

# NEOPLASTIC PROCESS OF T-CELL ORIGIN

T cell and NK cell neoplasms also include acute lymphoblastic and mature lymphoid neoplasms. They are relatively uncommon, but many of them are among the most aggressive of all lymphoid neoplasms. Some, however, have a more prolonged clinical course. Immunophenotypically, these neoplasms often show aberrant expression or loss of T cell markers that aid in the differential diagnosis. Additionally, they may be associated with a viral infection. Epstein-Barr virus (EBV) is most often associated with NK cell leukemias and extranodal NK/T cell lymphomas. Human T cell leukemia virus (HTLV-1) is etiologically linked to adult T cell leukemia/lymphoma. Besides morphologic, immunophenotypic, and genetic characteristics, clinical features play an important part in the definition of these diseases.

## T LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

### Case #14: T Lymphoblastic Leukemia/T Lymphoblastic Lymphoma

#### Clinical Vignette

This 20-year-old male presents with tissue mass. A lymph node biopsy sample is submitted for flow cytometric immunophenotyping using ClearLLab 10C Panels.

#### Flow Cytometric Immunophenotyping

##### B Cell Tube

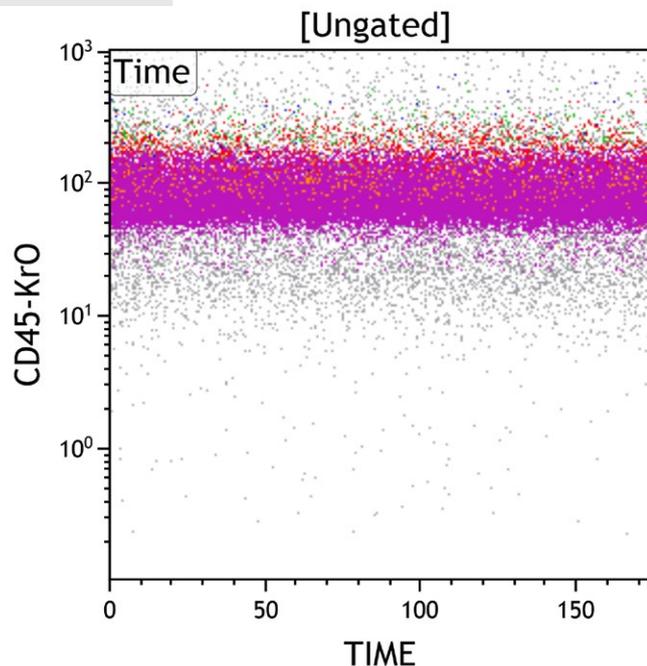


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

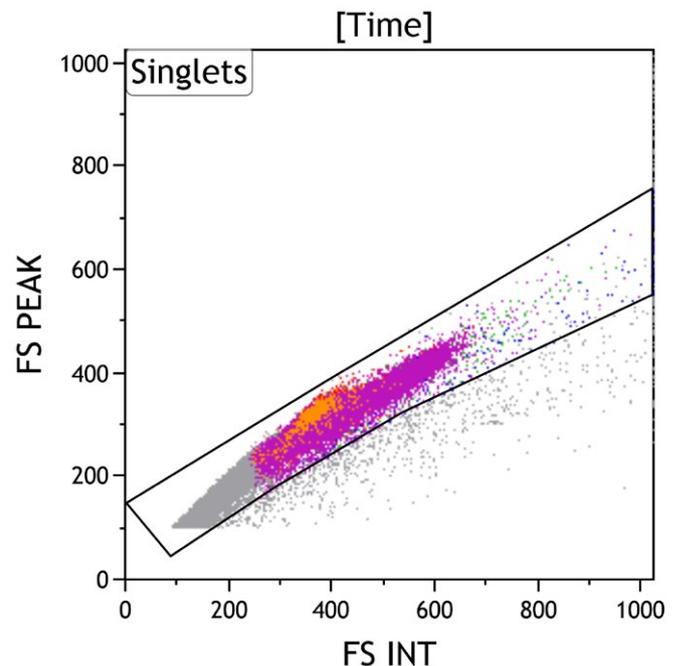


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

**B Cell Tube**

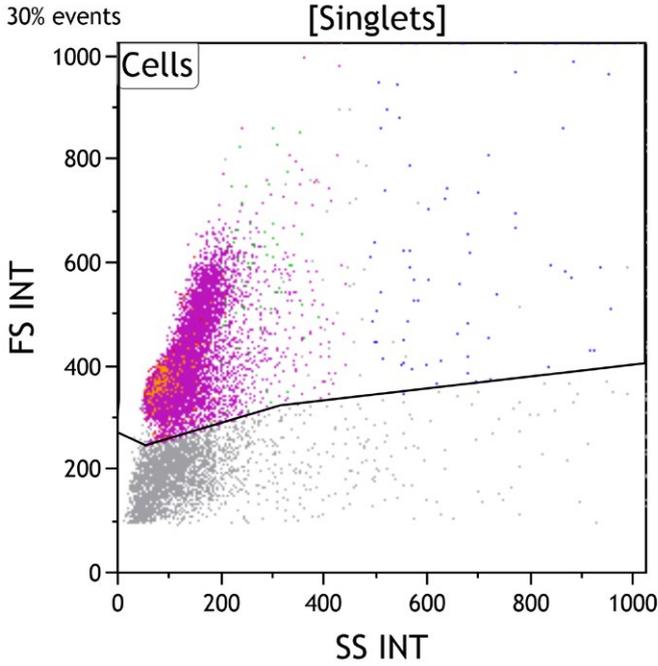


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

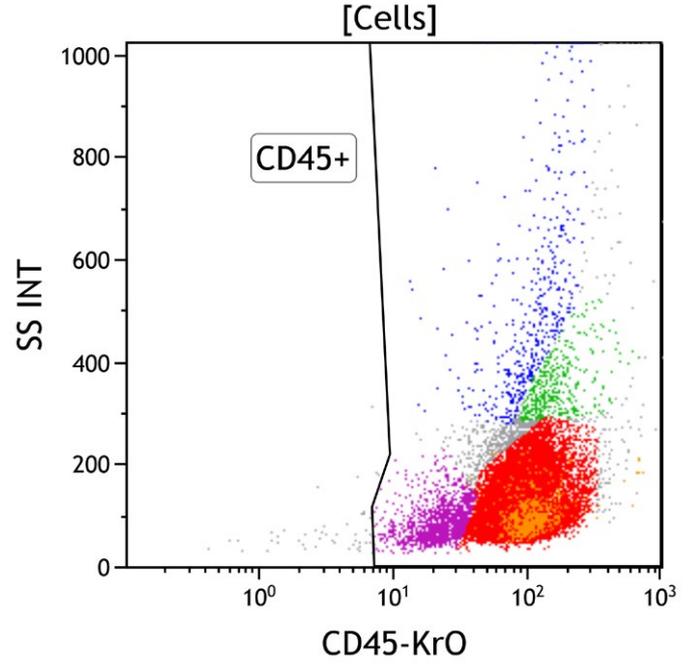


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

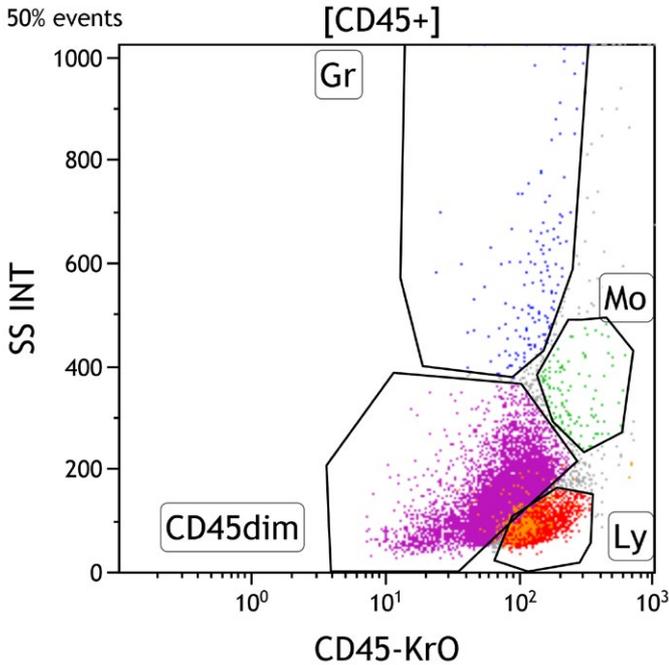


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red/orange), monocytes (Gate Mo, green), and granulocytes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

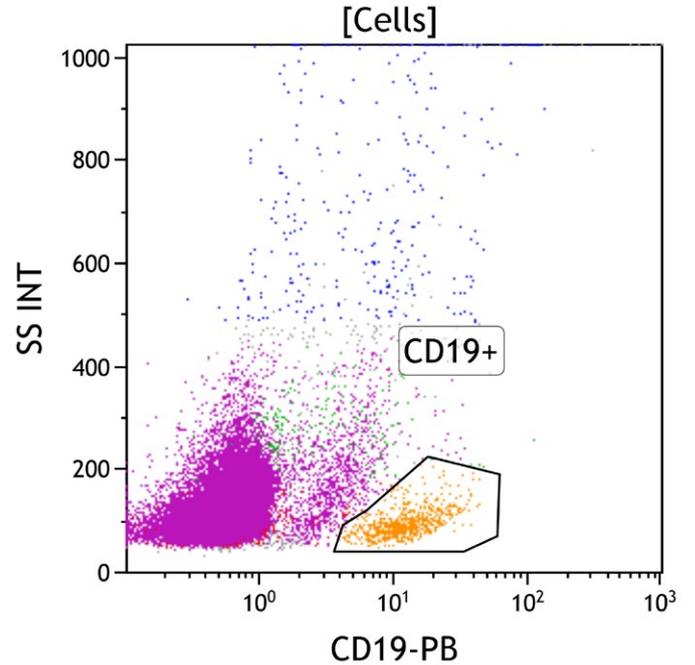


Figure 6: This CD19 vs Side Scatter dot plot shows events in the Cells gate. The CD19+ gate identifies CD19 positive cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express CD19.

**B Cell Tube**

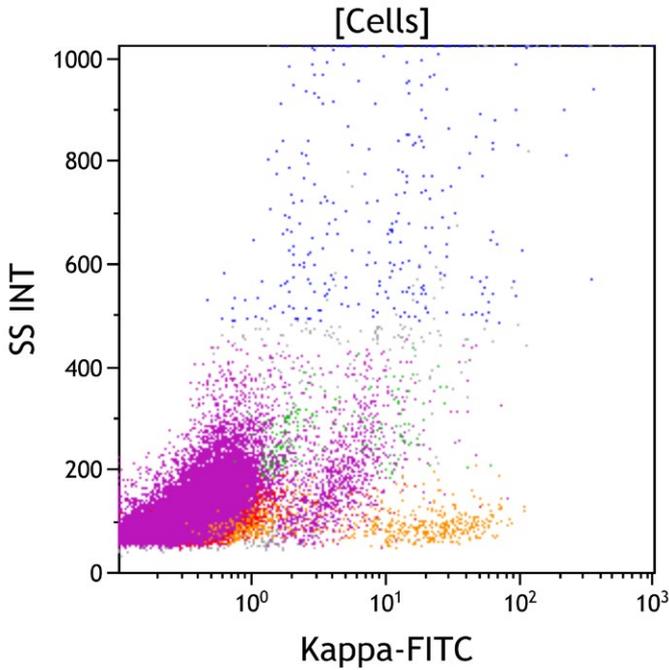


Figure 7: This Kappa vs Side Scatter dot plot shows all viable cells. Surface kappa light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of kappa positive B cells is normally greater than kappa negative (lambda positive) B cells. Apparent kappa positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The Kappa light chain positive cells are shown on the right side of the plot. Progenitors (purple) do not express kappa.

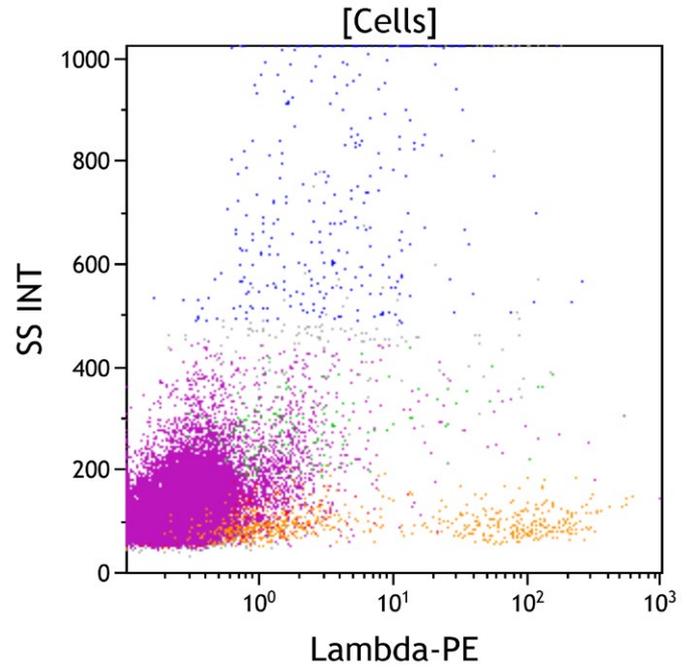


Figure 8: This Lambda vs Side Scatter dot plot shows all viable cells. Surface lambda light chain is expressed on mature B cells (orange) and late stage immature B cells. The proportion of lambda positive B cells is normally less than lambda negative (kappa positive) B cells. Apparent lambda positivity is seen on monocytes (green) due to Fc receptor-mediated binding of immunoglobulin. The lambda light chain positive cells are shown on the right side of the plot. Progenitors (purple) do not express lambda.

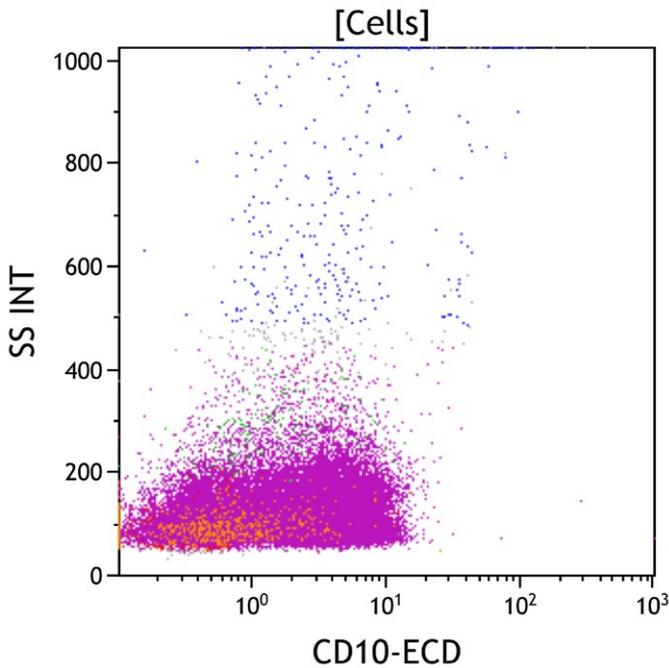


Figure 9: This CD10 vs Side Scatter dot plot shows all viable cells. CD10 is expressed on immature B cells, mature germinal center B cells, and mature granulocytes (blue). The granulocytes have high side scatter, in contrast to lymphoid cells that have low side scatter. Progenitors (purple) express partial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

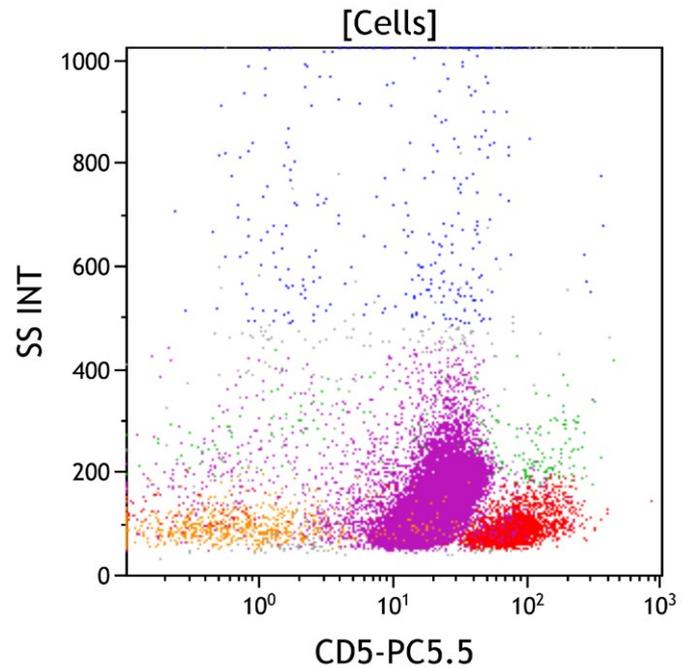


Figure 10: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on mature and immature T cells (red), as well as dimly on a subset of mature B cells (orange). These lymphoid cells typically have low side scatter. Progenitors (purple) express CD5 at a level lower than mature T cells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

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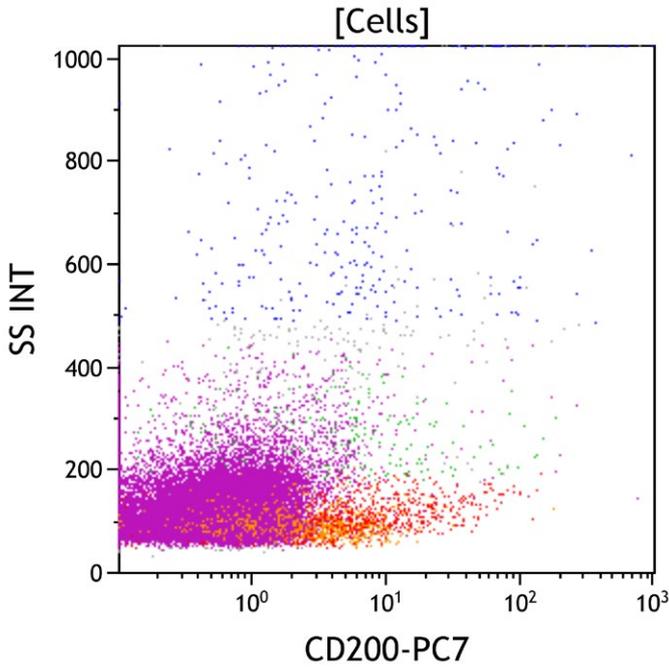


Figure 11: This CD200 vs Side Scatter dot plot shows all viable cells. CD200 is typically expressed on B cells, but is negative in some neoplastic B cells. It is especially useful in distinguishing mantle cell lymphoma (usually CD200 negative) from chronic lymphocytic leukemia/small lymphocytic lymphoma (usually CD200 positive). Progenitors (purple) do not express CD200.

