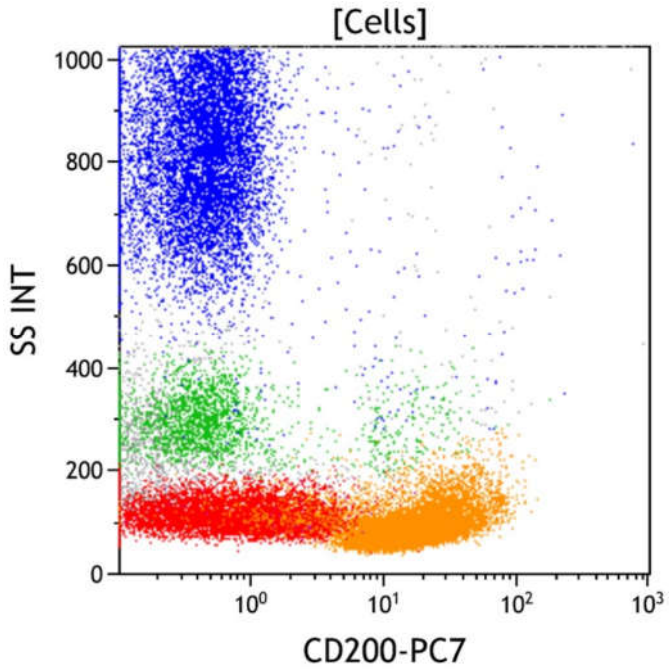

Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma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

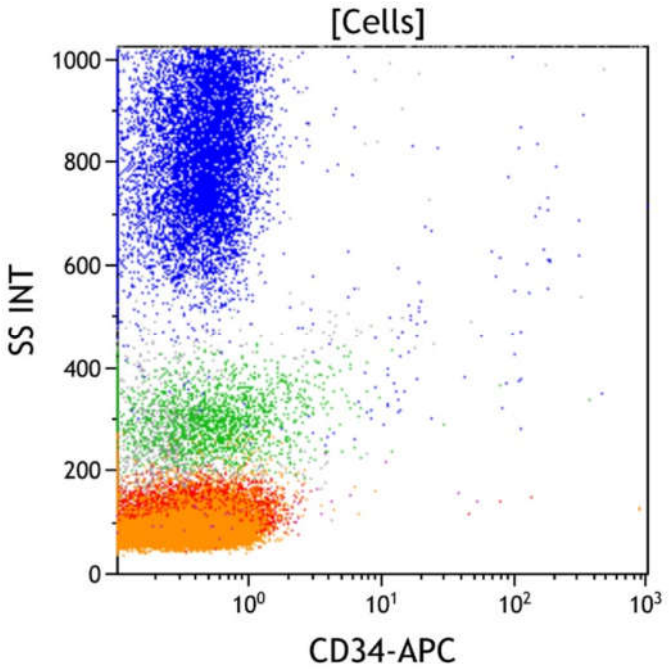

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The CD19 positive population (orange) is negative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

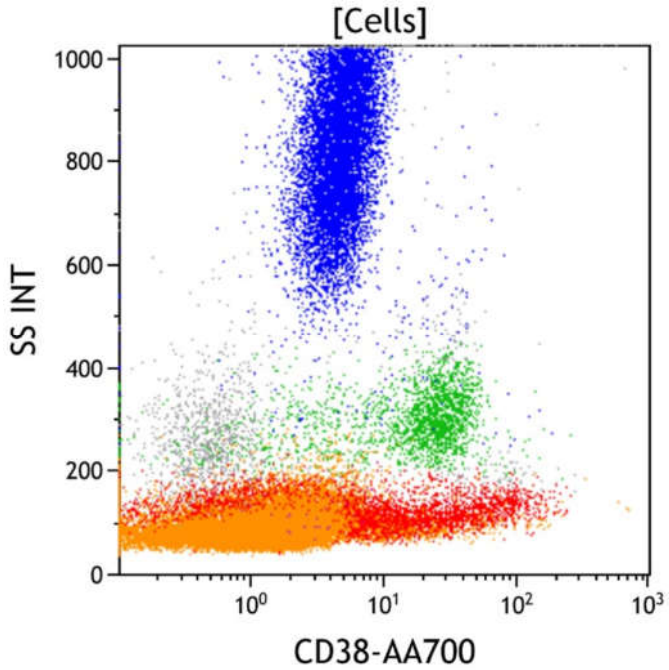

Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variably low level on activated mature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

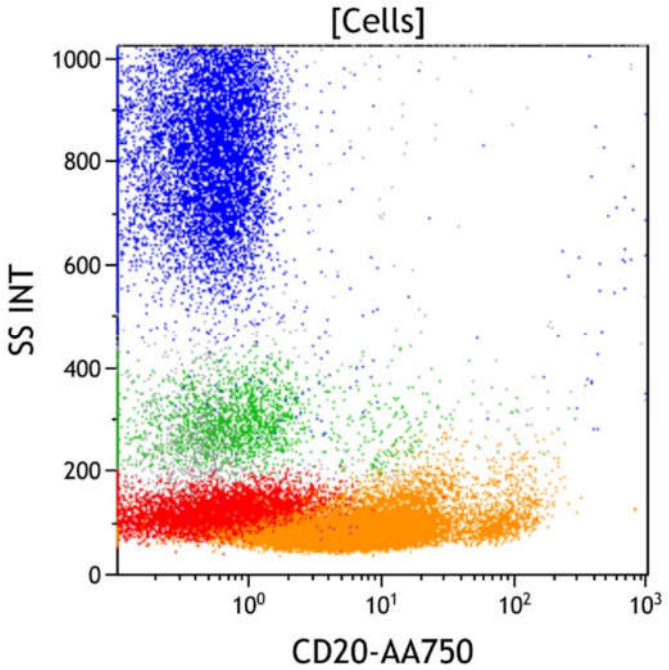

Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. The CD19 positive population (orange) displays variably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

B Cell Tube

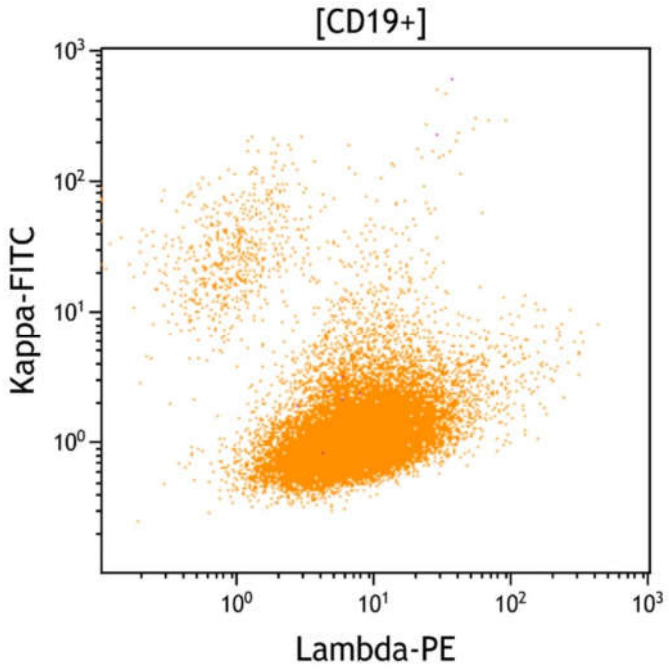

Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. Normal mature B cells are polyclonal, expressing either kappa or lambda light chain in a ratio of 1.4 with a range between 1 to 2. The CD19 positive cells (orange) predominantly have surface lambda light chain expression at a decreased level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

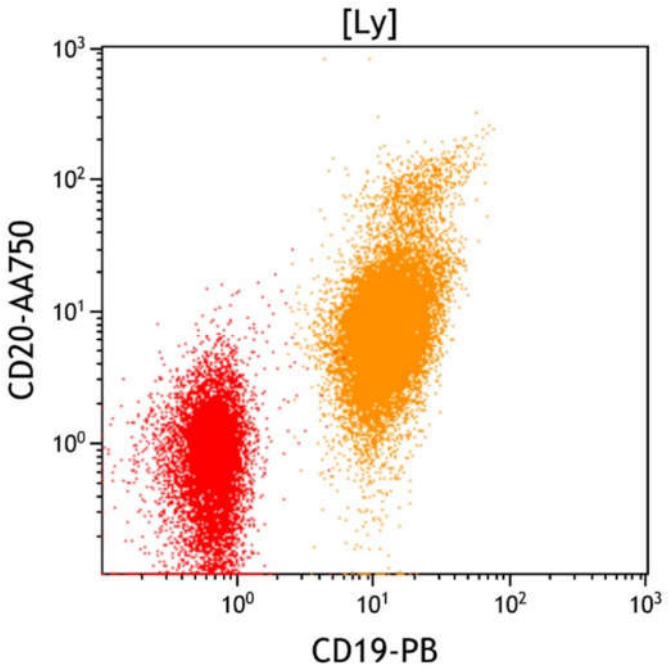

Figure 16. This CD19 vs. CD20 dot plot shows all cells in the lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Some neoplastic B cells may show decreased CD19 or CD20 expression. The clonal B cells (orange) display decreased CD19 and CD20 expression compared with the higher level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

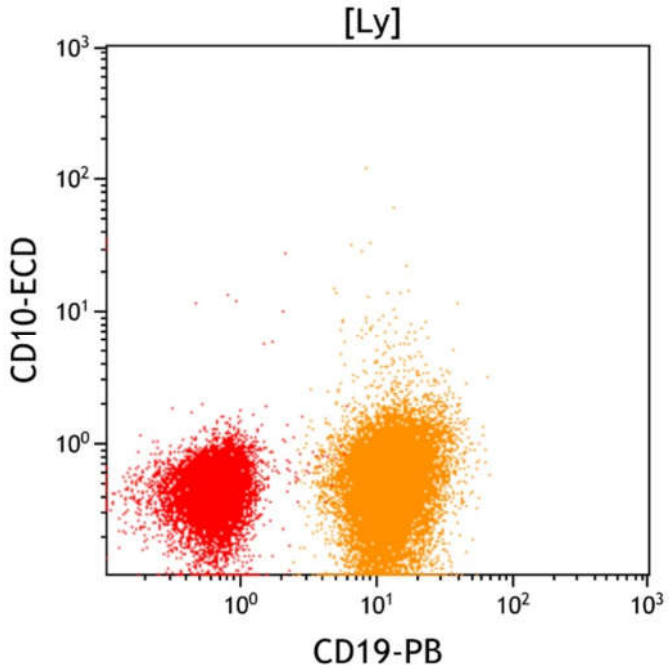

Figure 17. This CD19 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates.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

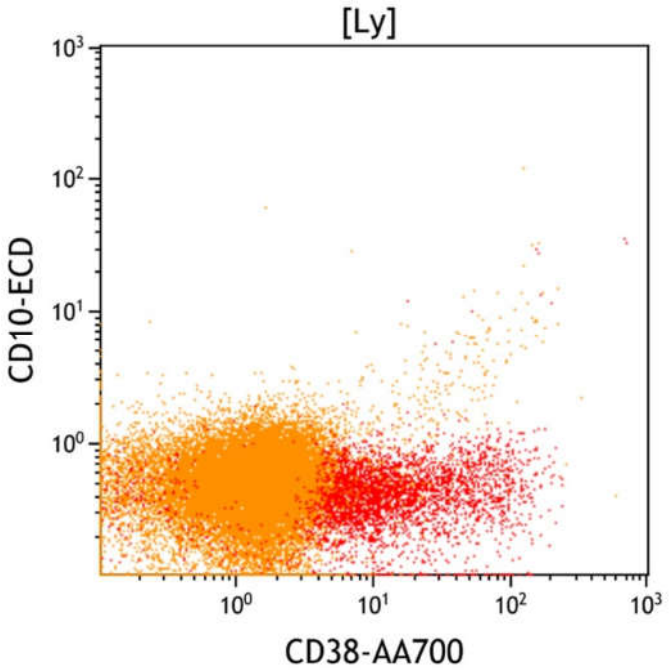

Figure 18. This CD38 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells are low to negative for CD38. T cells (red) show variable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

B Cell Tube

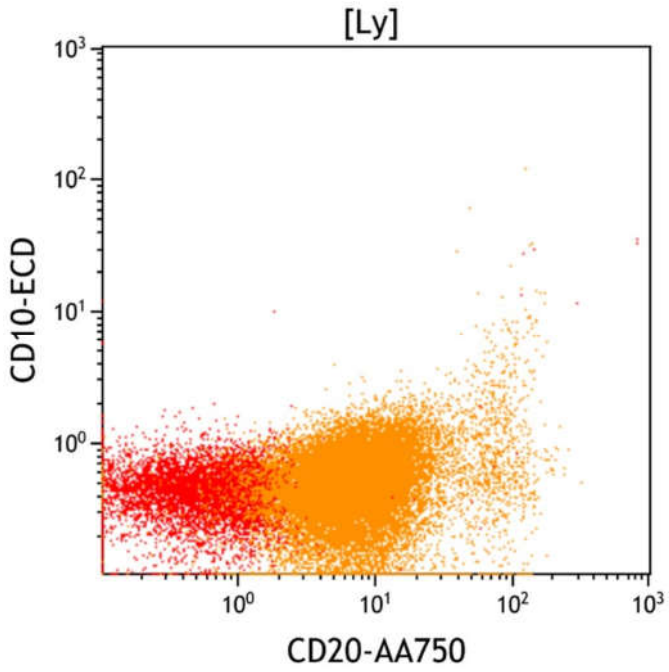

Figure 19. This CD20 vs CD10 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells uniformly express high-level CD20. The clonal B cell population (orange) displays decreased CD20 expression and is negative for CD10. The small population with normal CD20 expression (also in orange,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

