A 19-Color Multiparameter White Blood Cell Panel Designed for the Immunophenotyping of Normal and Malignant Leukocytes by Flow Cytometry

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Cell Analysis

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Abstract

Flow cytometry is an important tool for the diagnosis and classification of leukemia. While many leukocyte markers have been identified and are used to determine the exact subtypes of leukemia a patient may have, high-dimensional multicolor analysis has been limited by instrument capabilities. This has often resulted in splitting a valuable limited sample into multiple independent tests to fully define the patient's phenotype.

A 19-antibody white blood cell panel was developed using the ZE5™ Cell Analyzer from Bio-Rad Laboratories. With up to five lasers and 30 analysis parameters (of which 27 channels are for fluorescence detection), all the markers necessary to classify chronic/acute lymphoproliferative leukemia disorders can be combined into a single tube. Using this panel, a patient with chronic B-cell lymphocytic leukemia was successfully identified from a normal PBMC sample.

Introduction

With the advent of flow cytometry into the clinical arena as a diagnostic tool, high-dimensional multicolor analysis has become increasingly recognized as invaluable for the diagnosis and classification of leukemia. In the past, clinicians were restricted by the number of channels available to construct multicolor panels of leukocyte markers. As a result, defining populations of normal and aberrant blood cells often required splitting valuable samples into multiple tubes to achieve the needed resolution.

The ZE5™ Cell Analyzer, when configured with five lasers and 27 channels of fluorescence detection, allows all the markers necessary to classify chronic/acute lymphoproliferative leukemia disorders (CLL/ALL) to be mixed into a single tube. This panel combines 19 surface membrane markers of both cluster of differentiation and Ig species with TCR expression to successfully dissect aberrant leukocyte populations present in a patient with chronic B-cell lymphocytic leukemia (B-CLL).

Materials and Methods

Antibodies

CD3 BUV496, CD4 BUV661, CD19 BUV737, TCRgd BUV395, CD8 BV711, CD45 VioGreen, CD56 BV785, Kappa Light Chain BV421, CD7-BV650, HLADR VioBlue, CD117 BV605, CD33 FITC, CD34 PE-Vio770, CD64 PE-CF594, CD11b PE, CD14 PE-Cy5, CD20 APC-Vio770, CD38 APC-R700, Lambda Light Chain APC

Blocking Buffer

PBS + 50 µg/ml mouse, rat, rabbit IgG + 1% BSA + 1% glucose + 20 mM EDTA + 0.1% azide

Washing Buffer

PBS + 1% BSA + 1% glucose + 20 mM EDTA + 0.1% azide

Beads

AbC Total Antibody Compensation Bead Kit (Thermo Fisher Scientific)

Media

RPMI 1640 or DMEM + 10% FBS

Cell preparation

Normal PBMCs and B-CLL patient PBMCs were thawed and stained using standard procedures. Thawed samples were placed into 5 ml of prewarmed cell media in 50 ml polypropylene centrifuge tubes. Cells were counted and assessed for viability using the TC20™ Automated Cell Counter

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(Bio-Rad Laboratories) to obtain an accurate cell count and viability measurement for each sample. PBMCs were allowed to recover in culture media in a 37°C, 5% CO_2 incubator for at least an hour.

Cell Staining

The normal PBMC sample and the B-CLL PBMC patient sample (each sample contained 1 x 10^6 cells) were treated with blocking buffer for 10 min and then stained by adding a prepared cocktail containing all 19 antibodies. Antibody concentrations used were based on manufacturer recommendations. Samples were incubated on ice, protected from light, for 45 min to an hour. Cells were washed twice and resuspended into 1 ml of ice cold wash buffer. They were stored on ice and protected from light until acquisition on the ZE5 Cell Analyzer. Gating was carried out using FlowJo Software (Tree Star, Inc.). Stained compensation beads were used as single-stained controls (data not shown).

