Computational modelling of CAR T-cell therapy: from cellular kinetics to patient-level predictions

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Summary

Chimeric Antigen Receptor (CAR) T-cell therapy is characterised by the heterogeneous cellular kinetic profile seen across patients. Unlike traditional chemotherapy, which displays predictable dose-exposure relationships resulting from well-understood pharmacokinetic processes, CAR T-cell dynamics rely on complex biologic factors that condition treatment response. Computational approaches hold potential to explore the intricate cellular dynamics arising from CAR T therapy, yet their ability to improve cancer treatment remains elusive. Here we present a comprehensive framework through which to understand, construct, and classify CAR T-cell kinetics models. Current approaches often rely on adapted empirical pharmacokinetic methods that overlook dynamics emerging from cellular interactions, or intricate theoretical multi-population models with limited clinical applicability. Our review shows that the utility of a model does not depend on the complexity of its design but on the strategic selection of its biological constituents, implementation of suitable mathematical tools, and the availability of biological measures from which to fit the model.

Keywords: CAR T therapy; Cancer models; Computational biology; Pharmacokinetics; Cancer immunotherapy; Haematological cancer

Introduction

Over the past decade, immunotherapy has risen as one of the most promising strategies for cancer treatment. Adoptive cell transfer (ACT) therapies, using genetically modified T-cells expressing chimeric antigen receptors (CARs), have established a new paradigm in the management of lymphoproliferative neoplasms.[1] Particularly, CAR T-cells targeting CD19 and BCMA antigens have demonstrated long-lasting remission rates in the treatment of haematological malignancies,[2–4] leading to the approval of various CAR T-cell products.[5] Despite initial success of some anti-CD19 products, a substantial fraction of patients initially experiencing remission still encounter relapse within a year due to factors such as suboptimal distribution, proliferation and persistence of CAR T-cells[6]; checkpoint-induced immunologic exhaustion[7,8]; antigen loss; different levels of antigen and CAR expression in neoplastic and CAR T-cells[9]; and clonal diversity among patients treated with autologous CAR T products.[10,11]

As a live drug, CAR T-cell kinetics are governed by complex cellular dynamics that emerge from their interaction with a myriad of biological actors across the organism. Unlike traditional cytotoxic chemotherapy, which follows well-characterised dose-exposure relationships based on established pharmacokinetic (PK) principles of absorption, distribution, metabolism, and elimination of chemical molecules, CAR T therapy constitutes a new frontier for the field of pharmacology. Highly variable CAR T-cell kinetics, irrespective of dosing regimen, challenges the establishment of clear therapeutic guidelines to maximise response.[12,13] Such an intricate therapeutic landscape highlights the need for novel pharmacologic research to improve our understanding of CAR T-cell dynamics, which may eventually lead to predictable PK therapeutic windows.[12] From this perspective, understanding how baseline patient and product characteristics shape PK profiles and treatment response requires integrating knowledge from computational immunology, oncology, pharmacology, and population dynamics.

Since the approval of the first CAR T product,[14] numerous mathematical models have been published

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addressing haematological malignancies, such as leukaemia and lymphoma, as well as solid tumours.[15] Following the emergence of these models, several research groups have published reviews covering various aspects of the field: i) Chaudhury et al. introduced a modelling taxonomy for a set of relatively simple cellular kinetic models that describe *in vivo* dynamics of CAR T-cells and their interactions with cancer cells[16]; ii) Nukala et al. conducted a systematic review of modelling approaches addressing several aspects of CAR T-cell therapy including T-cell activation, T- and malignant cell population dynamics, therapeutic cost-effectiveness strategies, and patient survival[15]; iii) Qi et al. provided a focused review of the cellular kinetic phases of CAR T therapy (distribution, expansion, contraction, and persistence), examining the mechanisms underlying their complexity, the immunologic factors influencing each phase, and the mathematical modelling approaches used to characterise them[6]; iv) Mody et al. presented the perspective of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) on best practices and key considerations for clinical pharmacology and pharmacometric approaches to optimise CAR T and TCR T-cell therapy development[17]; and v) Kirouac, Zmurchok, & Morris utilised publicly available CAR T clinical data to illustrate how mathematical models can quantify the relationships between product characteristics, patient physiology, pharmacokinetics, and clinical outcomes, with the goal of contextualising data and translating product designs into effective clinical strategies.[18]

In this review, we examine computational models relevant to CAR T therapy across the fields of computational oncology, pharmacology, and immunology, while offering a novel perspective to classify and unify current and future modelling approaches. With this revision, we provide a comprehensive framework through which to understand CAR T mathematical models, focussing on three pivotal elements: i) the biological actors represented in the model (Biological constituents of CAR T-cell kinetics models); ii) the mathematical and computational tools employed to capture these biological constituents (Computational design of CAR T-cell kinetics models); and iii) the application given to these models based on the information available from reality (Application of CAR T-cell kinetics models). Beyond serving as a resource that consolidates current CAR T mathematical modelling knowledge in the early stages of the discipline, this paper seeks to serve as a platform to foster comprehension and interaction among biomedical researchers, clinicians, and modellers. Such a collaborative effort is essential to facilitate the challenging task of bringing modelling closer to the preclinical and clinical settings.

Biological constituents of CAR T-cell kinetics models

Models are simplified representations of reality and as such, their complexity depends on the extent to which they capture the intricacies of the systems that they represent. Tumour-immune dynamics involve interactions across multiple levels of biological organisation; however, most of the current CAR T mathematical modelling approaches focus on a single biological scale due to the inherent complexity of integrative multi-scale modelling (in design, clinical measurements, parameter fitting, and computational intensiveness).[19] Here, we introduce a novel classification system that categorises CAR T-cell kinetics modelling approaches based on the biological elements that they capture across different scales of biological organisation (subcellular, cellular, tissue, and organ). From this perspective, we characterise models based on: i) the number of cellular populations that they include (Number of cellular populations (cells)); ii) the tumour/CAR T-cell interaction habitats being studied (Cellular interaction habitats (tissues)); iii) the distribution of CAR T-cells across different body compartments (CAR T distribution (organs)); and iv) the inclusion of different subcellular concomitant factors that might influence CAR T therapy (Subcellular concomitant factors (molecules)). These modelling categories can be sorted by level of biological organisation, ranging from the sub-cellular scale to the organ level (Fig. 1). In addition to these four groups, modelling efforts can also include an additional organism level, when accounting for patient-specific characteristics (such as demographic, biometric, and genetic characteristics). Here, we delve into each of these modelling categories, highlighting the most paradigmatic examples from the current CAR T modelling literature. By reviewing these factors, our classification system serves as a tool to assess the complexity of CAR T-cell kinetics models through the lens of biological organisation.

Number of cellular populations (cells)

Based on the number of cell populations included, CAR T-cell kinetics models can be classified into those that focus exclusively on CAR T-cells (Pharmacokinetic modelling) and those that integrate multiple cellular populations to capture some of the immune dynamics following therapy (Pharmacokinetic/pharmacodynamic modelling (PK/PD)).

Pharmacokinetic modelling

PKs, often described as the effect of the body on a drug, involves the study of absorption, distribution, metabolization, and excretion of a pharmacologic substance in the organism. Since CAR T products are made from living cell populations rather than chemical compounds, CAR T therapy does not adhere to the conventional distribution, disposition and elimination pathways seen in traditional therapeutic molecules. As a result,

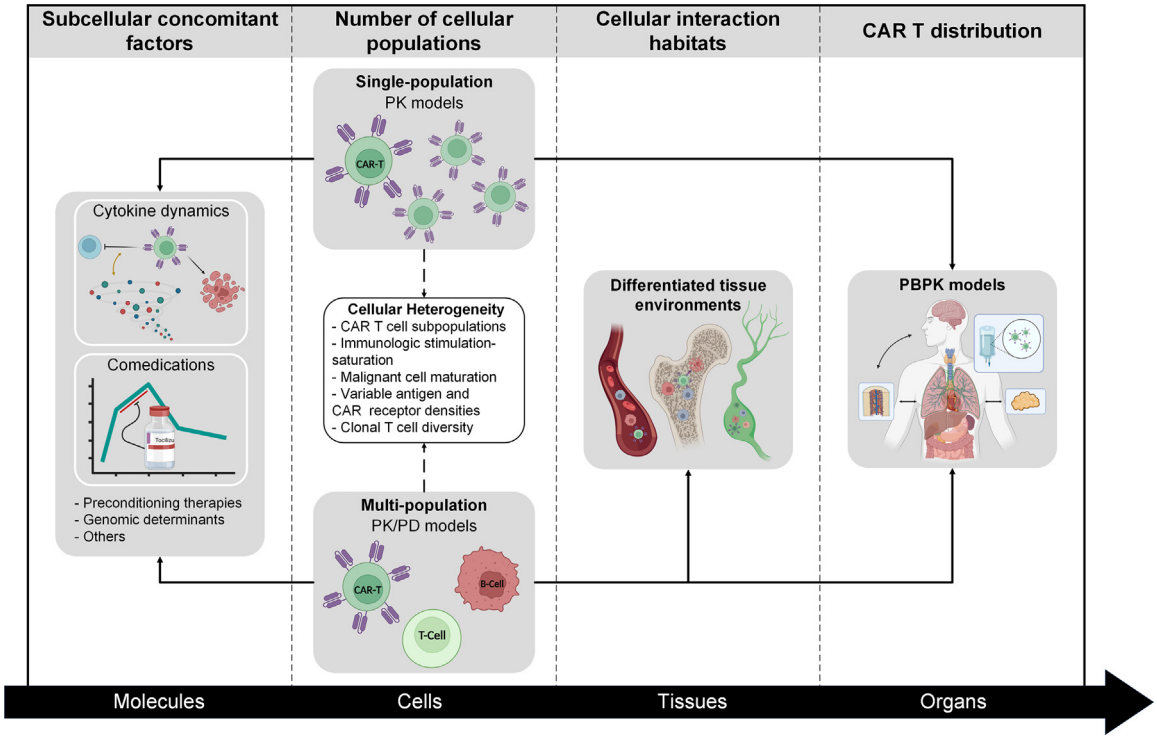

***Fig. 1:* Classification framework for CAR T-cell kinetics models based on biological scale.** This hierarchy highlights the essential components of CAR T-cell kinetics models at various biological levels. (**Subcellular concomitant factors**) At the smallest biological scale, CAR T-cell kinetics models can incorporate factors such as cytokine dynamics, concomitant medications, pre-treatment lymphodepletion strategies, and other subcellular determinants including genomic, proteomic, and molecular factors. (**Number of cellular populations**) At the cellular scale, single-population models represent CAR T-cell kinetics using traditional pharmacokinetic (PK) approaches, while multi-population models incorporate malignant cells and other relevant populations by implementing population dynamics approaches. Both approaches can further differentiate cell populations into phenotypic sub-groups to capture different cellular behaviours (Cellular heterogeneity box). (**Cellular interaction habitats**) While most multi-population models focus on cell interactions within the haematological environment, interactions in diverse tissue environments (such as bone marrow and lymphatic tissues) inevitably shape CAR T-cell dynamics. Consequently, multi-population interactions may be modelled differently depending on the specific tissue context in which they occur. (**CAR T distribution**) Both single and multi-population models can account for CAR T-cell distribution throughout the body using physiologically based PK (PBPK) models. These models facilitate the study of CAR T-cell delivery rates in tumour-containing anatomical compartments and off-tumour sites.

fundamental PK concepts, such as the relationship between clearance and the average drug concentration, do not apply in the same way.

