To the editor:

The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL

Minimal residual disease (MRD) monitoring via antigen receptor quantitative polymerase chain reaction (qPCR) is an important predictor of outcome in childhood acute lymphoblastic leukemia (ALL), is rigorously standardized within the EuroMRD consortium and has a greater sensitivity than flow cytometry (FC), which has been used in other trials.1 However, qPCR is laborious, expensive, and time consuming because of the development of patient-specific assays. MRD detection based on next-generation sequencing (NGS) of antigen receptor gene rearrangements is a promising tool that permits sequencing of large numbers of rearranged V-(D)-J segments and thus provides the picture of not only residual leukemia but also the normal immune repertoire of respective cells.2-5

To date, no correlation study on larger number of samples has been performed to determine how the discrepancies between NGS and qPCR data would affect clinical data. Moreover, there are no data investigating the impact of normal B-cell compartment reconstitution after induction treatment in ALL on prognosis. We developed a simple, cost effective, and easily adoptable approach using 2-round PCR amplifying virtually all immunoglobulin heavy chain (IgH) rearrangements6 and NGS on Ion Torrent/Proton sequencers (supplemental Methods; available on the Blood Web site). We compared the NGS-MRD results with current techniques7 in patients treated by Berlin-Frankfurt-Munster (BFM)-based protocol and investigated the changes in the treatment stratification using the new method.

Altogether, we sequenced 210 samples from 76 patients (62× day 15, 73× day 33, 75× day 78) with a median coverage of 729 842 reads per sample. The overall correlation of NGS- and qPCR-MRD in all samples was satisfactory (R² = 0.72; Figure 1A). A total of 32 samples (15%) were positive by NGS/negative by qPCR or vice versa, causing a shift in BFM risk stratification in 25 patients (33%), mainly between standard-risk (SR) and intermediate-risk (IR) groups in the low-positive patients. Five patients would be reassigned from the IR to the slow early responder (SER) group (4 of them relapsed), and 2 patients from SER to IR (1 of them relapsed).

NGS-MRD positivity at day 33 provided a more accurate prediction of relapse than qPCR-MRD positivity (Figure 1B-C) (5-year
NGS data provide information not only about MRD but also about the rest of the B-lymphoid repertoire. We assessed clonal heterogeneity of the IgH repertoire in investigated samples, using the Vidjil algorithm (supplemental Methods). Interestingly, the patients with relapse had significantly lower IgH repertoire diversity at days 33 and 78. Even after removal of patients with NGS-MRD higher than $10^{-4}$, the patients with lower clonal diversity at day 78 had significantly worse 5-year RFS than other patients ($P = .0005$; Figure 1F).

In conclusion, the NGS risk group assignment is partly different from current approaches. This is mostly because of discrepancies in low-positive samples in SR/IR groups, but with no negative impact on final outcome. The NGS even provided a more precise prediction of relapse than qPCR at day 33 in our limited cohort. However, a prospective validation study comparing both methods will be needed to definitely accept NGS as the replacement for qPCR. A redefinition of stratification criteria for childhood ALL will be only the final step of a complex process that has just started. The main challenge in NGS-MRD methodology is standardization aiming at highly reproducible results between different centers, as it was achieved previously within the Euro-MRD group for qPCR. A European network, the EuroClonality-NGS Consortium, has been formed to standardize the workflow of analytics, preanalytics, and bioinformatics. Before this is accomplished, the methodology described in our study can serve as a broadly accessible and relatively inexpensive noncommercial solution for centers that do not perform antigen receptor qPCR but want to start using benefits of MRD monitoring in their clinical setting.

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References


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