Clinical Pharmacology Perspectives for Adoptive Cell Therapies in Oncology

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Adoptive cell therapies (ACTs) have shown transformative efficacy in oncology with five US Food and Drug Administration (FDA) approvals for chimeric antigen receptor (CAR) T-cell therapies in hematological malignancies, and promising activity for T cell receptor T-cell therapies in both liquid and solid tumors. Clinical pharmacology can play a pivotal role in optimizing ACTs, aided by modeling and simulation toolboxes and deep understanding of the underlying biological and immunological processes. Close collaboration and multilevel data integration across functions, including chemistry, manufacturing, and control, biomarkers, bioanalytical, and clinical science and safety teams will be critical to ACT development. As ACT is comprised of alive, polyfunctional, and heterogeneous immune cells, its overall physicochemical and pharmacological property is vastly different from other platforms/modalities, such as small molecule and protein therapeutics. In this review, we first describe the unique kinetics of T cells and the appropriate bioanalytical strategies to characterize cellular kinetics. We then assess the distinct aspects of clinical pharmacology for ACTs in comparison to traditional small molecule and protein therapeutics. Additionally, we provide a review for the five FDA-approved CAR T-cell therapies and summarize their properties, cellular kinetic characteristics, dose-exposure-response relationship, and potential baseline factors/variables in product, patient, and regimen that may affect the safety and efficacy. Finally, we probe into existing empirical and mechanistic quantitative techniques to understand how various modeling and simulation approaches can support clinical pharmacology strategy and propose key considerations to be incorporated and explored in future models.

Adoptive cell therapies (ACTs) use immune cells that are isolated from the patient, optionally genetically engineered, substantially expanded, and infused back into the patient.[1] The current mainstream ACT is chimeric antigen receptor (CAR) T cell therapy, whereas other platforms, including engineered T cell receptor (TCR) T cell, tumor-infiltrating lymphocyte, natural killer cell, and allogeneic immune cell therapies are also under development. CAR T cell therapy uses T cells engineered to express transgenic artificial CARs targeting cell surface antigens,[2] whereas TCR-T cell therapy uses T cells engineered to express transgenic TCRs targeting antigenic peptides presented via major histocompatibility complex.[3] As of October 2021, five CAR Ts are approved by the US Food and Drug Administration (FDA): KYMRIAH (tisagenlecleucel), YESCARTA (axicabtagene ciloleucel), TECARTUS (brexucabtagene autoleucel), BREYANZI (lisocabtagene maraleucel), and ABECMA (idecabtagene vicleucel). The first four are anti-CD19+ CAR T cell therapies indicated for the treatment of relapsed/refractory hematological B-cell malignancies, and the last one (ABECMA) is an anti-BCMA CAR T cell therapy indicated for the treatment of relapsed/refractory multiple myeloma (MM). These engineered immune cells have high specificity for the tumor antigen, long persistence after a single infusion,[2] high sensitivity for re-activation even at low target antigen levels,[3] and promising activity across multiple tumor types,[4,5] making them ideal therapeutic "weapons."

Unlike traditional small molecules and protein therapeutics (SMs/PTs) that are either chemically synthesized or biologically manufactured, ACTs are comprised of a heterogeneous mixture of living cells from patients and can differ substantially among patients.[1] The engineering, processing, tumor biology, and host immune system together determine the interaction of ACTs with target.[1] Thus, the complexity of product manufacture and patient heterogeneity have more impact on the pharmacological activity of ACT compared with traditional drugs, which bring considerable challenges to clinical pharmacologists regarding selection of the optimal dose, patient, and regimen. As oncology treatment evolves to include these novel modalities, clinical pharmacology (CP) strategy must also evolve to meet the challenges. In this review, we discuss the unique kinetic, bioanalytical, clinical pharmacological, and modeling and simulation considerations for ACTs, summarize the characteristics of five FDA-approved ACTs, and identify novel CP strategies for clinical development.

KINETIC CONSIDERATIONS FOR ACTS

The central tenet of pharmacology necessitates deep understanding of drug kinetics as it is critically related to efficacy and toxicity. At the apparent level, the kinetic profiles of both SMs/PTs and ACTs are multiphasic; however, the underlying mechanisms are fundamentally different (**Table 1**). The dispositions of SMs/PTs are usually sufficiently described by two or three phases, driven by

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Table 1 Comparison from kinetic and clinical pharmacology aspects of small molecules, monoclonal antibodies, and T cell therapies

	Small molecule	Monoclonal antibody	T cell therapy
Pharmacokinetic/cellular kinetic aspect			
Absorption	Intestinal absorption if p.o.	Injection site absorption if s.c. or i.m.	Only relevant if regional delivery
Distribution	Various tissues/organs Many distribute intracellularly	Mainly limited to plasma, interstitial fluid, lymphatic system, and endothelial system TMDD Liver and reticuloendothelial system	T cells may traffic to secondary lymphoid tissues, general tissues, and tumor sites
Metabolism/catabolism	CYP enzyme-mediated metabolism Transporter-mediated uptake/efflux can be the rate-determining step	Catabolism No CYP enzyme-mediated metabolism	No enzyme-mediated metabolism or proteasome/lysosome-based catabolism
Excretion	Renal and biliary	Not applicable	Not applicable
CL	Dose/AUC	Dose/AUC	Not applicable T cells actively proliferate T cells may die through natural or contact-induced apoptosis
$t_{1/2}$	Hours	Days to weeks	Months, can be up to years Difficult to determine accurately with sparse sampling in terminal phase Difficult to interpret with potentially ongoing re-activation
Clinical pharmacology consideration			
Route of administration	Oral, i.v.	i.v., s.c., i.m.	i.v., regional
Dose frequency	Hourly to daily	Weekly to monthly	Often once in a lifetime
Dose proportionality	Mostly linear Nonlinear if complicated absorption or enzyme/transporter saturation	Mostly linear at high dose Usually nonlinear at low concentration (TMDD)	Not well established
Bioanalysis	Analyte: drug and metabolite Method: LC-MS	Analyte: antibody and anti-drug antibody Method: ELISA	Analyte: transgene, cell count, anti-drug antibody Method: qPCR, flow cytometry, cell-based and non-cell-based assays
Food/ARA effect	Yes	Not applicable	Not applicable
Formulation/excipient effect	Yes	May affect k_a and F for s.c. or i.m.	Not applicable
DDI	CYP enzyme/transporter-mediated inhibition and induction	Reversal of inflammatory disease state may indirectly cause DDI	Cytokine release may indirectly cause DDI
hERG and cardiotoxicity	Yes	Unlikely	Elevated cytokine; Cross-reactivity with epitope derived from protein expressed by cardiac tissue
Organ impairment	Yes	No	Unlikely
Immunogenicity	No	Yes	Yes
PK/PD relationship	Drug exposure at the site of action (plasma, interstitium, and intracellular space) drives PD PD typically does not affect PK	Drug exposure at the site of action (mainly plasma and interstitium) drives PD Target may also affect PK via TMDD	Cell exposure at the tumor site (blood, interstitium, lymph, and lymphoid tissues) drives PD Target also affects T cell proliferation via antigen stimulation

ARA, acid reducing agent; AUC, area under the curve; CL, clearance; DDI, drug-drug interaction; ELISA, enzyme-linked immunosorbent assay; F, bioavailability; hERG, human ether-à-go-go-related gene; k_a, absorption rate constant; LC-MS, liquid chromatography mass spectrometry; PD, pharmacodynamics; PK, pharmacokinetics; qPCR, quantitative polymerase chain reaction; $t_{1/2}$, terminal half-life; TMDD, target-mediated drug disposition.

absorption, distribution, metabolism/catabolism, and excretion. In contrast, cellular kinetic profiles generally have four phases: (1) rapid initial decline within hours after infusion due to cellular migration/extravasation into various organs/tissues; (2) exponential expansion due to both homeostatic and antigen-driven proliferation upon antigen recognition and cell activation; (3) contraction phase due to programmed apoptosis of activated cells, cell-to-cell contact mediated apoptosis, and decrease of antigen; and (4) persistence phase with measurable levels of cells months or even years after single infusion as a result of long-lived memory phenotypes or low-level constant proliferation/activation due to continual production of antigen.[6,7] Due to the intrinsic ability of cells to grow, some pharmacokinetic (PK) concepts/parameters like clearance and volume of distribution are not readily applicable. However, other parameters, such as peak plasma concentration (C_{max}), area under the curve (AUC), and half-life still provide meaningful characterization and assessment of cellular kinetics and consequently can be related to clinical outcomes.

