Sequencing of bone marrow DNA may predict leukemia relapse after CAR-T therapy with sufficient time to intervene

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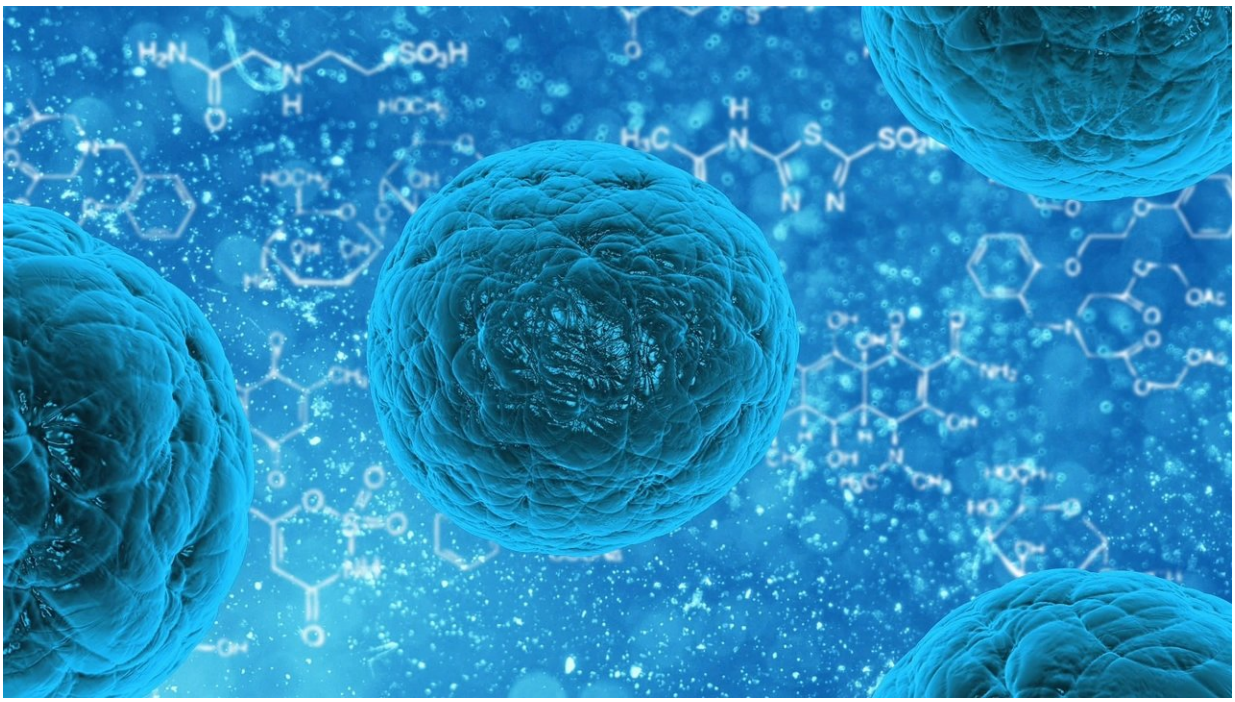

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In pediatric and young adult patients with acute lymphoblastic leukemia (ALL) treated with tisagenlecleucel (Kymriah), DNA sequencing-based detection of residual disease between three and 12 months accurately identified all patients who would eventually relapse, while other methods

were less predictive, according to a study published in *Blood Cancer Discovery*.

"This is the first paper to show an approach that identifies markers of relapse that are very specific, allowing clinicians to add additional therapy prior to relapse that will prevent it," said Michael Pulsipher, MD, lead author of the study and a professor of pediatrics and the division chief of Pediatric Hematology and Oncology at Intermountain Primary Children's Hospital and the Huntsman Cancer Institute of the University of Utah.

Tisagenlecleucel is a chimeric antigen receptor T-cell (CAR-T) treatment—a type of therapy in which patients' own T cells are harvested, reprogrammed to target a protein called CD19 that is expressed on the surface of cancer cells, and returned to the patient to fight ALL and lymphoma. Pulsipher said that over 80 percent of ALL patients treated with tisagenlecleucel experience a complete remission.

However, around half of patients who experience remission eventually relapse and require additional treatment, such as a bone marrow transplant. Accurately predicting relapse could allow patients who need a transplant to begin the process before the disease actually recurs.

Because the CD19 receptor is also expressed on normal B cells, treatment causes B-cell aplasia—the depletion of a patient's B cells. "Our current recommendation to centers giving tisagenlecleucel is to follow B cells in the blood monthly, using a standard test, as a way to predict patients at higher risk of relapse," said Stephan Grupp, MD, Ph.D., senior author of the study and a professor of pediatrics and the chief of Cell Therapy and Transplant at Children's Hospital of Philadelphia and the University of Pennsylvania. "Monitoring B-cell aplasia is not ideal because it only picks up part of the relapse risk." He noted that recurrence can sometimes occur in the absence of B-cell recovery, in the

form of tumor cells that do not express CD19 and can therefore evade functional CAR-T cells.

