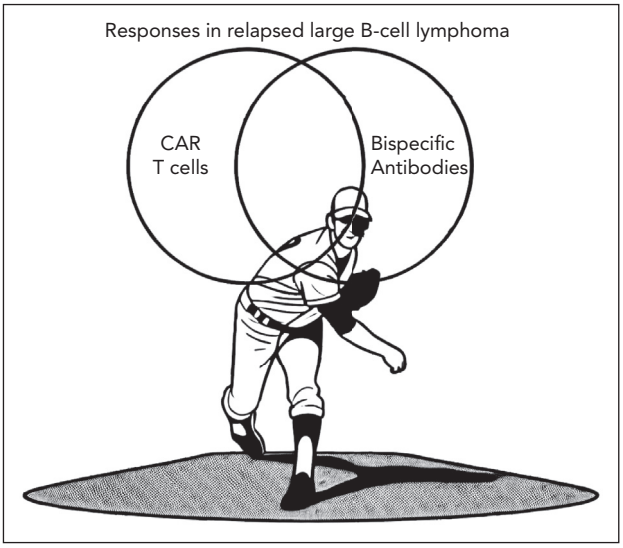

Venn diagram of lymphomas treated with CAR T-cell and bispecific therapies, demonstrating both overlapping and distinct responses.

therapy is critical. Third, this study partly addresses the emerging question: do resistance mechanisms to bispecific antibodies and CAR T-cells overlap or have important differences (see figure)? It appears that some patients who do not benefit from CAR T-cell therapy may benefit from odronextamab.

Odronextamab demonstrated notable efficacy in this difficult-to-treat population. However, limitations include the lack of a comparator arm to evaluate how odronextamab fares against other treatment options such as antibody-drug conjugates, allogeneic CAR T cells, bispecific antibodies combined with costimulatory molecules, small molecule inhibitors, or conventional chemotherapy. The schedule of odronextamab, twice weekly during the first cycle, a total of 27 intended infusions over 10 cycles, and indefinite therapy for responders, is cumbersome compared with other agents in clinical development for this disease indication. Additionally, patients with poor responses to CAR T-cell therapy, the biggest unmet need, also derived less benefit from odronextamab.

In conclusion, odronextamab is a solid base hit in patients who already have 2 strikes, progressive LBCL after chemoimmunotherapy and CAR T-cell therapy, with almost half of patients responding and a median response duration of over 14 months. Although future studies are warranted to further optimize outcomes, this study establishes odronextamab as a promising option for a population with limited alternatives.

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HEMATOPOIESIS AND STEM CELLS

Comment on Boudia et al, pages 1510

iPSCs unlock clues to pediatric AML onset

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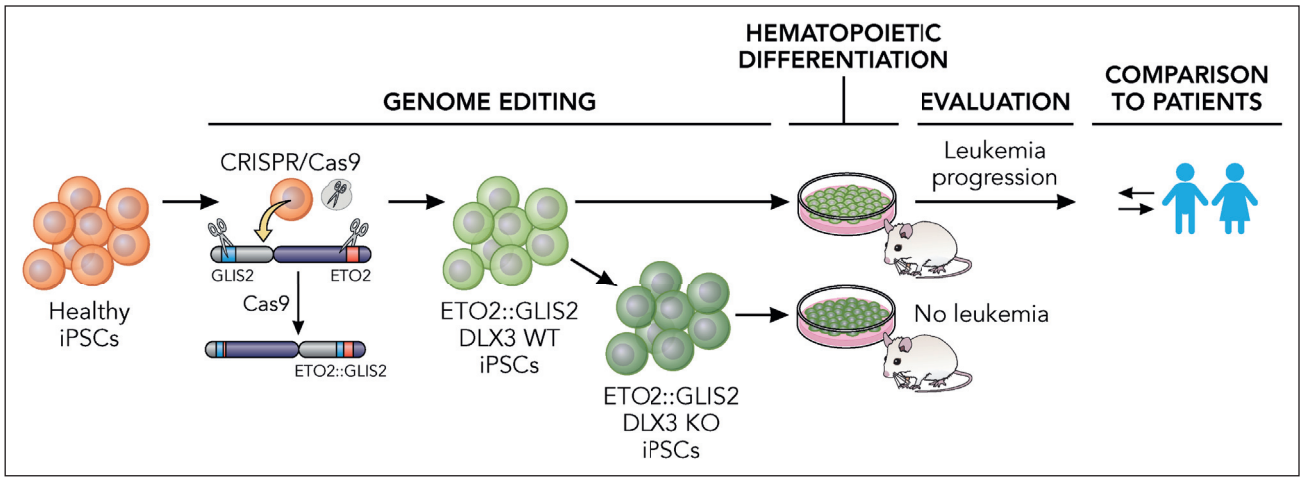
In this issue of *Blood*, Boudia and colleagues[1] introduce a human induced pluripotent stem cell (iPSC) model of ETO2::GLIS2-driven acute megakaryoblastic leukemia (AMKL). The model recapitulates the human disease, enabling a comprehensive molecular investigation that identifies osteogenic homeobox factor DLX3 as a critical player in the onset of leukemia.