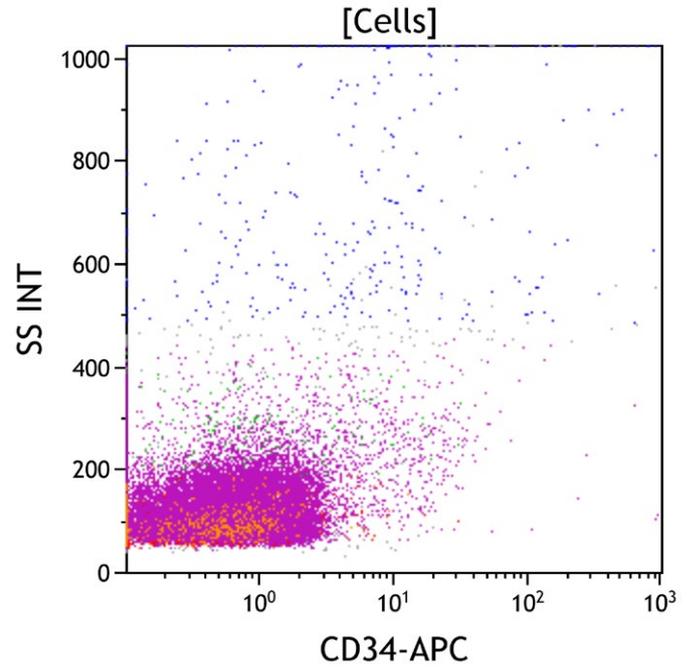


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). Progenitors (purple) do not express CD34.

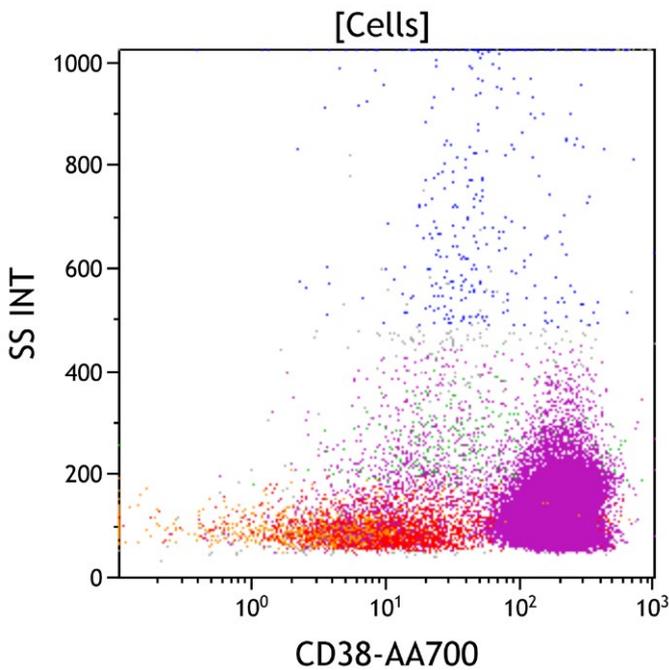


Figure 13: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes (green), and at a variable level on activated mature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

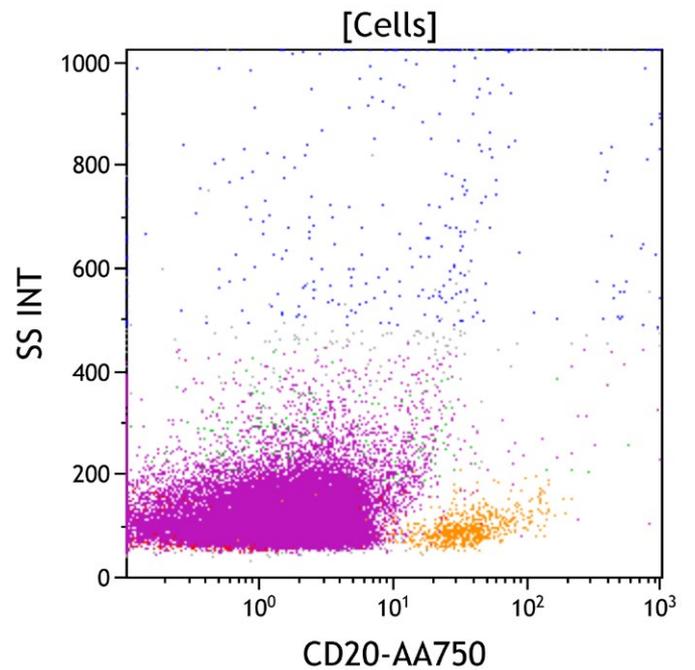


Figure 14: This CD20 vs Side Scatter dot plot shows all viable cells. CD20 is expressed on mature B cells (orange) and at a variably low level on a subset of mature T cells. It is variably expressed on later stage immature B cells. CD20 positive cells are usually in the lymphocyte gate with low side scatter. Progenitors (purple) do not express CD20, the apparent low-level positivity being increased is due to compensation background from the bright CD38.

**B Cell Tube**

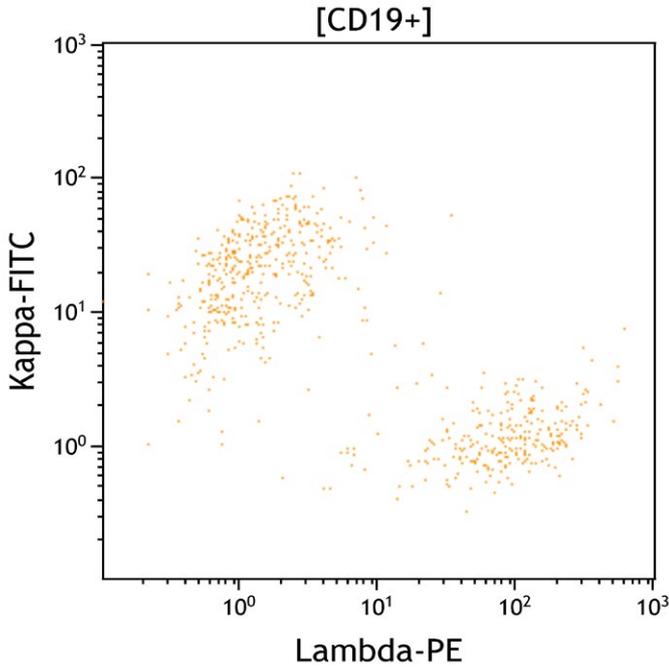


Figure 15. This Lambda vs Kappa dot plot shows all CD19+ cells. The early immature B cells do not express surface immunoglobulin light chains, i.e. negative for either kappa or lambda light chain. The mature B cells are polyclonal, expressing either kappa or lambda light chain. The normal kappa to lambda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

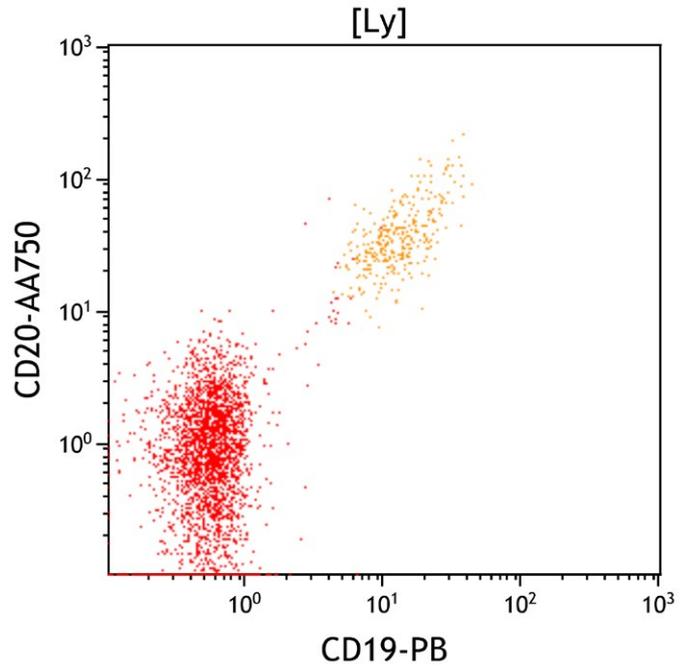


Figure 16. This CD19 vs. CD20 dot plot shows all cells in the Lymphocyte gate (Ly). Mature B cells express both CD19 and CD20 (orange). Immature B cells express CD19 and variably lower CD20. Some neoplastic B cells may show decreased CD19 or CD20 expression.

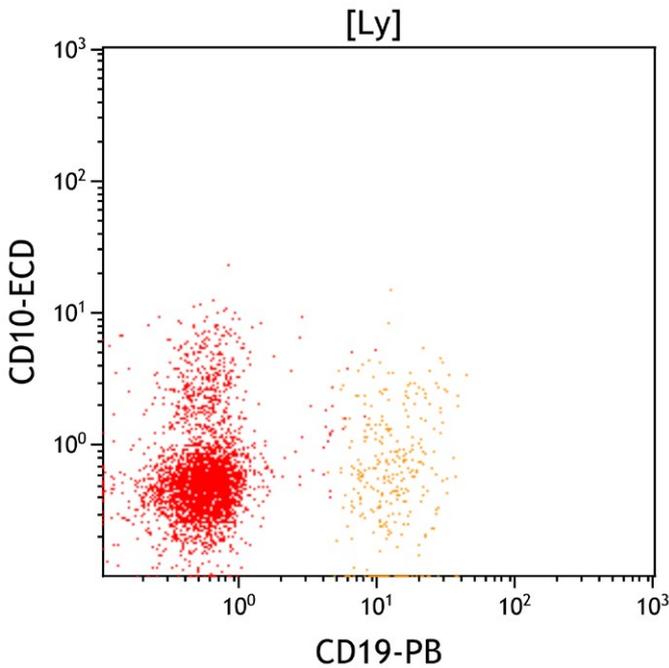


Figure 17. This CD19 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). B cells are CD19 positive (orange). CD10 positive mature B cells are distributed in germinal centers in lymph nodes and a small subset of late stage immature B cells is present in peripheral blood and bone marrow aspirates. Immature B cells are normally CD10 positive, but they are seen largely in the CD45 dim gate. This case displays extensive overlap among gates. The CD10 dim staining (red, upper left) is due to overlap of the progenitors in the Lymphocyte gate (Ly).

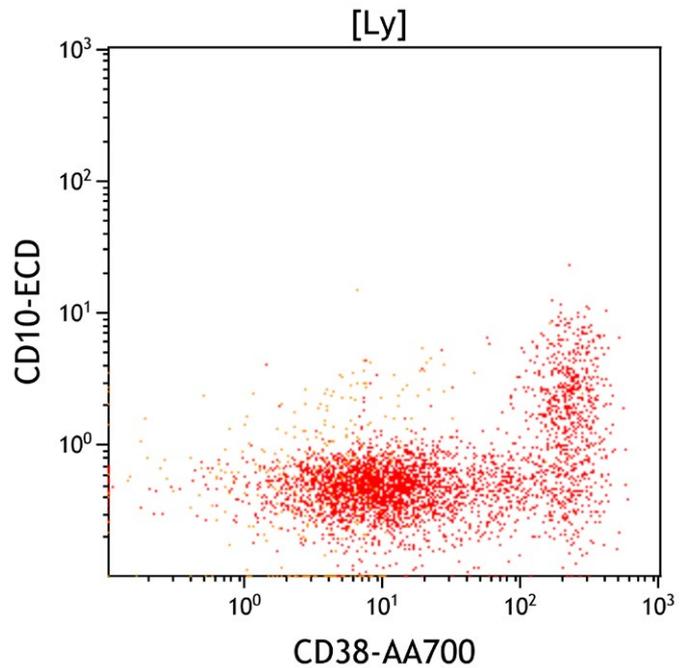


Figure 18. This CD38 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). CD38 is expressed at the highest level on plasma cells, at a moderate level on immature B cells and at a low level on germinal center B cells. Most mature B cells (orange) display low to absent expression of CD38. T cells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

**B Cell Tube**

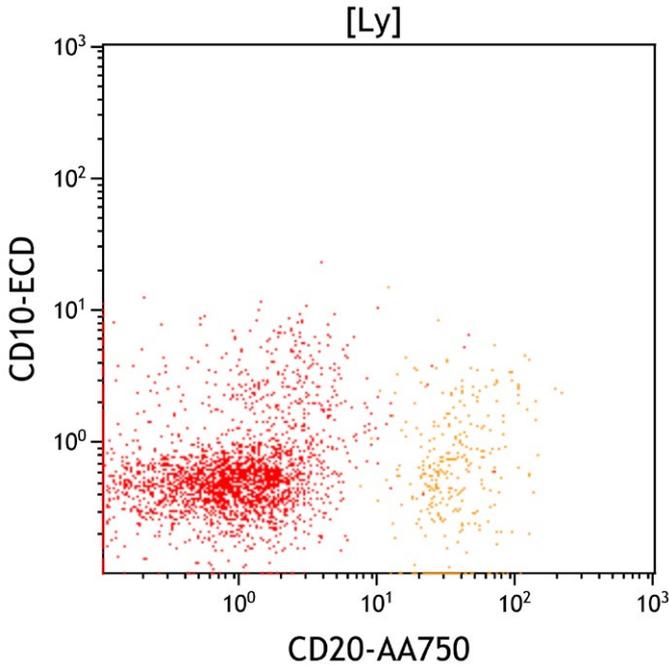


Figure 19. This CD20 vs CD10 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells uniformly express high level CD20. The rare CD10 positive B cells with variably decreased CD20 represent late stage immature B cells. This case displays extensive overlap among gates. The CD10 dim staining (red, upper left) is due to overlap of the progenitors in the Lymphocyte gate (Ly).

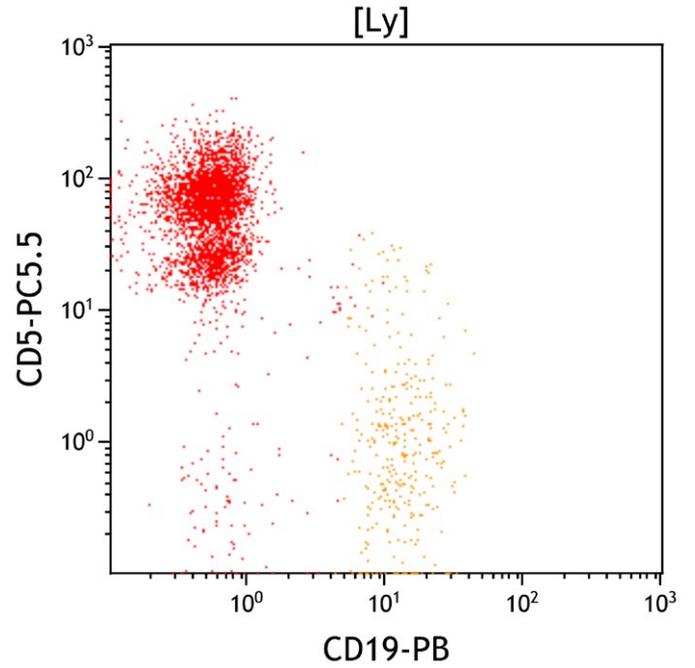


Figure 20. This CD19 vs CD5 dot plot shows all cells in the Lymphocyte gate (Ly). CD5 is expressed on T cells (red), variably expressed at a low level on a subset of normal mature B cells (orange), and expressed on some subtypes of neoplastic B cells. This case displays extensive overlap among gates. The intermediate CD5 staining (red, middle left) is due to overlap of the progenitors in the Lymphocyte gate (Ly).

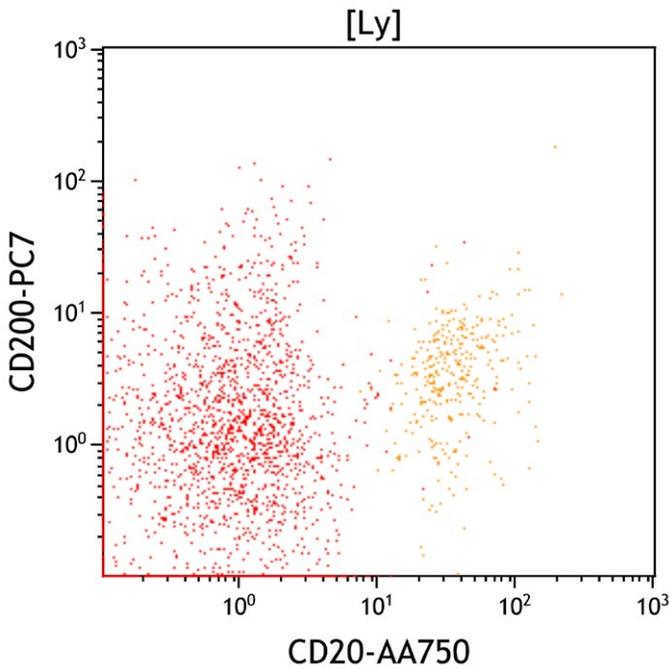


Figure 21. This CD20 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells express CD200 at a low to moderate level (orange).

