Editorial Commentary

Searching for the optimal sequence of dual-targeted CAR T cells in relapsed/refractory acute lymphoblastic leukemia

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Chimeric antigen receptor (CAR) T-cell therapy has transformed the treatment landscape for patients with relapsed or refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL) (1-5). In 2017, the Food and Drug Administration (FDA) approved tisagenlecleucel (Kymriah®) for the treatment of R/R B-ALL in patients 25 years old or younger following the landmark phase II ELIANA trial that demonstrated a complete remission (CR)/CR with incomplete count recovery (CRi) rate of 81% with 3-year event-free survival (EFS) of 44% (1,6). The relapse-free survival (RFS) with and without censoring for interim therapy [including hematopoietic stem cell transplantation (HCT)] was 52% and 48%, respectively (6), supporting the curative potential of CAR T cells in a proportion of children with R/R B-ALL. More recently, brexucabtagene autoleucel (Tecartus®) was approved for adults with R/R B-ALL based on the ZUMA-3 study that yielded a CR/CRi rate of 71% (95% CI: 57–82%) in adults with R/R B-ALL with an 18-month RFS rate of 35% for patients censored at subsequent allogeneic HCT and 42% for patients not censored at subsequent allogeneic HCT (3,7). The CD19 antigen is universally expressed on B-cell ALL cells in immunotherapy-naïve patients, and thus, it has been the main target for CAR T-cell therapy in this setting. This strategy of CD19 CAR T cell therapy has been explored extensively for nearly a decade since the earliest clinical trial results.

Notwithstanding the excellent response rate of CD19-targeted CAR T-cell therapy in R/R B-ALL, subsequent disease relapse remains unfortunately common with limited available salvage options. Treatment failure post CAR T-cell therapy is an area of extensive investigation with several described resistance mechanisms including diminished CAR T-cell persistence, targeted antigen loss on leukemic cells, intrinsic resistance of death signaling of tumor cells, and the complex and suppressing tumor microenvironment (1,8-10). Multiple strategies have been explored on how to improve the efficacy of current CAR T-cell platforms such as optimizing antigens selection for targeting, modulating costimulatory signaling, and enhancing lymphodepletion regimens to augment *in vivo* T cells response and persistence. Several studies are investigating the administration of CAR T-cell therapy in combination with radiotherapy, vaccines, checkpoint inhibition, and immunomodulatory agents (11). However, these efforts are hampered by tumor antigens heterogeneity, pre-existing divergent tumor clones, limitation in CAR T-cell trafficking, and premature T-cell exhaustion.

CD19-negative relapse after CD19-directed CAR T-cell therapy has been an area of interest in R/R B-ALL as it is observed in over 50% of relapses. The emergence of CD19-relapse has been linked to alternative splicing, immune selection, lineage switch, epitope masking, and methylation silencing (12). In attempt to address CD19-negative relapse, other leukemia-specific targets have been studied in patients who failed prior CD19-targeted immunotherapies.

CD22 antigen is an appealing target in R/R B-cell ALL as it is widely expressed in most cases and there is a strong success in targeting R/R B-ALL with inotuzumab, a CD22-antibody drug conjugate (13). CD22-directed CAR T-cell therapy was tested as a salvage strategy in patients who failed prior CD19-targeted immunotherapy, and although CD22-CAR T cell therapy has succeeded in producing remissions in the majority of treated cases, these remissions were not sustained and relapse occurred quickly (14-16). Targeting multiple antigens has also been investigated as a tactic to overcome antigen-negative relapse by early eradication of tumor heterogeneity and different pre-existed leukemia clones. Dual-targeted therapy was studied in various strategies, such as: mixing the infusion of two different antigen-targeted CAR T-cells (mixed CAR), co-expressing two different CARs on a single T-cell (bicistronic CAR), or modifying a single CAR to contain two separate single-chain variable fragments (scFv) in tandem (tandem CAR) (17). Administration of a CD19-/CD22-targeted CAR T-cell therapy has been demonstrated to be safe and effective but did not appear to be superior to single agent CD19-directed CAR T-cell therapy (18-20).