Treatment outcomes of pediatric leukemia have drastically improved in recent decades, with survival rates of childhood acute myeloid leukemia (AML) now reaching 75%.[2] However, some subtypes still suffer from a dismal prognosis. One of these subgroups is non–Down syndrome AMKL, an aggressive and rare form of AML most frequently observed in young children and rarely seen in adults. In 2012, a cryptic inversion of chromosome 16 was described.[3,4] The inversion leads to expression of the ETO2::GLIS2 (also known as CBFA2T3::GLIS2) oncofusion protein, which was shown to induce self-renewal capacity and differentiation toward the megakaryocyte lineage.[5] It was found in almost 20% of the pediatric non–Down syndrome AMKL cases and is associated with a particularly poor outcome, with an overall survival of only 15% to 30%.[5] Onset of this condition, often in very young children, implies that the fusion may occur during fetal life, and the developmental susceptibility of the oncofusion has been confirmed in an animal model of the disease.[6]

The biological factors that trigger onset of the disease are poorly understood, and fetal characteristics are difficult to capture experimentally. Infantile AMKL is a prime example, as it is associated with development. Thus, a modeling strategy should faithfully recapitulate the developmental context and express the oncogene at physiological levels in relevant cell types. Boudia et al used a CRISPR/Cas9 approach to introduce the clinically relevant genetic aberration in human iPSCs. iPSCs are generated by reprogramming somatic cells to a pluripotent state. When these cells differentiate toward the hematopoietic lineage, they recapitulate features of embryonic hematopoiesis. Thus, they provide a human source of cells suitable for gene editing. The genetic complexity and clonal composition of the disease can be captured by generating iPS cell lines from samples from leukemic patients.[7] Boudia and colleagues have instead introduced the oncofusion into otherwise healthy iPSCs, allowing them to study the effect of a single driver in a controlled fashion.

Bertuccio et al have previously presented a model of the ETO2::GLIS2-fusion based on iPSCs.[8] In that model, the oncofusion was placed under the control of the pan-hematopoietic *CD43* promoter in otherwise healthy iPSCs. Although the model replicated some characteristics seen in patients with AMKL, no disease development occurred upon transplantation to mice. Here Boudia and colleagues took on a different approach, using CRISPR/Cas9 technology to introduce the inversion into healthy iPSCs (see figure). By adding guide RNAs targeting the breakpoint regions, precise engineering of the fusion could be achieved. The fusion was thereby placed under the control of the endogenous *ETO2* promoter, replicating what is seen in patients. The ETO2::GLIS2 iPS-derived hematopoietic cells could give rise to AML in mice, and phenocopied cellular and molecular features observed in pediatric patients with AMKL. One notable finding was the high expression of the cell surface protein CD56 seen in the model. High expression of CD56, in combination with low or negative expression of CD38, CD45, and HLA-DR, defines a distinct aberrant immunophenotype known as RAM. The RAM phenotype has a dismal prognosis[9] and is often specifically associated with ETO2::GLIS2 AMKL. Identification of a RAM phenotype in a diagnostic pediatric AML sample often prompts targeted analysis of the ETO2::GLIS2 translocation when genome-wide testing is not available. Thus, a RAM phenotype has significant clinical relevance, and the fact that the model developed by Boudia et al expresses high levels of CD56 further enhances its clinical value.

The authors also monitored the development of disease and found progressive changes at the chromatin level. Aberrant expression of *DLX3*, a homebox factor also linked to osteosarcoma, was identified as an early event. When DLX3 was overexpressed in normal cord blood cells, a change in the balance between GATA and ETS motifs was seen, features also observed in AMKL patients. In addition, ETO2::GLIS2 iPSCs-derived hematopoietic cells deficient in DLX3 had lost their leukemia-initiating capacity. Because ETO2::GLIS2-driven AMKL is relatively resistant to conventional high-intensity chemotherapy, alternative treatment strategies are needed to improve outcome for these patients. Targeting of the folate receptor 1 (FOLR1) has shown clinical potential,[10] and the role of DLX3 in these leukemias, as demonstrated by Boudia et al, could pave the way for novel therapeutic approaches.



