Best Practices and Considerations for Clinical Pharmacology and Pharmacometric Aspects for Optimal Development of CAR-T and TCR-T Cell Therapies: An Industry Perspective

Hardik Mody[1], Ken Ogasawara[2], Xu Zhu[3], Dale Miles[1], Prathap Nagaraja Shastri[4], Jochem Gokemeijer[5], Michael Z. Liao[1], Sreeneeranj Kasichayanula[6], Tong-Yuan Yang[7], Nagendra Chemuturi[8], Swati Gupta[9], Vibha Jawa[2] and Vijay V. Upreti[10,*]

With the promise of a potentially "single dose curative" paradigm, CAR-T cell therapies have brought a paradigm shift in the treatment and management of hematological malignancies. Both CAR-T and TCR-T cell therapies have also made great progress toward the successful treatment of solid tumor indications. The field is rapidly evolving with recent advancements including the clinical development of "off-the-shelf" allogeneic CAR-T therapies that can overcome the long and difficult "vein-to-vein" wait time seen with autologous CAR-T therapies. There are unique clinical pharmacology, pharmacometric, bioanalytical, and immunogenicity considerations and challenges in the development of these CAR-T and TCR-T cell therapies. Hence, to help accelerate the development of these life-saving therapies for the patients with cancer, experts in this field came together under the umbrella of International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) to form a joint working group between the Clinical Pharmacology Leadership Group (CPLG) and the Translational and ADME Sciences Leadership Group (TALG). In this white paper, we present the IQ consortium perspective on the best practices and considerations for clinical pharmacology and pharmacometric aspects toward the optimal development of CAR-T and TCR-T cell therapies.

Over the past couple of decades, tremendous research efforts have led to accelerated development of adoptive cell therapies (ACTs), specifically for cancer therapy. ACTs utilize immune cells isolated from the patient (autologous) or a healthy donor (allogeneic), genetically engineered to be antigen-specific, substantially expanded *ex vivo*, and infused into the patient.[1,2] ACTs are of various types, including tumor infiltrating lymphocytes (TILs), engineered T cell receptor T cells (TCR-T), chimeric antigen receptor T cells (CAR-T), and chimeric antigen receptor NK cells (CAR-NK), along with others. Whereas other cell-based therapies are still under development, autologous CAR-T therapy has evolved as one of the expanded branches of cancer immunotherapy. Positive clinical outcomes in heavily pretreated patients suffering from hematological malignancies, such as relapsed/refractory B-cell malignancies, including leukemia, lymphoma, and multiple myeloma, have led to regulatory approvals of 4 CD19- and 2 BCMA-targeting CAR-T therapies.[3,4] Despite these successes, challenges continue to exist for autologous CAR-T therapies including relapse, on-target toxicities, less encouraging efficacy for solid tumors, long "vein-to-vein" time for terminally ill patients from leukapheresis to infusion, lack of flexibility for repeat dosing, high cost, and manufacturing hurdles.[5]

To address the above challenges, efforts are underway to optimize and improve the CAR engineering and design aspects for autologous and allogeneic therapies.[2] CARs are synthetic T-cell receptors with different functional domains. The first generation CAR included an extracellular antigen-binding domain (usually single-chain variable fragment (scFv) of an antibody) that is fused through the hinge and transmembrane domains to the intracellular signaling CD3ζ chain of the TCR complex.[6–9] Upon contact with the tumor cells, the CAR scFv recognizes and engages with the target antigen in a major histocompatibility complex (MHC)-independent manner, which leads to induction of key signaling events, including T-cell activation and proliferation, cytokine release, and eventually tumor cell lysis. However, transient T-cell proliferation and limited cytokine secretion led to the development of second- and third-generation CARs, which included co-stimulatory endo domains, such as CD28 or/and 4-1BB to improve *in vivo* proliferative capacity, persistence, and overall activity of CAR-Ts.[10–13] Next generation CAR-Ts are further modified to

[1]Clinical Pharmacology, Genentech, South San Francisco, California, USA; [2]Clinical Pharmacology, Pharmacometrics, Disposition and Bioanalysis, Bristol Myers Squibb, Lawrence Township, New Jersey, USA; [3]Quantitative Clinical Pharmacology, AstraZeneca, Boston, Massachusetts, USA; [4]Clinical Pharmacology and Pharmacometrics, Janssen R&D, LLC, Spring House, Pennsylvania, USA; [5]Discovery Biotherapeutics, Bristol Myers Squibb, Cambridge, Massachusetts, USA; [6]Clinical Pharmacology, Gilead Sciences, Foster City, California, USA; [7]Bioanalytical Discovery and Development Sciences, Janssen R&D, LLC, Spring House, Pennsylvania, USA; [8]Clinical Pharmacology, DMPK, Pharmacometrics, Moderna, Inc., Cambridge, Massachusetts, USA; [9]Development Biological Sciences, Immunology, AbbVie, Irvine, California, USA; [10]Clinical Pharmacology, Modeling & Simulation, Amgen, South San Francisco, California, USA. *Corresponding author: Vijay V. Upreti, PhD, FCP (vupreti@amgen.com)

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include bispecific or multispecific scFvs to target different tumor antigens for improving selectivity and specificity,[14] suicidal genes to trigger CAR-T depletion in case of toxicity, additional armoring to improve T-cell function, and/or to overcome immunosuppressive tumor microenvironment, such as IL-12 expression, along with other modifications.[15] However, such next generation engineering also comes with its own set of challenges (e.g., safety risk due to a new mode of action) and hence should be thoroughly investigated during the early development stage.

In addition to optimizing CAR design, other cell therapies including TCR-T or γδ T-cells, innate cells including natural killer (NK) cells, and allogeneic "off-the-shelf" therapies are under rapid investigation.[16] This paper is not meant to cover the perspectives on all different cell therapies; rather, it is focused on CAR-T therapies, general strategies to be considered for cell therapies, and some thoughts on how to adapt one type of therapy to another. For example, in contrast to CAR-Ts, which require target antigen on the cell surface, TCR-T therapies capitalize on the natural mechanisms of T-cells and recognize intracellular proteins presented as peptides on MHC (i.e., MHC-dependent mechanism).[17] The difference in the manner of function leads to specific considerations in the development of TCR-T therapy, which will be discussed in this paper.

The dose-exposure and exposure-response (safety and efficacy) relationships for ACTs is convoluted due to a variety of factors, including unique pharmacokinetic (PK) characteristics, intrinsic and extrinsic patient-related factors, and product-related factors due to heterogeneous T-cell population. Optimal dosing in the context of exposure-response relationships and identifying appropriate covariates of exposure or response are beginning to be explored and reported with varying degrees of model complexity.[18–21] Lack of relevant preclinical models continue to pose a major hurdle for preclinical to clinical translation that impacts first-in-human (FIH) study design and dose selection. Traditional quantitative tools in drug development leveraged for small molecules or biologics cannot be directly applied but rather require adaption and optimization for the development of ACTs.

To address clinical pharmacology-related challenges for ACTs, a pan-industry group comprising industrial scientists was assembled under the umbrella of the International Consortium for Innovation and Quality. The main focus of the group was to review clinical pharmacology learnings from existing data including approved CAR-T therapies, discuss considerations for FIH study design, including dose selection, review CAR-T PK/pharmacodynamic (PD) models published in the literature, and discuss unique considerations for TCR-T therapies. Although clinical pharmacology aspects for CAR-T therapies have been published from a couple of individual companies,[22,23] this white paper is the first pan-industry collective perspective on considerations for clinical pharmacology and pharmacometric aspects of CAR-Ts and TCR-Ts.

CHARACTERIZATION OF MULTIPHASIC CELLULAR KINETICS, DOSE-EXPOSURE, AND EXPOSURE-RESPONSE RELATIONSHIPS, AND IMPACTFUL FACTORS FOR CAR-T CELL THERAPIES

Characterization of cellular kinetics

Due to the "living drug" nature, CAR-T cells undergo *in vivo* expansion after infusion and exhibit unique PK (also termed as cellular kinetics (CKs)) behavior. The typical PK characteristics, such as absorption, distribution, metabolism, and excretion observed for small and large molecules are not applicable for CAR-Ts. Instead, CAR-T CK profile is considered to have up to four distinct phases: distribution, expansion, contraction and persistence with early distribution phase captured only through intensive sampling during early timepoints after infusion[24] (**Figure 1**). Such multiphasic CK profiles are quantitatively analyzed by polymerase chain reaction (PCR)-based methods, which measures T-cell as copies of CAR transgene per μg genomic DNA, and/or by flow cytometry, which measures the number of T-cells with surface-expressed transgenic CAR per μL of blood. Pros and cons of the two bioanalytical methods and the underlying mechanisms that drive the multiphasic CK profile were previously discussed.[25,26]

The PK terms of CK, exposure, and expansion are often used interchangeably for cell therapies, and may be characterized with non-compartmental analysis (NCA) or population-based PK approach. NCA can be used to compute CK parameters, such as maximum observed concentration (C_{max}), time of C_{max} (T_{max}), and partial area under the concentration-time curve (such as from time zero to 28 days after dosing ($AUC_{0\text{-}28d}$)) which characterizes cellular expansion. The first month after infusion was demonstrated to sufficiently capture the cell expansion phase and adequately reflected the overall exposure,[27] although different time intervals for AUC may be used depending on the clinical efficacy and safety end points.[28] Time of last quantifiable concentration (T_{last}) and terminal half-life ($t_{1/2}$) are reported as parameters for cellular persistence, however, these parameters should be interpreted with caution because they greatly depend on the follow-up time. Thus, for large variation in the follow-up time, T_{last} should be analyzed by appropriate time to event methods (e.g., Kaplan–Meier).[29] Other key NCA PK parameters, such as clearance and volume of distribution, are not applicable to CAR-Ts. Handling of data below the limit of quantification (BLOQ) should also be carefully examined, as it may introduce bias when characterizing the distribution phase followed BLOQ sample or when CAR-T is re-activated afterBLOQ. Persistence is also used for safety monitoring in the long-term follow-up period/study (up to 15 years after infusion). The US Food and Drug Administration (FDA) guidance[30] recommends the monitoring of persistence vector sequences until they become undetectable. When persistence vector sequences are detected in ≥ 1% of cells, the assessment of the vector integration pattern is recommended. Criteria of stopping the monitoring could also be implemented, such as when two consecutive samples become BLOQ. The C_{max} and AUC are the exposure metrics that are commonly used for exposure-response analyses. **Table 1** summarizes dose-exposure and exposure-response relationships observed in clinical studies of approved CAR-T therapies.

Dose-exposure-response relationships for efficacy and safety

Dose-exposure relationship. A clear dose-exposure relationship has not been generally demonstrated for approved CAR-T therapies (**Table 1**). No apparent relationship between dose and exposure (AUC or C_{max}) was observed across the studied dose

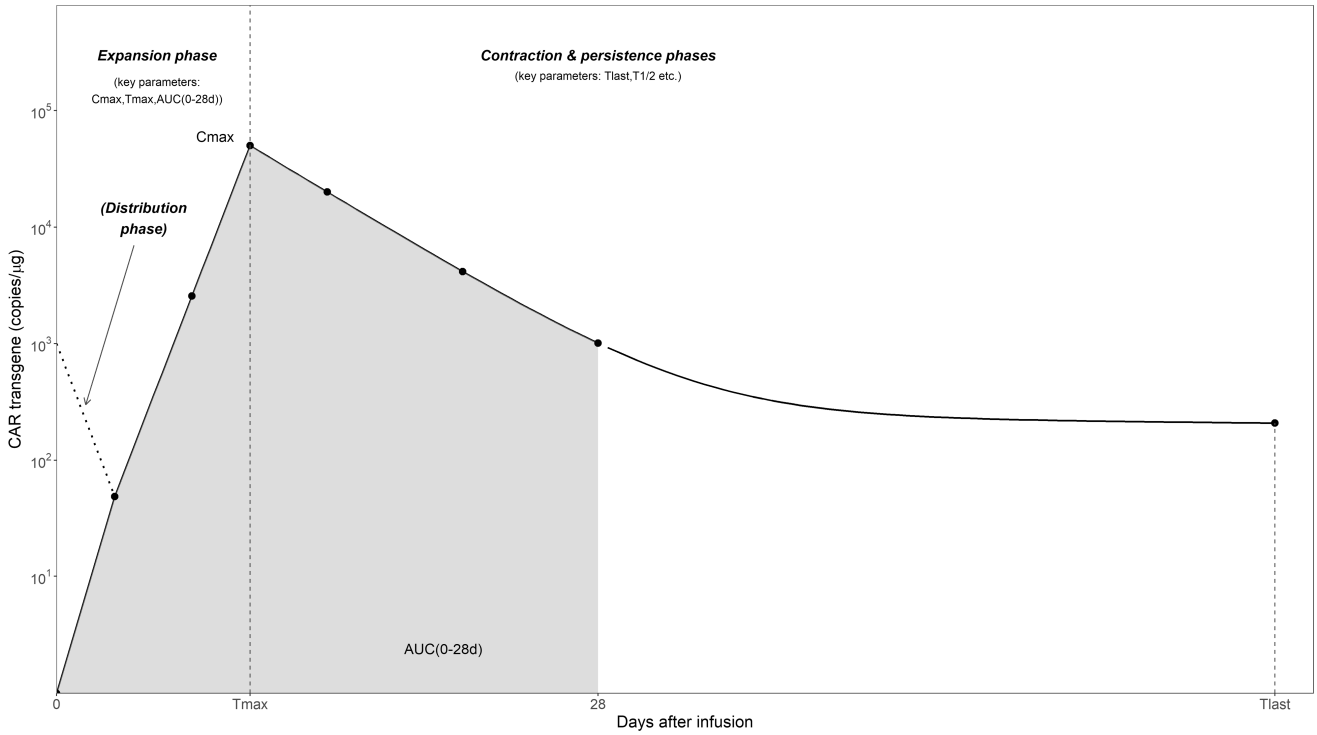

Figure 1 A typical CK (concentration-time) profile for a CAR-T therapy depicting its multiphasic nature and key CK parameters. $AUC_{0\text{-}28d}$, area under the concentration-time curve from time zero to 28 days after dosing; CK, cellular kinetic; C_{max}, maximum observed concentration; T_{max}, time of C_{max}.

range for tisagenlecleucel (tisa-cel)[31,32] or among target dose levels for lisocabtagene maraleucel (liso-cel),[33] with no clear relationship between dose and efficacy or safety. Idecabtagene vicleucel (ide-cel) exposure increased with increasing dose within the evaluated target dose range of $150–450 \times 10^6$ CAR+ T cells, however, the high interindividual variability resulted in substantial overlap of ide-cel exposure across the target dose levels.[34,35] A positive dose-efficacy/safety relationship was suggested for ide-cel based on the simulation of the established exposure-response models, which indicated a positive benefit–risk assessment for the range of exposures associated with the target dose range of $150–450 \times 10^6$ CAR+ T cells.[35] In contrast, a positive trend of dose-dependency for exposure or response up to a certain dose (beyond which the relationship plateaus) was seen in a number of early clinical studies.[34,36–38] A recent meta-analysis of published clinical studies also suggested threshold dose for optimal efficacy beyond which dose escalation is unlikely to result in improved efficacy but would be associated with a higher incidence of adverse events.[39] However, it is important to note that the described meta-analyses or comparison across different studies are difficult to interpret if the trials are not randomized, due to dose-exposure or dose–response relationships for CAR-T therapies being confounded by differences in the nature of products, dose ranges, sample size, and other patient- and product-related characteristics.

