Abstract: P421

Title: FLOW-CYTOMETRIC MRD DETECTION IN PEDIATRIC T-ALL: A CONSENSUS-BASED STANDARDIZED APPROACH

Abstract Type: Poster Presentation

Topic: Acute lymphoblastic leukemia - Clinical

Background:

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 15% of all newly diagnosed pediatric ALL patients. A risk-based stratification approach is successful in identifying patients at risk of relapse, whose treatment is very poor. The most important high-risk factor is measurable residual disease (MRD), which is measured by either polymerase chain reaction (PCR) or flow cytometry (FC).

Aims:

Within the international collaboration of AIEOP-BFM Flow laboratories, we aimed to develop and optimize a standardized approach for MRD measurement in T-ALL using a fixed analysis strategy. The developed tubes should be suitable for both 8-color and 12-color cytometers, according to the available optical configurations.

Methods:

Based on the survey among the groups, we reached a consensus on backbone and additional markers to be included in both 8-color and 12-color tubes for T-ALL MRD. The 8-color panel included 2 tubes with surface markers only and 1 tube with intracellular CD3, and the 12-color panel included 1 surface and 1 intracellular tube. Custom-manufactured tubes with dried antibodies for backbone markers were tested in parallel with local panels evaluated by a local expert ("local standard") in 8 laboratories. Standard operating procedure (SOP) including nucleated cell staining and lysing methodology was optimized, leading to the validation of the final third version of the SOP. A total of 64 diagnostic and 67 day 15 (d15) samples were acquired. We designed 3 fixed gating strategies ("fixed gating") to identify blast cells in parallel with global expert evaluation of T-ALL MRD tubes ("expert gating"). Each strategy consisted of (1) basic steps to identify T cells and (if possible) excluding NK cells (by e.g. CD7, CD5, CD16+CD56), (2) identification of atypical blasts with tube specific aberrant markers (e.g. CD48, CD34, CD99) and (3) fine tuning cluster gating (with e.g. CD4, CD8, CD5, CD45).

Results:

By analyzing the diagnostic samples, we proved that the optimized tubes allow the identification of blast cells. The proportion of blasts by expert gating correlated with the local standard value (Spearman $R=0.85$ for surface tubes of both 8-color and 12-color panels, $p<0.0001$). We then applied expert gating and fixed gating strategies to d15 samples (Figure 1). Expert gating correlated with the local standard (Spearman $R=0.9$ for all surface tubes, $p<0.001$). When we applied the fixed gating strategies and selected the one that best fitted the case, we reached Spearman $R=0.89$ ($p<0.0001$) for surface tubes. FC MRD results on d15 significantly correlated with PCR MRD values ($n=20$) when applying both expert and best fixed gating strategies (Spearman $R=0.74$ and $R=0.76$, respectively). A similar correlation was observed for PCR MRD with local standard analysis (Spearman $R=0.79$). With respect to 3 MRD categories (0-0.1%; 0.1-10%; 10-100%), 91% and 77% of patients would reach the same category as local standard using expert gating and best of fixed gating strategy, respectively. These findings could reflect the heterogeneity of the T-ALL immunophenotype, which cannot always be fully covered by the fixed approach.

Summary/Conclusion:

In conclusion, we have successfully developed a standardized approach for T-ALL MRD measurement to be used in the frame of BFM-oriented clinical trials.

Although T-ALL represents a subgroup of childhood ALL, thanks to the international collaboration, we were

able to collect a high number of samples suitable for assessment and validation of the analytical protocol. Our work shows that although the T-ALL is a heterogeneous disease and some cases still need an expert analytical approach, in most cases, fixed analysis leads to valid results.

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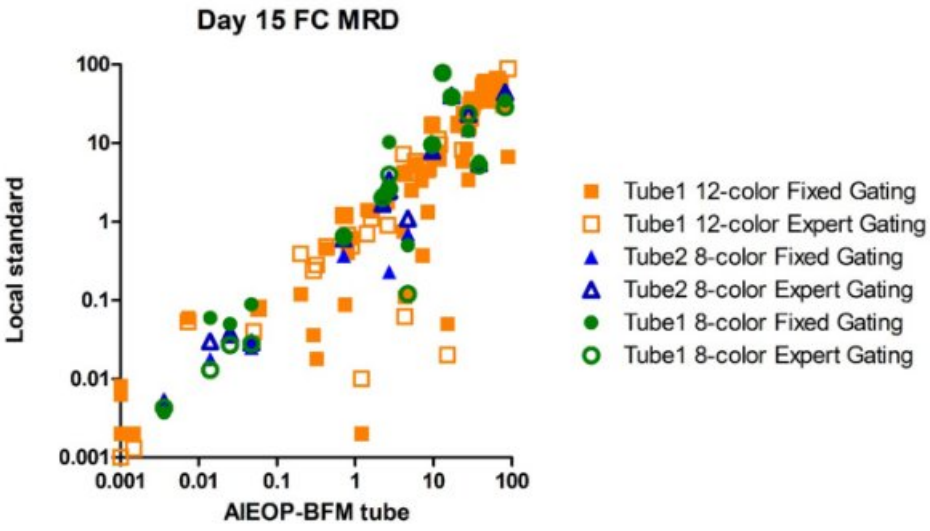

Keywords: T-ALL, Minimal residual disease (MRD), Flow cytometry, Standardization