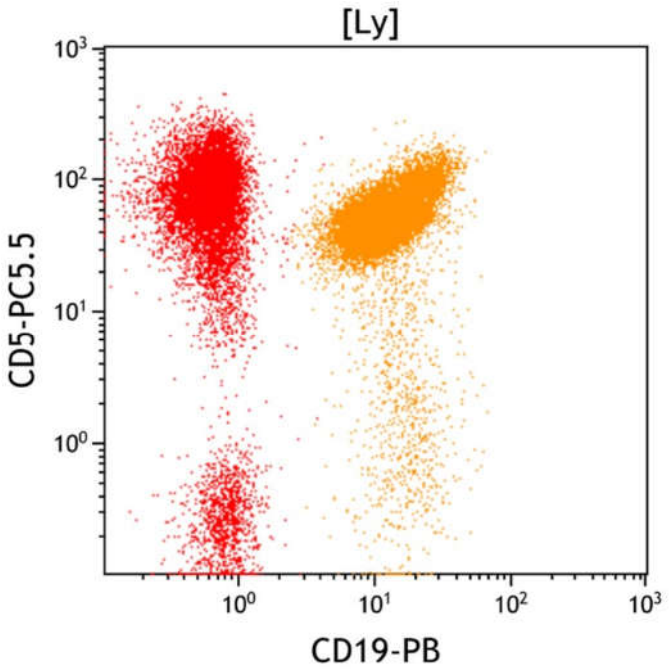

Figure 20. This CD19 vs CD5 dot plot shows all cells in the lymphocyte gate (Ly). CD5 is expressed on T cells (red, upper right), variably expressed at a low level on a subset of normal mature B cells, and expressed on some subtypes of neoplastic B cells. The clonal B cell population (orange) expresses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

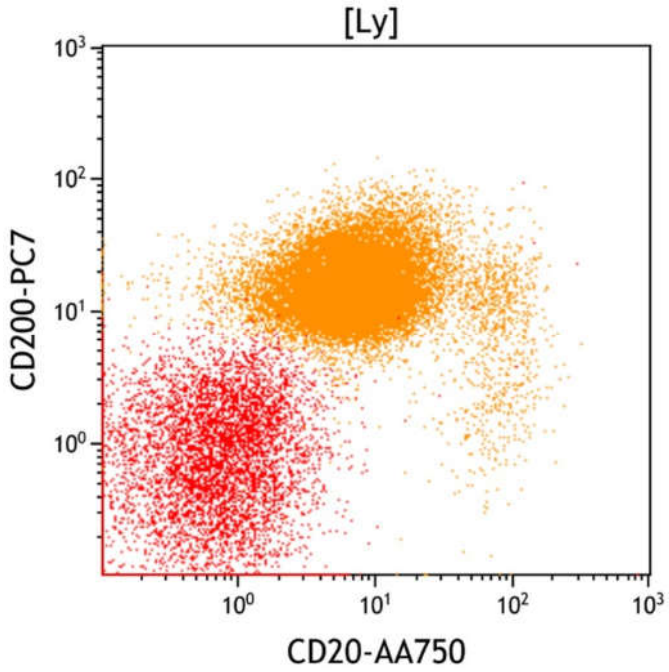

Figure 21. This CD20 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level. The clonal B cell population (orange) is positive for CD200 with decreased CD20. The small population with normal CD20 and CD200 expression (also in orange,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

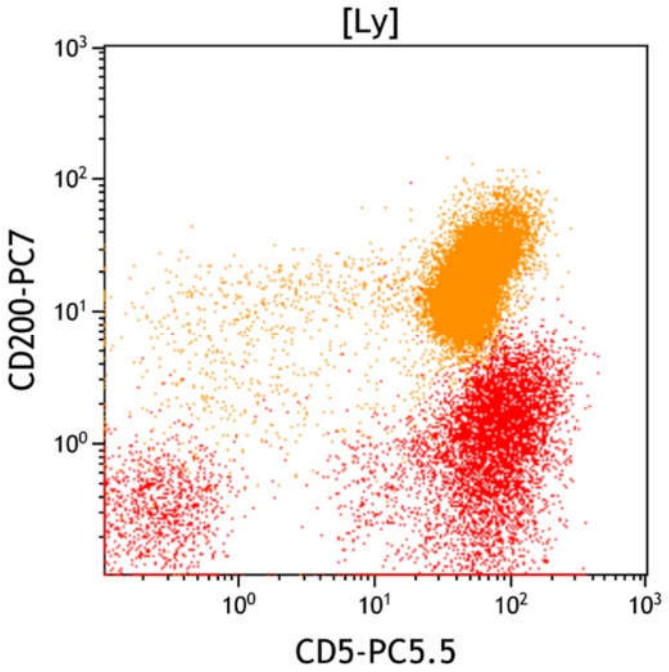

Figure 22. This CD5 vs CD200 dot plot shows all cells in the lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma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

T Cell Tube

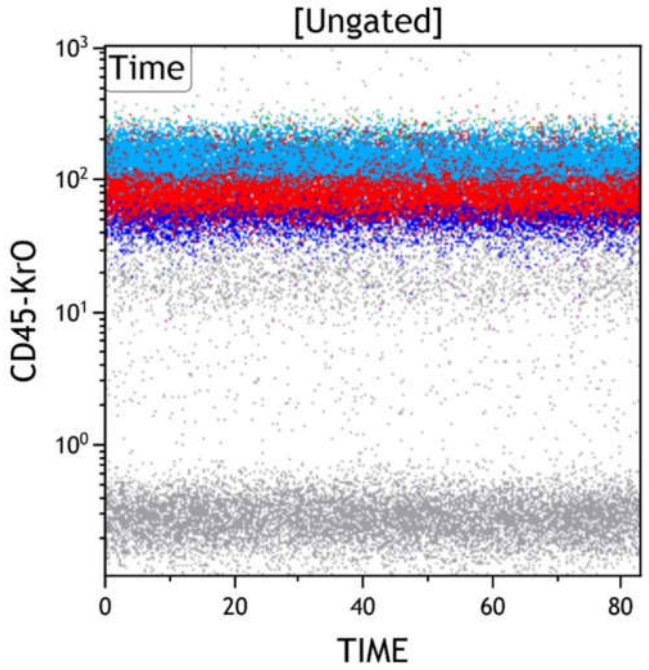

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

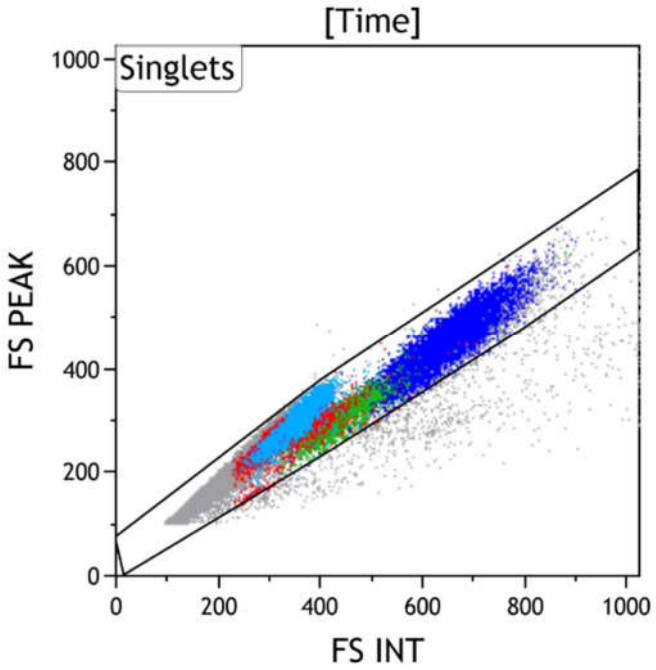

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

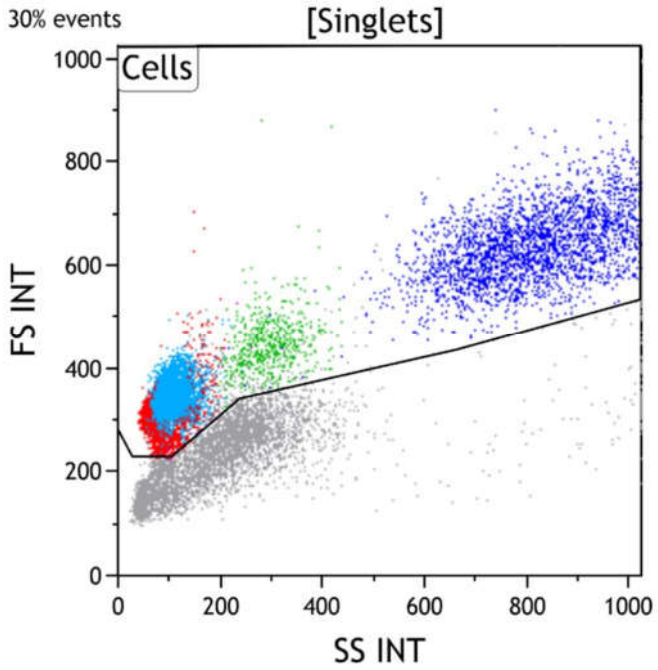

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

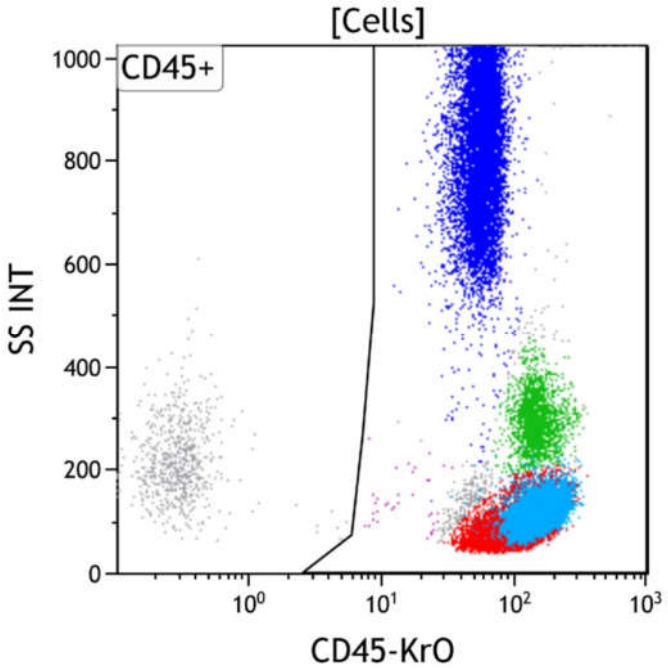

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

T Cell Tube

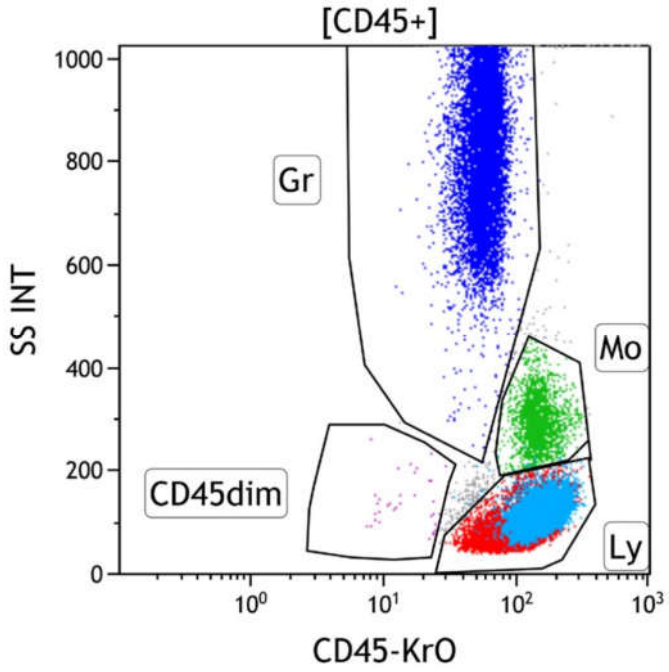

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, aqua/red), monocytes (Gate Mo, green), and granulocytes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

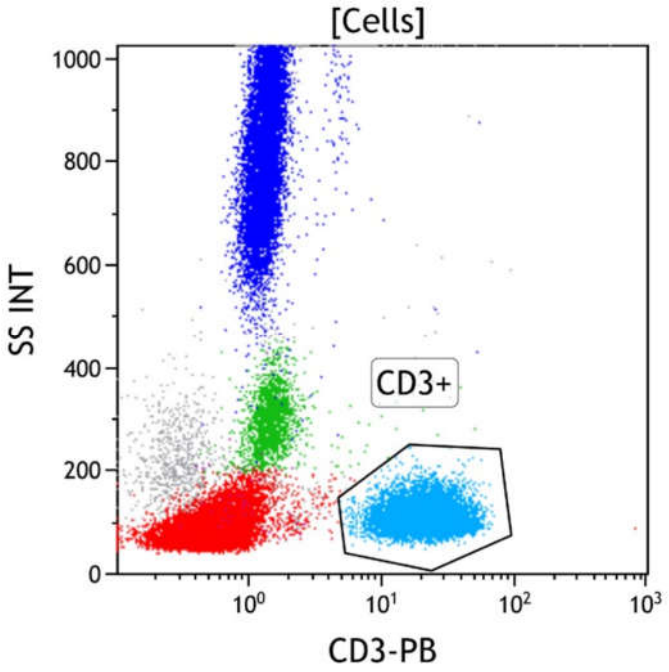

Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies surface CD3 positive cells (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells.

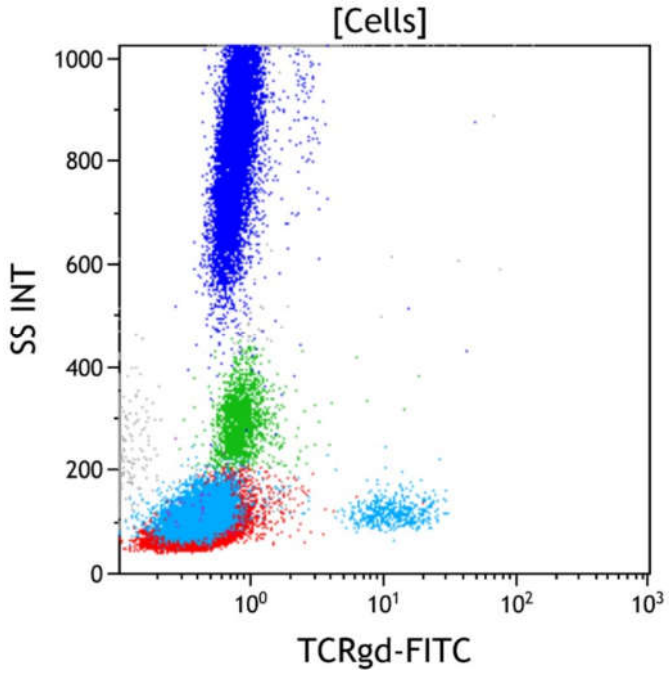

Figure 7: This TCRγδ vs Side Scatter dot plot shows all viable cells. TCRγδ is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua).

