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REVIEW

# Development of and insights from systems pharmacology models of antibody-drug conjugates

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**Abstract**

Antibody-drug conjugates (ADCs) have gained traction in the oncology space in the past few decades, with significant progress being made in recent years. Although the use of pharmacometric modeling is well-established in the drug development process, there is an increasing need for a better quantitative biological understanding of the pharmacokinetic and pharmacodynamic relationships of these complex molecules. Quantitative systems pharmacology (QSP) approaches can assist in this endeavor; recent computational QSP models incorporate ADC-specific mechanisms and use data-driven simulations to predict experimental outcomes. Various modeling approaches and platforms have been developed at the in vitro, in vivo, and clinical scales, and can be further integrated to facilitate preclinical to clinical translation. These new tools can help researchers better understand the nature and mechanisms of these targeted therapies to help achieve a more favorable therapeutic window. This review delves into the world of systems pharmacology modeling of ADCs, discussing various modeling efforts in the field thus far.

## INTRODUCTION

Antibody-drug conjugates (ADCs) are engineered immunoconjugate drugs composed of three core components: (1) a monoclonal antibody (mAb) and (2) one or more cytotoxic small molecules (known as *payloads* or *warheads*), attached via (3) a chemical linker (Figure 1). Predominantly developed as cancer therapies, this strategy aims to harness the advantages of both chemotherapeutics and biologics while minimizing their disadvantages. Small molecule chemotherapy drugs provide the desired cell-killing capabilities but do not discriminate between on-target and off-target cells, which can cause unnecessary damage to healthy tissue and harmful side effects.

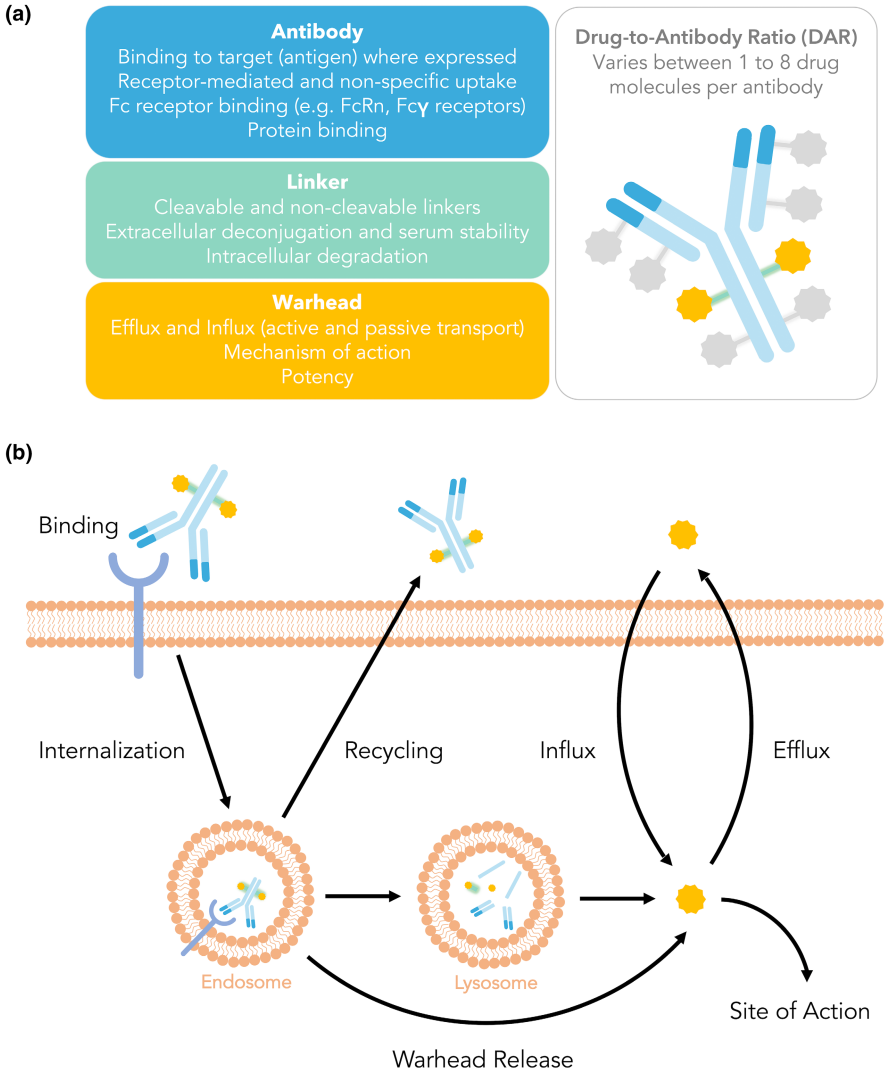


**FIGURE 1** Key ADC properties and mechanisms for QSP modeling. (a) The antibody, linker, and warhead components of ADCs each have different design properties that must be considered during modeling. Another key characteristic is the drug-to-antibody ratio (DAR), which typically varies between one and eight. (b) Key mechanisms of action of the ADC include binding to the target antigen, internalization into the cell, trafficking and recycling of the ADC, endosomal cleavage of the linker or lysosomal degradation of the ADC for warhead release, influx and efflux of the warhead, and cell killing effects at the site of action. ADC, antibody-drug conjugate; QSP, quantitative systems pharmacology.

Antibodies can target specific cells by binding to particular antigens on the cell surface but may lack the cytotoxicity to effectively destroy cells compared to chemotherapeutics.[1–3] ADCs, therefore, strive to achieve the best of both worlds, maximizing efficacy while minimizing toxicity.

This targeted drug delivery to selected cells while sparing others is remarkably similar to Nobel Laureate Paul Ehrlich's early 20th century concept of the "magic bullet" for treating human diseases.[4] The first animal studies of ADCs (in the 1960s) led to clinical trials in the 1980s; however, despite the promise of ADCs and several decades of development, success has been limited until recently. As of 2021, there have been 12 ADCs approved for clinical use, all for oncologic indications, with a majority receiving approval in 2019 and onward (Table 1). For other applications, such as immunomodulation, limited exploration has occurred in recent years.[18] Clinical development has been terminated for over 55 ADCs[19]; these failures often stem from narrow therapeutic windows (i.e., the separation between toxic and efficacious doses is small or absent).[20] Designing and engineering the ADC to expand the therapeutic window is no simple task. Yet, despite these hurdles, enthusiasm for ADCs remains high, with over 80 ADC candidates in nearly 600 ongoing clinical trials.[19] This is driven by new ADC technologies (e.g., novel conjugation techniques, warhead types, improved selection, and optimization of antibodies), translational and clinical development strategies (e.g., alternative dosing schedules, patient selection, improved use of biomarker data, and combination therapies), and an improved understanding of ADC therapeutic index.[19,20] These approaches will contribute to the development of the next generation of ADCs.

Optimization of ADC design is complex, as each subunit (antibody, linker, and warhead) can be considered both individually and in the context of the ADC as a whole.