BIOANALYTICAL CONSIDERATIONS FOR ACTS

To obtain cellular kinetic profiles, quantitative polymerase chain reaction (qPCR) and flow cytometry are the two most commonly used bioanalytical methods, measuring T cells as copies of transgene per μg genomic DNA and number of T cells per μL blood, respectively.[8] Although highly sensitive and easier to implement, qPCR may overestimate the number of CAR/TCR-expressing transgenic T cells, whereas flow cytometry is less sensitive and logistically more complicated but can provide absolute enumeration of transgenic T cells by detecting CAR/TCR as well as T-cell phenotype information. The qPCR and flow cytometry data were generally correlated for KYMRIAH[9] and BREYANZI; however, gene silencing/downregulation and other factors may limit the concordance between these methods, making comparison difficult across ACTs.[10] Interestingly, it is also feasible to develop qPCR assays for cellular kinetics with results reported as copies of transgene/μL blood, which eliminates the impact by differences in genomic DNA content in blood, especially due to lymphodepletion or rapid expansion of CAR T cells.[11] From a mechanistic modeling perspective, cellular kinetic data based on flow cytometry is preferred as units expressed as number of cells per unit volume are more interpretable, and it captures varying cell phenotypes which can be related to different species in the models and explain the heterogeneity in cellular kinetics and clinical outcome. However, validated flow assays may be limited in the number of fluorescent channels that can be monitored, and thus require careful selection of the appropriate markers. A detailed comparison between qPCR and flow cytometry is shown in **Table 2**.

Due to the unique kinetics of ACTs, novel sampling strategies should be applied. To characterize rapid initial distribution and expansion, daily samples over the first few days post infusion are important, with gradual reduction in frequency thereafter. Less frequent sampling may be justified after expansion phase/early contraction phase (usually > 28 days). The persistence phase can be characterized with weekly, monthly, and even quarterly sampling (**Table 3**). Immunogenicity can be positive for some ACTs based on cell-based assay or non-cell-based competitive ligand binding assay (**Table 3**), and may be induced by expression of artificial CAR, non-endogenous TCR, or residual non-human protein,[12] although *ex vivo* and *in vivo* cell expansion is expected to dilute the latter to very low levels. Currently, the clinical data suggest that immunogenicity does not affect cellular kinetics, clinical response and safety; therefore, baseline or treatment-induced positive patients should still benefit from the therapies (**Table 3**). Additional steps to mitigate immunogenicity can be considered, such as *in silico* analysis of the recombinant TCR/CAR. In addition, lymphodepletion regimen prior to infusion also contributes to low immunogenicity rates. Nonetheless, monitoring immunogenicity with frequent sampling and testing remains essential and valuable.

THE APPLICABILITY OF TRADITIONAL CP CONSIDERATIONS IN ACTS

Due to the unique properties and kinetics of ACTs, several traditional CP considerations/lessons learned from SM/PT are either unknown or less applicable. Some examples include: (1) the impact of renal and hepatic impairment on cellular kinetics has not been reported; (2) the effect from food, formulation, and acid reducing agents are not relevant due to the intravenous administration of ACT; (3) the traditional half-life driven dosing frequency may not be suitable as the memory cells can be reactivated; and (4) the concept of dose proportionality is less applicable because the drug can be generated *de novo* by cellular expansion driven by many factors (e.g., host immune status and tumor immunogenicity) that are unrelated to dose.

Nonetheless, some conventional CP considerations still apply to ACTs. For drug-drug interaction (DDI) considerations, engineered T cells do not directly interact with enzymes and transporters, and hence direct DDI with concomitantly administered drugs is not expected. However, T cell activation upon target engagement can lead to transient but drastic release of cytokines (e.g., interleukin 6 (IL-6)), which in turn may suppress/downregulate CYP450 enzyme expression, thereby elevating systemic concentrations for drugs that are substrates of the affected pathways.[13,14] Therefore, monitoring for CYP450 and transporter-related DDI may be warranted for ACTs, especially for concomitant drugs with narrow therapeutic indexes. Similarly, engineered T cells are not expected to directly interact with cardiac ion channels, suggesting minimal toxicity risk of disrupting the function of human ether-à-go-go-related gene cardiac potassium channel. However, high levels of pro-inflammatory cytokines have been correlated with QT prolongation in patients with inflammatory conditions.[15] It is unknown whether cell therapies could exert a similar indirect cytokine-mediated mechanism on the QT interval.

SUMMARIES FROM FDA-APPROVED ACTS

The core CP objectives are to characterize cellular kinetics, determine dose-exposure-response relationship, and identify key covariates affecting such relationship. **Table 3** summarizes these characteristics of five FDA-approved CAR T cell therapies based on their US prescription information and Biologics License Application, review in Clinical Pharmacology and Pharmacometrics (**Table 3**).[16–28] Across the five ACTs, the infused amount of CAR T cells is typically 0.2–5 × 10^8 cells/kg

Table 2 Comparison of bioanalytical aspects and strategies between qPCR and flow cytometry

	qPCR	Flow cytometry
Assay sensitivity[9]	Highly sensitive: LLOQ ≤ 50 copies of transgene/μg DNA	Sensitivity depends on the relative affinity of the CAR/TCR detection agent, i.e., anti-ID vs. dextramer
Sample matrix and storage[46,47]	Frozen whole blood	Whole blood at room temperature, run 24–72 hours for absolute cell quantitation
Sample batching[46,47]	Yes	No, must be analyzed within 1–2 days post collection to enable absolute cell quantitation
Detection[47]	Measures copies of transgene in the cell	Measures cells with surface expression of transgenic CAR/TCR, multiplexed with other markers of T-cell phenotype/subset
Post-treatment detection duration[8]	Can often detect transgene in circulation for years depending on cell persistence	Generally, can detect engineered T-cells in circulation for months to years, but may fall below assay sensitivity at later time points depending on cell persistence
Other considerations	The extent of transgene incorporation into genome is generally unknown. Does not measure expression level of the transgenic CAR/TCR on the cell surface. Applying newly developed qPCR method with volume-based unit enables more accurate evaluation of *in vivo* ACT kinetics due to reduced bias from processes such as lymphodepletion[11]	Does not provide direct quantitation of the extent/density of CAR/TCR expression on the cell surface. CAR/TCR downregulation upon T cell activation may lower the mean fluorescence intensity without affecting the cell count

ACT, adoptive cell therapy; anti-ID, anti-idiotype; CAR, chimeric antigen receptor; LLOQ, lower limit of quantification; qPCR, quantitative polymerase chain reaction; TCR, T cell receptor.

body weight for patients ≤ 50 kg, or $0.1–6 \times 10^8$ cells for patients > 50 kg, or in adults, or as maximum dose. The noncompartmental analysis (NCA) results show a median $AUC_{0-28\ days}$ of ~ 460–1100 days×cells/μL blood or $0.2–3 \times 10^6$ days × copies/μg gDNA, a median C_{max} of 40–90 cells/μL blood or $24–260 \times 10^3$ copies/μg gDNA, and a median time to C_{max} (T_{max}) around 8–15 days. The terminal half-life is hard to define and determine due to the possibility of continuous activation/proliferation and sparse sampling during persistence phase; nonetheless, CAR T cells have been reported to persist for up to several years, and population PK modeling showed an estimated terminal half-life ranging from 173 to 564 days (**Table 3**).