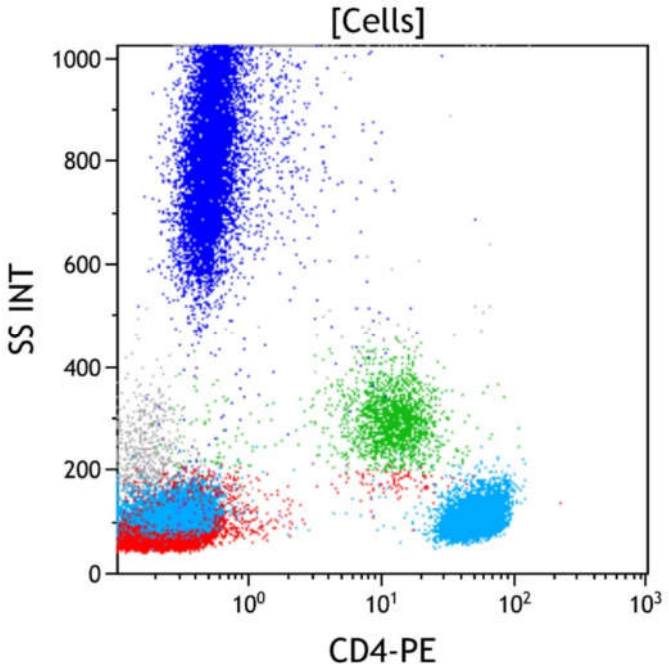

Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells.

T Cell Tube

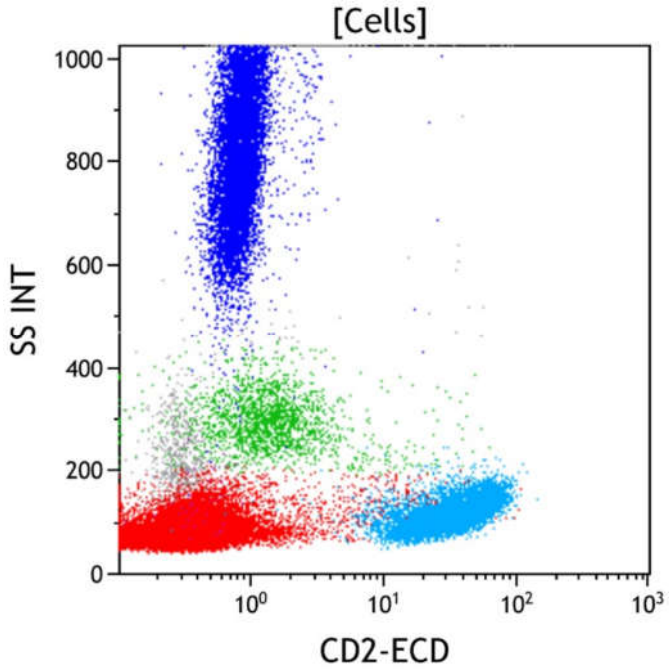

Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green).

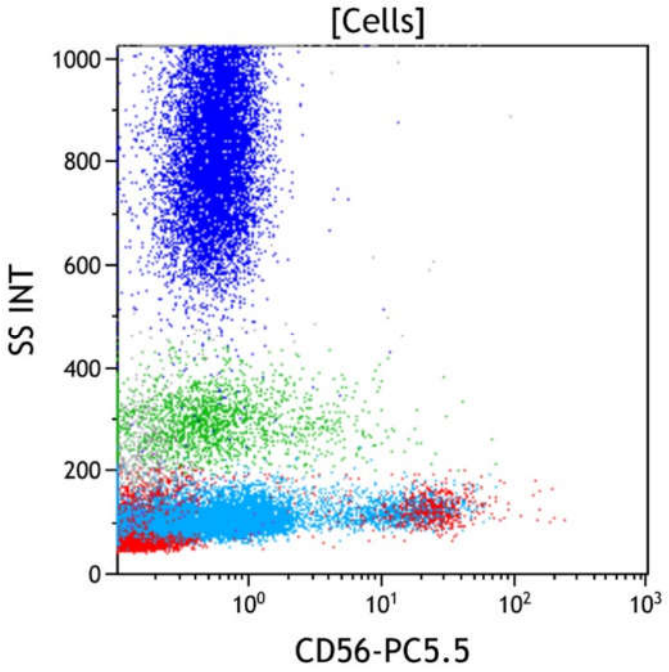

Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and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

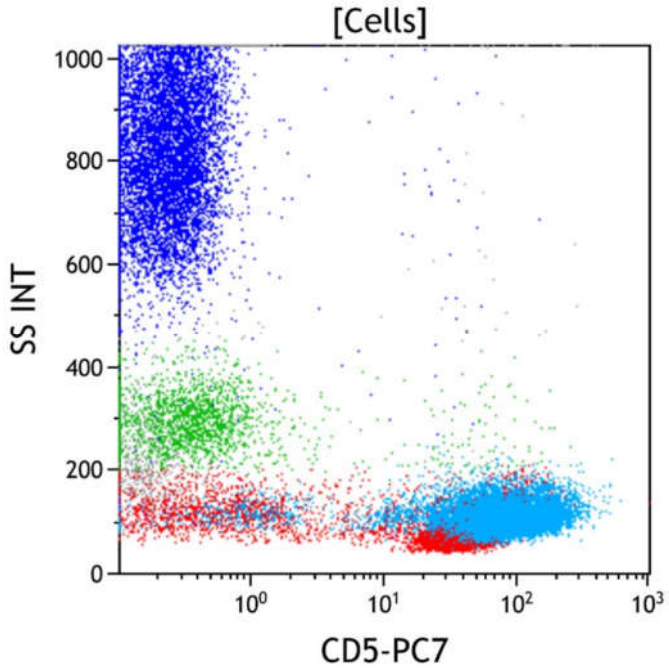

Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

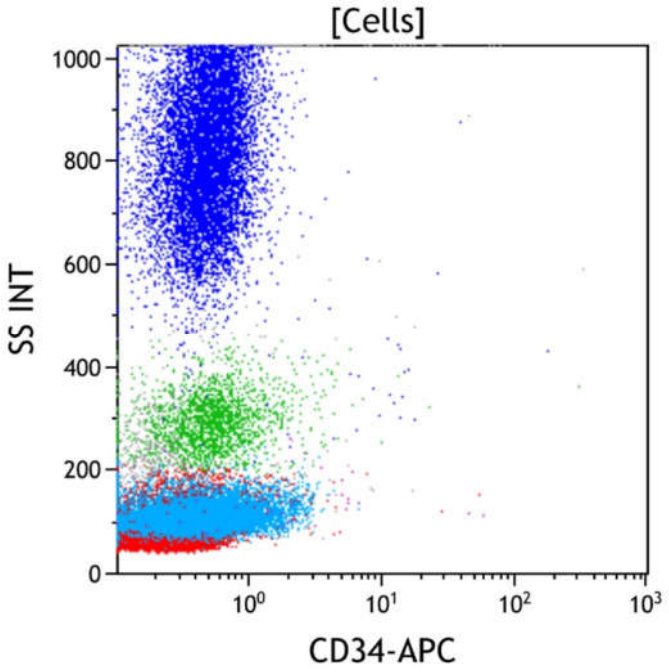

Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). The mature granulocytes, monocytes, and lymphocytes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

T Cell Tube

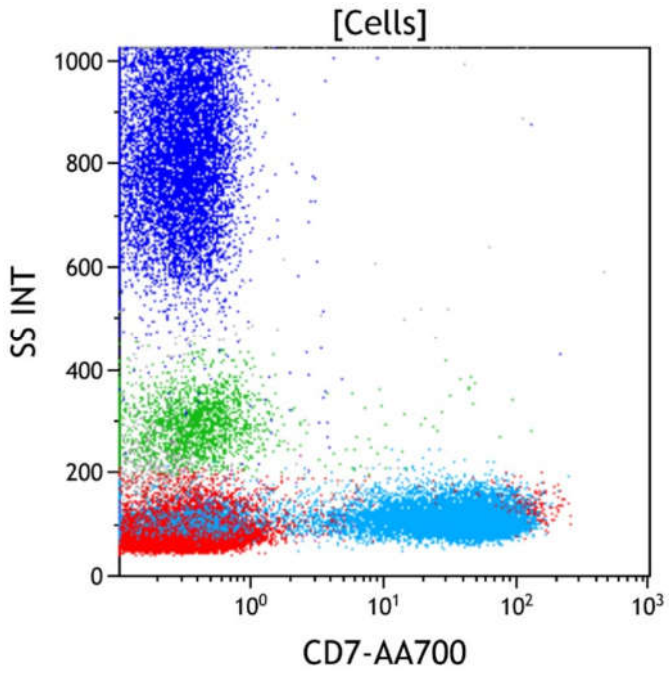

Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red), and dimly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

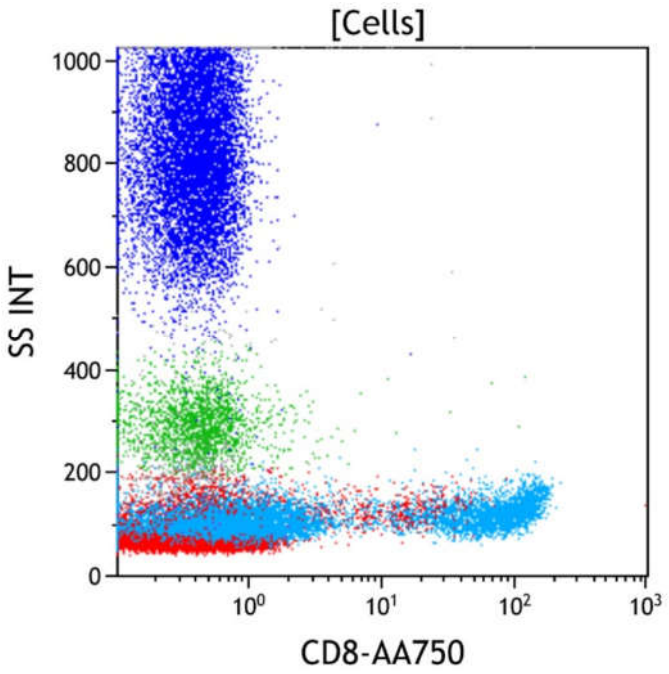

Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells.

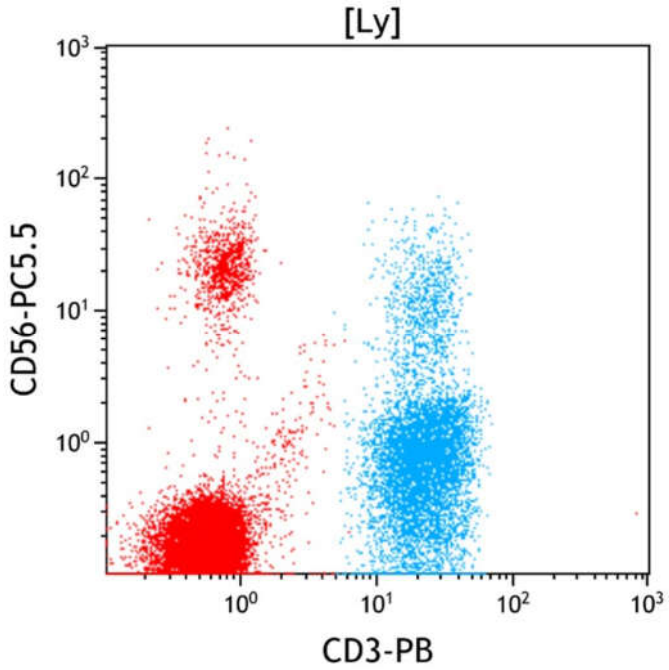

Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells (aqua) and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 on a major subset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

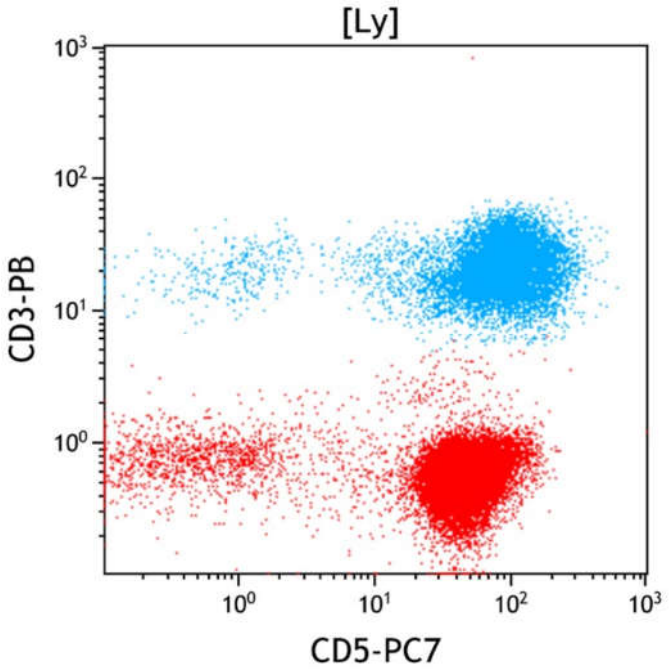

Figure 16. This CD5 vs CD3 dot plot shows all cells in the lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

