cytognos
Flow Cytometry Solutions

MINIMAL RESIDUAL DISEASE
BCP-ALL

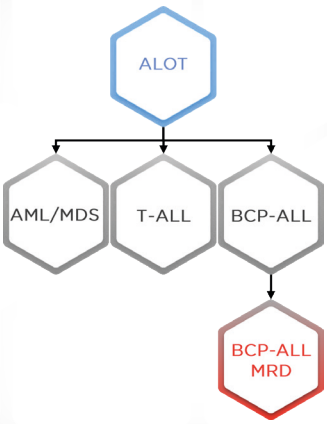
B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL)

Acute leukemias are marked by the diffuse replacement of bone marrow with abnormal immature and undifferentiated hematopoietic cells. Based on the origin of the abnormal hematopoietic cells involved, such as lymphoid, myeloid, mixed or undifferentiated, these disorders are classified accordingly.[1]

Acute Lymphoblastic Leukemia (ALL) is the most frequent type of leukemia in children but can also occur in adults.[2] In adults, 75% of cases develop from precursors of the B-cell lineage, called B-Cell Precursor (BCP) ALL, with the remainder of cases onsisting of malignant T-cell precursors.[3]

IMMUNOPHENOTYPING IN ACUTE LEUKEMIA WORKFLOW

Acute leukemia is a complex multifactorial disease and diagnosis requires an interdisciplinary approach, including review of symptoms and physical examination, blood testing, bone marrow biopsy, morphology, cerebrospinal fluid (CSF) evaluation, radiology, and genetic testing.[3, 4] Flow cytometry proves to be an important tool that is integral to leukemia diagnosis[5]: it facilitates the phenotypic characterization of leukemic cells at diagnosis, gives prognostic information relative to disease severity and allows monitoring of Minimal Residual Disease (MRD).[6, 7, 8, 9, 10, 11] Flow cytometry has been reported to be a sensitive method for the detection of MRD, in some cases providing sensitivity comparable to molecular tests.[6,11]



The EuroFlow™ Consortium has designed, tested and validated a set of 8-color antibody panels for the diagnosis, classification and MRD analysis of acute leukemia(s)[5,6]; used in combination with novel Infinicyt™ software tools, the multidimensional immunophenotypic characterisation of blast cells by flow cytometry is optimized.[12,13]

The Euroflow™ BCP-ALL MRD panel

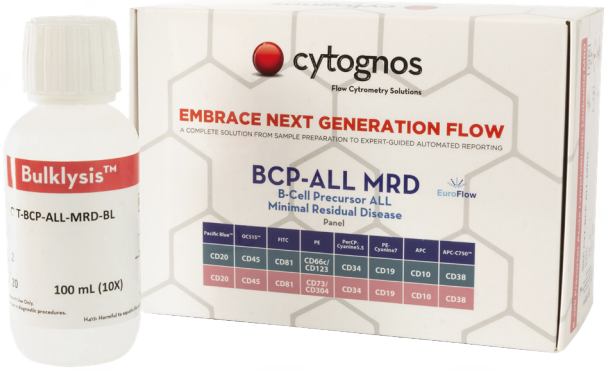
Although therapies have advanced to such an extent that survival of patients diagnosed with ALL has improved, relapses still occur in 20% of children and 40%-50% of adults.[2,14] The detection of MRD in ALL has proven to be a fundamental tool for guiding therapeutic decisions as it determines the response to initial treatment and the subsequent identification of risk groups, it allows surveillance of disease burden in relation to stem cell transplantation and early follow-up of relapse.[14]

The BCP-ALL MRD assay for bone marrow (BM) samples, as designed and validated by the EuroFlow™ Consortium, comprises two standardized antibody panels (tube 1 + tube 2) and Standard Operating Procedures (SOPs), targeting a sensitivity of at least 10^{-5} when more than 4 million BM cells are evaluated, which means comparable MRD detection as obtained with real-time quantitative polymerase chain reaction (RQ-PCR).[6]

BCP-ALL MRD	PB	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
Tube 1	CD20	CD45	CD81	CD66c+CD123	CD34	CD19	CD10	CD38
Tube 2	CD20	CD45	CD81	CD73+CD304	CD34	CD19	CD10	CD38

The BCP-ALL MRD reagent is composed of two 8-colour reagent combinations, sharing several common markers (backbone).

