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The origin of relapse in pediatric T-cell acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) comprises a group of hematologic neoplasms that arise from the malignant transformation of lymphoid B-lineage or T-lineage progenitor cells. ALL is the most common malignancy in childhood, accounting for almost 30% of pediatric cancers.[1] Considerable advances in the treatment of childhood ALL have been made in the past decades, and 5-year survival rates now exceed 85% in children. However, approximately 15%-20% of those ALL cases relapse and have a poor prognosis.[2,3] Because most intensive chemotherapy regimens have reached the limit of tolerability, current research efforts are focusing on identifying new targets for the development of more effective therapies, a strategy which requires a detailed understanding of the biology of these leukemias.

ALL is characterized by a multistep oncogenic process, in which a plethora of genomic lesions accumulate and cooperate to alter normal mechanisms that control cell cycle, proliferation, differentiation and survival of lymphocytes. Over the last decade, studies using gene expression profiling, DNA copy-number analyses, and next-generation sequencing have provided new insights into the pathogenesis and clinical behavior of ALL.[4] Furthermore, sequencing studies of matched diagnostic, remission and relapse samples have provided important insights into the correlation of the different mutations, clonal evolution, and treatment resistance.[4]

Using single nucleotide polymorphism array technology, Charles Mullighan and colleagues performed genome-wide copy number analysis and loss-of-heterozygosity (LOH) analysis on matched diagnostic and relapse pediatric ALL samples.[5] They observed a significant increase in the number of chromosomal deletions in B-ALL samples taken at relapse, but no significant changes were observed in T-ALL.[5] Based on a comparison of the chromosomal deletions identified in paired diagnosis-relapse samples, these investigators were able to conclude that in about half of the cases of B-ALL the relapse clone was derived from the major leukemic clone at diagnosis. However, in the other half of B-ALL cases, the relapse clone was derived from a pre-leukemic clone or a minor clone present at diagnosis, since the relapse clone had very few dele-

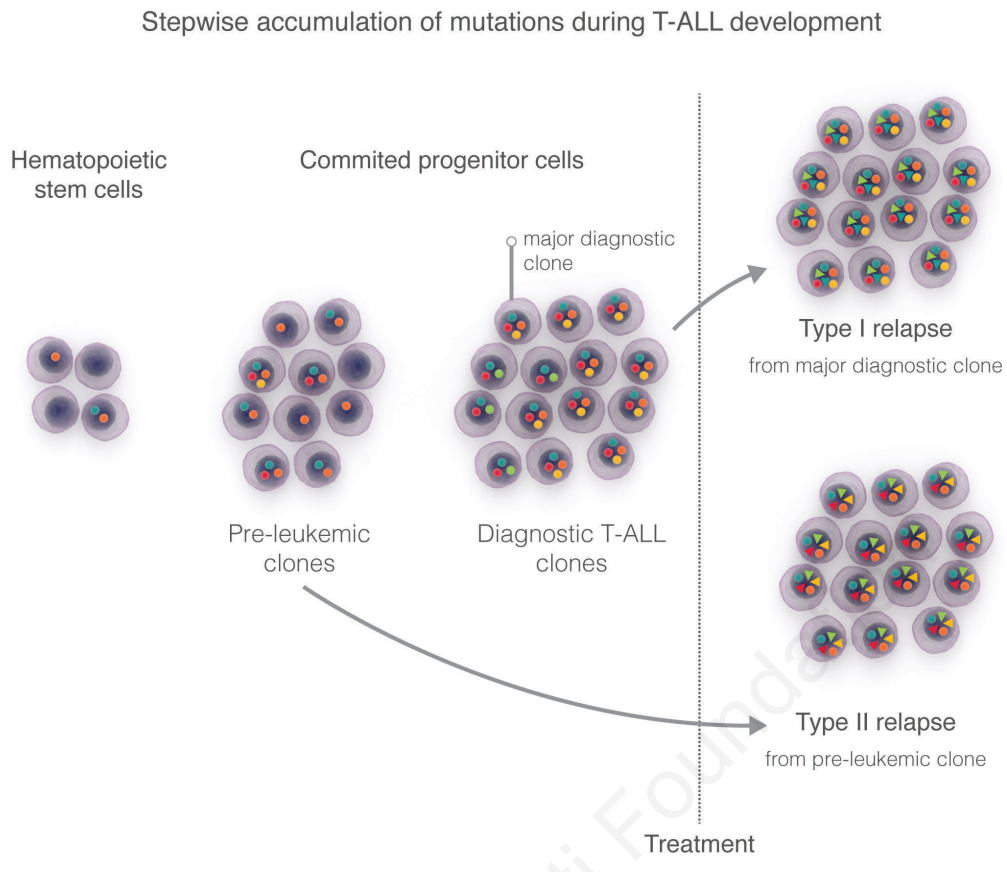

Figure 1. Schematic representation of clonal evolution during the development of T-ALL by the stepwise accumulation of additional mutations. As described by Kunz *et al.* in this issue, relapse clones can originate from pre-leukemic clones by the acquisition of new mutations or can originate from the major diagnostic clone by the acquisition of additional mutations.

tions in common with the major diagnostic clone. For T-ALL, such analysis was not informative, as there were insufficient copy number variations present.

