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CLINICAL TRIALS AND OBSERVATIONS

Comment on Neelapu et al, page 2307

Putting the pedal to the metal: axi-cel for LBCL

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In this issue of *Blood*, Neelapu et al[1] provide a 5-year follow-up of the pivotal phase 2 ZUMA-1 trial of axicabtagene ciloleucel (axi-cel) for relapsed and refractory large B-cell lymphoma (R/R LBCL), reporting that overall and disease-specific survival were sustained.

Preclinical studies have shown that second-generation anti-CD19 CAR (chimeric antigen receptor) T cells, which incorporate either a 4-1BB (19BBz) or a CD28 (1928z) costimulatory domain, have different tumoricidal kinetics. Although 19BBz CAR T-cells kill slower, they show better persistence. 1928z CAR T cells induce a more rapid tumor clearance. Axi-cel is the only 1928z CAR T-cell product for LBCL and was approved based on data from the ZUMA-1 trial.[2] So, would you expect a race car to be more durable than a road car? Probably not. But perhaps durability—or to rephrase it, persistence—is not necessarily needed when anti-CD19-directed CAR T cells race against LBCL.

Depending on their fitness, patients with R/R LBCL undergo either a salvage chemotherapy, followed by high-dose chemotherapy and autologous stem-cell transplantation, with curative intent or treatment with palliative intent. Despite intensive treatment, due to the aggressive course of their disease, patients with R/R LBCL often lose the race against their tumor and face a dismal prognosis. The pivotal ZUMA-1 trial found remarkable response rates in patients with R/R LBCL after second-line treatment (overall response rate = 83% and complete response rate [CR] = 58%) with a 24-month progression-free survival of 36%.[2] However, for treatment of R/R LBCL, there was no long-term follow-up data beyond 3 years available for any approved anti-CD19-directed CAR T-cell product, until the current publication.

Neelapu and colleagues report the 5-year long-term follow-up of the pivotal phase 2 ZUMA-1 trial, providing the updated efficacy data including time to progression, time to next therapy, and disease-specific survival. No formal statistical hypothesis was tested, and data were analyzed using descriptive statistics. Assessments after 24 months of follow-up data, including adverse events, were performed when clinically indicated as per institutional standard of care. Importantly, the clinical outcome was correlated with the axi-cel peak and the area under the curve from day 0-28 ($AUC_{0\text{-}28}$) detected in peripheral blood from 97 out of 101 treated patients.

Ongoing responses were present in 31% of patients (CR = 30%) after 5 years with a median progression-free survival of 5.9 months (95% confidence interval: 3.3-15.0 months) and median time to progression of 6.1 months (4.4-29.7 months). Disease-related death after 24 months occurred in 4% of patients (remaining 41% of disease-related deaths occurred within the first 24 months), which translates into a median overall survival of 25.8 months (12.8 months-not estimable [NE]) and a median disease-specific survival, which has not been reached (15.4 months-NE). In addition, a higher median CAR T-cell peak and higher median $AUC_{0\text{-}28}$ were associated with better clinical outcome.

Thus, long-term response was obtained in roughly one-third of patients with R/R LBCL—a disease with a historically grim outcome. More excitingly, real-world studies conducted independently in both the United States and France that included patients who did not meet the eligibility criteria of the ZUMA-1 trial confirmed initial response rates seen with axi-cel in the ZUMA-1 trial.[3,4] This indicates that the long-term responses reported by Neelapu and colleagues might be reproduced in our daily practice. In addition, axi-cel is approved as a second-line treatment for LBCL and is being evaluated as first-line treatment for high-risk LBCL,[5,6] giving axi-cel the opportunity to enter the road earlier and increasing the chance to win the race against LBCL in more patients.