Selection of the antigen target and optimization of the mAb is crucial. A recombinant immunoglobulin G (IgG) mAb serves as the base of the ADC and vehicle for the cytotoxic drug. The target antigen for the antibody should be abundantly expressed on the surfaces of tumor cells, but not on other cell types.[20] The choice of the

**TABLE 1** List of approved ADCS

| Drug name | Maker | Indication | Trade name | Year approved | Antibody target | Warhead class | Warhead mechanism of action | Linker | Has published QSP model |
|---|---|---|---|---|---|---|---|---|---|
| Gemtuzumab ozogamicin | Pfizer/Wyeth | Relapsed CD33-positive acute myeloid leukemia | Mylotarg | 2000, approval withdrawn 2010, re-approved 2017 | CD33 | Calicheamicins | Targets minor groove of DNA and causes strand scission | AcBut linker (4-[4′-acetylphenoxy] butanoic acid) | No |
| Brentuximab vedotin | Seattle Genetics, Millennium/ Takeda | Relapsed Hodgkin lymphoma and relapsed systemic anaplastic large cell lymphoma | Adcetris | 2011, expanded conditions in 2017 and 2018 | CD30 | MMAE | Inhibits cell division by blocking the polymerization of tubulin | Protease (cathepsin) cleavable linker (valine-citrulline) | Yes[5,6] |
| Trastuzumab emtansine | Genentech/Roche | HER2-positive metastatic breast cancer | Kadcyla | February 2013 | HER2 | Maytansinoid | Binds at plus ends of cellular microtubules and thereby inhibits cell division in the target tumor cells | Succinimidyl trans-4-(maleimidylmethyl) cyclohexane-1-carboxylate | Yes[7–16] |
| Inotuzumab ozogamicin | Pfizer/Wyeth | Relapsed or refractory B-cell acute lymphoblastic leukemia | Besponsa | August 2017 | CD22 (mostly expressed on B-cells) | Calicheamicins | Targets minor groove of DNA and causes strand scission | AcBut linker (4-[4′-acetylphenoxy] butanoic acid) | Yes[17] |
| Moxetumomab pasudotox | AstraZeneca | Relapsed or refractory hairy cell leukemia | Lumoxiti | September 2018 | CD22 (mostly expressed on B-cells) | Pseudomonas exotoxin (PE38) | Inhibits elongation factor-2, preventing elongation of polypeptides | Immunoglobulin genetically joined to immunotoxin | No |
| Polatuzumab vedotin-piiq | Genentech/Roche | Relapsed or refractory diffuse large B-cell lymphoma | Polivy | June 2019 | CD79B | MMAE | Inhibits cell division by blocking the polymerization of tubulin | Protease (cathepsin) cleavable linker (valine-citrulline) | No |
| Enfortumab vedotin-ejfv | Astellas, Seattle Genetics | Locally advanced or metastatic urothelial cancer | Padcev | December 2019 | Nectin-4 | MMAE | Inhibits cell division by blocking the polymerization of tubulin | Protease (cathepsin) cleavable linker (valine-citrulline) | No |
| Trastuzumab deruxtecan | AstraZeneca, Daiichi Sankyo | Unresectable or metastatic HER2-positive breast cancer | Enhertu | December 2019 | HER2 | Topoisomerase I inhibitor | Blocks the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome | Protease (cathepsin) cleavable tetrapeptide-based linker | No |
| Sacituzumab govitecan | Immunomedics | Triple-negative breast cancer with relapsed or refractory metastatic disease | Trodelvy | April 2020 | Trop-2 | SN-38 (topoisomerase I inhibitor) | Blocks the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome | Hydrolyzable linker (azido-PEG-lysyl-p-amidobenzyl alcohol) | No |

**TABLE 1** (Continued)

| Drug name | Maker | Indication | Trade name | Year approved | Antibody target | Warhead class | Warhead mechanism of action | Linker | Has published QSP model |
|---|---|---|---|---|---|---|---|---|---|
| Belantamab mafodotin | GlaxoSmithKline | Relapsed or refractory multiple myeloma | Blenrep | August 2020 | B-cell maturation antigen (BCMA or CD269) | Maleimidocaproyl monomethyl auristatin F (mcMMAF) | Inhibits cell division by blocking the polymerization of tubulin | Protease-resistant maleimidocaproyl linker | No |
| Loncastuximab tesirine | ADC Therapeutics | Relapsed or refractory large B-cell lymphoma | Zynlonta | April 2021 | CD19 (expressed in wide range of B cell hematological tumors) | Pyrrolobenzodiazepine (PBD) dimer | Causes formation of crosslinks in DNA, which blocks cell division and causes apoptosis | Cathepsin B-cleavable valine-alanine linker | No |
| Tisotumab vedotin-tftv | Seagen | Recurrent or metastatic cervical cancer | Tivdak | September 2021 | Tissue factor | MMAE | Inhibits cell division by blocking the polymerization of tubulin | Protease (cathepsin) cleavable linker (valine-citrulline) | No |

*Note:* List of Approved ADCs. Twelve antibody-drug conjugates have been approved for use by the FDA as of the end of 2021, with a noticeable increase in approvals since 2017. However, many of these ADCs do not yet have a published QSP model.

Abbreviations: ADCs, ADC, antibody-drug conjugate; FDA, US Food and Drug Administration; MMAE, monomethyl auristatin E; QSP, quantitative systems pharmacology.

target antigen is key, as target-mediated drug disposition (TMDD) plays an important role in defining the pharmacokinetics (PK) of the overall ADC.[21] Whereas ADC-antigen binding generally triggers internalization and facilitates delivery of the warhead to the site of action inside the cell, non-internalized ADCs can still produce strong cell-killing of the target cells and neighboring cells (bystander effect) by warhead release. Although antitumor activity of the naked mAb is not necessary, in some cases, the mAb can activate an immune response against the selected cells through antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis. One example is trastuzumab emtansine (T-DM1), which has DM1 warheads attached to the mAb trastuzumab (approved as a treatment in its own right) that targets HER2 receptors in HER2-positive breast cancer. Therefore, the collective antitumor effects of both the mAb and the warhead must be taken into account in such instances. Once the target antigen has been selected, the mAb itself can be further engineered to improve payload delivery (particularly via enhanced control of linker placement on the mAb) and to have high target-binding affinity, good retention, and low immunogenicity and cross-reactivity.[22] Modifying the mAb's ability to bind to Fc receptors (most notably neonatal Fc receptors or FcRns) can also alter the therapeutic index.[23] ADCs can bind to FcRns inside endosomes, allowing for recycling of the ADC back to the cell surface where the higher physiologic pH triggers unbinding from the FcRn.[22] This recycling mechanism impacts the PK profile of the ADC by reducing ADC clearance, which can help to improve the therapeutic index.[24]

Synthetic, covalent, chemical linkers connect the mAbs to the cytotoxic warheads to form the ADCs, which typically have a drug-to-antibody ratio (DAR) between one and eight, although most clinical-stage ADCs have an average DAR of 3.5–4.[20] Stability of the linker is crucial, as the ADC must hold onto its payload while in systemic circulation, only releasing the warhead once inside the appropriate cell. Preventing deconjugation in the circulation reduces off-target toxicity and increases delivery of the drug to the tumor. Both cleavable and noncleavable linkers have been explored, each with its own set of advantages and disadvantages. ADCs with linkers that are cleavable, via lysosomal proteases, acidic pH, or breakdown of disulfide bridges, run a higher risk of off-target toxicity, but may still be active for targets with poor internalization, whereas ADCs with noncleavable linkers must be internalized, so that the mAb can then undergo proteolytic degradation to release the warhead for action.[25] Another important consideration is the position of the linker on the mAb; control over the linker position enables site-specific conjugation of the warhead, allowing

for increased homogeneity of an ADC's DAR and higher consistency in the amount of warhead delivered to target cells.

