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One of the most significant innovations in personalized cancer medicine over the past decade has been the development of chimeric antigen receptor (CAR) T-cell therapy, in which a patient's own cells are engineered to becoming cancer-fighting weapons (**Figure 1**). Blending oncology, biotechnology, and immunology, CAR T-cell therapy has revolutionized the management of relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL), various B-cell non-Hodgkin's lymphomas (NHLs), and multiple myeloma (MM). Since 2017, there have been six CAR T-cell therapies that have been approved by the US Food and Drug Administration (FDA) for relapsed/refractory hematological malignancies. These include four products targeting CD19 for the treatment of B-cell NHL and B-ALL (tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, and lisocabtagene maraleucel) and two products targeting B-cell maturation antigen (BCMA) for MM (idecabtagene vicleucel and ciltacabtagene autoleucel). To date, all approved CAR T-cell therapies have been autologous products (i.e., engineered using the patient's own T-cells); however, development of "off-the-shelf" allogeneic products (derived from donors), including allogeneic CAR NK products is underway. As the field has grappled to optimize the CAR constructs, prolong duration of response, and minimize toxicities, such as cytokine release syndrome (CRS) and neurotoxicity,[1] it has been fascinating to observe how the principles of quantitative clinical pharmacology are being adapted and applied to understand the behaviors of a living drug. For example, a general model of *in vivo* cellular kinetics (CKs) of CAR T-cells has been proposed that involves initial rapid distribution following infusion, then subsequent expansion followed by biexponential decline.[2]

In this issue of *Clinical Pharmacology & Therapeutics* (*CPT*) Ogasawara and colleagues contribute to the growing understanding of the relationship between *in vivo* CAR T-cell expansion, antitumor efficacy, and toxicity.[3] The CKs of lisocabtagene maraleucel from patients treated in the TRANSCEND NHL 001 trial[4] were evaluated using two bioanalytical methods: quantitative polymerase chain reaction to measure the transgene and flow cytometric detection of the nonfunctional truncated epidermal growth factor receptor that is co-expressed along with the CD19-specific CAR from peripheral blood samples.[3] Reported CK parameters included maximum expansion (C_{max}), time to C_{max} (T_{max}), and area under the curve (AUC) for both 0–28 and 0–90 days post-infusion ($AUC_{0-28\ days}$ and $AUC_{0-90\ days}$). A high correlation between $AUC_{0-28\ days}$ and C_{max} or $AUC_{0-90\ days}$ was observed. Multivariable logistic regression analysis revealed an association between *in vivo* cellular expansion and both antitumor efficacy (overall response and complete response rates) and toxicity (CRS and neurologic events). This analysis further demonstrated that patient age and tumor burden were confounding variables. Of

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Figure 1 *Clinical Pharmacology & Therapeutics* July 2022 cover image: CAR T-cell therapy.

particular interest were the data obtained from patients who received a second infusion of the product, either as part of a planned two-dose schedule or as salvage therapy at the time of relapse. These studies revealed that the planned second dose administered on day 15 did not result in a higher C_{max}, whereas those who received a second infusion at the time of relapse achieved $C_{max}/AUC_{0-28\ days}$ values that were either equivalent or lower than what had been achieved after the initial infusion.

This work adds to the findings from other CAR T-cell trials that have explored potential dose/exposure-response relationships from the perspective of *in vivo* expansion and CKs. A CK analysis from patients treated on the JULIET study (tisagenlecleucel in diffuse large B-cell lymphoma), showed no difference in CK parameters between responders and nonresponders; although, there was a trend toward higher-than-median C_{max} being associated with longer duration of response.[5] However, there was an association between higher C_{max} (or $AUC_{0-28\ days}$) and rates of grade 3/4 CRS.[5] Similar studies with tisagenlecleucel in patients with B-ALL or chronic lymphocytic leukemia (CLL) demonstrated an association between C_{max}/AUC and response.[6,7] Expansion of axicabtagene ciloleucel in patients with B-NHL was associated with response and neurological events but not CRS.[8] In the MM setting, idecabtagene vicleucel C_{max} was associated with deeper response and longer progression free survival (PFS).[9] As 97% of patients with MM receiving ciltacabtagene autoleucel achieved a response, any potential correlation between expansion and response was not possible to discern; although, there appeared to be an association between expansion and severity of CRS.[10] A commonality among all of these studies is high interpatient variability. Less information is available regarding the kinetics of long-term persistence of various CAR T-cell products as well as the kinetics in other relevant compartments, such as the bone marrow, secondary lymphoid tissues, and cerebral spinal fluid.