Exposure-response relationship for efficacy. Exposure-response relationship for best overall response (i.e., responders vs. nonresponders) has been extensively investigated for the approved CAR-T therapies (**Table 1**). Although responders typically have higher expansion (C_{max} and $AUC_{0\text{-}28d}$) than nonresponders, in certain instances, no apparent differences in expansion between the two groups have also been observed (e.g., tisa-cel in 3L+ large B-cell lymphoma (LBCL)[32] and 3L+ follicular lymphoma (FL),[40,41] liso-cel in 2L LBCL[42,43]). Generally, T_{max} is similar in responders and nonresponders. Although longer persistence was observed in responders of tisa-cel for acute lymphoblastic leukemia (ALL)[31] and 3L+ LBCL,[32] this observation may be confounded by longer follow-up in responders compared with nonresponders and should be interpreted with caution. Higher cellular expansion was also associated with higher likelihood of achieving minimal residual disease negativity for ide-cel.[34] Interestingly, data from axicabtagene ciloleucel (axi-cel) showed that early expansion, rather than CAR-T cell concentrations after 3 months or beyond, correlated better with durable response[44] (ongoing response at least 1 year after axi-cel infusion). Exposure-response relationship for time-to-event end points (e.g., progression-free survival (PFS), event-free survival , and duration of response (DOR)) has also been assessed. Higher expansion is associated with longer PFS for liso-cel in 2L LBCL (transplantation not intended only)[43] and ide-cel in relapsed/refractory multiple myeloma.[34] Similarly, higher tisa-cel expansion is associated with longer DOR among responders in 3L+ FL.[41]

Overall, discrepant findings among different indications suggest that both the disease and product may impact the exposure-response relationship for CAR-T therapies (discussed further in the later sections). The differences across different indications or studies could

Table 1 Summary of dose-exposure and exposure-response relationships of six approved CAR-T therapies

CAR-T therapy	Indication	Approved dose (US)	Dose-exposure	Exposure-efficacy (BOR)	Exposure-safety (CRS)	Exposure-safety (NE)	Reference
Tisagenlecleucel	B-cell ALL (pediatric and young adult)	$0.2–5.0\times10^6$/kg (≤50 kg); $10–250\times10^6$ cells (>50 kg)	No apparent relationship	Higher expansion is associated with response (caution of limited number of nonresponders)	Higher expansion is associated with any grade CRS	Not available	31
	LBCL (3L+)	$60–600\times10^6$ cells	No apparent relationship	No apparent relationship	Higher expansion is associated with any grade CRS, grade ≥3 CRS	No apparent relationship between expansion and any grade NE, grade ≥3 NE	32
	FL (3L+)	$60–600\times10^6$ cells	No apparent relationship	No apparent relationship	Higher expansion is associated with any grade CRS	No apparent relationship between expansion and any grade NE	40,41
Axicabtagene ciloleucel	LBCL (3L+)	2×10^6/kg, maximum of 200×10^6 cells	Not applicable (one target dose)	Higher expansion is associated with response	No apparent relationship between expansion and grade ≥3 CRS	Higher expansion is associated with grade ≥3 NE	128,129
	LBCL (2L)		Not applicable (one target dose)	Higher expansion is associated with response	Higher expansion is associated with grade ≥2 CRS	Higher expansion is associated with grade ≥3 NE	130,131
	FL (3 L+)		Not applicable (one target dose)	NA (due to high ORR)	Higher expansion is associated with grade ≥3 CRS	Higher expansion is associated with grade ≥3 NE	132,133
Brexucabtagene autoleucel	r/r MCL	2×10^6/kg, maximum of 200×10^6 cells	Exposure increases within the dose range evaluated	Higher expansion is associated with response	Higher expansion is associated with grade ≥3 CRS	Higher expansion is associated with grade ≥3 NE	134,135
	r/r B-cell ALL (adult)	1×10^6/kg, maximum of 100×10^6 cells	No apparent relationship	Higher expansion is associated with response	Higher expansion is associated with grade ≥2 CRS	Higher expansion is associated with grade ≥3 NE	136,137
Lisocabtagene maraleucel	LBCL (3L+)	$50–110\times10^6$ cells	No apparent relationship	Higher expansion is associated with response	Higher expansion is associated with any grade CRS	Higher expansion is associated with any grade NE, grade ≥3 NE	19,27,33
	LBCL (2L)	$90–110\times10^6$ cells	Not applicable (one target dose)	No apparent relationship	Higher expansion is associated with any grade CRS	Higher expansion is associated with any grade NE	42
	LBCL (2L TNI)		Not applicable (one target dose)	No apparent relationship	No apparent relationship between expansion and any grade CRS	Higher expansion is associated with any grade NE	43

(Continued)

Table 1 (Continued)

CAR-T therapy	Indication	Approved dose (US)	Dose-exposure	Exposure-efficacy (BOR)	Exposure-safety (CRS)	Exposure-safety (NE)	Reference
Idecabtagene vicleucel	MM (5L+)	$300–460\times10^6$ cells	Exposure increases within the dose range evaluated	Higher expansion is associated with response	Higher expansion is associated with any grade CRS	No apparent relationship between expansion and any grade NE	34,35,38,138
Ciltacabtagene autoleucel	MM (5L+)	$0.5–1\times10^6$/kg, maximal of 100×10^6 cells	Not applicable (one target dose)	NA (due to high ORR)	Higher expansion is associated with any grade CRS	Higher expansion is associated with any grade NE	139,140

2L, second line; 3L+, third line or later; ALL, acute lymphoblastic leukemia; BOR, best overall response; CRS, cytokine release syndrome; FL, follicular lymphoma; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; NE, neurological event; ORR, overall response rate; r/r, relapsed or refractor; TNI, transplantation not intended.

also be due to small number of nonresponders (because of high overall response rate (ORR)) and/or smaller sample size relative to high interindividual variability in expansion parameters. Additionally, systemic exposure may not be reflective of CAR-T levels at the site of action (e.g., lymph nodes for lymphomas), hence, perhaps not reflective for possible efficacy responses which further complicates the interpretation of exposure-efficacy relationship.

Exposure-response relationship for safety. Cytokine release syndrome (CRS), resulting from CAR-T cell activation and subsequent release of cytokines, is an on-target primary safety concern for CAR-T therapy.[45] Additionally, neurological events (NEs) are another common safety concern, however, the underlying mechanism is not fully understood. Exposure-response relationships for CRS and NE have been extensively evaluated with higher expansion generally associated with higher grade or incidence of CRS and/or NE across different CAR-T therapies (**Table 1**). No apparent difference in the T_{max} of patients experiencing these safety events has been noted.

Dose-exposure-response relationship in solid tumor indication. Currently, there are no approved cell therapies in solid tumor indications, but clinical data for investigational assets are still emerging. Interestingly, some dose-dependency in kinetics has been reported,[46,47] as well as a trend of better efficacy in patients with higher expansion,[46,48–50] which is consistent with hematologic malignancies. However, cell therapy in solid tumor indications is further complicated due to T-cell activation in tumor tissue[51] and trafficking, and the heterogeneous and immunosuppressant tumor microenvironment. Another challenge is the potential on-target off-tumor toxicities due to expression of the target on healthy tissues, which can be lethal and/or observed even at starting cohorts during dose escalation in clinical studies. Clinical case studies that observed on-target off-tumor toxicities as well as potential approaches to address the same, including optimization of CAR domain and affinity, logic-gating approaches, controlling CAR-T cells postinfusion through safety switches, along with others, have been extensively reviewed recently and are beyond the scope of this paper.[52]

Multiple factors affecting dose-exposure-response relationship. The impact of patient-related intrinsic factors, such as disease burden, target expression, immune condition, and extrinsic factors, such as prior treatment, lymphodepletion, or other comedications, as well as product-related factors, such as T-cell phenotype, should be evaluated for potential impact on the dose-exposure-response relationship (**Figure 2**).

The impact of certain patient demographics (e.g., body weight, age, sex, and race) on CK has been evaluated using population modeling for tisa-cel (ALL),[18] liso-cel (3L+ LBCL),[19] and cilt-acabtagene autoleucel (cilta-cel).[20] None of these analyses found any patient demographics to have meaningful impact on CK. Specifically, body weight (BW) or body surface area (BSA) does not seem to be a significant covariate on CK or impact kinetics or efficacy,[19,24] and yet both fixed and BW-based dosing have been used for adults in hematologic malignancies or solid tumors.[53]

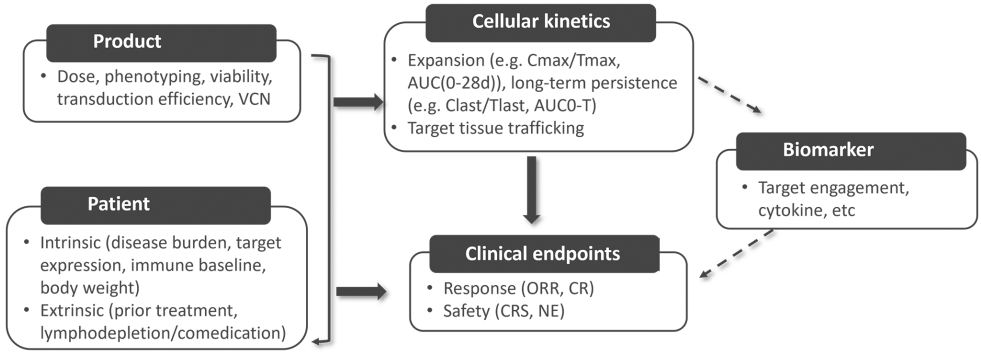

Figure 2 A schematic summarizing key product- and patient-related characteristics that can impact cellular kinetics and clinical biomarkers or end points of efficacy and safety. $AUC_{0\text{-}28d}$, area under the concentration-time curve from time zero to 28 days after dosing; C_{last}, last measurable concentration; C_{max}, maximum observed concentration; CR, complete response; CRS, cytokine release syndrome; NE, neurological event; ORR, overall response rate; T_{last}, time of last quantifiable concentration; T_{max}, time of C_{max}; VCN, vector copy number.

Tumor burden is generally known as a key baseline characteristic to have an impact on efficacy, safety, or CK of CAR-T therapies.[28,33,44] For liso-cel in 3L+ LBCL, patients with a high tumor burden had numerically lower response rates, higher incidence of CRS and NE, and higher cellular expansion than patients with a low tumor burden.[33] Exposure-response analysis of liso-cel in 3L+ LBCL suggested that tumor burden was confounding the relationship between cellular expansion and response, and high tumor burden does not necessarily translate to better responses.[27] On the other hand, high tumor burden appeared to be associated with CRS or NE partially through higher liso-cel expansion by high tumor burden.[27] For tisa-cel in diffuse large B-cell lymphoma, disease burden showed impact on safety while there was no clear impact on CKs.[32] Such complex impact of tumor burden on expansion or efficacy has been linked with T-cell exhaustion at high tumor burden, whereas there is a lack in antigen stimulation with low tumor burden.[54] Thus, it is crucial to comprehensively investigate the impact of tumor burden on CK and safety/efficacy in the context of exposure-response relationship.

Corticosteroids or tocilizumab are commonly used to mitigate CRS or NE caused by CAR-T therapies. Axi-cel data from the ZUMA-1 study showed no negative impact of low or high dose of corticosteroid use on CKs or efficacy, and prophylactic and earlier intervention actually resulted in a lower cumulative corticosteroid dose with better safety mitigation.[55] Population CK analysis also indicated no impact of corticosteroid or tocilizumab on the tisa-cel expansion rate.[18] On the other hand, lymphodepletion agents used as a conditioning regimen are known to improve CAR-T expansion, persistence, function, and efficacy, probably through eliminating sinks for homeostatic cytokines and immunosuppressive elements.[56–58]

T-cell phenotype (e.g., T-cell fitness), in a CAR-T product may have a significant impact on CKs or efficacy. Memory stem cell phenotype has been associated with higher response in patients.[59] A similar finding was reported for axi-cel, in which T-cell fitness indicated by shorter *ex vivo* doubling time and higher CCR7 + CD45RA- composition was associated with higher expansion ability *in vivo* and more durable response.[44] Similarly, intrinsic quality of T-cells obtained from patients, which is dependent on the age, previous lines of therapy, along with other factors; can also impact *in vivo* CAR-T expansion and response.[60] For instance, T-cells from patients with naïve and stem cell memory phenotype showed higher *in vitro* expansion. Whereas chemotherapy induced reduction of early lineage cells was associated with a decline in the *ex vivo* stimulation response.[60] In addition, CD4+:CD8+ ratio in the final product is one of the key product characteristics, and a defined composition of CD4+ and CD8+ CAR+ T-cells was associated with better expansion and efficacy in animal models.[61] However, among 6 approved CAR T-cell therapies, the CD4+:CD8+ ratio is not defined except for liso-cel.[29] In addition, for tisa-cel, no apparent association was observed between CD4+:CD8+ ratio in the final product and CKs or efficacy and safety.[31,32]

With the multiple confounders of dose-exposure relationship that include the large impact of patient and product characteristics on the CKs or response, dose may not be the most critical factor in impacting exposure or response, rather various product or patient-related factors may be more critical. Clinical decision is not limited to dose selection, rather it could also include factors such as patient selection, lymphodepletion optimization, and product characterization.

TRANSLATIONAL, DOSE SELECTION, AND FIH STUDY DESIGN CONSIDERATIONS

Preclinical-to-clinical translation

Immunotherapy poses several translational challenges due to difficulties in interspecies difference in the immune systems, mimicking expression and intrinsic properties of tumor antigens, tumor microenvironment, and disease and patient-dependent immune conditions including immune suppressive environment. Upon that CAR-Ts pose unique translational challenges attributed to their complex design that includes multiple domains (such as binding region,[62] co-stimulatory,[63] and activating domains) and variable phenotypic composition. Similarly, CK and clinical responses are dependent on a multitude of patient-specific factors (e.g., tumor burden and immune condition) and product-specific characteristics.

Relevant preclinical *in vitro* and *in vivo* models to characterize CAR-T cells in general are provided in the FDA guidance.[64]

Specifically, *in vitro* studies may enable the evaluation of novel CAR constructs (binding epitopes / affinities, spacers, and co-stimulatory domains, etc.) and how these features relate to function. *In vitro* functional assays to evaluate antigen-dependent CAR-T proliferation, killing potency, and cytokine induction provide valuable information on product activity or relative activity to other products. These studies should be performed across a range of effector to target (E:T) ratios to enable the evaluation of CAR-T sensitivity to tumor antigen expression. Advancements in building organoids and other 3D systems helps to further mimic tumor microenvironment. CAR-T therapies are often tested *in vivo* in the immune-deficient mice bearing human tumors to demonstrate proof of concept, however, the lack of interplay among complex human immune components in these mouse models limits its direct translatability to guide the FIH study. Alternate mouse models, such as syngeneic, genetically engineered, and/or humanized models, provide unique advantages and can be particularly helpful to elucidate mechanisms in the preclinical setting, but they all come with specific limitations. It is recommended that the CK features, such as antigen-dependent expansion and contraction, are characterized together with pharmacological end points, such tumor killing dynamics in preclinical mouse models. Generally, long-term persistence is not assessed in such *in vivo* studies due to Graft-vs.-host disease (GvHD). Multiple dose groups should be considered to allow early assessment of dose-dependency. Animal tumor models with different tumor antigen expression should also be considered to assess the antigen-dependency and impact of tumor target expression on CAR-T function.

In general, there is a lack of relevant animal models to assess CRS or neurotoxicity, which is commonly observed in the clinic. Therefore, preclinical safety studies in non-human primates for a novel CAR-T product is often not relevant during the preclinical evaluation, and such information is not used for FIH dose derivation either. *In vitro* / *ex vivo* assessments to evaluate toxicity and target expression using off-tumor tissues may inform the clinical safety monitoring plan.

Given there is no one *in vitro* or *in vivo* system that is completely translatable, it is important to carefully select the preclinical models based on the critical question that is addressed in the discovery and development of CAR-Ts. More work is necessary to understand basic mechanistic differences between *in vitro* / *in vivo* models and humans to build relevant translational PK/PD tools for projecting FIH doses.

FIH starting dose selection

It has been over a decade since the first CAR-T therapy was introduced in human studies and hundreds of clinical trials are currently active, yet there is no standardized approach toward FIH starting dose selection for CAR-Ts. Some of the traditional PK and starting dose approaches are not directly applicable due to limited relevance of preclinical models to the clinic and the living nature of CAR-T products, which are expected to expand at varying levels upon administration. The recent FDA guidance on development of CAR-Ts highlighted that experience from prior clinical studies with other CAR-T products can be used to inform starting doses with careful considerations, and preclinical studies may be used to inform the intended starting dose where there is acceptable risk. Our perspective is in line with the FDA guidance, and additional considerations and recommendations toward starting dose selection is provided.

Leveraging prior clinical information. It is important to perform a thorough review of internal and externally published information on prior clinical trials to inform FIH doses, clinical study design, dose limiting toxicities, and efficacy by disease categories and tumor target of interest (**Figure 3a**). An exploratory literature survey of FIH starting doses conducted by this IQ Working Group found that the FIH starting doses for CAR-T cells in hematological and solid tumor indications were generally similar (typical starting doses ranging around a million CAR positive T-cells per kg as starting dose), although differences were observed in the starting doses for TCR-T cell therapies when compared with CAR-T cell therapies, with starting doses for TCR-T cell therapies being typically higher than CAR-T cell therapies. As CAR-T cell therapies for solid tumor indications are still evolving and fully optimized dosing and optimal clinical efficacy for CAR-T cell therapies remains to be demonstrated for solid tumors, we hypothesize that the dose required in solid tumor indications to elicit optimal efficacy may trend relatively higher due to limited distribution of CAR-Ts to relatively confined, solid tumors vs. easier to access, hematological tumors. Additionally, the limited distributions of CAR-Ts at the solid tumor site may lead to lower E:T ratios that further limit CAR-T activity which may necessitate higher dose. Furthermore, there are differences even within hematological malignancies that can impact dose selection, including immune condition, antigen expression, and site of action considerations. Therefore, it is recommended to leverage clinical information from prior CAR-Ts with the same or similar indication and target expression properties. On top of that, product characteristics, such as specific CAR design, phenotype, and manufacturing conditions, along with others, may alter its activity, in which cases additional preclinical evaluation may be helpful.