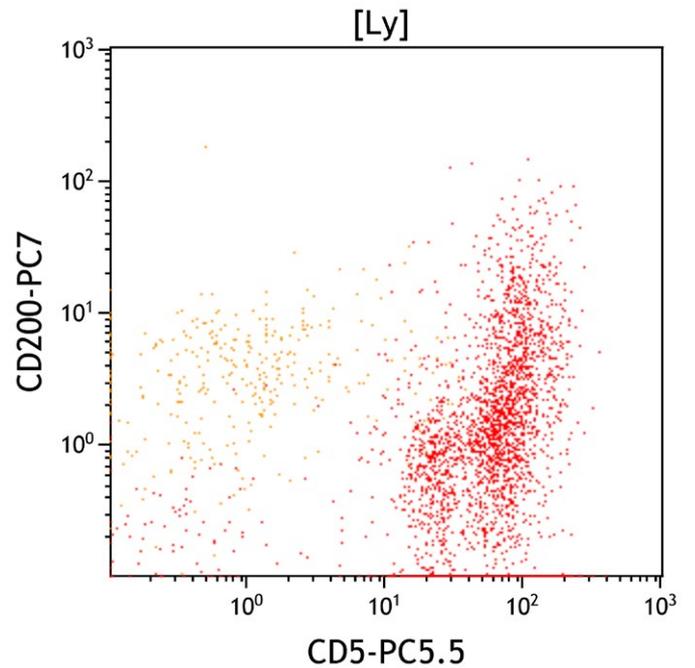


Figure 22. This CD5 vs CD200 dot plot shows all cells in the Lymphocyte gate (Ly). Most mature B cells normally express CD200 with a subset variably expressing CD5. Neoplastic B cells in chronic lymphocytic leukemia/small lymphocytic lymphoma typically express CD5 and CD200, whereas mantle cell lymphoma typically expresses CD5 but not CD200. This case displays extensive overlap among gates. The intermediate CD5 staining is due to overlap of the progenitors in the Lymphocyte gate (Ly).

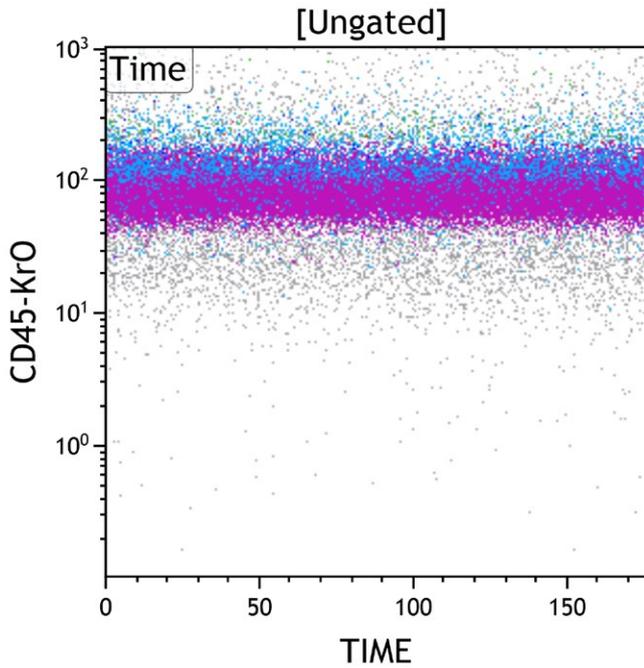


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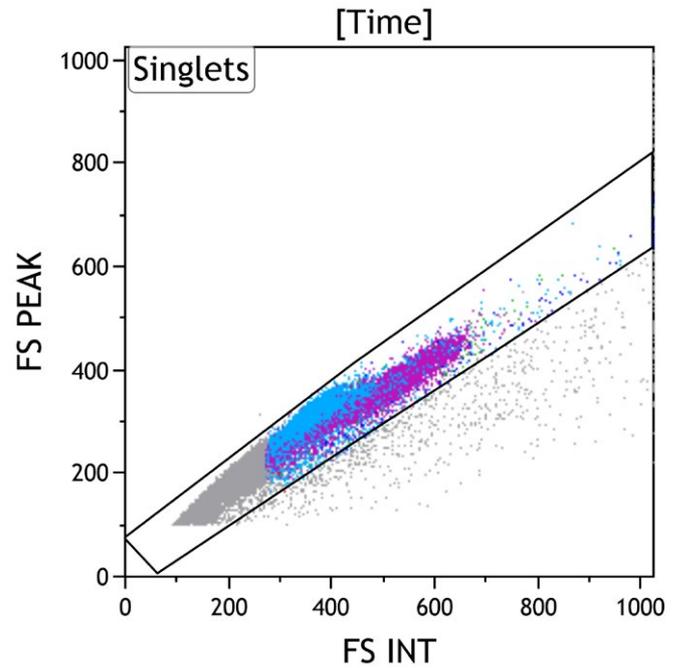


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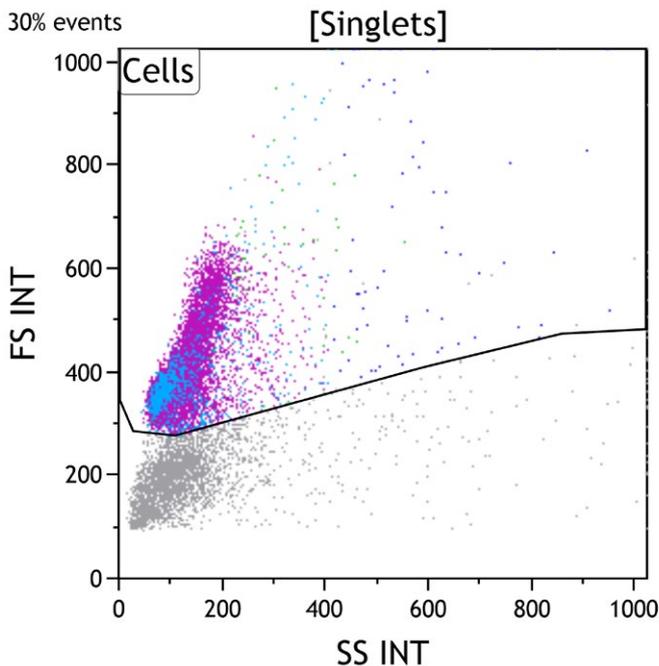


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

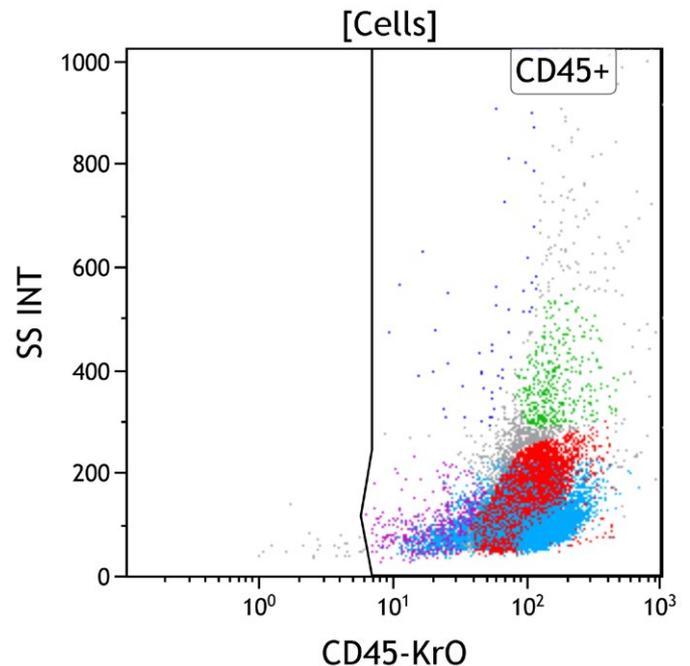


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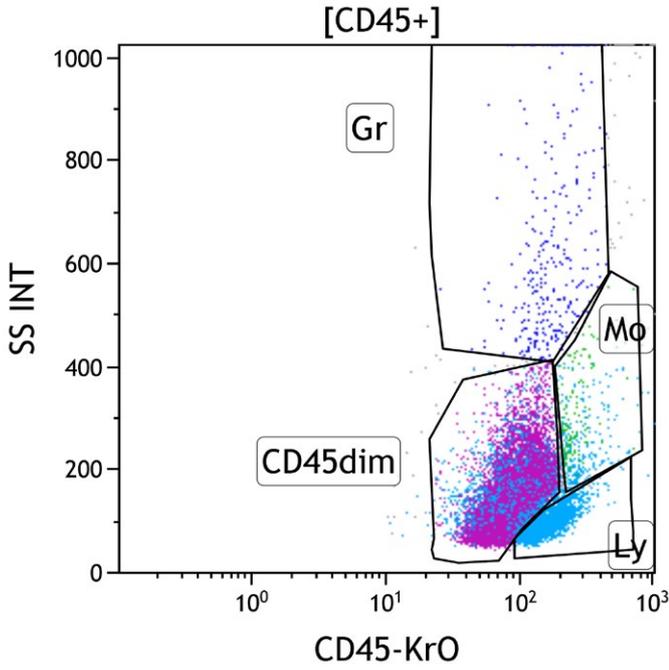


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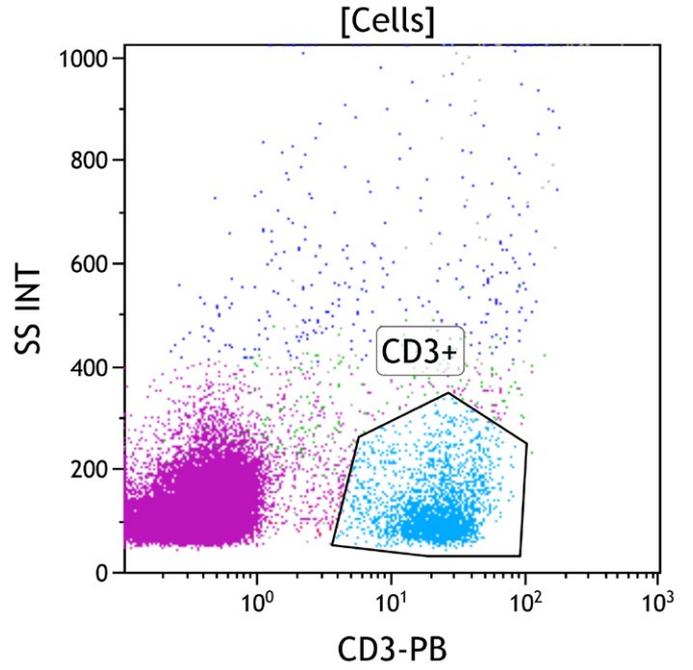


Figure 6: This CD3 vs Side Scatter dot plot shows all viable cells. The CD3+ gate identifies cells with surface CD3 expression (aqua). CD3 is highly specific for T cells, being expressed only on the surface of mature T cells and later stage immature T cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express surface CD3.

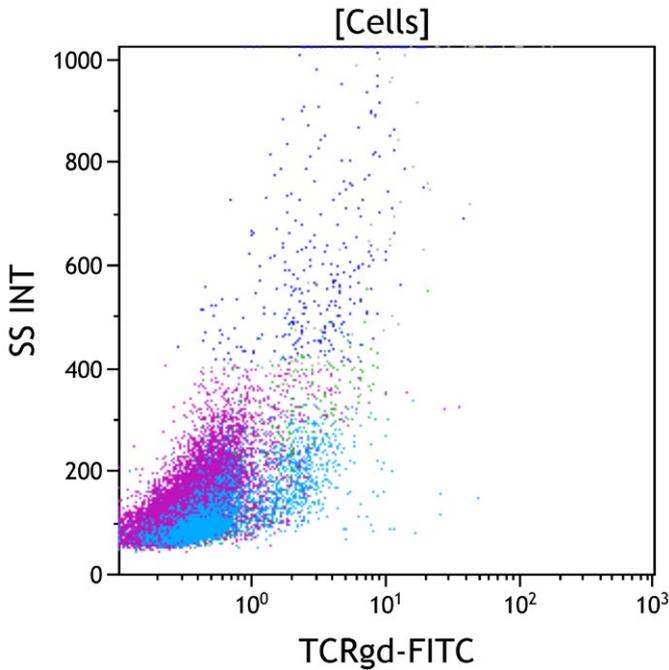


Figure 7: This TCR $\delta$  vs Side Scatter dot plot shows all viable cells. TCR $\delta$  is a subunit of T cell receptor and expressed on a small subset of cytotoxic T cells. These cells typically have low side scatter (aqua). Progenitors (purple) do not express TCR $\delta$ .

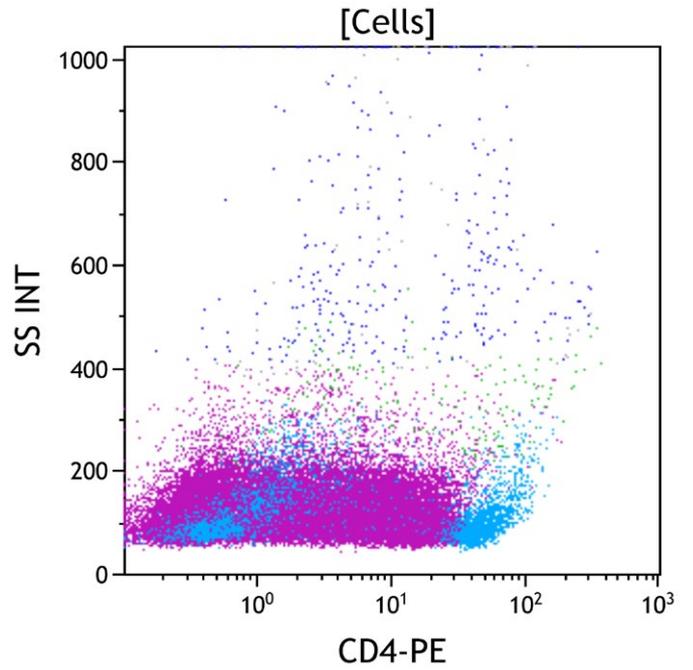


Figure 8: This CD4 vs Side Scatter dot plot shows all viable cells. CD4 is expressed on a subset of immature and mature T cells at a high level (aqua). CD4 is also expressed on monocytic cells (green) at a level lower than that of CD4 positive T cells. It is also expressed at a low level on immature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

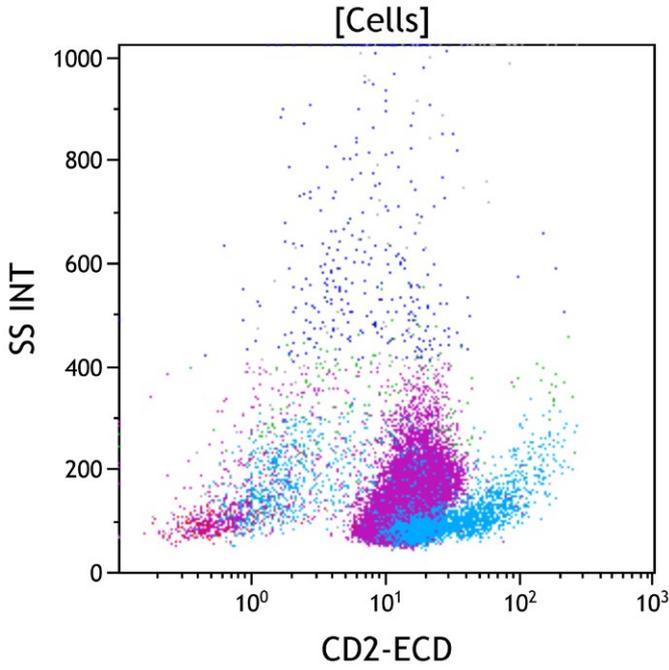


Figure 9: This CD2 vs Side Scatter dot plot shows all viable cells. CD2 is an antigen expressed by nearly all immature and mature T cells (aqua). CD2 is also expressed on NK cells (red) and at a low level on monocytes (green). Progenitors (purple) do express intermediate CD2.

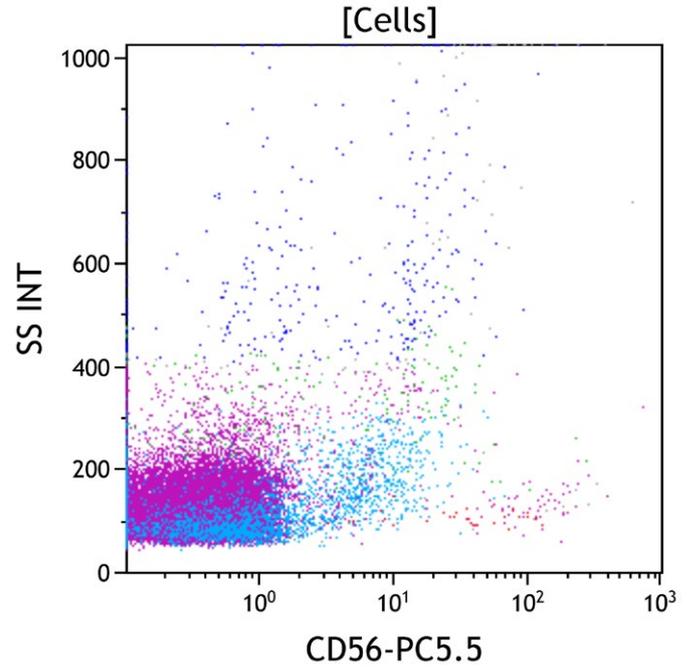


Figure 10: This CD56 vs Side Scatter dot plot shows all viable cells. CD56 is normally expressed on a major subset of NK cells (red), T cells with natural killer activity (NK/T cells), and many gamma/delta T cells (aqua). CD56 is also partially expressed on monocytic cells (green) in both reactive and neoplastic conditions. Progenitors (purple) do not express CD56.