T Cell Tube

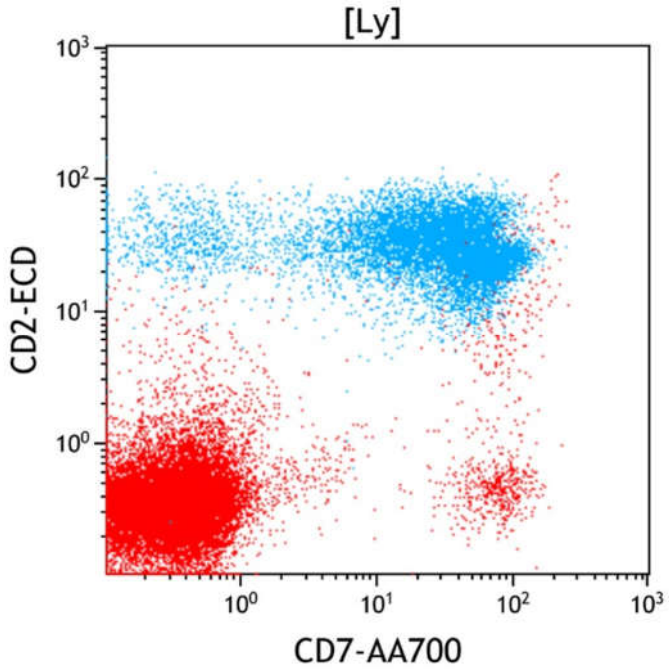

Figure 17. This CD7 vs CD2 dot plot shows all cells in the lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

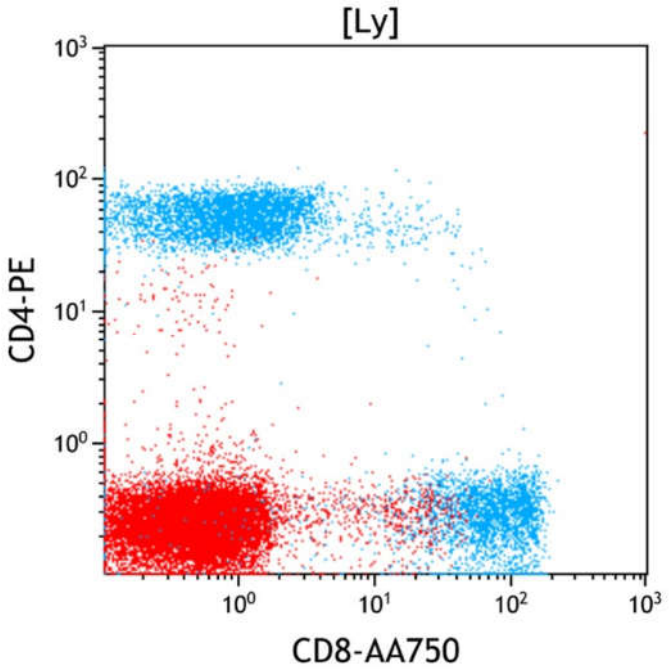

Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional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

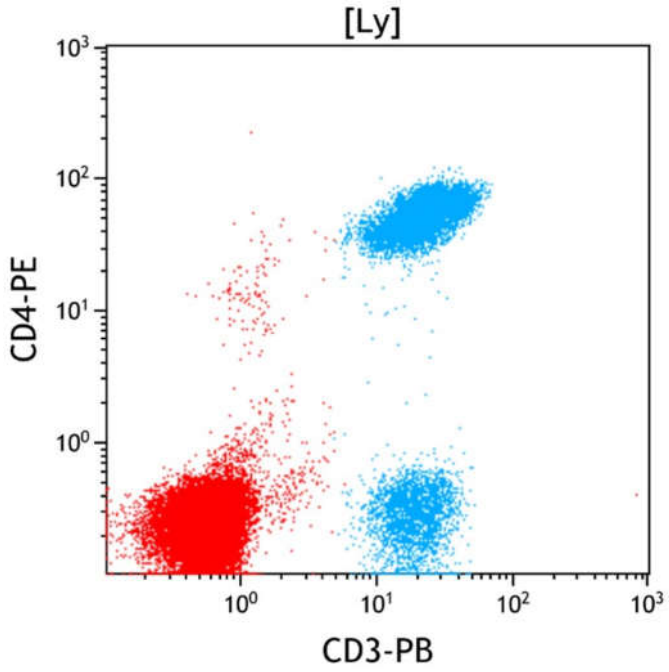

Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4. Of note, the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

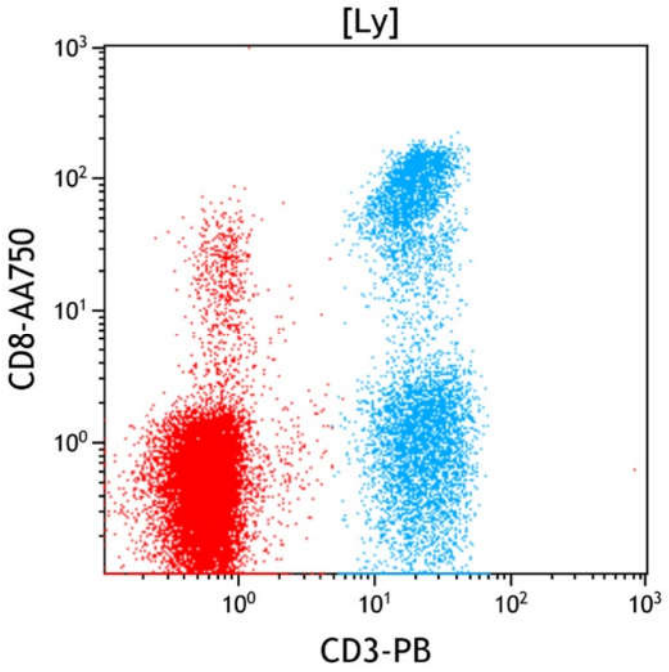

Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells (red, upper left) also expresses CD8 without CD3.

T Cell Tube

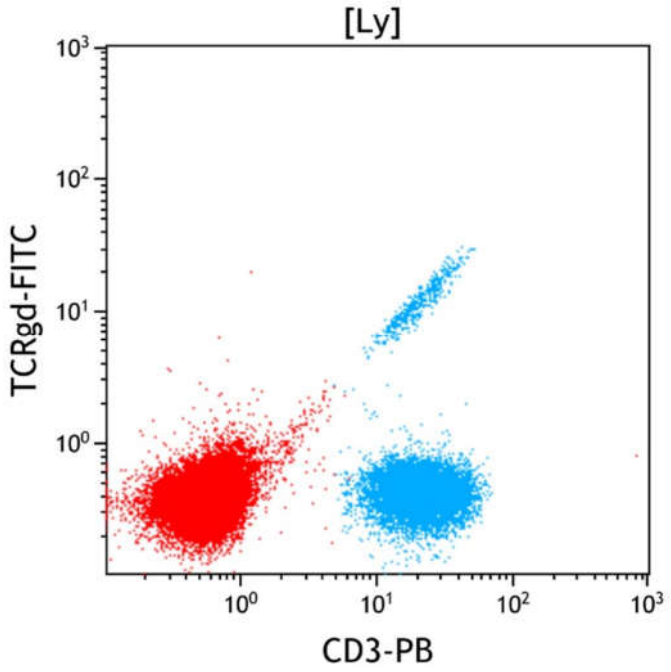

Figure 21. This CD3 vs TCRγδ dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR is due to their presence as a heterodimeric complex having a fixed 1:1 ratio, so increased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

M1 Cell Tube

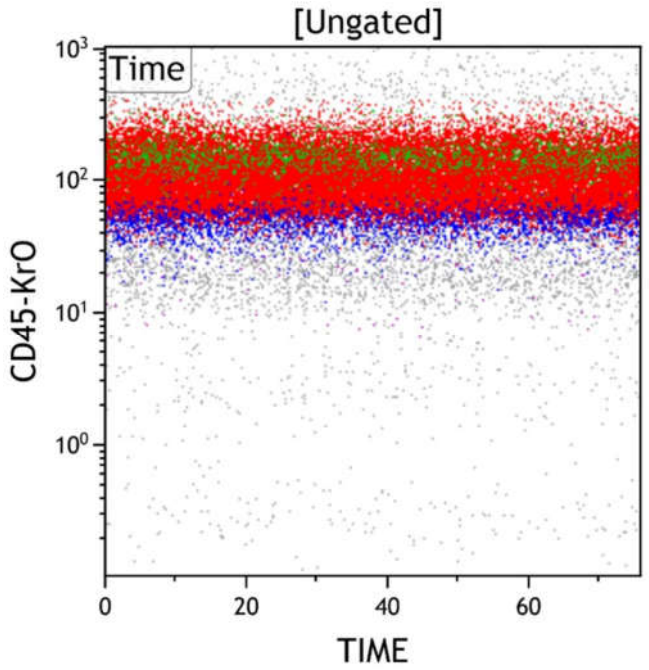

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

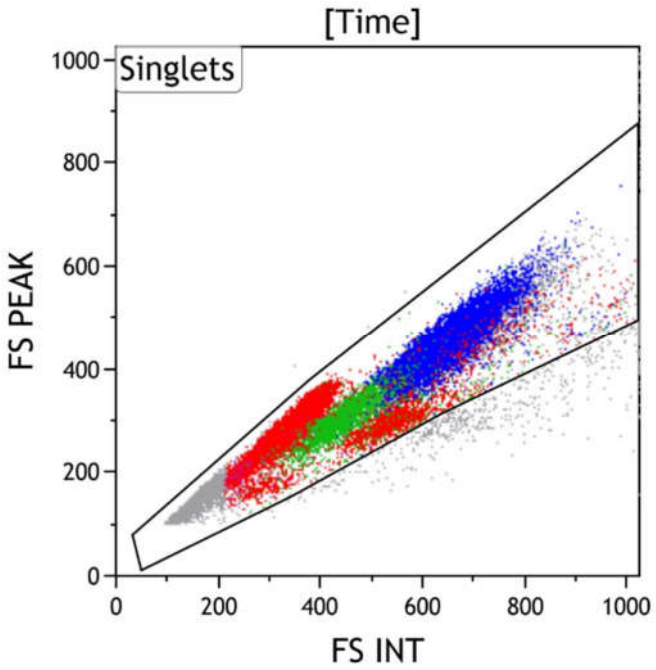

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

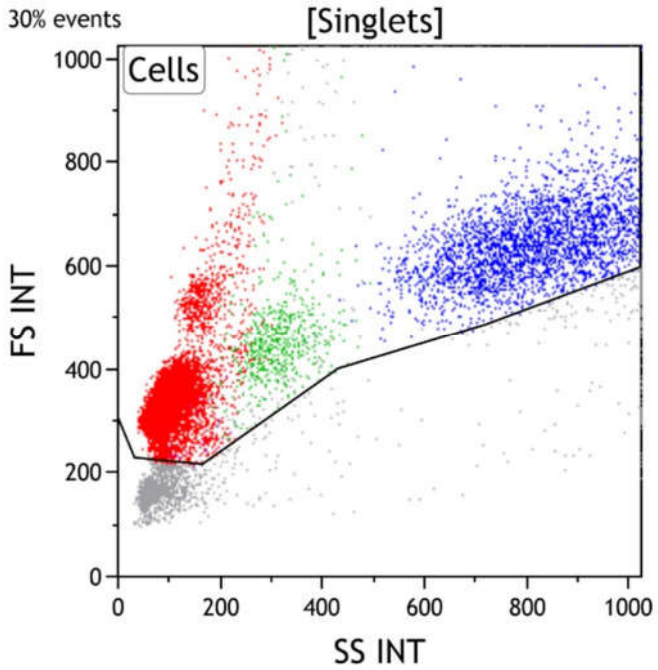

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

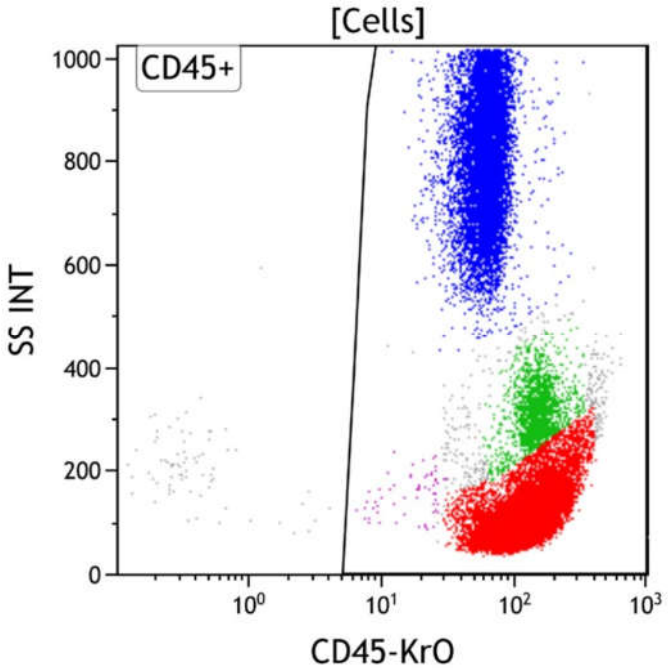

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

M1 Cell Tube

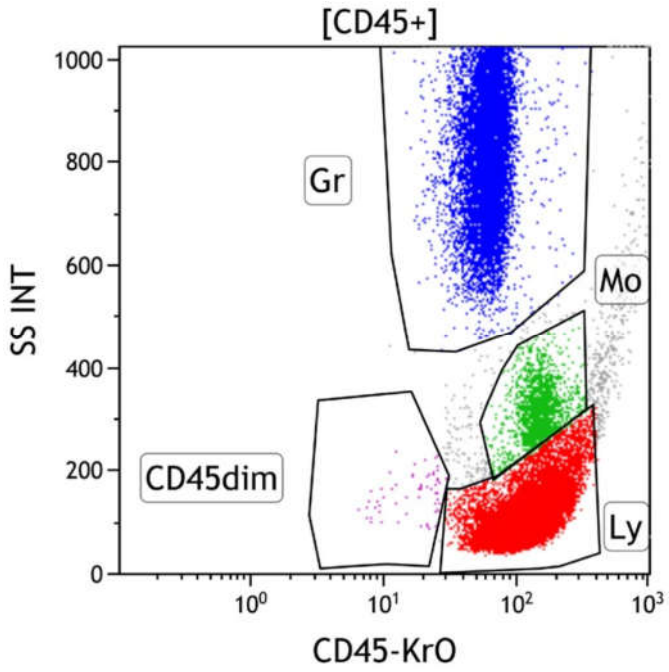

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

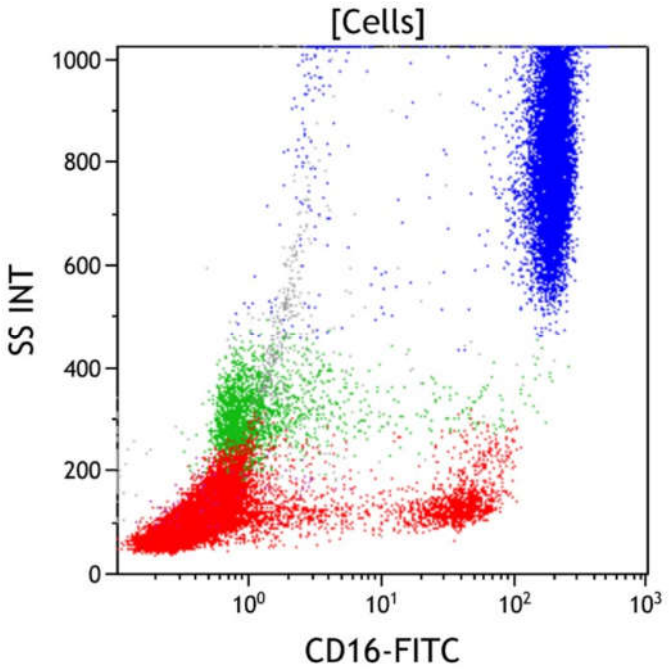

Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on granulocytes (blue). Most NK cells express CD16 (red, lower right), as do a subset of activated monocytes (green).