Models that focus solely on CAR T-cell kinetics are often based on traditional PK principles. In 2017, Mueller et al. implemented non-compartmental analysis (NCA) methods to characterise the *in vivo* kinetics of the CAR T product Tisagenlecleucel (tisa-cel) in patients with relapsed/refractory B-cell acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL).[20] NCA methods enable the estimation of PK parameters from product concentration-time profile measurements, without requiring the assumption of any specific compartmental model.[21] As such, NCA offer a quick method to evaluate drug exposure characteristics directly from empirical measurements, such as maximum concentration (C_{max}) and area under the curve (AUC), using methods like the trapezoidal rule to calculate the AUC, without the need to fit or select any model parameters.

Following these approaches, in 2018, Mueller et al. introduced the first CAR T compartmental analysis (CA) approach to describe the clinical pharmacology of tisa-cel in the same group of patients[22] (Supplementary Equation S9). In this study, quantitative polymerase chain reaction (qPCR) results of CAR presence in peripheral blood were modeled using a composite approach. This included an initial exponential growth phase until CAR T C_{max}, followed by a traditional two-compartmental PK model to describe the biphasic decline of CAR T-cells after C_{max} (Fig. 2a, bottom panel). In traditional two-compartment PK models, the biphasic reduction in product concentration is a consequence of a two-stage process: drug distribution from the central compartment to peripheral tissues, followed by drug elimination. In contrast, Mueller's biexponential

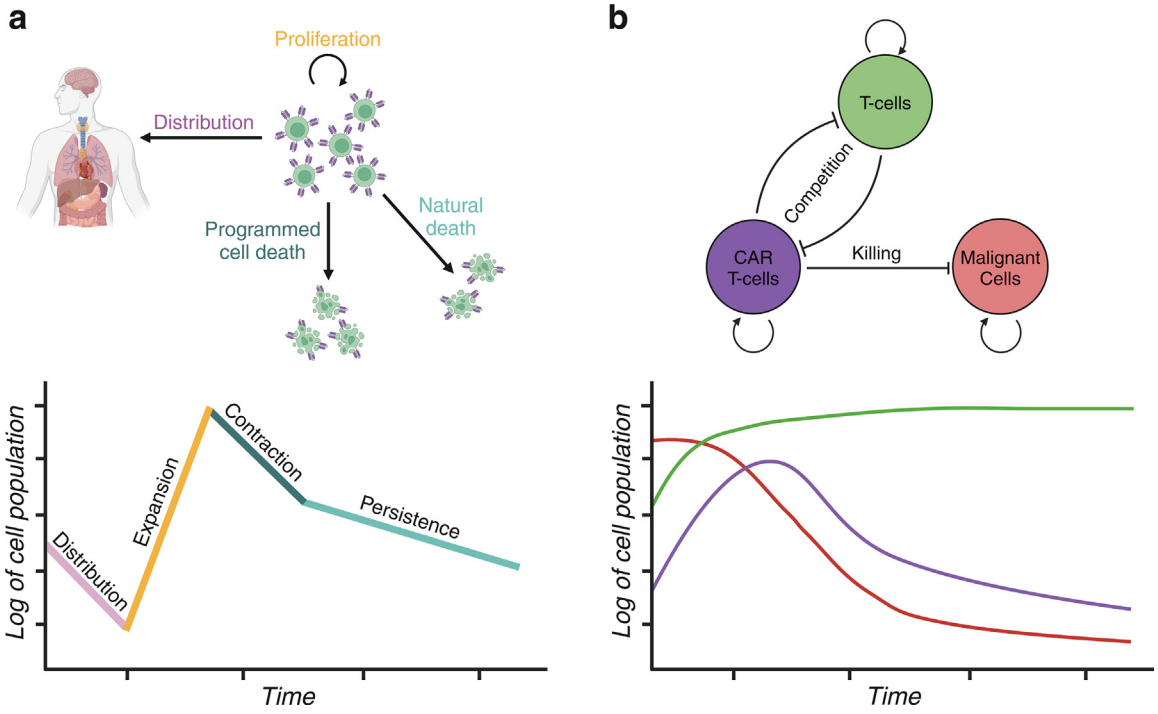

***Fig. 2:* Single- and multi-population CAR T-cell kinetics models.** (a) Single-population frameworks draw inspiration from traditional pharmacokinetic (PK) models, typically using ordinary differential equations. These equations characterise CAR T-cell dynamics through parameters governing distribution, proliferation, and death rates (including programmed cell death and natural death of persistent cells) as first-order processes (adapted from[6]). (b) Multi-population models, correspond to the pharmacokinetic/pharmacodynamic (PK/PD) counterpart, which inspire from mathematical oncology, immunology, and biology to capture the interactions arising among different cell populations. In this three-population model diagram, CAR T-cells compete with endogenous T-cells and exert cytotoxic effects on malignant cells. Feedback arrows represent exponential growth following simple mitotic behaviour (adapted from[23]).

approach attempts to replicate the observed dynamics in CAR T therapy, where programmed cell death following immune engagement with tumour cells and exhaustion drive the initial contraction phase, and the persistence of memory CAR T-cell characterises the slower death rate observed after the initial immune response (Fig. 2a, top panel).

While these approaches inspire from traditional PK model formulations, a range of methods and equations have been specifically adapted for this context.[6] Stein et al. were the first to extend previous immunologic modelling knowledge on T-cell immune response[24–28] by adapting De Boer's model of murine immune response to *Listeria monocytogenes* bacteria and *Lymphocytic choriomeningitis* virus[25] to the context of CAR T therapy.[29] This approach was implemented to characterise the impact of tocilizumab and corticosteroids to the *in vivo* expansion of tisa-cel for patients with ALL. As in Mueller's approach, effector cells initially abide to exponential growth until maximum concentration, followed by a biphasic decline. However, instead of using a traditional two-compartmental PK model, a two-cell population model is used to characterise rapid death of effector CAR T-cells following immunologic engagement, and progressive replacement by the memory compartment (Supplementary Equation S10).

Pharmacokinetic/pharmacodynamic modelling (PK/PD)

Pharmacodynamics (PDs), often described as the effect that the drug has on the body, is the study of the molecular, biochemical, and physiologic effects of a pharmacologic substance on the organism. As live drugs, CAR T therapies do not follow the conventional biochemical mechanisms of action observed in traditional therapeutic molecules. Instead, the pharmacologic response is linked to the complex cellular dynamics arising from interactions among CAR T-cells, malignant and healthy cells expressing the target antigen, the immune system, and other concomitant treatments. This means that fundamental PD concepts, such as the relationship between product concentration in the periphery and site of action, do not necessarily correlate with the treatment effect on disease burden, clinical response, and toxicities. PDs play a pivotal role in the quantitative evaluation of both treatment effectiveness and safety. All current multi-population CAR T-cell

kinetics models include a PD component related to the cytotoxic effect of CAR T-cells, which can be analysed separately. However, since these models account for both product kinetics and target-cell killing, this section jointly examines PKs and PDs.

Connecting PKs and PDs requires relating drug dynamics to the product's effects on the organism. Thus, CAR T PK/PD modelling involves studying both circulation and function of the therapeutic product in the patient organism. Since CAR T-cell dynamics are directly linked to their engagement with target antigens, they do not follow the conventional dose-effect relationships seen in traditional cytotoxic chemotherapy, where drug concentration is independent of tumour burden and immune dynamics. Instead, CAR T-cell levels are directly influenced by the concentration and interactions with target cells. This intricate connection between disease and CAR T kinetics means that a fundamental aspect of understanding the complex cellular dynamics arising from treatment lies in unravelling the interplay between tumour cell growth and death, as well as the expansion and contraction of CAR T-cells. Despite their utility, single-cell population models omit tumour evolution and interactions with all other immunologic actors that inevitably influence drug kinetics.[16,30] To integrate PK and PD frameworks, multi-population cellular models leverage knowledge from population dynamics modelling—commonly used in demographics, ecology, microbiology, and epidemiology—to capture interactions between malignant cells, CAR T-cells, and other cellular populations (Fig. 2b). Since the publication of the first CAR T multi-population model in 2019,[31] several research groups have included varying levels of biological complexity into their models. Here, we outline some of the biological building blocks seen across currently available multiple-population CAR T PK/PD models.

