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High-sensitivity next-generation sequencing MRD assessment in ALL identifies patients at very low risk of relapse

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Key Points

- Many patients who are "MRD negative" by standard MFC have clinically significant MRD that is detectable with an ultrasensitive NGS assay.
- Early achievement of MRD negativity using a high-sensitivity NGS assay identifies patients who have a very low risk of relapse.

Measurable residual disease (MRD) is highly prognostic for relapse and overall survival (OS) in acute lymphoblastic leukemia (ALL), although many patients with apparent "MRD negativity" by standard assays still relapse. We evaluated the clinical impact of a highly sensitive next-generation sequencing (NGS) MRD assay in 74 adults with ALL undergoing frontline therapy. Among remission samples that were MRD negative by multiparameter flow cytometry (MFC), 46% were MRD^+ by the NGS assay. After 1 cycle of induction chemotherapy, MRD negativity by MFC at a sensitivity of 1×10^{-4} and NGS at a sensitivity of 1×10^{-6} was achieved in 66% and 23% of patients, respectively. The 5-year cumulative incidence of relapse (CIR) among patients who achieved MRD negativity by MFC at complete remission (CR) was 29%; in contrast, no patients who achieved early MRD negativity by NGS relapsed, and their 5-year OS was 90%. NGS MRD negativity at CR was associated with significantly decreased risk of relapse compared with MRD positivity (5-year CIR, 0% vs 45%, respectively; $P = .04$). Among patients who were MRD negative by MFC, detection of low levels of MRD by NGS identified patients who still had a significant risk of relapse (5-year CIR, 39%). Early assessment of MRD using a highly sensitive NGS assay adds clinically relevant prognostic information to standard MFC-based approaches and can identify patients with ALL undergoing frontline therapy who have a very low risk of relapse and excellent long-term survival.

Introduction

Persistent or recurrent measurable residual disease (MRD) after initial therapy for acute lymphoblastic leukemia (ALL) is associated with high rates of relapses and poor overall survival (OS) across many retrospective and prospective studies, including in a large meta-analysis.[1-8] Although achievement of MRD negativity is associated with more favorable outcomes, this level of response is not fully protective against relapse. In many reports, patients who are "MRD^-" by conventional assays still have relapse rates of 25% and higher.[2,8,9] These relapses are presumably driven by very low levels of residual disease that persist below the level of detection of conventional assays and predispose to subsequent relapse. For patients with Philadelphia chromosome (Ph)-negative ALL, the most common MRD assays in clinical practice are a patient-specific quantitative polymerase chain reaction (PCR) assay to identify clonal

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immunoglobulin heavy chain (IGH) or T-cell receptor (TCR) rearrangements, which is used in many regions of Europe, and multiparameter flow cytometry (MFC), which is widely used in the United States and in other countries.[10] Standard MFC assays are capable of detecting residual leukemia cells in 1 out of 10 000 cells (1×10^{-4} or 0.01%), although some ≥8-color MFC assays may achieve a sensitivity of 10^{-5} with adequate cellular input.[11-13]

MRD assays that can achieve higher levels of sensitivity than conventional MFC and PCR will be better able to detect and quantify low levels of MRD and may further improve our prognostication in ALL. Highly sensitive next-generation sequencing (NGS)-based MRD assays have been developed that can detect MRD at a sensitivity of 1×10^{-6}, which is 1 to 2 logs deeper than standard MFC or PCR assays.[10] The clonoSEQ MRD assay is Food and Drug Administration–cleared for MRD detection in ALL, although the clinical utility of this assay compared with other standard MRD assays has not been robustly proven across clinical contexts. Previous studies have shown that this NGS assay can detect low levels of MRD in patients who were considered "MRD$^-$" by less sensitive MFC or PCR technologies.[14,15] It has also been shown to outperform conventional MRD assays and improve risk stratification in childhood ALL[14,16] and in patients with ALL who have undergone allogeneic stem cell transplantation (alloSCT).[15,17] However, there are limited data supporting the use of a high-sensitivity NGS-based MRD assay in adults with ALL undergoing frontline therapy and demonstrating its superiority compared with other standard-of-care MRD assays in this context. We therefore sought to evaluate the clinical utility of this NGS MRD assay in adult patients with newly diagnosed ALL, with the goal of determining whether achievement of MRD negativity with this high-sensitivity assay could identify patients who have a very low risk of relapse. We also sought to determine whether this NGS assay could provide additional prognostic information to standard MFC-based MRD assessment.

Methods

Study design and participants

This is a retrospective study evaluating the prognostic impact of a highly sensitivity NGS MRD assay in patients with Ph$^-$ ALL. Eligible patients received frontline ALL therapy at our institution between 2/2012 and 7/2018 using a backbone of either hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine) or mini–hyper-CVD (hyperfractionated cyclophosphamide, vincristine and dexamethasone alternating with methotrexate and cytarabine) and achieved complete remission (CR) as best response after 1 induction cycle. Patients were selected for inclusion based on the availability of a banked pretreatment bone marrow sample (for purposes of clonality determination) and at least 1 post-treatment remission bone marrow sample collected between 1 month and 4.5 months from the start of treatment (for purposes of MRD tracking). This study was conducted at a single academic center (The University of Texas MD Anderson Cancer Center). This study was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center and was conducted in accordance with the Declaration of Helsinki.