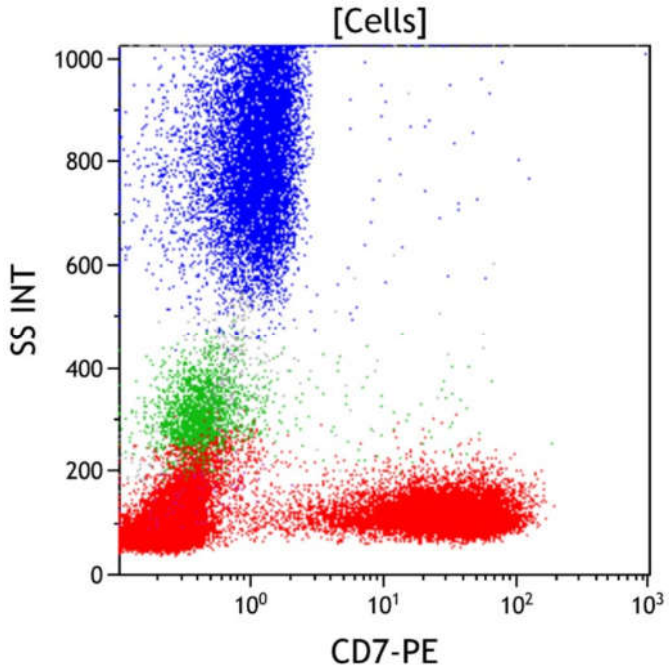

Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red lower, right),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

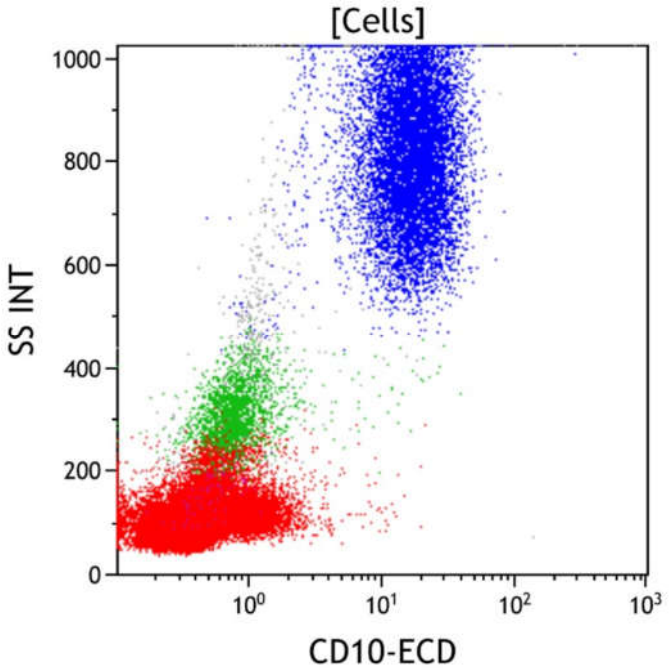

Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. The mature lymphocytes (red) show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

M1 Cell Tube

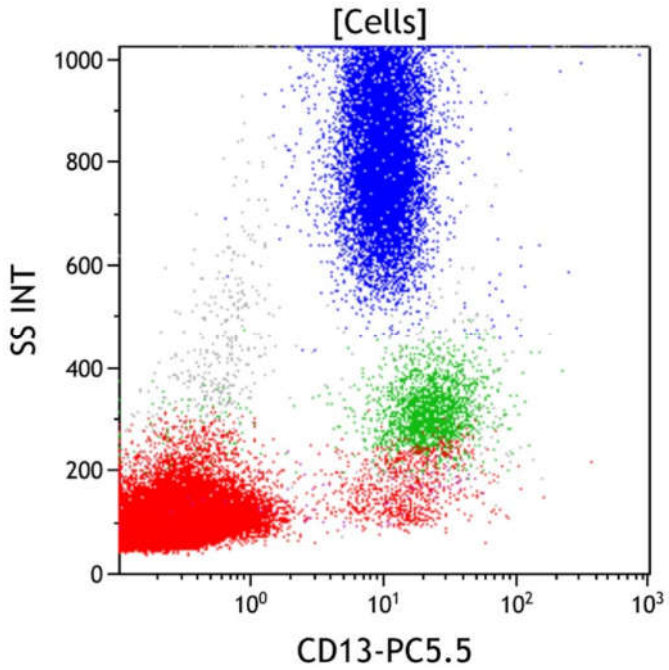

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue, with high side scatter) and on mature monocytes (green).

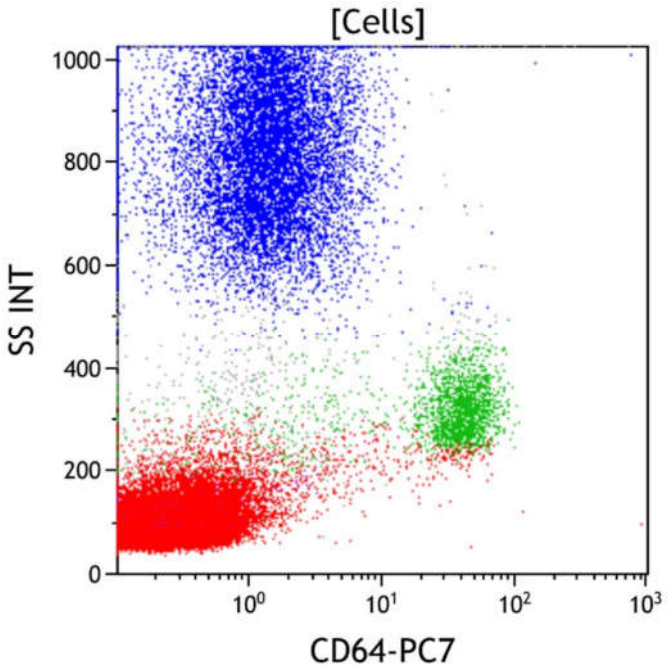

Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on immature and mature monocytes (green). CD64 is not well expressed on resting mature granulocytes (blue), but increases in expression with granulocyte activation. CD64 is not expressed on lymphoid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

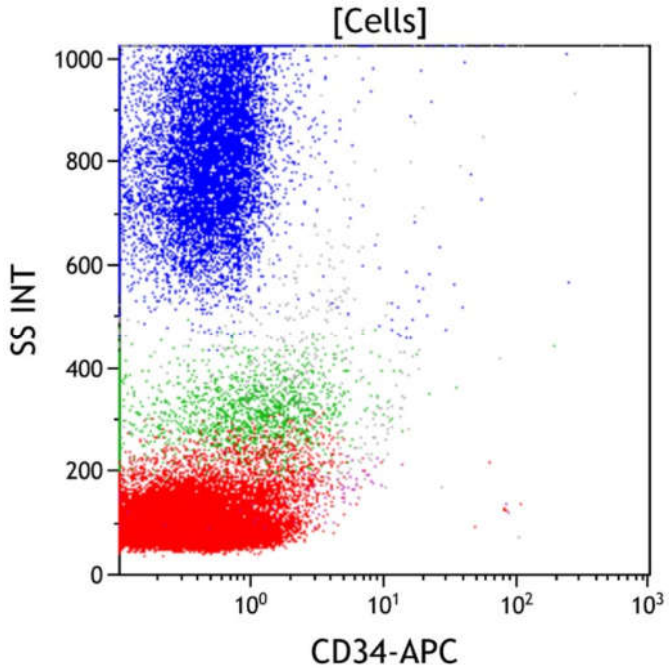

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are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

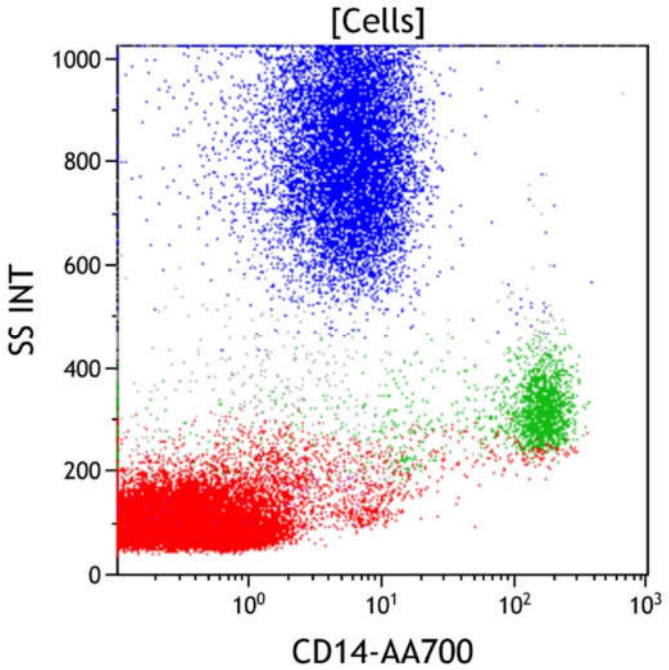

Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on granulocytes (blue) at a low level.

M1 Cell Tube

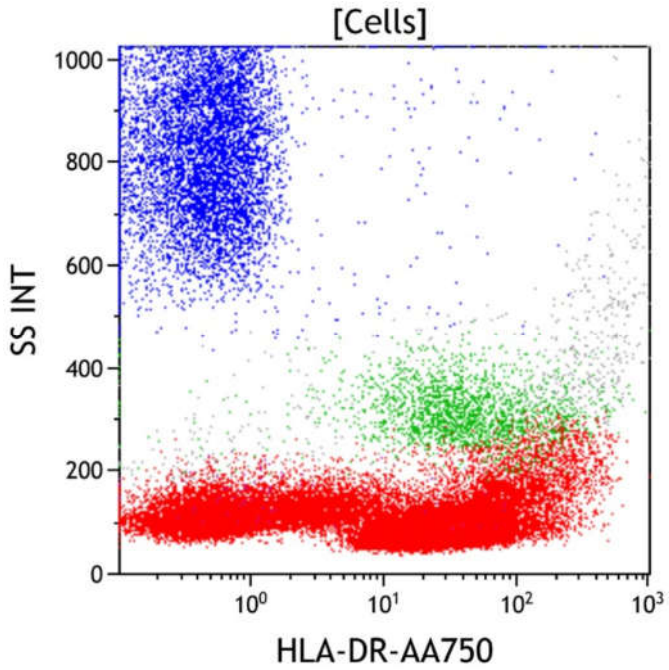

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature B cells (red) and immature B cells, and activated T cells (red).

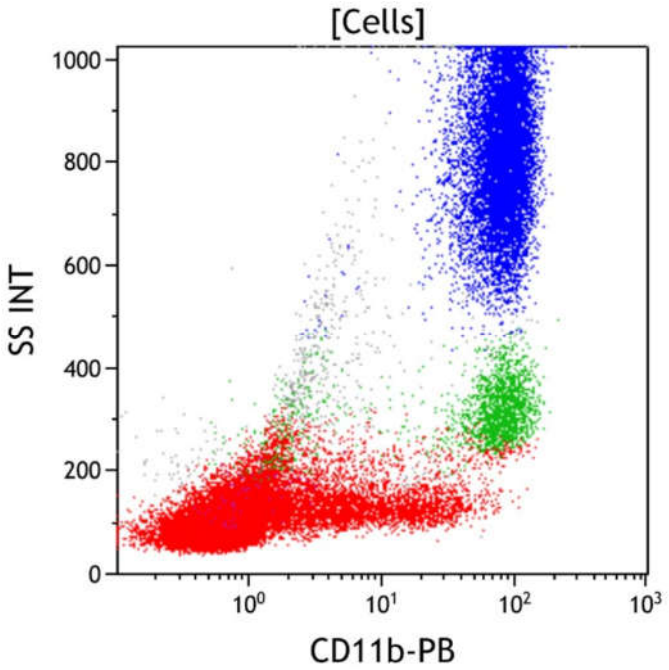

Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on granulocytes (blue) and on monocytes (green). CD11b is also expressed on NK cells (red, lower right) and basophils.

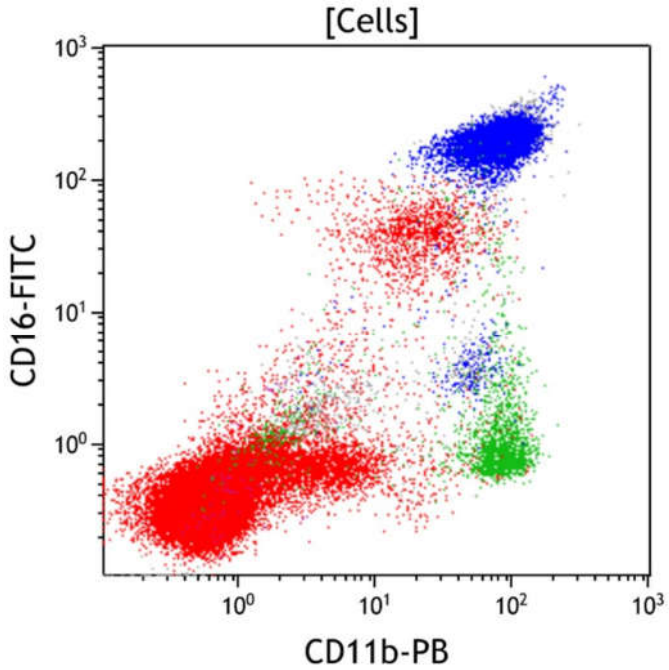

Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes (green), granulocytes (blue), and NK cells (red). CD16 is expressed on granulocytes (blue) and a subset of NK cells (red).