Interestingly, another anti-CD19 CAR T-cell product—lisocabtagene maraleucel (liso-cel), which has a 4-1BB costimulatory domain—shows comparable initial response rates as axi-cel in R/R LBCL and is approved for second- and third-line treatment of R/R LBCL.[7] Although long-term data for liso-cel are pending, the promising early results challenge the necessity of reliance on CD28-mediated costimulation for anti-CD19 CAR T cells in LBCL. They also raise the difficult issue of an exclusive response in at least some individuals with LBCL to one or the other product. For example, antigen density varies dramatically in LBCL when compared with healthy B cells, and in CD19 low tumors, 1928z CAR T cells have more favorable outcome compared with 19BBz CAR T cells.[8]

To conclude, more stringent and consistent molecular profiling of tumor samples[9] along with careful patient stratification and continued in-depth CAR T-cell product characterization[10] are needed in future clinical trials to help select the right CAR T-cell product for individuals with LBCL and to improve existing CAR T-cell products. The 5-year follow-up of axi-cel in R/R LBCL provided by Neelapu and colleagues in this issue of *Blood* is reassuring and confirms that current and future efforts in the CAR T-cell field are paving the road to success for LBCL treatment. In other words, time to put the pedal to the metal.

Conflict-of-interest disclosure: The author has received honoraria from Novartis and Miltenyi Biotec as well as travel support from Gilead. ■

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https://doi.org/10.1182/blood.2023020188

GENE THERAPY

Comment on Calabria et al, page 2316

Intrathymic AAV gene delivery

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In this issue of *Blood*, Calabria et al show that intrathymic (IT) delivery of adeno-associated virus (AAV) vectors results in a site-specific integration within T-cell receptor (TCR) genes close to DNA breaks created by recombination-activating gene (RAG) enzymes during the variable, diversity, and joining [V(D)J] recombination.[1]

With tremendous advances in molecular diagnostic techniques, an increasing number of causative gene defects have been discovered in human diseases. Gene therapy (GT), which aims to fix gene defects, promises a potential cure for these diseases. AAV vectors are the leading platforms for in vivo GT. They have a high transduction efficiency, a broad range of target tissues, and a good safety profile. Because AAV vectors present predominantly episomal, they are not useful in treating highly proliferative cells such as hematopoietic and lymphoid cells owing to the dilution effect.[2,3]

Pouzolles et al injected AAV-ZAP-70 into the thymi of ZAP-70-deficient mice and found that IT injection of AAV-ZAP-70 transduced up to 5% of thymocytes, resulting in the rapid development of functional T lymphocytes and rapid reconstitution of the thymic medulla (within 10 days). The gene-corrected T lymphocytes persisted in the periphery for more than 40 weeks after treatment.[4] Genomic integration was detected in the treated mice. In this issue of *Blood*, the same research team published the results of their study on the AAV integration profile in lymph nodes, spleens, livers, and brains of ZAP-70-deficient mice treated with IT AAV-ZAP70 injection.[1] This study also included some wild-type mice with AAV-GFP IT injection and treated MeCP2 deficiency mice. They found that more than 90% of integrations in T lymphocytes were clustered within the TCR α, β, and γ genes. The insertion sites were mapped to DNA breaks created by enzymatic activity of RAGs during V(D)J recombination. In contrast, AAV integrations in the liver and brain were distributed across the entire mouse genome.[1] This characteristic integration profile is likely related to the tendency of AAV vectors to integrate at DNA breakage sites,[5] which may not occur with other types of vectors.[6]

These findings demonstrate that IT AAV delivery can take advantage of the physiological DNA breaks generated during TCR V(D)J gene recombination, overcome the limitation of AAV vectors, deliver therapeutic transgenes into the genome of T lymphocytes, and achieve a long-term therapeutic outcome (see figure). If the integration interferes with TCR gene rearrangement, transduced thymocytes with no functional TCR will most likely die instead of becoming malignant. Therefore, insertional mutagenesis and genotoxicity are not likely to be an issue in this gene delivery approach. Because transduced thymocytes go through the selection process in the thymus and may develop tolerance to the antigens of the AAV capsid and transgene, immunogenicity seems less likely to be a problem. IT AAV gene