An iPSC model of ETO2::GLIS2-driven acute megakaryoblastic leukemia. Boudia et al genome edited healthy iPSCs to express the ETO2::GLIS2 oncofusion. After differentiation to blood, cells were transplanted to mice and leukemia initiation and progression was evaluated. The features observed were compared with patient samples. The authors also generated ETO2::GLIS2 iPSCs deficient in DLX3 (KO), which resulted in loss of the leukemia-initiating ability in mice. iPSC, induced pluripotent stem cell; KO, knockout; WT, wild-type. Professional illustration by Patrick Lane, ScEYEnce Studios.

The work elegantly demonstrates how human iPSCs can be used to model the initiation of pediatric leukemia. By studying the ETO2::GLIS2 fusion in otherwise healthy cells, the onset of disease could be explored. Despite the challenges in generating transplantable iPS-derived hematopoietic cells, and the addition of only a single mutation, an engraftable leukemia was observed. Of clinical importance, the model may evolve into a drug screening platform for the development of novel treatment strategies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Yu et al, page 1526

Prizloncabtagene autoleucel: a new CAR T cell for B-NHL

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In this issue of *Blood*, Yu et al reported promising clinical results of prizloncabtagene autoleucel (prizlon-cel), a next-generation chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory B-cell non-Hodgkin lymphomas (r/r B-NHL).[1]

The advent of anti-CD19 CAR T-cell therapy has revolutionized the treatment landscape for relapsed/refractory large B-cell lymphomas (r/r LBCL) and, in general, for r/r B-NHL. Long-term survival outcomes of 30% to 40% have been reported in pivotal clinical trials with tisagenlecleucel (tisa-cel), axicabtagene ciloleucel (axi-cel), lisocabtagene maraleucel (liso-cel) with these results confirmed in real-world settings.[2-5] These advances have enabled treatment for a patient population previously limited to palliative care. Unfortunately, the majority of patients who receive such therapies experience relapse or disease progression within the first 6 to 12 months after infusion. Anti-CD19 CAR T-cell failure is associated with a grim median survival of just 6 months.[6] Furthermore, few of these patients are eligible for additional treatments, with an even smaller subset able to undergo more advanced or intensive therapies, such as potentially curative allogeneic hematopoietic-cell transplantation. Therefore, it is of paramount importance to enhance the efficacy of CAR T-cell therapy. But what strategies have been employed to improve efficacy?

Two main approaches have been adopted. The first involves using CAR T-cell therapies earlier in the treatment sequence. For example, axi-cel and liso-cel have been tested as second-line treatments for high-risk LBCL in the ZUMA-7 and TRANSFORM trials, respectively.[7,8] Other trials are exploring the use of currently available anti-CD19 CAR T-cell therapies or newer cell therapies as first-line treatments. The second approach focuses on improving the efficacy of CAR T-cell therapy itself. Several methods have been investigated. One strategy is to reduce manufacturing time, addressing the fact that many patients progress before receiving CAR T-cell therapy. These efforts include testing in vivo CAR T-cell expansion and allogeneic "off-the-shelf" products. Another strategy involves modifying the CAR T-cell product, for example, using specific T-cell subpopulations (eg, adjusting the CD4:CD8 ratio) for manufacturing CAR T cells. Advances in transfection techniques, such as replacing lentiviral vectors with transposon- or CRISPR-based methods, have also been explored. Additionally, modifications to the CAR construct itself, either in its extracellular portion (eg, bispecific or bicistronic CARs) or its intracellular portion (eg, third- or fourth-generation CAR T cells), are being tested. A third approach involves combining CAR T-cell therapy with synergistic drugs, such as ibrutinib.

The results of the phase 1 trial of prizlon-cel, a bispecific CAR T-cell therapy targeting both CD19 and CD20, conducted by Yu and colleagues, represent a promising strategy for advancing CAR T-cell therapy in B-NHL (with 92% of study patients with LBCL). Prizlon-cel directly addresses antigen heterogeneity and loss, which are key drivers of resistance in single-antigen CAR T-cell therapies. Dual targeting of CD19 and