requires extrapolation of currently available data over a long period. Therefore, follow-up of the current study is needed to confirm that the presented results are achieved in practice. Such a dynamic assessment of the cost-effectiveness of treatments, implementing continuous updates of new evidence and insights, might be a valuable approach to ensure rapid access to promising new treatments while ensuring affordable healthcare.

Cost-effectiveness analysis uses QALYs that combine length and quality of life (QoL). In the analysis of Yamamoto and colleagues, length of life is similar (0.1), meaning QALY gains are driven by differences in QoL. Ideally, QoL values should be treatment specific; however, QoL data are often lacking, as in this study. Instead, the data were derived from the literature (ie, representing older regimens in UK patients).[7] Moreover, the assumption of higher QoL during maintenance therapy is crucial. In fact, the conclusion of the authors, that the key driver of QALYs gained is first-line PFS, only holds if their assumption (ie, that QoL during first-line PFS is substantially better than QoL for second-line PFS) is correct. Given the relevance, the collection of QoL data should be a research priority in future trials.

In conclusion, Yamamoto and colleagues add to the field of cost-effectiveness analyses in MM by broadening the study to include sequential treatments and by using MRD as an endpoint. This is of utmost importance because in many parts of the world, ensuring (financial) access to novel treatments leading to a longer and valuable life is limited by financial constraints.[8] Timely cost-effectiveness evidence should help with the optimal use of scarce resources and price negotiations, improving access for individual patients. Nevertheless, such analyses are only as good as the data underlying them. Often, assumptions must be made that substantially affect outcomes. Thus, it is critical that dynamic assessments using different endpoints over time and solid MRD and QoL data are collected during clinical trials and population-based registries.

Conflict-of-interest disclosure: H.M.B. reports consulting or advisory role for Pfizer (paid to institute) and research funding from BMS-Celgene (paid to institute). S.Z. reports consulting or advisory role for BMS, Janssen-Cilag, Takeda, Sanofi, and Oncopeptides (all paid to institute) and research funding from Janssen-Cilag and Takeda (all paid to institute). ■

REFERENCES

1. Yamamoto C, Minakata D, Koyama S, et al. Daratumumab in first-line therapy is cost-effective in transplant-eligible patients with newly diagnosed myeloma. *Blood*. 2022; 140(6):594-607.
2. Zheng Y, Pan F, Sorensen S. Modeling treatment sequences in pharmacoeconomic models. *PharmacoEconomics*. 2017;35(1): 15-24.
3. Attal M, Lauwers-Cances V, Hulin C, et al; IFM 2009 Study. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. *N Engl J Med*. 2017;376(14): 1311-1320.
4. Patel KK, Giri S, Parker TL, Bar N, Neparidze N, Huntington SF. Cost-effectiveness of first-line versus second-line use of daratumumab in older, transplant-ineligible patients with multiple myeloma. *J Clin Oncol*. 2021;39(10):1119-1128.
5. Dimopoulos MA, Oriol A, Nahi H, et al; POLLUX Investigators. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016; 375(14):1319-1331.
6. Blommestein HM, Franken MG, van Beurden-Tan CHY, et al. Cost-effectiveness of novel treatment sequences for transplant-ineligible patients with multiple myeloma. *JAMA Netw Open*. 2021;4(3):e213497.
7. Hatswell AJ, Burns D, Baio G, Wadelin F. Frequentist and Bayesian meta-regression of health state utilities for multiple myeloma incorporating systematic review and analysis of individual patient data. *Health Econ*. 2019; 28(5):653-665.
8. Seefat MR, Cucchi DGJ, Dirven S, Groen K, Zweegman S, Blommestein HM. A systematic review of cost-effectiveness analyses of novel agents in the treatment of multiple myeloma. *Cancers (Basel)*. 2021;13(22): 5606.

DOI 10.1182/blood.2022016958

IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Diorio et al, page 619

The uncut version: base-edited allo-CAR T cells

Dimitrios Laurin Wagner | Charité - Universitätsmedizin Berlin

In this issue of *Blood*, Diorio et al[1] harness base editing technology to develop a potent and complex gene-edited CD7-specific chimeric antigen receptor (CAR) T-cell product for off-the-shelf use in patients with T-cell leukemia and other CD7$^+$ malignancies. Their manufacturing platform showcases the potential of base editing for future progress toward safer and more accessible CAR T-cell therapies.

After the success of personalized CAR T-cell therapy in B-cell malignancies, the field expanded to develop CAR technology for other malignant diseases. With some CAR T-cell products moving toward use earlier in treatment, there is an increasing interest in overcoming the logistic hurdles involved in personalized cell manufacturing by establishing allogeneic off-the-shelf solutions.[2] Herein, gene silencing with programmable nucleases (eg, via Zinc finger nucleases, TALEN, CRISPR-Cas9) has become an essential tool to facilitate engineering of T cells with the desired attributes.

Conventional programmable nucleases allow gene silencing by forcing DNA double-stranded breaks (DSBs) at coding regions of the targeted gene. Repeated cuts and error prone DNA repairs promote small insertions and deletions that induce frameshift mutations and disrupt protein expression. Despite achieving highly efficient gene knockouts, repetitive cutting by nucleases can induce genetic rearrangements such as inversions, larger deletions, and even complete loss of chromosomes.[3] To overcome the challenges of safe allogeneic CAR (allo-CAR) T-cell therapy, multiple genetic modifications will be required to eliminate the risk of graft-versus-host disease and allogeneic cell immunogenicity, which limit CAR T-cell persistence and antitumor efficacy.[4] However, simultaneous targeting of multiple genes with nuclease-assisted gene disruption can create myriad translocations and genetic rearrangements with unknown long-term consequences.