Table 1. Nineteen-Antibody White Blood Cell Panel

Marker	Cell Distribution	Description
CD3	Mature T-cells and thymocytes	T-cell activation signaling and regulation of TCR expression
CD4	T-helper cells, regulatory T-cells, monocytes, and macrophages	T-cell activation, thymic differentiation, and receptor for HIV
CD19	B-cells (but not plasma cells) and follicular dendritic cells	Regulator of B-cell development, activation, and differentiation
TCRgd	T subset	Antigen recognition
CD8	Thymocyte subsets and cytotoxic T-cells	Coreceptor for MHC class I molecules
CD45	Hematopoietic cells (not erythrocytes and platelets)	Critical for B- and T-cell receptor–mediated activation. Also required for thymic selection
CD56	NK, T subset, neurons, some large granular lymphocyte leukemias, myeloid leukemias	Adhesion
Kappa light chain	Immunoglobulin light chain	Antibodies are produced by B lymphocytes, each expressing only one class of light chain. Once set, light chain class remains fixed for the life of the B lymphocyte. In a healthy individual, the total kappa to lambda ratio is roughly 3:1
CD7	Thymocytes, T-cells, natural killer cells, and pluripotent hematopoietic stem cells	T-cell costimulation. Interacts with SECTM1
HLA-DR	MHC class II cell surface receptor	Presentation of peptides to CD4+ T lymphocytes
CD117	Hematopoietic stem cells and progenitors	Receptor for stem cell factor or c-kit ligand
CD33	Monocytes, granulocytes, mast cells, and myeloid progenitors	Lectin activity and adhesion. A receptor that inhibits the proliferation of normal and leukemic myeloid cells
CD34	Hematopoietic stem cells and progenitors and capillary endothelial cells	Cell adhesion (via L-selectin). Possible role in early hematopoiesis through mediation of the attachment of stem cells to the bone marrow extracellular matrix or directly to stromal cells
CD64	IgG Fc receptor monocytes and macrophages	Binds to the Fc region of IgG with high affinity
CD11b	Granulocytes, monocytes, natural killer cells, T- and B-cells, and dendritic cells	Integrin alpha-M
CD14	Monocytes, macrophages (myelomonocytic cells), Langerhans cells, and granulocytes	Receptor of complex of LPS and LBP
CD20	B-lymphocyte surface antigen B1 and T- and B-cell subsets	B-cell activation and proliferation
CD38	Variable expression levels on most hematopoietic and some nonhematopoietic cells. High levels on plasma cells, early T- and B-cells, activated T-cells and germinal center B-cells	Regulator of cell activation, proliferation, and adhesion
Lambda light chain	B-cells	Antibodies are produced by B lymphocytes, each expressing only one class of light chain. Once set, light chain class remains fixed for the life of the B lymphocyte. In a healthy individual, the total kappa to lambda ratio is roughly 3:1

Results

PBMCs from a healthy normal donor were stained using a 19-color panel and run on the ZE5 Cell Analyzer. Results demonstrated that all of the common markers were easily identifiable with no need for FMO controls.

PBMCs from a chronic lymphocytic leukemia patient were then stained with the same 19-color panel. Though the majority of cell populations remained unchanged in this panel, they could be useful for identification of other leukemia subtypes in additional samples. The panel easily identified the patient sample as a chronic B-cell leukemia based on:

1. The expression of CD19 along with an aberrant expression of surface kappa light chain and lambda light chain, indicating a monoclonal B-cell population (100% expression of only one type light chain).
2. Lowered expression of CD38 on CD20-expressing cells.

Normal White Blood Cell Immunophenotype

General Subset Identification

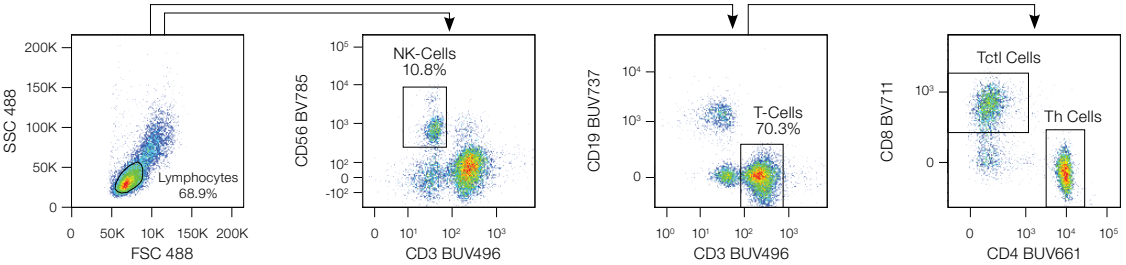


Fig. 1. Normal peripheral blood subset analysis shows typical distribution of all common markers. These panels show NK-cell, B-cell, and T-cell sets, along with T-cell subsets of T-helper (Th) and cytotoxic T (Tctl) cells.

