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OMIP-010: A New 10-Color Monoclonal Antibody Panel for Polychromatic Immunophenotyping of Small Hematopoietic Cell Samples

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• **Key terms**
OMIP; 10-color flow cytometry; immunophenotyping; leukemia/lymphoma; small cell samples

Purpose and Appropriate Sample Types

The 10-color panel consisting of 15 monoclonal antibodies (mAbs) is developed to detect leukemia and lymphoma cells in small cell samples [hypoplastic bone marrow (BM), fine needle aspirates, or cerebral spinal fluids (CSFs)]. MAbs conjugates were selected to identify populations of distinct cell lineages and to determine stages of differentiation based on specific antigen expression patterns. As such, conjugates containing the same fluorochrome could be combined. This panel is tested on peripheral blood (PB), BM, and CSF and provides a strong improvement of diagnostic potential.

Background

Most immunophenotypic analyses are performed using up to five-color panels and are usually adequate in samples containing high cell numbers. Samples that contain few cells and samples with minimal residual disease need high-level multicolor analyses to gather sufficient data for diagnostic purposes (1). However, the commercial availability of fluorochrome-labeled mAbs can hinder the formation of multicolor panels. Using CD45-Krome Orange (2), we defined a 10-color panel with 15 mAbs for the detection of aberrant cells in small samples. The other 14 mAbs were selected based on their sensitivity of recognition for different cell lineages and developmental stages. MAbs to low-density antigen were conjugated to bright dyes. In some cases, two mAbs were conjugated to the same fluorochromes and combined where each mAb was independently directed against antigens expressed exclusively on different cell populations.
Conjugates were titrated to find the adequate concentration, thereby maximizing the signal-to-noise ratio. Subsequently, PMT settings and compensation of spectral overlap were performed on each single conjugate. All incubations were performed at room temperature in the dark. This panel enables a reliable clinically diagnostic immunophenotyping of small cell samples with more accurate results and, as such, improves patient care.

Table 1. Summary for OMIP-010

Purpose	Improvement of phenotyping of leukemia and lymphoma cells in small cell samples by a panel consisting of 10 colors and 15 mAb
Species	Human
Cell types	Fresh CSF, BM, PB
Cross-references	None

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Table 2. Reagents used in OMIP-010

LASER	MAB	CLONE	FLUOROCHROME	SOURCE[a]
Blue (488 nm)	CD34	581	FITC	BC
	Ig-kappa	Polyclonal rabbit antihuman	FITC	DAKO
	CD7	8H8.1	PE	BC
	Ig-lambda	Polyclonal rabbit antihuman	PE	DAKO
	CD10	ALB1	ECD	BC
	CD4	T4	PECy5.5	BC
	CD56	NKH-1	PECy7	BC
	CD117	4G7	PECy7	BC
Violet (405 nm)	CD15	80H5	PB	BC
	CD20	HRC20	PB	BC
	CD45	J33	KO	BC
Red (638 nm)	CD3	UCHT1	APC	BC
	CD33	D3HL60.251	APC	BC
	CD8	T8	APC-Ax700	BC
	CD19	J4.119	APC-Ax750	BC

APC, allophycocyanin; Ax, Alexa; Cy, cyanin; ECD, energy coupled dye (PE coupled to Texas Red); FITC, fluorescein isothiocyanate; KO, Krome Orange; PB, Pacific blue; PE, R-phycoerythrin.

[a] BC, Beckman Coulter, Marseille, France; DAKO, DAKO Glostrup, Denmark.