Among them, CD19, CD45, CD34, CD10 and CD20 are known to allow appropriate gating of BCP, characterization of several BCP subpopulations and discrimination between normal and malignant BCPALL cells.[6] Since CD66c and CD123 are both virtually negative on normal/reactive BCP cells[15,16] they are combined in the same PE channel, leaving a position available in the panel for additional markers.[6]



To optimize the composition of the BCP-ALL MRD panel with additional markers, the separation between normal and pathological populations was scored to reach the optimal combination to enhance the separation of these two entities (Figure 1).[6] Further evaluation on 78 BCPALL patients, showed that CD38 (~35% of cases), CD66c/CD123 (~30%), and CD81 (~19%) improved the separation between normal/reactive and malignant BCP cells as compared with the 5 backbone markers only. Based on the level and frequency of overexpression of CD73 and CD304 (~20% and ~40%) and their stability during follow-up, these were added to the final panel. Because of high background levels when combining these last 2 markers with CD66c and CD123 in a single fluorescence channel, a second tube was designed, identical to the first tube but with CD73/CD304 instead of CD66c/CD123 in the PE channel. Together, the two 8-color antibody tubes allowed separation between normal and malignant BCP cells in 99% of studied patients.[6]

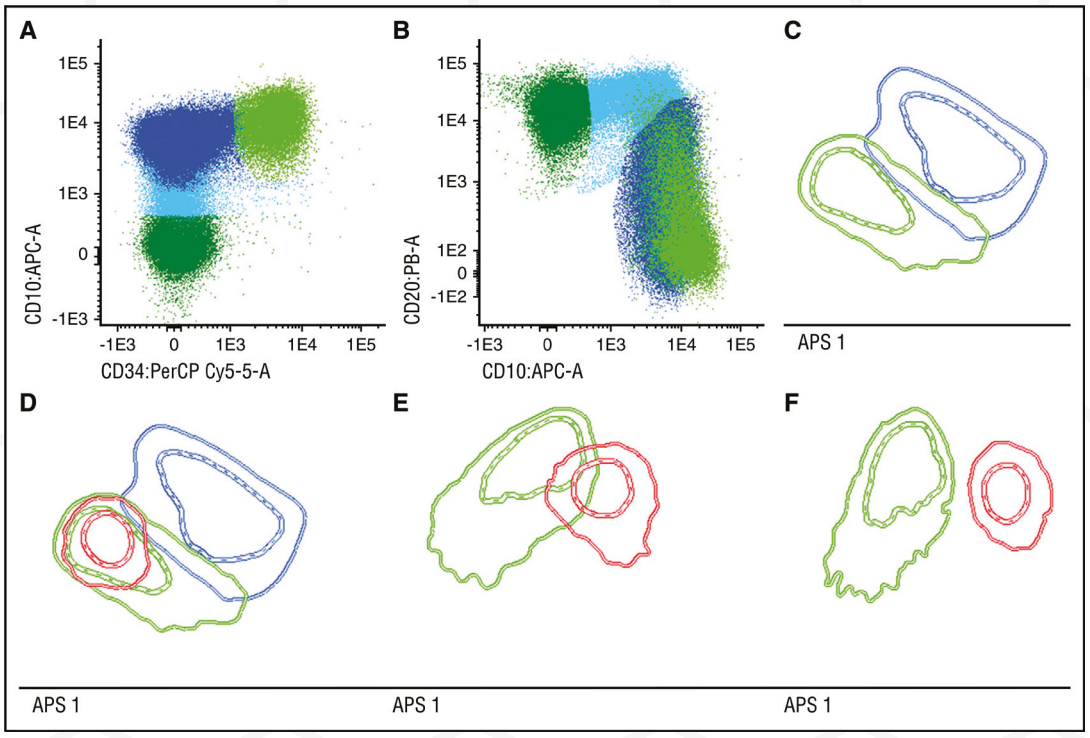


***Figure 1**[6] shows the strategy followed by the EuroFlow™ Consortium to optimize the composition of the BCP-ALL MRD panel. **Figures A and B** show the different subpopulations of B cells (CD19+) from various normal/reactive or regenerating bone marrow samples. **Figure C** represents a view of the Automatic Population Separator (APS) plot for the most immature BCP populations (pre-B-I in light green and pre-B-II in blue). The dotted line represents 1 standard deviation of the cases being plotted and the solid line 2 standard deviations. In **Figure D**, each individual BCP-ALL case was plotted in a fixed APS together with the normal populations. Then, each BCP-ALL case was visualized in separate unfixed APS with the nearest normal population, using the backbone markers (**Figure E**) and the 8 markers (**Figure F**). The separation between normal and pathological populations was scored to reach the optimal combination to enhance the separation of these two entities.*

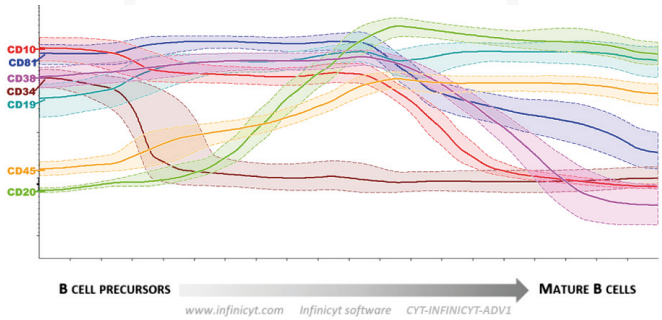


Figure 2 *shows the maturation pattern of normal BCP cells in BM when using tools available in the Infinciyt™ software.*

For flow cytometric MRD data analysis using the Euroflow™ BCP-ALL MRD panel, the presence of abnormal leukemic BCP in a given BM sample can be determined by looking at the changes in the pattern expression of the markers. The marker expression on normal BCP in BM as well as their maturation pattern can be found in the Infinciyt™ software (Figure 2) (for more information refer to the Infinicyt™ software Instruction for Use (IFU)).