Leveraging preclinical evaluations. There is a continuous interest in exploring preclinical experimental and *in silico* models to inform FIH study design for CAR-Ts. Particularly, use of preclinical studies may be helpful when selecting a starting dose for a next generation CAR-T product targeting an antigen with limited prior experience in the clinic. Potential approaches leveraging preclinical results along with prior clinical learnings to select FIH starting dose are summarized in **Figure 3**.

In an empirical approach, attempts can be made to allometrically scale CAR-T doses from preclinical models to humans (**Figure 3b**). For example, BSA-based scaling to convert the mouse efficacious doses to human equivalent dose have been considered based on internal discussions within this IQ Working Group. Such empirical scaling can serve as a starting point for preclinical to clinical efficacious dose translation. However, without considering the CK differences expected between mouse and patients with cancer this approach can be misinformed and there is no current mechanistic hypothesis that

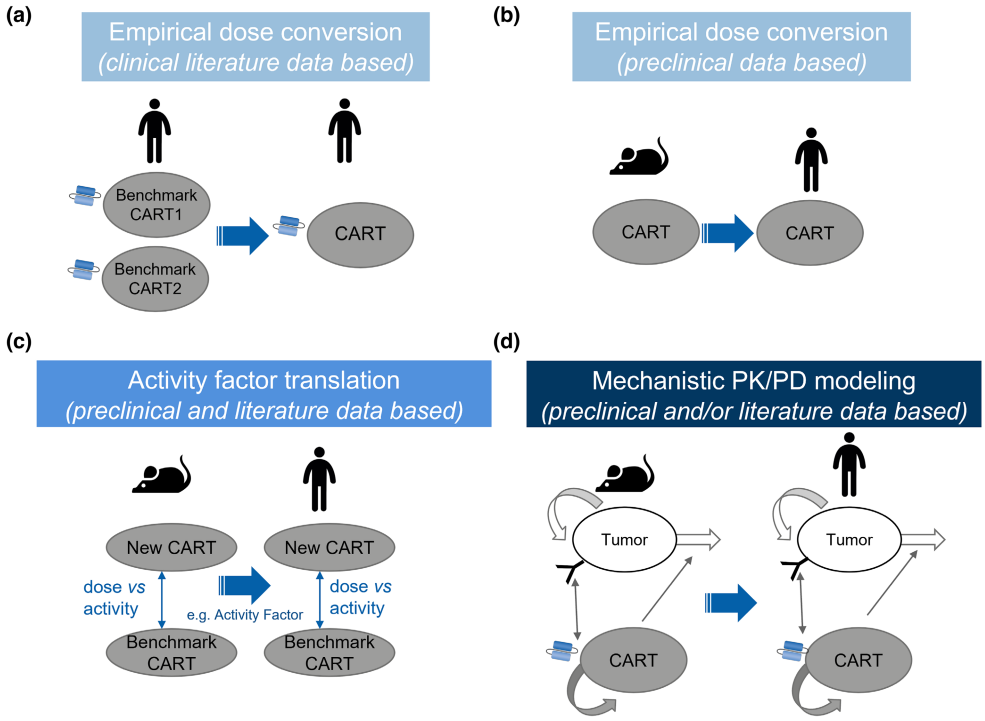

Figure 3 Schematic representation of empirical dose conversion directly from clinical literature (**a**) or preclinical data (**b**), activity factor (**c**), and mechanistic PK/PD modeling (**d**) approaches to project first-in-human starting dose for CAR-Ts. PK/PD, pharmacokinetic/pharmacodynamic.

would suggest why a BSA-based approach is relevant for CAR-T products.

Another approach of scaling preclinical doses to the clinic is based on leveraging results from a benchmark CAR-T. This approach leverages an "activity factor" determined from prior preclinical and clinical data with the benchmark CAR-T and the preclinical data of new CAR-T[65] (**Figure 3c**). For this approach, it is recommended that the benchmark CAR-T should bind to the same or functionally similar target and have clinical safety or efficacy data available. The activity factor is derived by statistical analysis or empirical modeling comparing the benchmark CAR-T with the new CAR-T in preclinical animal efficacy models. Finally, leveraging the clinical dose-safety or dose-efficacy information of the benchmark CAR-T, a dose predicted to be tolerable and with some clinical activity can be derived for the new CAR-T. This approach was applied to two CAR-Ts with different targets and indications, and cellular expansion, early clinical activity was observed in the first few patients with tolerability allowing further dose escalation.[66,67] The major limitation of this approach is that it is applicable only to new CAR-Ts when clinical information from other benchmark CAR-T therapy with the same or functionally similar target or for the same indication is available. In addition, the approach assumes that the derived activity factor is translatable from preclinical studies to the clinic and that the preclinical model can be used to rank order novel CAR-T products. However, these assumptions may not always hold true given the known lack of translatability of preclinical animal models. Therefore, extrapolating the approach to other CAR-T products should be made with caution based on the totality of information.

With emerging trials of novel targeted products, there is a need in establishing a translational approach to characterize the interplay between CAR-T expansion and tumor killing and predict CAR-T performance in human based on animal data with reasonable assumptions (**Figure 3d**). A multiscale, mechanistic PK/PD model has been used to describe such interaction between tumor and CAR-T.[68,69] Certain key model parameters (CAR binding, CAR-T expansion, and tumor killing) informed or calibrated based on preclinical *in vitro* and *in vivo* data were used to capture the clinical data. Additional case studies on PK/PD models have been summarized later. Although progress made in such mechanistic models are encouraging, these approaches need further validation with reverse translation assessments.

Overall, selection of FIH starting dose based on prior clinical studies that takes disease category, target properties, and CAR design into consideration is commonly used. Application of preclinical assessments to de-risk the selected dose for a novel target and experiments that benchmark with a CAR-T product with prior clinical results can inform FIH doses by taking totality of data into consideration. However, one must be cautious of the limitations of the preclinical models used to compare activity between multiple CAR-T products given the limited translatability.

FIH study design considerations

In addition to the starting dose selection, other FIH dosing-related considerations include (i) dosing based on BW or BSA or fixed dose, (ii) dose fractionation or disease burden-based dosing, (iii) single dose vs. repeat dosing, (iv) dose escalation strategy, and (v) selection of lymphodepletion regimen.

The dose of CAR-T therapy can be based on viable, CAR positive, T-cells as a fixed dose or normalized to BW or BSA of the patient. Although both strategies have been used in adult patients for both hematological and solid tumor indications, BW-based dose is more commonly used in pediatric indications for safety considerations. Based on available published clinical data, neither BW nor BSA have been found to be significant covariates on cellular expansion or contraction, nor have they been indicated to impact exposure vs. efficacy relationship for CAR-Ts.[19,24,53] Hence, a fixed dose approach to initiate a FIH trial is the preferred and recommended strategy as it can simplify manufacturing process and reduce the risk of dosing errors. As clinical data develops using the fixed dosing approach, it is highly recommended to assess the BW or BSA as a covariate with emerging clinical data during development to further justify the strategy. When body size is identified as a covariate for CK and for response (safety and efficacy based on preliminary E-R relationships) from early phase trial(s) data, body-size-based dosing should be implemented for late phase/registrational trials, otherwise late phase development should be continued with fixed dosing.

Dose fractionation or tumor burden-based dosing strategy[70,71] may be introduced in patients with high risk of CRS or neurotoxicity for safety mitigation. A recent meta-analysis across 18 clinical studies suggested that dose fractions over 2–3 days may mitigate safety risk especially in patients with high disease burden.[72] Such a risk-based dose fractionation approach may improve safety while retaining efficacy. A similar risk-stratified, tumor-burden-based dosing was beneficial to balance safety and efficacy in a clinical study, with lower dosing in patients with high tumor burden to mitigate toxicity and higher dosing in patients with low tumor burden to ensure target engagement and tumor clearance.[36] However, lower dosing in high tumor burden patients may also result in insufficient CAR-T expansion leading to suboptimal efficacy in clearing the high tumor burden. Besides, dose fractionation or tumor-burden-based dosing may pose operational challenges in the clinic or difficulty in assessing the dose-expansion or dose–response relationships or restrict patient selection. Therefore, it is suggested to evaluate and optimize the benefit of risk-based dosing strategy through controlled studies.

Autologous CAR-Ts have so far largely been a single dose treatment paradigm. Whereas a significant number of patients relapse after a single dose of CAR-T therapies, clinical outcomes after repeat dosing are not completely understood with variable data emerging from limited patients in the clinical studies.[37,73] Interestingly, retrospective analysis on the outcomes after second CAR-T infusions, in patients who relapsed or became refractory after the first dose, suggested that repeat dosing may be beneficial in a subset of patients.[74,75] Optimized lymphodepleting regimen before the first infusion as well as an increased CAR-T dose for the second infusion were associated with higher CAR-T persistence as well as higher ORRs and prolonged PFS after the second infusion.[74] However, repeat dosing may not be feasible for autologous CAR-Ts due to manufacturing challenges, cost of goods, requirement of lymphodepletion prior to each dose, and development of immunogenicity, along with other factors. As the allogeneic "off-the-shelf" cell therapies are being developed, repeat dosing will likely be more feasible, and may be become necessary if persistence is attenuated relative to autologous CAR-Ts. Clinical pharmacology strategies will need to adapt based on emerging clinical learnings to rationalize multiple dosing regimen, dosing frequency, need for lymphodepletion prior to each dose, and associated immunogenicity or GvHD risks associated with allogeneic therapies.

Traditional oncology FIH study dose escalation designs based only on toxicity may not be informative in guiding cell therapy dose escalation.[76] For traditional oncology therapeutics, such as chemotherapy, toxicity and efficacy are both highly dose correlated. However, for cell therapies, the starting dose is often already within the active dose range and efficacy can be as quickly observed as the toxicity. Dose to efficacy and safety relationships are not always linear and it is not uncommon to see efficacy and safety signals overlap at the starting dose itself or in early cohorts. With dose escalation, higher doses may not necessarily link to better efficacy, whereas target-related toxicity, such as CRS and neurotoxicity, is overall well-managed, therefore, the dose-efficacy and dose-toxicity relationships may be shifted for cell therapy.[23,39,77] Establishing a toxicity and efficacy probability interval (TEPI) is one of the optimal approaches for cell therapies as it integrates both safety and efficacy data to maximize the therapeutic benefit for patients. Similarly, Bayesian Optimal Interval (BOIN)-guided escalation strategy perhaps might be more appropriate than using Bayesian logistic regression models (BLRMs) or 3 + 3 designs.[78]

As described in previous sections, CAR-Ts are a complex modality where dose is just one of the many factors that affect efficacy and safety. Dose selection should consider both efficacy and safety, and the patient and product factors that potentially impact dose–response relationships. Due to the large interpatient variability and relatively small cohorts, it may be hard to select one optimal dose from escalation and more than one cohort could be expanded to further optimize the recommended phase II dose (RP2D).[38,79] On top of that, dose for cellular therapies may not be the most critical impact factor always in determining efficacy or safety, therefore the decision for clinical development could be based on a variety of other factors, for example, dose range, exposures, product characterization criteria, and/or patient selection based on target expression.

Finally, both the lymphodepletion agents and the doses should be deliberately selected based on learnings from other CAR-T clinical trials and optimized in early clinical cohorts.

MODELING AND SIMULATION STRATEGIES FOR DISCOVERY AND DEVELOPMENT OF CAR-TS

Significant efforts are initiated toward exploring modeling and simulation (M&S) approaches through different stages of discovery and development of CAR-T therapies–from characterization of leads to supporting FIH to clinical development and beyond (**Figure 4**). Due to the "living nature" of the product, the concept of mass-balance does not apply for CAR-T therapies. Hence, the traditional compartmental PK models of small or large molecules are not directly applicable, but rather adapted to capture the multiphasic CK profile for CAR-Ts.[18] Given the availability of diverse datasets related to patient- and product-specific characteristics, biomarker, safety, and efficacy, multiple modeling approaches can

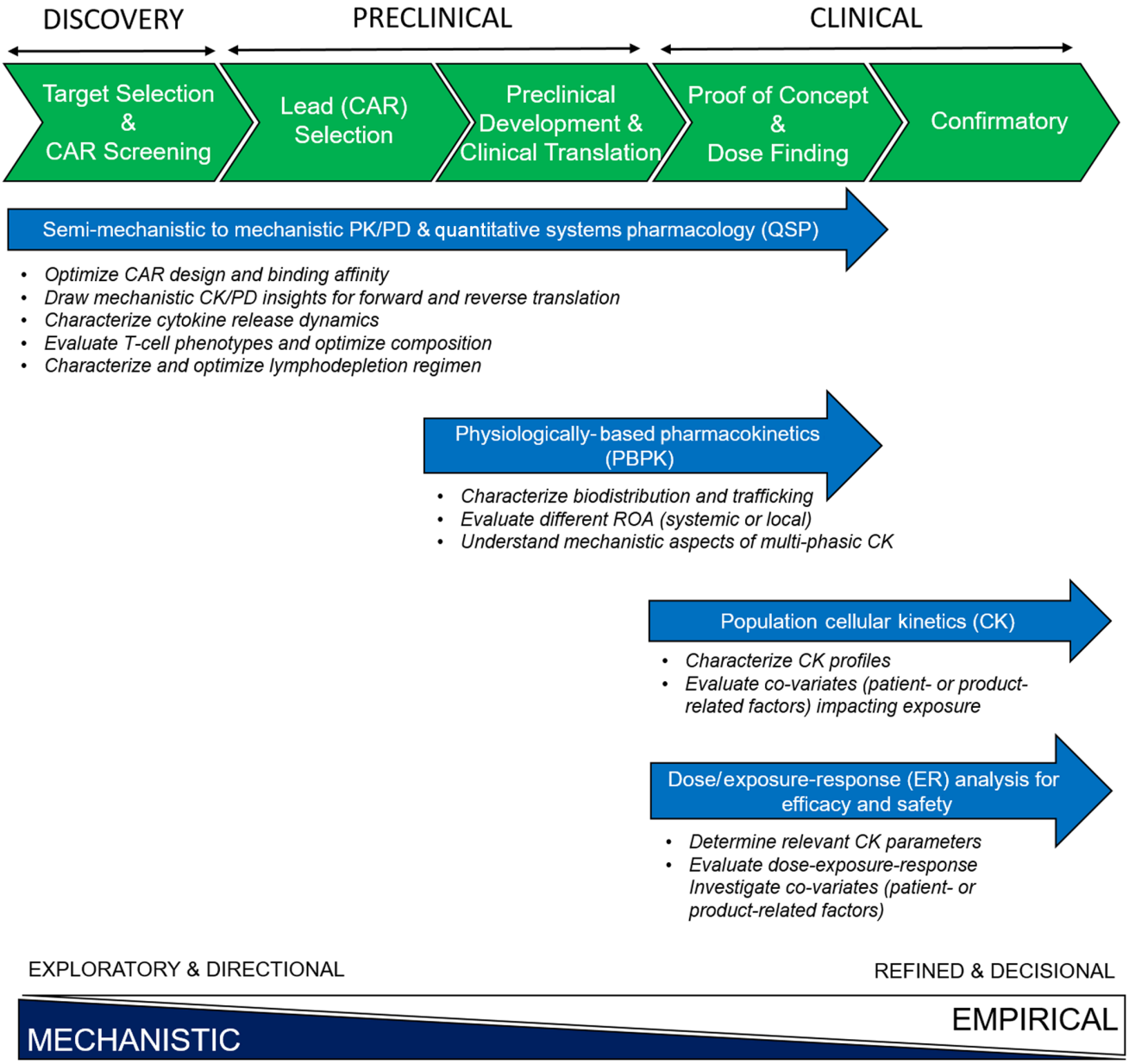

Figure 4 Modeling and simulation approaches to inform preclinical and clinical development of CAR-T therapies. PD, pharmacodynamic.

be applied to integrate the rich multidimensional data. Fit-for-purpose utilization of different M&S approaches depending on the scope, availability of different datasets, stage of the program, model assumptions, and limitations while acknowledging the gaps in the field, will be useful for the successful implementation of model-informed drug development (MIDD) for cell therapies.

The scientific questions should drive the appropriate level of complexities in the models. For instance, empirical models with concepts of compartmental modeling can describe essential elements of the CK profile, and infer effector and memory subsets based on differential half-life without directly measuring them. Supported with additional early or late clinical datasets of patient and product characterization, such models can be very useful in evaluating and explaining the variability in CKs and better assessing the dose-exposure-response relationships. If the goal of the modeling is to predict distribution of T-cells to the site of action, or to understand various T-cell subsets interplay with the tumor, a more mechanistic physiologically-based pharmacokinetic (PBPK) or quantitative systems pharmacology (QSP) model can provide important additional insights into the complex CAR-T and tumor interactions and impact of confounding factors. Yet, such approaches also require high amount of T-cell phenotype, distribution, binding, activation, and other functional data, which may not be always available. Here, semimechanistic models may provide a good compromise for describing expansion and activation of T-cells subsets to optimize phenotypic composition, for characterizing cytokine release dynamics to understand CRS, or for optimizing lymphodepletion regimen prior to CAR-T therapy,

without requiring a fully bottom-up approach. A combination of empirical exposure-response analysis, population CK, and semi-mechanistic modeling can be used real-time with emerging data during early clinical development to better understand large interpatient variability in limited number of patients and making informed decisions for dose optimization, mitigating risk, and impacting future study design.