The cytotoxic agent (warhead) is a chemotherapy drug, optimized for high potency. As they lack specificity to tumor cells, warheads depend on the antibody to deliver them to the correct tissue. The mechanism of action of the drug used can vary, although many warheads bind to DNA or microtubules to cause cell death. These warheads can also serve as substrates for efflux transporters, which enable these drugs to escape the target cells and harm nearby healthy tissue (known as the bystander effect).[2] Whereas these bystander effects undercut the ADC's specificity and delivery of warhead to the target cells, they can also be beneficial, such as in solid tumors with heterogeneous expression of the target antigen, enabling the warhead to reach tumor cells that do not express the target antigen. Most ADCs currently in clinical trials use a limited number of drug families as warheads (calicheamicins, auristatins, maytansinoid, topoisomerase I inhibitors, and pyrrolobenzodiazepines), as the warhead must fulfill numerous and sometimes contradictory criteria, such as high potency, high relative hydrophobicity, and having a suitable location for attachment of the linker.[20] The potency of these warheads can be modified, as can the number of warheads per ADC (DAR). Determining the best combination of DAR and potency to maximize efficacy and minimize toxicity is a key challenge in designing the ADC.

In combining the antibody, linker, and warhead, the challenge is to maximize efficacy and minimize toxicity. This task calls for a deep understanding of the biological and pharmacological systems, processes, and mechanisms at play. Seeking answers through experimental methods alone can be laborious, expensive, or even infeasible. Computational modeling can probe questions and enhance insight through quantitative simulation of drug action and performance. Researchers have often used of PK and pharmacodynamic (PD) models, such as physiologically-based pharmacokinetic (PBPK) models, to aid in the drug development process. In particular, quantitative systems pharmacology (QSP) approaches integrate mechanistic knowledge with biomedical data at multiple scales to construct an interpretable and predictive model.[26,27] Hence, QSP models are tools that allow for maximum use of available preclinical and clinical data to improve understanding of the mechanism and derive hypotheses (Figure 2).

Due to the complexity of ADCs, the breakdown of an ADC molecule generates many different analytes, which can make data collection difficult. When using experimental data for parametrization, certain key analytes must be measured. Each of these different bioanalytical measurements are crucial to developing robust QSP models of ADCs. For instance, in order to define the PK and exposure-response relationships, it is recommended to measure the levels of either conjugated antibody (antibody with at least 1 warhead attached) or antibody-conjugated drug (total warhead conjugated to antibody), plus total antibody and unconjugated drug.[28] Typically, these analytes are measured in the plasma, tumor, and non-target tissues that are common sites of toxicity, as these measurements are important for determining therapeutic index and to model on-target and off-target effects.

Use of QSP approaches has increased in recent years, particularly to support decision making in drug development, drug approvals, and clinical practice.[29] A survey with respondents from over 30 pharmaceutical companies indicated the use of nonclinical QSP modeling in a majority of the companies in various therapeutic areas (with autoimmune disorders and oncology having the most QSP support), and this trend of increased QSP modeling applications is expected to continue.[30] Efforts to build QSP models of ADCs not only arise from biotechnology and pharmaceutical companies, but also from academic researchers, as well as academia-industry collaborations. Different types of models, including PK, PD, and spatially detailed models have been developed for different purposes and to answer different questions. In addition, they have been applied to understand various ADCs and to simulate different scenarios, including in vitro cell culture, preclinical animal experiments, and clinical trials in humans.

Previous reviews have described a variety of PK-PD models applicable to ADCs at the discovery, preclinical development, and clinical development stages of drug development.[31] In this review, we examine computational models of ADCs classified within the umbrella of systems pharmacology with a focus on mechanism-based models,[32] mainly those that build upon known cellular and intracellular processes of ADCs. Apart from one paper, we describe studies focused on modeling efficacy rather than toxicity.

We will highlight some of the key systems pharmacology models for ADCs developed in the past several years, describing model development and progression, key findings, and examples of model applications (Table 2). These models are organized in four key areas, grouped by their respective focuses, approaches, and insights (as noted in Figure 3): cellular mechanisms; spatial representation (including tumor heterogeneity); preclinical translation; and clinical translation. Several models cover more than one of these areas; where relevant, we have included them in more than one category, or focused mainly on their main contribution to one specific category.

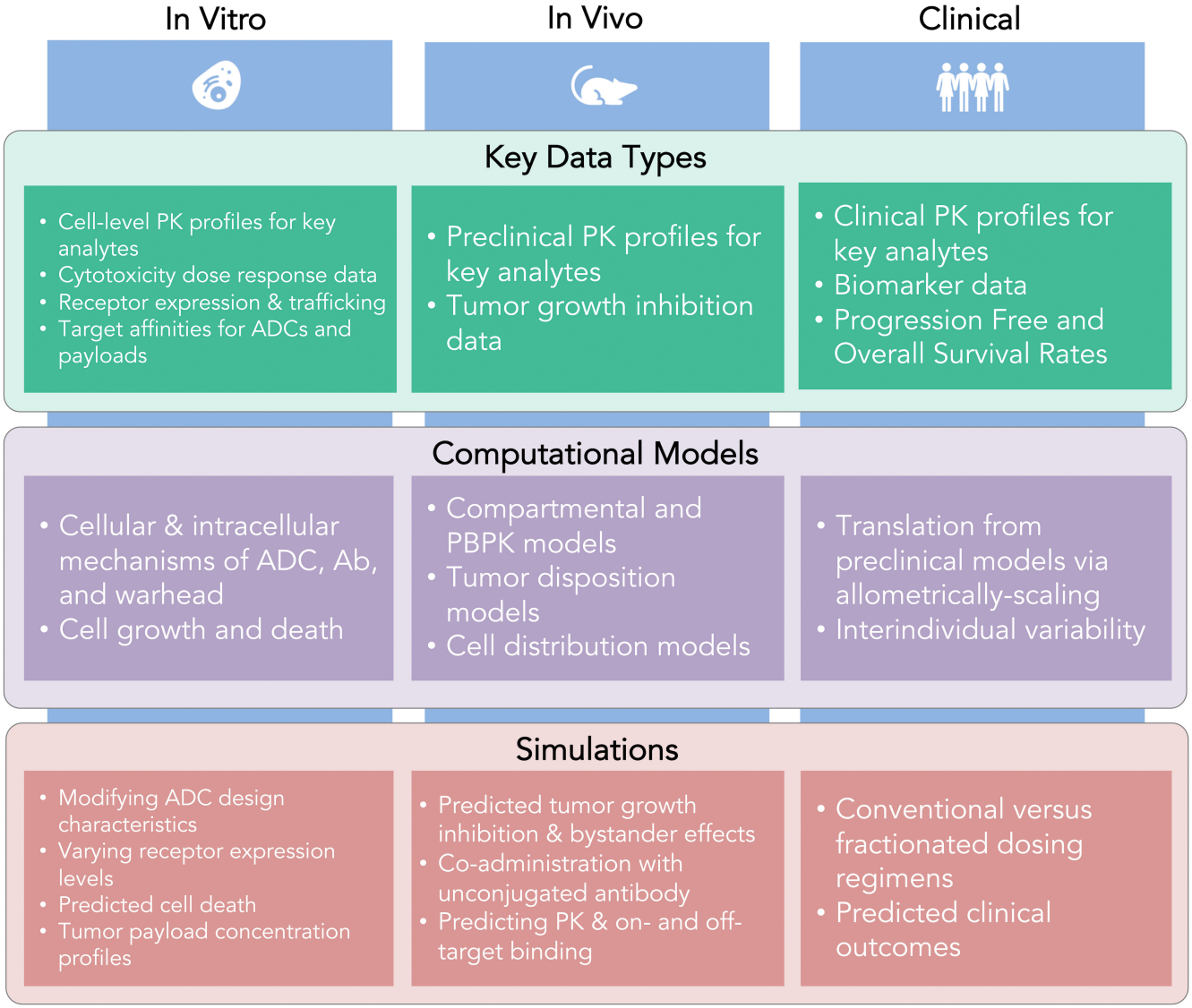


**FIGURE 2** Structure and key considerations for QSP modeling of ADCs. During QSP modeling of ADCs, the relevant data types may vary between different biological scales, as do the structures of the computational models themselves. Subsequently, the resulting simulations enable the exploration of different phenomena at the in vitro, in vivo, and clinical scales. Ab, antibody; ADC, antibody-drug conjugate; PBPK, physiologically-based pharmacokinetic; PK, pharmacokinetic.