The dose-exposure relationships are inconsistent across ACTs. As summarized in **Table 3**, KYMRIAH and BREYANZI showed no relationship, whereas TECARTUS and ABECMA suggested a trend of higher exposure with increasing dose. Repeated doses or retreatment of ACTs may have substantially attenuated cellular kinetics compared with the first dose, as shown for BREYANZI. For the dose-response relationship, both KYMRIAH and ABECMA showed positive trends between dose and response rate, and between dose and CRS probability; however, data for other ACTs are generally lacking. Overall, dose-response relationships have not been well-established across approved ACTs, suggesting that factors other than dose may be important to predicting safety and efficacy of ACTs. In addition, number of subjects for some dose levels were too limited to be sufficiently powered to detect dose-exposure/efficacy/safety relationships. For example, in study BB2121-CRB-40, ABECMA was dosed at 0.5×10^8 cells for only 3 subjects (**Table 3**). For KYMRIAH, despite of the wide range of dose tested ($0.2–5 \times 10^6$ cells/kg) for patients ≤ 50 kg, the median dose was 3.1×10^6 cells/kg,[7] suggesting that majority of subjects received a dose toward the higher end, and relatively fewer subjects were tested with low dose levels. Taken together, the study design, including the dose selection and subject enrollment at different dose levels, play important roles to understand the dose-exposure/efficacy/safety relationships. The statistical significance and clinical relevance of the current positive/negative/unclear findings in dose dependency need to be interpreted with caution due to the limited number of subjects, potential lack of power, and possible false positives/negatives.

In contrast, positive exposure-response relationships appear for all five ACTs for at least one indication. Median AUC and C_{max} were higher by ~ 100% (e.g., KYMRIAH, YESCARTA, and BREYANZI) to ~ 700% (e.g., TECARTUS) in responders compared with nonresponders. For safety, AUC and C_{max} were trending higher in subjects with grade 3+ cytokine release syndrome (CRS) compared with subjects with grade < 3 CRS for most CAR T cell therapies, especially in TECARTUS where a 300% higher exposure was observed. A similarly positive exposure-response relationship was also observed for neurological toxicity for three of the five CAR T cell therapies (YESCARTA, TECARTUS, and BREYANZI). However, exposure-response relationships alone may be insufficient to guide clinical development of optimal therapy because exposure cannot be controlled directly as dose-exposure relationships are not well-established.

Baseline characteristics traditionally evaluated as covariates in PK analyses have also been assessed for potential impact on cellular kinetics and clinical outcomes. As shown in **Table 3**, the younger age group (< 65 years old) receiving TECARTUS and BREYANZI showed numerically higher exposure than the older age group (> 65 years old), CAR T cells in patients with acute lymphoblastic leukemia (ALL) also showed younger patients had

Table 3 A Summary of all currently FDA-approved CAR T cells

Drug[a]	KYMRIAH[18,19,28] (tisagenlecleucel)	YESCARTA[16,20,21] (axicabtagene ciloleucel)	TECARTUS[22,24] (brexucabtagene autoleucel)	BREYANZI[17,23,26] (lisocabtagene maraleucel)	ABECMA[25,27] (idecabtagene vicleucel)
Initial US approval	2017	2017	2020	2021	2021
Target	CD19	CD19	CD19	CD19	BCMA
Indication	Adult 2L + R/R large B cell lymphoma Pediatric and young adult R/R B-cell ALL	Adult 2L+ R/R large B cell lymphoma Adult 2L+ R/R follicular lymphoma	Adult R/R mantle cell lymphoma	Adult 2L+ R/R large B cell lymphoma	Adult 4L+ R/R multiple myeloma
Design	Second generation Antigen-binding domain: anti-CD19 scFV Hinge region: CD8α Transmembrane domain: CD8α Co-stimulatory domain: CD137 (4-1BB) Activation domain: CD3ζ	Second generation Antigen-binding domain: anti-CD19 scFV Hinge region: CD28 Transmembrane domain: CD28 Co-stimulatory domains: CD28 Activation domain: CD3ζ	Second generation Antigen-binding domain: anti-CD19 scFV Hinge region: CD28 Transmembrane domain: CD28 Co-stimulatory domains: CD28 Activation domain: CD3ζ	Second generation Antigen-binding domain: anti-CD19 scFV Hinge region: IgG4 Transmembrane domain: CD28 Co-stimulatory domains: CD137 (4-1BB) Activation domain: CD3ζ Co-expression: truncated EGFRt	2nd generation Antigen-binding domain: anti-BCMA scFV Hinge region: CD8α Transmembrane domain: CD8α Co-stimulatory domains: CD137 (4-1BB) Activation domain: CD3ζ
Key study and dose range/level[b]	For both studies CCTL019B2202 (n = 61) and CCTL019B2205J (n = 29): 0.2 to 5.0 × 10^6 cells/kg for ≤ 50 kg 0.1 to 2.5 × 10^8 cells for > 50 kg	ZUMA-1 Phase I: 1.1–2 × 10^6 cells/kg (n = 7) Phase II (cohorts 1 and 2): 2 × 10^6 cells/kg (n = 101)	ZUMA-2 Cohort 1: 2 × 10^6 cells/kg (n = 60) Cohort 2: 0.5 × 10^6 cells/kg (n = 14)	TRANSCEND NHL 001 (017001) Dose level 1 (single): 0.5 × 10^8 cells (n = 40) Dose level 1 (double): 0.5 × 10^8 cells (n = 5) Dose level 2 (single): 1 × 10^8 cells (n = 166) Dose level 3 (single): 1.5 × 10^8 cells (n = 32)	Study BB2121-MM-001: 1.5 × 10^8 cells (n = 4) 3 × 10^8 cells (n = 70) 4.5 × 10^8 cells (n = 54) Study BB2121-CRB-401: 0.5 × 10^8 cells (n = 3) 1.5 × 10^8 cells (n = 18) 4.5 × 10^8 cells (n = 38) 8 × 10^8 cells (n = 3)
Approved dose	For lymphoma: 0.6 to 6 × 10^8 cells For ALL: 0.2 to 5.0 × 10^6 cells/kg for ≤ 50 kg 0.1 to 2.5 × 10^8 cells for > 50 kg	2 × 10^6 cells/kg (max: 2 × 10^8)	2 × 10^6 cells/kg (max: 2 × 10^8)	0.5 to 1.1 × 10^8 cells 1:1 ratio of CD4:CD8	3 to 4.6 × 10^8 cells
Immunogenicity[c]	Pretreatment: 86% to 91.4% positive Treatment induced: 5% positive	Pretreatment: 0% positive (cell-based assay) Treatment induced: 0% positive (cell-based assay)	Pretreatment: 0% positive (cell-based assay) Treatment induced: 0% positive (cell-based assay)	Pretreatment: 11% positive Treatment induced: 11% positive	Pretreatment: 3% positive Treatment induced: 47% positive
Cellular kinetics[d]					
$AUC_{0–28\ days}$[e]	See below for subgroup data	462.3 (5.1–14,329.3) days × cells/μL (median (min–max))	1,136.6 (1.8–27,700) days × cells/μL (median (min–max))	214,283 (77,282–689,752) days × copies/μg gDNA (median (Q1–Q3))	3,088,455 (190) days × copies/μg gDNA (geometric mean (CV%))