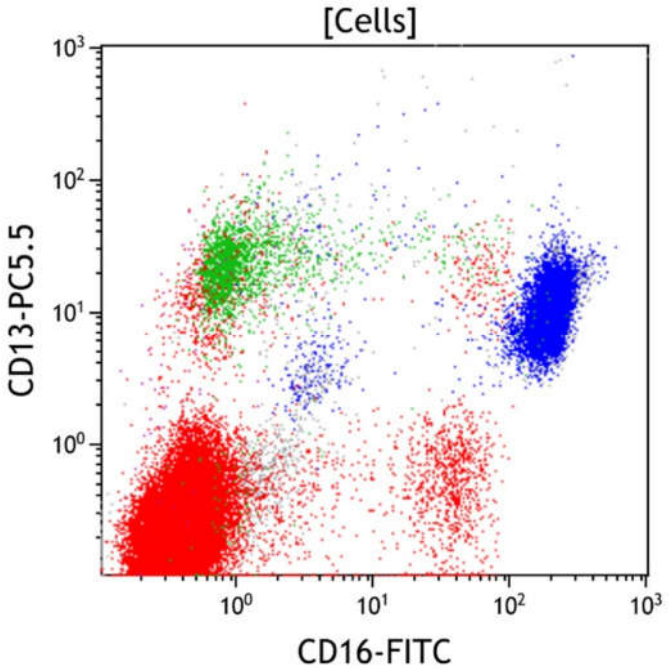

Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD16 is expressed on granulocytes (blue) and a subset of NK cells (red, lower right).

M1 Cell Tube

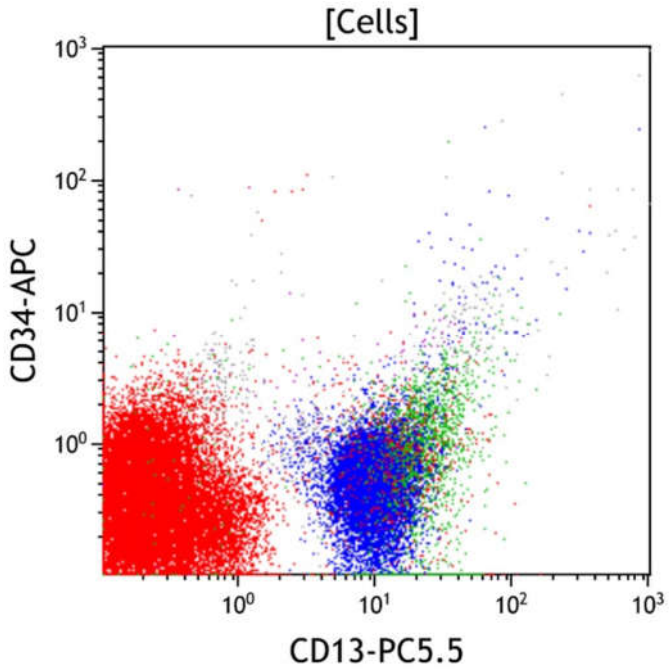

Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors.

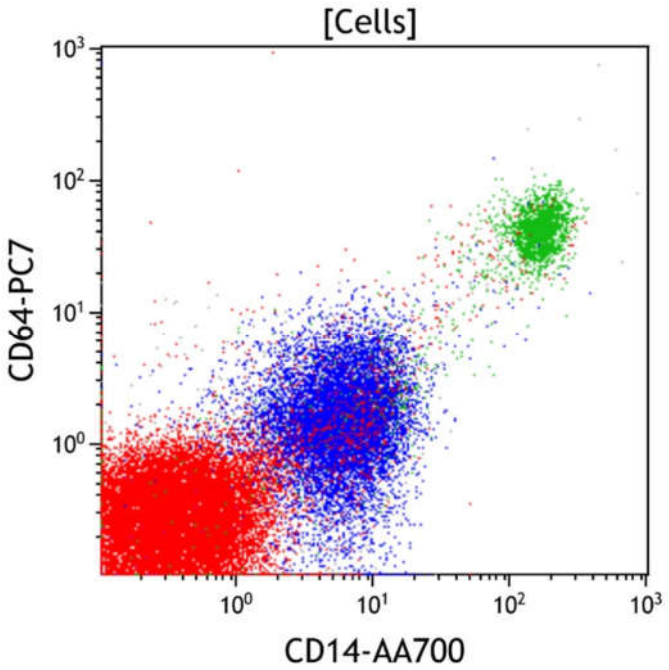

Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes (green) and at a lower level on granulocytes (blue). CD14 is expressed at a high level on monocytes and a lower level on granulocytes.

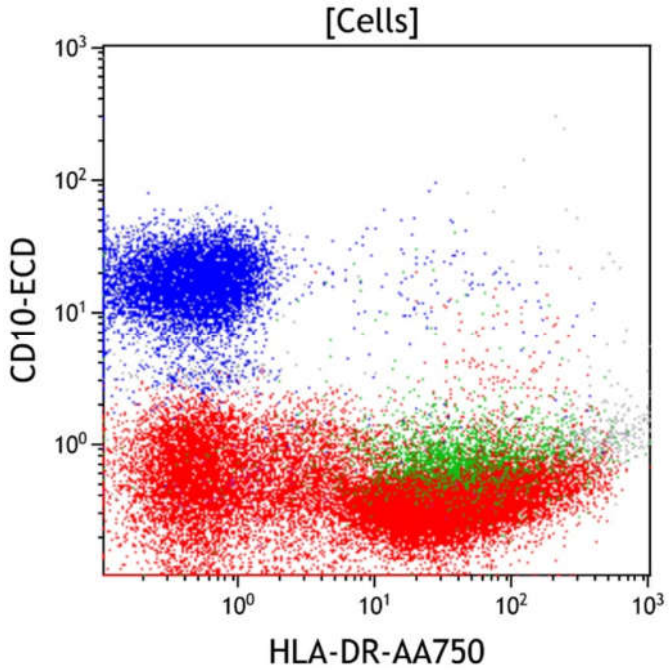

Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes (green), B cells (red, lower right), plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed on granulocytes (blue).

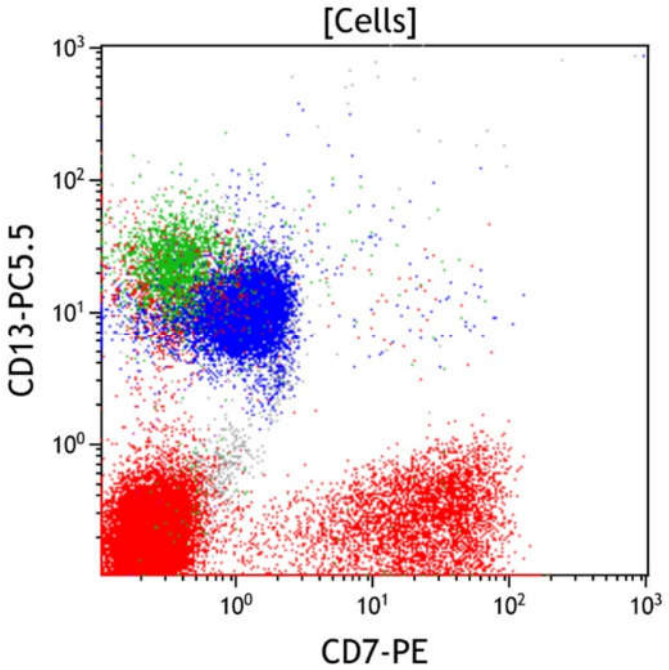

Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells (red, lower right). CD13 is expressed on granulocytes (blue), monocytes (green), basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen.