## GLOSSARY OF MODELED ADCs

Anti-5T4 ADC (A1mcMMAF): an in-house ADC targeting 5T4, an oncofetal antigen expressed on tumor-initiating cells.

Brentuximab vedotin (SGN-35): CD30-targeting antibody linked to monomethyl auristatin E (MMAE) warheads via valine-citrulline linkers, used for treatment of relapsed Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL).

Inotuzumab ozogamicin: CD22-targeting antibody linked to N-Ac-γ-calicheamicin DMH molecules for targeting B cell malignancies such as non-Hodgkin's lymphoma (NHL) and acute lymphocytic leukemia (ALL).

Trastuzumab emtansine (T-DM1): HER2-targeting antibody covalently linked to emtansine (DM1) warheads approved for use to treat HER2+ breast cancer.

Trastuzumab-vc-MMAE (T-vc-MMAE or T-MMAE): consists of MMAE warheads conjugated to trastuzumab with valine-citrulline peptide linkers, often used as a tool ADC.

Trastuzumab maytansinoid: a HER2-targeting ADC similar to T-DM1 (DM1 is a cytotoxic maytansinoid), which is used clinically for treating HER2+ breast cancer.

Anti-STEAP1-vc-MMAE ADC (DSTP3086S): STEAP1-targeting antibody linked to monomethyl auristatin E (MMAE) warheads via valine-citrulline linkers, for targeting prostate cancer.

**TABLE 2** List of ADC QSP models

| Model | Ref | Title | Group | ADC modeled | Scale | Key insights |
|---|---|---|---|---|---|---|
| Shah et al. (2012) | [5] | Bench to bedside translation of antibody drug conjugates using a multiscale mechanistic PK/PD model: a case study with brentuximab-vedotin | Pfizer | Brentuximab-vedotin | In vitro/in vivo/clinical | This model is one of the first QSP models tailored for ADCs using cell-level mechanisms that lays the foundation more many future models, and provides a strategy for preclinical to clinical translation by using preclinical data to predict clinical response. Disposition of the ADC and payload were identified as key processes; for instance, drug efflux rate was found to be an important parameter that is often overlooked |
| Haddish-Berhane et al. (2013) | [7] | On translation of antibody drug conjugates efficacy from mouse experimental tumors to the clinic: a PK-PD approach | Pfizer | T-DM1 and an anti-5T4 ADC (A1mcMMAF) | In vivo/clinical | Comparison of three transduction models representing tumor growth inhibition enabled the development a hybridized model that could more accurately predict cell growth and killing. The authors also presented the "tumor static concentration" criteria that can be used as a measure of efficacy for an ADC |
| Shah et al. (2014) | [33] | A priori prediction of tumor payload concentrations: preclinical case study with an auristatin-based anti-5 T4 ADC | SUNY Buffalo, Pfizer | Anti-5T4 ADC (A1mcMMAF) | In vitro/in vivo | This is a mechanism-based PK model of A1mcMMAF (based on Shah et al. 2012) that can be used to predict tumor concentrations of the ADC and payload. The authors noticed that the sensitivity of several key model outputs is dose-dependent, and found that payload dissociation and tumor size were key parameters |
| Bender et al. (2014) | [8] | A mechanistic PK model elucidating the disposition of trastuzumab emtansine (T-DM1), an ADC for treatment of metastatic breast cancer | Genentech | T-DM1 | In vivo | Two PK modeling approaches using preclinical data were explored; the first approach incorporates stepwise deconjugation of the small molecule drug from the main trastuzumab body, and is one of the first models of ADC to do so. However, as this is very data-intensive, a second approach using a reduced model with a single deconjugation parameter was also proposed for situations when less analytical data is available |
| Vasalou et al. (2015) | [34] | A mechanistic tumor penetration model to guide ADC design | Novartis | General ADC framework | In vitro/in vivo | One of the most detailed mechanistic models for ADCs at the time, this ADC model framework includes ADC binding and payload release kinetics, receptor dynamics, systemic distribution, vascular permeability, and interstitial transport. The highly customizable nature enables parameters to be adjusted based on the characteristics of the ADC, target receptor, and tumor. The researchers found tumor attributes that could decrease ADC efficacy (e.g., high receptor expression causing a binding site barrier) and strategic ADC properties that could overcome them (e.g., using antibodies with slightly lower affinities to overcome this barrier) |

(Continues)

**TABLE 2** (Continued)

| Model | Ref | Title | Group | ADC modeled | Scale | Key insights |
|---|---|---|---|---|---|---|
| Maass et al. (2016) | [35] | Determination of cellular processing rates for a trastuzumab-maytansinoid ADC highlights key parameters for ADC design | MIT, Pfizer | Trastuzumab-maytansinoid ADC (TM-ADC) | In vitro | Researchers developed a set of generalizable techniques to parametrize a computational model of the cellular processing of ADCs, including ADC binding to the target antigen, receptor-mediated internalization, proteolytic ADC degradation, payload efflux, and payload binding to the intracellular target. The resulting kinetic model can be incorporated into larger PK-PD models as described in the companion paper (Singh et al. 2016a). Internalization and efflux rates were found to be key parameters that influence levels of payload delivery |
| Singh et al. (2016a) | [9] | Evolution of ADC tumor disposition model to predict preclinical tumor PKs of trastuzumab-emtansine (T-DM1) | SUNY Buffalo, MIT, Pfizer | T-DM1 | In vitro/in vivo | Using the parameters derived from the in vitro experiments as described in Maass et al. 2016, the authors integrated this cell-level mechanistic model with a tumor disposition model. They found that receptor-mediated endocytosis and passive diffusion contributed differently to intracellular drug exposure at the different scales, and that drug exposure in the system is sensitive to deconjugation and diffusion of the drug across the membrane of the tumor cell |
| Betts et al. (2016) | [17] | Preclinical to clinical translation of ADCs using PK-PD modeling: a retrospective analysis of inotuzumab ozogamicin | Pfizer, Janssen, Bristol-Meyers Squibb | Inotuzumab ozogamicin, a CD22-targeting ADC | In vitro/in vivo/ clinical | This multiscale, mechanism-based PK-PD model includes ADC disposition and clearance in the plasma and tumor, cellular-level mechanisms, and tumor growth and inhibition. Model analysis showed that tumor growth, ADC PK, and payload efflux to be sensitive parameters and potentially more useful than antigen expression as a predictor of outcome. Model simulations also showed that while a more conventional dosing regimen works well for NHL, fractionated dosing may provide improved results for ALL |
| Cilliers et al. (2016) | [10] | Multiscale modeling of ADCs: connecting tissue and cellular distribution to whole animal PKs and potential implications for efficacy | Univ. of Michigan | T-DM1 | In vitro/in vivo | This multiscale model is the first to integrate cellular mechanisms with a PB-PK model to characterize T-DM1. Notably, the tumor compartment was represented by a Krogh cylinder tissue model, enabling representation of tissue-scale distributions of ADCs and antibodies, which is not reflected in the typical "well-stirred" compartments in PBPK models. They found antibody co-administration can help to improve ADC penetration into the tumor, by overcoming the binding site barrier. An analysis of six publications suggested that at a constant dose of a sufficiently potent small molecule, ADCs with a lower DAR and higher antibody dose were generally more successful in reducing tumor growth than those with a with a higher DAR and lower antibody dose |