(Continued)

Table 3 (Continued)

Drug[a]	KYMRIAH[18,19,28] (tisagenlecleucel)	YESCARTA[16,20,21] (axicabtagene ciloleucel)	TECARTUS[22,24] (brexucabtagene autoleucel)	BREYANZI[17,23,26] (lisocabtagene maraleucel)	ABECMA[25,27] (idecabtagene vicleucel)
C_{max}[f]	See below for subgroup data	41.9 (0.8–1,513.7) cells/μL (median (min–max))	88.6 (0.16 – 2,589.5) cells/μL (median (min–max))	23,964 (8,159–78,748) copies/μg gDNA (median (Q1–Q3))	256,333 (165) copies/μg gDNA (geometric mean (CV%))
T_{max}	See below for subgroup data	8 days (median (min–max))	15 (8–31) days (median (min–max))	12 (10–15) days (median (Q1–Q3))	11 (7–28) days (median (min–max))
$t_{1/2}$[g]	PopPK model-derived terminal (β) phase half-life was 220 days	PopPK model-derived terminal (β) phase half-life was 173 days	N.R.	PopPK model-derived terminal (β) phase half-life was 564 days	N.R.
PK sampling time	Days 1, 4, 7, 11, 14, 21, 28 and then at months 3, 6, 9, 12, and then every 6 months until month 60	Days −5, 7, 14, and 28, and months 3, 6, 9, 12, 15, 18, and 24	Pre-dose, days 7, 14, 28, month 3, then every 3 months through month 24 and annually	Pre-dose, days 3, 7, 10, 14, 21, 28, 60, 90, 180, 270, 365, and 1.5 years and 3 years	Pre-dose, days 2, 4, 7, 9, 11, 14, 21, and months 1, 2, 3, 6, 12
Dose-kinetics relationship[h]	No clear relationship	N.R.	Comparing 0.5×10^6 cells/kg to 2×10^6 cells/kg, the $AUC_{0–28\ days}$ and C_{max} were ~ 40% lower; cell persistence also tends to be worse after 15 months	No clear relationship	The geometric mean of C_{max} and $AUC_{0–28\ days}$ showed a positive trend with dose
Demographics-kinetics relationship	Younger age group showed higher AUC and C_{max} Gender and race showed no clear relationship with AUC and C_{max}	Age and gender showed no significant association with AUC and C_{max}	Comparing age < 65 to age > 65, median AUC was 80% higher (1,640 vs. 877) and C_{max} was 90% higher (113 vs. 74), numerically Gender showed no clear association with AUC and C_{max}	Comparing age < 65 to age > 65, median AUC was 130% higher (312,636 vs. 135,843) and C_{max} was 206% higher (43,440 vs. 14,195) Gender and body weight showed no clear association with cellular kinetics	Gender, race, and age showed no significant relationship with kinetics Patients with lower body weight had higher expansion, but not considered clinically relevant
Baseline tumor burden-kinetics relationship	Comparing high to low tumor burden, AUC was 243% higher, and C_{max} was 144% higher	No clear relationship	No clear relationship	Comparing SPD > 50 cm^2 to SPD < 50 cm^2, median AUC was 186% higher (523,567 vs. 183,268) and C_{max} was 145% higher (53,399 vs. 21,760)	No clear relationship
Immunogenicity-kinetics relationship	No clear relationship	No clear relationship	No clear relationship	No clear relationship	No clear relationship
Dose/cellular kinetics-response relationship					
Dose-efficacy relationship[h]	Response probability showed an initial increasing trend as dose increases, plateaued around 2.0×10^6 cells per kg	N.R.	N.R.	No clear relationship	ORR showed a positive trend with cell dose

(Continued)

Table 3 (Continued)

Drug[a]	KYMRIAH[18,19,28] (tisagenlecleucel)	YESCARTA[16,20,21] (axicabtagene ciloleucel)	TECARTUS[22,24] (brexucabtagene autoleucel)	BREYANZI[17,23,26] (lisocabtagene maraleucel)	ABECMA[25,27] (idecabtagene vicleucel)
Dose-safety relationship[h]	Grade ≥ 3 CRS probability showed a modest increasing trend as dose increases	N.R.	N.R.	N.R.	All grade CRS incidence rate showed a positive trend with cell dose
Cellular kinetics-efficacy relationship	For ALL, comparing responders to nonresponders, geometric mean $AUC_{0-28\ days}$ was 104% higher (318,000 vs. 156,000 days × copies/μg gDNA), geometric mean C_{max} was 74% higher (34,700 vs. 20,000 copies/μg gDNA) For DLBCL, geometric mean $AUC_{0-28\ days}$ and C_{max} are comparable between responders and nonresponders	For LBCL: comparing responders to nonresponders, median $AUC_{0-28\ days}$ was 151% higher (557 vs. 222), median C_{max} was 106% higher (43.6 vs. 21.2) For FL: median $AUC_{0-28\ days}$ and C_{max} are comparable between responders and nonresponders	Comparing responders to nonresponders, median $AUC_{0-28\ days}$ was 775% higher (1,487 vs. 170), median C_{max} was 753% higher (102.4 vs. 12)	Comparing responders to nonresponders, median $AUC_{0-28\ days}$ was 76% higher (273,552 vs. 155,240), median C_{max} was 128% higher (35,335 vs. 15,527)	Comparing responders to nonresponders, median $AUC_{0-28\ days}$ was 582% higher (4,769,184 vs. 699,720), median C_{max} was 418% higher (375,224 vs. 72,397)
Cellular kinetics-CRS relationship	Higher CRS grade was associated with higher $AUC_{0-28\ days}$ and C_{max}	Comparing subjects with grade 3+ CRS to subjects with grade 1 and 2 CRS, median $AUC_{0-28\ days}$ (601.5 vs. 453.4) and median C_{max} (41.9 vs. 38.7) were not statistically significantly different	Comparing subjects with grade 3+ CRS to subjects with grade ≤ 2 CRS, median $AUC_{0-28\ days}$ was 297% higher (2,312 vs. 583), median C_{max} was 280% higher (203 vs. 53.4)	Comparing subjects with any grade CRS to subjects without CRS, median $AUC_{0-28\ days}$ was 141% higher (380,671 vs. 157,771), median C_{max} was 131% higher (43,756 vs. 18,937)	Comparing subjects with any grade CRS to subjects without CRS, median $AUC_{0-28\ days}$ was 431% higher, median C_{max} was 330% higher
Cellular kinetics-neurologic event relationship	No clear relationship	Comparing subjects with grade 3+ neurologic events to subjects with grade ≤ 2 neurologic events, median $AUC_{0-28\ days}$ was 157% higher (826.3 vs. 321.2), median C_{max} was 106% higher (63.5 vs. 30.9)	Comparing subjects with grade 3+ neurologic events to subjects with grade ≤ 2 neurologic events, median $AUC_{0-28\ days}$ was 370% higher (2,312 vs. 492), median C_{max} was 463% higher (250 vs. 44.4)	Comparing subjects with any grade neurologic events to subjects without neurologic events, median $AUC_{0-28\ days}$ was 288% higher (622,692 vs. 160,666)	No clear relationship

