**Introduction**

The development of B lymphocytes in the bone marrow is well-studied due to the importance of understanding the dysregulation that occurs in leukemias and other diseases of the hematopoietic system. This development occurs in a well-defined order that can be defined by the sequential up- and down-regulation of markers that can be subdivided into distinct stages. These stages remain fairly consistent throughout normal ontogeny, but vary with the percentage of cells in a particular stage depending on exposure to antigens and individual genetics.

Historically, in the scientific literature there have been discrepancies and inconsistencies in the timing of the up-regulation or down-regulation of some markers in relationship to other markers. It is important to have a clear picture of these relationships in normal bone marrow ontogeny. Here we present a normal progenitor model that was constructed using Probability State Modeling and analysis of data from sixteen normal samples.

Probability State Modeling, or PSM, employs algorithms that allow one to model any number of flow cytometry data files and calculate the average expression of markers as well as the correlations between samples and directionality of the markers. With PSM we can perform an unattended analysis of each data file. This reduces the subjectivity that arises during traditional gating analysis.

Using typical B-Cell lineage markers, CD45, CD19, CD10, CD34, CD38, and CD20, we were able to clearly define four distinct stages of development of B-cells and generate correlation statistics to support the transitions between these stages. We were also able to determine the expression profiles of several other markers, CD12, CD22, CD44, CD9, that contribute to the development of B-Cells in the marrow.

Furthermore, using the same markers and a subset of the data files, we were able to statistically define three very early preceding stages of lymphocyte development and the coordinated expression of markers defining these stages.

![Image of flow cytometry data analysis](image)

**Materials and Methods**

Data analysis was performed using GemStone™ Probability State Modeling and Graphics Software program version 1.0.115. Flow Cytometry listmode data files for the study were selected from our library of data files based on criteria summarized in Table 1 below. Briefly, files must include the markers CD19, CD45, CD10, CD34, and CD38 for staging, have an adequate number of events, and unequal separation between positive and negative peaks.

A normal B-cell developmental model was constructed using Gemstone and Probability State Modeling by selecting for the CD10 positive to dim events and low SSC. Stratiﬁcation for staging was done with CD10, CD45, CD38 and CD44. Each file was analyzed unattended with statistics collected during the analysis. Correlation coefﬁcients were calculated to measure the modulation of each marker to determine coordinated changes in expression patterns. Based on these statistics an averaged model was created.

A normal progenitor model was constructed in a similar manner; however, progenitors were selected as CD34 positive. CD38 low to intermediate intensity and SSC low to intermediate intensity. Stratiﬁcation was determined with CD38, CD10 and CD19 to identify the stages of very early B-cell development. Fewer files were used in this section due to inadequate numbers of events in the omitted files.

**Results**

![Image of flow cytometry data analysis](image)

**Progenitor Stage Analysis**

Representative summary plot of an unattended analysis for one study file. This PSM overlay plot summarizes the modulation of CD34, CD45, CD20, CD10 and CD38 during B-ontogeny. Modelling reduces the listmode data to a set of critical control points that can be used to quantify the relative order of marker changes. The listmode events have been selected for CD10 dim to positive and SSC lymphocytes (see Materials and Methods for details). When CD34 down-regulates, usually CD45-up regulates slightly (see open triangles for control points a and b). When CD45 up-regulates for the second time, CD20-up regulates (control points c and d). Finally, when CD10 down-regulates, CD38 also down-regulates (control points e and f). These locations are quantified by the PSM model as cumulative percentages and summarized for all suitable files in Table 3.

**Figure 2. B-Cell Stage Analysis**

Representative summary plot of an unattended analysis for one study file. This PSM overlay plot summarizes the modulation of CD34, CD45, CD20, CD10 and CD38 during B-ontogeny. Modelling reduces the listmode data to a set of critical control points that can be used to quantify the relative order of marker changes. The listmode events have been selected for CD10 dim to positive and SSC lymphocytes (see Materials and Methods for details). When CD34 down-regulates, usually CD45-up regulates slightly (see open triangles for control points a and b). When CD45 up-regulates for the second time, CD20-up regulates (control points c and d). Finally, when CD10 down-regulates, CD38 also down-regulates (control points e and f). These locations are quantified by the PSM model as cumulative percentages and summarized for all suitable files in Table 3.