STANDARDISED OPERATING PROCEDURES FOR MRD evaluation

Flow cytometry immunophenotyping results are highly dependent on the protocols used for sample processing. For this reason, the BCP-ALL MRD assay was designed in collaboration with the EuroFlow™ Consortium and the protocol was developed following general EuroFlow™ standardized recommendations and Standard Operating Procedures* (SOPs) for flow cytometers equipped with three-laser (blue, red, violet). Using these fully standardized SOPs for all BCP-ALL MRD samples and upon evaluation of sufficient cells (> 4 million, preferably more), a sensitivity comparable to RQ-PCR (golden standard methodology) was reached.[6]

During early phases of disease treatment, the cellularity of BM patient samples is usually low and using the standard methodology requiring the direct staining of 100 µL of whole BM will not allow the acquisition of the millions of cells needed to reach similar sensitivity as obtained by RQ-PCR. Therefore, the BCP-ALL MRD SOP as developed with the Euroflow™ Consortium, consists of a new erythrocyte lysing procedure (using BulkLysis™) to lyse sufficiently large volumes of BM and resuspend the resulting leukocytes in a small volume of washing buffer suitable for staining. This new protocol allows for the staining of 10 million cells in 100 ·L of cell suspension without compromising the data quality and as such enables the earlier mentioned sensitivity of at least 10^{-5}.[6]

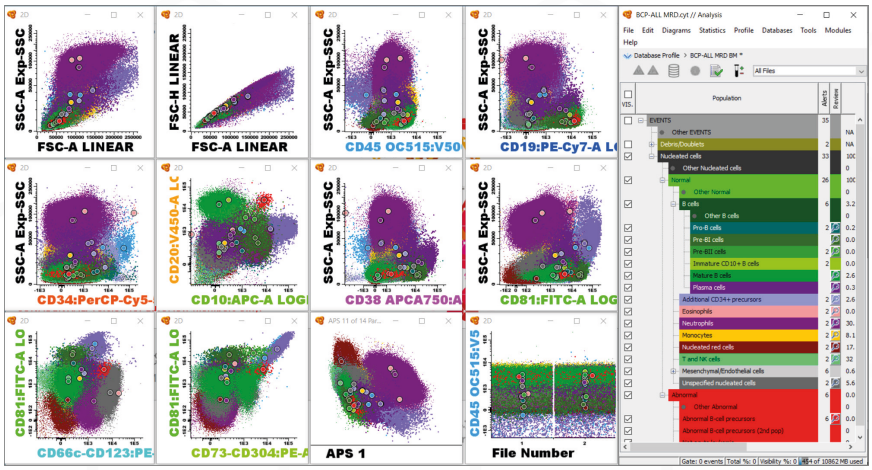
*The corresponding SOPs may be found at www.euroflow.org.

Infinicyt™ data analysis and reference databases

Delivery of timely and accurate results is important for appropriate patient care. Therefore, it is essential that flow cytometry data analysis and result reporting is accurate, objective, and specific to the clinical questions, and that the data are processed and reported rapidly. The number of immunophenotypic markers that can be evaluated in 8-color assays and the high number of cells to be acquired to achieve the desired assay sensitivity, clearly increases the complexity of data analysis.

EuroFlow™ developed a database containing representative flow cytometry data sets from normal healthy BM samples processed in different standardized centers. The database (available through Infinicyt™) when used with files which follow EuroFlow™ SOPs allows for a more automated analysis of the complete BM sample[12,13].

The BCP-ALL-MRD database is designed to be used with Infinicyt™ Automated Gating and Identification (AG&I) tool13 in order to provide a more automated analysis of the sample. Normal populations are identified comparing them with the reference database while events which differ from normal need to be confirmed by the user. All the information from the analysis and deviations from the normality ranges are automatically included in the results report (Figure 3).



In summary, the EuroFlow™ BCP-ALL MRD panel, with the SOPs and the database, is a powerful tool that helps the users to generate reproducible and objective data also when handling complex patient samples.[6,12,13]

Figure 3 *displays the result of the automatic analysis in Infinicyt ™ of a sample stained with the BCP-ALL-MRD panel. The image shows the entire sample analyzed, with the alert column warning of deviations of the populations from the normal BM database.*

CONCLUSION

The BCP-ALL MRD assay, used in combination with Infinicyt™ software, enables labs to qualitatively identify and discriminate BCP-ALL cells from normal/reactive BCP in BM samples from treated BCP-ALL patients,[6] ultimately supporting good patient outcomes.[17]

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Ordering information:

Product	Reference	Format	Size
BCP-ALL-MRD kit	CYT-BCP-ALL-MRD	Lyophilized	20 test

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