

A SINGLE 8-COLOR FLOW-CYTOMETRIC IMMUNOSTAINING ALLOWS DELINEATION OF BOTH TYPICAL MYELOID AND LYMPHOID ACUTE LEUKEMIA AND UNDIFFERENTIATED/IMMATURE ACUTE LEUKEMIA : ON BEHALF THE EuroFlow CONSORTIUM

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BACKGROUND

The development of standardized 8-color flow cytometry (FC) combined with bio-informatic merging of data from multiple tubes/cases allows multiparameter evaluation of surface and intracellular proteins at the single cell level, while maintaining all the advantages of FC diagnostics (rapidity, evaluation of cellular heterogeneity, application to minimal residual disease, etc).

AIMS

Within the European EuroFlow program, we designed an 8-color Acute Leukemia Orientation Tube (ALOT) for fast and efficient evaluation of patients with suspected acute leukemia in order to choose appropriate, more detailed immunophenotypic panels. (Fig. 1)

Violet Laser (405 nm)		Blue Laser (488 nm)				Red Laser (633 nm)	
Pacific Blue cyCD3	Pacific Orange CD45	FITC cyMPO	PE cyCD79a	PerCP Cy5.5 CD34	PE-Cy7 CD19	APC CD7	APC H7 smCD3
UCHT1 (BD Biosciences)	HI30 (Invitrogen)	MPO-7 (Dako)	HM57 (Dako)	8G12 (BD Biosciences)	J3.119 (Beckman Coulter)	eBio124-1D1 (eBioscience)	SK7 (BD Biosciences)

Fig 1: Antibody combination of the ALOT.

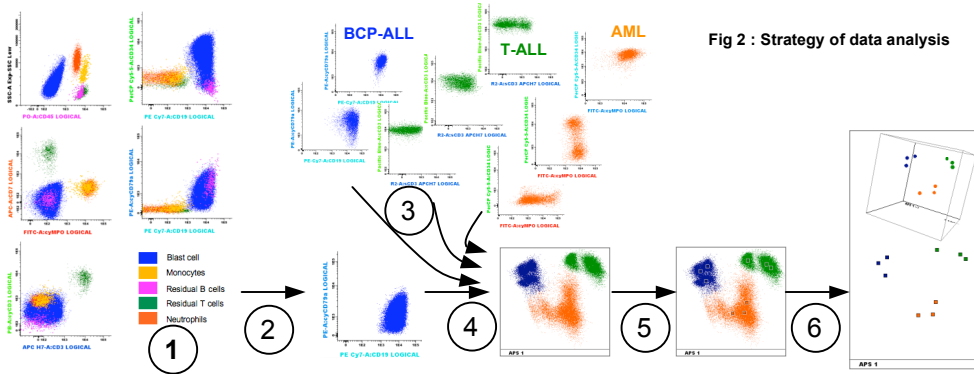
METHODS

A - PATIENTS / SAMPLES AND FC ANALYSIS

A total of 157 acute leukemia samples (105 BM, 45 PB, 7 others) were analyzed with ALOT by FC (FACS Canto II, BD Biosciences), in 8 different centers, using the EuroFlow standard operating procedures (SOP) for instrument settings, which justified comparison of intermachine results. Conventional diagnostic procedures for lineage assessment of blast cells were used at each center in parallel.

B - BIOINFORMATIC PROCESSING OF DATA

ALOT data were centrally collected and processed using the Infinicyt software (Cytognos SL, Salamanca) as shown in Fig.2.



- Flow analysis** : Gating of the blast cell population
- Extraction** : Creation of a FCS file containing information only on the blast population
- Merging** : Pool of the information on blast populations from different samples in a single « virtual » merged tube/data file
- APS (Automated Population Separation)** : Unsupervised classification of the different leukemias by principal component analysis (PCA)
- Mean calculation** : each leukemic sample is represented by its mean, that represents the centre of gravity of the blast population over the 10 parameters measured
- Cluster analysis and pattern recognition** : each leukemic sample is now represented by only one dot (mean value), and clusters are identified by the software.