TABLE 2 (Continued)

| Model | Ref | Title | Group | ADC modeled | Scale | Key insights |
|---|---|---|---|---|---|---|
| Singh et al. (2016b) | 36 | Quantitative characterization of in vitro bystander effect of ADCs | SUNY Buffalo | Trastuzumab-vc-MMAE | In vitro | To explore the rate and extent of the bystander killing in a heterogeneous system, the authors used a co-culture experimental system and discovered a positive correlation between bystander effects and increased receptor expression levels, a substantial time delay before bystander killing occurred in the antigen negative cells, and evidence that bystander killing may decrease as the population of antigen positive cells shrinks. Based on this data, they developed a novel PD model to predict these bystander effects, integrating cell distribution models that represented the antigen positive and negative cells in the system |
| Sukumaran et al. (2017) | 37 | Development and translational application of an integrated, mechanistic model of ADC PKs | Genentech | DSTP3086S (anti-STEAP1-vc-MMAE) | In vitro/in vivo/clinical | This mechanism-based platform model to predict PK behavior of MMAE-based ADCs includes DAR-dependent clearance and explicit representation of all DAR species for the ADC, including sequential deconjugation as a higher DAR converts to a lower DAR species; the model showed that as DAR increases, antibody clearance increases sharply. The authors integrated rodent and cynomolgus monkey PK profiles into a cross-species model, which successfully captured PK profiles of the different analytes, as well as measurements from a phase I clinical trial following allometric scaling of appropriate parameters |
| Singh and Shah (2017a) | 11 | Application of a PK-PD modeling and simulation-based strategy for clinical translation of ADCs: a case study with trastuzumab emtansine (T-DM1) | SUNY Buffalo | T-DM1 | In vivo/clinical | Using the PK-PD modeling approach described in Betts et al. 2016 along with the preclinical tumor disposition model from Singh et al. 2016a, the authors developed a translated PK-PD model and conducted a case study with T-DM1, simulating clinical trials to predict PFS and ORRs. The simulated results were comparable to those from three separate trials, and suggested that a fractionated dosing regimen may provide a more substantial improvement in ORR than increasing the clinically approved dose |
| Ait-Oudhia et al. (2017) | 6 | A mechanism-based PK-PD model for hematological toxicities induced by ADCs | Univ. of Florida, SUNY Buffalo | Brentuximab vedotin (SGN-35) and adotrastuzumab emtansine (T-DM1) | In vivo | Researchers developed mechanism-based PK-PD models to assess the hematological toxicities of T-DM1 and SGN-35, building two compartmental models with linear elimination and first order payload release, which were able to accurately reflect the PK profiles and ADC-induced hematological toxicities of both ADCs. They also simulated the effects of the linker design on the associated myelosuppression by changing the payload release rate constant, which found hematotoxicity may be improved by a fourfold increase in the deconjugation rate of T-DM1, or a 70% decrease in that of SGN-35 |

(Continues)

**TABLE 2** (Continued)

| Model | Ref | Title | Group | ADC modeled | Scale | Key insights |
|---|---|---|---|---|---|---|
| Singh and Shah (2017b) | [38] | Measurement and mathematical characterization of cell-level PKs of ADCs: a case study with Trastuzumab-vc-MMAE | SUNY Buffalo | Trastuzumab-vc-MMAE | In vitro | To quantify the cell-level PKs of the tool ADC T-vc-MMAE, the authors conducted cellular disposition studies in low-HER2 and high-HER2 expressing cell lines, using three main analytical methods to measure concentrations for three key analytes (unconjugated drug, total drug, and total antibody). They used this extensive data to estimate rates for payload influx, efflux, and ADC intracellular degradation, building a novel single-cell disposition model to describe the three key analytes. Their global sensitivity analysis revealed ADC internalization and degradation rates, HER2 expression, and payload efflux to be key parameters influencing intracellular MMAE exposure |
| Khera et al. (2018) | [12] | Computational transport analysis of ADC bystander effects and payload tumoral distribution: implications for therapy | Univ. of Michigan | Trastuzumab-vc-MMAE and T-DM1 | In vitro/in vivo | Building on Cilliers et al. 2016, this computational model focuses on ADC solid tumor distribution and bystander effects, predicting payload distribution as a function of antibody dose, payload dose, and payload properties. The team found that direct cell killing (via receptor-mediated ADC uptake) to be more efficient than bystander killing, though the properties of the payload are an important factor in determining this. The model can be used to identify the optimal ADC dosing and payload physiochemical properties to improve delivery throughout the tumor and maximize efficacy |
| Shah et al. (2018) | [13] | Establishing IVIVC for ADC efficacy: a PK-PD modeling approach | SUNY Buffalo, Pfizer | 19 different ADCs, including T-DM1 and others with similar mechanisms of action | In vitro/in vivo | Data for 19 ADCs were used to establish an IVIVC between the in vitro and in vivo efficacy of an ADC. The authors developed a simple PK-PD model characterized using experimental data to calculate the TSC at both the in vitro and in vivo scales. The in vitro and in vivo TSCs had a positive linear relationship, and were used to establish the IVIVC, which can be used to rapidly identify promising early-stage ADC candidates and help to optimize the design of preclinical studies |
| Singh and Shah (2019) | [39] | A "Dual" cell-level systems PK-PD model to characterize the bystander effect of ADC | SUNY Buffalo | Trastuzumab-vc-MMAE | In vitro | To examine the in vitro bystander effects of ADC, the authors developed a cell-level systems PK-PD model for two cell lines (high and low HER2 expressing) by integrating their previously published cell-level PK model (Singh and Shah 2017b) to the cell-distribution PD model (Singh et al. 2016b). The models for both cell types were mechanistically integrated to describe the bystander effects, and the subsequent dual model was able to reasonably reflect the observed experimental data, suggesting that a similarly high tubulin occupancy by MMAE was required to achieve the desired cytotoxic effects in both cell lines |

TABLE 2 (Continued)