Similar mechanistic modeling approaches can also be used for preclinical to clinical (forward) and reverse translation with relevant preclinical and clinical data, or to enable insights from systems with complex interdependent interactions (e.g., combination therapy). Once built, such platform models can also potentially guide optimization of CAR design and lead selection during early stages of discovery when data availability are generally minimal. **Table 2** highlights CAR-T models published in the literature, with model structures, key learnings, and potential applications. More technical modeling details were discussed extensively in recent publications,[80,81] hence only an overview is provided below.

In addition to NCA, nonlinear mixed-effect population modeling can be used to characterize CKs, investigate dose-exposure-response relationship, and evaluate impact of covariates. Stein *et al.*[18] published the first population CK model to characterize the clinical CK profiles of tisa-cel and to evaluate the impact of extrinsic and intrinsic factors, specifically CRS-treating therapies on *in vivo* expansion kinetics. Similar population modeling and covariate assessment was carried out by Ogasawara *et al.*[19] for liso-cel, Wu *et al.*[20] for cilta-cel, and Mu *et al.*[21] for CT103A CAR-T therapies. Such quantitative model-based approaches supported regulatory approvals of CAR-T therapies.[82–86] Liu *et al.*[24] further utilized the CK model to characterize individual datasets from diverse patient population across seven different clinical trials and indications. Here, model-based meta-analysis was able to systematically characterize and compare the CK profiles across different targets/tumor types and evaluate different factors affecting clinical outcomes in humans.

Semimechanistic to mechanistic CK/PD and QSP modeling approaches can be used to draw mechanistic insights of tumor-CAR-T interaction and understand the impact of different variables (e.g., binding affinity, target density, CAR density, tumor burden, etc.) which may be helpful for *in vitro* to *in vivo* correlation and preclinical to clinical translation. Sahoo *et al.*[87] developed the Chimeric Antigen Receptor T-cell treatment Response in GliOma (CARRGO) model to evaluate the kinetics of CAR-T-mediated killing of glioma tumors. Such model-based analysis can be potentially used to project and optimize therapy outcomes/responses in patients based on individual patient tumor characteristics, including growth rate and antigen expression levels. Similarly, a three-compartment mathematical CAR-T model was developed to understand the immunotherapy effect of CAR-Ts on tumor suppression and to characterize the long-term immunological memory in cancer.[88–90] The authors leveraged the model to evaluate the impact of different doses, dose fractionation, and tumor burden on tumor responses, including elimination, equilibrium (dormancy), and escape. Similar mechanistic modeling approaches have been used to understand the impact of the CAR-T, leukemic, and B-cell dynamics on the treatment outcomes including, responses, side-effects, relapses, and associated critical factors in patients with B-cell ALL [91,92] or to characterize the BCMA targeting CAR-Ts and tumor dynamics in multiple myeloma indication.[69] A recent publication from Kirouac *et al.*[93] described T-cell activation and regulation by tumor to transit among memory, effector, and exhausted status, leading to tumor cell killing by the effectors. The model was trained with clinical datasets in a machine learning approach and was able to accurately predict the clinical responses. Overall, such modeling approaches can be further developed and explored to predict therapy outcomes (efficacy) for different scenarios.

Another good example of QSP model application is in characterizing cytokine release and CRS. It is important to understand the dynamics of cytokine release as they are the main drivers of CRS, a major anticipated on-target toxicity for CAR-T therapies. Hardiansyah *et al.*[94] was able to characterize the clinical CK and the associated cytokine release. The model also aimed to investigate the convoluted relationship between CAR-T dose, baseline disease burden, CK, and cytokine elevation. Similarly, Hanson *et al.*[95] investigated the interactions among CAR-T cells, tumor cells, host immune system, inflammatory cytokines, and associated toxicity with the help of mechanistic, mathematical modeling. *In silico*-based simulations demonstrated patient's tumor burden (rather than cell dose) to be highly correlated with cytokine release and toxicity, which is consistent with Hardiansyah's findings. Such model-based platforms can help in better understanding the impact of different mechanistic aspects or variables (e.g., dose, tumor burden, CK, etc) on clinically observed side effects and aiding personalized dosing.

Different T-cell subsets show different functional features (e.g., the naïve population has higher proliferation potency while the effector population has cytotoxic potency). Mueller-Schoell *et al.* incorporated different CAR-T phenotypes in a population QSP model to characterize the multiphasic CAR-T CK profiles in patients with non-Hodgkin's lymphoma and investigated the impact of CAR-T product phenotypic composition on survival outcomes.[96] Based on modeling analysis, the authors proposed that a clinical composite score of maximum naïve CAR-T cells normalized to baseline tumor burden can be used as a potential predictor for survival outcomes in patients. Similarly, Paixao *et al.*[97] leveraged a CAR-T cell phenotypic model to better understand multiphasic CAR-T profile, variabilities associated with patient and product heterogeneities, as well as impact on long-term therapy outcomes. Such quantitative frameworks can be potentially explored to evaluate impact of T-cell subsets, optimize CAR-T product phenotypic composition, and predict clinical outcomes after accounting for anticipated variabilities for a patient population.

Lymphodepletion conditioning regimen prior to ACTs is critical to create immunosuppressive environment for autologous T-cell expansion. Several models have been published to characterize the impact of current preconditioning lymphodepletion regimens on CKs and its optimization to improve clinical outcomes, although each model focuses on slightly different mechanisms of the lymphodepleting process. Owens *et al.* assumed the lymphodepleting effect through saturating fractional cell-kill terms on CAR-T cells,[98] whereas Kimmel *et al.* assumed a competitive relationship

Table 2 Summary of CAR-T models published in the literature focusing on model structure, applications, and key learnings

Model type	Authors	Key features of the model	Cell therapy / indication	Datasets	Application	Key learnings
Empirical NLME population modeling and covariate evaluation	Stein *et al.* (2019)[18]	Concept of compartmental modeling includes compartments of effector and memory CAR-T cells to characterize the different phases of CKs using piecewise function	Tisa-cel (anti-CD19 CAR-T) for B-cell ALL	• Cellular kinetics in blood by qPCR or flow; • Product or patient characterization (if applicable)	• To characterize the clinical CK profiles to capture different phases of CKs • To evaluate the impact of intrinsic (e.g., tumor burden) and extrinsic (e.g., CRS-treating therapies) patient-related and product-related (e.g., dose, CD4:CD8) on clinical outcomes • To compare clinical CK profiles across multiple trials and indications, and compare parameter differences among responders vs. nonresponders and hematological malignancies vs. solid tumors	• The model estimated doubling time or half-life for different phases, and lag phase duration or mean transit time (where applicable) • No impact of any of the covariates on the peak CAR-T levels • The multiphasic CK profile was similar for all tumor types with higher C_{max} in responders and in hematological malignancies • No correlation of dose and baseline tumor burden showed with CAR-T expansion or response • CD4:CD8 ratio of the CAR-T product close to one in responders and higher/diverse in nonresponder
	Liu *et al.* (2021)[24]		Anti-CD19, anti-BCMA, anti-EGFR CAR-T across multiple trials and indications			
	Ogasawara *et al.* (2021)[19]		Liso-cel (anti-CD19 CAR-T) for LBCL			
	Wu *et al.* (2022)[20]	Lag time and four transit compartments to capture redistribution and expansion, and two compartments with fast and slow (effector and memory) decline rate	Cilta-cel (anti-BCMA CAR-T) for MM			
Mechanistic PK/PD modeling to capture CAR-T and tumor interactions	Sahoo *et al.* (2020)[87]	Includes temporal interaction between tumor and CAR-T cells; can include features such as CAR-T cell activation, proliferation, exhaustion, or inhibition by tumor-induced immunosuppression or include different T-cell phenotypes along with tumor cell growth and killing	Anti-IL13Rα2 CAR-T for GBM	• *In vitro* functional data including T-cell activation and tumor cell killing • *In vivo* CAR-T kinetics and tumor killing dynamics (preclinical mouse studies)	• To characterize the kinetics of CAR-T cell activation, expansion or exhaustion; and tumor cell killing mediated by CAR-T cells • To assess additional impactful factors (e.g., dose, tumor characteristics such as growth rate and antigen level) on CAR-T function • To characterize CAR-T cell expansion and tumor growth inhibition kinetics in preclinical mouse models • To enhance mechanistic understanding and determine critical drug- and system-specific parameters impacting dose-exposure-response relationship for CAR-Ts • Reducing and optimizing the number of *in vivo* experiments with *in silico* tests to select specific scenarios that could be tested	• CAR-T dose was negatively correlated with the killing rate and positively correlated with the CAR-T proliferation and exhaustion rate • Killing rate showed a decreasing trend while exhaustion rate increased with an increase in antigen expression levels • A balance between proliferation and exhaustion rate is more critical than killing rate for treatment outcomes in GBM

(Continued)

Table 2 (Continued)

Model type	Authors	Key features of the model	Cell therapy / indication	Datasets	Application	Key learnings
	Rodrigues *et al*. (2020)[90] Barros *et al*. (2020)[89]		Two CAR-T receptors (19BBz and CAR-T 123) and different tumor targets (HDML-2 and RAJI cell lines) from immunodeficient mouse models			• Effector T cells into memory phenotype as CAR-T and tumor cells get cleared • Re-challenging with new tumor cells triggered differentiation of memory to effector T cells • Tumor growth rate, CAR-T proliferation, and CAR-T inhibition by tumor-induced immunosuppression as critical factors for the success of CAR-T therapy
	Singh *et al*. (2021)[69]	Assumes binding interaction to form CAR-target complex which drives tumor cell killing and CAR-T expansion; PK model with blood and tissues compartments, with memory and effector CAR-T cells	Anti-BCMA CAR-T (Ide-cel) for MM	• CAR-T kinetics and tumor lesion dynamic change (clinical) • Disease related biomarker (if applicable)		• Baseline tumor burden is a more critical factor for *in vivo* CAR-T expansion rather than the actual cell dose • Model-based virtual patient simulations suggest a steep dose-exposure-response relationship with a "threshold dose" beyond which the relationship is flat
Mechanistic PK/PD modeling including cytokine production and focus on safety	Hardiansyah *et al*. (2019)[94]	Effector and memory T-cells distributed in blood and tissue compartments; include features of cytokine release and cascade, tumor killing driven by activate T-cells	Anti-CD19 CAR-T for CLL	Cytokine dynamics along with other relevant functional data	• To capture complex relationships between CAR-T and cytokine release, and the impact of dose and disease burden on cytokines • To simulate the interplay among different phenotype of CAR-T cells, other immune cells, tumor cells, and inflammatory cytokines • To investigate the impact of patient's tumor burden and dose on inflammatory toxicity	• CAR-T expansion, target cell killing (efficacy), and magnitude of cytokine release or elevation is more correlated with baseline tumor burden and not the cell dose
	Hanson *et al*. (2016)[95]	In silico, include features of B-cell (target cell) dynamics, cytokine release, T-cell differentiation, and lymphocyte dynamics	Anti-CD19 CAR-T for B-ALL			

(Continued)

Table 2 (Continued)

Model type	Authors	Key features of the model	Cell therapy / indication	Datasets	Application	Key learnings
PBPK model for T-cell distribution	Khot *et al.* (2019)[100]	Includes 12 major tissues and tumor compartment, blood and lymph flows, vascular and extravascular sub compartments for tissues, T-cell extravasation and recirculation, T-cell elimination (via lungs), and retention factors for certain tissues	Cr-51 labeled T-cells injected in the melanoma xenograft model	CAR-T distribution kinetics into tissues	• To characterize the whole body PK, CKs, and T-cell distribution to various tissues of exogenously injected T-cells in preclinical mouse model	• T-cells rapidly accumulated (>90%) in spleen, liver, lungs, kidneys, bones, and lymph nodes with low (<1%) distribution to the tumors • Transmigration rates were higher for tissues with higher T-cell accumulation with retention factors estimated for spleen, liver, and kidneys to characterize the steady state accumulation
	Singh *et al.* (2021)[69]	Includes 10 major tissues (including tumor), similar model structure as Khot *et al.* with additional features of T-cell activation by tumor antigen, T-cell elimination via liver linked with PD model	Anti-EGFR and anti-CD19, anti-BCMA, anti-HER2 in multiple mouse xenograft models		• To characterize *in vivo* cellular expansion kinetics and tissue distribution (including tumor) in mouse xenograft models • PBPK/PD models to establish PK (*in vivo* expansion kinetics) and PD (tumor growth inhibition) relationship • To investigate IVIVC for killing	• IVIVC determined *in vitro* killing parameters to be ~10–20-fold higher as compared to *in vivo* estimates • Sensitivity analysis showed tumor burden was a more sensitive parameter as compared with cell dose for cellular expansion kinetics; CAR affinity, CAR density, antigen density positively correlated with CAR-T expansion
	Tsai *et al.* (2022)[101]	Minimal PBPK model feature to access local delivery (pleural or liver) of CAR-Ts; adopted from full PBPK/PD models of but also includes pleural or liver tumor compartment	Local delivery of CAR-Ts targeting pleural and liver cancers		• To investigate the impact of dosing route (local delivery for pleural and liver cancers) on CAR-T expansion kinetics, distribution, and tumor killing • To identify key factors impacting CAR-T expansion and killing after local delivery	• Liver model simulations demonstrated significant increases in tumor infiltration and earlier CAR-T expansion with local delivery as compared with i.v. • Tumor local blood flow rate was identified as a sensitive parameter that impacted CAR-T efficacy
	Brown *et al.* (2021)[102]	Account for different species including human, mouse, rat; similar PBPK modeling framework with maximum delivery rate to specific organs and tumor tissues estimated	Allogeneic NK cells in renal cell carcinoma		• To investigate typical perfusion and maximum delivery rates of CAR-T cells to different normal organs and tumor tissues • To compare delivery rates across species	• Maximum delivery rates estimated 100,000-fold higher (where doses are typically only 10–100 lower) in mice as compared with that in humans • Higher effective doses may be required for efficacy for solid tumors in humans due to the differences in the delivery rates

(Continued)

Table 2 (Continued)

Model type	Authors	Key features of the model	Cell therapy / indication	Datasets	Application	Key learnings
Mechanistic PK/PD modeling on interaction of host immune and CAR-T cells to characterize and optimize lymphodepletion therapy	Owens *et al.* (2021)[98]	Includes both normal T cells and CAR-T cells, and suppressive effect of CAR-T cells through saturating fractional cell-kill or competition with normal T cells	Simulations for treatment plans of conditioning and CAR-T therapy consistent with anti-CD19 CAR-T therapy (Tisa-cel and Axi-cel)	CAR-T CKs, host normal T-cell kinetics, and other relevant cytokines (e.g., IL-7)	• To investigate the impact of preconditioning lymphodepletion and CAR-T therapy on tumor-immune interaction and success of CAR-T therapy • To simulate different treatment plans of lymphodepletion therapy and CAR-T cell doses, and other factors that impact clinical outcomes	• Preconditioning therapy is required for CAR-T therapy • Appropriate lymphodepletion therapy and regimen can reduce CAR-T cell dose required. Optimization of recovery period between lymphodepletion therapy and CAR-T infusion is the key • Different delivery schedules can impact patient outcomes even with the same dose of conditioning and CAR-T therapy
	Kimmel *et al.* (2021)[58]		Anti-CD19 CAR-T (Axicel) for LBCL			• CAR-T cell expansion is mediated by immune reconstitution dynamics following lymphodepletion therapy and competition between normal and CAR-T cells • Model-based simulations suggest lymphodepletion before second infusion might be necessary
	Derippe *et al.* (2022)[99]	Includes CAR-T cells divided into different phenotypes following a progressive differentiation model; CAR-T cell expansion and elimination by host T cells mediated through cytokine e.g., IL-7 (relevant for allogeneic context)	Allogeneic anti-CD19 CAR-T (UCART19) for B-cell ALL		• To understand the interplay between host-immune system, IL-7, and CAR-T CKs • To quantitate CAR-T elimination via host immune cells in an allogeneic context	• Model captured variability in the CKs by incorporating the IL-7 and dynamics between host immune and CAR-T cells • Need of alemtuzumab (anti-52 mAb) along with Flu/Cy for CAR-T expansion • High impact of memory phenotype on CAR-T expansion and persistence

(Continued)

Table 2 (Continued)

Model type	Authors	Key features of the model	Cell therapy / indication	Datasets	Application	Key learnings
Mechanistic PK/PD modeling including T-cell phenotype	Kimmel *et al*. (2020)[141]	Includes co-evolution, dynamics and interactions for normal memory T cells, memory and effector CAR-T, and tumor cells	Anti-CD19 CAR-T (Axi-cel) for LBCL	CAR-T CKs and phenotyping data along with other relevant functional data	• To quantitate sources of variability or factors that impact tumor response and clinical outcomes of CAR-T therapy and rank order them • To predict treatment outcomes (e.g., PFS) under different treatment scenarios (e.g., tumor load, cell composition, lymphodepletion)	• CAR-T product with a more memory phenotype can improve clinical outcomes • CAR-T expansion is impacted by immune reconstitution, antigen load, and cytokines after lymphodepletion therapy • Decreasing tumor mass with the help of other therapies before CAR-T infusions may help in increasing the durability of responses
	Mueller-Schoell *et al*. (2021)[96]	Kinetics and dynamics of different phenotypes of CAR-T (naïve, central memory, effector memory, and effector) with progressive differentiation and their interactions with tumor cells	Anti-CD19 CAR-T (Axi-cel) for LBCL		• To characterize different CAR-T phenotypes and tumor burden in patients and associated variability • To investigate factors (patient-related or therapy/product-related) especially phenotyping that impacted clinical outcomes such as CAR-T expansion, patient survival, and long-term responses	• Maximum CAR-T expansion normalized to baseline tumor burden is a better predictor of clinical outcome • Day 7 CD4+/CD8+ ratio and previous autologous stem cell transplantation identified as co-variates significantly impacting maximum expansion rate • A clinical composite score (CCS) of "maximum naïve T-cell concentration /Baseline tumor burden" with cutoff values were proposed for early survival
	Paixao *et al*. (2022)[97]		Anti-CD19 CAR-T for B-ALL, CLL, DLBCL and MCL			• Model predicted CAR-T exposure (area under the concentration-time curve for the first 28 days) and fraction of non-exhausted cells as a joint predictor matrix for treatment outcomes

ALL, acute lymphoblastic leukemia; CK, cellular kinetic; CLL, chronic lymphocytic leukemia; C_{max}, maximum observed concentration; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; IVIVC, *in vitro* to *in vivo* correlation; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; NLME, nonlinear mixed-effect; PBPK, physiologically-based pharmacokinetic; PFS, progression-free survival; PK/PD, pharmacokinetic/pharmacodynamic; qPCR, quantitative polymerase chain reaction.