Another approach with dual-targeted CAR T cell therapy that was studied is the sequential administration of CD19-CAR and CD22-CAR T-cells. While early results have demonstrated promising leukemia-free survival (LFS) rates of 53–68% with low rates of antigen-negative relapse, CAR T-cell persistence was limited, and therefore, this was translated into frequent antigen-positive relapse (21,22). In one particular study by Pan *et al.* (23), CD22-directed CAR T-cell therapy was infused to 20 R/R B-ALL patients in CR when the first infusion of CD19-directed CAR T-cells was undetectable. The median interval between the two CAR T-cell infusions was 1.7 months with all patients sustained negative measurable residual disease (MRD) remission at the time of CD22 CAR T-cell infusion. Overall, the study demonstrated 12-month LFS of 80% and 18-month overall survival (OS) rate of 92% despite no patients have received consolidation with allogeneic HCT. Among the three patients who experienced relapse, all had CD19 loss including one patient with concurrent CD19/CD22 downregulation. Considering the importance of CAR T-cell persistence to provide an ongoing immune surveillance to prevent antigen-positive relapse, the limited nature of CAR T-cell persistence seen in this trial, as well as the need for repeating administration of the lymphodepletion regimen which may further eradicate the earlier CD19 CAR T-cells, brings to question the response durability of sequential administration strategy. Similar sequential strategy was also explored by Yan *et al.* in the relapsed B-ALL post-transplant setting with all 22 evaluable patients achieved MRD negative remission following infusion (24).

In this study by Wang *et al.*, a novel approach of concurrent administration of CD19- and CD22-targeted CAR T-cells at the same time was utilized with the premise of reducing the risk of antigen-escape relapse and avoiding administering a second lymphodepletion regimen as to was done in prior trials of sequential CAR T cells infusions (25). In this phase II trial of 225 evaluable children (age ≤21 years of age) with R/R B-ALL (n=194) or extramedullary relapse (n=31), CD19 and CD22 CAR T-cells were co-administered together at a 1:1 ration. The MRD-negative CR rate was 99%, with promising remission durability on a short follow up. The 12-month EFS was 74% for all responders, and this was 69% in patients treated for hematologic relapse without consolidative allogeneic HCT, 95% for isolated testicular relapse, and 69% for isolated central nervous system (CNS) relapse. The 12-month EFS was superior in patients who received consolidation with allogeneic HCT compared to those who did not (85% *vs.* 69%), however, there was no significant difference in 12-month OS.

Furthermore, 43 (22%) patients experienced subsequent relapse, including 17 relapses as CD19-negative disease and only one case of CD22-negative relapse. Most CD19-negative relapse (n=16) had retained CD22 expression. There were 24 (56%) patients relapsed with antigen positive ($CD19^+/CD22^+$) disease. In an exploratory analysis of 21 patients with relapsed disease, CD19-targeted CAR T cells were lost in all of 11 patients with $CD19^+CD22^+$ relapse and in 4 of 9 patients with $CD19^-CD22^+$ relapse, but not in the single patient with $CD19^-CD22^-$ relapse. However, CD22-targeted CAR T cells were lost universally in all 21 relapsed patients. Using quantitative polymerase chain reaction to detect the CAR transgene completed in 76 patients, the authors found that expansion occurred earlier for CD19CAR than for CD22CAR T-cells (peaked at mean ± SE: 7.3±0.5 *vs.* 10.9±0.9 days; P=0.0013). The CD19 CAR T-cells also had a more robust expansion for a longer duration than CD22 CAR T-cells. The authors postulated that the lack of expansion and persistence of CD22 CAR T-cells could be explained by lower CD22 expression compared to CD19 antigen expression on leukemic blasts or by poor CD22-scFV signaling activity. These observations could also potentially be explained by diminished CD22 antigen density, which has been shown to be associated with increased relapse after CD22-directed CAR T-cell therapy (14).

This trial additionally enrolled a cohort (n=31) of isolated extramedullary relapse and treated these patients with a higher dose of CAR T-cells to overcome the concern of low antigen stimulation in extramedullary sites. The recommended dose for patients with hematologic relapse was 5.0×10^6 CAR T cells/kg, whereas patients with isolated extramedullary relapse received a dose between 5.0×10^6 CAR T cells/kg and 1×10^7 CAR T cells/kg. This strategy was effective in demonstrating 100% CR rate without local irradiation. Among patients with CNS (n=10) and isolated testicular relapse (n=20), 12-month EFS were 69% and 95%, respectively.