| Model | Ref | Title | Group | ADC modeled | Scale | Key insights |
|---|---|---|---|---|---|---|
| Singh et al. (2019) | 40 | A cell-level systems PK-PD model to characterize in vivo efficacy of ADCs | SUNY Buffalo | Trastuzumab-valine-citrulline-monomethyl auristatin E (T-vc-MMAE) | In vitro/in vivo | By integrating the previous single-cell PK-PD model (Singh and Shah, 2019) with tumor distribution, the group developed an in vivo systems PK-PD model that similarly predicts T-vc-MMAE efficacy as a function of intracellular target occupancy. The high-HER2 expressing tumors had higher exposures to total trastuzumab, unconjugated MMAE, and total MMAE compared to the low-HER2 expressing tumors, as well as higher tubulin occupancy. However, the plasma PK of all ADC analytes and prolonged retention of MMAE were similar between both tumor types |
| Singh et al. (2020a) | 14 | Antibody co-administration as a strategy to overcome binding-site barrier for ADCs: a quantitative investigation | SUNY Buffalo, Univ. of Michigan | T-DM1, T-vc-MMAE | In vitro/in vivo | Using two trastuzumab-based ADCs (one with and one without bystander effects), the researchers conducted in vivo experiments and developed a semimechanistic PK-PD model to evaluate the effects of ADC doses with antibody co-administration (at 1, 3, or 8-fold higher antibody) or without. Co-administration improved efficacy in tumors with high antigen expression levels, but had limited or negative effect on tumors with lower antigen expression and for ADCs with bystander effects |
| Menezes et al. (2020) | 15 | An agent-based systems pharmacology model of the ADC kadcyla to predict efficacy of different dosing regimens | Univ. of Michigan | T-DM1 | In vitro/in vivo | This hybrid agent-based model is the first QSP model of ADCs to incorporate heterogeneity in the tumor microenvironment, including variation in blood vessel density. The model shows that antibody carrier doses can increase efficacy when the additional cells reached by the ADC overcome the diminished payload uptake caused by the presence of the unconjugated antibody. Fractionated dosing is shown to be less effective than a single dose for co-administration, but it can be useful when the increased tolerability is needed |
| Sharma et al. (2020) | 41 | Evaluation of quantitative relationship between target expression and ADC exposure inside cancer cells | SUNY Buffalo | T-vc-MMAE | In vitro | To study the link between antigen expression levels and ADC exposure in tumor cells, the authors measured the PK profiles and internalization rates of T-vc-MMAE, and receptor expression for four different HER2-expressing cell lines. The data was used to calibrate their previous cell-level systems PK model (Singh and Shah 2017b) by fitting intracellular degradation rates for two cell lines. They found a strong linear correlation between HER2 expression levels and ADC exposure in tumor cells, and an inverse relationship between HER2 expression level and internalization rate |

(Continues)

**TABLE 2** (Continued)

| Model | Ref | Title | Group | ADC modeled | Scale | Key insights |
|---|---|---|---|---|---|---|
| Singh et al. (2020b) | [42] | Evolution of the systems PK-PD model for ADCs to characterize tumor heterogeneity and in vivo bystander effect | SUNY Buffalo | T-vc-MMAE | In vitro/in vivo | The researchers used a joint experimental-computational approach to explore the significance of heterogeneous bystander effects of ADCs in vivo by conducting mouse tumor xenograft studies at varying ADC dosages, measuring plasma and tumor PK as well as tumor growth inhibition. This systems PK-PD model was built upon their previous models to account for different cell populations and revealed that fractionated dosing may improve ADC efficacy and bystander effect |
| Menezes et al. (2022) | [16] | Simulating the selection of resistant cells with bystander killing and antibody co-administration in heterogeneous human epidermal growth factor receptor 2–positive tumors | Univ. of Michigan | T-DM1, T-MMAE | In vitro/in vivo | The authors extended their previous hybrid agent-based model to incorporate angiogenesis, heterogeneous receptor expression, tumor cell sensitivity to payloads, and bystander payload that can diffuse to surrounding cells. Using this model, they investigated the effectiveness of co-administration of unconjugated antibody with ADC, as well as bystander killing. Simulations using this model showed both T-DM1 and T-MMAE benefitted from co-administration, including in tumors with intrinsic resistance to the payload. Additionally, whereas co-administration was particularly effective for payloads without bystander effects, such as T-DM1, this benefit was reduced with lower receptor expression |

*Note*: List of ADC QSP Models. A total of 23 models are covered in this review. Whereas the selected models are not exhaustive, it provides a comprehensive overview of the key insights gained from QSP models thus far.

Abbreviations: ADC, antibody-drug conjugate; ALL, acute lymphocytic leukemia; DAR, drug-to-antibody ratio; IVIVC, in vitro-in vivo correlation; MMAE, monomethyl auristatin E; NHL, non-Hodgkin's lymphoma; ORR, objective response rate; PBPK, physiologically-based pharmacokinetic; PD, pharmacodynamic; PFS, progression-free survival; PK, pharmacokinetic; TSC, tumor static concentration; QSP, quantitative systems pharmacology.

| Model | PK | PD | Cellular Mechanisms | Spatial Representation | Preclinical & Clinical Translation |
|---|---|---|---|---|---|
| Shah et al 2012 | 2 compartment model | Cell distribution model | ADC-Ag binding, internalization, efflux | Krogh cylinder model | Model values derived from mouse and patient data |
| Vasalou et al 2015 | 2 compartment model | Hill equation with proliferating and quiescent cells | Binding, internalization, receptor & ADC-Ag trafficking/recycling, degradation, efflux, influx | Krogh cylinder model | Parameterized for mouse xenograft |
| Cilliers et al 2016 | PBPK model | N/A | ADC-Ag binding, internalization, degradation, receptor recycling | Krogh cylinder model | Parameterized for mouse xenograft |
| Menezes et al 2020 | 2 compartment model | Cell death represented by Michaelis-Menten Equation | ADC-Ag binding, internalization, degradation, efflux, receptor recycling | 2D agent-based model | Parameterized for mouse xenograft |

**FIGURE 3** Characteristics of selected of systems pharmacology models of ADCs. Here, we highlight four examples from the 23 models covered in this review, for which key model characteristics are listed for comparison. In addition to exploring the PK and PD aspects of these models, we will focus on insights gained in four categories as noted on the figure: cellular mechanisms, spatial representation, preclinical translation, and clinical translation. The selected models each contributed significant insights in at least one of these categories, exemplifying the variety of insights that can be gained from QSP modeling. ADC, antibody-drug conjugate; N/A, not applicable; PBPK, physiologically-based pharmacokinetic; PD, pharmacodynamic; PK, pharmacokinetic; QSP, quantitative systems pharmacology.

## DEVELOPMENT OF SYSTEMS PHARMACOLOGY MODELS

### Cellular mechanisms

#### Mechanistic modeling of brentuximab vedotin in cell culture[5]

One of the first system pharmacology models of ADCs was developed for the ADC brentuximab vedotin.[5] Using experimental data from multiple sources for calibration and verification, the model captured the PKs (i.e., distribution) of the ADC and of warhead at the cellular level both in vitro and in vivo, and was able to predict tumor warhead concentrations and tumor growth inhibition. The model of in vitro cell culture used simplifying assumptions for some mechanisms, such as representing the multiple steps of bound ADC internalization and release of intracellular warhead as a single step. The model also included extracellular ADC binding to the antigen, and extracellular warhead escaping from inside the cell. In vitro experiments were simulated using data from an existing study in two CD30+ cell lines, and the simulated results were compared to data from a separate experimental study. In later models and publications, more mechanistic detail was added, as we will see below. We will also discuss this paper further in the *Clinical Translation* section.

#### Comparing and refining pharmacodynamic models of cell growth and killing[7]

Researchers developed refined models of cell killing by comparing three existing representative PD models of tumor growth inhibition.[7] These models represent tumor volume in a series of transit compartments to link the PKs to the tumor growth response. The existing models had differing cell growth and killing functions, but none fully captured the patterns seen in the data. Thus, the authors proposed new hybrid functions based on these three models, combining exponential, linear, and logistic cell growth and a saturable Michaelis–Menten equation for cell killing. They also introduced the concept of "tumor static concentration" (TSC) to represent the minimum inhibitory concentration (i.e.,

the concentration of drug at which tumor size neither grows nor shrinks). The TSC criteria acts as an efficacy index and was calculated for the existing models and for the novel hybrid models. This optimized PD model was later incorporated into several future ADC QSP models.[11,13,17] This paper is discussed further in the *Clinical Translation* section.