B-Cell Subset Identification

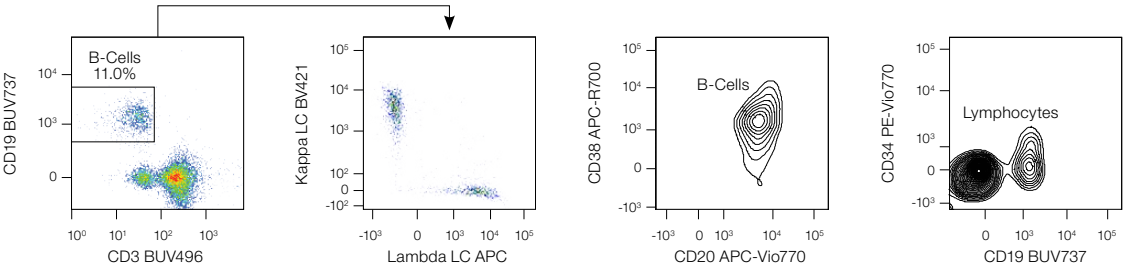


Fig. 2. Normal peripheral blood subset analysis of B-cell subset. These panels show the distribution of surface membrane kappa and lambda light chains, CD20 with CD38 co-expression, and CD19 with CD34 co-expression.

Myeloid-Cell Subset Identification

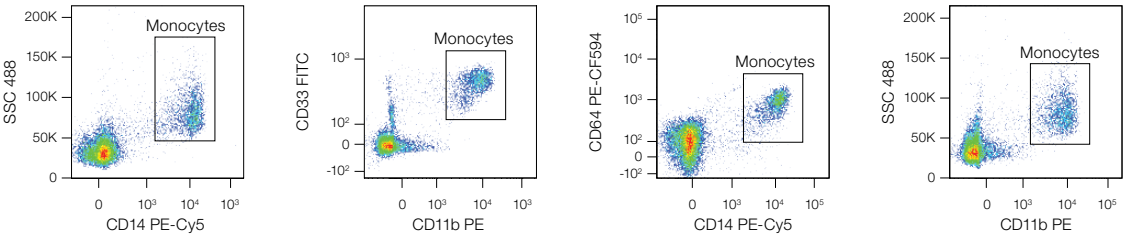
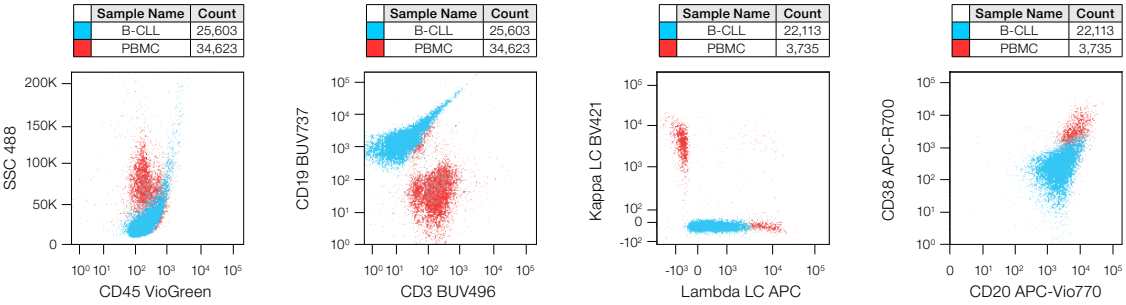


Fig. 3. Normal peripheral blood subset analysis of myeloid-cell subset. These panels show the expected distribution of CD14, CD11b, CD64, and CD33 antibodies.