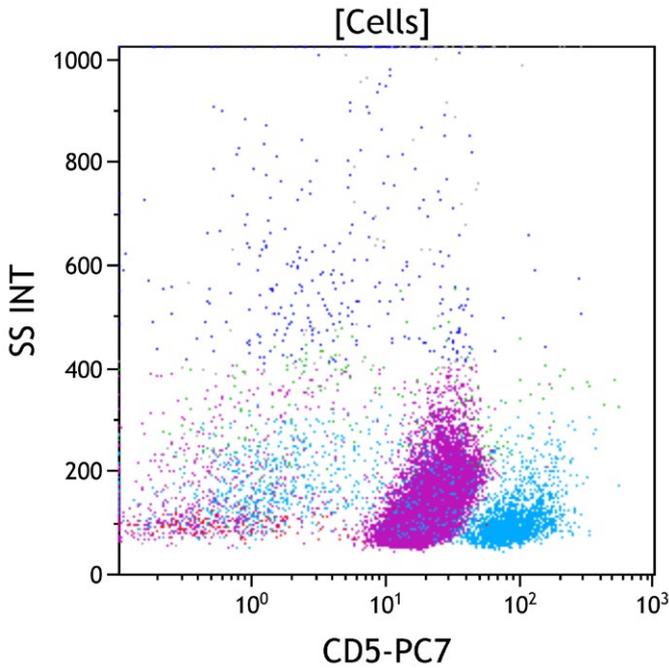


Figure 11: This CD5 vs Side Scatter dot plot shows all viable cells. CD5 is expressed on most immature and mature T cells (aqua), and at a low level on a subset of mature B cells. Very early immature T cells and gamma/delta T cells typically have little to no CD5 expression. A small subset of NK cells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

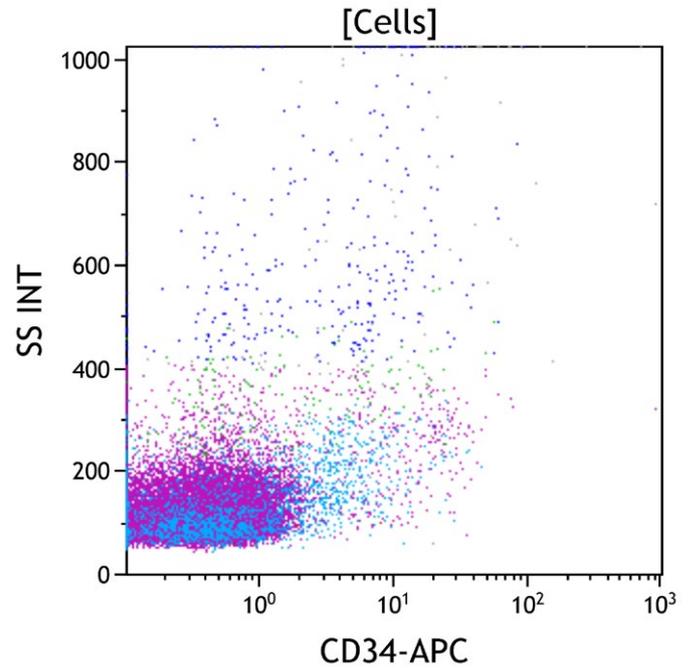


Figure 12: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Progenitors (purple) do not express CD34.

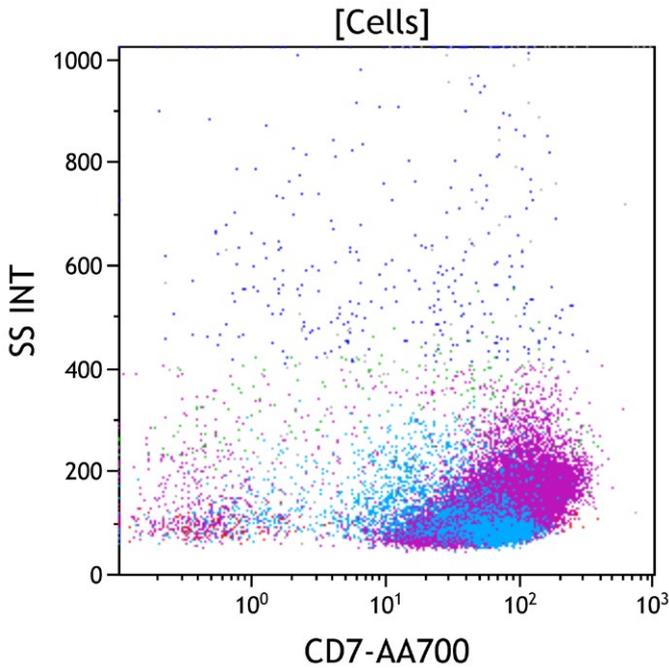


Figure 13: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (aqua). It is also uniformly expressed on NK cells (red, lower right),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

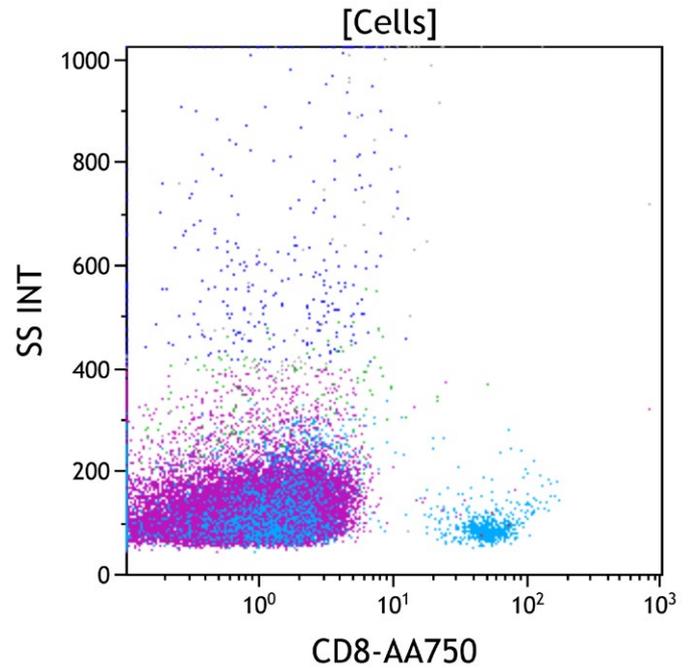


Figure 14: This CD8 vs Side Scatter dot plot shows all viable cells. CD8 is expressed on a subset of immature and mature T cells (aqua) and defines the cytotoxic mature T cell population. It is variably expressed at a low level on NK cells and gamma-delta T cells. Progenitors (purple) do not express CD8.

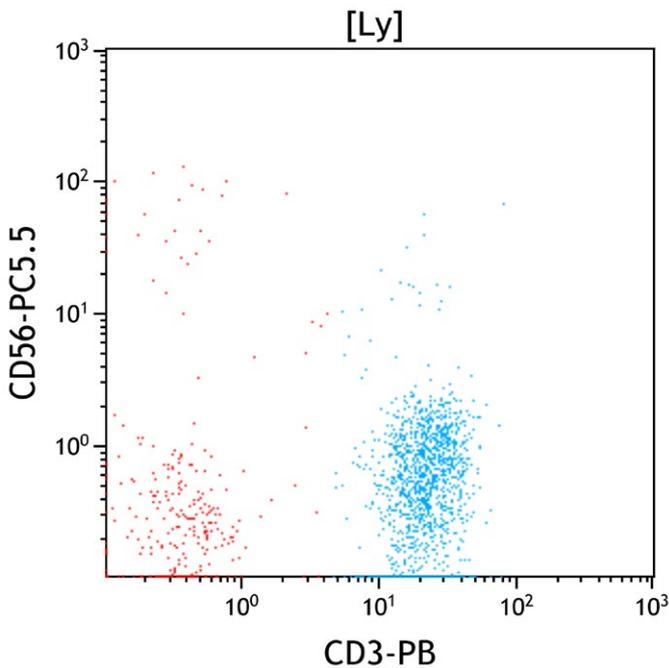


Figure 15. This CD3 vs CD56 dot plot shows all cells in the lymphocyte gate (Ly). T cells are defined by CD3 expression, as it is present on all mature T cells and not expressed by cells of other lineages. NK cells by definition do not express surface CD3, but do express CD56 is on a major subset (red,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

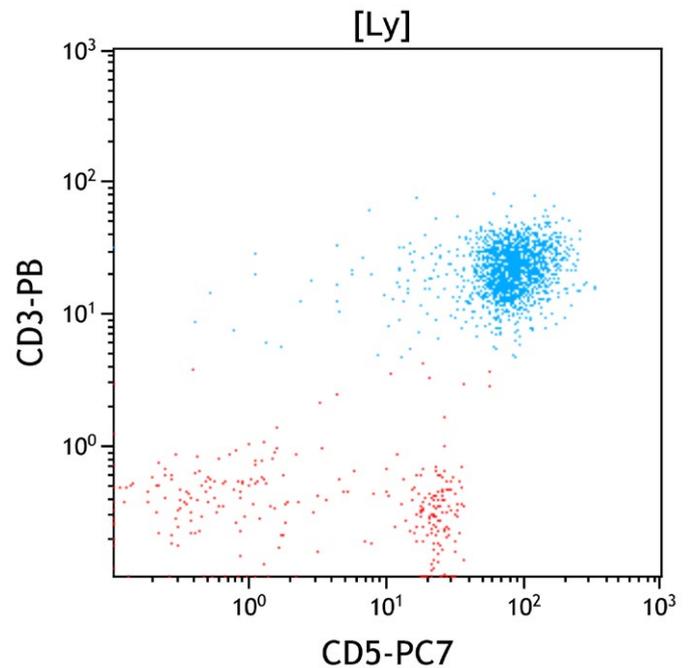


Figure 16. This CD5 vs CD3 dot plot shows all cells in the Lymphocyte gate (Ly). CD3 and CD5 are coexpressed on most mature T cells (aqua), although a small subset of cytotoxic T cells having a large granular lymphocyte morphology often shows reduced to absent expression of CD5. CD5 is not expressed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

**T Cell Tube**

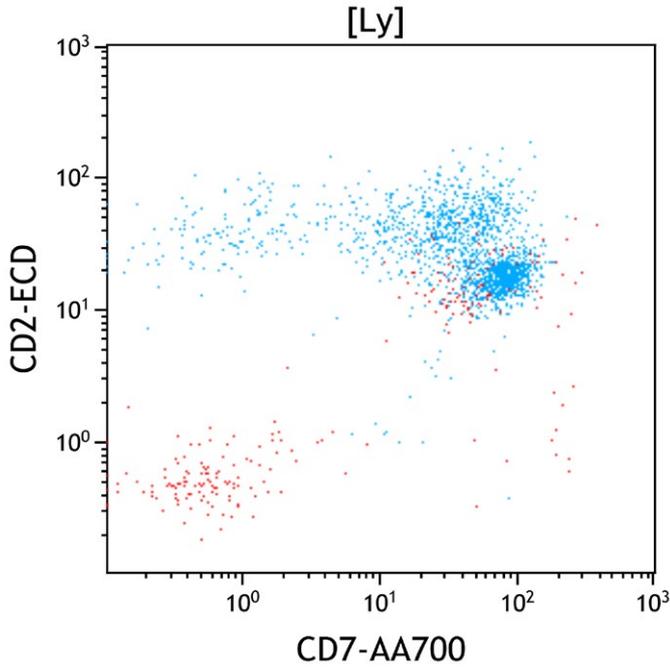


Figure 17. This CD7 vs CD2 dot plot shows all cells in the Lymphocyte gate (Ly). CD2 and CD7 are coexpressed on the large majority of mature T cells (aqua) and NK cells (red, upper right).

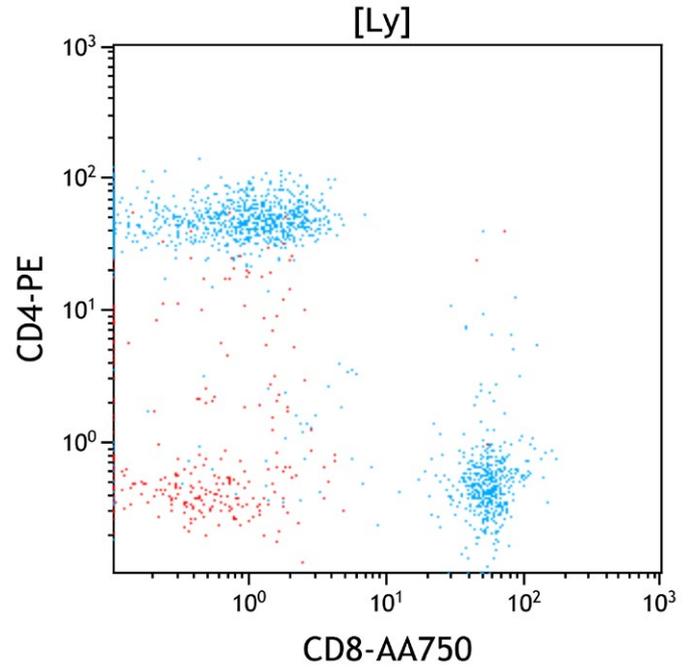


Figure 18. This CD8 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). CD3 positive T cells (aqua) contain CD4 positive (helper) and CD8 positive (cytotoxic) subsets. CD4 positive T cells usually outnumber CD8 positive T cells with a CD4:CD8 ratio of 1:1 to 4:1 in peripheral blood. Occasional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

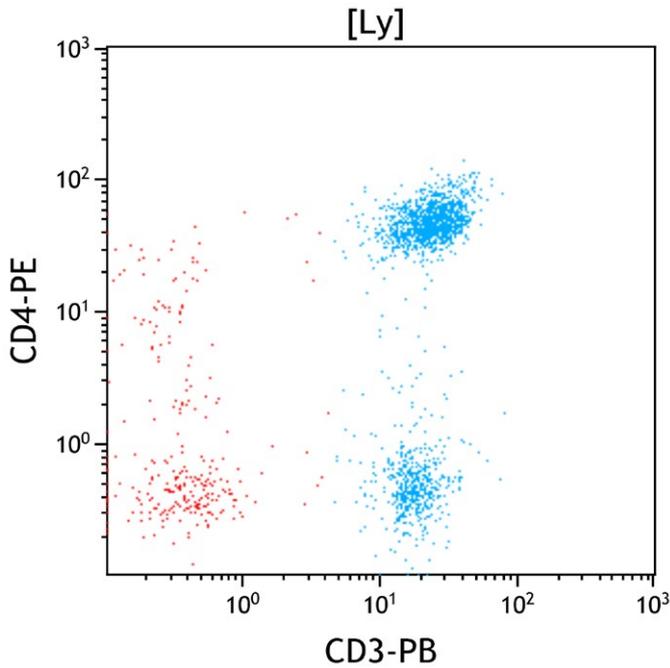


Figure 19. This CD3 vs CD4 dot plot shows all cells in the lymphocyte gate (Ly). All CD4 positive T cells express CD3. Monocytes and plasmacytoid dendritic cells express CD4 at a lower level than CD4 positive T cells and lack CD3 expression. NK cells lack expression of both CD3 and CD4.

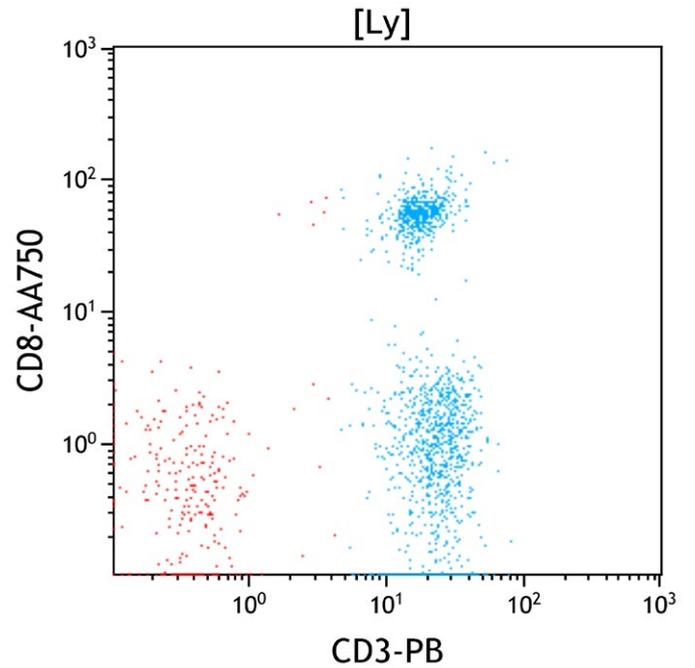


Figure 20. This CD3 vs CD8 dot plot shows all cells in the lymphocyte gate (Ly). All CD8 positive T cells express CD3 (aqua). A small subset of NK cells also expresses CD8 without CD3.

T Cell Tube

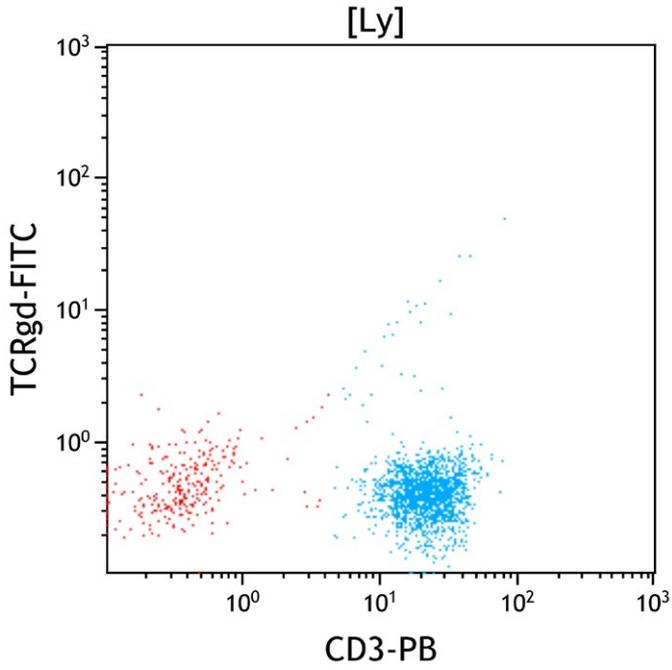


Figure 21. This CD3 vs TCR $\delta$  dot plot shows all cells in the lymphocyte gate (Ly). A small subset of T cells express TCR gamma/delta, which is co-expressed with CD3. The highly linear relationship between CD3 and TCR $\delta$  is due to their presence as a heterodimeric complex having a fixed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