Base editing is a technology that couples the programmable nature of the CRISPR-Cas9 system with avoidance of DNA DSBs during genetic modification. By fusing a single-strand DNA (ssDNA) deaminase enzyme to a catalytically inactive Cas9 variant, only an ssDNA cut (nick) is induced. The Cas9-mediated nicking of the genomic DNA exposes a short stretch of ssDNA to the attached deaminase which then converts the selected bases within their target window. Since the original report on cytosine base editors (CBEs) in 2016, which force targeted cytosine-to-thymine base conversions,[5] iterative improvements have yielded novel versions of base editors that reduce unwanted byproducts, improve the targeting scope, and allow editing of different bases.[6] Previous work has demonstrated that CBEs can be used to induce stop codons or disrupt splice sites to thwart effective protein expression in T cells.[7]

Diorio et al used a messenger RNA–encoded fourth-generation CBE to efficiently silence 4 different genes in more than 90% of the cells after a single electroporation. Building on previous work published in *Blood*,[8] they chose to disrupt CD7 expression to avoid fratricide (self-killing) after subsequent transduction with a CD7-specific CAR-encoding lentivirus. *TRAC* gene knockout was performed to eliminate potential alloreactive T-cell receptors and avoid graft-versus-host disease. Additional silencing of the endogenous immune checkpoint programmed cell death protein 1 (PD1; encoded by *PDCD1*) was introduced to improve antitumor performance in immunosuppressive environments. *CD52* deletion should enable conditioning for patients with an anti-CD52 monoclonal antibody to enhance initial CAR T-cell engraftment and synergistically target $CD52^+$ malignant blasts. In line with previous studies, multigene editing with CBEs did not induce detectable translocations in karyotyping and targeted sequencing assays in the modified T cells.[7,9]

Protocols to generate off-the-shelf CAR T-cell products aim to optimize CAR T-cell yields to result in many doses per production cycle. Diorio et al demonstrate that base editing enabled higher cell viability, better proliferation, and optimized CAR T-cell yields when compared with the conventional CRISPR-Cas9 nuclease system. This may be explained by reduced DNA damage response observed after quadruple editing with CBEs and better cellular fitness in the absence of gross genetic rearrangements. With overall yields between 16×10^9 and 17.2×10^9 CAR^+ T cells, a single manufacturing run may provide up to 200 administrations in adult patients (assuming a treatment dose of 1×10^6 CAR^+ T cells per kilogram of body weight in a cohort of patients averaging 80 kilograms each). Therefore, we can assume significantly lower cost per dose in contrast to personalized ex vivo manufacturing.

A high degree of reproducibility during allogeneic cell manufacturing is paramount to deliver consistent clinical outcomes. To date, only a few studies with gene-edited allo-CAR T-cell products have investigated donor-to-donor variability regarding product potency. In the article by Diorio et al, 3 independent clinical-scale manufacturing runs displayed low inter-donor variability regarding base editing outcome, cell yields, and even in vivo performance. Donor-dependent differences were observed during in vitro cytokine release assays and CD4:CD8 ratios in the final products. Future studies may identify donor- or product-dependent factors that are associated with better outcome.

Donor-derived CD7-specific CAR T cells have already been reported to induce high rates of complete remission in patients with leukemia.[10] Extensive in vivo modeling using human T-cell leukemia cell lines and patient-derived xenografts in immunodeficient mice attest to the high antitumor efficacy of the base-edited CD7-specific CAR T-cell products. Notably, Diorio et al did not include modifications that alter HLA expression to improve the persistence of the CAR T cells. Instead, they chose a formulation of edits to create a cellular product for potent antitumor attack as an effective means of bridging to curative allogeneic stem cell transplantation. Pending favorable risk-benefit analysis after finalization of a molecular off-target analysis, this new off-the-shelf CD7 CAR T-cell product will complement the clinical trial pipeline, and it may ultimately expand the treatment options for patients who have treatment refractory T-cell malignancies.

Conflict-of-interest disclosure: D.L.W. has filed patent applications for gene editing and cell therapies. ■

REFERENCES

1. Diorio C, Murray R, Naniong M, et al. Cytosine base editing enables quadruple-edited allogeneic CART cells for T-ALL. *Blood*. 2022;140(6):619-629.
2. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discov*. 2020;19(3):185-199.
3. Leibowitz ML, Papathanasiou S, Doerfler PA, et al. Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. *Nat Genet*. 2021;53(6):895-905.
4. Wagner DL, Fritsche E, Pulsipher MA, et al. Immunogenicity of CAR T cells in cancer therapy. *Nat Rev Clin Oncol*. 2021;18(6):379-393.
5. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016;533(7603):420-424.
6. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat Biotechnol*. 2020;38(7):824-844.
7. Webber BR, Lonetree CL, Kluesner MG, et al. Highly efficient multiplex human T cell engineering without double-strand breaks using Cas9 base editors. *Nat Commun*. 2019;10(1):5222.
8. Gomes-Silva D, Srinivasan M, Sharma S, et al. CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. *Blood*. 2017;130(3):285-296.
9. Georgiadis C, Rasaiyaah J, Gkazi SA, et al. Base-edited CAR T cells for combinational therapy against T cell malignancies. *Leukemia*. 2021;35(12):3466-3481.
10. Pan J, Tan Y, Wang G, et al. Donor-derived CD7 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia: First-in-human, phase I trial. *J Clin Oncol*. 2021;39(30):3340-3351.

DOI 10.1182/blood.2022016852