Although a relationship between cell dose administered and response has been demonstrated, this is generally limited to responses being dependent on a certain dose threshold, and above that threshold dose-dependency is not consistently seen.[9,11–14] In addition,

consistent correlations between dose administered and subsequent expansion have not been observed.[3,12,13] In a previously published population CK analysis of lisocabtagene maraleucel, a discernible dose-exposure relationship for cellular expansion could not be observed in the tested administered dose range of 44–156 × 10^6 CAR T-cells.[15] Indeed, the inability to discern dose-exposure relationships with CAR-T therapy is reconciled well in simulations from systems pharmacology models[16] as well as mechanism-informed modeling of clinical CK of multiple CAR-T products.[17] The interpretation of the results of exposure-response analyses for cell therapies like lisocabtagene maraleucel may thus not be entirely analogous to that applicable for small molecules or biologics. Whereas the multivariable exposure-response relationships described by Ogasawara *et al*.[3] advance our understanding of the importance of CAR-T expansion on clinical outcomes in the context of other sources of variability, quantitative translation to dose selection decisions may not be straightforward. Typically, the purpose of an exposure-response analysis conducted as part of a clinical pharmacology plan in drug development is to quantify the relationship between the systemic exposure of a therapeutic and the associated clinical outcomes (efficacy or toxicity). Together with an understanding of the dose-exposure relationship and associated intrinsic and extrinsic sources of variability in systemic exposures, the desired therapeutic dose range can be inferred and tailored as appropriate across populations and clinical contexts of use. Given that the inability to discern dose-exposure relationships is not uncommon with CAR-T therapies owing to far greater contribution of biological variability to CAR-T expansion than the administered dose, clinical pharmacologists will need to rethink strategies for informing dose optimization. This will require continued evaluation of the translational fidelity of systems pharmacology models as such models should in principle help explain and extend inference from clinically observed exposure-response relationships to optimize CAR-T precision therapy. Additional challenges with pharmacometric analyses of exposure-response relationships for CAR-T therapies include the need to carefully consider the impact of immortal time bias[18] when dealing with efficacy end points like duration of response or overall survival. Although less of a concern when using early "landmark" measures of cellular expansion as the exposure metric (e.g., AUC over 28 days following infusion) for relationships to response rates, these pharmacostatistical considerations are important if the question being asked involves the relationship of CAR-T persistence to response duration or survival outcomes in time-to-event analyses.

Ultimately the *in vivo* expansion of the CAR T-cell product appears to be just one of the factors that influences short- and/or long-term efficacy. Other potential contributors include the starting substrate, the phenotypes produced during the manufacturing process, pretreatment lymphodepletion regimens, the cell dose administered, the medications received after CAR T-cell administration, and the long-term persistence (and phenotype) of the engineered cells. From the starting substrate perspective, the collected T-cells may vary widely among individuals due to numbers and types of prior therapies, contributing to prolonged lymphopenia and/or suppressed T-cell function. For example, in the phase II study of idecabtagene vicleucel in patients with relapsed/refractory MM, the patient population had a median of six prior lines of therapy, with a range from 3 to 16 prior lines.[9] Thus, the quality of the collected T-cells would be expected to be very heterogeneous. On a broader level, the relative numbers of transduced CD4+ and CD8+ T-cells may be of importance (and lisocabtagene maraleucel has separate manufacturing processes and infusions for CD4+ and CD8+ T-cells), but more sophisticated immunophenotyping may be more informative. In a study involving CAR T-cell therapy in patients with CLL, it was found that collected T-cells with a higher frequency of CD27+CD45RO-CD8+ T-cells had memory-like characteristics and were associated with sustained remission.[19] Manipulation of the T-cells during manufacturing may elicit a superior phenotype, such as enrichment of memory-like T-cells and relative depletion of senescent cells. This approach is being evaluated with the bb21217 anti-BCMA product, which involves the use of *ex vivo* culture of the cells with a phosphoinositide 3-kinase inhibitor. Preliminary results from the ongoing phase I study of this product suggest that patients with higher than median number of CD8+ CAR T-cells expressing CD27/CD28 had significantly longer duration of response compared to patients with lower than median values ($P = 0.0024$).[20]