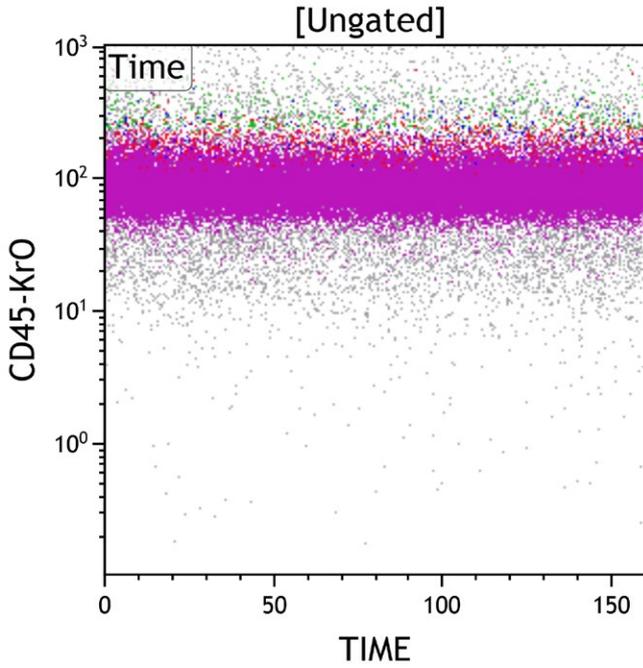


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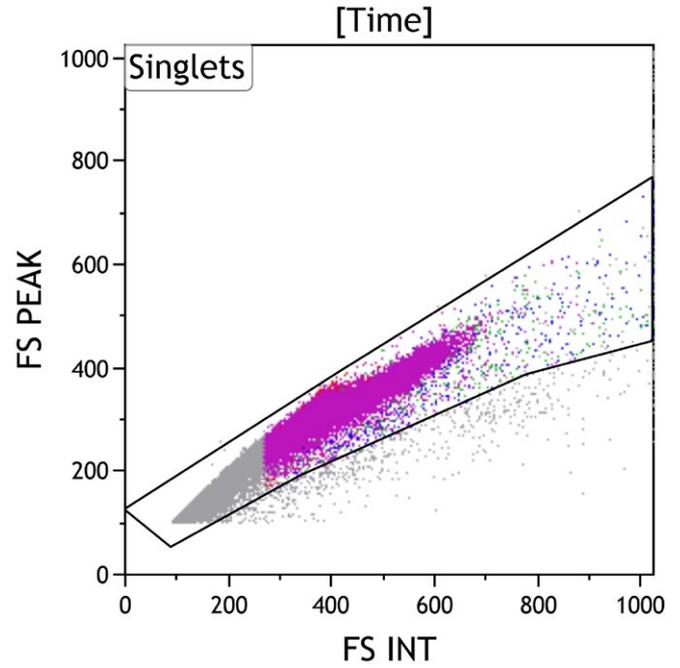


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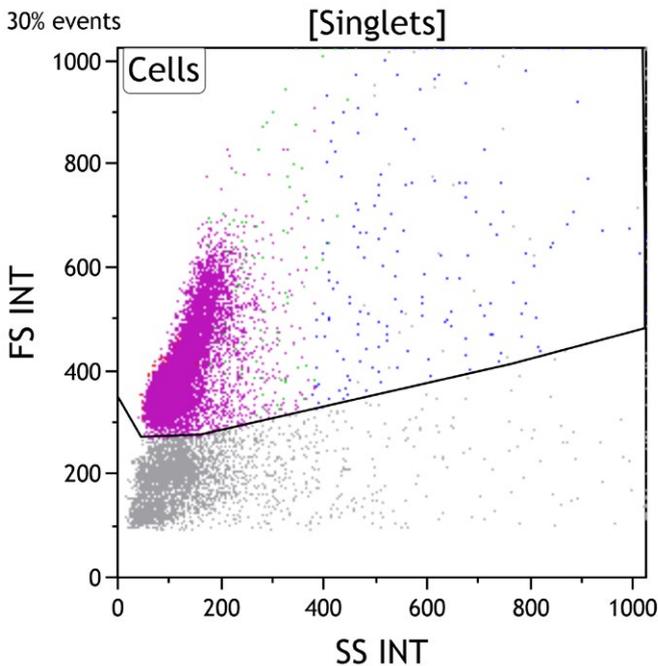


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter with increased side scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic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

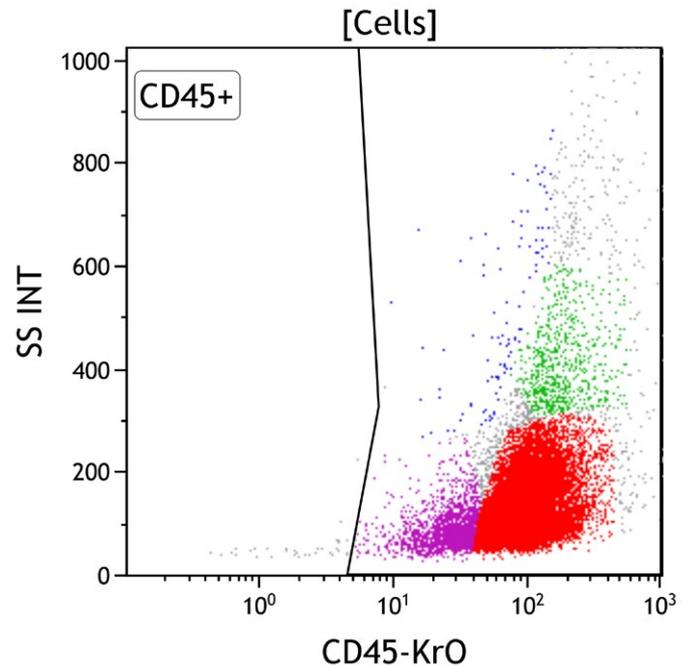


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

**M1 Cell Tube**

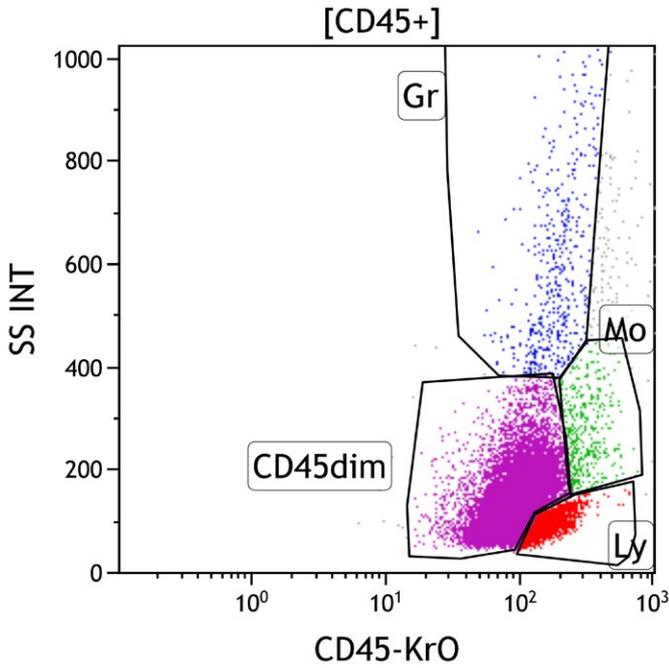


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

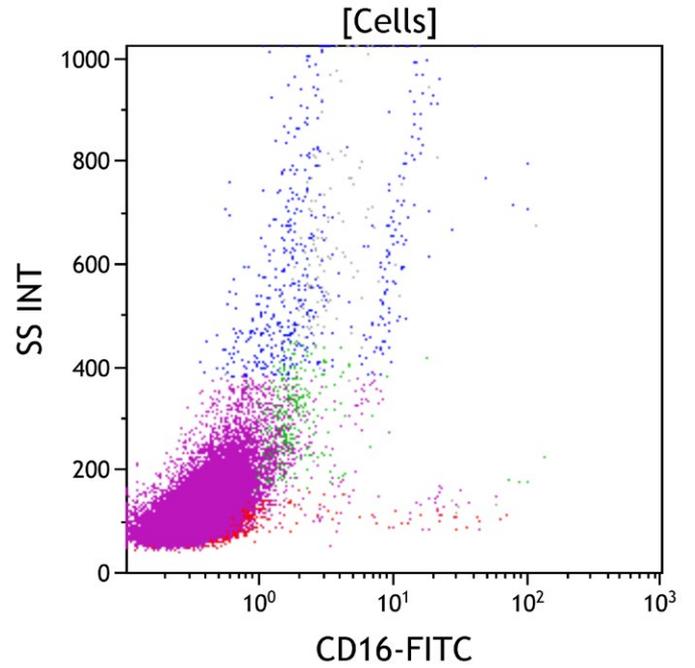


Figure 6: This CD16 vs Side Scatter dot plot shows all viable cells. CD16 is expressed at its highest level on mature granulocytes and at a variably lower level on metamyelocytes and bands (blue). Most NK cells express CD16 (red), as do a subset of activated monocytes (green). Progenitors (purple) do not express CD16.

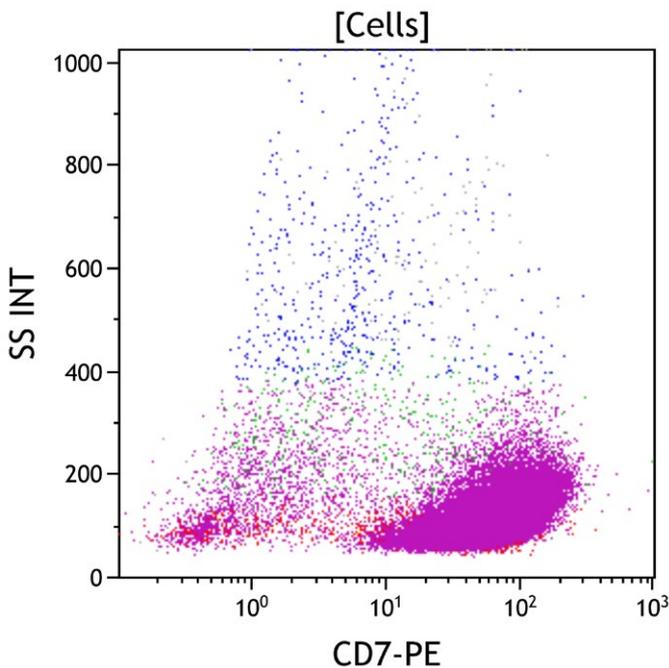


Figure 7: This CD7 vs Side Scatter dot plot shows all viable cells. CD7 is expressed on immature T cells at the earliest stage of T cell development and persists throughout T cell maturation to be variably expressed on mature T cells (red). It is also uniformly expressed on NK cells (red), and dimly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

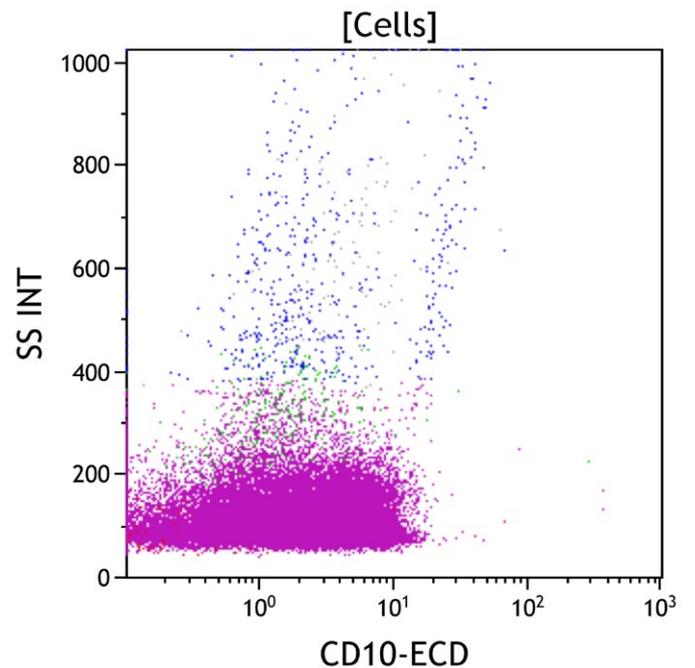


Figure 8: This CD10 vs Side Scatter dot plot shows all viable cells. Progenitors (purple) express variable CD10.

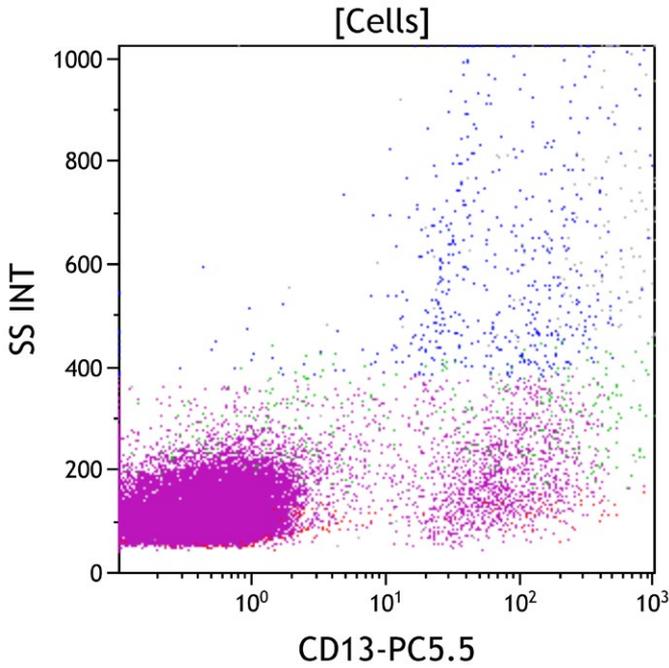


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes (green) and a lower level on immature monocytes with variable expression on myeloid progenitors. Progenitors (purple) do not express CD13.

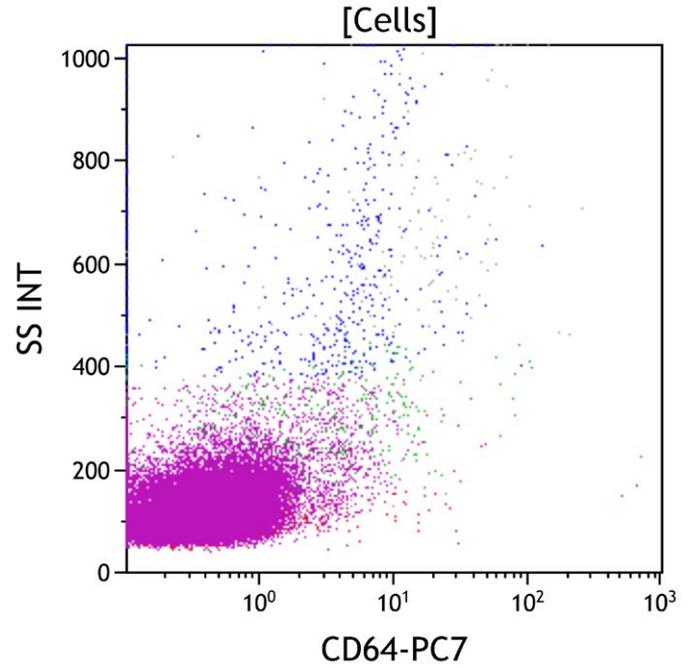


Figure 10: This CD64 vs Side Scatter dot plot shows all viable cells. CD64 is expressed at its highest level on mature and immature monocytes (green) and at a slightly lower level on promyelocytes and myelocytes (blue). CD64 is not well expressed on resting mature granulocytes (blue), but increases in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

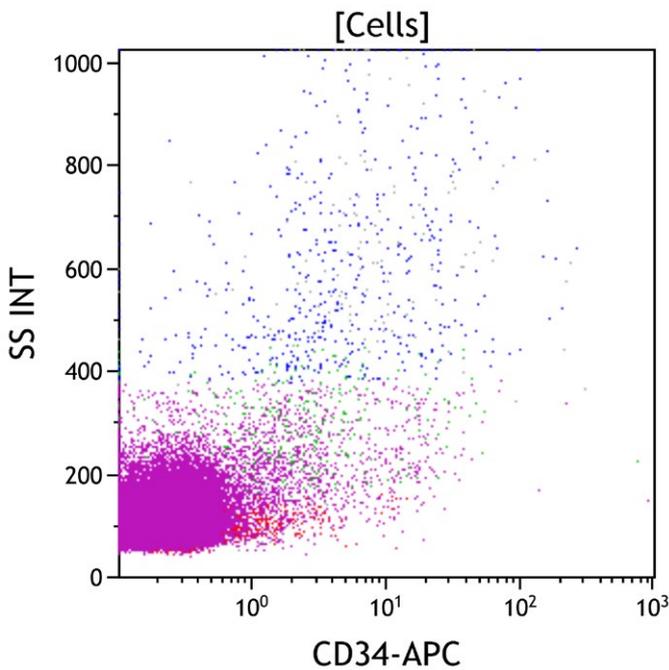


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is expressed on hematopoietic stem cells, early myeloid progenitors (myeloblasts), and immature B and T cells (lymphoblasts). CD34 positive progenitors typically have low to intermediate side scatter in the CD45 dim gate with immature B cell progenitors having lower side scatter than immature myeloid progenitors. Mature granulocytes, monocytes, and lymphocytes are negative for CD34. Progenitors (purple) do not express CD34.

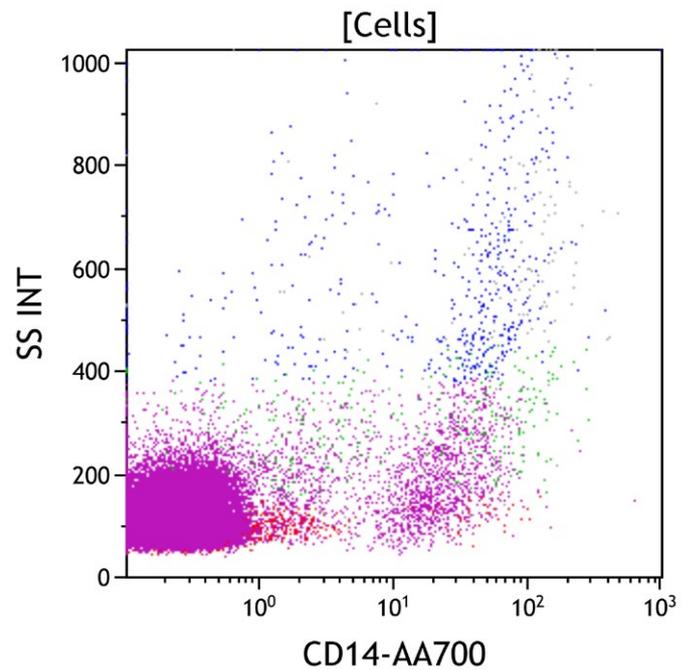


Figure 12: This CD14 vs Side Scatter dot plot shows all viable cells. CD14 is expressed at a high level on mature monocytes (green) and at a variably lower level on immature monocytes. CD14 is also expressed on mature granulocytes at a low level. Progenitors (purple) do not express CD14.