"A key goal for our patients and families is not only to define which patients are at increased relapse risk and need further therapy, but also to identify patients heading toward longer-term benefit who might not need further therapies such as transplant," Grupp said.

Liquid biopsies that investigate markers in the blood or bone marrow have increased in popularity, but these methods have not been directly compared. In this study, Pulsipher and colleagues investigated the predictive value of flow cytometry—a technique that interrogates proteins on the cell surface—and next-generation DNA sequencing minimal residual disease (NGS-MRD) monitoring, using blood and bone marrow samples collected from the ELIANA and ENSIGN phase II clinical trials at one, three, six, nine, and 12 months after tisagenlecleucel infusion. For flow cytometry, cells were analyzed for the presence of CD9, CD10, CD13, CD19, CD20, CD22, CD33, CD34, CD38, CD45, CD58, CD66c, and CD123. For NGS, the gene sequences of IgH, IgK, and IgL were analyzed for rearrangements and translocations.

The researchers found that, while flow cytometry could detect approximately one cancer cell per 10,000 blood cells, NGS-MRD was far more sensitive, with the ability to detect one cancer cell per 1 to 10 million blood cells, depending on the number of cells in the sample. This resulted in 131 percent more positive samples detected via NGS-MRD compared with flow cytometry.

NGS-MRD of bone marrow samples was more accurate in predicting relapse than flow cytometry. Of patients with any detectable disease DNA at three or six months post-infusion, 100 percent experienced either a relapse or progression to another therapy, with the exception of

one patient lost to follow-up. NGS-MRD detection was also able to find those at risk well in advance of relapse. Those who had NGS-MRD positivity at the lowest levels relapsed a median of 168 days after the positive test, and the assay detected 100 percent of the relapses. By contrast, flow cytometry was positive at a median 52 days prior to relapse and missed 50 percent of the relapses.

These data suggest that the more sensitive NGS measurements detect disease with sufficient lead time prior to relapse to allow repeat sampling and/or coordination of therapeutic interventions, Pulsipher said.

NGS-MRD was also more accurate than B-cell aplasia at predicting relapse. Three months post-treatment, B-cell recovery was not predictive of recurrence, while patients with a positive NGS sample had a 12-fold higher risk of recurrence.

Pulsipher recommended that NGS-MRD should complement monitoring of B-cell aplasia, rather than replacing it, however. He explained that, for some patients, 28 days may not be sufficient for CAR-T cells to rid the body of all primary disease, meaning these patients may test positive at one month but negative at three months. Therefore, the predictive value of NGS-MRD was lower at the one-month time point, reflecting a 4.87-fold increased risk for positive patients. B-cell recovery had a higher predictive value early after treatment, with positive patients experiencing a 3.33-fold higher risk of recurrence, which Pulsipher noted was insufficient on its own, due to the method's inability to identify tumor cells lacking the CD19 receptor.

Overall, Pulsipher and colleagues recommended that patients who lose B-cell aplasia within six months of therapy or present with NGS-MRD-positive disease in the marrow within the first year after therapy receive additional treatment to prevent relapse.

Pulsipher noted that his team is currently working on a prospective clinical trial to determine the feasibility of intervention before a patient experiences a recurrence based on the measurements from NGS-MRD testing.

In a commentary related to this study, Sara Ghorashian, BM BCh, Ph.D., and Jack Bartram, BM BCh, pediatric hematologists at the Great Ormond Street Hospital for Children in London, wrote, "To date there have been no reports systematically exploring the use of molecular MRD assessment following therapy with tisagenlecleucel. There is utility in the data Pulsipher et al. provide, comparing [flow cytometry] with NGS-MRD beyond the already-established increased sensitivity of the latter, but importantly, also exploring the optimal threshold for positivity and lead time to relapse from a positive result to obtain the best biomarker of relapse risk."

They added, "Whist it is clear from these data that NGS-MRD has the potential to be a powerful predictor of relapse post infusion of tisagenlecleucel, to take these data forward, the wider applicability of this approach in a multi-centre prospective validation is needed.

Limitations of this study include a relatively small number of samples assessed by NGS-MRD (just under 400), especially from peripheral blood, and the fact that samples were available for relatively few time points (one, three, six, nine, and 12 months).

More information: Next-Generation Sequencing of Minimal Residual Disease for Predicting Relapse after Tisagenlecleucel in Children and Young Adults with Acute Lymphoblastic Leukemia, *Blood Cancer Discovery*, DOI: 10.1158/2643-3230.BCD-21-0095