Diseased White Blood Cell Immunophenotype



Overlay of total population of normal PBMCs with B-CLL sample, showing almost complete dominance of lymphocyte population based on CD45 and side scatter.

Same total population now overlaid with CD3 and CD19, showing nearly complete dominance of CD19-positive B-cell population in the B-CLL sample.

Overlay of B-cell gated populations from normal PBMCs and B-CLL sample. Normal PBMC B-cells will display a ratio of surface membrane Ig light chain of kappa to lambda. The B-CLL sample shows a single chain expression, indicating a monoclonal population of B-cells.

Overlay of B-cell gated populations from normal PBMCs and B-CLL sample. Normal PBMC B-cells will have a relatively high expression of CD20 and CD38. The same gated population from a B-CLL patient shows a diminished CD38 expression.

Fig. 4. Aberrant expression of CD19 and surface membrane Ig light chain lambda and reduced expression of CD38 on CD20-positive B-cells are indicative of chronic B-cell lymphocytic leukemia.

Diseased White Blood Cell Immunophenotype

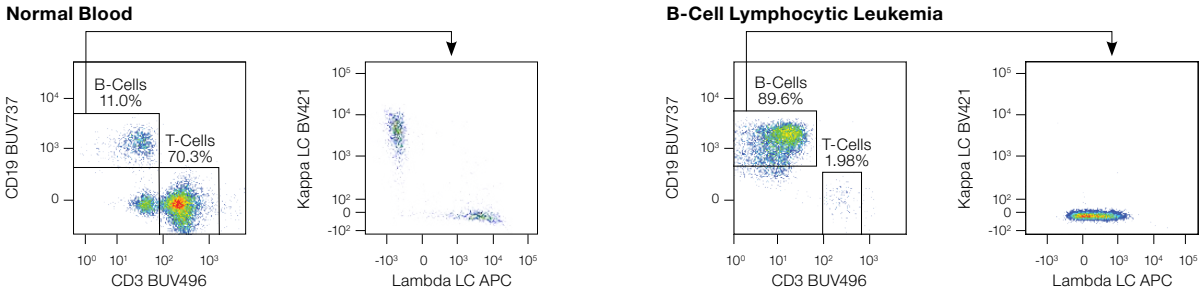


Fig. 5. Diseased peripheral blood subset analysis comparing the profile of normal blood to that of a patient with chronic B-cell lymphocytic leukemia. Note the increase in the B-cell subset along with single light chain distribution in the disease state.

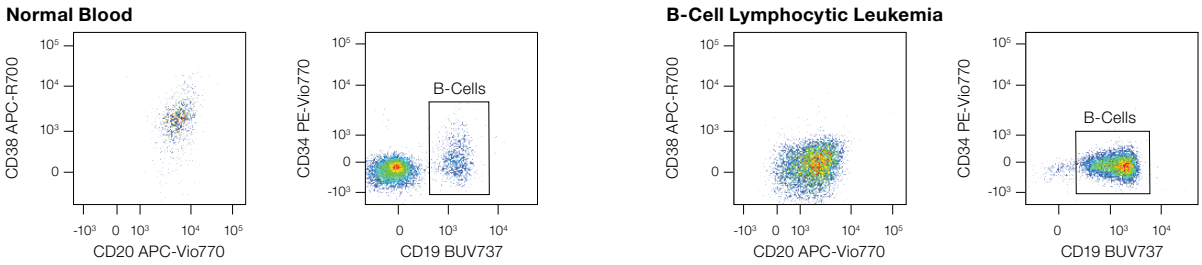


Fig. 6. Diseased peripheral blood subset analysis comparing the profile of normal blood to that of a patient with chronic B-cell lymphocytic leukemia. Note the loss of CD38 expression along with an increase in overall B-cell population in the disease state.

Conclusions

- Large-panel multiparameter design and analysis is straightforward using the ZE5 Cell Analyzer's 27 fluorescent detection channels
- A 19-color panel, designed from existing literature, could allow classification of CLL/ALL disorders utilizing a single tube with the combined antibodies on the ZE5 Cell Analyzer

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