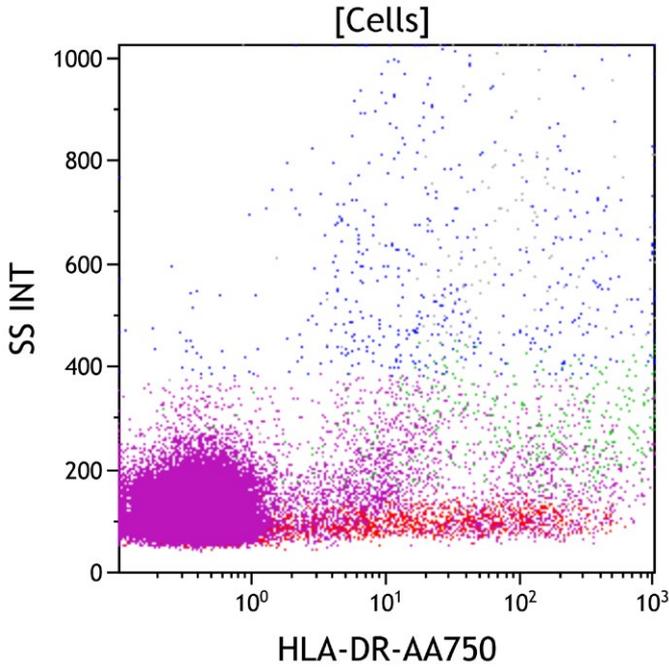


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes (green) and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature (red) and immature B cells, and activated T cells (red). Progenitors (purple) do not express HLA-DR.

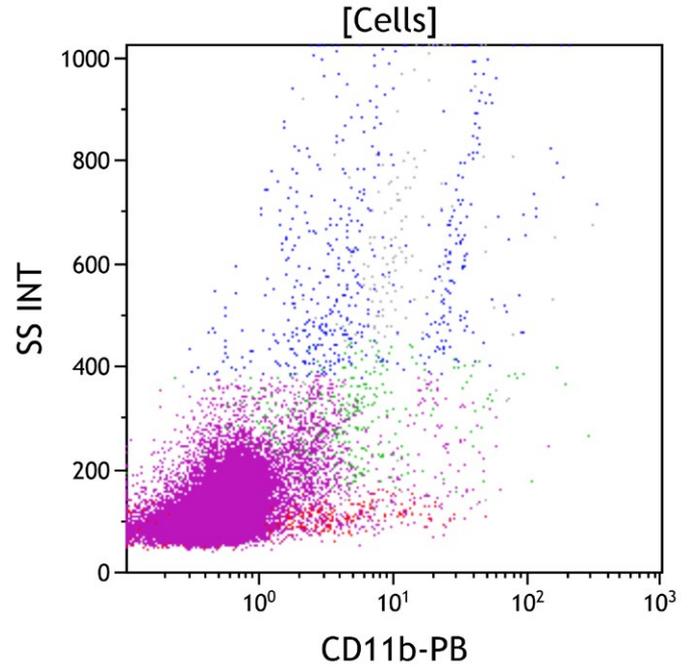


Figure 14: This CD11b vs Side Scatter dot plot shows all viable cells. CD11b is expressed on maturing granulocytes beginning at the late promyelocyte stage (blue), and on monocytes (green). CD11b is also expressed on NK cells (red) and basophils. Progenitors (purple) do not express CD11b.

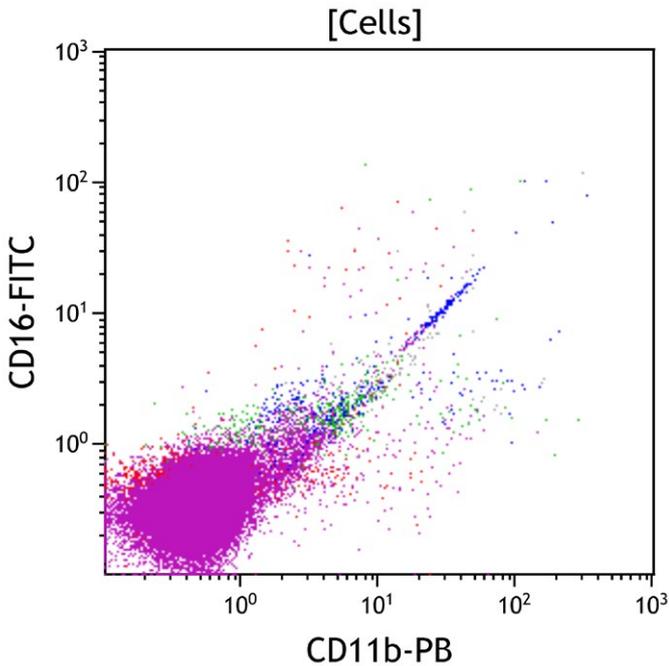


Figure 15. This CD11b vs CD16 dot plot shows all viable cells. CD11b is expressed on monocytes, immature and mature granulocytes and NK cells. CD16 is expressed on immature and mature granulocytes and a subset of NK cells. During granulocytic maturation, most promyelocytes lack CD11b and CD16 and acquire CD11b as they mature toward myelocytes. CD16 is then acquired at a low level on metamyelocytes and progressively increases with maturation to mature granulocytes, where it is expressed at its highest level. Progenitors (purple) do not express CD11b or CD16.

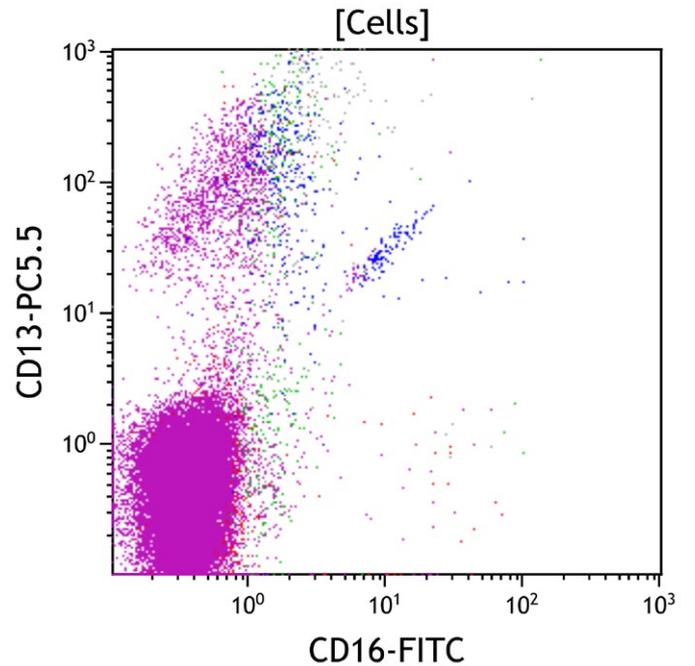


Figure 16. This CD16 vs CD13 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD16 is expressed on maturing granulocytes and a subset of NK cells. During granulocytic maturation, CD13 is expressed variably by promyelocytes without CD16 and lose CD13 as they mature to myelocytes. Myelocytes then simultaneously acquire CD13 and CD16 as they mature from metamyelocytes having low CD16 to mature granulocytes having high expression of both CD13 and CD16. Progenitors (purple) do not express CD16 or CD13.

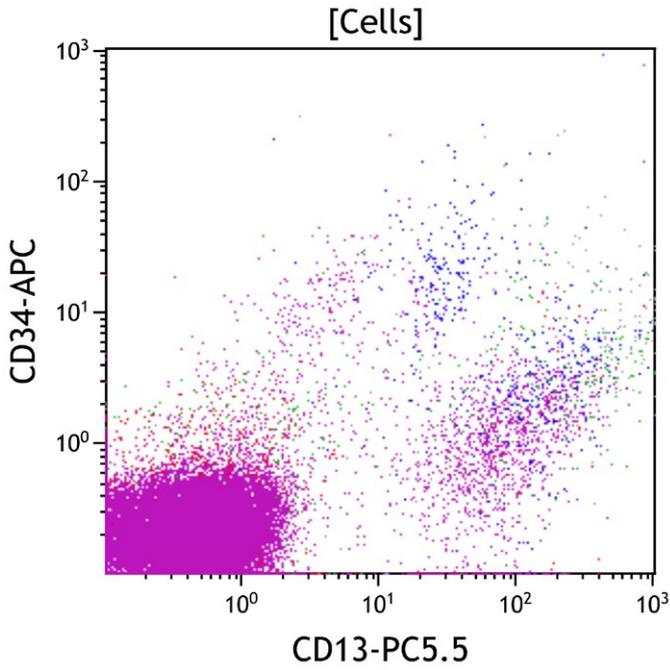


Figure 17. This CD13 vs CD34 dot plot shows all viable cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. CD34 is expressed on early hematopoietic progenitors. CD13 is not well expressed on CD34 positive B cell progenitors or mature lymphoid cells.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

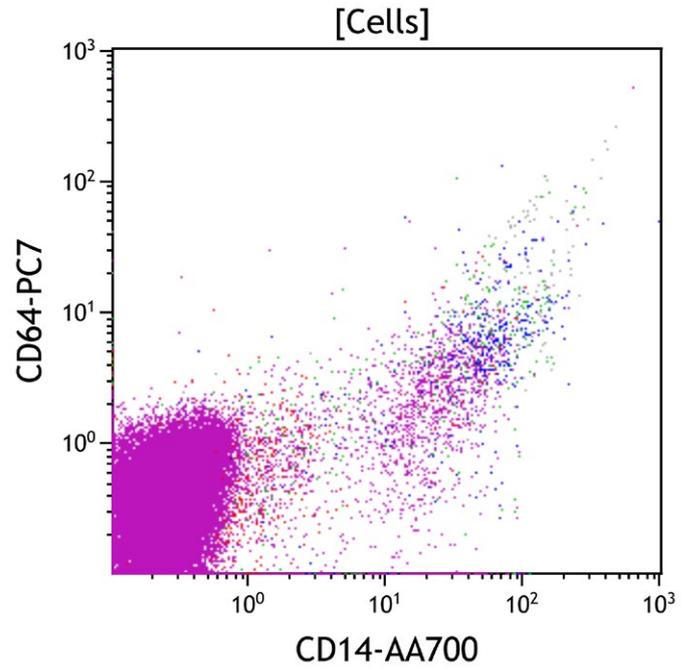


Figure 18. This CD14 vs CD64 dot plot shows all viable cells. CD64 is expressed at a high level on monocytes and at a lower level on maturing granulocytes. CD14 is expressed at a high level on mature monocytes and a lower level on mature granulocytes. Immature monocytes show high expression of CD64 without CD14 and progressively acquire CD14 during maturation to mature monocytes while retaining high-level CD64. Immature granulocytes express moderate CD64 without CD14 and acquire CD14 and lose CD64 at transition to mature granulocytes. Progenitors (purple) do not express CD14 or CD64.

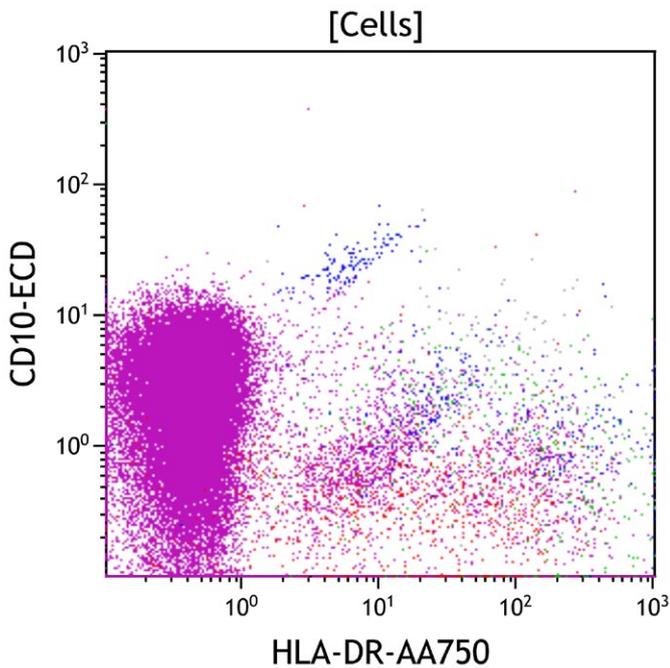


Figure 19. This HLA-DR vs CD10 dot plot shows all viable cells. HLA-DR is expressed on monocytes, B cells, plasmacytoid dendritic cells, and CD34 positive progenitors. CD10 is expressed by mature granulocytes (blue) and immature B cells. Immature B cells express both CD10 and HLA-DR. The abnormal progenitors (purple) express variable CD10 without HLA-DR.

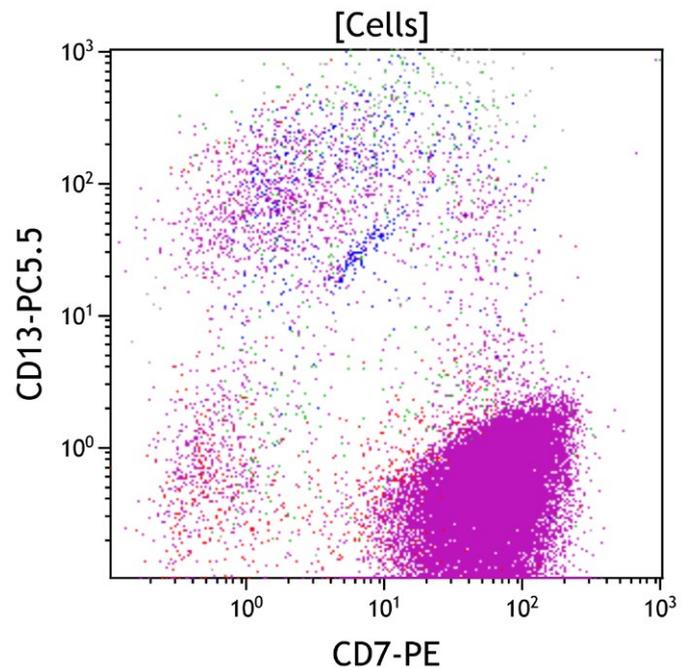


Figure 20. This CD7 vs CD13 dot plot shows all viable cells. CD7 is expressed on T cells and NK cells. CD13 is expressed on maturing granulocytes, monocytes, basophils, and CD34 positive progenitors. Coexpression of CD13 and CD7 is generally not seen. The abnormal progenitors (purple) express bright CD7 without CD13.

M2 Cell Tube

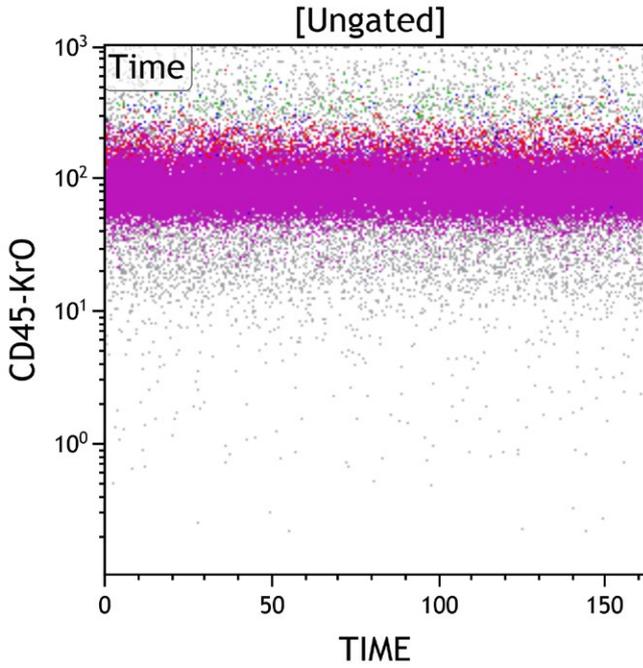


Figure 1: This Time vs CD45 dot plot is ungated and shows all events collected sequentially. This plot is intended to evaluate for fluidic perturbation during sample acquisition. Stable acquisition is represented by a uniform pattern of events over time. Events that deviate from the stable pattern can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

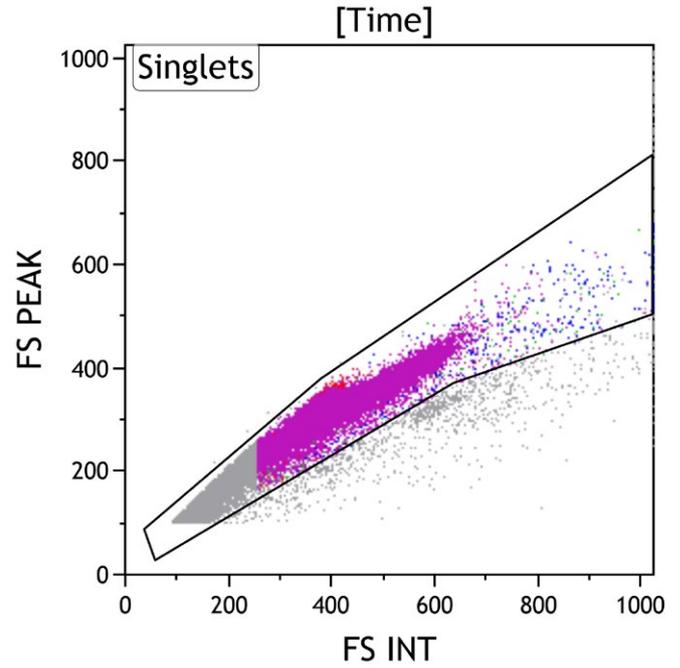


Figure 2: This FS INT vs FS PEAK dot plot shows events in the Time gate. This plot is intended to exclude cell doublets or aggregates. Singlet events show a linear relationship for INT vs PEAK and are included in the Singlets gate, while doublets lie outside the linear relationship.