Malignant cell killing (functional response). Multi-population CAR T models draw inspiration from traditional predator-prey models in ecology to characterise how CAR T-cells eliminate target cells (Fig. 2b). Unlike conventional predators, CAR T-cells do not depend on nutrients from their targets to grow. Instead, they rely on immunological signalling pathways, such as antigen presentation and cytokine dynamics. The simplest forms of these models are based on Lotka–Volterra equations[32,33] which use a mass action law (Supplementary Equation S11) to characterise the joint probabilities of cell-to-cell binding, recognition, and effective killing. In predator-prey theory, the time required for a predator to complete the cycle from prey detection to consumption is known as the handling time.[32,33] Lotka–Volterra models assume negligible handling time, as they imply no immunological limitations to the killing process. As a result, the rate of target cell elimination is directly proportional to the number of target cells (Supplementary Figure S2). This rate, known in predator-prey theory as the functional response,[34] describes how CAR T-cells kill as a function of prey density. Other multi-population models differ from the classic Lotka-Volterra approach by incorporating non-constant killing rates governed by stimulation-saturation laws, which depend on factors such as antigen and antibody cell density or the presence of certain cytokines. Researchers implementing these types of non-linear approaches draw from mathematical ecology—such as Holling Type II and Type III functional responses[34]—as well as biochemistry, and pharmacological tradition. Kimmel et al. and Liu et al. applied Michaelis–Menten kinetics—comparable to a Type II functional response in predator-prey theory—to characterise the saturation effect of B-ALL cell concentration on CAR T-cell killing efficacy[23,35] (Supplementary Equation S12 and Supplementary Figure S2). This functional response is approximately linear at low tumour volumes, similar to Lotka–Volterra models, but plateaus as tumour burden increases, reflecting the growing difficulty of eliminating malignant cells. This saturating effect is particularly relevant when modelling the tumour microenvironment, where cancer cells employ immunoediting strategies to evade immune recognition and destruction. In 2023, Kirouac et al. used the Hill equation—anaogous to a Type III functional response—to model both the saturation and stimulation effects of malignant cells on CAR T-cells. Unlike Michaelis–Menten kinetics, which capture only saturation effects, the Hill equation accounts for response sensitivity as a function of factors such as target antigen density[36] (Supplementary Equation S16 and Supplementary Figure S2). This approach may be particularly useful to model scenarios where the sensitivity is not directly proportional to antigen concentration, such as when tumour burden is too low or inaccessible to CAR T-cells to trigger immune engagement.

Antigen stimulation on CAR T growth (numerical response). In predator-prey theory, predator populations typically grow in proportion to their predation success rate, a concept known as the numerical response. In contrast, immune cell proliferation is driven by immunological signalling pathways rather than nutrients derived from target cell destruction. As a result, antigen stimulation is a key determinant of CAR T PK profile.[5,37,38] Several researchers have accordingly incorporated CAR T-cell growth rates that depend on antigen sensing. In 1995, De Boer and Perelson introduced the mathematical concept of antigen stimulation-saturation to characterise T-lymphocyte proliferation in response to peptide sensing on antigen-presenting cells.[26] In CAR T therapy, this approach was first applied in 2019 by

Hardiansyah and Ng, who used a Michaelis–Menten model to regulate how long-lived memory cells convert into effector CAR T-cells as a function of B-cell concentration in CLL.[31] Later, studies by Paixao et al., Martínez-Rubio et al., Liu et al., Santurio et al., and Sison et al. adopted similar Michaelis–Menten frameworks to model how activated CAR T-cells proliferate and transition into memory cells as a function of antigen levels.[35,39–42] In 2022, Kimmel et al. explored incorporating a functional dependence on both tumour cell burden and total T-cell counts but ultimately adopted a growth rate function based solely on T-cell burden, as it provided better model fits[23] (Supplementary Section B). Later, Kirouac et al. applied the Hill equation—widely used in pharmacology and biochemistry—to model CAR T memory self-renewal, differentiation, proliferation, effector exhaustion, and memory cell recovery[36] (Supplementary Equation S16). These stimulation-saturation frameworks have allowed for the integration of dynamic processes such as T-cell memory self-renewal, proliferation and regeneration, effector exhaustion, and cytokine release.[43] Depending on the malignancy and CAR T product studied, researchers have employed different conceptions of antigen drivers. Kimmel et al. excluded all CD19 cell sources other than malignant cells, assuming that diffuse large B-cell lymphoma (DLBCL) patients have effectively zero normal B-cells following lymphodepleting chemotherapy.[23] In contrast, Martínez-Rubio et al. considered the total $CD19^+$ cell count in ALL as the sum of leukaemic B-cells and non-malignant B-cell populations (Supplementary Equation S15).[40] Kirouac et al. incorporated antigen stimulation via a surrogate transient compartment representing total B-ALL cells, independent of CD19 expression, introducing a time delay between tumour burden changes and CAR T-cell response (Supplementary Equation S17).[36] Liu et al. modelled CAR T proliferation and differentiation as $CD19^+$ dependant, incorporating both $CD19^+$ and $CD19^-$ cells, to account for both positive and negative relapses while accounting for bystander killing.[35] Santurio et al. studied antigen-negative relapse by modelling tumour cells with varying antigen expression levels, which may develop temporary resistance due to phenotypic changes.[41] Lastly, Li et al. investigated the effects of single versus multiple CAR T-cell bindings to glioma cells to determine expansion rate conditions leading to treatment success.[44]

Malignant cell growth. Since a crucial aspect of oncologic PDs involves characterising treatment effects on tumour regression, CAR T PK/PD modelling requires some understanding of haematological and solid tumour growth dynamics. In mathematical oncology, tumour growth is typically described by two primary paradigms: exponential and sigmoidal models (Appendix A and Supplementary Figure S1). The exponential model assumes unbounded neoplastic growth (Supplementary Equation S1), which has been observed *in vitro* and *in vivo* in certain non-solid cancers, such as murine leukaemia.[46–48] In contrast, sigmoidal models incorporate growth limitations due to cell-to-cell competition for resources and space. While exponential and Gompertz models (a type of sigmoidal model; Supplementary Equation S4) have influenced some of the currently validated chemotherapeutic scheduling strategies (see Log-kill and Norton-Simon hypothesis in Appendix A), malignant growth does not adhere to a universal law. Instead, diverse growth dynamics have been observed across *in vitro* and *in vivo* experiments, among different malignancies, and even within clonal subpopulations of the same tumour (Fig. 3c). Recently, Gruber et al. used CLL as an informative model for untreated cancer progression and identified both exponential and sigmoidal growth patterns among patients as well as within malignant clonal subsets of the same patient.[49]

Given the variability in tumour growth dynamics across and within malignancies, CAR T-cell kinetics models must assess whether the assumptions and simplifications made are adequate for the intended objectives. Since currently approved CAR T products primarily target haematological malignancies, improving our understanding of both solid and non-solid tumour growth is fundamental to adapt modelling approaches and treatment strategies to each context. Moreover, late patient presentation and the rapid initiation of antineoplastic treatments following diagnosis limit the availability of clinical data points for studying naturally progressing tumours.

T-cell competition. While some multi-population models consider CAR T-cell growth independent of non-modified T-cells,[31,35,40,45] others incorporate a limitation in product expansion, hypothesising that CAR T and endogenous T-cells compete for the same ecological niche.[23] The model presented in 2021 by Kimmel et al. illustrates this type of approach, integrating: i) simple exponential malignant cell growth, ii) Michaellis–Menten predator-prey interactions, and iii) competition between CAR T and endogenous T-cells via sigmoidal growth laws[23] (a combination of Supplementary Equations S11 and S12). In this model, both endogenous and CAR T-cells follow mixed Gompertz growth patterns, constrained by a theoretical haematological carrying capacity (K), which represents the maximum T-cell burden the blood can support.

Cellular heterogeneity

Single- and multi-population modelling approaches often consider CAR T-cells and target cells as single unified populations, thereby overlooking cellular phenotypic and genotypic diversity. In this section, we review several of the main factors of cellular

heterogeneity that may be incorporated into CAR T kinetics models.

Diversity in CAR T-cell phenotypes. To try and more accurately reproduce CAR T dynamics, both single- and multi-population models incorporating the subdivision of CAR T-cells into distinct lymphocyte subpopulations have been developed. These models differentiate CAR T-cells based on phenotype, creating specific compartments to capture their differentiated properties, such as rapidly dying effector CAR T-cells and slowly contracting memory cells due to natural cell death (Fig. 3a). Understanding the contribution of these phenotypes is important, as treatment success has been shown to be related with characteristics directly linked to T-cell phenotypes. These include the capacity to self-renew and persist, the ability to traffic and penetrate tumour sites, and the capability to exert effector functions while resisting exhaustion induced by immunosuppression.[50] There is currently intense interest in generating CAR T-cell products with selected phenotypes that maximise antitumour activity. Initially, effector cells were thought to be best for CAR T therapies due to their potent killing capacity. However, recent research has shown that a less differentiated T-cell phenotype may lead to superior anti-cancer activity. As a result, current CAR T therapies are focussing on using less differentiated T-cells enriched with memory cells.[50]

The model published by Stein et al., assesses the impact of comedications to treat CRS on CAR T-cell kinetics, serving as an illustrative example of a single-population model that differentiates CAR T-cells into effector and memory compartments. This model represents the initial exponential growth of effector cells until maximal CAR T expansion, followed by a first-order conversion of effector cells into memory cells. This results in a rapid decline of effector cells after peak expansion due to activation-induced cell death, while memory cells experience a slower decline, reflecting sustained CAR T persistence. For multi-population approaches, different models have explored different conceptions of CAR T phenotype diversity. In 2022, Liu et al. grouped CAR T-cells into two groups: activated and non-activated, simplifying phenotype differentiation into cells participating in cytotoxic activity and those with potential to participate.[35] Similar approaches, such as those published by Martínez-Rubio et al. and Hardiansyah et al., distinguish CAR T-cells into effector (or activated) and memory phenotypes.[31,40] In both models, activated CAR T-cells are considered to distribute between peripheral blood and tissues (or bone marrow, in Martínez-Rubio et al.'s model).