ACT, adoptive cell therapy; ALL, acute lymphoblastic leukemia; AUC, area under the plasma concentration-time curve; $AUC_{0-28\ days}$, AUC from zero to 28 days; CAR, chimeric antigen receptor; C_{max}, maximum plasma concentration; CRS, cytokine release syndrome; CV%, coefficient of variation; DLBCL, diffuse large B cell lymphoma; EGFRt, truncated epidermal growth factor receptor; FDA, US Food and Drug Administration; FL, follicular lymphoma; LBCL, large B-cell lymphoma; N.R., not reported (or information difficult to access/extract); ORR, overall response rate; PK, pharmacokinetics; R/R, relapsed/refractory; scFV, single chain fragment variable; SPD, sum of the products of diameters; $t_{1/2}$, terminal half-life; T_{max}, time to reach C_{max}.

[a]Information of all drugs in this table was extracted from US Prescribing Information, Clinical Pharmacology Biologics License Application (BLA) Review and Pharmacometrics Review documents, accessed in 2021. [b]The studies listed here include both supportive and pivotal studies included in BLA review. Interpretation of pooled results from multiple studies should require caution due to inter-study difference and variability. Numbers of subject (*n*) shown here are for subjects with available PK data, and are subject to certain clinical data cutoff dates. [c]Immunogenicity data were based on different assays including cell-based assay and non-cell-based competitive ligand binding assay, therefore interpretation and comparison across all five ACTs need additional considerations. [d]Cellular kinetic parameters shown in this section are based on approved dose. [e]Units for AUC are days × copies of transgene/μg of genomic DNA for KYMRIAH, BREYANZI, and ABECMA for all sections in this table; units for AUC are days × cells/μL of blood for YESCARTA and TECARTUS for all sections in this table. [f]Units for C_{max} are copies of transgene/μg of genomic DNA for KYMRIAH, BREYANZI, and ABECMA for all sections in this table; units for C_{max} are cells/μL of blood for YESCARTA and TECARTUS for all sections in this table. [g]The population pharmacokinetic (PopPK) model-derived half-life values should be interpreted with caution as the median follow-up time could be much shorter than the half-life value. [h]The statistical significance and clinical relevance of dose-kinetics, dose-efficacy, and dose-safety relationship needs to be interpreted with caution due to limited dose levels being tested, limited number of subjects at certain dose levels, and large interindividual variability in the cellular kinetics and clinical outcomes.

higher fold expansion compared to older patients,[29] whereas age seems not to affect exposure of YESCARTA and ABECMA. Further, higher baseline tumor burden was correlated with higher exposure in KYMRIAH and BREYANZI, but not in YESCARTA, TECARTUS, and ABECMA. Some patients had either pre-existing antibodies against the CAR or generated treatment-induced anti-CAR antibodies. However, pre-existing and treatment-induced immunogenicity did not impact cellular kinetics and clinical outcomes. Further, immunogenicity assays may need improvement to assess the binding to membrane-bound CAR or TCR on T cells in its natural environment.

Overall, there is a lack of consistent findings across five approved ACTs in dose dependency and baseline variable effect, suggesting that factors related to heterogeneity in the infusion product may be critical. Application of advanced quantitative techniques may elucidate key drivers for efficacy and safety and provide additional insights into dose-exposure and exposure-response relationships.

MODELING AND SIMULATION STRATEGIES OF ACT

To gain insight into the dose-exposure-response relationship in ACTs, a nonlinear mixed-effect (NLME) population modeling approach can be used to quantify the interindividual variability (IIV) and identify key covariates explaining the IIV. Although classical PK models for SM/PT capture absorption, distribution, and elimination continuously, the NLME models for ACT use empirical piecewise functions that separate the expansion phase from contraction/persistence phase using a critical timepoint (e.g., T_{max}) and have been applied to both CAR T cell[7,30] and TCR T cell therapies.[31] The piecewise NLME model has been shown to adequately describe multiphasic dispositions of T cells with population parameters and IIVs estimated with reasonably high precision. The modeling results were used to enhance understanding of dose-exposure-response relationships, and relate covariates to key parameters (e.g., expansion and contraction rate constant, and empirical Bayes estimates of C_{max}). For example, Stein *et al.*[7] explored the potential effect of sex, age, weight, race, previous stem cell transplant, lymphodepletion therapy, CRS treatment, transduction efficiency, cell viability, and dose of infused cells on KYMRIAH exposure in patients with ALL; nonetheless, no statistically significant covariate was identified and the dose-exposure and baseline factor-exposure relationships remain unclear. In addition, Liu *et al.*[30] conducted similar NLME modeling with much more comprehensive datasets of multiple ACTs in varying cancer types, their results suggest the dose of ACT, baseline tumor burden, and CD4:CD8 ratio do not significantly correlate with kinetic parameters or clinical outcome. Interestingly, the C_{max} in responders is much higher than the C_{max} in nonresponders with ALL, chronic lymphocytic leukemia (CLL), MM, and non-small cell lung cancer, which is in line with the general observation via the NCA approach for approved ACTs (**Table 3**). Further, solid tumor indications, specifically glioblastoma, appear to have lower C_{max} and proliferation rate constants than hematological tumors (ALL, CLL, and MM), which might be attributed to limited T-cell tissue penetration and tumor infiltration in glioblastoma.

From both NCA based (**Table 3**) and the NLME model (**Table 4**) based results, the dose-exposure relationship remains vague, and no baseline parameter has been identified to consistently explain variability in either cellular exposure or clinical outcome. This challenge is actually not surprising given the empirical nature of NCA and NLME approaches, and the complex nature of biology, immunology, and pathophysiology involved in the interplay between T cells and tumor. Three main limitations have been identified for the current NLME models: (1) T cells were treated as one homogeneous kind, measured as copies of transgene/μg gDNA, which lack the considerations for the heterogeneity of T cells with different phenotypes, subsets, and levels of exhaustiveness; (2) tumor component was not incorporated, however, the cellular kinetic phases, such as proliferation (antigen-based T cell activation), contraction (antigen disappearance/elimination), and persistence (continuous antigen production) phases, are all directly driven by the antigen amount; (3) other mechanistically important players, such as cytokines, were not considered, despite cytokine modulation of the immune system-tumor interaction through various feedback and feedforward pathways.

