

IN THE SPOTLIGHT

Genomics, Transcriptomics, and Minimal Residual Disease Detection: The Winning Team to Guide Treatment of Acute Lymphoblastic Leukemia



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Summary: Cytogenetics supported by additional molecular analyses and minimal residual disease detection have been successfully combined to improve the outcome of childhood acute lymphoblastic leukemia (ALL). Results from the St. Jude Total Therapy Study 16 demonstrate that some of the recently identified ALL subtypes can further guide risk stratification.

See related article by Jeha et al., p. 326 (8).

Childhood acute lymphoblastic leukemia (ALL) is the most common pediatric cancer and is now associated with good treatment response and long-term survival for most patients. Over the past 50 years, the 5-year overall survival has increased from less than 50% to more than 90% (1). This major improvement has been achieved by optimizing chemotherapy regimens, improved supportive care, and better risk stratification based on genetics, clinical characteristics at diagnosis, and early treatment response measured by minimal residual disease (MRD). However, despite this success, there are still subtypes of ALL with less favorable outcome. Identification of these patients is important at diagnosis or early during treatment to intensify therapy or to offer novel (experimental) therapies.

ALL therapy consists of intensive multiagent chemotherapy and is typically composed of remission induction followed by postinduction consolidation including central nervous system (CNS) prophylaxis, delayed intensification, and eventually maintenance therapy. Monitoring of MRD during the first weeks and months of treatment has shown to be the most sensitive and specific predictor of relapse risk in children with ALL (2). More recently, several study groups showed that treatment response measured by MRD can be used to adapt treatment intensity. The ALL10 study from the Dutch Childhood Oncology Group (DCOG) demonstrated that chemotherapy could be reduced in patients with ALL with undetectable MRD levels at end of induction without negative effect on the survival rate (3). In addition, the same study illustrated

that outcomes of patients with ALL with intermediate or high levels of MRD at end of induction and consolidation could be improved with therapy intensification (3). These findings were also confirmed in the UKALL 2003 study, which showed that therapy reduction could be done safely in patients with ALL with favorable MRD at end of induction, while children with persistent MRD at the end of induction had benefit from intensified postremission therapy (4). Importantly, the UKALL 2003 study demonstrated that the relapse risk associated with a specific MRD level varied according to the genetic subgroup (4). A more refined risk stratification can thus be achieved by integration of genetic subtype and MRD levels during treatment, illustrating the importance of identifying all clinically relevant subtypes in ALL.

Next-generation sequencing has revolutionized the discovery of novel chromosomal rearrangements, gene fusions, and mutations that remained undetectable by conventional karyotyping and previous research efforts. For ALL, RNA sequencing has been a key technology to further identify and characterize biologically meaningful subtypes, because this technology allows for the simultaneous detection of fusion transcripts, gene expression levels, splicing defects, and mutations/indels (in expressed genes). In this way, ALL cases with similar gene expression patterns can be identified even if there are no common fusion genes present. An example of this is the recent discovery of the *ETV6*–*RUNX1*-like subgroup that is characterized by a gene expression pattern similar to *ETV6*–*RUNX1* cases but without that fusion gene (5–7). These cases have the gene expression pattern in common as well as high frequency of *ETV6* and *IKZF1* mutations. Also, the *BCR*–*ABL1*-like subgroup was initially characterized on the basis of gene expression profiling, and later found to contain cases with rearrangements of *CRLF2*, *EPOR*, or tyrosine kinases such as *JAK2*, *ABL1*, *ABL2*, *CSF1R*, and *PDGFRB* (7). These *BCR*–*ABL1*-like patients have the same poor outcome as the fusion-positive *BCR*–*ABL1*-positive ALL patients and can benefit from the addition of a tyrosine kinase inhibitor to the treatment regimen. In recent years, such transcriptomic approaches have led to the identification of several (often rare) novel ALL subtypes (5–7). The clinical significance of

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these newly identified subgroups remained, however, somewhat unclear because these included patients with ALL from different clinical trials with different treatment regimens.

In this study by Jeha and colleagues, 598 patients with ALL were included between 2007 and 2017 in the St. Jude Total Therapy Study 16 (ClinicalTrials.gov: NCT00549848; ref. 8). This is a risk-directed treatment protocol based on well-recognized genetic abnormalities identifiable by conventional cytogenetic analysis and MRD response during the first weeks of treatment. The objective of this study was to evaluate if a higher dose of polyethylene glycol-conjugated asparaginase (PEG-asparaginase; 3,500 IU/m² vs. conventional dose of 2,500 IU/m²) and if early intensification of intrathecal chemotherapy for a subgroup of patients with increased risk of CNS relapse would improve systemic and CNS outcome (9). Higher doses of PEG-asparaginase failed to improve outcome, but the study showed that additional intrathecal therapy during early induction contributed to improved CNS control without excessive toxicity for patients presenting with features associated with increased risk of CNS relapse (9). The clinical data of the 598 ALL cases treated with MRD-based therapy in the Total Therapy Study 16 were reanalyzed by Jeha and colleagues to determine the prognostic and therapeutic implications of all the currently known ALL subtypes identifiable by conventional cytogenetic analysis and RNA sequencing.