Since an important factor in treatment success involves having active CAR T-cells, several modelling approaches have incorporated the loss of CAR T-cell function due to upregulation of exhaustion markers. To

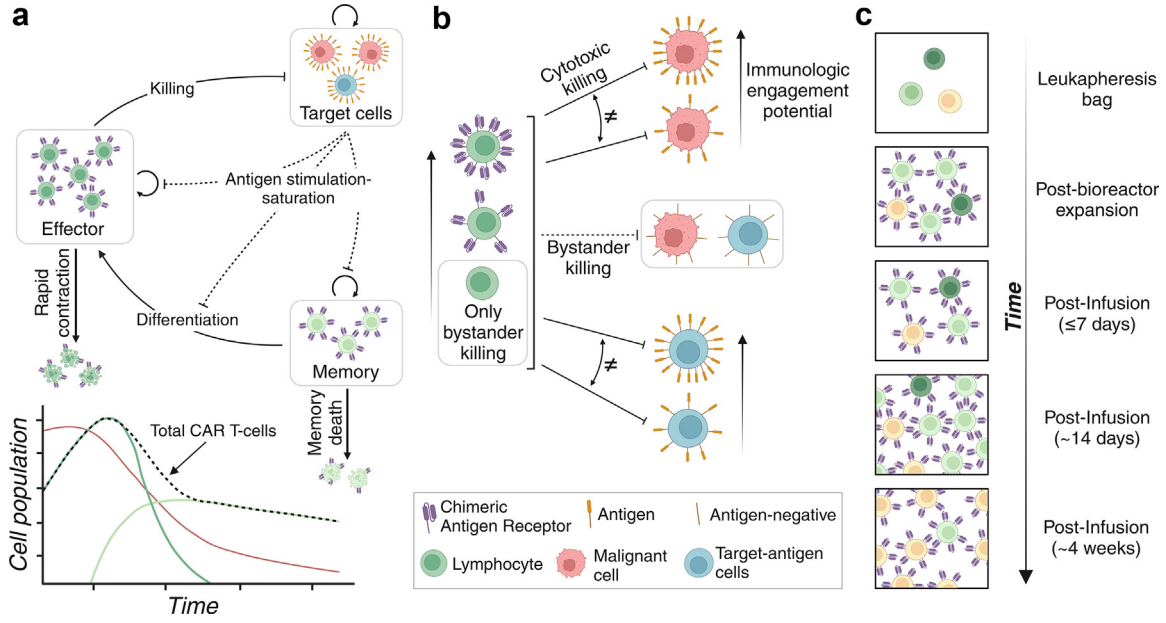

Fig. 3: **Cellular heterogeneity.** (a) Effector and memory CAR T-cell subpopulations exhibit distinct cellular dynamics, with immune engagement between tumour and effector cells regulating the stimulation or inhibition of memory cell proliferation and differentiation. (b) Differences in antigen and CAR receptor densities on malignant and CAR T-cell membranes influence their immunologic engagement potential, thereby impacting cellular-level kinetics. (c) Clonal diversity in CAR T products is highest before infusion, gradually declining over time as smaller, faster proliferating clones become dominant (adapted from[6]).

account for the saturation effect in target cell killing caused by CAR T exhaustion, multi-population models such as those by Paixao et al., Prybutok et al., and Kirouac et al. have included a specific compartment for this phenotype.[36,39,51] Paixao et al. showed that the combined assessment of $AUC_{0–28d}$ and the fraction of non-exhausted cells can serve as a predictive marker for varying treatment responses. Other approaches, like the CARRGO model (standing for Chimeric Antigen Receptor T-cell treatment Response in GliOma) by Sahoo et al. and the CAR T resistance model by Santurio et al., represent CAR T exhaustion without a dedicated compartment for this phenotype. Instead, these models characterise the reduction in CAR T-cell functionality by adjusting transition rate parameters, such as decreasing the growth rate or increasing the mortality rate of CAR T-cells.[41,52] A limitation of this approach is that exhaustion is not modelled as a separate compartment but through the modification of specific parameters that restrict CAR T-cell populations.

As illustrated by these models, different CAR T-cell phenotype compartments and differentiation rules can be implemented as our understanding of T-cell phenotypes continues to evolve. In recent years, various conceptual models of T-cell differentiation have been proposed to explain the biological basis of phenotype diversity. Tantaloo et al. work describing different hypotheses of T-cell phenotype differentiation, serves as a useful reference for modellers exploring compartmental model designs that classify CAR T-cells into different phenotypic groups.[50] Despite these efforts, designing and fitting models with distinct CAR T phenotypes remains challenging, since markers for these phenotypes are rarely measured in most clinical contexts.

Differences in CAR and antigen expression. Current modelling approaches often assume uniform antigen and CAR construct expression across malignant and CAR T-cells. However, several researchers have shown that high levels of CAR expression can lead to increased tonic signalling, up-regulation of exhaustion markers, enhanced *in vitro* cytotoxicity, and reduced *in vivo* bone marrow infiltration. Rodriguez-Marquez et al., studied the impact of CAR density on the functionality of BCMA CAR T-cells *in vivo* and *in vitro*[9] (Fig. 3b). Their findings suggest that patients treated with high-density BCMA CAR T products tend to exhibit worse clinical outcomes across various haematological malignancies. Despite this evidence, such hypotheses await further empirical validation and are still infrequent in computational modelling studies. In 2024, Santurio et al. developed a model to study how antigen density, including transient and permanent antigen loss due to treatment pressure and mutations, affects cellular dynamics and leads to diverse treatment response scenarios.[41] The authors adapted a previous multidrug resistance chemotherapy model by Greene et al.,[53] which used integral-partial differential equations to characterise how cell density and mutations influence solid tumour growth, to represent antigen expression density on the surface of tumour cells as a continuous variable. To date, no CAR T-cell models have studied the influence of CAR expression density on cellular dynamics.

In addition to studying CAR and target-antigen density, most models also ignore antigenic escape, which is a critical factor in CAR T treatment relapse. Beyond the model published by Santurio et al., the ODE-based model by Liu et al. incorporates a specific compartment for antigen-negative cells to account for antigen-mediated relapse mechanisms.[35] While this model accounts for antigen-loss, it does not incorporate the variability in antigen density across tumour cells, as seen in Santurio et al.'s model.

T-cell clonotypic diversity. Current computational models also overlook the impact of T-cell clonal diversity in the leukapheresis bag on CAR T-cell proliferation, persistence, and response after infusion (Fig. 3c). Some researchers suggest that distinct kinetic patterns among CAR T-cell clones may explain the weak correlation seen between dose and expansion, as a small subset of highly proliferative clones can drive most of the product's expansion.[6,10,54] Future modelling approaches could benefit from experimental techniques such as cellular barcoding of both product and tumour clones prior to infusion, aiding in the understanding of CAR T clonotypic fitness inequalities and their correlation with CAR T PK parameters.[55]

Target-cell maturation. Different haematological malignancies exhibit distinct oncogenic profiles based on the origin and maturation stage of the first cancer cell. Most CAR T-cell kinetics models currently simplify this complexity by assuming that all antigen-presenting cells share equivalent phenotypes and uniformly express the target antigen, regardless of maturation stage. This assumption overlooks how CAR T-cell dynamics may vary based on the maturation stage from which the first malignant cell originates. For instance, in diseases such as ALL, malignant B-cells may express the CD19 antigen in mature B-cell blasts but not in the bulk of leukaemia stem cells driving disease progression, that may be more immature. This disparity directly affects the maturation stages at which CAR T-cells can recognise and eliminate leukaemic cells (Supplementary Figure S3). Consequently, some CAR T-cell kinetics models may need to incorporate disease-specific characteristics, such as target cell maturation, to more accurately reproduce real tumour/CAR T interactions. Models based on this maturation-dependant antigen expression hypothesis, imply that predator-prey dynamics can only emerge within cell subsets where the

target antigen is expressed. In the model developed by Martínez-Rubio et al., the authors integrated healthy B-cell development into a predator-prey framework but excluded the maturation of malignant cells, representing leukaemic cells as a single antigen-expressing compartment.[40] To the best of our knowledge, no CAR T-cell kinetics models have yet incorporated malignant cell maturation stages in their design.

Cellular interaction habitats (tissues)

Depending on the development stage and specific physiopathology of each haematological malignancy, cancer cell concentrations vary across different body tissues (Supplementary Figure S3). In certain diseases, such as non-Hodgkin lymphoma, malignancies can exhibit characteristics of both solid and liquid tumours, with tumour cells being present in circulation, bone marrow, and lymph nodes. Beyond inherent trafficking differences across various tissues where CAR T/tumour interactions occur, the nature of these interactions—including their likelihood and the strength of the immunologic response—is influenced by the tissue microenvironment in which they unfold. Consequently, multi-population CAR T-cell kinetics models may incorporate different tumour growth dynamics, functional responses, and numerical response laws (see Pharmacokinetic/pharmacodynamic modelling (PK/PD)), depending on the tissue in which these cellular interactions take place.

Leukaemias, lymphomas, and myelomas are diseases of the bone marrow, and from that perspective, peripheral blood is only one of the many markers of disease burden. Consequently, CAR T-cells in the blood may not always be representative of the clones interacting with neoplastic cells at the primary site of action. Most therapeutic outcome measures—such as CAR T, lymphocyte, malignant cell, and cytokine counts—are derived from peripheral blood samples. However, multiple researchers have highlighted the importance of tracking bone-marrow dynamics for both tumour and CAR T-cells.[23,29,56] In this regard, modelling CAR T/tumour interactions exclusively within the haematological compartment may not always be representative of reality. Some models have incorporated CAR T/tumour interaction environments beyond the haematological domain,[40,45] but these approaches still lack empirical validation. Notably, Martínez-Rubio et al. developed a mathematical model integrating B-cell development and T-cell responses to describe the interplay between continuously renewing B-cells, CAR T-cells, and leukaemic cells in both the bone marrow and peripheral blood.[40] This model accounts for the maturation dynamics of healthy B-cells as well as the differentiation of CAR T-cells into effector and memory subpopulations.

When specifically modelling CAR T/tumour interactions, those occurring in peripheral blood are typically assumed to be instantaneously well-mixed and kinetically homogeneous throughout the entire haematological compartment. This assumption implies that the probability of interaction is equal across all modelled cells—a concept known in network theory as a complete (or homogeneous) contact topology. While this assumption may be reasonable to model the well-mixed dynamics of circulating cells in the blood, it may not adequately capture interactions occurring in solid tissue environments such as the bone marrow, lymph nodes, or tumour microenvironment. To better represent CAR T/tumour interactions in solid-tissues, modelling approaches could benefit from mathematical frameworks that incorporate alternative contact topologies. These methods could account for spatial constraints, where not all malignant cells are equally accessible to CAR T-cells. For instance, in the tumour microenvironment, cells near the tumour surface are more likely to interact with CAR T-cells, than those within the interior of the tumour mass requiring cell infiltration. Despite these biological complexities, current CAR T models predominantly rely on the complete contact topology assumption, which is inherently embedded in the functional forms typically used in predator-prey modelling approaches (Appendix B).

