Sanquin
Blood Supply

EUROFLOW AND SANQUIN PRODUCTS

Laboratory diagnostics of hematological malignancies has three major applications:
1. establishing the diagnosis;
2. prognostic (sub)classification;
3. evaluation of treatment effectiveness via detection of "minimal residual disease" (MRD).

Over the past decade, several molecular techniques have brought new insights into classification and monitoring of treatment effectiveness. However, they have several major disadvantages: they frequently are time consuming (1-3 days or more), not applicable in all categories of patients, and cannot focus on cellular subpopulations without preceding purification steps.

Flow cytometric immunophenotyping is the sole technique that fulfils the requirements of high speed, broad applicability at diagnosis and during follow-up, and accurate focusing on the malignant cell population using membranebound and intracellular proteins as targets.

However, innovations are needed in flow cytometry, such as development of novel antibodies, 8-color immunostaining protocols, and novel flow cytometry software for fast and easy interpretation of complex data and for automated pattern recognition, which are all key objectives of the EuroFlow consortium (EUFP6 project LSHB-CT-2006-018708: "Flow cytometry for fast and sensitive diagnosis and follow-up of haematological malignancies").

The EuroFlow consortium consists of two SME's and twelve diagnostic research groups, which are regarded as experts in the fields of flow cytometric and molecular diagnostics. Consequently the EuroFlow members are working on:

1. development of new software for fast and easy handling of large data sets and for integration of 8-color stainings into a single multicolor data file (INFINICYT™);

2. development of standardized 8-color antibody panels for fast and easy flow cytometric diagnosis and classification of hematological malignancies as well as for sensitive monitoring of patients for evaluation of treatment effectiveness;

3. development of multiplex immunobead assays for detection of fusion proteins and oncoproteins per disease category (particularly ALL and AML) ;

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4. development of software for automated pattern recognition of normal, reactive, and aberrant (malignant) leukocyte populations in blood and bone marrow;

5. creation of a large data base with hundreds of well-defined normal, reactive and malignant cell samples, which can be used as ready-to-use template for fully automated comparison with newly analyzed patient samples.

The INFINICYT software can automatically combine the immunophenotypic information of the selected cell populations from multiple tubes according to the so-called nearest neighbor calculations in which individual cells are matched with corresponding individual cells according to their backbone markers and scatter profile. This INFINICYT procedure transforms the 8-color EuroFlow panels into 12, 16, or ≥ 20-color immunostainings, dependent on the number of tubes per panel and the number of backbone markers per tube.

In a number of panels Sanquin participates with unique products:
- In the AML/MDS panel: CD16 FITC labeled (product code M1604), CD36 FITC labeled (product code M1613) and CD41 FITC labeled (product code M1674)
- In the T-CLPD panel: GranzymeB PE labeled (product code M2289)

The 8 fluorochromes have been selected in several testing rounds for brightness, limited spectral overlap and limited need for compensation, stability, etc. After multiple testing rounds the EuroFlow Consortium has chosen for the following fluorochromes: Pacific Blue, Pacific Orange, fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyl protein/cyanine 5.5 (PerCp-Cy5.5), PE-Cy7, allophycocyanine (APC), and APC-H7.
The EuroFlow antibody panels, the EuroFlow SOP, and the INFINICYT software can be used in combination with all flow cytometers that allow 8-color immunostainings.

In September 2012 in a special issue of Leukemia the following article was published: "EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes."
Here the validated EuroFlow 8-color antibody panels for immunophenotyping of hematological malignancies are presented.

The single-tube screening panels and multi-tube classification panels fit into the EuroFlow diagnostic algorithm with entries defined by clinical and laboratory parameters.
Two groups of markers are combined in each 8-color tube:
- backbone markers to identify distinct cell populations in a sample,
- markers for characterization of specific cell populations.

The characterization markers were positioned according to the diagnostic utility of the combined markers. Each proposed antibody combination was tested against reference databases of normal and malignant cells from healthy subjects and WHO-based disease entities, respectively.
The EuroFlow studies resulted in validated and flexible 8-color antibody panels for multidimensional identification and characterization of normal and aberrant cells, optimally suited for immunophenotypic screening and classification of hematological malignancies.