**Figure 3. Normal B-Cell Model**

Based on the B-Cell stages results, a “normal” B-cell model was constructed that constrained appropriate marker modulations to the two critical control points shown in Figure 2 and Table 2 (a, c, and e). Markers that were widely represented in this study (CD10, CD34, CD22 and CD38) were placed on the nearest “normal” stage boundary. The PSM overlay plot shown above is the average of all files in the unattended analysis. The end of the B1 stage is defined by the down-regulation of CD34 along with TdT, while CD45, CD31, and CD22-up regulate slightly. Not shown here is a slight down-regulation of CD38 at the end of B3 at the end of B1. The up-regulation of CD38 and CD44 up-regulate. The transition of B3 to B4 is marked by the down-regulation of CD10, CD38 and CD41, and the up-regulation of CD20 and CD44.

**Figure 4. Panel A:** A typical expression pattern for CD38. CD38 usually up-regulates slightly in B1 and is very heterogeneous upon down-regulation.

**Panel B:** When CD20 up-regulates at the end of B1 it becomes very heterogeneous, acting much like an activation marker. Upon entering the B4 stage, it slightly down-regulates.

**Panel C:** CD9 staining is very heterogeneous in stages B1 and B4.

**Table 1. Summary of all files used in study.** To be included in this study files had to at least have staining for CD19, CD45, CD10, CD34, CD38 and CD20. The units for the locations are cumulative percentages. The recorded results for all 15 samples and all markers are represented for each recorded stage for both CD34 (a and b) and the up-regulation of CD38 (c and d) are placed on the nearest “normal” stage boundary. The down-regulation of CD34 (see e and f) and the up-regulation of CD10 (see g) are defined as the end of the transition of B3 to B4 (purple, not shown).

**Table 2. B-Cell Stage Results.** All files were analyzed unattended with a B-Cell model to obtain critical stage locations for markers CD34, CD45, CD10, CD38, and CD20. The units for the locations are cumulative percentages. The recorded results for all 15 samples and all markers are represented for each recorded stage for both CD34 (a and b) and the up-regulation of CD38 (c and d) are placed on the nearest “normal” stage boundary. The down-regulation of CD34 (see e and f) and the up-regulation of CD10 (see g) are defined as the end of the transition of B3 to B4 (purple, not shown).

**Table 3. Progenitor Stage Results.** Files with enough events to analyze were modeled to obtain critical stage locations for markers CD34, CD19, CD10 and CD38. The units for the locations are cumulative percentages. The recorded results for all 15 samples and all markers are represented for each recorded stage for both CD34 (a and b) and the up-regulation of CD38 (c and d) are placed on the nearest “normal” stage boundary. The down-regulation of CD34 (see e and f) and the up-regulation of CD10 (see g) are defined as the end of the transition of B3 to B4 (purple, not shown).

**References**


**Conclusion**

Using Probability State Modeling we have mathematically determined the normal developmental stages of B-Cells in human bone marrow as early progenitor cells as well as when they are committed to the B-Cell lineage. This study demonstrates the utility of PSM to statistically analyze any number of high-dimensional cytometry data files and present the results in a clear and concise manner. With an averaged model we are able to generate correlation coefficients that provide confirmation to the coordinated expression of markers.

This approach is a valuable step in evaluating the dysregulation of maturation patterns that occur in disease states, marrow regeneration, and in detection of minimal residual disease. With this information in hand we will be better able to model and enumerate disrupted changes in antigen expression in hopes to define more clearly diagnostic and prognostic outcomes in the near future.

This work has been submitted to Cytoometry for publication in an upcoming special issue on computational data analysis.