Apart from differences in CAR T-cell trafficking and tumour interaction probabilities across tissue environments, one of the major challenges of CAR T therapy for solid tumours is the immunosuppressive capacity of the tumour microenvironment. In contrast to haematological malignancies, immune suppression in solid tumours significantly impairs CAR T-cell function by limiting their ability to expand, persist, infiltrate, exert cytotoxic activity, and recruit additional immune cells. To address these challenges, Léon-Triana et al. performed *in silico* experiments using a virtual CAR T product designed for the treatment of glioblastoma. This CAR T model targets two antigens: an off-tumour antigen (such as CD19 on B-cells) and a glioblastoma-associated antigen.[57] The rational behind this dual-targeting strategy is to enhance the initial off-tumour CAR T expansion, persistence, and trafficking from the stimulation of CD19-expressing cells in circulation, which may potentially improve overall treatment response. In this approach, the authors incorporated CAR T dysfunction due to tumour immunosuppression using a Michaelis–Menten inactivation term. They also modelled CAR T growth as a Michaelis–Menten type stimulation by both tumour and off-tumour antigens, while accounting for natural CAR T-cell death, exponential tumour growth, and Lotka–Volterra cell killing. Similarly, Paixão et al. employed s simpler modelling strategy by introducing a linear inhibition coefficient to represent CAR T dysfunction due to tumour interactions, which provides a straightforward method to capture immunosuppressive effects in solid tumours.[39]

CAR T distribution (organs)

Following intravenous infusion, CAR T-cells are rapidly distributed throughout the body, leading to a transient decline in circulating cell counts. This is followed by progressive recovery driven by antigen-mediated expansion. While clinical sampling frequencies are often insufficient to fully capture the initial distribution and expansion phases, studies have reported variations in distribution kinetics across different diseases—for instance, a rapid distribution in ALL versus a more sustained distribution in DLBCL.[58] Similar to traditional PK modelling, where plasma drug levels are often represented by a multi-phasic decline reflecting drug distribution and equilibrium (as seen in multi-compartmental PK models), some CAR T-cell models have adopted a similar approach. For example, in 2022, Paixao et al. developed a multi-phasic CAR T-cell dynamics model that characterised interactions between multiple CAR T-cell phenotypes and tumour cells. This approach includes a two-compartmental structure to distinguish between engrafted and distributed effector cells.[39]

In certain haematological disorders, CAR T-cells have shown preferential distribution towards tumour-containing anatomical compartments.[59] Effective treatment response depends on successful CAR T delivery, expansion, and interaction with malignant cells within these compartments. CAR T/tumour engagement is a fundamental driver of expansion, influenced by various patient- and product-specific factors. Under similar dosing strategies, a reduced CAR T/tumour engagement probability (as discussed in Cellular interaction habitats (tissues)), may result in an insufficient number of accessible target cells to drive the initial expansion necessary for therapeutic response. In this context, physiologically based pharmacokinetic (PBPK) modelling can help estimate typical perfusion rates of both healthy and tumour tissues, informing the delivery rates required for effective treatment. Brown et al. applied a PBPK model to compare maximum delivery rates in mice and humans, reporting up to a 10,000-fold higher CAR T-cell delivery per unit volume of target tissue in mice, while doses in preclinical murine models are typically only 10-100-fold lower than those in the clinic.[60] These findings suggest that achieving similar treatment responses in solid tumours may require higher doses or alternative treatment schedules compared to those used for haematological malignancies and *in vivo* studies.[5,61,62]

While further innovations in measuring CAR T distribution are still awaited, PBPK methods can be useful for identifying trafficking differences among effector populations, informing the limits of immune cell delivery across different organs and tumour types, and driving the quantitative estimation of first-in-human dosing and treatment schedules. This can lead to optimal cellular delivery to tumour-containing anatomical compartments. Additionally, PBPK models can help identify kinetic profiles associated with CAR T levels in certain body compartments that may lead to off-tumour related toxicities.

Preclinical evidence has also suggested that the biodistribution and effector function of CAR T-cells can be impaired by the presence of circulating antigen targets, which hinder CAR T-cell trafficking to the bone marrow and other tumour sites.[37,63,64] Modelling strategies that simulate different time windows between lymphodepletion and CAR T infusion may help elucidate potential strategies to reduce such sequestration. However, the phenotypic characteristics that enable individual CAR T-cell to successfully biodistribute and exert their effector functions are still unknown. Studying CAR T distribution is crucial for identifying organs where the product may accumulate, potentially leading to on-target off-tumour toxicities.

In contrast to antigen sequestration, the model by León-Triana et al. (described in Cellular interaction habitats (tissues)) investigates how an experimental *in silico* CAR T product targeting both off-tumour and tumour-specific antigens could potentially overcome immunosuppression in solid tumours such as glioblastoma.[57] The authors characterise how CAR T-cells initially expand from their interaction with CD19 expressing B-cells in peripheral blood and bone marrow, before trafficking to the brain, where glioblastoma cells reside. In this experiment, trafficking from the periphery to the tumour compartment is described using a simple first-order law, which represents the average fraction of CAR T-cells crossing the blood-brain barrier and infiltrating the tumour site. Although not explicitly labelled as such in the original article, this model can be considered a simple PBPK model, studying CAR T-cell trafficking across two compartments: the periphery (including peripheral blood and bone marrow) and the brain, where tumour cells reside.

Subcellular concomitant factors (molecules)

The previous classification categories have focused on mathematical models that use the cell as their basic biological unit, meaning these models only represent biological dynamics occurring at this cellular scale or higher. Still, CAR T-cell kinetics are inevitably influenced by subcellular factors that contribute to the observed cellular dynamics. Several authors have increased the resolution of CAR T-cell kinetics models to include molecular-scale determinants, such as cytokine dynamics, as well as the use of comedications before (such as preconditioning therapy[65]) and after treatment.[29,66,67]

CAR T-cell expansion comes with a concomitant risk of severe cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), particularly in patients with high tumour burden.[20] In standard care using CD19-specific CAR T-cells, higher CAR T expression and response correlate with an

increased risk of CRS and ICANS.[68] CRS typically precedes peak CAR T-cell expansion, while ICANS follows CAR T-cell concentration closely.[69] A deeper understanding of CAR T-cell kinetics is essential to develop strategies to modulate the inflammatory responses associated with CAR T-cell treatment. Although not clinically validated, several mathematical models have been developed to describe various aspects of T-cell and cytokine interactions. Yiu et al. created a mathematical model to describe the kinetics of cytokine storm during CRS using limited time-series data.[70] Hardiansyah and Ng[31] established a quantitative systems pharmacology model linking CAR T-cell kinetics to cytokine release, while also showing that disease burden is associated with CAR T-cell expansion. Based on existing clinical data, Zhang et al.[71] developed a mechanistic model incorporating a simplified cytokine network to investigate how CAR T and B-ALL cell interactions activate monocytes and trigger excessive cytokine release. Although the underlying biology of CRS is still not fully understood, improving its management through mathematical and computational approaches could lead to better risk prediction and improved mitigation strategies to prevent serious complications.

Patients undergoing CAR T therapy who experience severe CRS have shown to benefit from tocilizumab treatment and, in some cases, corticosteroids, which are known to reduce circulating T-cells[72] and induce apoptosis.[73] Mueller et al.[20] previously reported that patients receiving tisa-cel who were treated with a combination of tocilizumab and/or steroids for severe CRS still exhibited sustained CAR T expansion and persistence. In 2019, Stein et al. employed a modelling approach to assess how anti-inflammatory treatments influence the expansion profile and overall cell kinetics in patients receiving tisa-cel.[29] The primary aim of this model was to examine the differences in peak levels and expansion rates of tisa-cel in patients who received various anti-inflammatory regimens, with different combinations and schedules of tocilizumab and corticosteroids, compared to patients who did not require these treatments. In this context, modelling approaches that can accurately characterise the impact of these treatments on CAR T-cell dynamics are valuable tools for optimising therapeutic strategies.

Computational design of CAR T-cell kinetics models

In this section, we provide a framework for understanding the methodological approaches used to capture the biological constituents described in the proceeding section (Biological constituents of CAR T-cell kinetics models). To this end, we have classified CAR T-cell kinetics modelling design principles based on: i) the depth of biological understanding upon which the model structure is built (From description to comprehension); ii) whether biological constituents are captured using a top-down or bottom-up approach (From populations to cells); and iii) whether random effects are included or excluded from the model (From determinism to stochasticity).

From description to comprehension (empiric and mechanistic models)

Depending on their purpose, mathematical models incorporate different depths of understanding of the modelled reality. Models that provide detailed theoretical representations of a system's underlying mechanism are known as mechanistic (or explicative) models. In contrast, empirical models (also known as descriptive or statistical models) focus on capturing the observed quantitative dynamics without explicitly accounting for the biological mechanisms driving these patterns. The choice between these approaches depends largely on the available knowledge of the system and specific research objectives. In practice, most models integrate elements of both mechanistic and empirical thinking. Rather than forming a strict dichotomy, models exist along a continuum ranging from empirical to theoretical conceptions (Fig. 4).