M2 Cell Tube

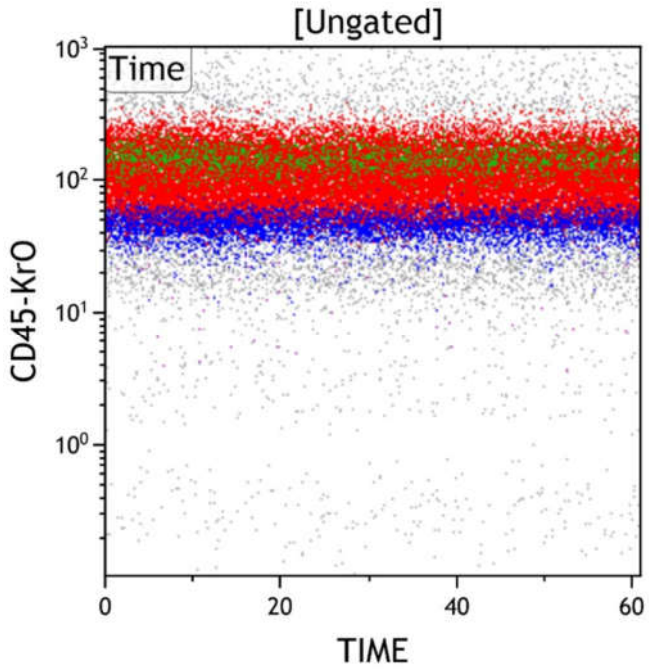

Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

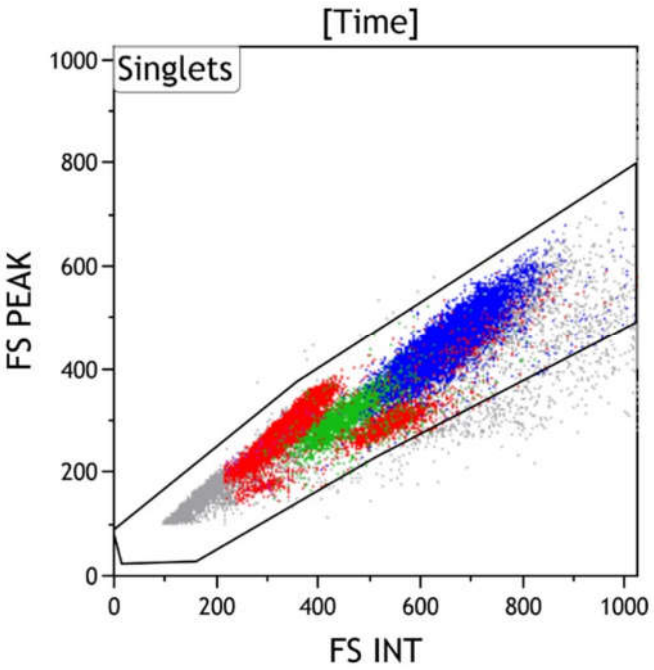

Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

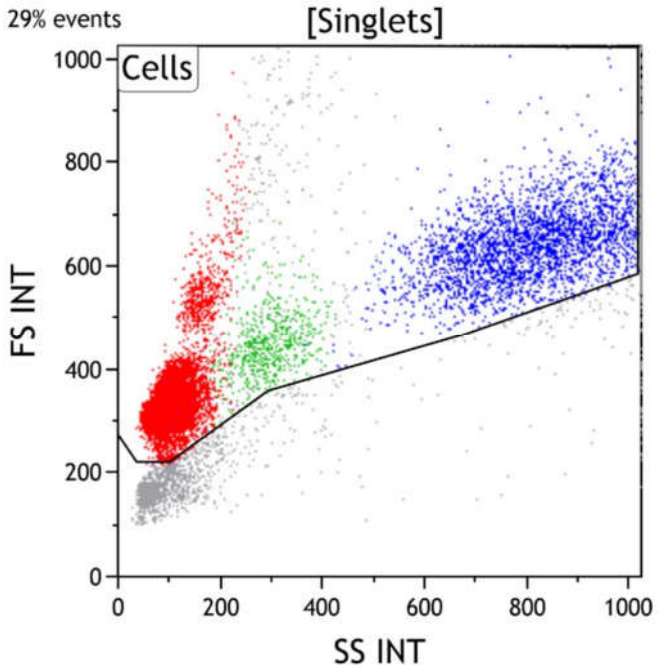

Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

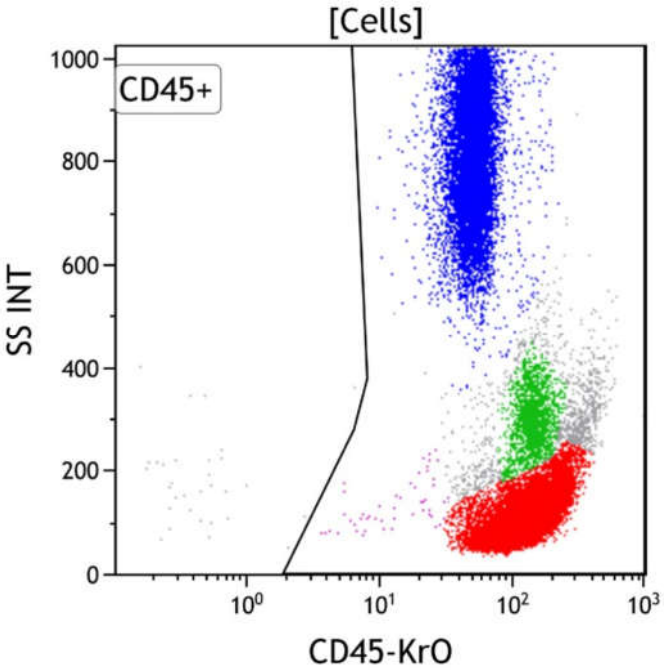

Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

M2 Cell Tube

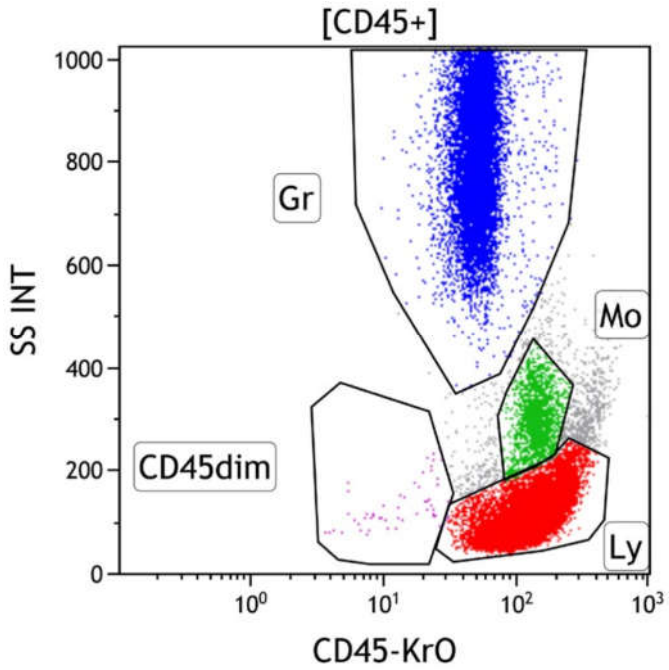

Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

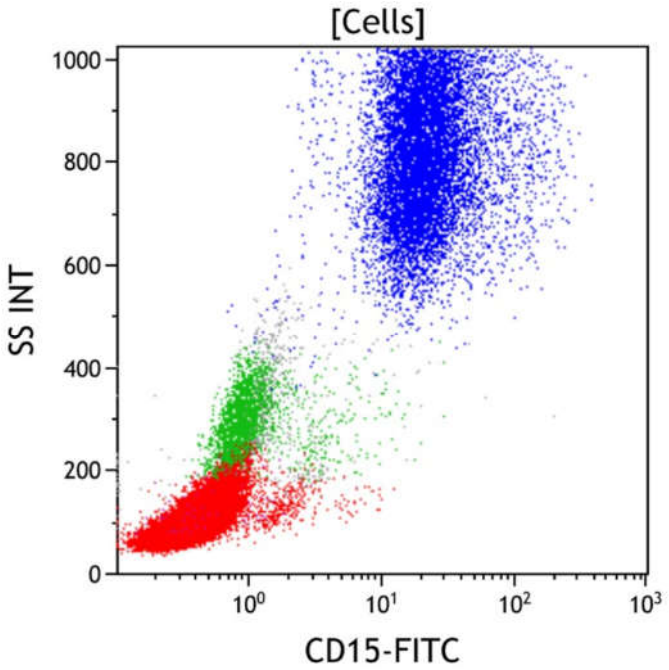

Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on granulocytes (in blue) and at a lower level on monocytes (green).

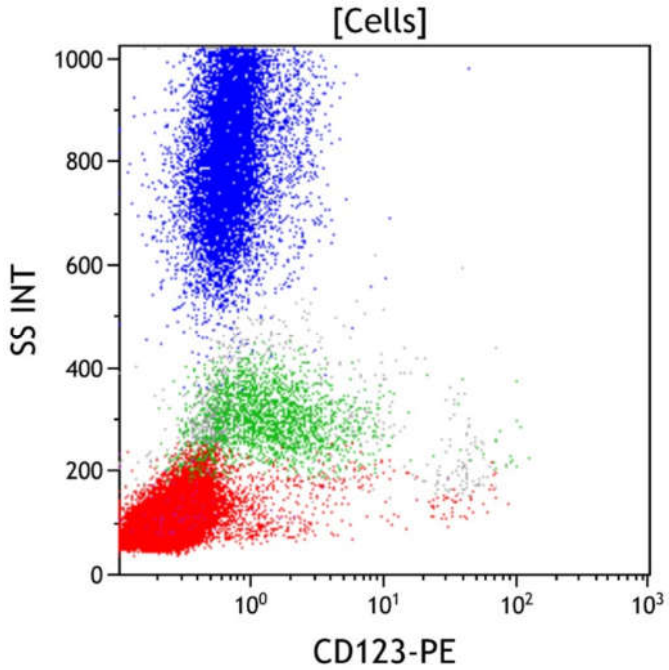

Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes (green).

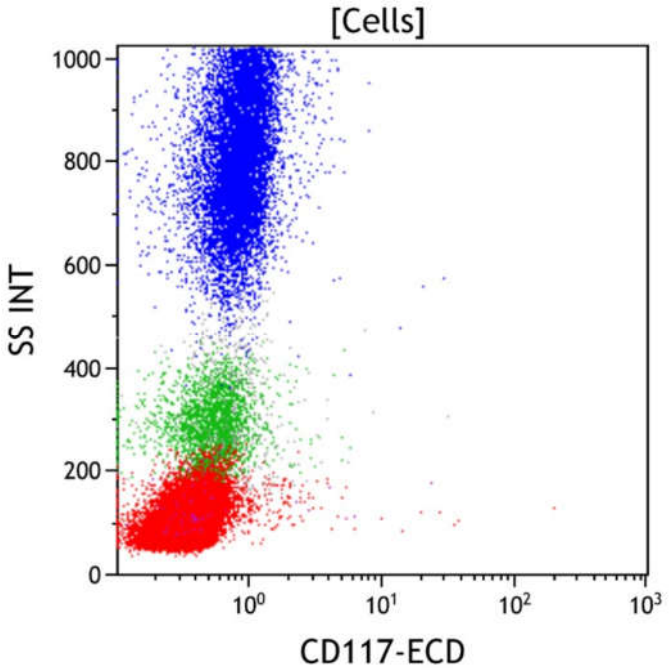

Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells. CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma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

M2 Cell Tube

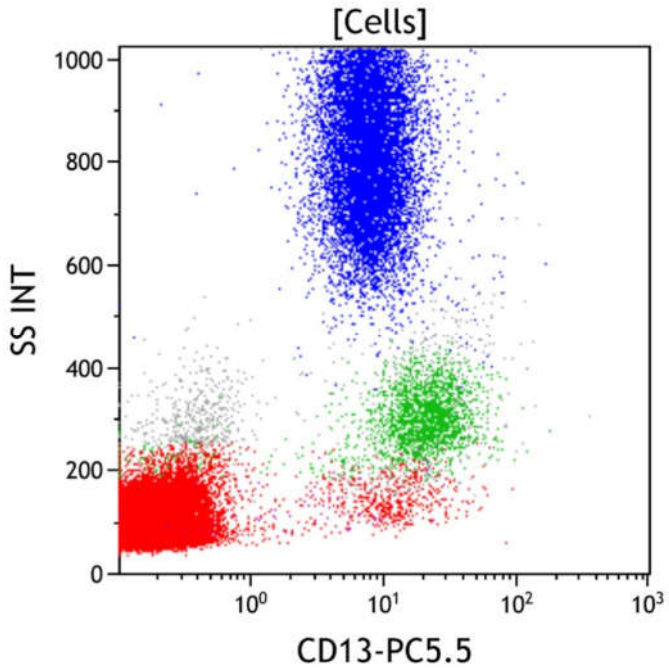

Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on granulocytes (blue) and on mature monocytes (green).

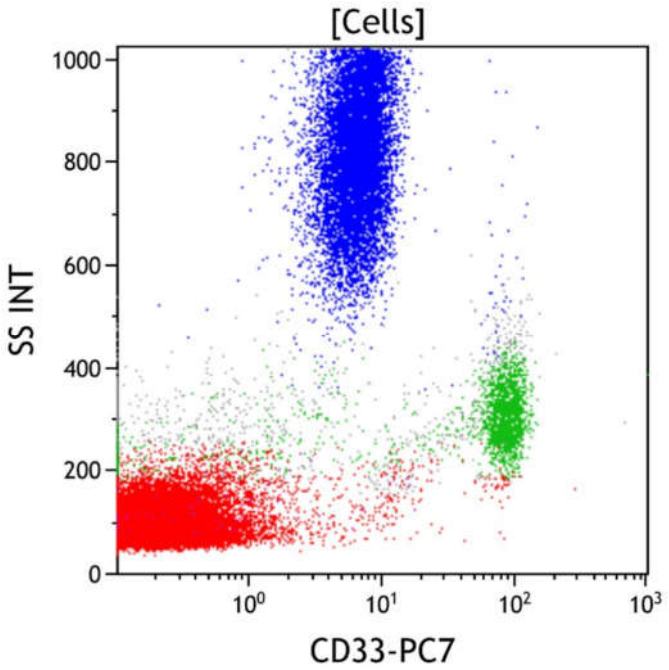

Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on monocytes (green) and at a lower level on granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors.

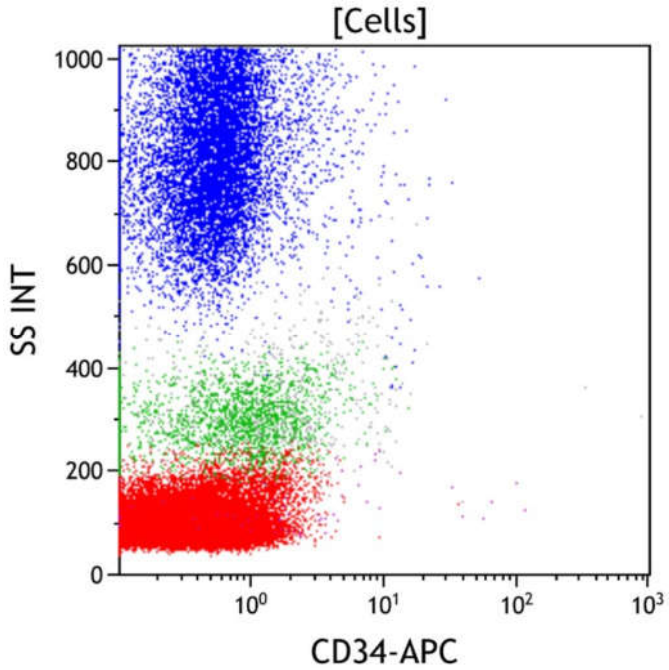

Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Mature granulocytes, monocytes, and lymphocytes are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

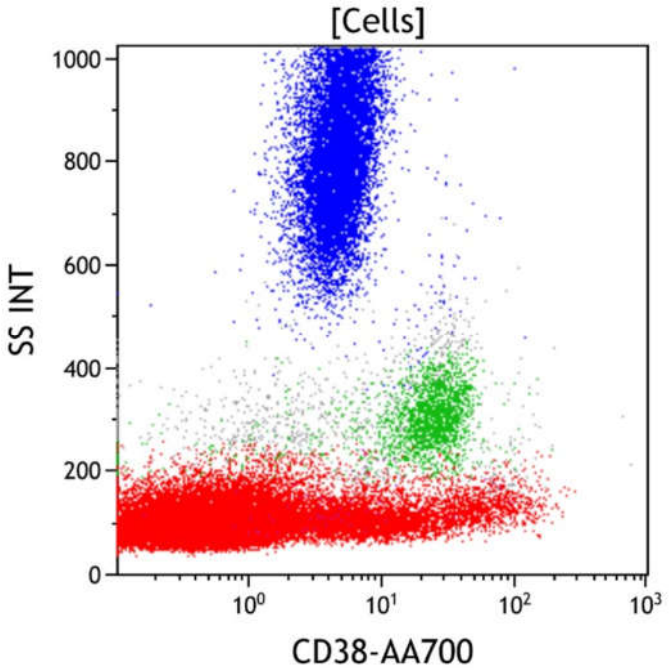

Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors and monocytes (green), and at a variable level on activated lymphocytes (red).

M2 Cell Tube

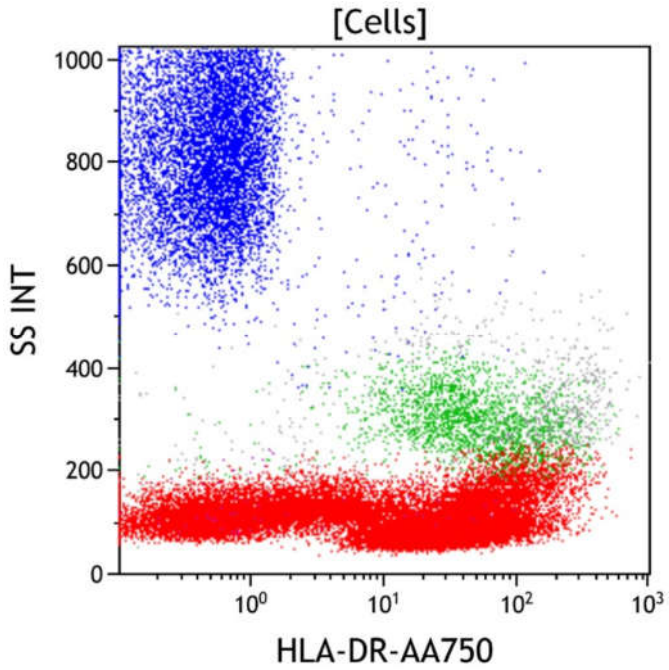

Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, immature and mature B cells (red), and activated T cells (red).

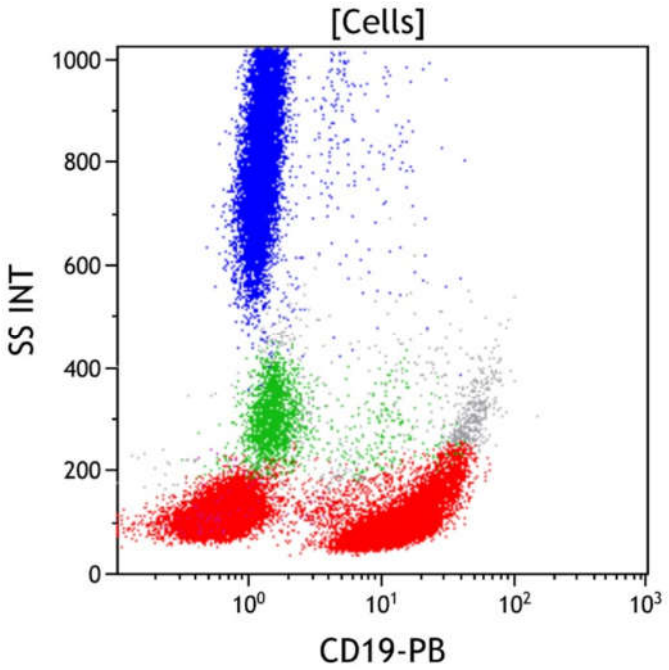

Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on immature and mature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. The mature B cell population (red) is relatively expanded compared with normal.