Table 3 Key differentiators between TCR-T and CAR-T potentially important to clinical pharmacology analyses

Attribute	TCR-T	CAR-T	Clinical pharmacology considerations for TCR-T
Construct for antigen recognition	• Endogenous TCR (native or engineered for enhanced affinity) • Potential for mispairing of alpha or beta chains of the new TCR with the corresponding cognate beta or alpha chains of endogenous TCRs.[119]	• Single chain fragment variable with antibody like binding (although alternative constructs in development[142])	• Potentially lower risk of off-target toxicities due to TCR negative selection (for fully endogenous TCR), which could support higher doses • Potential for a mispaired TCR that is self-reactive (if endogenous TCR not knocked out or rendered incapable of pairing).[119] • May need to characterize/monitor TCR-T cells with incomplete endogenous TCR knockout.
Targeting	• Targets rich repertoire of intracellular or cell surface cell proteins presented as MHC presented peptides[143] • HLA restricted[a] • Cut's target population at least in half when targeting a single HLA haplotype[144] • MHC restriction may favor CD8+ or CD4+ T-cells (for a single peptide–MHC class I or II target). • Requires lower antigen density • e.g., minimum of 4+ pMHC-TCR complexes[118] • At least a log-lower for a synaptic killing event for TCR-T vs. CAR-T[118] • Lower affinity (1–100 μM). High importance of binding half-life and avidity[103,118,145]	• Largely limited to cell surface antigens (but potential to recognize peptide–MHC)[143,146] • MHC independent (No HLA restriction) • Requires higher antigen density • e.g., 100+ targets/cell for cell killing[62,118] • Potentially even higher threshold required for cytokine release (~5,000 targets/cell)[118] • Antigen targets at higher levels for CAR-T vs. TCR-T (e.g., 20,000 to 50,000 sites per cell vs. 100–1,000 sites per cell)[143] • Higher affinity (<10–100 nM)[103,118]	• Ability to target intracellular antigens provides TCR-T with an advantage in solid tumors, where there is the greatest unmet need • Greater need to characterize biodistribution at the site of action • Challenging to accurately measure p-MHC target expression, which limits ability to model and predict target interactions • Hard to translate binding affinities form *in vitro* to *in vivo*. Higher reliance on functional avidity • Low affinity/high avidity makes it challenging to quantitate and characterize TCR-T therapies based on TCR-antigen interactions
Co-stimulatory signals for activation	• Endogenous	• Engineered into the CAR-T construct	• Potentially lower CRS (due to lower *in vivo* expansion) supporting higher doses and lowering the need for fractionated doses • Larger role for tumor microenvironment considerations in modeling efforts
Cell killing	• Synapse with classical with 3-ring structure[118] • Relatively slower off-rate	• Synapse that is disorganized and multi-focal[3] • CAR-T cells may directly contribute to target cell antigen loss resulting in lower antigen density (eg, trogocytosis)[118] • Faster proximal signaling, more rapid killing, and much faster off-rate/detachment from target[118] • Involves FAS/FAS-L axis for inducing apoptosis[118]	• Mechanistic models need to account for potentially lesser signal strength and slower ramp-up of the cell killing effect
Stage of development	• Experimental	• Experimental and commercial (6 approved therapies)	• Opportunity to apply learnings from CAR-T to TCR-T
Immunogenicity	• Lower risk due to fully human, native TCR (unless affinity enhanced)	• Higher risk due to non-endogenous protein constituting the CAR	• Although lower risk, still need to assess immune response against the engineered TCR and against the TCR-T cells, and potentially, any residual biological material from the cell processing (eg, Cas9)[147]

MHC, major histocompatibility complex.
[a]Except for gamma-delta T-cells, which do not require peptide MHC expression.

Table 4 Summary published TCR-T studies with at least 10 patients across multiple doses

Study/therapy/target	Indication(s)	Dose level (transduced cells)	Dose-exposure	Dose-efficacy	References
Phase I/CD4+ and CD8+ TCR-T (affinity enhanced)/ MAGE-A4 HLA-A*02+	Esophageal gastric, head and neck, melanoma, NSCLC, ovarian, urothelial, MRCLS, SS	DL1: 0.08–0.12×10^9 (N=3)[a] DL2: 0.5–1.2×10^9 to (N=3)[a] DL3: 1.2–6.0×10^9 (N=3)[b] EXP: 1.2–10×10^9 (N=29)[b,c] Two patients received a second cell infusion after disease progression and confirmed response	Ovarian cancer: Trend toward higher peak transduced cells (VCN/ug DNA) with higher dose, with greatest increase between DL1 and DL2. Group 2 and 3/expansion confounded by more intensive LD regimen for higher dose cohorts	ORR (all PR): 9/38 (24%), with 6/16 (44%) in SS. All responses in DL3, 2/9 received 1,800 mg/m^2/ day cyclophosphamide lympodepletion[c]	120
Phase 2 (SPEARHEAD-1) CD4+/CD8+ TCR-T (affinity enhanced)/ MAGE-A4 HLA-A*02+	Advanced SS or MRCLS	DL: 1.0-10×10^9 (N=52)[b]	Insufficient data–although the authors separate by high and low doses within the overall range, the results are potentially confounded by selecting for patients with better quality infusion product at the higher doses	Insufficient data–same potential confounding as observed for dose – exposure	148–151
Phase 1 (SURPASS)/CD4+ and CD8+ TCR-T (affinity enhanced)/ MAGE-A4 w/CD8 HLA-A*02+	MAGE-A4+ tumors including urothelial carcinoma and ovarian cancer	DL1: 0.8–1.2×10^9[b] DL2: 1.2–6×10^9[b] EXP: 1.2–10×10^9[b] N=44 patients total. Distribution among dose cohorts not reported	Not reported	Not reported	152–156
Phase 1/CD3+ (including CD8+) TCR-T (affinity enhanced)/ MAGE-A10 HLA-A*02+	Head and neck, melanoma, or urothelial tumors	DL1: 0.08–0.12×10^9 (N=3)[a] DL2: >1.2–6×10^9 (N=3)[b] EXP: 1.2 to 15×10^9 (N=7)[b]	Trend toward higher peak transduced cells (VCN/ug DNA) with increasing dose	Insufficient data – no tumor responses	121 Note: Multiple tumor types
Phase 1/ CD4+ and CD8+ TCR-T (affinity enhanced)/ AFP HLA-A*02:01+	Advanced HCC	DL1: 0.08–0.12×10^9 (N=2)[d] DL2: 0.5–1.2×10^9 to (N=3)[d] DL3: 1.2–6.0×10^9 (N=3)[b] EXP: 1.2–10×10^9 (N=13)[b]	Insufficient data–results are broken out by actual doses received, not assigned dose cohort, and weaker lymphodepletion for lower dose cohorts, leading to potential confounding	Insufficient data–same potential confounding as for dose-exposure.	157–159

Table 4 (Continued)

Study/therapy/target	Indication(s)	Dose level (transduced cells)	Dose-exposure	Dose-efficacy	References
Phase 1 (IMA203-101)/ Affinity enhanced CD8+ TCR-T/ PRAME HLA-A*02:01+	Multiple advanced solid cancers	DL1: $0.04–0.06\times10^9/m^2$ cells ($N=3$)[h] DL2: $0.12–0.18\times10^9/m^2$ cells ($N=7$)[h] DL3: $0.20–0.48\times10^9/m^2$ cells ($N=3$)[h] DL4: $\leq1.2\times10^9/m^2$ cells ($N=1$)[h] Includes two enrichment cohorts (EC1, $N=3$, and EC2, $N=1$), but the dose levels have not been disclosed	Not reported	Overall response (PR only) in 8 of 16 patients). Insufficient data to identify a dose–response relationship	160
Phase 1/ Native CD4+ and CD8+ TCR-T/ MAGE-A4 HLA-A*24:02+	Recurrent esophageal cancer	Assigned dose: DL1: 0.2×10^9 cells ($N=3$) DL2: 1×10^9 cells ($N=4$) DL3: 5×10^9 cells ($N=3$) Actual dose of transduced cells: DL1: $0.012–0.015\times10^9$ cells ($N=3$) DL2: $0.068–0.13\times10^9$ cells ($N=4$) DL3: $0.26–0.95\times10^9$ cells ($N=3$) No lymphodepletion	Trend toward higher peak cell levels with increasing dose level, albeit over a relatively lower range of transduced cell doses compared with other TCR-T studies	No objective responses, potentially due to lack of LD or low number of transduced cells	123
Phase 1/ Native CD4+ and CD8+ Native TCR-T/ HPV-16 E7 HLA-A*02:01$^+$	Metastatic HPV-associated epithelial cancers	DL1: 1×10^9 cells ($N=3$)[f] DL2: 10×10^9 cells ($N=3$)[f] DL3: 120×10^9 cells ($N=6$)[f] Following cell therapy infusion, IL-2 (aldesleukin) was given every 8 hours for 0 to 11 doses.	Trend toward increasing TCR-T cell levels with increasing dose level Multiple LD used, but no imbalances across dose cohorts However, the number of aldesleukin (IL-2) doses appeared generally greater for the lower dose cohorts, which could confound interpretation (e.g., if IL-2 were to negatively impact T cells through exhaustion mechanisms)	Responses in 6 of 12 patients, no clear dose–response trends	122

(Continued)

Table 4 (Continued)

Study/therapy/target	Indication(s)	Dose level (transduced cells)	Dose-exposure	Dose-efficacy	References
Phase 1/Native CD4+ and CD8+ TCR-T/MART-1 HLA-A*02:01+	Progressive metastatic melanoma	DL1: $N=3$[g] DL2: $N=11$[g] DL3: $N=4$[g] Actual doses of transfused ranging from 0.5 to 34.4×10^9 cells, with high overlap across dose cohorts	Insufficient data – High overlap in actual doses across dose cohorts Differences in number of transduction cycles and *ex vivo* culture time across dose cohorts Potential confounding due to differences in IL-2 administration across cohorts	Insufficient data – only two PR and no CRs	161
Phase 1a/Native CD4+ and CD8+ Native TCR-T and CRISPR KO of TCRα,β /≤3 personalized neoantigens with HLA matched to personalized neoantigen.	Metastatic solid tumor as follows: urothelial carcinoma, melanoma, NSCLC, head and neck squamous cell carcinoma, colorectal cancer, ovarian cancer, hormone-receptor positive breast cancer, triple-negative breast cancer, or prostate cancer	DL1: 0.4×10^9 cells ($N=6$)[e] DL2: 1.3×10^9 cells ($N=6$)[b] DL3: 4×10^9 cells ($N=4$)[b]	Insufficient data –LD, manufacturing process, and co-administration of IL-2 favor higher dose cohorts.	Insufficient data–same potential confounding as for dose-exposure.	162

AFP, alpha-fetoprotein; CR, complete response; DL, dose level; EXP, expansion; HCC, hepatocellular carcinoma; LD, lymphodepletion; MRCLS, myxoid/round cell liposarcoma; NSCLC, non-small cell lung cancer; ORR, overall response rate; PR, partial response; SS, synovial sarcoma; VCN, vector copy number.

[a]Lymphodepletion: Cyclophosphamide (600 mg/m^2/day) for 3 days and fludarabine (30 mg/m^2/day) for 3 days. [b]Lymphodepletion: Cyclophosphamide (600 mg/m^2/day) for 3 days and fludarabine (30 mg/m^2/day) for 4 days. [c]Lymphodepletion: Cyclophosphamide (1,800 mg/m^2/day) for 2 days and fludarabine (30 mg/m^2/day) for 4 days (7 patients). [d]Lymphodepletion: Cyclophosphamide (500 mg/m^2/day) for 3 days and fludarabine (20 mg/m^2/day) for 3 days. [e]Lymphodepletion: Cyclophosphamide (300 mg/m^2/day) for 3 days and fludarabine (30 mg/m^2/day) for 3 days. [f]Lymphodepletion: Cyclophosphamide (30 mg/kg/day or 60 mg/kg/day) for 2 days and fludarabine (25 mg/m^2/day) for 5 days. [g]Lymphodepletion: Cyclophosphamide (60 mg/kg/day or 60 mg/kg/day) for 2 days and fludarabine (25 mg/m^2/day) for 5 days. [h]Lymphodepletion: Cyclophosphamide (500 mg/m^2/day) for 4 days and fludarabine (30 mg/m^2/day) for 4 days.

between host T-cells and infused CAR-T cells.[58] Derippe *et al*.[99] assumed the suppression effect of host immune system on CD19-targeting allogeneic CAR-T (UCART19) cells through cytokine regulation like IL-7 in adult patients with B-cell ALL and quantitatively captured the beneficial effects of lymphodepletion therapy in the context of allogeneic products. Enhancing our understanding on these aspects will be more significant given the emergence of allogeneic CAR-T therapies as host immune cells may play a bigger role in CAR-T elimination and hence optimization of lymphodepletion regimens would be critical, especially considering the early clinical trials that are currently exploring feasibility and/or necessity of repeat dosing with allogeneic products.

It is important to investigate the distribution of CAR-Ts to the site of action, especially for solid tumor indications and in cases where on-target off-tumor toxicities are anticipated due to target expression on normal tissues. Khot *et al*. investigated whole body PK as well as Cr-51-labeled mouse T-cell distribution to various tissues in a melanoma xenograft mice model,[100] and developed a full PBPK model to characterize the T-cell distribution data. However, the study used untargeted T-cells whose distribution patterns to tumors or target expressing tissues may vary as compared with targeted T-cells. The PBPK model further developed by Singh *et al*. was able to characterize antigen-dependent expansion and tissue distribution of multiple, targeted CAR-Ts and subsequently linked to PDs for better understanding the relationship between *in vivo* expansion kinetics and tumor growth inhibition in mouse xenograft studies.[68] Interestingly, Tsai *et al*. recently further adopted some features of the full PBPK/PD models to develop a minimal PBPK/PD model and characterized CAR-T distribution, proliferation, and tumor dynamics after local delivery of CAR-Ts for pleural and liver tumors in mouse models.[101] Although limitation exists due to key model assumptions and data requirement, such a modeling framework can further support the development of local delivery of CAR-Ts for solid tumors. Informed by the minimal PBPK/PD model, the authors suggested local delivery to be more effective for CAR-Ts in solid tumors as compared with systemic delivery. This is somewhat consistent with Brown *et al*.[102] who earlier leveraged PBPK modeling approaches and suggested that higher human doses may be required to drive efficacy for solid tumors based on the comparison of effective CAR-T delivery rates to tumors in humans as compared with mouse models. The authors concluded that quantitation of species-specific and organ-specific differences in the delivery rates as well as homing of the delivered CAR-T cells to specific organs are critical to overcome the challenges associated with the solid tumor indications. Thus PBPK/PD modeling approaches can help to establish CK/PD relationship in preclinical setting and potentially scale for clinical predictions. Given the lack of biodistribution data from the clinic, these research efforts and conclusions rely heavily on mouse models and assumes that such models fully recapitulate clinical conditions.