All CAR T-cell modelling approaches discussed in Biological constituents of CAR T-cell kinetics models operate along this empirical-to-mechanistic spectrum. At the empirical end, methods such as NCA, which do not require the assumption of a specific compartmental model (as implemented by Mueller et al.[20,22]), contrast with single-population models that incorporate basic biological assumptions regarding T-cell transitions between effector and memory phenotypes (Stein et al. model; Supplementary Equation S10). On the mechanistic side, multi-population models aim to capture the complex dynamics arising from interactions between different immunologic populations. Based on the biological properties outlined in Cellular heterogeneity, we identified the following models as paradigmatic examples of single and multi-population models: Stein et al. as a simple CA PK model including CAR T-cell heterogeneity (effector and memory compartments); Hardiansyah et al. as a classic application of Lotka–Volterra type malignant cell killing[31]; Kimmel et al. as a model incorporating T-cell competition[23]; Kirouac et al. as an approach including antigen stimulation[36] (Supplementary Equation S14); Liu et al. as a multi-population model representing CAR T-cell heterogeneity[35]; Martínez-Rubio et al. as an approach capturing benign B-cell maturation and distinct tumour/CAR T-cell interaction habitats[40]; Brown et al. as a PBPK model for CAR T biodistribution[60]; and Hardiansyah et al. and Zhang et al. as multi- and single-population models incorporating cytokine dynamics.[31,71] Based on the characteristics of these examples, we propose a semi-quantitative framework to describe CAR T-cell models based on the number of biological layers (as described

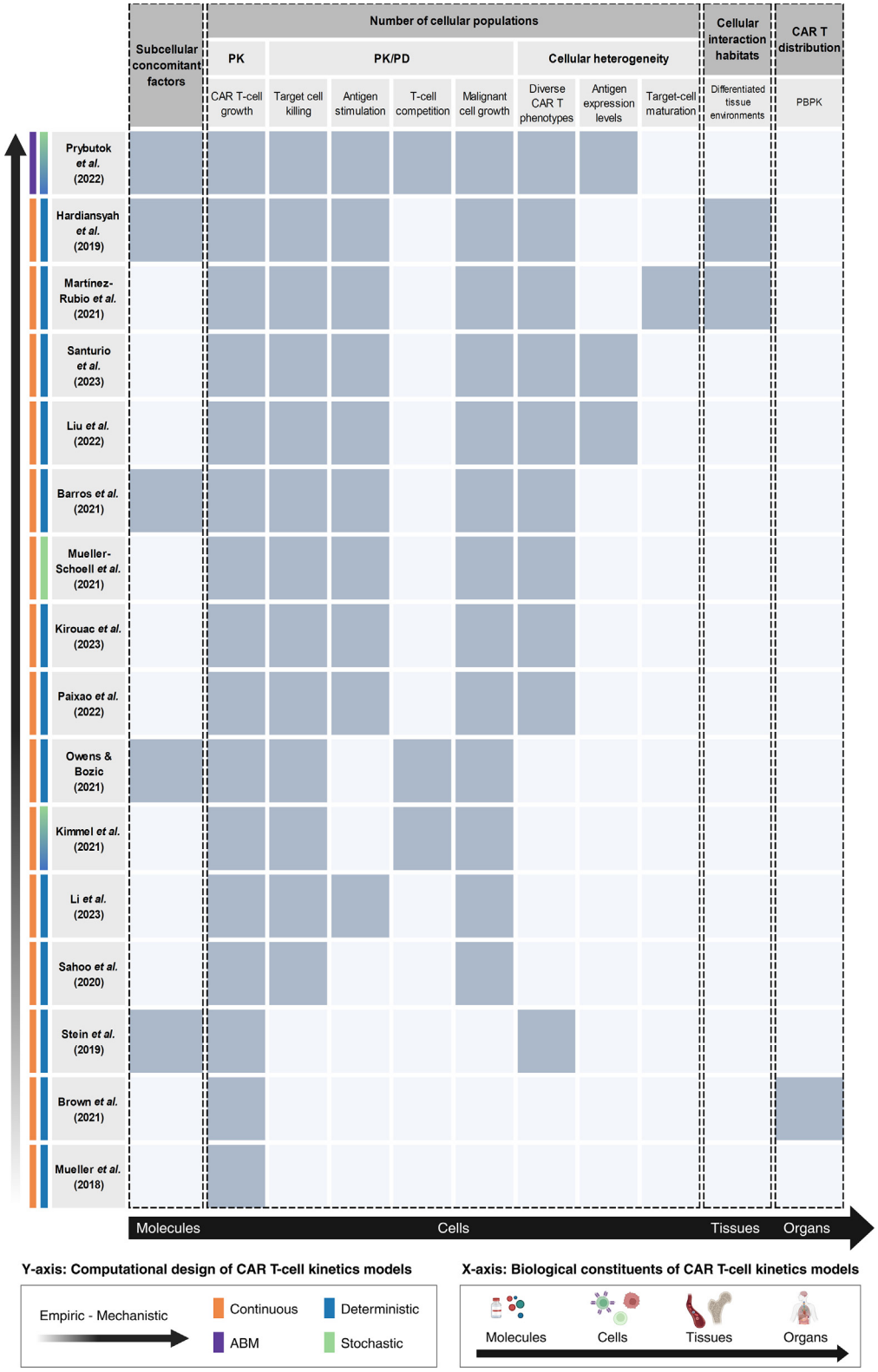

Fig. 4: **Taxonomic classification of CAR T mathematical models based on its biological constituents and computational design.** This figure illustrates the incorporation of varying degrees of biological comprehension and the use of different computational approaches of the most

in Cellular heterogeneity) captured by each modelling approach. Using this biological complexity index, we classify key CAR T-cell modelling examples along this empiric-to-mechanistic scale (X-axis in Fig. 4).

Overall, the choice between an empirical or mechanistic modelling approach depends on the specific objectives of its application. When the goal is to improve biological understanding of the underlying system, the model should incorporate some of the mechanisms and entities that constitute the real system. Conversely, if prediction is the primary objective, a model may deviate from biological reality in its design, as long as it provides sufficient predictive accuracy.

From populations to cells (continuous and agent-based models)

Depending on the treatment of the modelled agents, population dynamics models can be classified as continuous or discrete. Continuous models take a top-down approach, analysing the entire population of the system to determine transition rates that drive system dynamics at the population-level. In contrast, agent-based models (ABMs) follow a discrete, bottom-up approach, simulating individual-level transitions and interactions (such as those of immune cells in CAR T modelling) to derive the emergent population-level dynamics through computer simulations (Supplementary Figure S5).

Current CAR T modelling approaches are primarily based on continuous models using ordinary differential equations (ODEs), which capture the principles governing cellular dynamics at the population-level. These models are typically calibrated using experimental measurements from peripheral blood samples and, less frequently, from bone marrow biopsies as markers of cellular populations (Supplementary Figure S6). While ODE-based models can account for intrinsic cellular characteristics, such as CAR affinity and antigen density, by considering average abundances of the studied populations,[43] they are limited in their ability to capture single-cell characteristics and specific cell-to-cell immunologic interactions.[74]

In 2022, Prybutok et al. introduced the first model to incorporate discrete methods, creating a hybrid CAR T modelling approach that combined continuous methods—such as partial differential equations to control nutrient diffusion, stoichiometric equations to determine cellular glucose and oxygen uptake, and ODEs to model TGFα and IL-2—with a discrete ABM. This model examined how variations in product and tumour characteristics (e.g., dose, $CD4^+$:$CD8^+$ ratio, CAR-antigen affinity, and CAR and antigen expression levels) individually and collectively impact treatment outcomes.[51] Later, Giorgadze et al. incorporated variations in antigen and tumour cell distribution in another ABM to reflect the microenvironment complexity of solid tumours and to investigate conditions that are prone to success or failure in CAR T treatment for breast cancer.[75]

In this context, we have classified the models reviewed in Fig. 4 based on how model agents are treated, using these two categories: Continuous and ABM, as shown on the Y-axis of the Fig. 4. Discrete modelling approaches in CAR T therapy allow for experimentation with cellular heterogeneity before committing to costly and time-consuming *in vitro* and *in vivo* modelling. These approaches hold potential for multi-scale modelling, enabling the gradual incorporation of mechanisms responsible for cellular dynamics at different biological levels, which can then be validated by comparing simulation results with experimental observations. However, in contrast to ODE-based models, quantitatively validating ABMs remains challenging due to unknown biological mechanisms, limited empirical data for model calibration, the abundance of parameters, and computational intensive simulations.

From determinism to stochasticity

Depending on the inclusion or exclusion of random processes, models can be classified as deterministic or stochastic. Deterministic models rely on fixed functions, producing a single simulation outcome for a given initial state of the system. In contrast, stochastic models introduce randomness, leading to a distribution of possible outcomes for the same set of initial conditions. The motivation for incorporating uncertainty into these models can be twofold: to replicate the inherently non-deterministic nature of many real-world systems, or to account for our incomplete understanding of reality.

Dynamic systems, including those governing CAR T-cell kinetics, can be modelled using both deterministic and stochastic approaches. Therefore, modellers must decide whether incorporating stochasticity into the model contributes to the research objectives. In general, when dealing with large populations, the individual stochastic effects of the system's agents tend to average out, converging to the global behaviour that deterministic models aim to reproduce. In the context of CAR T

paradigmatic examples available in the current CAR T modelling literature. **(X-axis)** Sorts biological constituents of CAR T-cell kinetics models based on their biological scale. **(Y-axis)** Continuous and categorical classification of models based on its computational design (as described in Computational design of CAR T-cell kinetics models). The three classification groups of this axis describes CAR T-cell kinetics models based on: i) the depth of biological comprehension from which models are built by using a continuous scale that goes from the simplest empiric to the most complex mechanistic models; ii) the treatment of the model agents (cells) which may be modelled as a unified population (continuous) or as discrete entities (agent based-models [ABMs]); and iii) the exclusion or inclusion of random processes (deterministic or stochastic models).

therapy, most of the published continuous models are governed by deterministic ODEs, which produce identical treatment outcomes for a given patient and treatment regime. In 2021, Kimmel et al. found that their multi-population ODE model, while accurately depicting the initial stages of the observed CAR T-cell dynamics, failed to reproduce the durable responses observed in the clinic.[23] The authors found that within the framework of their model, tumour eradication was deterministically unstable—meaning that, in the long run, all virtual patients would relapse. They hypothesised that stochastic effects might play an import role in tumour extinction when tumour populations become small. Consequently, they proposed a hybrid deterministic–stochastic approach, in which the system is simulated deterministically for large cell populations, but when the tumour burden reaches a certain population threshold, random effects are introduced to account for stochastic tumour extinction events in small populations.

Another approach which has been used to introduce stochasticity in CAR T-cell models are nonlinear mixed-effects (NLME) models. NLME models combine three submodels: the structural, statistical, and covariate submodels. This approach allows the model to account for variability in both parameters and data observations—while covariate modelling helps explain inter-individual variability by identifying baseline covariates that influence the model's outcomes. In 2021, Mueller-Schoell et al. developed a NLME model based on a deterministic multi-population CAR T model as its structural component to characterise fixed effects.[76] This approach allowed researchers to describe the evolution of different CAR T phenotypes (naïve, central memory, effector memory, and effector CAR T-cells) and tumour volume measurements in clinical data, while also identifying factors contributing to the large inter-individual variability in CAR T-cell expansion and patient survival.