## Assessing tumor penetration using a customizable model platform with more detailed ADC receptor trafficking[34]

In 2015, Vasalou et al. developed a mechanistic ADC model framework that includes ADC binding and payload release kinetics, receptor dynamics, systemic distribution, vascular permeability, and interstitial transport.[34] This model incorporated more detailed mechanisms of receptor trafficking than most models at the time, including intracellular trafficking between endosomes and lysosomes, recycling of the ADC-receptor complex, and release of the warhead into the cytosol. The inclusion of these mechanisms allowed the authors to study ADC efficacy as a function of payload cleavage and intracellular kinetics. For instance, simulations demonstrated that ADCs with endosomal rather than lysosomal warhead release had elevated payload concentrations, leading to increased shrinkage of the tumor. Whereas these simulations were conducted for a generic ADC, the model is designed to be highly customizable, with parameters that can be adjusted based on the characteristics of the ADC, target receptor, and tumor. This flexibility enables the model to serve as a platform for better interpretation of experimental data, selection of tumor properties, and optimization of ADC design. This detailed mechanistic model was paired with a Krogh cylinder model to describe solid tumor penetration in a mouse model; the spatial components are discussed below in the *Spatial Effects* section.

## Experimental techniques to parameterize computational models with cellular and intracellular mechanisms for trastuzumab maytansinoid[35]

As models become more detailed, experiments are needed to identify parameters. The authors developed a set of generalizable techniques to parametrize a computational model of the cellular processing of ADCs, using trastuzumab maytansinoid (which is used clinically for treating HER2+ breast cancer) as the model ADC.[35] These methods were based on flow cytometry and fluorescence imaging, and were used to quantify the processes of ADC binding to target antigen, receptor-mediated internalization, proteolytic ADC degradation, efflux of the warhead, and effector complex formation via warhead binding to the intracellular target. The experiments were performed in three high-HER2-expressing cell lines: BT-474, NCI-N87, and SK-BR-3. The internalization, degradation, and efflux rate constants were identified, and following a local sensitivity analysis with 10% perturbations from the established parameters, they determined internalization and efflux rates to be key parameters that influence levels of warhead delivery. The resulting kinetic model of cellular-level processes can be incorporated into larger PK-PD models, and, indeed, were, as described in a companion paper[9] which we discuss in a later section below.

## Extending a PK-PD model of T-DM1 to incorporate more intracellular mechanisms, including ADC degradation and passive diffusion[9]

Using the parameters derived from the in vitro experiments, as described in the previous paper,[35] Singh et al.[9] used the model to characterize pharmacokinetics of T-DM1 in three HER2+ cell lines. The model also improved on the previous model[35] of ADC with the addition of more intracellular details, including intracellular ADC degradation and passive diffusion of unconjugated drug across tumor cells. This cellular model was integrated with a tumor drug disposition model, enabling the prediction of tumor warhead concentrations in the mouse xenografts. To quantify the ADC cellular processes, the authors analyzed the relative contribution of the antigen-mediated and passive diffusion pathways in producing unconjugated drug inside the cell. This analysis was performed for both the in vitro and in vivo systems, finding that receptor-mediated endocytosis and passive diffusion contributed differently to intracellular drug exposure at the different scales. Passive diffusion was the more prominent pathway in vitro, whereas receptor-mediated intake had a higher contribution in vivo. The global and local sensitivity analyses also showed that drug exposure in the system is sensitive to deconjugation and diffusion of the drug across the membrane of the tumor cell, which is consistent with the results found in this group's prior work. The authors also proposed an ideal system PK model for intracellular processing of ADCs, which involves more mechanistic details on specific intracellular compartments early endosomes, late endosomes, recycling endosomes, and lysosomes; however, the data to achieve this was not available.

## Exploring the effects of bystander killing and tumor heterogeneity using a co-culture system[36]

To better understand the rate and extent of the bystander killing in a heterogeneous system, this model focused on the HER2-targeting Trastuzumab-vc-MMAE (T-vc-MMAE) as an example of an ADC that exhibits bystander effects.[36] Using a co-culture system comprising HER2-negative cells (GFP-MCF7) and HER2-positive cells with different levels of receptor expression (NCI-N87, BT474, and SKBR3) to represent tumor heterogeneity, they identified a positive correlation between bystander effects and increased receptor expression levels (i.e., HER2-negative cells were more likely to be killed by bystander effects if the HER2-positive cells they were cultured with had higher levels of HER2). They also observed a substantial time delay before bystander killing occurred in the antigen-negative cells. Further analysis of the co-culture system also suggested that bystander killing may decrease as the population of antigen positive cells shrinks. Based on these data, they developed a novel PD model to capture bystander effects, integrating cell distribution models that represented the antigen-positive and -negative cells in the system. This model could be integrated with a systems PK model for ADCs to link the systemic ADC concentrations and predict the outcomes from bystander effects.

## Cellular PK model of trastuzumab-vc-MMAE suggests that intracellular exposure of the warhead is dictated by antigen expression, internalization, degradation, and efflux[38]

Singh and Shah sought to quantify the cellular PK of the HER2-targeting ADC trastuzumab-valine-citrulline-monomethyl auristatin E (T-vc-MMAE), which consists of MMAE warheads conjugated to trastuzumab with valine-citrulline peptide linkers.[38] Conducting cellular ADC disposition studies in low-HER2 expressing (GFP-MCF7) and high-HER2 expressing (NCI-N87) cell lines, they incubated the cells with MMAE or T-vc-MMAE for 2 h, and used three main analytical methods to measure unconjugated drug, total drug, and total antibody concentrations (liquid chromatography–tandem mass spectrometry, a forced deconjugation method, and an enzyme-linked immunosorbent assay respectively). Although similar levels of MMAE accumulated in both cell lines following MMAE exposure, the NCI-N87 cells had much higher intracellular exposure of MMAE following T-vc-MMAE exposure. This extensive data allowed them to estimate MMAE influx rates, MMAE efflux rates, and T-vc-MMAE intracellular degradation rates, and to develop a novel single-cell drug disposition model to describe the three analytes (unconjugated drug, total drug, and total antibody). Their global sensitivity analysis revealed ADC internalization and degradation rates, HER2 expression, and MMAE efflux to be key parameters that dictated intracellular exposure to MMAE. This single-cell model provided a solid foundation for further exploring the bystander effects of ADCs, as demonstrated in further studies by this group.[39,41]

## Building a cell-level systems PK-PD model to describe in vitro bystander effects using intracellular target occupancy[39]

As an extension of their previous cellular ADC disposition study,[38] Singh and Shah developed a cell-level systems PK-PD model to examine the in vitro bystander effects of ADCs, using T-vc-MMAE, which is known to have bystander effects, as the representative ADC.[39] These bystander effects are often desirable in a heterogeneous tumor environment, allowing for improvement of the overall ADC efficacy in cells with different target receptor expression levels. The team conducted in vitro experiments in high-HER2 expressing cells (NCI-N87), low-HER2 expressing cells (GFP-MCF7), and co-cultures with both cell lines to study these bystander effects. PK-PD models with cellular mechanisms were developed for each cell type by integrating their previously published cell-level PK model[38] to the cell-distribution PD model,[36] and the simulations captured the intracellular target (tubulin) occupancy following exposure to T-vc-MMAE. The PK-PD models for both cell types were then mechanistically integrated to describe the bystander effects, and the subsequent dual model was able to reasonably reflect the observed experimental data, demonstrating that a similarly high tubulin occupancy by MMAE was required to achieve the desired cytotoxic effects in both cell lines. Compared to previous models that explored bystander effects, the single-cell framework for this model enables multiple cell populations to be represented, and can be incorporated with a tumor drug disposition model to predict bystander effects in vivo.