Indeed, the fitness and heterogeneous characteristics of both initial leukapheresis product and final infusion product are critically important. For leukapheresis products, the frequency of stem cell memory phenotype was higher in responding patients,[32] and a higher CD4+/CD8+ ratio may correlate with better expansion for some indications.[33] For infusion products, single cell analysis of CD19 CAR T cell product derived from patients with non-Hodgkin's lymphoma showed polyfunctional T cells deploying specific cytokine and chemokine profiles, which significantly were associated with clinical efficacy and safety outcomes.[34] Furthermore, in CD19 CAR T cells derived from patients with CLL, gene set scores based on early memory/memory and low glycolysis/exhaustion phenotypes were associated with improved complete/partial response. *Ex vivo* inhibition of CAR T cell glycolysis during manufacturing was associated with higher CAR T cell proliferative capacity upon restimulation with CD19 expressing tumor cells.[32] Taken together, the heterogeneity of T cells is likely to be responsible for the highly variable cellular kinetics and clinical outcomes. To capture this, quantitative systems pharmacology (QSP) modeling can be a valuable tool to understand the differential proliferation, phenotype conversion, apoptosis, and tumor killing processes for various species of T cells at the same time. The challenge of this approach is the requirement of enormous T cell data measured by flow cytometry for each kind/species, as well as longitudinal data of tumor dynamics to capture the antigen-dependent T cell kinetics. A recent model by Mueller-Schoell *et al.*[35] incorporated four phenotypes, including naïve, central memory, effector memory, and terminally differentiated effector T cells and enabled both T cell-mediated tumor killing as well as tumor-dependent T cell proliferation.[35] However, due to the sparse data from only 19 patients, several parameters, including tumor growth rate, homeostatic proliferation rate constants, and death rate constants, are unidentifiable, and only two parameters were allowed to consider IIV during fitting. Nonetheless, this provides a great foundation for future work considering both

Table 4 A list of modeling approaches to support clinical pharmacology strategies

Cell product	Indication	Model feature	Modeling and simulation approach	How does the model support clinical pharmacology?	What does the model show?
Anti-NY-ESO-1 TCR-T[31]	Myeloma and sarcoma	1) One T cell type 2) One compartment 3) Two phases: proliferation and decay 4) Piecewise function	1) Empirical NLME modeling	1) To characterize the cellular kinetics	1) The average decay (terminal phase) half-life of the anti-NY-ESO-1 TCR-T was estimated as 83.9 days (95% CI: 15–153 days)
Tisagenlecleucel (anti-CD19 CAR-T)[7]	B cell CLL	1) Two T cell types: effector and memory 2) One compartment 3) Three phases: expansion, contraction, persistence 4) Piecewise function	1) Empirical NLME modeling 2) Covariate analysis	1) To characterize the cellular kinetics 2) To quantify the IIV 3) To explore covariates affecting kinetics, especially the impact of CRS treatment (e.g., tocilizumab and corticosteroids) on expansion	1) The doubling time, initial decline half-life, and terminal half-life of tisagenlecleucel were estimated as 0.78, 4.3, and 220 days, respectively 2) CRS treatment (e.g., tocilizumab and corticosteroids) did not affect the expansion rate of tisagenlecleucel 3) No covariate was found to significantly impact the cellular kinetics of tisagenlecleucel
anti-CD19, anti-BCMA, and anti-EGFR CAR-T[47]	DLBCL, CLL, ALL, MM, NSCLC, GBM	1) Two T cell types: effector and memory 2) Two compartments 3) Four phases: distribution, expansion, contraction, persistence 4) Piecewise function	1) Empirical NLME modeling 2) Covariate analysis	1) To characterize the cellular kinetics 2) To investigate the difference in CAR-T cellular kinetics across varying indications 3) To explore covariates affecting the response of CAR-T therapies, such as cell number and CD4:CD8 ratio of infusion product	1) A higher cell proliferation rate and capacity, and a lower contraction rate was found in responders compared to nonresponders 2) Dose, baseline tumor burden, and CD4:CD8 ratio in the infusion product had no significant relationship with cellular kinetics
anti-CD19 CAR-T[37]	B cell CLL	1) Two T cell types: effector and memory 2) Two compartments 3) Key mechanisms: • T cell: distribution, proliferation, tumor-dependent conversion and reactivation, death • Cytokine: baseline production, release from activated T cells, elimination, and IL-10 inhibition • Tumor: replication, death, killing by activated T cells	1) Mechanistic QSP modeling 2) Simulation of various disease burden and cell dose scenarios	1) To characterize the kinetic profiles of both T cell and cytokines 2) To understand the impact of disease burden and CAR-T dose on cytokine release	1) A higher baseline tumor burden was associated with higher cytokine 2) CAR-T dose alone only had a minor effect on cytokine release

(Continued)

Table 4 (Continued)

Cell product	Indication	Model feature	Modeling and simulation approach	How does the model support clinical pharmacology?	What does the model show?
anti-CD19 CAR-T[35]	NHL	1) Four T cell type: naïve, central memory, effector memory, and effector T cells 2) One compartment 3) Key mechanisms: • T cell: homeostatic proliferation, tumor-dependent proliferation, conversion, and death • Tumor: logistic growth and T cell killing from all phenotypes • No cytokine	1) Mechanistic QSP modeling with IIV estimation of key parameters 2) Covariate analysis 3) Survival prediction	1) To characterize the cellular kinetics of different phenotypes and tumor dynamics 2) To understand the difference between low and normal proliferation groups and identify the reason 3) To propose a kinetics- and tumor-based clinical composite score to predict early survival	1) A low T cell maximum expansion capacity and no previous autologous stem cell transplantation were associated with poor prognosis 2) A clinical composite score of "C_{max} of naïve CAR-T cell/ baseline tumor burden" was proposed as a predictor for survival, with a cutoff value of 0.00136
anti-BCMA, anti-HER2, anti-CD19 and anti-EGFR CAR-T[38]	Various	1) One T cell type 2) Multiple compartments in the final PBPK/PD model 3) Key mechanisms: • T cell: circulation, transmigration, target engagement, expansion, and hepatic elimination • Cytokine: released by CAR-target complex • Tumor: linear/exponential growth with 4-step signal transduction of killing	1) Multiscale mechanistic PK/PD modeling with IIV estimation of key parameters: • cell level PD model • PBPK model in mouse • PBPK/PD model in mouse 2) Simulation of cellular kinetic profiles and tumor growth inhibition under various tumor burden and cell dose scenarios	1) To enhance the understanding of key determinants of PK and PD of CAR-T 2) To facilitate the design of lead CAR-T candidates 3) To enable preclinical-to-clinical translation for FIH	1) The relationships between key parameters (e.g., CAR affinity (k_{on} and k_{off}), CAR density, and antigen density) and extent of T cell expansion and overall tumor volume were better appreciated 2) CAR-T cells may have a steep dose-exposure relationship 3) C_{max} was more sensitive to tumor burden than T cell dose
anti-BCMA CAR-T[39]	MM	1) Two T cell types 2) Two compartments 3) Key mechanisms: • T cell: distribution, target engagement, expansion, conversion, and elimination • Tumor: tumor growth and death, biomarker (M-protein, sBCMA) production, and degradation	1) Multiscale mechanistic PK/PD modeling with IIV estimation of key parameters: • Cell level PD model • PK/PD model in mouse and human 2) Simulation of biomarker dynamics and clinical outcomes under different dose scenarios	1) To enhance the understanding of key determinants of PK and PD of CAR-T 2) To enable preclinical-to-clinical translation 3) To assess the impact of dose and tumor burden on cellular kinetics, biomarker dynamics, and clinical response	1) The preclinical and translational PKPD relationship effectively described the kinetic profile of CAR-T cell, soluble BCMA, and serum M-protein in human 2) The clinical PKPD model sufficiently described the categorical response data and PFS rate for patients 3) CAR-T cells may have a steep dose-exposure-response relationship, and C_{max} was more sensitive to tumor burden than dose

(Continued)

Table 4 (Continued)