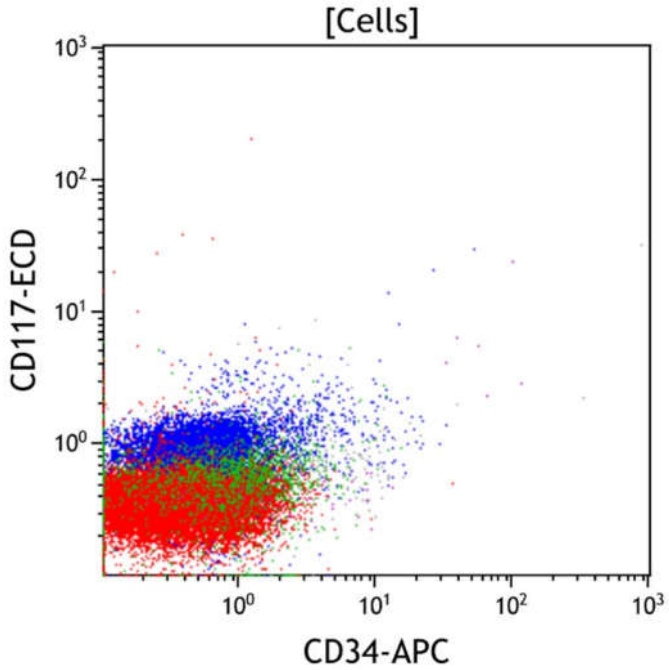

Figure 15. This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors.

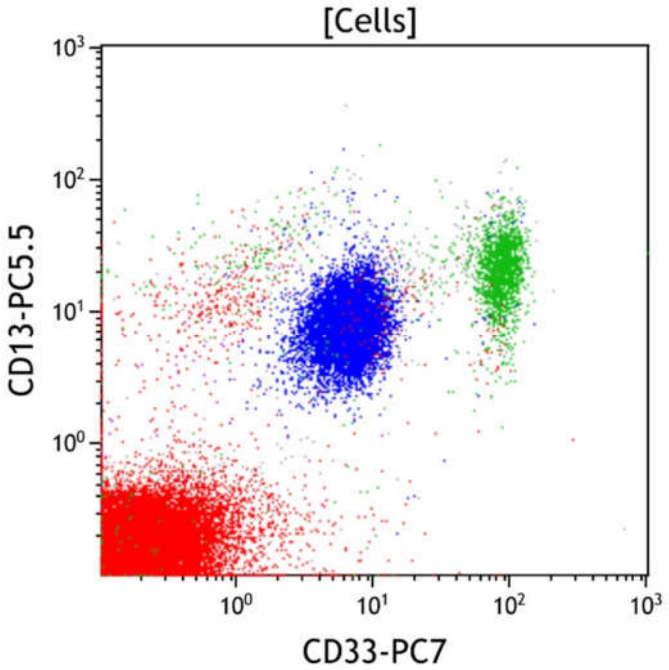

Figure 16. This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes (green), granulocytes (blue), basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Lymphocytes largely do not express either CD13 or CD33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

M2 Cell Tube

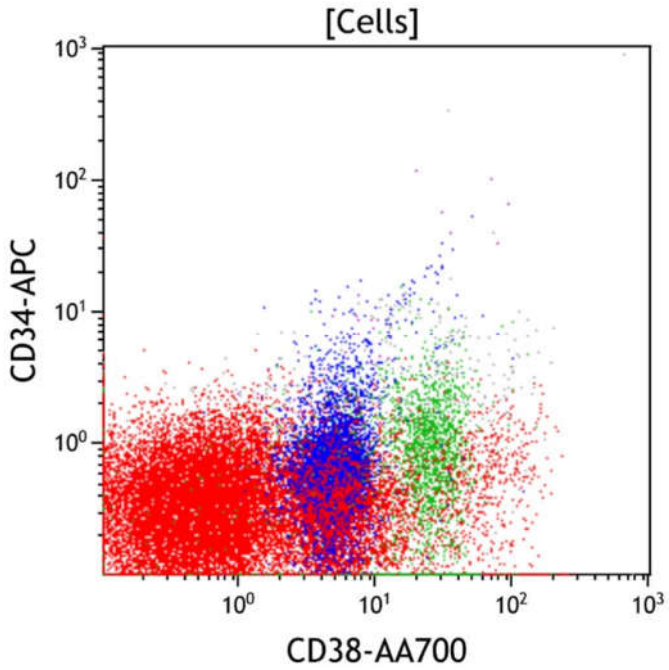

Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Mature granulocytes (blue), monocytes (green), and lymphocytes (red) are negative for CD34.

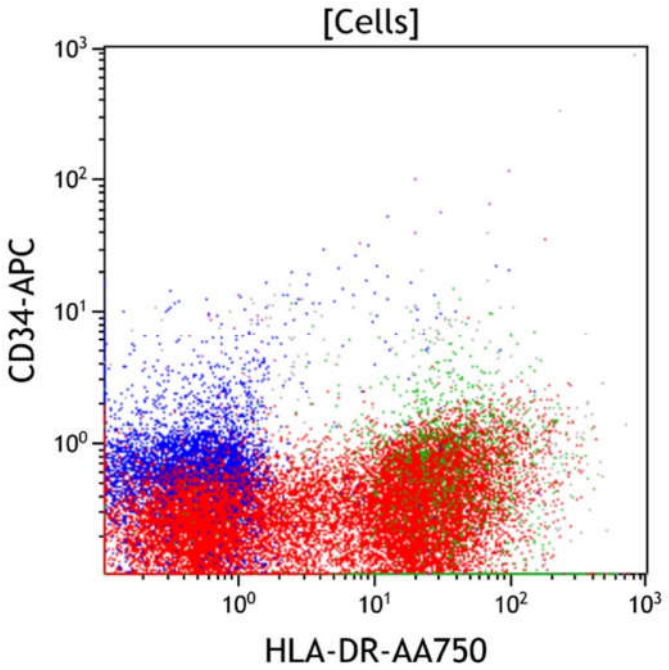

Figure 18. This HLA-DR vs CD34 dot plot shows all viable cells. HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors.

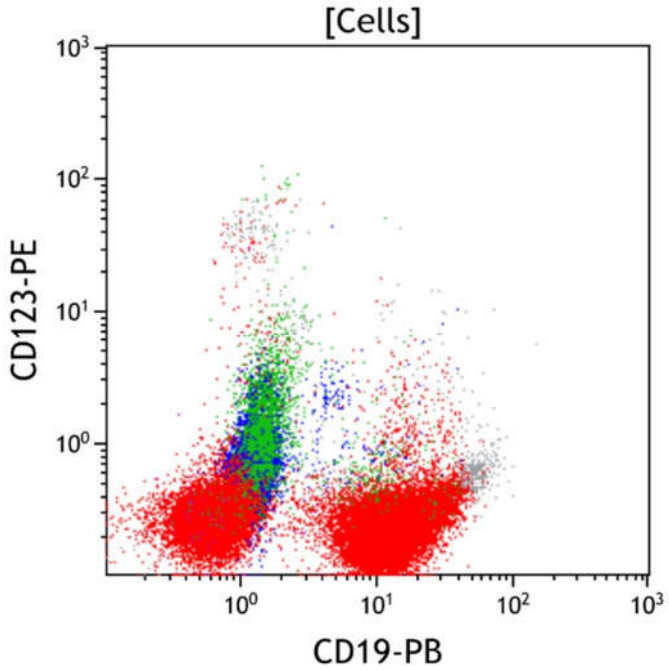

Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells (red, lower middle) normally do not express significant CD123.

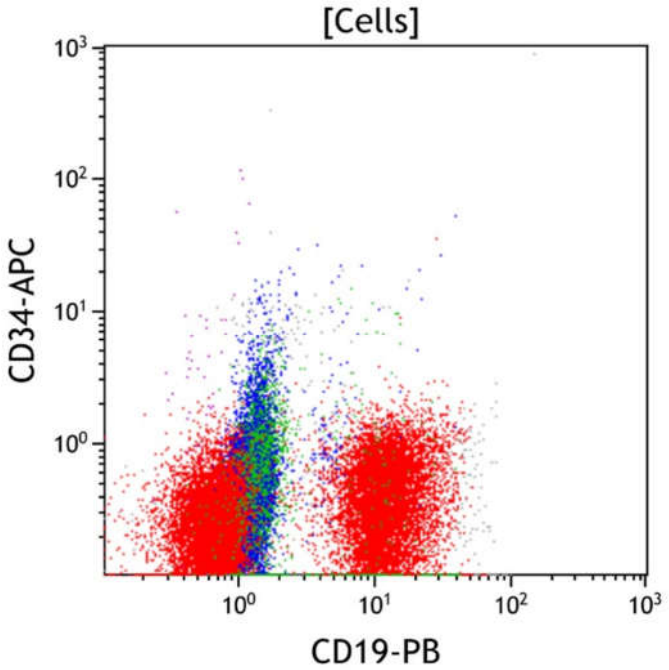

Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Mature CD19 positive B cells (red, lower middle) do not express CD34.

M2 Cell Tube

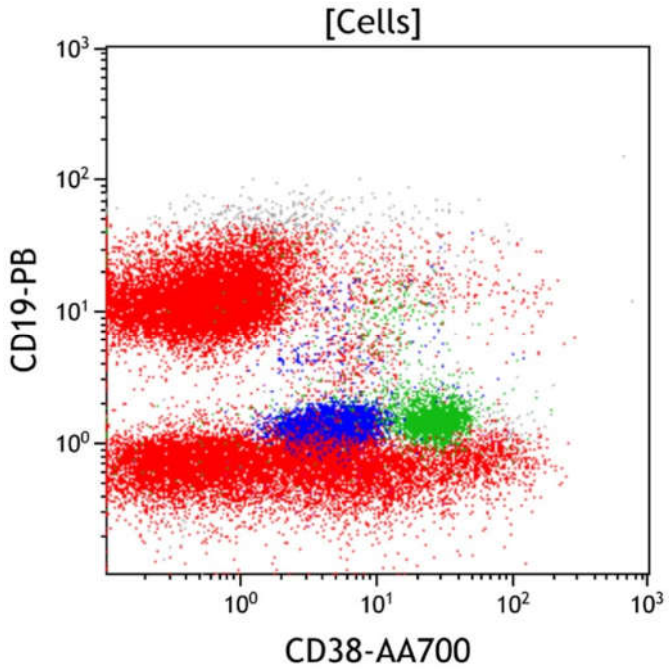

Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. The CD19 positive B cells (red, right middle) have low to absent CD38 expression.

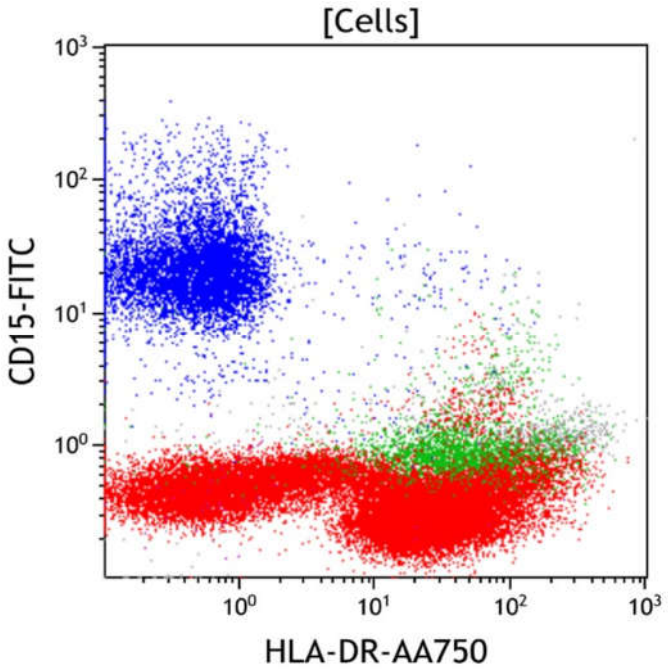

Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on granulocytes (blue) and monocytes (green). HLA-DR is expressed on B cells (red, lower right), monocytes (green), plasmacytoid dendritic cells, and CD34 positive progenitors. Granulocytes (blue) do not express HLA-DR.

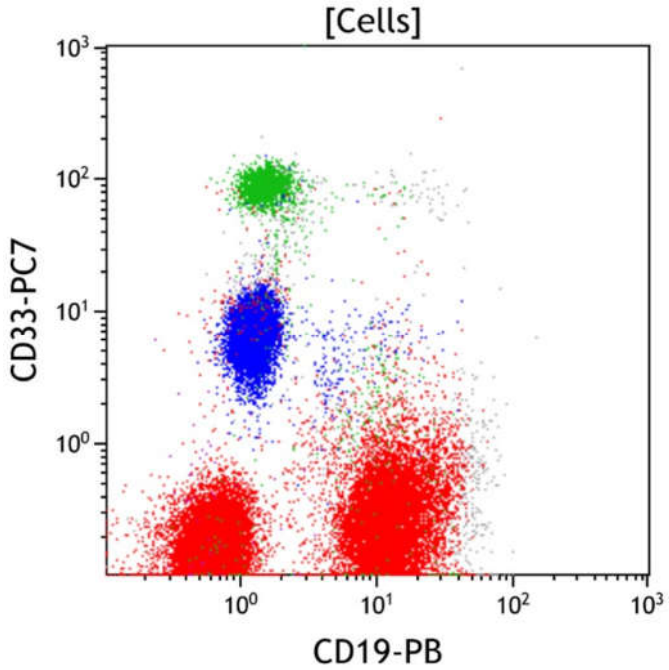

Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells. CD33 is expressed by monocytes (green) and granulocytes (blue). Mature CD19 positive B cells (red, lower middle) do not normally express significant CD33.

Results of Flow Cytometric Immunophenotyping

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

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