In addition to above, modeling approaches are also being explored to understand binding kinetics for CAR binder selection and optimize CAR design, characterize on-target off-tumor effects, impact of dual targeting, understand CAR-T signaling, and optimize combination with other modalities.[59,62,63,102–109]

Although various quantitative CAR-T models have been explored in recent years, to date, their actual utility in real drug development remains limited. This is due to lack of relevant preclinical models, convoluted dose-exposure-response relationship, wide interpatient variability, and many patient-specific and product-specific factors that impact functionality of CAR-T therapy. So far, application of the pharmacometric approaches for CAR-T clinical development have been limited to NCA or population CK modeling along with dose-exposure-response analysis where covariates or sources of variability for exposure are identified in guiding dose optimization. However, quantitative translation for dose optimization may be more challenging for CAR-Ts given the large interpatient variability coming from the multitude of factors that impact CAR-T expansion. Here, mechanistic modeling can aid in better understanding of drivers for exposure and response and can explain the variability to better guide dose selection. However, dedicated future efforts are warranted in validating mechanistic modeling approaches in dosing recommendations for specific patient populations, and *a priori* predicting clinical outcomes of CAR-Ts. As discussed earlier, the current starting dose selection strategy is highly empirical and based on clinical experience with existing cell therapies in the clinic. Here, there is an opportunity for mechanism-based modeling approaches to enable preclinical to clinical translation and guide optimal dose selection for FIH study. Besides, accumulating preclinical and clinical data from investigational cell therapies can significantly help in building the models and validating the parameter estimates for forward and reverse translation. Similarly, model-based meta-analysis of existing preclinical and clinical literature data can also be very helpful to leverage learnings for newer CAR-T programs in development using totality of evidence approach.[24,110] Given the availability of rich multidimension datasets from small number of patients, especially during early clinical development, it is also essential to use advanced analytics, such as machine learning and artificial intelligence-based, dimensionality-reduction approaches to disentangle correlated variables, understand the causal relationship and key drivers of safety and efficacy, and eventually identify the optimal combination of factors that may help predict outcomes for a given CAR-T product. Learnings can be leveraged, at least partially if not fully, for other cell therapy platforms, such as allogeneic or other cell types (such as NK, γδ T-cells, etc.). Overall, the clinical pharmacologist and pharmacometrician needs to have the expertise and knowledge to work at the interface of the many functions associated to propose the right questions, relevant data, and integrate models with reasonable assumption and appropriate level of details.

UNIQUE IMMUNOGENICITY AND BIOANALYTICAL CHALLENGES AND CONSIDERATIONS FOR CAR-T CELL THERAPIES

As all bio-therapeutics, CAR-T cells also have the potential to elicit immune responses in patients which can impact safety, alter the exposure, or neutralize the ability of the CAR domain to engage with the tumor antigen. The different CAR domains that can be associated with potential risks include: (i) extracellular antigen recognition domain (ECD; e.g., scFv with a linker), (ii)

hinge and transmembrane domain (e.g., CD8α and CD28), (iii) intracellular CD3ζ domain, and (iv) co-stimulatory domains (e.g., CD28 and 4-1BB). Although the ECD can be presented in context of both class I MHC leading to a cellular CTL (cytotoxic T lymphocyte) and class II MHC leading to a T-cell effector (CD4 T helper) mediated immune response; the rest of the intracellular domains pose a risk when presented through a class I pathway. The nonhuman portions of the chimeric receptor, scFv CDR's, and domain junctions can be associated with neo-epitope content. The membrane proximate presentation of the chimeric receptor, as well as soluble receptor resulting from regular cell turnover, can result in T cell driven humoral/anti-drug antibody (ADA) responses. The gene editing components and genomes and proteins from viral vectors used to introduce genes to further enhance the T-cell function and reduce transplantation-related challenges can also prime the innate phase immune responses. The gene edited cell variants and culture contaminants that carryover from the process development (during *ex vivo* expansion) and aggregated/clumped cells during infusion have also been implicated in priming the early phase immune response. Both the cell mediated response and ADA mediated complement activation have the potential to decrease expansion and persistence of the CAR-Ts as well as neutralize CAR function resulting in decreased efficacy. An adaptive cell mediated T-cell response (CTL and effector Th) can have a long-term memory component that is more likely to have a significant impact on CK and efficacy during retreatment or serial treatment with CAR-T of shared sequence.

A risk-based streamlined bioanalytical strategy to assess ADA response would include an assessment of binding antibodies to the ECD domain comprising of the scFv region of CAR. As the ECD domain contains the region that binds to the tumor antigen target, evaluating any immune reactivity against this domain should be adequate to understand impact on efficacy (CAR-T expansion and persistence that relates to loss of response). Further characterization of the binding ADA through onset, kinetics, and magnitude may help to understand maturity of the response. ADA is unlikely to interfere with CAR-T quantitative PCR (qPCR)-based PK assays but have the potential to interfere with flow-based assays that are used to monitor CKs. However, in most cases, the latter assay is not used for primary PK parameter determination. The development of cell based neutralizing antibodies may be challenging due to lack of well-characterized cell lines that can evaluate loss of functional response subsequent to inhibition of CAR-T binding to the target antigen. The cellular (CTL and effector) responses may help understand any loss of efficacy due to elimination of CAR-T cells. However, such assays have proven difficult to deploy in clinical setting and are challenging to develop due to lack of patient derived cells as well as the impact on quality of such samples due to processing, shipping, and handling. Besides, these patients have gone through multiple lines of debilitating treatments that impacts sample integrity. Logistical, sensitivity, variability, and cell viability challenges have made it difficult to generate robust cellular immunogenicity data for CAR-Ts.

The immunogenicity of currently approved CAR-T therapies for B-cell targeting hematological malignancies does not seem to have an apparent impact on cell expansion, safety, and efficacy.[29,111–115] The onset of cellular immune response tends to be around 3–6 months postinfusion, which does not impact the expansion phase of the cells and target engagement. Although cellular immunogenicity may impact the long-term cellular persistence, to date, there are no data to indicate that it is associated with any impact on clinical responses. Additionally, the overall humoral response may be diminished for approved B-cell targeting CAR-T therapies due to a reduced likelihood of an ADA-mediated immune response. The immunogenicity rates observed with CD19 or BCMA targeted CAR-T therapies were similar irrespective of murine or humanized scFv regions in the CAR domains. Hence, for such indications, a simplified immunogenicity assessment would entail evaluating binding antibodies to the ECD domain. The lack of impact on efficacy provides a rationale for not performing neutralizing antibody assessments. Similarly, the lack of CTL mediated killing negates the need to perform a cell mediated immunogenicity assessments.

Reverse translational approaches evaluating the binding affinity of ADAs from clinical subjects as well as cellular immune response to probe the memory component of human T-cells help to further optimize sample collections and an overall understanding of the immune response to CAR-Ts. As the industry moves towards next generation multi-domain CARs, non-B-cell targets, and solid tumor indications, it will be important to monitor immunogenicity, as humoral responses may be more robust and not influenced by inhibiting the B-cell pharmacological targets. The role of innate responses in priming the adaptive response due to the extraneous residuals and impacting safety and efficacy also needs further delineation.

UNIQUE CLINICAL PHARMACOLOGY CONSIDERATIONS FOR TCR-T CELL THERAPIES

Although the cell therapy landscape is dominated by CAR-T cell therapies, multiple TCR-T cell therapies are also in active investigation with ~ 214 therapies in development as of April 16, 2021.[116] TCR-T therapies may potentially overcome some of the current limitations of CAR-T therapies, including the requirement for surface presentation of target antigens. Whereas excellent cell surface antigens have been identified and exploited in hematological malignancies, it has been challenging to similarly identify surface antigens that are broadly expressed in solid tumors while showing minimal or no expression in important normal tissues.[117] TCR-T cell therapies can recognize intracellular proteins presented MHC–peptide complexes, which greatly expands the number of potential targets, albeit at in a restricted patient population due to genetic polymorphisms in the antigen-presenting HLA. This increased target repertoire may enable identification of antigens that are both pervasive within, and selective for, solid tumors.

TCR-T cell and CAR-T cell therapies show many similarities in their pharmacology that supports extrapolation of clinical pharmacology strategies for CAR-T to TCR-T. Both therapies have a similar mechanism of action, including target engagement leading to synapse formation, with the ability to perform serial killing via directed release of cytolytic proteins (e.g., perforin and granzymes). Both therapies are subject to regulation via checkpoint inhibitors.[118]

However, there are key differences between TCR-T and CAR-T that are important to consider when developing clinical pharmacology strategies (**Table 3**). These differentiators can impact the features incorporated into PK/PD model, including biodistribution aspects, types, and mechanisms of toxicity, and the degree to which nonclinical studies can inform clinical studies (**Table 4**).

First, TCR-T therapies generally incorporate a native or engineered TCR that relies on endogenous signaling mechanisms, which may provide some safety advantages over CAR-T cells. In consequence, in order to accurately predict both toxicity and efficacy, the microenvironment surrounding TCR-peptide–MHC interactions may have greater importance in pharmacometric models of TCR-T therapies. Furthermore, if the endogenous TCR is knocked out (e.g., to prevent TCR alpha/beta mispairing[119]), additional T-cell populations within the infusion product may need to be characterized/monitored (e.g., cells with both endogenous and transduced TCR, or cells with neither transduced not endogenous TCRs).

Second, a higher proportion of TCR-T therapies are in active development for targets in solid tumors, vs. hematological malignancies,[116] possibly owing to the greater unmet need and opportunity in solid tumors, coupled with the larger repertoire of potential antigens. Given the predominance of TCR-T investigations in solid tumors, an understanding of T-cell biodistribution and activity at the site of action may play a more critical role in informing clinical pharmacology strategies.

Third, the number of infused TCR-T vs. CAR-T cells is generally higher, with the TCR-T doses frequently consisting of billions of infused cells (e.g., 1×10^9 to 100×10^9, see **Table 4**). Although head-to-head studies have not been performed between TCR-T and CAR-T, these generally higher doses may be motivated by potentially lower postinfusion expansion, biodistributional limitations, and/or differences in the tumor microenvironment for TCR-Ts vs. CAR-Ts, especially when native TCRs (as opposed to engineered TCRs with enhanced affinity) are used to target solid tumors.

To ascertain if either dose-exposure or dose–response relationships could be identified, several key TCR-T studies incorporating multiple dose levels with at least 10 or more subjects were selected for evaluation (**Table 4**). Of these, four studies were identified that showed apparent trends toward increasing postinfusion transduced cell exposure with increasing dose, that were not otherwise impacted by factors that could confound associations of dose with either exposure or outcome in these early phase studies (e.g., changes across dose levels in lymphodepletion, manufacturing process, IL-2 co-administration, tumor types, or subject to selection bias such that patients with poor *ex vivo* expansion or inferior T-cell fitness are excluded from higher dose cohorts, see **Table 4** for examples).

All 4 studies showing positive dose-exposure relationships were phase I and used both CD8+ and CD4+ transduced T-cells; 2 studies used an affinity enhanced or engineered TCR, and 2 used a native TCR construct (**Table 4**). Studies using an affinity enhanced TCR investigated a broad range of dose levels, and included transduced cell doses substantially lower than 1×10^9 (MAGE-A4[120] and MAGE-A10[121]). Overall, 9 of 38 (24%) patients responded (all partial response) for the MAGE-A4 targeting TCR with all responses occurring at the highest dose levels. There were no tumor responses for the MAGE-A10 targeting TCR. For the two native TCRs showing positive dose-exposure relationships, one study investigated a relatively higher transduced cell dose range (HPV-16 E7, 1×10^9 to 120×10^9[122]), and the other a low transduced cell dose range (MAGE-A4, $0.012–0.015 \times 10^9$ to $0.26–0.95 \times 10^9$ cells[123]). No dose-efficacy relationships were seen for either study, although 6 of 12 patients responded using the MAGE-A4 therapy.

Fourth, translating nonclinical *in vivo* data to inform clinical CK/PD, safety and efficacy is generally more challenging for TCR-T vs. CAR-T. Given the requirement for MHC-I peptide presentation, syngeneic mouse models cannot recapitulate the human TCR-MHC-I interaction, unless humanized. Although efforts to make transgenic humanized mouse models have been made, these need further development.[124] Without such models, syngeneic models are limited to elucidating off-target toxicities[125] and evaluating synergies with other immunotherapies, such as immune checkpoint inhibitors.[124] Xenograft models can be used to study proof of concept TCR-T efficacy and screen mutant neoantigen-specific TCRs,[126] but it can be hard to delineate between rejection and actual TCR-T efficacy.[124] Given translational limitations, there has been a high reliance on *in vitro* data to support FIH studies for TCR-T therapies. This includes *in vitro* pharmacology to assess TCR specificity and potency (peptide pulsed and endogenous) and phenotype characterization. Safety assessment may be based on target RNA levels in tumor vs. primary cells from various organs, altered peptide scans and reactivity against potential off-target peptides, activity against primary cells from normal tissues, and alloreactivity against B-cells expressing a range of HLA complexes. Safety assessment may include events related to gene editing (off-target edits and chromosomal translocations).

Fifth, translating and modeling *in vitro* binding affinity/avidity is also more challenging for TCR-T vs. CAR-T, although modeling advances have started to elucidate differences between the two therapies.[103] TCR-T therapies show some divergence between affinity and T-cell activity, and the weak affinity of a single TCR-peptide–MHC interaction often require tetramers or hexamers to generate sufficient binding affinity for reliable measurement. It can also be very challenging to measure accurately peptide expressed via MHC on the cell surface. For these reasons, measure of functional avidity (i.e., T-cell fitness/activity at varying concentrations of peptide epitope as a half-maximal effective concentration) is often used for translating *in vitro* to *in vivo*.[127] From a modeling perspective, TCR-T and CAR-T may share common critical mechanisms and biology, including cell expansion/retraction in periphery due to homeostatic mechanisms, cellular trafficking to tumor, lymphoid, and potentially normal tissues expressing target. Both have antigen-triggered activation, proliferation, and serial killing. Both TCR-T and CAR-T cells may undergo apoptosis/persistence/biodistribution, with the distribution of T-cell phenotypes being potentially important (T_{SCM}, T_{CM}, T_{EFF} etc.). However, several unique aspects should be considered when modeling TCR-T cells. TCR-T models relay on functional avidity/cell killing in contrast to CAR-T models which can be built based on CAR binding affinity. TCR-T models may need to incorporate features that describe endogenous co-stimulatory interactions, leading to a potentially

attenuated T-cell response that may be more impacted by the tumor microenvironment. TCR-T models may have different biodistribution requirements, especially when targeting solid tumors.

Overall, whereas TCR-T and CAR-T therapies share many similarities, pharmacometric models for TCR-T may need to include the higher complexity associated with the tumor microenvironment and signaling and costimulatory signals, and may have a higher reliance on clinical and *in vitro* data, given the challenges in translating TCR interactions with peptide MHC characterized by low affinity and high avidity.

SUMMARY AND CLOSING REMARKS

To conclude, cell therapies, such as CAR-Ts and TCR-Ts, are considered as "living biologics" as they undergo *in vivo* expansion and exhibit unique multi-phasic CK profile.

- NCA or population-based PK approach can be used to characterize CK with C_{max} and $AUC_{0\text{-}28d}$ exposure metrics usually used for exposure-response analyses. Other PK parameters, such as T_{last} and $t_{1/2}$ should be interpreted with caution, whereas other typical PK parameters, such as clearance and volume of distribution, are not relevant for cell therapies.
- The dose-exposure-response relationship for CAR-T and TCR-T is confounded by multiple patient- and product-related characteristics. Hence, patient-specific intrinsic (e.g., tumor burden, target expression, and immune cell fitness) and extrinsic factors (e.g., prior lines of therapy, lymphodepletion, and CRS treating therapies), as well as product-specific characteristics (e.g., cell phenotype and CD4:CD8 ratio) should be evaluated for potential impact on dose-exposure-response (efficacy and safety) relationship and support clinical decisions.
- Prior clinical experience with other relevant CAR-T and TCR-T products along with relevant preclinical studies should be used to design FIH study and dose selection. More efforts are required to build translational PK/PD modeling tools to inform FIH doses and understand mechanistic differences between preclinical models and humans.
- The dose of CAR-T therapy should be based on viable, CAR-positive, T-cells either as a fixed dose or normalized to body size. It is recommended to assess BW or BSA as a covariate from early phase trials to justify the dosing strategy. It is suggested to evaluate the benefits of risk-based dosing strategies, including dose fractionation or tumor-burden based dosing where applicable.
- The selection of specific lymphodepleting agents and their doses should be based on prior clinical learnings and further optimized during early cohorts.
- Traditional oncology FIH dose escalation studies based on only toxicity may not be helpful for cell therapies. Instead TEPI is one of the optimal approaches as it integrates both safety and efficacy data to maximize the therapeutic benefit for patients. Similarly, BOIN-guided escalation strategy might be more appropriate than BLRM or 3 + 3. Additionally, it may be hard to select an optimal dose from escalation, and dose expansion could be extended to more than one cohort for RP2D selection.
- Fit-for-purpose mindset for the utility of different M&S approaches depending on the scope, scientific questions, data availability, and stage of the program will be the key for the successful implementation of MIDD for cell therapies. Although empirical compartmental approaches can be used to characterize CK profiles, on the other hand, semimechanistic, PBPK or QSP modeling approaches can be leveraged to gain further mechanistic insights. Clinical pharmacologist and pharmacometrician should have the expertise to work at the interface of cross-functional teams, frame the right questions, and integrate models with relevant data and assumptions.