Cell product	Indication	Model feature	Modeling and simulation approach	How does the model support clinical pharmacology?	What does the model show?
Cr-51 labeled T cells[40]	Melanoma	1) One T cell type 2) Multiple compartments 3) Key mechanisms: • T cell: circulation, transmigration, retention, elimination	1) PBPK modeling in mouse 2) Parameters for transmigration and retention in different tissues/organs were estimated	1) To characterize the cellular kinetics physiologically in mouse 2) To quantify T cell distribution to various tissues and organs 3) To understand T cell infiltration into tumor	1) The model optimized transmigration rate and retention factors of different tissues/organs with good confidence 2) The biodistribution coefficient (tissue/blood AUC ratio) is high in the spleen, liver, lungs, kidneys, and bones
anti-ErbB2 CAR-T[48]	Breast cancer	1) One T cell type 2) One compartment 3) Key mechanisms: • T cell: tumor-dependent proliferation and death • Tumor: growth and T cell killing	1) Predator-prey variant model 2) Simulation of tumor growth inhibition under different dosing scenarios	1) To predict response of CAR-T therapy in preclinical model 2) To determine the minimal effective dose	1) The model recapitulated *in vivo* tumor growth results in previous experiments, and predicted results of new therapy prospectively in murine model 2) The model estimated that using 1–10% of the full CAR-T dose in preclinical studies can achieve comparable efficacious results to full dose

ALL, acute lymphocytic leukemia; CAR-T, chimeric antigen receptor T cell; CI, confidence interval; CLL, chronic lymphocytic leukemia; C_{max}, peak plasma concentration; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; FIH, first-in-human; GBM, glioblastoma multiforme; IIV, interindividual variability; MM, multiple myeloma; NHL, Non-Hodgkin lymphoma; NLME, nonlinear mixed effects models; NSCLC, non-small cell lung carcinoma; PBPK, physiologically-based pharmacokinetics; PD, pharmacodynamics; PFS, progression-free survival; PK, pharmacokinetics; QSP, quantitative system pharmacology; TCR-T, T cell receptor T cell.

heterogeneity of ACTs with differential properties of varying T cell species and the T cell-tumor interaction.

Besides T cell heterogeneity and T cell-tumor interaction, it is equivalently important to understand the cytokines and other immunomodulators as some of them are key drivers for CRS and neurotoxicity. To identify key predictors associated with CRS, statistical models, including the regression model and the decision-tree model, were developed by evaluating 43 cytokines from 51 patients with ALL after receiving anti-CD19 CAR T cell therapy.[36] Using IFNγ, sgp130, and IL1RA data, the regression model successfully predicts patients that develop grades 4–5 CRS with 86% sensitivity and 89% specificity. Owing to the key roles of cytokines, QSP modeling[37] could also be developed to incorporate cytokine release of IFNγ, IL6, and IL10, and the modeling results suggest that cytokine elevation may be related to baseline tumor burden more than the dose of infused cells. Collectively, the predictive statistical model and cytokine-integrated QSP model can be valuable for developing risk mitigation strategies and managing severe toxicities. Other models, such as mechanistic PK/pharmacodynamic (PD) models have also been implemented where data from *in vitro* assays and *in vivo* xenograft studies were used to understand how receptor density, receptor affinity, and antigen density affect T-cell kinetics and tumor depletion.[38,39] This method may facilitate product design in the discovery phase and preclinical to clinical translation in the early development phase to guide dosing strategy. Finally, physiologically-based PK modeling has been applied to understand *in vivo* T-cell distribution into animal tissues to study tissue penetration, which could be valuable to solid tumor indications.[40]

In summary, piecewise empirical NLME models can be used to characterize T cell kinetics and to identify key covariates affecting the exposure. Due to the heterogeneity of infused T cells and the complicated immune-tumor interaction, QSP modeling may have special advantages to deconvolute the confounding results produced by multiple T cell species. Further, connecting cytokines with T cells and tumors may enable better prediction of cytokine release, and statistical models can be leveraged to predict severe CRS probability to mitigate toxicity risk. Finally, mechanistic PK/PD models and physiologically-based PK models may also provide insights into *in vitro*-to-*in vivo* translation and solid tumor indications. **Table 4** provides a summary of ACT models that incorporated T cell kinetics and conducted fitting or validation using observed data. More mechanistic and systemic models are needed that characterize T cell tumor distribution and heterogeneity in conjunction with tumor dynamics, tumor microenvironment, as well as other key players, such as endogenous T cells, macrophage, and cytokine signaling (both activating and inhibitory). Two other reviews have also provided valuable insights into T cell modeling.[41,42]

NOVEL CP CONSIDERATIONS AND FUTURE DIRECTIONS

ACT is a complex and novel modality that has shown tremendous promise for treating cancer, with five FDA-approved CAR T cell therapies so far. Along with the technology and regulatory guidance, the CP aspect of this new treatment modality is still evolving. As the core purpose of CP is to determine the best dose/product (i.e., what) for the most appropriate patient (i.e., who) with the optimal conditioning/administration regimen (i.e., how), it is critical to understand the complex interaction between T cells and host biology/disease to identify key factors that we can control and relate to safety and efficacy. Several control points include product characteristics (i.e., dose, fitness, phenotype, and subset of both apheresis product and infusion product), patient characteristics (i.e., demographic information, tumor type, tumor burden, and tumor microenvironment[2]), and regimen characteristics (timing and type of conditioning therapy, comedications, single vs. multiple infusion, and intravenous vs. regional injection). The large number of ACT control points demonstrate the uniqueness of this therapeutic modality compared with SM/PT, necessitating close collaboration of CP with biomarker, bioanalytical, chemistry, manufacturing, and control, and clinical science and safety teams. As our understanding of these control points improves and/or more homogenous cellular products are developed, clinical pharmacologists will have opportunities to better guide dose selection (first-in-human), escalation/expansion (phase I), and determination (pivotal) to optimize ACT development.

The current indications for approved ACTs are limited to hematological malignancies, whereas application to solid tumor remains challenging. For solid tumors, circulating T cells may not reflect T cell expansion and persistence at the site of action. Indeed, limited clinical data show rapid disappearance of administered radiolabeled tumor-infiltrating lymphocytes from blood within 72 hours (< 1% of total infused radioactivity), but sustained levels in the lungs, liver, and spleen for more than 13 days.[43] Local delivery of engineered T cells to the intended site of action may help overcome distributional limitations. Importantly, the presence of T cells in tumor tissue may not be sufficient to drive response if the T cells are excluded or spatially distributed such that they are not able to access all tumor cells in the tissue.[44] So far, research on biodistribution of engineered T cell therapy is limited in humans as a result of radioactivity/cell number ratio changes over time, leakage of radiotracers from cells, and toxicity.[45] Developing safe and controllable tracers and improving technology for tracer tracking are important to elucidating the relationship between tissue exposure and response.

To ensure optimal clinical development, clinical pharmacologists can contribute by applying quantitative methodologies, including: (1) translational PK/PD modeling to optimize product, patient, and regimen characteristics before the first-in-human study; (2) real-time statistical/machine learning approaches to forecast severe toxicity probability and mitigate risk during the clinical study; and (3) NLME and QSP modeling to gain deep insight and design future studies more appropriately with optimal selection of dose, patient, and regimen. Given the complexity and cost of delivering these therapies, initial studies may generate rich, robust, and multilevel datasets of T cell, biomarker, safety, and efficacy data, but in a relatively small number of patients. Therefore, integration of data across internal ACTs and externally published ACTs will be critical to drawing conclusions and informing decision making. As the field evolves and additional key drivers of safety and efficacy are elucidated, banking and preserving tissues may prove valuable as samples can be re-assayed for new biomarkers or other characteristics. These

quantitative methodologies and infrastructure are anticipated to transfer well to the next generation of cell therapies, including TCR-T, NK-T, and off the shelf/allogeneic cell systems and individualized therapies targeting tumor-specific private neo-antigens.

