



Next generation sequencing as the new gold standard for minimal residual disease detection in B-ALL

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Cheng *et al.* (1) in their study explored the use of the next-generation sequencing (NGS) technology to evaluate post-remission minimal residual disease (MRD) in B acute lymphoblastic leukaemia/lymphoma (B-ALL) patients and its potential to be implemented in clinical settings. The authors propose an NGS-based method with an applicability of around 90% of B-ALL patients and a sensitivity of up to 10^{-6} and losing only 6.25% of the cases where leukaemia-specific clonotypes were not identified.

The relevance of using the strategies of MRD in the clinical setting is (I) the improvement in the prognosis of adult B-ALL and the adjustment of treatment for each patient. (II) MRD levels appear to be the most powerful tool to indicate or not allogeneic stem cell transplant in first CR.

Several studies in both child B-ALL and adult B-ALL (2-10), have demonstrated a strong correlation between MRD and risk of relapse. These studies have a different sensitivity and have been done in different time points of therapy (post-induction or post-consolidation). MRD assessment at the end of induction therapy is useful for recognizing patients with low risk of relapse, and MRD at the end of consolidation therapy is useful for identifying patients with high risk of relapse (2).

Post-induction MRD levels using multiparametric flow cytometry (MFC; whose sensitivity is $<0.1\%$) was identified as an independent predictor of relapse regardless of whether the patient was classified in a standard or high-risk group at diagnosis (n=116) (10). The MRC UKALL/ECOG

study (3) (n=161) observed a longer remission free survival (RFS) in patients with MRD negativity ($<0.01\%$) (71% *vs.* 15%, $P=0.0002$). In another study headed by MD Anderson Cancer Center (MDACC) investigators, MRD status stabilised by MFC (sensitivity of 0.01%) in 340 B-ALL patients was identified as a prognostic marker associated with improved disease-free survival (DFS) and overall survival (OS) (5).

Finally, Ribera *et al.* (9) published a study with 282 B-ALL patients in CR, that shows poor MRD clearance was the only prognostic factor for DFS and OS. Prognosis for Ph-negative high risk-ALL in adolescents and adults with a good early response to induction and low flow-MRD levels by MFC (with a sensibility of 10^{-4}) after consolidation is quite favourable when allogeneic stem cell transplant (SCT) is avoided.

Besides post-induction and post-consolidation MRD using ASO-PCR in Ph-negative B-ALL patients both standard and high-risk was the only factor associated with remission duration and survival in a study of German Multicenter Study Group for Adult ALL (GMALL). In this study the authors evaluated the potential advantage of intensifying treatment with the incorporation of allogeneic SCT based on post-consolidation MRD status. Patients without molecular remission had a better probability to keep complete remission if they were consolidated with SCT (66% *vs.* 12%, $P<0.0001$) (8). Bassan *et al.* showed that post-consolidation MRD (7) status has prognostic significance and it allows to the adjustment of post-

consolidation treatment. Patients with MRD <0.01% received maintenance chemotherapy for 2 years, whereas patients with MRD \geq 0.01% were eligible to undergo allogeneic SCT.

MRD is defined as the presence of leukemic cells not detected by conventional morphologic method (2). These include: (I) identification of leukaemia-associated immunophenotype by MFC; (II) the detection of leukemic-specific rearrangements of immunoglobulins and T-cell receptor (Ig/TCR); (III) the detection of fusion gene transcripts by qPCR; and (IV) strategies of amplicon-based NGS of Ig/TCR rearrangements. All these methods have advantages and disadvantages but NGS have shown more precision for relapse prediction in B-ALL (11,12) and in other haematological pathologies associated with B-cell clonality such as multiple myeloma (MM) (13).

There are commercially available NGS-based Igs clonality assays, and Adaptive Biotechnologies (clonoSEQ) and Invivoscribe (LymphoTrack) are the companies to represent the largest commercial NGS B cell clonality assay market share (14). But other 'in-house' MRD tests with an affordable and flexible design are being used (13).

MRD by NGS has shown to be more specific and more sensitive in the detection of multiple Ig receptor rearrangements compared to qPCR in a work of 30 B-ALL patients who underwent SCT (n=228) (15). This result has also been confirmed in acute myeloid leukaemia (AML) and our group has recently published an NGS based method that simplifies and standardizes MRD evaluation, with high applicability. It also improves upon MFC and qPCR to predict the outcome of AML patients, with a sensitivity of 10^{-4} for SNV mutations and 10^{-5} for insertions/deletions. In this study, MRD positive status by NGS was the only independent factor conferring risk of relapse (HR =3.76, P=0.012) (16).

NGS is a promising tool for MRD detection and could be the new gold standard for MRD detection in B-ALL. However, before the implementation of NGS-MRD methods in clinical practice, the workflow step by step needs to be standardized (spike-in calibrators, quality controls, sample preparation, bioinformatics pipeline, and guidelines for correct data interpretation) (12). Initially, the optimal biological sample is bone marrow (BM) *vs.* peripheral blood (PB). MRD levels in B-ALL tend to be 1–3 logs upper in BM than PB (17,18). And, as is well known, DNA amount input is critical to reach a good sensitivity (19), 1–5 μ g DNA is the input amount recommended by Cheng *et al.* The coverage is also important, given that a read is interpreted as

a DNA molecule, a minimum of 1 million reads are needed to evaluate 1 million DNA molecules if you want to reach a sensitivity around 10^{-6} . NGS technology, in particular, introduces errors into DNA sequence as a consequence of DNA amplification, so the use of polymerases with high fidelity is required to minimize this weak point associated with this technology. Obviously, the use of spike-in calibrators and quality control is recommended to ensure the veracity of the results, but it is not mandatory if the analyst provide an accurate bioinformatics pipeline able to address this problem. At present, the great inconvenient for NGS is the analysis of data and the interpretation of the results. An appropriate bioinformatic program to evaluated MRD levels able to discriminate MRD positive/negative patients is necessary. Cheng *et al.* used LymphoTrack-Miseq software for the identification of VDJ sequences of clones with 5% or more of total reads at diagnosis. However, they used the sequence of VDJ junction which confers a greater sensitivity than an entire VDJ sequence for MRD tracking. But there are also some freeware tools to identify the specific clonotype by read-for-read assessment (IMGT/HighVQuest, MixCR) (14).

In conclusion, MRD by NGS could be the new gold standard for MRD detection in B-ALL. The detailed work described by Cheng *et al.* shows that this NGS method has good sensitivity, reproducibility and above all, it can easily be implemented in clinical settings. This work also shows that it is a more specific method in the identification of leukemic cells than other conventional MRD methods.

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Footnote

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Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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