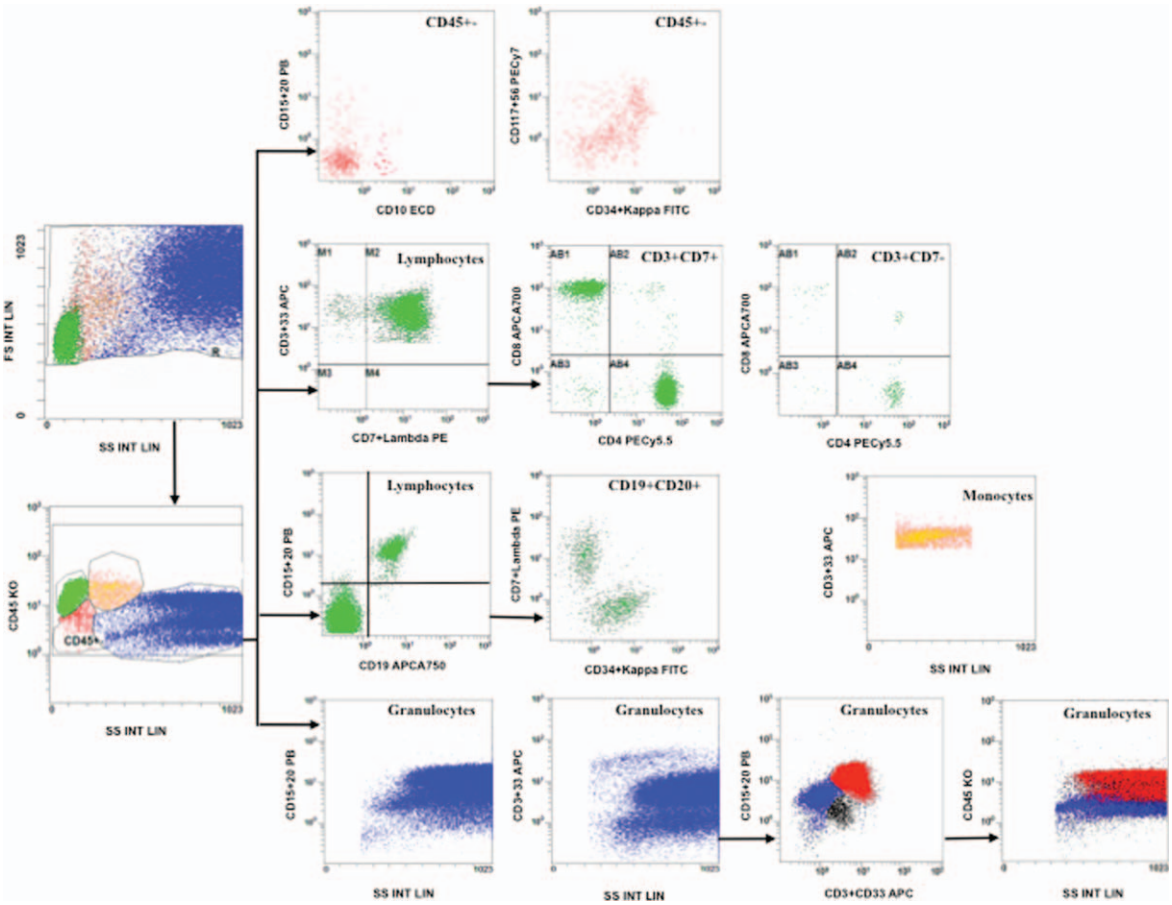

Figure 1. Staining and evaluation protocol of OMIP-010. Using the correct gating procedure information about lymphocyte subsets, monocytes, myeloid cells, precursors, and aberrant cell populations are obtained. A normal BM aspirate was filtered and was subsequently incubated with the 15-mAb combination. Cell populations were analyzed as follows: in the FS/SS plot, debris was excluded from further evaluation. The BM cell populations were plotted in a CD45-KO/SS plot and different subpopulations were gated. Each subpopulation was further evaluated in a double fluorescence plot using the displayed markers. 1. CD45dim/SSlow (progenitors): CD15+CD20/CD10: different B-cell precursors; CD34+Ig-kappa-/CD117+CD56: lymphoid and myeloid progenitors. 2. CD45+/SS− (lymphocytes): CD3+CD33/CD7+Ig-lambda: T-cell subsets; within CD3+CD33: CD4 and CD8 T cells; CD117+CD56/CD3+CD33: T cells, NK cells, and NK-T cells. 3. CD45+/SS− (lymphocytes): CD15+CD20/CD19: B cells; within CD19+CD20: Ig-lambda and Ig-kappa B-cell subsets. 4. CD45+/SSdim (monocytes): CD3+CD33/SS: CD33++ monocytes. 5. CD45±/SS+ (granulocytes): CD15+CD20/SS and CD3+CD33/SS: different myeloid populations. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

Similarity to Published OMIPs

None to date.

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