Multiparameter flow cytometry for MRD assessment

MRD assessment was performed on fresh bone marrow aspiration samples using 6-color multiparameter flow cytometry (MFC) as described previously.[18] MRD was defined in comparison with known patterns of antigen expression by normal maturing B-cell precursors (hematogones). Analysis was performed on at least 200 000 cells, and a minimum of 20 events was required to constitute a leukemic blast population. The analytic sensitivity of the assay was 0.01%. Per our institutional practice, MFC for MRD was assessed with each remission bone marrow, generally every 1 to 3 months for the first year of treatment. MFC MRD information was available from the clinical laboratory and was available to clinicians for decision making.

NGS assay for MRD assessment

The clonoSEQ MRD assay (Adaptive Biotechnologies Co., Seattle, WA) is an NGS-based immunosequencing assay that was used for this analysis. Pretreatment genomic DNA derived from stored bone marrow specimens was sequenced using multiplex PCR assays composed of primers targeted to the variable genes (forward primers) and joining genes (reverse primers) of the CDR3 region within immunoglobin and TCR genes. For B-cell tumors, sequences of the IGH, IGK, and IGK receptor genes, as well as IGH/BCL1 and IGH/BCL2 translocations, were assessed. For T-cell tumors, separate assays were used to assess the CDR3 regions of TCR-γ (TCRG) and TCR-β. Because 30% to 50% of patients with B-cell ALL have a trackable TCR sequence,[19] samples for which a trackable B-cell receptor sequence was not identified were reflexed for analysis of TCRG followed subsequently by TCRB. After the PCR and NGS steps were completed, the data were analyzed via proprietary algorithms to mitigate residual amplification bias that may be introduced by the PCR reactions.[20,21] Using high-throughput NGS, the index sequence(s) identified in the diagnostic sample were specifically searched for and quantified, if present, in the remission bone marrow. This assay has an analytic sensitivity of 0.0001% (1×10^{-6}), with a sensitivity that is dependent on the cellular input. To achieve 95% confidence that the frequency of the clone of interest is $<10^{-6}$ in a remission sample, an input of 1.9 million cells was required. NGS-based MRD assessment was performed retrospectively, and this information was therefore not available to clinicians for decision-making.

Response and outcome definitions

CR was defined as 5% or fewer blasts in the bone marrow with an absolute neutrophil $\geq 1 \times 10^9$/L and a platelet count of $\geq 100 \times 10^9$/L. Relapse was defined as recurrence of bone marrow blasts >5% or extramedullary ALL. Cumulative incidence of relapse (CIR) was calculated from the time of CR until relapse, censored for death in CR or if the patient was alive at last follow-up. OS was calculated from the time of treatment initiation until death from any cause, censored if the patient was alive at last follow-up. Survival estimates were not censored at the time of alloSCT.

Statistical methods

Patient characteristics were summarized using median (range) for continuous variables and frequencies (percentages) for categorical variables. To compare 2 groups, Fisher's exact test was performed for categorical variables, and Wilcoxon rank-sum test was performed for continuous variables. The Kaplan-Meier method was used to

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estimate the probabilities for RFS and OS, and differences between groups were evaluated with the log-rank test. The Gray's test was used to compare cumulative incidence probabilities between groups. All statistical analyses were performed using GraphPad Prism 9.

Results

Calibration of ID samples

We identified 90 patients with Ph^- ALL who underwent frontline ALL therapy at our institution and had adequate banked pretreatment samples for NGS MRD analysis. Clonality of the baseline sample was able to be determined in 81 patients (overall calibration rate, 90%). Among these 81 baseline samples, 76 calibrated on BCR, 0 calibrated on TCRB, and 5 calibrated on TCRG. The median number of dominant sequences per baseline sample was 2 (range, 1-9). The characteristics for patients who underwent successful identification calibration and those who did not are shown in supplemental Table 1. The ID calibration rate was higher in patients with B-cell ALL than in those with T-cell ALL (96% vs 60%, respectively; $P < .001$). Among the 6 patients with early T-cell precursor ALL, only 3 (50%) calibrated, which may be reflective of the earlier stage of development of these blasts, which may manifest with absence of a clonal TCR.[22,23]