Whole-exome sequencing of paired diagnosis-relapse B-ALL cases provided increased resolution to study relapse-specific changes and led to the identification of somatic mutations in *NT5C2* or *PRPS1* genes as a cause of relapse. *NT5C2* encodes a 5'-nucleotidase and the mutations were shown to confer increased enzymatic activity and resistance to treatment with nucleoside analog therapies.[6,7] Similarly, *PRPS1* encodes a phosphoribosyl pyrophosphate synthetase enzyme implicated in *de novo* purine synthesis and the PRPS1 mutants drive constitutive *de novo* purine synthesis. Ma and colleagues confirmed the frequent mutation of *NT5C2* in relapsed B-ALL and identified additional signaling pathways that are frequently mutated, including *CREBBP*, *WHSC1*, *TP53*, *USH2A*, *NRAS* and *IKZF1*.[8]

While we have a comprehensive picture of the genomic complexity of T-ALL at diagnosis,[9-12] data on relapsed T-ALL cases are limited. In this issue of Haematologica, Kunz and colleagues explore the genetic basis and clonal evolution of diagnosis and relapse samples in pediatric T-ALL.[13] They performed an integrated genomic analysis of 13 T-ALL cases at the time of diagnosis, during remission and at relapse. They showed that all 13 relapse samples had at least some mutations in common with the diagnostic clone, confirming that all relapses were related to the diagnostic clone. There were, however, two different patterns of relapse in T-ALL: about half of the relapse samples (type 1) were characterized by the presence of additional mutations on top of the mutations present in the major diagnostic clone, while the other half of relapse samples (type 2) harbored totally new mutations and were not similar to the diagnostic clone (Figure 1). These two types of relapse show strong similarity to the mechanisms of relapse described for B-ALL, with the difference that in B-ALL novel chromosomal deletions are often acquired at relapse, while this is not common in T-ALL.

On average, Kunz *et al.* found that samples at first diagnosis carried 11.5 somatic single nucleotide variants and indels, similar to the findings of previous studies.[9] Matched samples at the time of relapse, however, carried 26 somatic single nucleotide variants and indels, showing a significant increase in the mutational load at relapse. This is somehow unexpected. If relapse occurs as a consequence of the fact that the leukemia clone is not completely eradicated, then the leukemia could just grow back without the need to acquire more mutations. Even if a relapse originates from a pre-leukemic clone, that clone would need to acquire new mutations, but no more than is needed for a T-ALL at first diagnosis. A possible expla-

nation for the increased mutational load at relapse is the incorporation of mutations during chemotherapy. Indeed, Kunz *et al.* observed that mutations at the central position of the trinucleotides TCA and GCT were significantly more frequent among relapse-specific single nucleotide variants. This could be linked to an effect of the drug cytarabine (arabinofuranosyl cytidine), which is incorporated into DNA instead of the normal deoxycytidine and as a consequence causes problems during DNA replication of rapidly dividing cells. Thus, at least part of the additional mutations present at relapse could be therapy induced.

Methylome analysis revealed that overall DNA methylation levels hardly differ between primary leukemia and relapse. However, a set of promoters was found to be recurrently hypomethylated in relapse samples compared to samples taken at initial diagnosis. These results suggest that DNA methylation changes may cooperate with mutational events in driving T-ALL relapse, although correlation analyses between methylation and gene expression profiles would be necessary to further clarify this issue. It will also be of interest to determine whether there is any link between the acquisition of mutations in DNA methylases or TET proteins and observed differences in methylation patterns.

Most interestingly, and similar to the situation in relapsed B-ALL, *NT5C2* was identified as a recurrently mutated gene associated with relapse in five of 13 cases and in both type 1 and type 2 relapse samples. A somewhat unexpected finding, however, was that *NT5C2* mutations can be subclonal at relapse, indicating that the *NT5C2* mutation is not the initiating event of relapse and only one of the factors contributing to therapy resistance. Also, *NT5C2* mutations were identified in subclones at diagnosis in two of the cases, indicating that such mutations may also provide a benefit. No other recurrent mutations were detected at relapse. Furthermore, and in agreement with previous studies,[5] the number of copy number alterations did not differ between diagnosis and relapsed samples, indicating that such deletions or duplications do not contribute significantly to relapse in T-ALL.

Of interest, Kunz *et al.* show that relapse-specific alterations tend to activate general mechanisms of carcinogenesis rather than known leukemia-specific drivers. Their observations suggest that T-ALL development requires specific genetic changes that affect hematopoietic precursors and T-cell-specific pathways, while disease progression is associated with the acquisition of mutations in more general oncogenic pathways. In addition, there are mutations such as those in *NT5C2* that confer direct resistance to drug treatment.

These novel results provide important insights into the genetic basis of relapse in pediatric T-ALL. In addition, the data reveal some of the direct consequences of chemotherapy on DNA and identify mechanisms by which T-ALL cells can become resistant to steroid treatment.

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