As CAR T-cells represent a therapy that can replicate and sustain itself over time, an

improved understanding of the mechanisms driving *in vivo* CAR T-cell expansion and persistence is needed. There has been much scrutiny over whether therapies administered to manage CAR T-cell-induced CRS can abrogate expansion and efficacy. In general, the literature to date has suggested that receipt of corticosteroid therapy post-CAR T-cell administration does not negatively impair *in vivo* expansion or efficacy; although, one study found that use of corticosteroid treatment within 7 days of CAR T-cell infusion was associated with shorter PFS.[21–24] In the population CK model of lisocabtagene maraleucel, treatment with tocilizumab and/or corticosteroids was identified as a covariate associated with a higher C_{max} and $AUC_{0\text{-}28\ days}$.[15] However, as these metrics of expansion were also associated with CRS or neurological events necessitating administration of tocilizumab and/or corticosteroids, the interpretation of causality in covariate analyses is not straightforward, and the authors ultimately concluded that the covariates did not have a meaningful impact on lisocabtagene maraleucel CKs.[15] Whether other immunomodulatory agents (e.g., lenalidomide) can enhance the cytolytic activity and persistence of CAR T-cell therapy is currently under investigation. Finally, long-term follow-up (7 years) of 2 patients with CLL who received CD19 CAR T-cells demonstrated changes over time in the phenotypes of the T-cells as well as the numbers and identities of the clones that contributed to long-term CAR T-cell persistence.[25]

Ogasawara *et al*.'s finding that a second administration of lisocabtagene maraleucel 2 weeks after the first dose did not alter C_{max}[3] further highlights the differences between cellular therapy and traditional therapeutics and the need for more extensive exploration of the factors controlling *in vivo* expansion. Determining the mechanisms for tumor resistance to CAR T-cell therapy remains an active area of investigation and is likely multifactorial, related to problems with CAR T-cell persistence and/or long-term cytotoxic activity, the tumor microenvironment as well as tumor-intrinsic mechanisms, such as loss of antigen expression or aberrant apoptotic machinery.[26,27]

The science behind cellular therapy is continuing to rapidly evolve, leading to the development of novel approaches, such as dual-targeting CAR T-cells (including T-cells co-expressing different CARs vs. mixtures of different CAR T products), armored CAR T-cells (engineered to secrete cytokines or antibody-like proteins), allogeneic CAR T-cells, CAR-NK cells, regional delivery of CAR T-cells, and combining CAR T-cell therapy with chemotherapeutic or immunotherapeutic agents. The field of clinical pharmacology will face new challenges to fully delineate the pharmacokinetics, pharmacodynamics, and pharmacometrics as these novel approaches reach human testing. Whereas pharmacokinetics for cellular therapy has evolved into CK, it is evident that understanding exposure-response relationships will require more than simply counting how many engineered cells are present at any given timepoint post-infusion. The inherent complexity of T-cell biology coupled with alterations in CAR T-cell phenotypes following infusion will require sophisticated molecular and cellular interrogation strategies to capture the phenotypic and functional characteristics of these living drugs. Collaboration with immunologists, systems pharmacologists, data scientists, and others working on cellular therapy will be critical.

Innovative modalities, such as cell and gene therapies, are transforming the therapeutic landscape in oncology and other disease areas, resulting in a spectrum of novel treatment options. This requires clinical pharmacologists to adapt and develop new skill sets, knowledge, and expertise. *CPT* aspires to continue to develop as the pre-eminent destination journal for research and educational publications in this area. To that end, the journal plans to devote the September 2023 themed issue to "Clinical Pharmacology of Novel Therapeutic Modalities" and a Call for Papers will be issued in the coming months.

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