While still uncommon in CAR T modelling literature, stochastic dynamics are a fundamental aspect of the previously described ABMs (From populations to cells [continuous and agent-based models]). These models incorporate random effects into their transition and interaction rules.[51,75]

Based on the inclusion of some of the described stochastic processes in CAR T-cell kinetics models, we have categorised the models presented in Fig. 4 into deterministic and stochastic categories, as shown on the Y-axis of the figure.

Application of CAR T-cell kinetics models

Once a model has been built, its application depends on two primary factors: the objectives being pursued and the feasibility to obtain model parameters. CAR T-cell kinetics models can be classified as either quantitative or qualitative. Quantitative models use initial conditions and numeric parameters—either derived from biological rates available in the literature or estimated by fitting the model to empirical data—to generate numerical simulations. In contrast, qualitative models rely on theoretical mathematical analyses that do not require numerical parameters to study the system's dynamics. These methods analyse the behaviour and properties of the model's equations using mathematical theory rather than direct numerical simulations.

Qualitative theory of differential equations enables the exploration of fundamental properties of the system, such as the existence of steady states, equilibrium points, and stability, without the need to numerically solve the model. For example, the models published in 2021 by León-Triana et al. and the two by Pérez-García et al. used qualitative analysis to investigate the properties and equilibrium points of the system, mathematically describing the longitudinal dynamics of B cells, leukaemic clones and CAR T-cells without relying on empirical data or biological rates from the literature.[45,57,77] While qualitative methods are limited to theoretical analysis, they provide a valuable framework to generate new hypotheses about the fundamental laws of the system, that might otherwise be difficult to conceptualise without a mathematical representation of the system.

Quantitative frameworks can be further classified based on the sources of information used to obtain numerical parameters. Current modelling strategies primarily rely on blood sample measurements as surrogate markers for CAR T/tumour dynamics across the entire organism. Peripheral blood and bone marrow are the main accessible sources for most CAR T programmes, meaning that dynamics in non-accessible tissues remain unmeasured and hidden from empirical validation (Supplementary Figure S6). In this context, approaches using empirical data must evaluate whether the established modelling objectives can be achieved given the available sampling frequency, the measured compartments, the laboratory analytical techniques used, and the reliability of the methods used to infer cell dynamics in non-accessible compartments. Table 1 reviews the key models discussed in this review (those outlined in Fig. 4), categorising them by the origin of parameters (literature-based or fitted) and, for models with fitted parameters, by the source of the data used to identify parameters prior to simulations.

Quantitative approaches can also be classified based on the amount of time-course experimental data used to identify model parameters. Approaches that use complete time-course product-level data (also known as drug monitoring approaches) can be applied in both the preclinical and clinical setting.[52]

In the preclinical context, *in silico* modelling can be useful, since testing every new CAR design approach *in vitro*, *in vivo*, and *ex vivo* is costly, time-consuming, and labour intensive. In 2021, Barros et al. developed a three-population ODE based model (CARTmath) to

	Model objective	Included cell populations	Model type	Computational design	Parameter sources	Data sources	CAR T product	Target disease	Measurement type
Prybutok et al. (2022)	Investigate how clinically relevant design choices and inherent tumor features, such as dose, CD4+:CD8+ ratio, CAR-antigen affinity, cancer and healthy cell antigen expression, individually and collectively impact treatment outcomes.	CD4+ and CD8+ CAR T-cells, and target-expressing healthy and cancer cells.	Multi-population PK/PD	Hybrid model based on a discrete ABM (although it also includes ODEs and PDEs to characterise some continuous processes)	Pre-clinical, clinical, estimated, and assumed by modellers.	Literature search of parameters.	Non-specific	Tissue healthy and tumour cells growing in a *in silico* dish.	Non-fitted parameters
Hardiansyah et al. (2019)	Describe the complex relationships of CAR T and pro inflammatory cytokines associated with CRS for CAR T therapy.	Effector and memory CAR T-cells in both peripheral blood and tissue, and B-ALL cells. Including the influence of certain cytokines.	Multi-population PK/PD	System of ODEs	Clinical	Data from 2 patients of a previously published work including information on inflammatory cytokines and blood kinetic data of CAR T-cells.	anti-CCD19	CLL	Patient-level blood samples
Martínez-Rubio et al. (2021)	Describe the interaction between cancerous cells, healthy B cells and CAR T-cells, with the purpose of reproducing and shedding light on the clinical features typically observed in clinical trials.	Memory and activated CAR T-cells, and tumour and healthy B-cells in blood and bone marrow.	Multi-population PK/PD	System of ODEs	Clinical	Literature search of parameters which have been experimentally fitted using clinical data.	Non-specific	B-ALL	Non-fitted parameters
Santurio et al. (2023)	Investigate how antigen-mediated resistance mechanisms affect therapy outcomes by describing the level of antigen expression of tumor cells.	Effector and memory CAR T-cells, and antigen-positive or negative tumour cells.	Multi-population PK/PD	System of PDEs	Clinical	Clinical data from 18 patients accross multiple publications in the literature.	CD28- or 4-1BB-based CD19 CAR T-cells	B-ALL, CLL, MCL and DLBCL	Patient-level blood samples
Liu et al. (2022)	Recapitulate key cellular mechanisms and dynamics during treatment including characteristics of responding and non-responding patients, and CD19+ and CD19-negative relapse.	Non-activated and activated CAR T-cells, and CD19+ and CD19- B-ALL cells.	Multi-population PK/PD	System of ODEs	Clinical	Data collected from multiple clinical studies available in the literature, representing a cohort of 209 patients.	Multiple anti-CD19 products	B-ALL	Population and patient-level blood samples
Barros et al. (2021)	Develop a simple mathematical model to describe tumor response to CAR T-cell immunotherapy in immunodeficient mouse models.	Effector and memory CAR T-cells, and tumour cells.	Multi-population PK/PD	System of ODEs	Pre-clinical	Results of previous *in vivo* murine models performed by research groups at University of Pennsylvania and Baylor College of Medicine.	anti-CD123 CAR T-cells	HDLM-2 and RAJI tumour cell lines	*In vitro* and *in vivo* data
Mueller-Schoell et al. (2021)	Characterize typical and individual concentration-time profiles of four different CAR T phenotypes and patient/therapy-related factors associated with poor survival.	Naïve, central memory, effector memory and effector CAR T-cells, and CD19+ metabolic tumour cells.	Multi-population PK/PD	NLME model including system of ODEs and statistical submodel	Clinical	Cohort of 24 adult patients trated at MD Anderson Cancer Center.	Axicabtagene ciloleucel	B-cell NHLs: DLBCL, PML and transformed FL	Patient-level blood samples

(Table 1 continues on next page)

	Model objective	Included cell populations	Model type	Computational design	Parameter sources	Data sources	CAR T product	Target disease	Measurement type
(Continued from previous page)									
Kirouac et al. (2023)	Quantify the relationships between CAR T product characteristics, patient physiology, pharmacokinetics, and clinical outcomes in different haematological malignancies.	Memory, effector and exhausted CAR T-cells, and tumour B-cells.	Multi-population PK/PD	System of ODEs	Clinical	PK and tumor dynamic profiles of 38 patients digitized from a clinical study published by the University of Pennsylvania.	Tisagenlecleucel	B-ALL and CLL	Patient-level blood samples
Paixao et al. (2022)	Study the multiphasic CAR T-cell dynamics when interacting with tumor cells, when considering patient and product heterogeneities.	Effector, memory and exhausted CAR T-cells, and tumour cells.	Multi-population PK/PD including distribution of effector CAR T-cells	System of ODEs	Clinical	Three data sets from the literature on anti-CD19 CAR T-cell therapy.	Non-specific	ALL, CLL, DLBCL, and MCL	Patient-level blood samples
Owens & Bozic (2021)	Test different chemotherapy and CAR T therapy treatment regimens on tumor-immune interactions.	CAR T-cells, normal T-cells, and tumour cells.	Multi-population PK/PD	System of ODEs	Clinical	Litreature search of parameters for 4 theoretical patients.	Non-specific	DLBCL, CLL and melanoma	Patient-level blood samples
Kimmel et al. (2021)	Understand T and CAR T-cell dynamics when interacting with malignant B-cells *in vivo*.	CAR T-cells, normal T-cells, and tumour B-cells.	Multi-population PK/PD	Hybrid model (System of ODEs and stochastic approach)	Clinical	Population-level ZUMA-1 clinical trial data.	Axicabtagene ciloleucel	DLBCL	Population-level blood samples
Li et al. (2023)	Develop a model to characterise multiple CAR T-cells binding to single glioma cells.	CAR T and glioma cells.	Multi-population PK/PD	System of ODEs	Pre-clinical	Data from multiple *in vitro* co-culture experiments.	Anti-IL13Rα2 CAR T-cells	Glioblastoma	*In vitro* images & *in vitro* single-cell population analysis
Sahoo et al. (2020)	Explore the kinetics of CAR T-cell killing in glioma.	CAR T and glioma cells.	Multi-population PK/PD	System of ODEs	Pre-clinical & Clinical	*In vitro* co-culture experiments & Data of 1 glioma patient.	Anti-IL13Rα2 CAR T-cells	Glioblastoma	Single-cell *in vitro* & single-patient data
Stein et al. (2019)	Characterise the impact of tocilizumab and corticosteroids to the *in vivo* expansion of CAR T-cells.	Effector and memory CAR T-cells, including the impact of tocilizumab and corticosteroids on expansion.	PK	System of ODEs	Clinical	90 patients from ELIANA and ENSIGN clinical trials.	Tisagenlecleucel	B-ALL	Patient-level blood samples
Brown et al. (2021)	Quantify and compare the delivery rates of CAR T-cells across organs and species.	CAR T-cells in different anatomical compartments.	PBPK	System of ODEs	Random selection of pre-clinical and clinical parameters.	Literature search of parameters.	Non-specific	Multiple solid tumours	Non-fitted parameters
Mueller et al. (2018)	Describe the clinical pharmacology of tisa-cel.	CAR T-cells.	Classic PK two-compartment analysis	Piece-wise equation	Clinical	91 patients from ELIANA and ENSIGN clinical trials.	Tisagenlecleucel	B-ALL	Patient-level blood samples

This table illustrates how different quantitative CAR T modelling approaches use information to infer model parameters based on the objective of the model and data availability. Acronyms: NLME: nonlinear mixed effects, MCL: mantle cell lymphoma, PK: pharmacokinetics, PD: pharmacodynamics, ABM: agent-based model, ODE: ordinary differential equation, PDE: partial differential equation, ALL: acute lymphoblastic leukaemia, CLL: chronic lymphocytic leukaemia, NHL: non-Hodgkin lymphoma, DLBCL: diffuse large B-cell lymphoma, PML: primary mediastinal lymphoma, FL: follicular lymphoma.

