IN THE SPOTLIGHT

Deciphering the Clonal Origin of Relapsed Acute Lymphoblastic Leukemia in Children

Seishi Ogawa[1,2,3]

Summary: In this issue of *Blood Cancer Discovery*, Waanders and colleagues characterize somatic alterations in a large cohort of relapsed pediatric acute lymphoblastic leukemia (ALL). This comprehensive genomic analysis reveals mutations distinctly associated with primary disease versus response to therapy. In the reconstructed clonal evolution scenarios, relapsed leukemic cells propagate from clones already expanded at diagnosis and rarely from unexpanded dormant ancestral clones. The information gleaned through subclonal mutation analysis at diagnosis may help to estimate relapse risk and select therapeutic options with minimal relapse potential. High prevalence of hypermutation patterns among repeatedly relapsing ALL cases suggests that activating antitumor immunity has a potential to benefit this group of patients.

See related article by Waanders et al., p. 96 (2).

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer (1). Once showing an invariably dismal prognosis, most cases with pediatric ALL now can be cured with intensive chemotherapy with or without tyrosine kinase inhibitors; during the past 40 years, 5-year overall survival has dramatically improved from less than 10% in the late 1960s to over 90% in the most current studies (1). However, as many as 10% of pediatric ALL cases experience a relapsed disease with poor prognosis, with relapsed ALL being the second leading cause of cancer-related deaths in children, even with the advent of immunotherapy using chimeric antigen receptor (CAR)–T cells (1). Together with rare cases of primary refractory diseases, relapsed ALL represents a major challenge to improve outcome of pediatric (and also adult) ALL. This is at least partly due to the fact that our knowledge about ALL relapse is still very limited. What are the genetic alterations responsible for relapsed diseases, what is their clonal origin, what mutational processes are involved therein, and most importantly, what molecular events are targetable for therapy? These are the key questions to be answered to better manage, predict, or even prevent relapse to further improve clinical outcome of pediatric ALL.

In this issue of *Blood Cancer Discovery* (2), Waanders and colleagues addressed these questions through investigating 92 cases with relapsed pediatric ALL, including 67 B-progenitor (B-ALL), and 25 early T-cell precursor (n = 7) or other T-lineage (n = 18) ALLs, in which longitudinal samples, including diagnostic and relapse samples, were analyzed for somatic events, combining genome and transcriptome sequencing with SNP array–based copy number analysis. This work considerably expands the scale and resolution of earlier studies tackling this question (3, 4). Through this comprehensive characterization of the largest relapsed ALL cohort to date, Waanders and colleagues delineated a mutational landscape of relapsed ALL with their clonal structure and evolutional trajectories, providing an important clue to understand how relapse-fated clones survive initial therapy and ultimately give rise to relapsed diseases, in terms of its clonal origin and somatic mutations, and how these findings can be utilized for early detection/prediction and therapy of relapsed ALL.

FEATURES OF SOMATIC MUTATIONS IN RELAPSED ALL

In terms of somatic mutation, relapsed ALL had substantially different mutational profiles from those seen at initial diagnosis. For example, 74% of all mutations in relapsed ALL, including single-nucleotide variations (SNV) and indels, were absent from diagnostic samples but newly acquired. In total, 50 genes were significantly enriched as mutational targets in relapsed diseases in that mutations in these genes were newly acquired or increased their allele frequency at relapse. Among these, 29 are known to be involved in ALL pathogenesis but more enriched in relapse than diagnosis samples, where they might have persisted from diagnosis or newly mutated at relapse, although six (*NT5C2, LRP1B, USH2A, APC2, PIK3R4*, and *NCOR2*) were exclusively found at relapse, while mutations in other genes, including *TPTRT, ROBO2*, and *TRRAP*, persisted from diagnosis to relapse. Other mutations enriched in relapsed samples involved the RAS pathway and epigenetic regulators. Although newly acquired at relapse in some cases, RAS pathway mutations are also present at the time of diagnosis, particularly in B-ALL. In the latter cases, they frequently appear as multiple independent RAS pathway mutations in multiple subclones, which converged into one or two clonal mutations at relapse, most likely as a result of clonal selection during initial therapy. Epigenetic regulators enriched in relapse

[1]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [2]Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto, Japan. [3]Department of Medicine, Center for Hematology and Regenerative Medicine, Karolinska Institute, Stockholm, Sweden.