It is the sincere hope of this IQ Working Group that the described clinical pharmacology and pharmacometric considerations and best practices for optimal development of CAR-T and TCR-T cell therapies, will aid in the acceleration of the development and availability of these potentially curative therapies for patients with cancer.

CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

1. Kruger, S.F., Cadilha, B.L., von Bergwelt-Baildon, M., Endres, S. & Kobold, S. Challenges in clinical trial design for T cell-based cancer immunotherapy. *Clin. Pharmacol. Ther.* **107**, 47–49 (2020).
2. Bashor, C.J., Hilton, I.B., Bandukwala, H., Smith, D.M. & Veiseh, O. Engineering the next generation of cell-based therapeutics. *Nat. Rev. Drug Discov.* **21**, 655–675 (2022).
3. Mullard, A. FDA approves fourth CAR-T cell therapy. *Nat. Rev. Drug Discov.* **20**, 166 (2021).
4. Mullard, A. FDA approves first BCMA-targeted CAR-T cell therapy. *Nat. Rev. Drug Discov.* **20**, 332 (2021).
5. Schultz, L. & Mackall, C. Driving CAR T cell translation forward. *Sci. Transl. Med.* **11**, eaaw2127 (2019).
6. Akhoundi, M., Mohammadi, M., Sahraei, S.S., Sheykhhasan, M. & Fayazi, N. CAR T cell therapy as a promising approach in cancer immunotherapy: challenges and opportunities. *Cell. Oncol. (Dordr.)* **44**, 495–523 (2021).
7. Hanssens, H., Meeus, F., De Veirman, K., Breckpot, K. & Devoogdt, N. The antigen-binding moiety in the driver's seat of CARs. *Med. Res. Rev.* **42**, 306–342 (2022).
8. Sterner, R.C. & Sterner, R.M. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* **11**, 69 (2021).
9. Tokarew, N., Ogonek, J., Endres, S., von Bergwelt-Baildon, M. & Kobold, S. Teaching an old dog new tricks: next-generation CAR T cells. *Br. J. Cancer* **120**, 26–37 (2019).
10. Ajina, A. & Maher, J. Strategies to address chimeric antigen receptor tonic signaling. *Mol. Cancer Ther.* **17**, 1795–1815 (2018).
11. Kawalekar, O.U. *et al*. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity* **44**, 380–390 (2016).
12. Long, A.H. *et al*. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat. Med.* **21**, 581–590 (2015).
13. Zhao, Z. *et al*. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. *Cancer Cell* **28**, 415–428 (2015).
14. Furqan, F. & Shah, N.N. Multispecific CAR T cells deprive lymphomas of escape via antigen loss. *Annu. Rev. Med.* **74**, 279–291 (2023).

15. Lin, H., Cheng, J., Mu, W., Zhou, J. & Zhu, L. Advances in universal CAR-T cell therapy. *Front. Immunol.* **12**, 744823 (2021).
16. Caldwell, K.J., Gottschalk, S., Talleur, A.C. & Allogeneic, C.A.R. Cell therapy-more than a pipe dream. *Front. Immunol.* **11**, 618427 (2020).
17. Liu, Y. *et al*. TCR-T immunotherapy: the challenges and solutions. *Front. Oncol.* **11**, 794183 (2021).
18. Stein, A.M. *et al*. Tisagenlecleucel model-based cellular kinetic analysis of chimeric antigen receptor-T cells. *CPT Pharmacometrics Syst. Pharmacol.* **8**, 285–295 (2019).
19. Ogasawara, K., Dodds, M., Mack, T., Lymp, J., Dell'Aringa, J. & Smith, J. Population cellular kinetics of Lisocabtagene Maraleucel, an autologous CD19-directed chimeric antigen receptor T-cell product, in patients with relapsed/refractory large B-cell lymphoma. *Clin. Pharmacokinet.* **60**, 1621–1633 (2021).
20. Wu, L.S. *et al*. Population-based cellular kinetic characterization of ciltacabtagene autoleucel in subjects with relapsed or refractory multiple myeloma. *Clin. Transl. Sci.* **15**, 3000–3011 (2022).
21. Mu, W. *et al*. A model perspective explanation of the Long-term sustainability of a fully human BCMA-targeting CAR (CT103A) T-cell immunotherapy. *Front. Pharmacol.* **13**, 803693 (2022).
22. Huang, W. *et al*. Clinical pharmacology perspectives for adoptive cell therapies in oncology. *Clin. Pharmacol. Ther.* **112**, 968–981 (2022).
23. Kast, J. *et al*. Recent advances and clinical pharmacology aspects of chimeric antigen receptor (CAR) T-cellular therapy development. *Clin. Transl. Sci.* **15**, 2057–2074 (2022).
24. Liu, C. *et al*. Model-based cellular kinetic analysis of chimeric antigen receptor-T cells in humans. *Clin. Pharmacol. Ther.* **109**, 716–727 (2021).
25. Hays, A., Durham, J., Gullick, B., Rudemiller, N. & Schneider, T. Bioanalytical assay strategies and considerations for measuring cellular kinetics. *Int. J. Mol. Sci.* **24**, 695 (2022).
26. Qi, T., McGrath, K., Ranganathan, R., Dotti, G. & Cao, Y. Cellular kinetics: a clinical and computational review of CAR-T cell pharmacology. *Adv. Drug Deliv. Rev.* **188**, 114421 (2022).
27. Ogasawara, K. *et al*. In vivo cellular expansion of lisocabtagene maraleucel and association with efficacy and safety in relapsed/refractory large B-cell lymphoma. *Clin. Pharmacol. Ther.* **112**, 81–89 (2022).
28. Mueller, K.T. *et al*. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. *Blood* **130**, 2317–2325 (2017).
29. Bristol Myers Squibb Breyanzi U.S. prescribing information. <https://packageinserts.bms.com/pi/pi_breyanzi.pdf> (2022). Accessed April 17, 2023.
30. U.S. Food & Drug Administration Long term follow-up after administration of human gene therapy products–guidance for industry. <https://www.fda.gov/media/113768/download> (2020). Accessed April 17, 2023.
31. Mueller, K.T. *et al*. Clinical pharmacology of Tisagenlecleucel in B-cell acute lymphoblastic leukemia. *Clin. Cancer Res.* **24**, 6175–6184 (2018).
32. Awasthi, R. *et al*. Tisagenlecleucel cellular kinetics, dose, and immunogenicity in relation to clinical factors in relapsed/refractory DLBCL. *Blood Adv.* **4**, 560–572 (2020).
33. Abramson, J.S. *et al*. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet* **396**, 839–852 (2020).
34. Munshi, N.C. *et al*. Idecabtagene Vicleucel in relapsed and refractory multiple myeloma. *N. Engl. J. Med.* **384**, 705–716 (2021).
35. Connarn, J.N. *et al*. Characterizing the exposure-response relationship of idecabtagene vicleucel in patients with relapsed/refractory multiple myeloma. *CPT Pharmacometrics Syst. Pharmacol.* https://doi.org/10.1002/psp4.12922 (2023).
36. Turtle, C.J. *et al*. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J. Clin. Invest.* **126**, 2123–2138 (2016).
37. Turtle, C.J. *et al*. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med* **8**, 355ra116 (2016).
38. Raje, N. *et al*. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N. Engl. J. Med.* **380**, 1726–1737 (2019).
39. Rotte, A., Frigault, M.J., Ansari, A., Gliner, B., Heery, C. & Shah, B. Dose-response correlation for CAR-T cells: a systematic review of clinical studies. *J. Immunother. Cancer* **10**, e005678 (2022).
40. Fowler, N.H. *et al*. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat. Med.* **28**, 325–332 (2022).
41. European Medicines Agency Kymriah-H-C-4090-II-0044, EPAR–assessment report–variation. <https://www.ema.europa.eu/en/documents/variation-report/kymriah-h-c-4090-ii-0044-epar-assessment-report-variation_en.pdf> (2022). Accessed April 17, 2023.
42. Kamdar, M. *et al*. Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial. *Lancet* **399**, 2294–2308 (2022).
43. Sehgal, A. *et al*. Lisocabtagene maraleucel as second-line therapy in adults with relapsed or refractory large B-cell lymphoma who were not intended for haematopoietic stem cell transplantation (PILOT): an open-label, phase 2 study. *Lancet Oncol.* **23**, 1066–1077 (2022).
44. Locke, F.L. *et al*. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv.* **4**, 4898–4911 (2020).
45. Roberts, Z.J., Better, M., Bot, A., Roberts, M.R. & Ribas, A. Axicabtagene ciloleucel, a first-in-class CAR T cell therapy for aggressive NHL. *Leuk. Lymphoma* **59**, 1785–1796 (2018).
46. Narayan, V. *et al*. PSMA-targeting TGFbeta-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nat. Med.* **28**, 724–734 (2022).
47. Koyama, T. *et al*. Interim results of a first-in-human phase 1 dose-escalation trial of TAK-102, a glypican-3 targeted armored chimeric antigen receptor T-cell immunotherapy in patients with advanced solid tumors. *J. Immunother. Cancer* **10**, A770 (2022).
48. Adusumilli, P.S. *et al*. A phase I trial of regional mesothelin-targeted CAR T-cell therapy in patients with malignant pleural disease, in combination with the anti-PD-1 agent pembrolizumab. *Cancer Discov.* **11**, 2748–2763 (2021).
49. Qi, C. *et al*. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. *Nat. Med.* **28**, 1189–1198 (2022).
50. Zhou, D. *et al*. Clinical pharmacology characterization of AMG 119, a chimeric antigen receptor T (CAR-T) cell therapy targeting Delta-like ligand 3 (DLL3), in patients with relapsed/refractory small cell lung cancer (R/R SCLC). *Clin. Pharmacol. Ther.* **111**, S61 (2022).
51. Skovgard, M.S. *et al*. Imaging CAR T-cell kinetics in solid tumors: translational implications. *Mol. Ther. Oncolytics* **22**, 355–367 (2021).
52. Flugel, C.L. *et al*. Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. *Nat. Rev. Clin. Oncol.* **20**, 49–62 (2023).
53. He, J.Z. *et al*. A consideration of fixed dosing versus body size-based dosing strategies for chimeric antigen receptor T-cell therapies. *Clin. Pharmacol. Drug Dev.* **11**, 1130–1135 (2022).
54. Li, X. & Chen, W. Mechanisms of failure of chimeric antigen receptor T-cell therapy. *Curr. Opin. Hematol.* **26**, 427–433 (2019).
55. Oluwole, O.O. *et al*. Prophylactic corticosteroid use in patients receiving axicabtagene ciloleucel for large B-cell lymphoma. *Br. J. Haematol.* **194**, 690–700 (2021).
56. Neelapu, S.S. CAR-T efficacy: is conditioning the key? *Blood* **133**, 1799–1800 (2019).
57. Hirayama, A.V. *et al*. The response to lymphodepletion impacts PFS in patients with aggressive non-Hodgkin lymphoma treated with CD19 CAR T cells. *Blood* **133**, 1876–1887 (2019).

58. Kimmel, G.J., Locke, F.L. & Altrock, P.M. The roles of T cell competition and stochastic extinction events in chimeric antigen receptor T cell therapy. *Proc. Biol. Sci.* **288**, 20210229 (2021).
59. Fraietta, J.A. *et al*. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat. Med.* **24**, 563–571 (2018).
60. Das, R.K., Vernau, L., Grupp, S.A. & Barrett, D.M. Naive T-cell deficits at diagnosis and after chemotherapy impair cell therapy potential in pediatric cancers. *Cancer Discov.* **9**, 492–499 (2019).
61. Sommermeyer, D. *et al*. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. *Leukemia* **30**, 492–500 (2016).
62. Liu, X. *et al*. Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice. *Cancer Res.* **75**, 3596–3607 (2015).
63. Singh, N. *et al*. Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. *Nat. Med.* **27**, 842–850 (2021).
64. U.S. Food & Drug Administration Considerations for the development of chimeric antigen receptor (CAR) T cell products–draft guidance for industry. <https://www.fda.gov/media/156896/download> (2022). Accessed April 17, 2023.
65. Zhu, X. *et al*. Novel benchmark scaling method for first-in-human dose of CAR-T therapies. *Mol. Ther.* **30**, 524 (2022).
66. Flinn, I.W. *et al*. A first-in-human study of YTB323, a novel, autologous CD19-directed CAR-T cell therapy manufactured using the novel T-charge TM platform, for the treatment of patients (pts) with relapsed/refractory (r/r) diffuse large B-cell lymphoma (DLBCL). *Blood* **138**, 740 (2021).
67. Sperling, A.S. *et al*. Phase I study of PHE885, a fully human BCMA-directed CAR-T cell therapy for relapsed/refractory multiple myeloma manufactured in <2 days using the T-charge TM platform. *Blood* **138**, 3864 (2021).
68. Singh, A.P. *et al*. Development of a quantitative relationship between CAR-affinity, antigen abundance, tumor cell depletion and CAR-T cell expansion using a multiscale systems PK-PD model. *MAbs* **12**, 1688616 (2020).
69. Singh, A.P. *et al*. Bench-to-bedside translation of chimeric antigen receptor (CAR) T cells using a multiscale systems pharmacokinetic-pharmacodynamic model: a case study with anti-BCMA CAR-T. *CPT Pharmacometrics Syst. Pharmacol.* **10**, 362–376 (2021).
70. Frey, N.V. *et al*. Optimizing chimeric antigen receptor T-cell therapy for adults with acute lymphoblastic leukemia. *J. Clin. Oncol.* **38**, 415–422 (2020).
71. Xu, J. *et al*. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 9543–9551 (2019).
72. Frigault, M., Rotte, A., Ansari, A., Gliner, B., Heery, C. & Shah, B. Dose fractionation of CAR-T cells. A systematic review of clinical outcomes. *J. Exp. Clin. Cancer Res.* **42**, 11 (2023).
73. Gardner, R.A. *et al*. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood* **129**, 3322–3331 (2017).
74. Gauthier, J. *et al*. Factors associated with outcomes after a second CD19-targeted CAR T-cell infusion for refractory B-cell malignancies. *Blood* **137**, 323–335 (2021).
75. Holland, E.M. *et al*. Efficacy of second CAR-T (CART2) infusion limited by poor CART expansion and antigen modulation. *J. Immunother. Cancer* **10**, e004483 (2022).
76. Li, D.H., Whitmore, J.B., Guo, W. & Ji, Y. Toxicity and efficacy probability interval design for phase I adoptive cell therapy dose-finding clinical trials. *Clin. Cancer Res.* **23**, 13–20 (2017).
77. Dasyam, N., George, P. & Weinkove, R. Chimeric antigen receptor T-cell therapies: optimising the dose. *Br. J. Clin. Pharmacol.* **86**, 1678–1689 (2020).
78. Kurzrock, R., Lin, C.C., Wu, T.C., Hobbs, B.P., Pestana, R.M. & Hong, D.S. Moving beyond 3+3: the future of clinical trial design. *Am. Soc. Clin. Oncol. Educ. Book* **41**, e133–e144 (2021).
79. U.S. Food & Drug Administration Summary basis for regulatory action–ABECMA. <https://www.fda.gov/media/147627/download> (2021). Accessed April 17, 2023.
80. Chaudhury, A. *et al*. Chimeric antigen receptor T cell therapies: a review of cellular kinetic-pharmacodynamic modeling approaches. *J. Clin. Pharmacol.* **60**(Suppl 1), S147–S159 (2020).
81. Nukala, U., Rodriguez Messan, M., Yogurtcu, O.N., Wang, X. & Yang, H. A systematic review of the efforts and hindrances of Modeling and simulation of CAR T-cell therapy. *AAPS J.* **23**, 52 (2021).
82. Belov, A., Schultz, K., Forshee, R. & Tegenge, M.A. Opportunities and challenges for applying model-informed drug development approaches to gene therapies. *CPT Pharmacometrics Syst. Pharmacol.* **10**, 286–290 (2021).
83. Madabushi, R., Seo, P., Zhao, L., Tegenge, M. & Zhu, H. Review: role of model-informed drug development approaches in the lifecycle of drug development and regulatory decision-making. *Pharm. Res.* **39**, 1669–1680 (2022).
84. U.S. Food & Drug Administration KYMRIAH (tisagenlecleucel). <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah-tisagenlecleucel> (2022). Accessed April 17, 2023.
85. U.S. Food & Drug Administration *YESCARTA (axicabtagene ciloleucel)*. <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta-axicabtagene-ciloleucel> (2022). Accessed April 17, 2023.
86. U.S. Food & Drug Administration TECARTUS (brexucabtagene autoleucel). <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus-brexucabtagene-autoleucel> (2022). Accessed April 17, 2023.
87. Sahoo, P. *et al*. Mathematical deconvolution of CAR T-cell proliferation and exhaustion from real-time killing assay data. *J. R. Soc. Interface* **17**, 20190734 (2020).
88. Barros, L.R.C., Paixao, E.A., Valli, A.M.P., Naozuka, G.T., Fassoni, A.C. & Almeida, R.C. CARTmath-A mathematical model of CAR-T immunotherapy in preclinical studies of hematological cancers. *Cancers (Basel)* **13**, 2941 (2021).
89. Barros, L.R.C., Rodrigues, B.J. & Almeida, R.C. CAR-T cell goes on a mathematical model. *J. Cell Immunol.* **2**, 31–37 (2020).
90. Rodrigues, B.J., Barros, L.R.C. & Almeida, R.C. Three-compartment model of CAR T-cell immunotherapy. *bioRxiv* https://doi.org/10.1101/779793 (2020).
91. Leon-Triana, O. *et al*. CAR T cell therapy in B-cell acute lymphoblastic leukaemia: insights from mathematical models. *Commun Nonlinear Sci. Numer Simul.* **94**, 105570 (2021).
92. Liu, L. *et al*. Computational model of CAR T-cell immunotherapy dissects and predicts leukemia patient responses at remission, resistance, and relapse. *J. Immunother. Cancer* **10**, e005360 (2022).
93. Kirouac, D.C., Zmurchok, C., Deyati, A., Sicherman, J., Bond, C. & Zandstra, P.W. Deconvolution of clinical variance in CAR-T cell pharmacology and response. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-023-01687-x (2023).
94. Hardiansyah, D. & Ng, C.M. Quantitative systems pharmacology model of chimeric antigen receptor T-cell therapy. *Clin. Transl. Sci.* **12**, 343–349 (2019).
95. Hanson, S. *et al*. Toxicity management in CAR T cell therapy for B-ALL: mathematical modelling as a new avenue for improvement. *bioRxiv* https://doi.org/10.1101/049908 (2016).
96. Mueller-Schoell, A. *et al*. Early survival prediction framework in CD19-specific CAR-T cell immunotherapy using a quantitative systems pharmacology model. *Cancers (Basel)* **13**, 2782 (2021).
97. Paixao, E.A., Barros, L.R.C., Fassoni, A.C. & Almeida, R.C. Modeling patient-specific CAR-T cell dynamics: multiphasic kinetics via phenotypic differentiation. *Cancers (Basel)* **14**, 5576 (2022).
98. Owens, K., Bozic, I. & Modeling, C.A.R. T-cell therapy with patient preconditioning. *Bull. Math. Biol.* **83**, 42 (2021).
99. Derippe, T. *et al*. Mechanistic Modeling of the interplay between host immune system, IL-7 and UCART19 Allogeneic CAR-T cells in adult B-cell acute lymphoblastic leukemia. *Cancer Res. Commun.* **2**, 1532–1544 (2022).