Table 1: **Categorisation of quantitative CAR T-cell kinetics models based on parameter sources.**

characterise the response of non-solid tumours to CAR T-cells in virtual immunodeficient mouse models.[67] In emerging clinical trials, *in silico* modelling can help facilitate a safer transition to the clinic by supporting the quantitative selection of first-in-human doses (Fig. 5a). Several published models have utilised retrospective *in vitro* and *in vivo* experimental data from previous works to fit CAR T and tumour population dynamics.[43,52,67,78] Importantly, Sahoo et al. developed the CARRGO model to study CAR T treatment responses in glioma, using specifically generated experimental data from an *in vitro* real-time cell analyser that measures single-cell characteristics.[52] This data was then used to estimate parameters for the mathematical model which was then applied to human data.

In the clinical context, drug monitoring approaches can be implemented retrospectively or at the bedside (Fig. 5b). Retrospective population PK and PK/PD approaches use time-concentration data from previously treated patients to examine the relationships between dose, exposure, and clinical outcomes at the population level, while also exploring the impact of patient variability on CAR T dynamics. To calibrate model parameters, most of the current models using clinical data rely on patient-level or aggregated data extracted from previously published clinical trials. This data is often obtained with specialised software tools that extract data points from the published time-concentration plots, as seen in models such as those by Liu et al. and Kirouac et al.[36,58] Other approaches, with direct access to clinical data, have also calibrated model parameters using population-level cell measurements from peripheral blood samples, as seen in the model published by Kimmel et al.,[23] or by using patient-level measurements to perform individual model fits, as shown in the model published by Stein et al.[29]

In contrast to retrospective approaches, therapeutic cell monitoring (TCM) methods can also be applied at

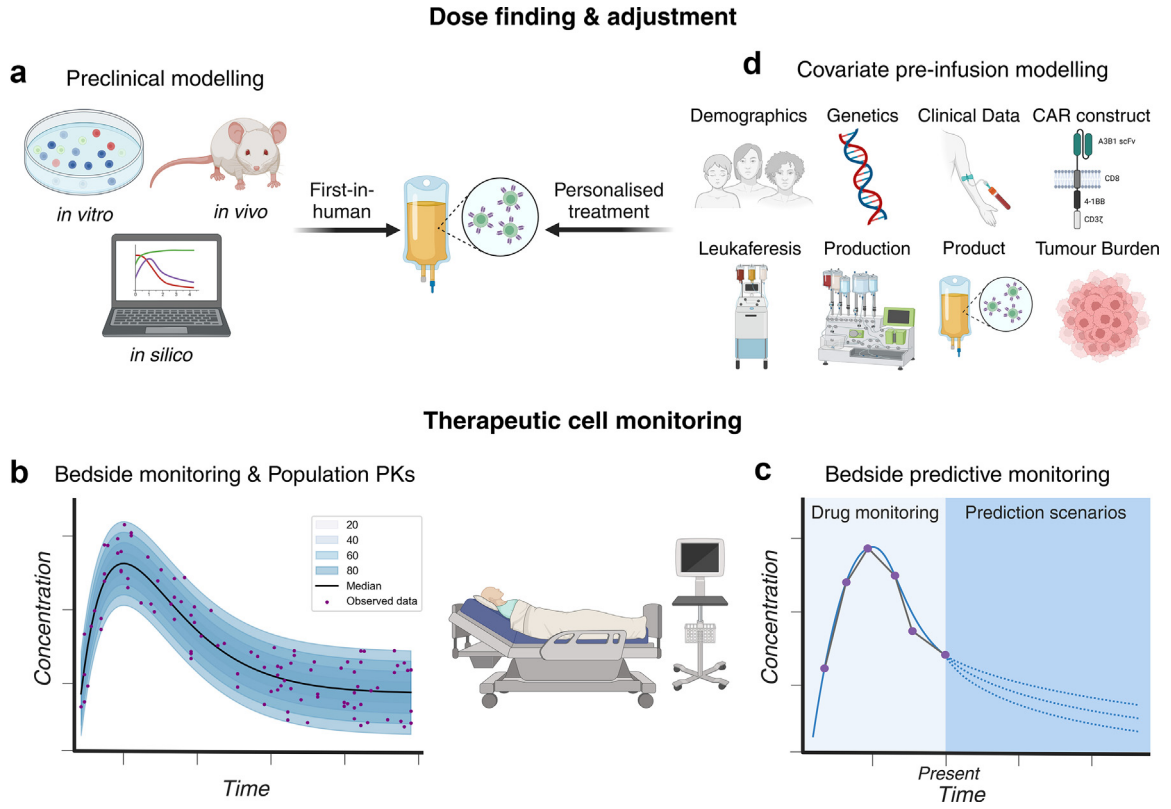

***Fig. 5:* Quantitative implementations of CAR T-cell kinetics models based on the information used to fit the model parameters.** (a) Integrating *in vitro* and *in vivo* model outcomes with computational modelling approaches can support quantitative evaluations during product development stages, aiding the transition from the preclinical to clinical settings. (b) Non-compartmental and compartmental pharmacokinetic (PK) models have potential to support the analysis of serum CAR T levels at the population or bedside, facilitating population monitoring and clinical decision making. (c) Bedside predictive therapeutic drug models employing Bayesian methods can be used to link the observed PK profile over a certain time period with different PK prediction scenarios to improve clinical management. (d) Identification of covariates with predictive value for the observed CAR T PK profile may elucidate interpatient and product heterogeneity, enabling dose and fractioning adjustments that improve treatment outcomes.

Search strategy and selection criteria

We conducted a non-systematic review of a knowledgeable selection of articles in the field, obtained by exhaustively searching in PubMed, Google Scholar, and other search engines. Search terms included, but were not limited to, 'Computational CAR T model', 'Mathematical CAR T model', 'Computational CAR T model', 'CAR T-cell modelling', 'CAR T-cell kinetics', and 'CAR T-cell pharmacology'. Our search was limited to peer-reviewed articles published in English.

the bedside to analyse product kinetics and quantitatively support clinical management. Bedside predictive TCM (also known as *a posteriori* modelling) uses bedside data along with PK information from previously treated patients to customise dosing regimens through Bayesian prediction of individual patient dynamics (Fig. 5c). While TCM has played a significant role in treatment allocation in oncology,[79] its application in cancer cell therapies is still pending.

Finally, predictive dose adjustment models (also known as *a priori* modelling) rely on pre-infusion data to account for interpatient variability and predict individual PK dynamics (Fig. 5d). These approaches should consider multiple factors, including the CAR construct design, manufacturing process, bridging chemotherapy, conditioning lymphodepletion, cell phenotype characteristics, tumour burden dynamics, and intrinsic and extrinsic patient factors.[17] By integrating these variables, dosing regimens could potentially be optimised to prevent underexposure or overexposure to CAR T therapy. Still, the impact of many of these covariates on CAR T-cell kinetics remains poorly understood. Combined with the early stage of CAR T-cell kinetics modelling as a field, this limitation has made the application of covariate-based modelling unfeasible to date.

Conclusions

Over the past decade, computational modelling has made significant strides in advancing our understanding of CAR T-cell therapy. As regulatory agencies increasingly recognise its value in characterising the heterogeneous cellular kinetics of CAR T therapy, the need for collaboration across biomedical, clinical, and computational research continues to grow. However, constructing accurate CAR T-cell kinetics models remains challenging due to our incomplete understanding of the complex pharmacology of CAR T therapy and the difficulty of obtaining *in vivo* measurements across key compartments, such as the bone marrow, lymphoid organs, and the tumour microenvironment. Successful implementation now depends on achieving a balanced integration of research objectives, biological knowledge, mathematical design, and access to empirical data.

Outstanding questions

Given the highly interconnected tumour-immune dynamics in CAR T therapy, modelling the safety and efficacy of CAR T-cell therapies requires a deep understanding of the complex tumour-immune interactions that emerge after therapy, along with the ability to measure key biological processes. Therefore, the future of CAR T-cell modelling depends on advancing our knowledge of CAR T biology and developing reliable and sufficiently rich analytical measurements. To support this, research protocols should prioritise the quantitative utility of biological data. By designing studies with sufficient sampling frequency and standardised analytical methods, modellers can enhance the accuracy and translatability of computational models, ultimately improving their role in guiding the development of CAR T therapies and clinical decision-making.

While current single-population models based on peripheral blood samples may not fully capture the biological complexity of CAR T therapy, they have effectively described key PK aspects of therapy. Conversely, multi-population PK/PD models have made progress in representing certain mechanistic aspects of CAR T-cell behaviour, yet they remain limited by the challenge of obtaining sufficiently detailed *in vivo* measurements to fit and validate model parameters. Although mechanistic models hold theoretical promise, clinically motivated research should be cautious about constructing overly complex models with unvalidatable parameters.

The future clinical value of computational approaches depends on close collaboration between clinicians, biomedical researchers, and modellers. Such interdisciplinary efforts are essential to move beyond the descriptive nature of current CAR T-cell kinetic models and achieve the level of precision seen in traditional chemotherapeutic PK/PD modelling. In doing so, computational modelling can play a pivotal role in optimising treatment strategies, guiding dose management and selection, and enabling bedside therapeutic monitoring of CAR T-cell therapies.

Contributors

Adrià Murias-Closas: Writing–original draft, Writing–review & editing, Visualization, Conceptualization. Clara Prats: Writing–review & editing. Gonzalo Calvo: Writing–review & editing. Daniel López-Codina: Writing–review & editing, Conceptualization, Supervision. Eulàlia Olesti: Writing–review & editing, Visualization, Conceptualization, Supervision. All authors read and approved the final version of the manuscript.

Declaration of interests

The authors report no conflicts of interest in this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2025.105597.

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