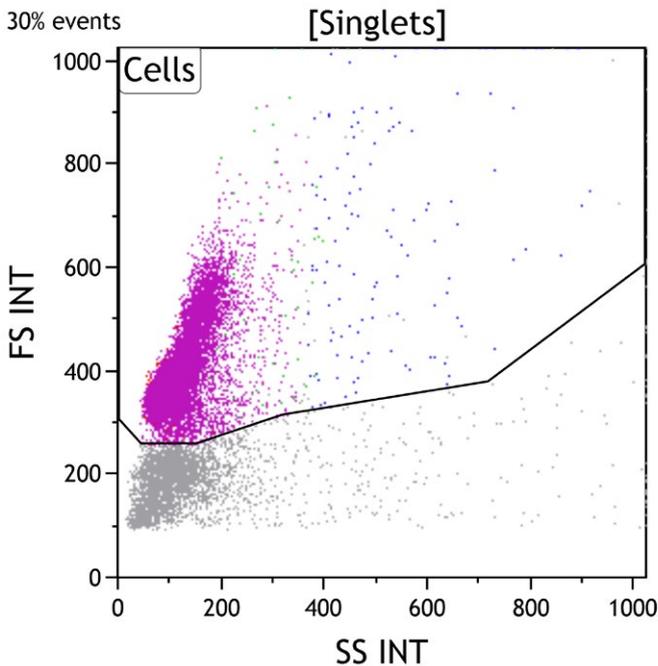


Figure 3: This Side Scatter vs Forward Scatter dot plot shows events in the Singlets gate. This plot is intended to exclude cell debris, which usually has decreased forward scatter. Early apoptotic cells also have mildly increased side scatter while late apoptotic and necrotic cells have variably decreased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

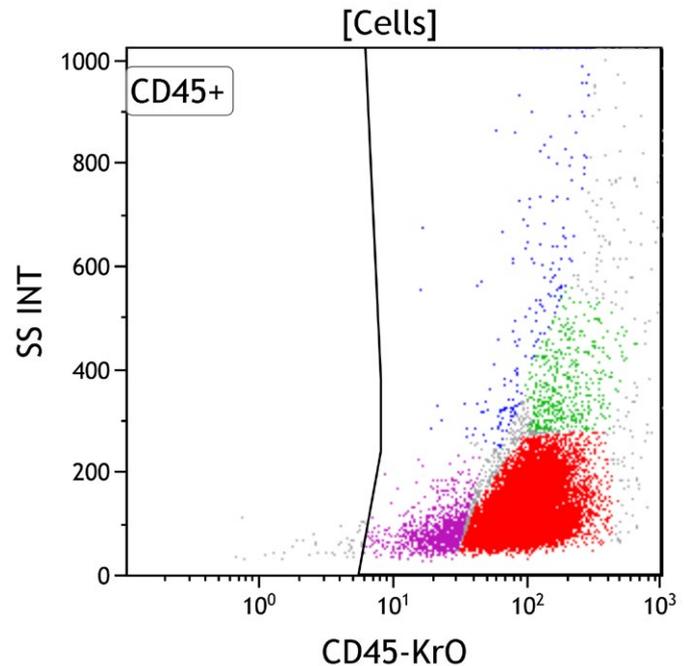


Figure 4: This CD45 vs Side Scatter dot plot shows events in the Cells gate. This plot is intended to highlight various subsets of white blood cells, which are gated as CD45 positive. The CD45 negative population usually includes red blood cells, platelet aggregates, tissue debris or non-hematopoietic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

M2 Cell Tube

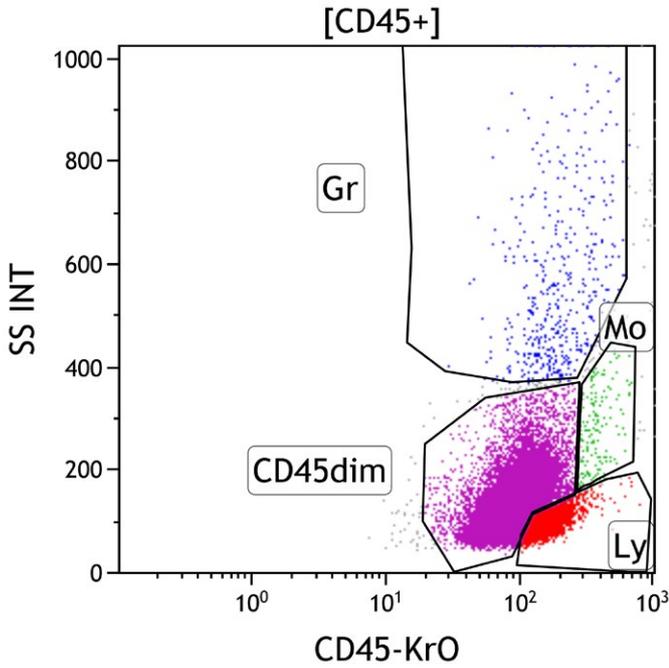


Figure 5: This CD45 vs Side Scatter dot plot shows events in the CD45+ gate. This dot plot permits distinction of several white cell populations typically found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate Ly, red), monocytes (Gate Mo, green), and granulocytes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

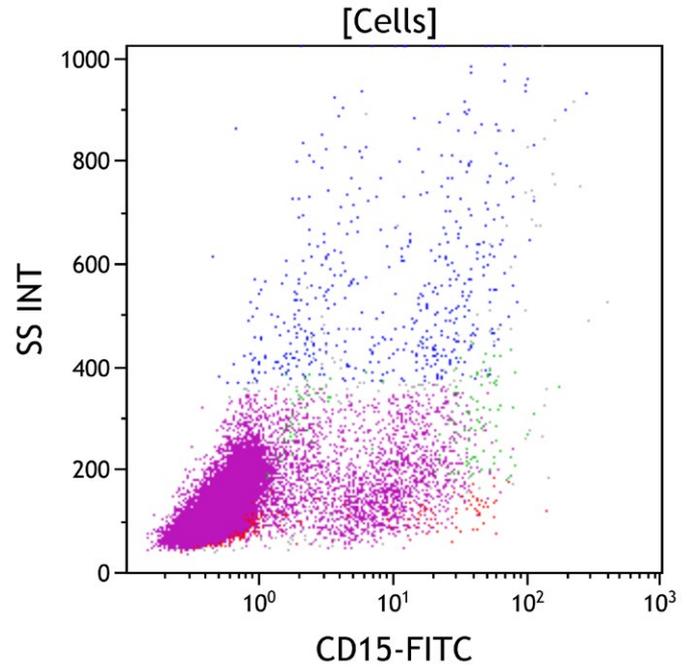


Figure 6: This CD15 vs Side Scatter dot plot shows all viable cells. CD15 is expressed on immature and mature granulocytes (blue) from the time of early commitment to myelomonocytic maturation. CD15 is also expressed at a lower level on monocytes. Progenitors (purple) do not express CD15.

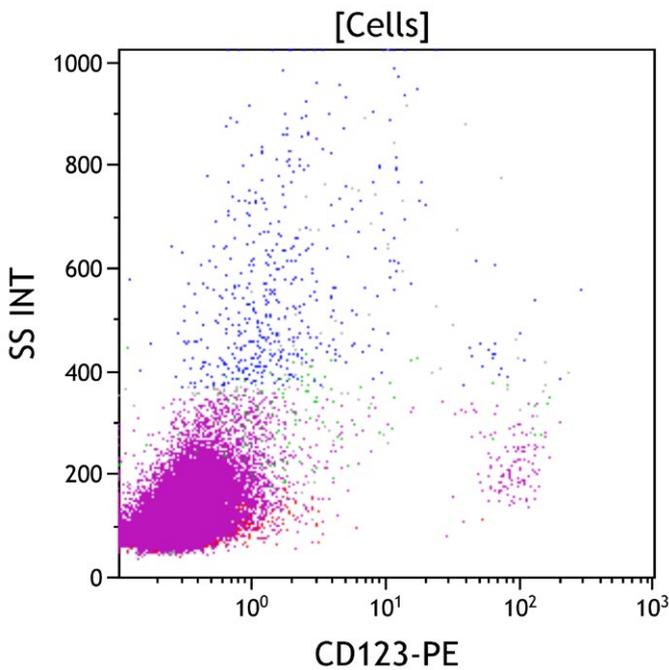


Figure 7: This CD123 vs Side Scatter dot plot shows all viable cells. CD123 is expressed at a high level on basophils and plasmacytoid dendritic cells and at a lower level on CD34 positive myeloid progenitors and monocytes. Progenitors (purple) do not express CD123.

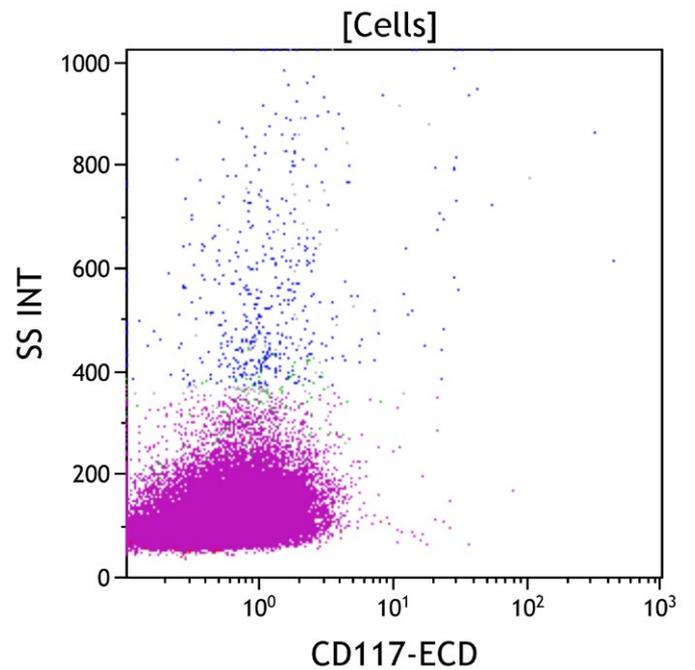


Figure 8: This CD117 vs Side Scatter dot plot shows all viable cells. CD117 is expressed variably on CD34 positive myeloid progenitors, early promyelocytes, and early erythroid precursors, and at a high level on mast cells (blue right). CD117 is also expressed on subsets of reactive NK cells and neoplastic plasma cells. Progenitors (purple) do not express CD117.

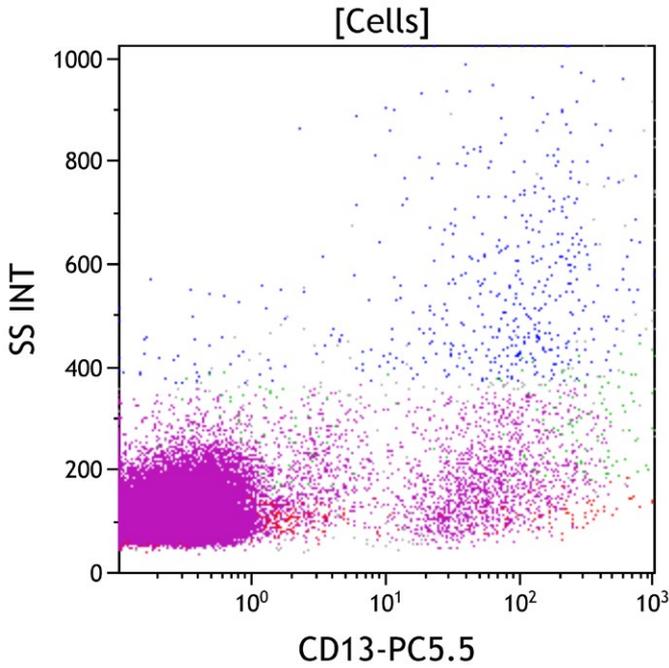


Figure 9: This CD13 vs Side Scatter dot plot shows all viable cells. CD13 is expressed on maturing granulocytes with variable intensity dependent on maturational stage (blue). It is expressed at a variably high level on promyelocytes and a low level on myelocytes with increasing expression as they mature toward granulocytes, where it is expressed at a high level. CD13 is expressed at a high level on mature monocytes and a lower level on immature monocytes with variable expression on myeloid progenitors. Progenitors (purple) do not express CD13.

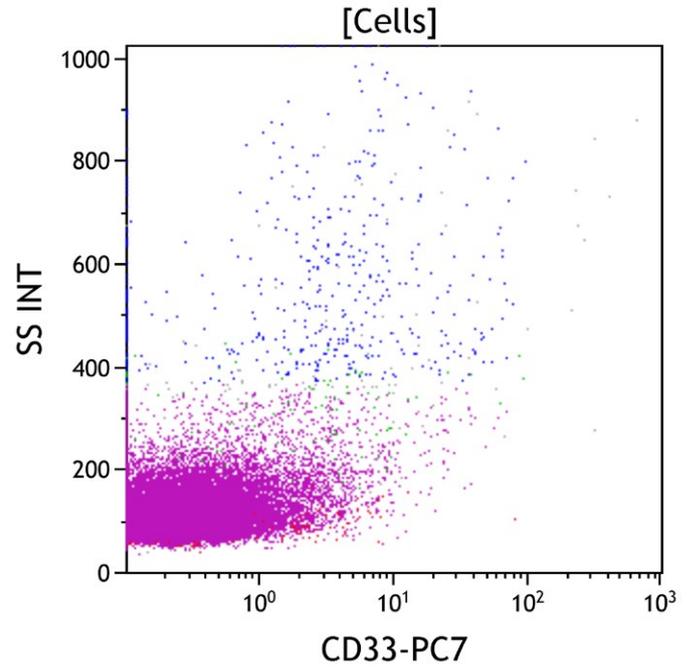


Figure 10: This CD33 vs Side Scatter dot plot shows all viable cells. CD33 is expressed at its highest level on immature and mature monocytes, at a slightly lower level on immature granulocytes, and at the lowest level on mature granulocytes (blue). CD33 is also expressed on basophils, a subset of NK cells, and a subset of CD34 positive myeloid progenitors. Progenitors (purple) do not express CD33.

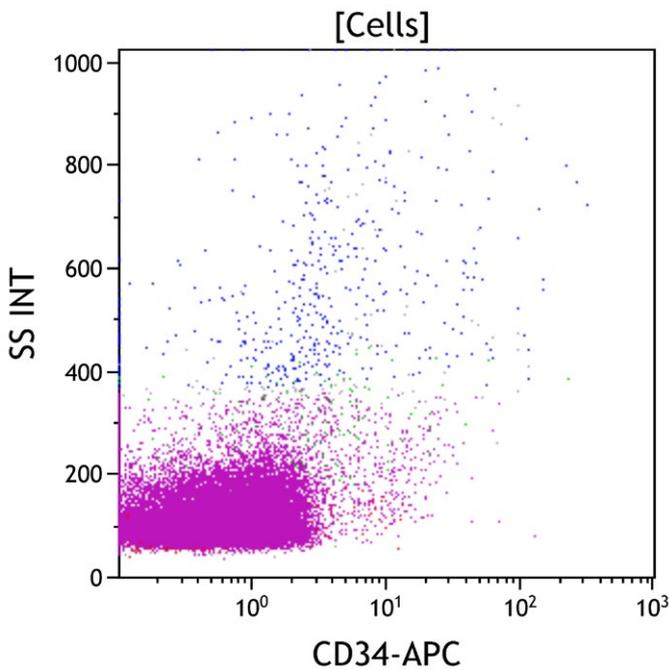


Figure 11: This CD34 vs Side Scatter dot plot shows all viable cells. CD34 is a marker of early hematopoietic progenitors. It is typically expressed on myeloid blasts, immature B and T cells (lymphoblasts). CD34-positive blasts typically have low to intermediate side scatter in the CD45 dim gate. Progenitors (purple) do not express CD34.

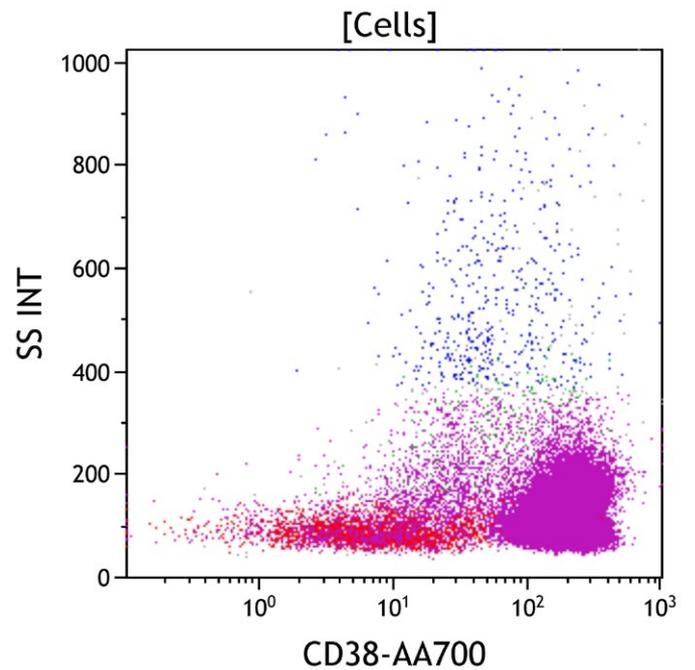


Figure 12: This CD38 vs Side Scatter dot plot shows all viable cells. CD38 is an activation marker. It is expressed at the highest level on plasma cells, at a moderate level on immature myeloid and lymphoid progenitors, at a low level on monocytes, and at a variably low level on activated mature lymphocytes.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

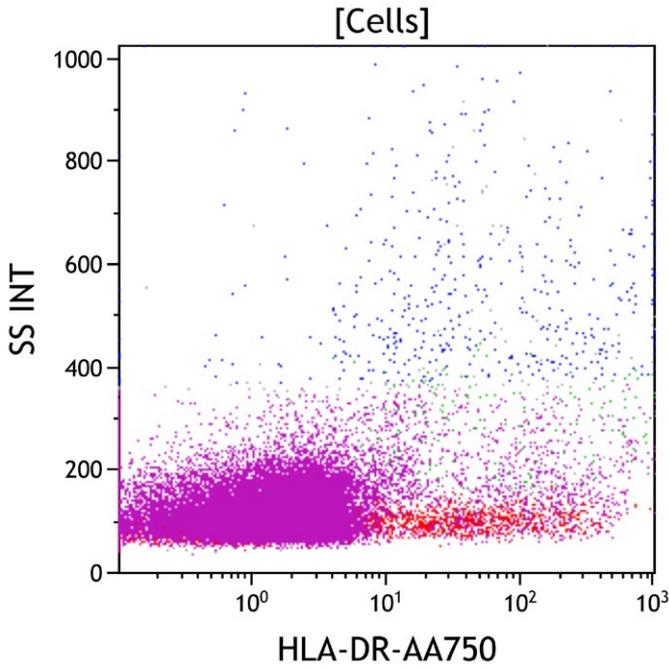


Figure 13: This HLA-DR vs Side Scatter dot plot shows all viable cells. HLA-DR is expressed on antigen presenting cells including monocytes and plasmacytoid dendritic cells. It is also expressed on CD34 positive progenitors, mature and immature B cells, and activated T cells. Progenitors (purple) do not express HLA-DR.