Corresponding Author: Seishi Ogawa, Kyoto University School of Medicine, Kyoto 113-8655, Japan. Phone: 813-5800-9046; Fax: 813-5800-9047; E-mail: sogawa-tky@umin.ac.jp

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samples included *PRDM2, PHF19, TET3*, and *SIN3A*, and other genes, many of which have not been reported in ALL.

ORIGIN OF RELAPSED CLONES

The authors also investigated the clonal origin of relapse. Although a previous study had addressed this issue relying on copy number abnormalities (CNA) as detected by SNP array analysis (5), the clonal origin was more accurately determined here using integrated genomics and xenografting with limiting dilution of diagnostic and relapsed samples. In accordance with the previous observation based on SNP array analyses, half of the relapses originated from a minor subclone already present at diagnosis, while one fourth originated from a major diagnostic clone. Relapse was polyclonal in a minority cases, where multiple subclones at diagnosis contributed to the relapse (18%), as typically seen in the case of ALL harboring multiple RAS pathway–mutated subclones. In the remaining rare cases, relapse actually represented a second primary leukemia, which represented an independent leukemia caused on the basis of germline predisposition or developed from a common ancestral clone sharing the same gene fusion or mutations. The origin of relapsed ALL was further evaluated using limiting dilution followed by xenografting (6) of paired diagnosis and relapse samples from 8 patients, which was able to successfully separate two closely related clones. Importantly, relapse almost always propagated from a clone already present at the time of diagnosis, which was explicitly demonstrated by sensitively detecting mutations inherent to the relapse-fated clones using droplet digital PCR (ddPCR) between diagnosis and relapse. This is in line with the mechanism of resistance to TKI, reported for many cancer types (7). In an accompanying article, the authors isolated and characterized these drug-tolerant clones using extensive xenografting of paired diagnostic and relapse samples. Of note, those drug-tolerant clones had distinct engraftment and metabolic properties and transcriptionally displayed enrichment for chromatin remodeling, mitochondrial metabolism, proteostasis programs, and an increase in stemness pathways (8). These findings suggest that sensitive detection of shared mutations can be used for monitoring the persistence of relapse-fated leukemic cells and during complete remission (CR) for early detection of relapse.

FREQUENT HYPERMUTATIONS IN RELAPSED DISEASES

Another new finding of potential clinical importance, which has not been well recognized and is rather unexpected, is high frequency of hypermutations in relapsed diseases. Defined by an inflection at 85 mutations/exome, approximately 1.3 mutations/Mb, hypermutations were found in only 3% of diagnostic samples but at significantly higher frequency in 17% of first relapses and as high as 64% of second relapses, regardless of molecular subtype of ALL. Analysis of mutational signatures delineated several mechanisms implicated in the hypermutations in relapsed ALL, including endogenous activation of cytosine deaminase such as AID/APOBE and biallelic inactivation of mismatch repair (MMR) genes. The most significant implication of frequent hypermutations in relapsed ALL is a possibility that many relapsed ALL could be responsive to treatments activating anticancer immunity such as checkpoint inhibitors. High mutational burden correlates with immunogenicity and with clinical response to immune checkpoint blockade in many cancer types, presumably due to mutagenesis-derived neoantigens. Consistent with this possibility, the neoantigen-predicting algorithm employed in this study identified HLA-restricted neoantigens encoded and expressed by hypermutated ALL cells, which make them potentially recognizable by cancer-specific cytotoxic T cells. Thus, a testable clinical question emerging from this work is whether hypermutated relapsed ALL is amenable to therapies activating antileukemia immunity.

QUESTIONS UNSOLVED

Despite comprehensive detection of somatic alterations associated with relapsed ALL, a critical question remains to be answered: What is the mechanism of progressive therapy resistance in relapsed diseases? Except for rare mutations implicated in the resistance to glucocorticoids and thiopurines, it is largely unknown in most instances what mutations are responsible for the selection of relapsed clones and explain therapy resistance, and what is the underlying mechanism. Answering these questions will be essential for developing novel agents to overcome the resistance. Hypermutation, which is seen in increasing frequency with tumor recurrence, might partly explain this, while posing another set of questions. How hypermutated relapse clones evade or escape antitumor immunity despite the predicted abundance of neoantigens and whether the immune response can be harnessed to eradicate the relapsed clones via checkpoint inhibition or other means, are among the important issues to be addressed in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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