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1. Milone, M.C. & Bhoj, V.G. The pharmacology of T cell therapies. *Mol. Ther. Meth. Clin. Dev.* **8**, 210–221 (2018).
2. Norelli, M., Casucci, M., Bonini, C. & Bondanza, A. Clinical pharmacology of CAR-T cells: Linking cellular pharmacodynamics to pharmacokinetics and antitumor effects. *Biochim. Biophys. Acta* **1865**, 90–100 (2016).
3. 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. *Cells* **9**, 1720 (2020).
4. Zhang, J. & Wang, L. The emerging world of TCR-T cell trials against cancer: a systematic review. *Technol. Cancer Res. Treat.* **18**, 1533033819831068 (2019).
5. Newick, K., O'Brien, S., Moon, E. & Albelda, S.M. CAR T cell therapy for solid tumors. *Annu. Rev. Med.* **68**, 139–152 (2017).
6. 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).
7. 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).
8. Shi, J. *et al*. Bioanalysis in the age of new drug modalities. *AAPS J.* **23**, 64 (2021).
9. 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).
10. Ellis, J. Silencing and variegation of gammaretrovirus and lentivirus vectors. *Hum. Gene Ther.* **16**, 1241–1246 (2005).
11. Yamamoto, S. *et al*. Quantitative PCR methodology with a volume-based unit for the sophisticated cellular kinetic evaluation of chimeric antigen receptor T cells. *Sci. Rep.* **10**, 17884 (2020).
12. Gorovits, B. & Koren, E. Immunogenicity of chimeric antigen receptor T-cell therapeutics. *BioDrugs* **33**, 275–284 (2019).
13. Lee, J.I., Zhang, L., Men, A.Y., Kenna, L.A. & Huang, S.M. CYP-mediated therapeutic protein-drug interactions: clinical findings, proposed mechanisms and regulatory implications. *Clin. Pharmacokinet.* **49**, 295–310 (2010).
14. Liptrott, N.J. *et al*. The impact of cytokines on the expression of drug transporters, cytochrome P450 enzymes and chemokine receptors in human PBMC. *Br. J. Pharmacol.* **156**, 497–508 (2009).
15. Sordillo, P.P., Sordillo, D.C. & Helson, L. Review: the prolonged QT interval: role of pro-inflammatory cytokines, reactive oxygen species and the ceramide and sphingosine-1 phosphate pathways. *In Vivo* **29**, 619–636 (2015).
16. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Pharmacometric Review, Application Number 125643, YESCARTA (axicabtagene ciloleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta-axicabtagene-ciloleucel>. Accessed October 26, 2021.
17. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Clinical Pharmacology/Pharmacometrics Review, Application Number 125714, BREYANZI (lisocabtagene maraleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/breyanzi-lisocabtagene-maraleucel>. Accessed October 26, 2021.
18. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Prescribing Information, Application Number 125646, KYMRIAH (tisagenlecleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah-tisagenlecleucel>. Accessed April 22, 2021.
19. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Clinical Pharmacology BLA Review, Application Number 125646, KYMRIAH (tisagenlecleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah-tisagenlecleucel>. Accessed July 25, 2021.
20. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Prescribing Information, Application Number 125643, YESCARTA (axicabtagene ciloleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta-axicabtagene-ciloleucel>. Accessed April 22, 2021.
21. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Clinical Pharmacology BLA Review, Application Number 125643, YESCARTA (axicabtagene ciloleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta-axicabtagene-ciloleucel>. Accessed July 25, 2021.
22. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Clinical Pharmacology BLA Review, Application Number 125703, TECARTUS (brexucabtagene autoleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus-brexucabtagene-autoleucel>. Accessed July 25, 2021.
23. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Clinical Pharmacology BLA Review, Application Number 125714, BREYANZI (lisocabtagene maraleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/breyanzi-lisocabtagene-maraleucel>. Accessed July 25, 2021.
24. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Prescribing Information, Application Number 125703, TECARTUS (brexucabtagene autoleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus-brexucabtagene-autoleucel>. Accessed July 25, 2021.
25. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Clinical Pharmacology BLA Review, Application Number 125736, ABECMA (idecabtagene vicleucel) <https://www.fda.gov/vaccines-blood-biologics/abecma-idecabtagene-vicleucel>. Accessed July 25, 2021.
26. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Prescribing Information, Application Number 125714, BREYANZI (lisocabtagene maraleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/breyanzi-lisocabtagene-maraleucel>. Accessed July 25, 2021.
27. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Prescribing Information, Application Number 125736, ABECMA (idecabtagene vicleucel) <https://www.fda.gov/vaccines-blood-biologics/abecma-idecabtagene-vicleucel>. Accessed July 25, 2021.

28. Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration, Final Report-Pharmacometrics Analysis of Tisagenlecleucel-T, Application Number 125646, KYMRIAH (tisagenlecleucel) <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah-tisagenlecleucel>. Accessed October 26, 2021.
29. Itzhaki, O. *et al*. Head-to-head comparison of in-house produced CD19 CAR-T cell in ALL and NHL patients. *J. Immunother. Cancer* **8**, e000148 (2020).
30. Liu, C. *et al*. Model-based cellular kinetic analysis of chimeric antigen receptor-T cells in humans. *Clin. Pharmacol. Ther.* **109**, 716–727 (2021).
31. Stadtmauer, E.A. *et al*. CRISPR-engineered T cells in patients with refractory cancer. *Science* **367**, EABA7365 (2020).
32. 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).
33. Cohen, A.D. *et al*. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. *J. Clin. Invest.* **129**, 2210–2221 (2019).
34. Rossi, J. *et al*. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. *Blood* **132**, 804–814 (2018).
35. 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).
36. Teachey, D.T. *et al*. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Cancer Discov.* **6**, 664–679 (2016).
37. Hardiansyah, D. & Ng, C.M. Quantitative systems pharmacology model of chimeric antigen receptor T-cell therapy. *Clin. Transl. Sci.* **12**, 343–349 (2019).
38. 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).
39. 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).
40. 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).
41. 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).
42. 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).
43. Fisher, B. *et al*. Tumor localization of adoptively transferred indium-111 labeled tumor infiltrating lymphocytes in patients with metastatic melanoma. *J Clin. Oncol.* **7**, 250–261 (1989).
44. Carstens, J.L. *et al*. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. *Nat. Commun.* **8**, 15095 (2017).
45. Krekorian, M. *et al*. Imaging of T-cells and their responses during anti-cancer immunotherapy. *Theranostics* **9**, 7924–7947 (2019).
46. Mika, T. *et al*. Digital-droplet PCR for quantification of CD19-directed CAR T-cells. *Front. Mol. Biosci.* **7**, 84 (2020).
47. Demaret J. *et al*. Monitoring CAR T-cells using flow cytometry. *Cytometry B Clin. Cytom*. **100**, 218–224 (2021). http://dx.doi.org/10.1002/cyto.b.21941
48. Globerson Levin, A., Kronik, N., Shiloach, T., Waks, T., Eshhar, Z. & Vainstein, V. Less is more: reducing the number of administered chimeric antigen receptor T cells in a mouse model using a mathematically guided approach. *Cancer Immunol. Immunother.* **69**, 1165–1175 (2020).