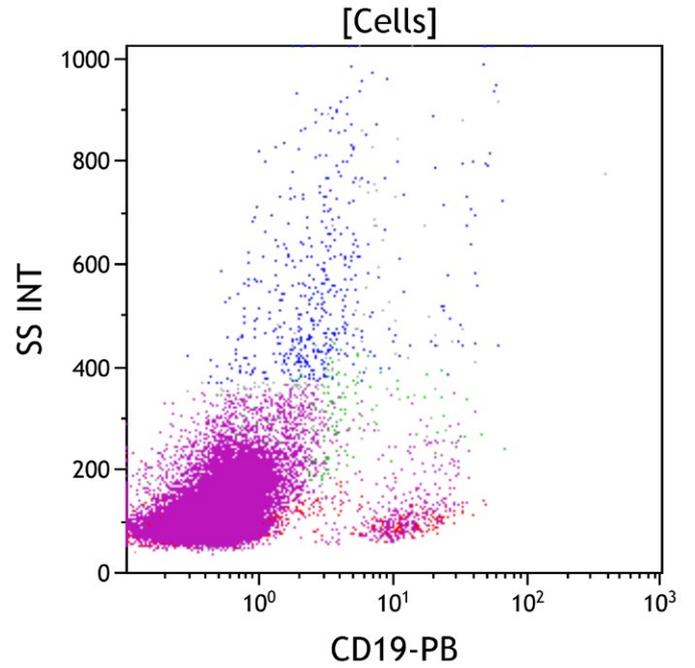


Figure 14: This CD19 vs Side Scatter dot plot shows all viable cells. CD19 is expressed on mature and immature B cells, as well as most plasma cells. These cells typically have low to moderate side scatter. Progenitors (purple) do not express CD19.

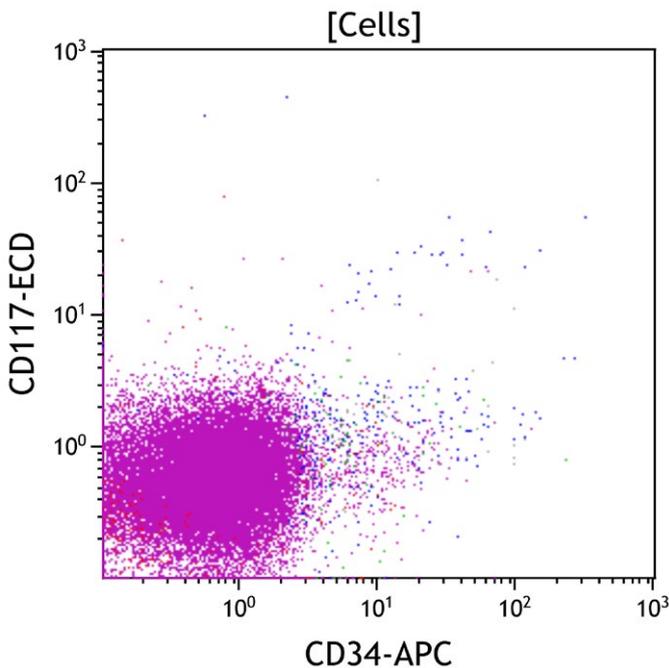


Figure 15: This CD34 vs CD117 dot plot shows all viable cells. CD34 is expressed on myeloid blasts and early immature B cell precursors. CD117 is expressed on myeloid blasts, promyelocytes, and early erythroid precursors, but negative on early B cell precursors. The progenitors (purple) do not express CD34 or CD117.

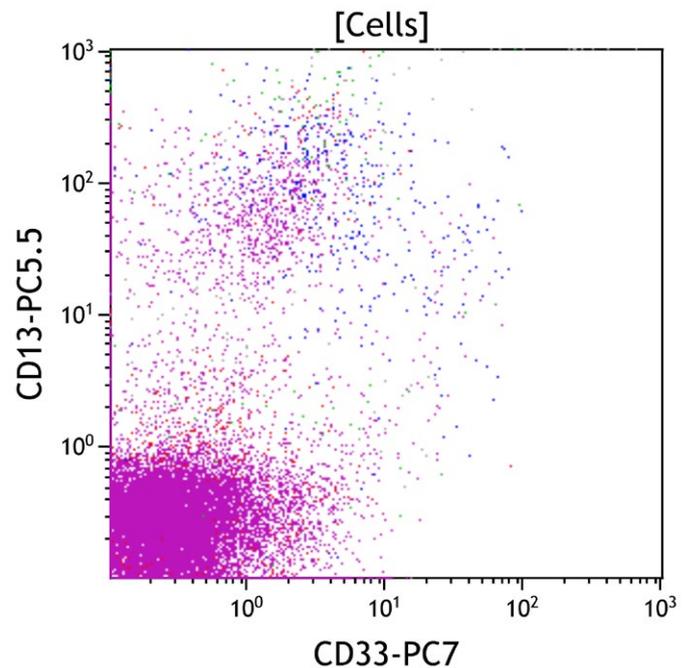


Figure 16: This CD33 vs CD13 dot plot shows all viable cells. CD33 and CD13 are expressed on monocytes, maturing granulocytes, basophils, and CD34 positive progenitors. Monocytes express CD33 at a uniformly high level with more variable CD13. Immature granulocytes express higher CD33 and lower CD13 than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

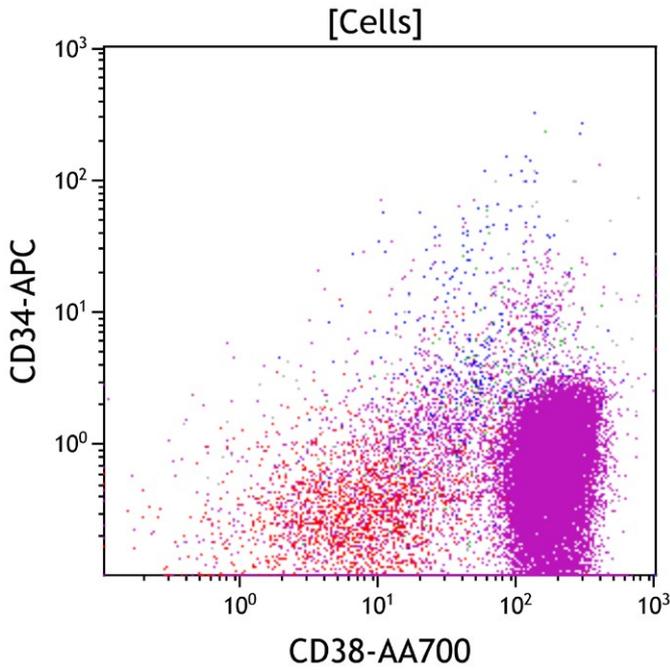


Figure 17. This CD38 vs CD34 dot plot shows all viable cells. CD38 is uniformly expressed on lineage committed early progenitors having variable CD34. Hematopoietic stem cells have the highest level of CD34 with variably decreased CD38. Progenitors (purple) express bright CD38 without CD34.

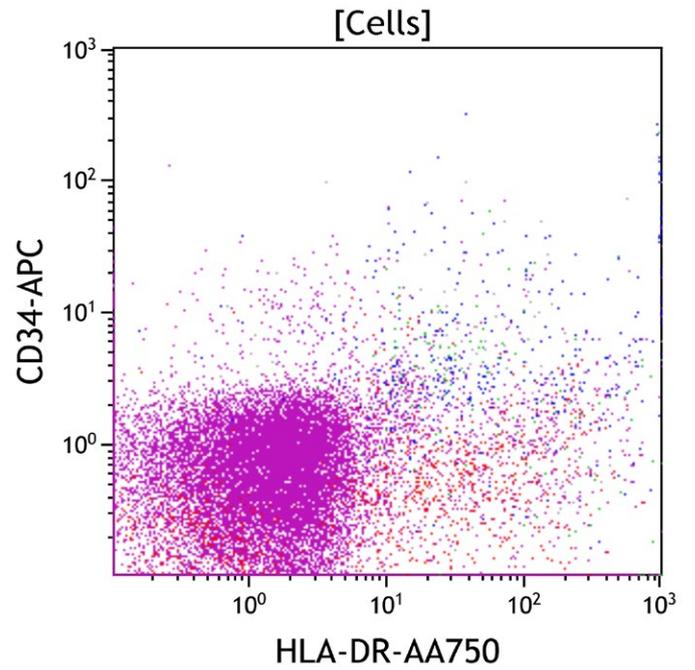


Figure 18. This HLA-DR vs CD34 plot shows all viable cells. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. CD34 is expressed on early progenitors. Early progenitors variably express both CD34 and HLA-DR with the highest level of HLA-DR seen on early monocytes. Progenitors (purple) do not express HLA-DR or CD34.

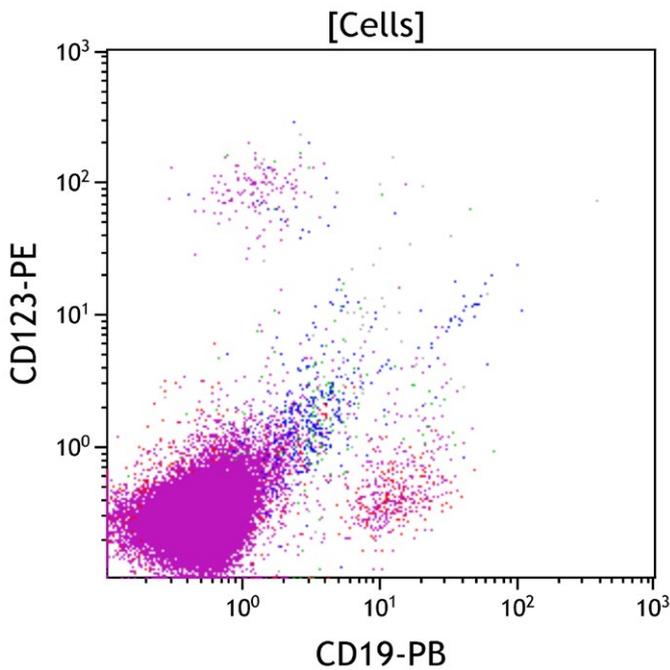


Figure 19. This CD19 vs CD123 dot plot shows all viable cells. CD19 is expressed on B cells. CD123 is expressed on basophils, plasmacytoid dendritic cells, monocytes (green), and CD34 positive progenitors. CD19 positive B cells normally do not express significant CD123. CD123 positive basophils and plasmacytoid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

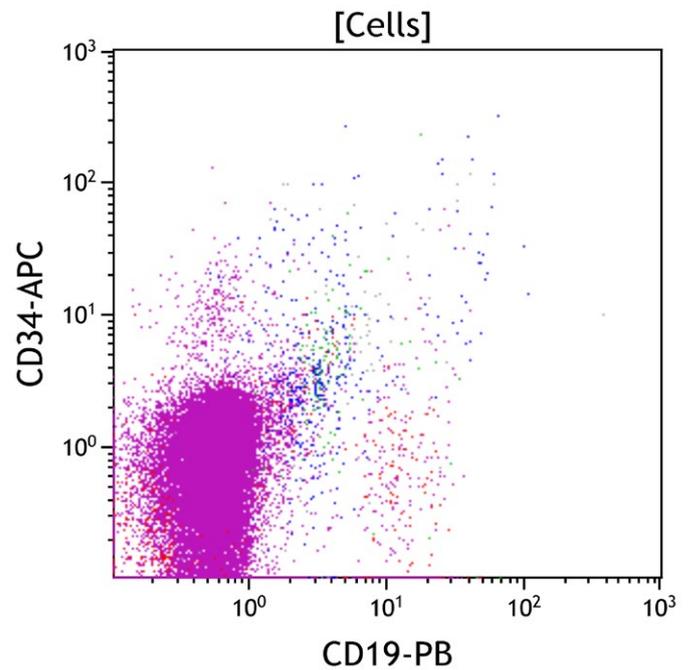


Figure 20. This CD19 vs CD34 dot plot shows all viable cells. CD19 is expressed on B cells. CD34 is expressed on early progenitors. Early immature B cells are positive for both CD19 and CD34, while later stage B cells do not express CD34. CD34 positive myeloid progenitors do not express CD19. Progenitors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

M2 Cell Tube

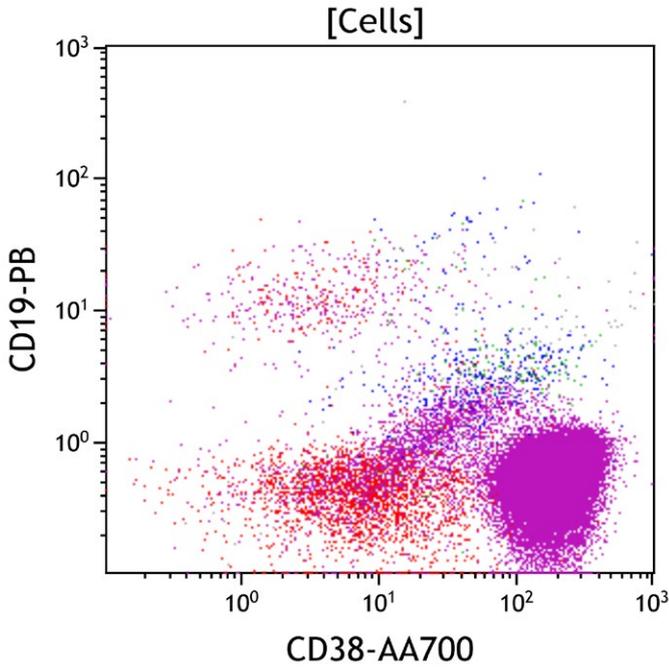


Figure 21. This CD38 vs CD19 dot plot shows all viable cells. CD38 is uniformly expressed on plasma cells and lineage committed early progenitors. Most of the progenitors in this sample are B cell progenitors expressing CD19 and intermediate CD38. Mature CD19 positive B cells show lower expression of CD38 (red left). The apparent CD19 positivity on maturing granulocytes (blue) is due to autofluorescence, largely from eosinophils. Progenitors (purple) express bright CD38 without CD19.

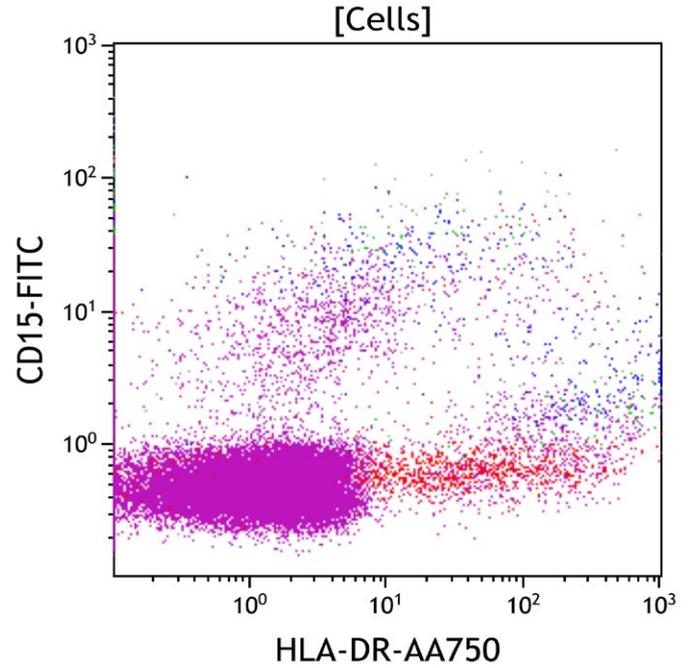


Figure 22. This HLA-DR vs CD15 dot plot shows all viable cells. CD15 is expressed on maturing granulocytes and monocytes. HLA-DR is expressed on B cells, monocytes, plasmacytoid dendritic cells, and CD34 positive progenitors. Maturing granulocytes do not express HLA-DR, except the earliest forms where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

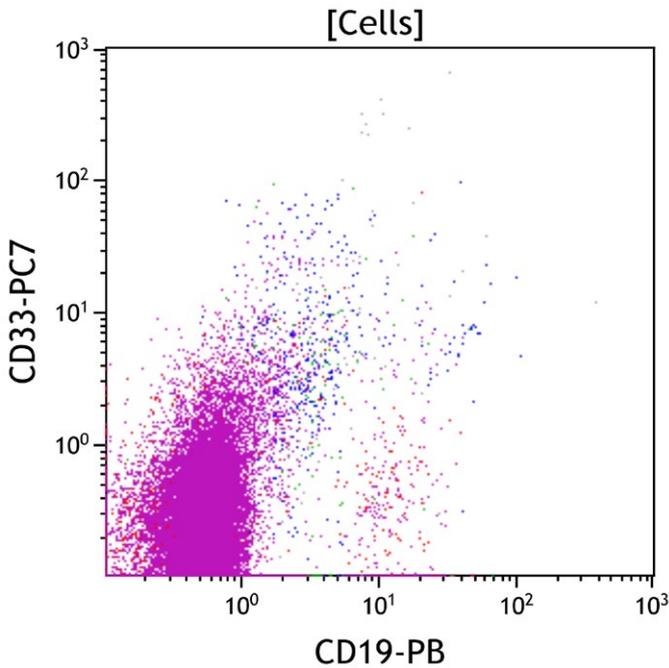


Figure 23. This CD19 vs CD33 dot plot shows all viable cells. CD19 is expressed on B cells (red and purple). CD33 is expressed by monocytes (green) and maturing granulocytes (blue). CD19 positive B cells normally do not express CD33. The apparent CD19 positivity on maturing granulocytes (blue) is due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

## Results of Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with expression of intermediate CD2, variable CD4, intermediate CD5, bright CD7, dim CD10, bright CD38, intermediate CD45 and are negative for surface CD3, CD8, CD19, CD34, CD56, CD117 and other B cell or myeloid antigens. Compared with normal immature T cells, the expression of CD4 and CD10 without CD8 and the bright CD38 is abnormal.

The immunophenotype of the abnormal population is in keeping with abnormal immature T cells, i.e. T-lymphoblasts. The findings are consistent with a diagnosis of T-lymphoblastic leukemia/lymphoma. Additional testing for cytoplasmic CD3 and TdT could be performed to confirm T cell lineage and immaturity, respectively.