Persistent B-cell aplasia is used as a surrogate marker for the continuing presence of CAR T-cell and reflection of anti-CD19 CAR T-cells activity and functionality. Prior studies have correlated loss of B-cell aplasia within 3–6 months of infusion with a higher risk of relapse, and the persistence of B-cell aplasia for 9–12 months with a lower risk of relapse (26). All patients in this study by Wang *et al.* all 181 patients who were analyzed by day 28 post infusion had B-cell aplasia. Median time to B-cell recovery was 74 days (interquartile range, 47.8–97.8). As shown in some prior studies (27), persistence of B-cell aplasia was found to be predictive of durable EFS. The cumulative incidence of loss of B-cell aplasia by 6 months post infusion was 60%. The EFS rate was 100% for patients with B-cell aplasia persisted for at least 6 months after infusion. Accordingly, multivariate analysis demonstrated that persistence of B-cell aplasia for at least 6 months post infusion was associated with better EFS rates.

Coadministration of CD19 and CD22-directed CAR T-cells shows promise in reducing relapse. While activity appears favorable compared to CD19-CART cell therapy in this setting (1), the safety profile was acceptable with rates of grade ≥3 cytokine release syndrome (CRS) and neurotoxicity of 29% and 4%, respectively. Nonetheless, there were 2 fatal incidents related to neurotoxicity and one death related to CRS. Study investigators acknowledged the higher rate of seizures (grade ≥3 =14%) compared to other studies, and this was attributed potentially to rapid expansion of CAR T cells and lack of prophylactic antiseizure medications.

Adding to the encouraging results of this study, the median time between enrollment and infusion of fresh CAR T cells was only 7 days, and this is an important advancement in R/R B-ALL in which lengthy manufacturing time of CAR T cells usually leads to high dropout rate as the consequence of disease progression and/or complications from interim bridging therapies. In contrast to ELIANA study where 18.5% (18 of 97) of the enrolled patients dropped out prior to infusion (1), only 2.6% (6 of 231) of eligible patients dropped out from Wang *et al.* study, further contributing to the remarkable results. However, the low dropout rate could be also attributed to the fact that the majority of enrolled patients in this study received coadministration CAR T-cell infusion in their first relapse (70%), as in contrast to the ELIANA study in enrolled patients had more advanced disease.

Whereas strategies investigating the optimal development of bicistronic or tandem CAR strategies are ongoing, Wang *et al.* study is the largest to date of the coadministration approach. Nonetheless, CD19 antigen loss was observed in over third of relapses following co-administration of CD19-CD22 CAR T cell therapy in this study, and thus, this approach unlikely will sufficiently address the CD19-negative relapse issue in R/R B-ALL. Furthermore, superior RFS was observed among responders who underwent consolidation with allogeneic HCT and this could be related to the short persistence (median was <3 months) of CAR T cells compared to tisagenlecleucel. However, it is crucial to have an updated longer follow up for patients treated on the study who did not undergo allogeneic HCT to identify which patients potentially cured with co-administration of CD19/CD22 CAR T cell therapy despite lack of CAR T cells persistence. Sustaining negative MRD at 3 months post CAR T cell infusion by a highly sensitive assay has shown a promise in predicting durable responders, regardless of B-cell aplasia (28). This tool could be useful to use with dual CAR T cell targeting therapy to stratify patients who are in need for a consolidation with allogeneic HCT. As the cost of ALL treatment is raising with the integration of expensive novel therapies one could question the cost-effectiveness of manufacturing two CAR products for each single patient in this setting. Data demonstrating a strong advantage of dual-targeting CAR over CD19-CAR T cell therapy are warranted to justify the cost of such approach.

This trial by Wang *et al.* offers great insight into the optimal sequence of administering two CAR T cells in R/R B-ALL in a safe and effective approach. Studies directly comparing this approach to single CAR T cell infusion and to other combination sequences are warranted to optimize CAR T cell treatment in R/R B-ALL.