100. Khot, A., Matsueda, S., Thomas, V.A., Koya, R.C. & Shah, D.K. Measurement and quantitative characterization of whole-body pharmacokinetics of exogenously administered T cells in mice. *J. Pharmacol. Exp. Ther.* **368**, 503–513 (2019).
101. Tsai, C.H., Singh, A.P., Xia, C.Q. & Wang, H. Development of minimal physiologically-based pharmacokinetic-pharmacodynamic models for characterizing cellular kinetics of CAR T cells following local deliveries in mice. *J. Pharmacokinet. Pharmacodyn.* **49**, 525–538 (2022).
102. Brown, L.V., Gaffney, E.A., Ager, A., Wagg, J. & Coles, M.C. Quantifying the limits of CAR T-cell delivery in mice and men. *J. R. Soc. Interface* **18**, 20201013 (2021).
103. Harris, D.T. *et al*. Comparison of T cell activities mediated by human TCRs and CARs that use the same recognition domains. *J. Immunol.* **200**, 1088–1100 (2018).
104. Dushek, O. *et al*. Antigen potency and maximal efficacy reveal a mechanism of efficient T cell activation. *Sci. Signal* **4**, ra39 (2011).
105. Leon-Triana, O., Perez-Martinez, A., Ramirez-Orellana, M. & Perez-Garcia, V.M. Dual-target CAR-Ts with on- and off-tumour activity may override immune suppression in solid cancers: a mathematical proof of concept. *Cancers (Basel)* **13**, 703 (2021).
106. Santurio, D.S. & Barros, L.R.C. A mathematical model for on-target off-tumor effect of CAR-T cells on gliomas. *Front. Syst. Biol.* **2**, 1–15 (2022).
107. Rohrs, J.A., Zheng, D., Graham, N.A., Wang, P. & Finley, S.D. Computational model of chimeric antigen receptors explains site-specific phosphorylation kinetics. *Biophys. J.* **115**, 1116–1129 (2018).
108. Cess, C.G. & Finley, S.D. Data-driven analysis of a mechanistic model of CAR T cell signaling predicts effects of cell-to-cell heterogeneity. *J. Theor. Biol.* **489**, 110125 (2020).
109. Adhikarla, V. *et al*. A mathematical Modeling approach for targeted radionuclide and chimeric antigen receptor T cell combination therapy. *Cancers (Basel)* **13**, 5171 (2021).
110. Upreti, V.V. & Venkatakrishnan, K. Model-based meta-analysis: optimizing research, development, and utilization of therapeutics using the totality of evidence. *Clin. Pharmacol. Ther.* **106**, 981–992 (2019).
111. Bristol Myers Squibb Abecma U.S. prescribing information. <https://packageinserts.bms.com/pi/pi_abecma.pdf> (2021). Accessed April 17, 2023.
112. Janssen Biotech Inc Carvykti U.S. prescribing information. <https://www.janssenlabels.com/package-insert/product-monograph/prescribing-information/CARVYKTI-pi.pdf> (2023). Accessed April 17, 2023.
113. Kite Pharma Inc Tecartus U.S. prescribing information. <https://www.gilead.com/-/media/files/pdfs/medicines/oncology/tecartus/tecartus-pi.pdf> (2021). Accessed April 17, 2023.
114. Kite Pharma Inc Yescarta U.S. prescribing information. <https://www.gilead.com/-/media/files/pdfs/medicines/oncology/yescarta/yescarta-pi.pdf> (2022). Accessed April 17, 2023.
115. Novartis Pharmaceuticals Corporation Kymriah U.S. prescribing information. <https://www.kymriah-rems.com/siteassets/kymriah-rems_pi_may-2022.pdf> (2022). Accessed April 17, 2023.
116. Upadhaya, S., Yu, J.X., Shah, M., Correa, D., Partridge, T. & Campbell, J. The clinical pipeline for cancer cell therapies. *Nat. Rev. Drug Discov.* **20**, 503–504 (2021).
117. Wagner, J., Wickman, E., DeRenzo, C. & Gottschalk, S. CAR T cell therapy for solid tumors: bright future or dark reality? *Mol. Ther.* **28**, 2320–2339 (2020).
118. Strohl, W.R. & Naso, M. Bispecific T-cell redirection versus chimeric antigen receptor (CAR)-T cells as approaches to kill cancer cells. *Antibodies (Basel)* **8**, 41 (2019).
119. Bethune, M.T. *et al*. Domain-swapped T cell receptors improve the safety of TCR gene therapy. *Elife* **5**, e19095 (2016).
120. Hong, D.S. *et al*. Autologous T cell therapy for MAGE-A4(+) solid cancers in HLA-A*02(+) patients: a phase 1 trial. *Nat. Med.* **29**, 104–114 (2023).
121. Hong, D.S. *et al*. Phase 1 clinical trial evaluating the safety and anti-tumor activity of ADP-A2M10 SPEAR T-cells in patients with MAGE-A10+ head and neck, melanoma, or urothelial tumors. *Front. Oncol.* **12**, 818679 (2022).
122. Nagarsheth, N.B. *et al*. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. *Nat. Med.* **27**, 419–425 (2021).
123. Kageyama, S. *et al*. Adoptive transfer of MAGE-A4 T-cell receptor gene-transduced lymphocytes in patients with recurrent esophageal cancer. *Clin. Cancer Res.* **21**, 2268–2277 (2015).
124. Siegler, E.L. & Wang, P. Preclinical models in chimeric antigen receptor-engineered T-cell therapy. *Hum. Gene Ther.* **29**, 534–546 (2018).
125. Srivastava, S. *et al*. Immunogenic chemotherapy enhances recruitment of CAR-T cells to lung tumors and improves antitumor efficacy when combined with checkpoint blockade. *Cancer Cell* **39**, 193–208 (2021).
126. Wei, T. *et al*. Generation of neoantigen-specific T cells for adoptive cell transfer for treating head and neck squamous cell carcinoma. *Onco. Targets. Ther.* **10**, 1929726 (2021).
127. Campillo-Davo, D., Flumens, D. & Lion, E. The quest for the best: how TCR affinity, avidity, and functional avidity affect TCR-engineered T-cell antitumor responses. *Cell* **9**, 1720 (2020).
128. Neelapu, S.S. *et al*. Axicabtagene Ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med* **377**, 2531–2544 (2017).
129. U.S. Food & Drug Administration. *Clinical Pharmacology BLA Review - Yescarta*. <https://www.fda.gov/media/109140/download> (2017). Accessed April 17, 2023.
130. Locke, F.L. *et al*. Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma. *N Engl J Med* **386**, 640–654 (2022).
131. U.S. Food & Drug Administration. *April 1, 2022 Clinical Pharmacology Memo - YESCARTA*. <https://www.fda.gov/media/157676/download> (2022). Accessed April 17, 2023.
132. European Medicines Agency. *Yescarta-H-C-004480-II-0042 EPAR - assessment report - variation*. <https://www.ema.europa.eu/en/documents/variation-report/yescarta-h-c-004480-ii-0042-epar-assessment-report-variation_en.pdf> (2022). Accessed April 17, 2023.
133. Jacobson, C.A. *et al*. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. *Lancet Oncol* **23**, 91–103 (2022).
134. U.S. Food & Drug Administration. *Clinical Pharmacology BLA Review - Tecartus*. <https://www.fda.gov/media/141164/download> (2020). Accessed April 17, 2023.
135. Wang, M. *et al*. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. *N Engl J Med* **382**, 1331–1342 (2020).
136. Shah, B.D. *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* **398**, 491–502 (2021).
137. U.S. Food & Drug Administration. *October 1, 2021 Clinical Pharmacology Memo - TECARTUS*. <https://www.fda.gov/media/157660/download> (2021). Accessed April 17, 2023.
138. U.S. Food & Drug Administration. *Clinical Pharmacology BLA Review - Abecma*. <https://www.fda.gov/media/147781/download> (2021). Accessed April 17, 2023.
139. Berdeja, J.G. *et al*. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet* **398**, 314–324 (2021).
140. European Medicines Agency. *Carvykti : EPAR - public assessment report*. <https://www.ema.europa.eu/en/documents/assessment-report/carvykti-epar-publicassessment-report_en.pdf> (2022). Accessed April 17, 2023.
141. Kimmel, G.J., Locke, F.L. & Altrock, P.M. Response to CAR T cell therapy can be explained by ecological cell dynamics and stochastic extinction events. *bioRxiv*, 717074 (2020).
142. Pluckthun, A. Designed ankyrin repeat proteins (DARPins): binding proteins for research, diagnostics, and therapy. *Annu Rev Pharmacol Toxicol* **55**, 489–511 (2015).
143. Dubrovsky, L. *et al*. T cell receptor mimic antibodies for cancer therapy. *Oncoimmunology* **5**, e1049803 (2016).

144. Allele Frequency Net Database. *Allele*Frequencies in Worldwide Population*. <http://www.allelefrequencies.net/hla6006a.asp?hla_selection=A*02:01>. Accessed April 17, 2023.
145. Kalergis, A.M. *et al*. Efficient T cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex. *Nat Immunol* **2**, 229–234 (2001).
146. He, Q., Liu, Z., Liu, Z., Lai, Y., Zhou, X. & Weng, J. TCR-like antibodies in cancer immunotherapy. *J Hematol Oncol* **12**, 99 (2019).
147. Stadtmauer, E.A. *et al*. CRISPR-engineered T cells in patients with refractory cancer. *Science* **367** (2020).
148. D'Angelo, S.P. *et al*. SPEARHEAD-1: Preliminary Translational Insights from a Phase 2 Trial of Afamitresgene Autoleucel (Formerly ADP-A2M4) in Patients with Advanced Synovial Sarcoma or Myxoid/Round Cell Liposarcoma. (2021 CTOS Virtual Annual Meeting, 2021).
149. D'Angelo, S.P. *et al*. Identification of response stratification factors from pooled efficacy analyses of afamitresgene autoleucel ("Afami-cel" [Formerly ADP-A2M4]) in metastatic synovial sarcoma and myxoid/round cell liposarcoma phase 1 and phase 2 trials. *Journal of Clinical Oncology* **40**, 11562 (2022).
150. Van Tine, B.A. *et al*. Final Analysis from SPEARHEAD-1 Cohort 1 of Afamitresgene Autoleucel ("Afami-cel" [Formerly ADP-A2M4]) in Advanced Synovial Sarcoma and Myxoid/Round Cell Liposarcoma. (2022 CTOS Annual Meeting, Vancouver, BC, Canada, 2022).
151. ClinicalTrials.gov. Spearhead 1 Study in Subjects With Advanced Synovial Sarcoma or Myxoid/Round Cell Liposarcoma. <https://clinicaltrials.gov/ct2/show/NCT04044768>. Accessed April 17, 2023.
152. Hong, D. *et al*. Initial safety, efficacy, and product attributes from the SURPASS trial with ADP-A2M4CD8, a SPEAR T-cell therapy incorporating an affinity optimized TCR targeting MAGE-A4 and a CD8α co-receptor. *Journal for ImmunoTherapy of Cancer* **8**, A231 (2020).
153. Aggen, D.H. *et al*. Preliminary clinical outcomes of ADP-A2M4CD8, a next-generation autologous T-cell receptor T-cell therapy, in patients with advanced urothelial cancer. *Journal of Clinical Oncology* **41**, 517 (2023).
154. Blum Murphy, M.A. *et al*. Safety and efficacy from the phase 1 SURPASS trial of ADP-A2M4CD8, a next-generation T-cell receptor T-cell therapy, in patients with advanced esophageal, esophagogastric junction, or gastric cancer. *Journal of Clinical Oncology* **41**, 349 (2023).
155. Hong, D.S. *et al*. Updated safety and efficacy from SURPASS, the phase I trial of ADP-A2M4CD8, a next-generation autologous T-cell receptor T-cell therapy, in previously treated patients with unresectable or metastatic tumors. *Ann Oncol* **33**, S331–S355 (2022).
156. Moore, K. *et al*. 2022-LBA-414-ESGO Preliminary clinical outcome of ADP-A2M4CD8, a next-generation autologous T-cell receptor T-cell therapy, in patients with advanced epithelial ovarian cancer. *International Journal of Gynecologic Cancer* **32**, A472–A473 (2022).
157. Meyer, T. *et al*. Overall Safety and Efficacy from the Phase 1 Trial of ADP-A2AFP SPEAR T-Cells in Patients With Advanced Hepatocellular Carcinoma or Other Cancer Types Expressing Alpha-Fetoprotein. (16th ICLA Annual Conference 2022, Madrid, Spain, 2022).
158. Sangro, B. *et al*. LB012 - Data from the third dose cohort of an ongoing study with ADP-A2AFP SPEAR T cells. *J Hepatol* **73**, S122 (2020).
159. Sangro, B. *et al*. Data from the third dose cohort and expansion phase of a Phase 1 trial of ADP-A2AFP SPEAR T-cells for patients with hepatocellular carcinoma and other cancer types expressing alpha-fetoprotein. 15th ICLA Conference, Virtual (2021).
160. Wermke, M. *et al*. 959 Safety and anti-tumor activity of TCR-engineered autologous, PRAME-directed T cells across multiple advanced solid cancers at low doses – clinical update on the ACTengine® IMA203 trial. *Journal for ImmunoTherapy of Cancer* **9**, A1009 (2021).
161. Morgan, R.A. *et al*. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* **314**, 126–129 (2006).
162. Foy, S.P. *et al*. Non-viral precision T cell receptor replacement for personalized cell therapy. *Nature* **615**, 687–696 (2023).