Study population characteristics

Among the 81 patients who underwent successful identification calibration, 74 had at least 1 remission sample for NGS MRD assessment. These 74 patients with complete ID and MRD information are the primary subjects of this analysis, and their baseline characteristics are shown in Table 1. Thirty-four patients (46%) had at least 1 poor-risk baseline feature, defined as either poor-risk cytogenetics (ie, low hypodiploidy/near triploidy, complex cytogenetics, or *KMT2A* rearrangement), CRLF2 positivity by MFC, or a *TP53* mutation. Fifty-one patients (69%) received a hyper-CVAD–based regimen, and 23 patients (31%) received a lower-intensity mini–hyper-CVD-based regimen as frontline ALL therapy. Specific treatment regimens are shown in supplemental Table 2. Four patients who received hyper-CVAD (8% of hyper-CVAD–treated patients) and 19 patients who received mini–hyper-CVD (83% of mini-hyper-CVD–treated patients) also received inotuzumab ozogamicin as part of their frontline regimen; no patients received blinatumomab as part of their frontline regimen. The median duration of follow-up for the entire study population is 64 months (range, 17-103 months). Twenty-three patients (31%) underwent alloSCT in first remission. At last follow-up, 18 patients (25%) had relapsed, and 26 patients (35%) had died. Of note, there was no significant difference in CIR or OS in the patients evaluated in this study compared with a cohort of 89 patients with ALL who received frontline therapy during the same time period at our institution and in whom banked samples were not available.

Correlation of MFC MRD and NGS MRD

The rates of MRD negativity at 3 different time points (at CR [ie, after the first induction course, or 3 to 4 weeks into therapy], at 1.5-3 months from the start of treatment, and at 3.5 to 4.5 months from the start of treatment) are shown in Figure 1. Forty-three patients had an MFC and NGS MRD sample available at CR, 46 patients had samples available at 1.5 to 3 months, and 34 patients had samples available at 3.5 to 4.5 months. The rate of MRD negativity by MFC was 72%, 72%, and 85%, respectively, at these 3 time points. The NGS MRD assay was performed on a total of 121 remission samples. Among 28 samples that were MRD^+ by MFC, all were confirmed to also be MRD^+ by NGS. Among 93 samples that were MRD^- by MFC, 23 were indeterminate for NGS MRD at 10^{-6} due to inadequate DNA/cellular input, and 70 were able to be classified as either MRD^+ or MRD negativity by the NGS assay. Among these 70 samples that were MRD^- by MFC and were evaluable for MRD assessment by NGS at 10^{-6}, 32 (46%) were MRD^+ by NGS. The median level of detectable MRD by the NGS assay among these discordant cases (ie, MRD^- by MFC and MRD^+ by NGS) was 0.0027% and was significantly lower than in the concordant cases (ie, MRD^+ by both MFC and NGS) where the median level of MRD was 0.125% ($P < .0001$). The discordance rates between MFC and NGS were higher at earlier timepoints than at later timepoints (60% at CR, 52% at 1.5 to 3 months, and 25% at 3.5 to 4.5 months), suggesting that NGS MRD assessment may have more utility when performed earlier in the treatment course.

Association of baseline characteristics and likelihood of MRD response

The baseline characteristics of patients who were MRD^- (n = 10), MRD^+ (n = 27), or MRD indeterminate (n = 6) at 10^{-6} by NGS at the time of CR are shown in supplemental Table 3. Interestingly, the rates of NGS MRD negativity were similar in patients who had at least 1 poor-risk disease feature and those who did not (24% vs 22%, respectively; $P = .89$). Among the 4 patients with a poor-risk baseline feature who achieved MRD negativity by NGS, 1 patient had complex cytogenetics, 1 patient had low hypodiploid karyotype with a *TP53* mutation, 1 patient had CRLF2 overexpression by MFC and a *TP53* mutation, and 1 patient had CRLF2 overexpression by MFC and a *JAK2* mutation. Similar rates of MRD negativity by NGS at CR were also observed between patients who received a hyper-CVAD or mini-hyper-CVD–based regimen (23% for both groups).

Impact of MRD status by MFC at CR

MRD negativity by MFC at the time of CR (ie, after 1 induction course) was achieved in 49 of the 74 (66%) evaluable patients with NGS MRD information. Eleven (22%) of these MRD^- patients underwent alloSCT in first remission. Despite achieving early MRD negativity by MFC, 12 patients still relapsed, with a median time to relapse of 16.3 months (range, 6.2 to 48.2 months). The 5-year CIR and OS rates for patients who were MRD^- by MFC at CR were 29% and 68%, respectively (supplemental Figure 1). Similar rates of relapse were observed in patients treated with either hyper-CVAD or mini–hyper-CVD regimens who were MRD^- by MFC (5-year CIR rates, 30% and 24%, respectively).

Impact of MRD status by NGS at CR

Ten patients of the 43 patients (23%) with an evaluable CR sample for NGS MRD analysis achieved NGS MRD negativity at 10^{-6}. Achieving MRD negativity by NGS at the time of CR better predicted the likelihood of durable remission than did achieving MRD negativity by MFC. None of the 10 patients who achieved NGS MRD negativity at 10^{-6} at the time of CR subsequently relapsed (CIR rate, 0%), and their 5-year OS rate was 90%. Of note, 1 patient who was 69 years of age achieved NGS MRD negativity at CR but died in remission after 7 months. All other patients who achieved NGS MRD negativity at CR were alive and in ongoing

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