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Footnote

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References

1. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med 2018;378:439-48.
2. Park JH, Rivière I, Gonen M, et al. Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. N Engl J Med 2018;378:449-59.
3. Shah BD, Ghobadi A, Oluwole OO, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet 2021;398:491-502.
4. Aldoss I, Khaled SK, Wang X, et al. Favorable Activity and Safety Profile of Memory-Enriched CD19-Targeted Chimeric Antigen Receptor T-Cell Therapy in Adults with High-Risk Relapsed/Refractory ALL. Clin Cancer Res 2023;29:742-53.
5. Pasquini MC, Hu ZH, Curran K, et al. Real-world evidence of tisagenlecleucel for pediatric acute lymphoblastic leukemia and non-Hodgkin lymphoma. Blood Adv 2020;4:5414-24.
6. Laetsch TW, Maude SL, Rives S, et al. Three-Year Update of Tisagenlecleucel in Pediatric and Young Adult Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia in the ELIANA Trial. J Clin Oncol 2023;41:1664-9.
7. Shah BD, Ghobadi A, Oluwole OO, et al. Two-year follow-up of KTE-X19 in patients with relapsed or refractory adult B-cell acute lymphoblastic leukemia in ZUMA-3 and its contextualization with SCHOLAR-3, an external historical control study. J Hematol Oncol 2022;15:170.
8. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015;385:517-28.
9. Schultz LM, Eaton A, Baggott C, et al. Outcomes After Nonresponse and Relapse Post-Tisagenlecleucel in Children, Adolescents, and Young Adults With B-Cell Acute Lymphoblastic Leukemia. J Clin Oncol 2023;41:354-63.
10. Si Lim SJ, Grupp SA, DiNofia AM. Tisagenlecleucel for treatment of children and young adults with relapsed/refractory B-cell acute lymphoblastic leukemia. Pediatr Blood Cancer 2021;68:e29123.
11. Al-Haideri M, Tondok SB, Safa SH, et al. CAR-T cell combination therapy: the next revolution in cancer treatment. Cancer Cell Int 2022;22:365.
12. Lemoine J, Ruella M, Houot R. Born to survive: how cancer cells resist CAR T cell therapy. J Hematol Oncol 2021;14:199.
13. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med 2016;375:740-53.
14. Fry TJ, Shah NN, Orentas RJ, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat Med 2018;24:20-8.
15. Pan J, Niu Q, Deng B, et al. CD22 CAR T-cell therapy in refractory or relapsed B acute lymphoblastic leukemia. Leukemia 2019;33:2854-66.
16. Zhu H, Deng H, Mu J, et al. Anti-CD22 CAR-T Cell Therapy as a Salvage Treatment in B Cell Malignancies Refractory or Relapsed After Anti-CD19 CAR-T therapy. Onco Targets Ther 2021;14:4023-37.
17. Guo Z, Tu S, Yu S, et al. Preclinical and clinical advances in dual-target chimeric antigen receptor therapy for hematological malignancies. Cancer Sci 2021;112:1357-68.
18. Spiegel JY, Patel S, Muffly L, et al. CAR T cells with

dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. Nat Med 2021;27:1419-31.
19. Cordoba S, Onuoha S, Thomas S, et al. CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial. Nat Med 2021;27:1797-805.
20. Roddie C, Lekakis LJ, Marzolini MAV, et al. Dual targeting of CD19 and CD22 with Bicistronic CAR-T cells in Patients with Relapsed/Refractory Large B Cell Lymphoma. Blood 2023;141:2470-82.
21. Wang N, Hu X, Cao W, et al. Efficacy and safety of CAR19/22 T-cell cocktail therapy in patients with refractory/relapsed B-cell malignancies. Blood 2020;135:17-27.
22. Liu S, Deng B, Yin Z, et al. Combination of CD19 and CD22 CAR-T cell therapy in relapsed B-cell acute lymphoblastic leukemia after allogeneic transplantation. Am J Hematol 2021;96:671-9.
23. Pan J, Zuo S, Deng B, et al. Sequential CD19-22 CAR T therapy induces sustained remission in children with r/r B-ALL. Blood 2020;135:387-91.
24. Yan N, Wang N, Wang G, et al. CAR19/22 T cell cocktail therapy for B-ALL relapsed after allogeneic hematopoietic stem cell transplantation. Cytotherapy 2022;24:841-9.
25. Wang T, Tang Y, Cai J, et al. Coadministration of CD19- and CD22-Directed Chimeric Antigen Receptor T-Cell Therapy in Childhood B-Cell Acute Lymphoblastic Leukemia: A Single-Arm, Multicenter, Phase II Trial. J Clin Oncol 2023;41:1670-83.
26. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 2014;6:224ra25.
27. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 2013;368:1509-18.
28. Pulsipher MA, Han X, Maude SL, et al. Next-Generation Sequencing of Minimal Residual Disease for Predicting Relapse after Tisagenlecleucel in Children and Young Adults with Acute Lymphoblastic Leukemia. Blood Cancer Discov 2022;3:66-81.

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