Risk classification in the St. Jude Total Therapy Study 16 was performed on the basis of immunophenotype [B-cell ALL (B-ALL), T-cell ALL (T-ALL), early T-cell precursor ALL (ETP-ALL)], age, blood leukocyte count, DNA index (to identify those cases with high hyperdiploidy), the presence of specific chromosomal rearrangements (*ETV6-RUNX1*, *BCR-ABL1*, *KMT2A* rearrangements), and MRD levels. MRD was measured by flow cytometry in blood on day 8 and in bone marrow on day 15 and day 42 (end of remission induction). RNA-sequencing data were available for 502 cases, which allowed the authors to further refine these cases and identify patients with new ALL subtypes (8). Of note, RNA sequencing was only used retrospectively to assign cases to all the new subtypes; it was not used prospectively to help determine the risk groups. This was also not possible in 2007 when the study was initiated, as the new subtypes were only defined in recent years.

About half of the ALL cases in this study (302 of 598) were part of the *ETV6-RUNX1* ($n = 128$), high-hyperdiploid ($n = 154$), or *DUX4*-rearranged ($n = 20$) subtypes, and these B-ALL cases showed the highest overall survival rates (95%–98%) and the lowest relapse rates (0%–3%). None of these cases were treated as high risk and none of these cases had MRD levels $\geq 1\%$ at day 42 (end of remission induction). Moreover, the majority of these cases were treated as low risk, but that was less clear for the *DUX4*-rearranged subtype where relatively more cases were treated as standard risk. Despite the usually good prognosis of *ETV6-RUNX1* and high-hyperdiploid cases, 7 patients with *ETV6-RUNX1* and 37 with high-hyperdiploid subtypes received standard-risk treatment because of day 15 MRD $\geq 1\%$, resulting in intensification of treatment. On the other hand, none of the 95 patients with *ETV6-RUNX1* or high-hyperdiploid ALL relapsed if they had day 8 MRD $< 0.01\%$ in blood and received low-risk therapy. These data suggest that this latter group of ALL patients could be considered for further treatment reduction to avoid unnecessary side effects of the treatment.

One tenth of patients in this trial (58 of 598) were treated as high risk. This group included all *BCR-ABL1*-cases, all ETP cases, 35% of *KMT2A*-rearranged cases, 20% of *BCR-ABL1*-like, and 16% of T-ALL cases. These are all subtypes with known increased relapse risk, and despite the intensified treatment for these subtypes also with the addition of dasatinib for patients with *BCR-ABL1*-positive ALL, 5-year event-free survival (EFS) and overall survival were below 82% with increased risk for relapse.

From the recently identified new subgroups, *ETV6-RUNX1*-like, *MEFD2*-rearranged, and *PAX5*-altered (*PAX5alt*) showed higher relapse risk, even if the patient had achieved day 42 MRD $< 0.01\%$. Overall, the *KMT2A*-rearranged, *ETV6-RUNX1*-like, and *MEFD2*-rearranged subtypes (together 7% of the ALL cases) had the lowest 5-year EFS of 64% to 67%.

Seventeen percent of the leukemias were of T-cell origin and were classified as T-ALL or ETP-ALL. These patients had a 5-year EFS of about 80%. In the T-ALL group, several subtypes can be defined on the basis of the expression of transcription factors or fusion genes. When looking at these subtypes, it became clear that patients from the *HOXA* or *LMO1/LMO2* subtypes were more often treated as high risk due to day 42 MRD $\geq 1\%$ and these cases had higher risk of relapse compared with the other T-ALL subtypes. All 10 ETP-ALL cases in the study were treated as high risk, according to observations that this was a poor prognostic subtype in previous studies, which now resulted in a similar 5-year EFS and risk for relapse between T-ALL and ETP-ALL patients.

The results of this study confirm, once again, that molecular analyses to define the ALL subtypes in combination with MRD assessment at different time points during treatment are important to guide treatment choices. Importantly, the data further indicate that some of the more recently identified ALL subtypes also have prognostic value and should be taken into account. However, incorporation of this knowledge in current treatment of patients with ALL requires a fast and accurate assignment of each new case to one of the ALL subtypes. This is relatively easy if clear cytogenetic markers or cell-surface markers exist, but is more difficult when gene-expression signatures need to be used, such as for example to identify the *BCR-ABL1*-like or *ETV6-RUNX1*-like subtypes. Of the new subtypes, *DUX4*-rearranged ALL is clearly among the most favorable subtypes (EFS 95%), while *PAX5alt* can be considered as intermediate-risk group (EFS 83%). Also, the new subtypes *ZNF384*-rearranged, *NUTM1*-rearranged, and *PAX5P80R* are considered here to have intermediate-risk EFS rates. A high frequency of relapse was observed in the new subtypes *MEFD2*-rearranged, *ETV6-RUNX1*-like, and *PAX5alt*, and even MRD $< 0.01\%$ on day 42 did not guarantee absence of relapse in these subtypes. These subtypes may require additional molecularly targeted therapy or immunotherapy to improve outcome.

A reliable molecular analysis of ALL, including the capability to detect rare subtypes, in combination with MRD assessment remains essential to guide optimal risk-adapted treatment. This not only leads to improved survival of patients at higher risk, but also reduces morbidity for those patients where less intensive treatment is equally effective (10).

Authors' Disclosures

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