RESULTS

1 - Pairwise PCA-based comparison of well characterized leukemias enables recognition of 3 well separated disease clusters (Fig. 3)

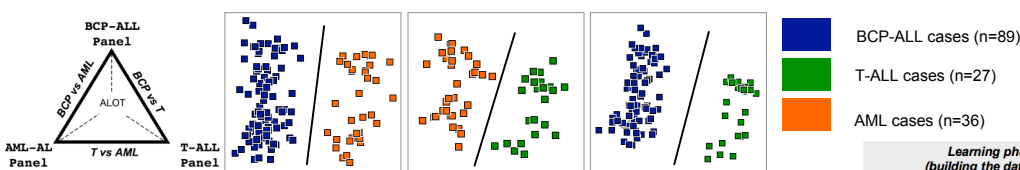


Fig. 3 : Based on the ALOT combination, all typical cases of AL (152/157) can be recognized with the new approach. No case was misclassified.

2 - Atypical acute leukemias (2 AML, 3 undifferentiated AL) clusters (Fig. 4)

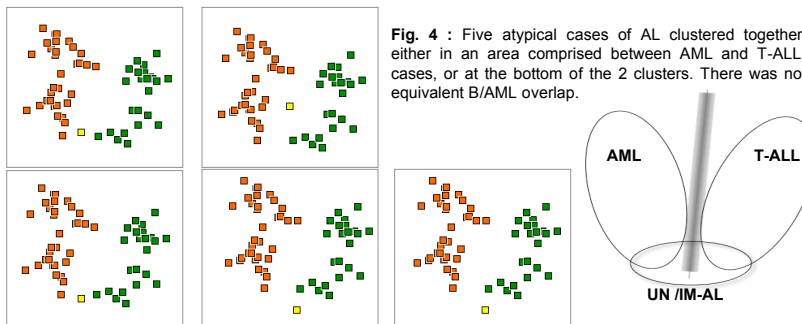


Fig. 4 : Five atypical cases of AL clustered together either in an area comprised between AML and T-ALL cases, or at the bottom of the 2 clusters. There was no equivalent B/AML overlap.

CONCLUSION / FUTURE DIRECTIONS

1 - Toward automated analysis : « help to diagnosis tool »

Based on well defined cases, the Infinicyt software tools used are able to predict the immunophenotypic lineage-subtype of individual acute leukemia cases (automated pattern recognition) (Fig. 5)

2 - Screening of atypical acute leukemias : undifferentiated/ immature AL

The single 8-color tube allows delineation of UN/IM acute leukemia which will benefit from specific characterization immunophenotypic strategies.

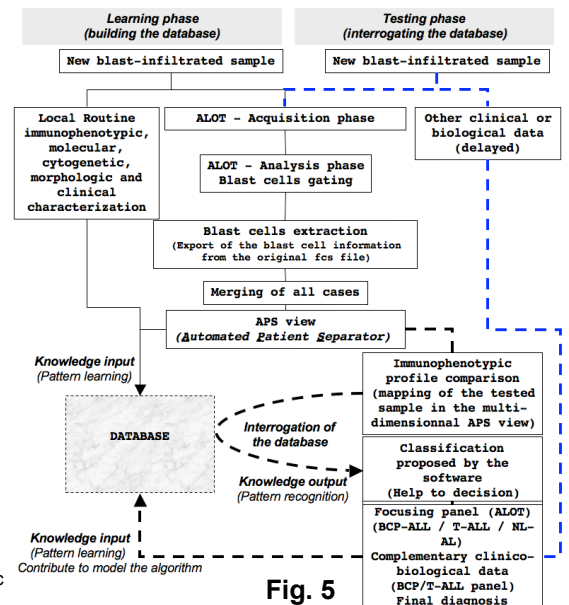


Fig. 5