## Optimizing parameters for an existing cell-level systems PK model for trastuzumab-vc-MMAE[41]

Sharma et al. measured the PK profiles and internalization rates of T-vc-MMAE, and receptor expression for four different HER2-expressing cell lines (with differing expression levels) to study the relationship between antigen expression levels and ADC exposure in

tumor cells.[41] Using these data to calibrate the cellular PK model previously developed by their group,[38] the authors fitted intracellular degradation rates for two cell lines (SKBR-3 and MDA-MB-453). They found a strong linear correlation between HER2 expression levels and ADC exposure in tumor cells, and an inverse relationship between HER2 expression level and internalization rate. This inverse relationship may be due to the increased recycling of the HER2 complexes in high HER2-expressing cell lines as compared to low HER2-expressing cell lines, as seen in another experimental study.[43]

## Spatial effects

Some of the models discussed previously include a spatial component to the model,[5,34] typically to describe drug penetration in a solid tumor. Most of these models used Krogh cylinder geometry to represent drug distribution from a cylindrical blood vessel into a surrounding idealized cylinder of tumor tissue, based on previously published models.[44,45] The Krogh cylinder model enables representation of tissue-scale distributions of the ADC and antibodies, which is not reflected in the typical homogenous or "well-mixed" compartments found in most compartmental or PBPK models. These spatial effects are further explored into the following models.

### Using a customizable model platform with a Krogh cylinder model to explore the effects of tumor vascularization and the binding site barrier[34]

As an example of insights gained from these spatial models, the Vasalou 2015 model[34] discussed in the *Cellular Mechanisms* section incorporated detailed mechanisms of receptor trafficking paired with Krogh cylinder geometry, varying the Krogh cylinder radius to simulate tumors with differing levels of vascularization. They found that given the same ADC dose, tumors with higher degrees of vascularization can be reduced more quickly than tumors with less vascularization. Through their simulations, the researchers identified tumor attributes that would contribute to decreased ADC efficacy, and also tested ADC design scenarios to overcome these barriers. As an example, high receptor expression levels in the tumor can cause a "binding site barrier" when there is also rapid internalization and low recycling rates – in other words, the ADC cannot penetrate as deeply into the tumor because it binds to (and is internalized by) cell-surface receptors close to the vasculature. However, antibodies with slightly lower affinities may allow for "looser" binding to overcome the "binding site barrier," and therefore penetrate deeper in the tumor.

### Investigating antibody-ADC co-administration to enhance tumor penetration of T-DM1[10]

Cilliers et al. developed a multiscale model of T-DM1, integrating cellular mechanisms with a PBPK-based model to characterize the systemic drug disposition kinetics and heterogeneous tumor distribution of this ADC.[10] The model was developed using experimental data on ADC distribution in mouse xenograft models. At the cellular scale, the model includes binding, internalization, and degradation of both the ADC and unconjugated mAb. This was incorporated into a PBPK model that tracks systemic distribution of the ADC and mAb, and was validated experimentally. The tumor compartment was represented by a Krogh cylinder tissue model with permeability and diffusion. This was the first group to use this model to examine spatial effects of tumor drug disposition alongside the effects of co-administration of ADC with unconjugated mAb; the unconjugated mAb was administered alongside the ADC at varying ratios both in silico and in vivo using immunofluorescence imaging. The authors found that such carrier doses can significantly help to improve penetration of the ADC into the tumor by overcoming the binding site barrier. Additionally, they explored the effects of DAR on tumor penetration by analyzing data from six publications, finding that the effect was sufficiently large such that at a constant dose of a sufficiently potent small molecule, ADCs with a lower DAR and a higher co-administered antibody dose were generally more successful in reducing tumor growth than those with a higher DAR and lower antibody dose; DAR-dependent clearance and deconjugation may also be key contributors to this phenomenon. Used in conjunction with experimental data, this model can aid in exploring and understanding the impacts of the multiple mechanisms behind ADCs.

### Using computational models to identify the optimal ADC dosing and warhead properties and assess the role of bystander effects on ADC efficacy[12]

Khera and colleagues expanded on their previous computational model[10] to focus on ADC distribution within solid tumors and the role of bystander effects

on efficacy.[12] The model predicts warhead distribution as a function of antibody dose, warhead dose, and warhead properties. In particular, as heterogeneous tumor distribution of the ADC is linked to decreased efficacy, increasing the antibody dose can increase tumor penetration, which decreases the heterogeneity of drug concentration and increases the resulting efficacy. By simulating warheads with bystander effects (MMAE) and those without (DM1), the team also found direct cell killing (via target antigen-mediated uptake of ADC) to be more efficient than bystander killing, although the properties of the warhead (including lipophilicity, molecular weight, radius, diffusivity, half-life, Damköhler number, and reported bystander effects) are an important factor in determining whether it will be effective for bystander killing. Thus, this model can be used to identify the optimal ADC dosing and warhead physiochemical properties to improve delivery throughout the tumor and maximize efficacy.

## Antibody co-administration may be synergistic in tumors with high antigen expression but not in those with low antigen expression[14]

Earlier models had explored antibody co-administration with ADCs to improve tumor penetration[10] but had not explored the specific scenarios in which this strategy would be most beneficial. To quantitatively explore ADC-antibody co-administration as a method to overcome the binding site barrier phenomenon, researchers conducted in vivo experiments and QSP modeling using T-DM1 and T-vc-MMAE.[14] Whereas both ADCs have trastuzumab as the antibody carrier, T-vc-MMAE is known to exhibit bystander effects while T-DM1 does not. Tumor growth inhibition data from mouse xenograft models carrying high HER2 (NCI-N87 cells) and low HER2 (MDA-MB-453 cells) was used to build a semimechanistic PK-PD model to evaluate the effects of doses with trastuzumab co-administration (at 1, 3, or 8-fold higher antibody) or without. Using an interaction parameter to measure the benefit, the authors found the ADC interaction with the carrier dose was synergistic in high-antigen-expressing tumors, whereas in low-antigen-expressing tumors (and warheads that exhibit bystander effect), the interactions had an additive or less than additive benefit. Thus, the researchers conclude that whereas the ADC-antibody co-administration approach can be useful in improving ADC effectiveness in some situations, it should not be applied without a cost–benefit analysis.

## Agent-based model of T-DM1 to represent tumor heterogeneity and simulate antibody co-administration[15]

Menezes et al. developed a hybrid agent-based model to capture the effects of different T-DM1 treatment regimens on a tumor subsection.[15] The model includes central and peripheral tissue compartments, with tumor cells as individual agents on a grid system undergoing cell division and both natural and drug-induced cell death. Notably, this is the first systems pharmacology model of ADCs to not only capture drug PK-PD and cell dynamics, but also incorporate heterogeneity in the tumor microenvironment, including variation in blood vessel density. This contrasts previous ADC models that used the Krogh cylinder model to represent the tumor compartment; which both can portray the heterogeneous tissue distribution of the ADC, Krogh cylinders reflect a homogenous tumor cell population, whereas the agent-based model enables cell-level heterogeneity in the microenvironment and vasculature to be included. Much like the Cilliers 2016 model,[10] the researchers also explore the use of a trastuzumab carrier dose in conjunction with T-DM1 to improve ADC tumor disposition. The model shows increased efficacy in instances where the increased number of cells reached by the ADC overcomes the diminished uptake of the warhead caused by the presence of the unconjugated antibody, which matches experimental data from NCI-N87 mouse xenograft tumors. Additionally, whereas fractionated dosing is shown to be less effective than a single dose for co-administration, it can be useful when the increased tolerability enables a higher ADC dosage.

## Expanding the agent-based model to quantify the effectiveness of antibody co-administration and bystander killing[16]

Recently, Menezes et al. extended their hybrid agent-based model described above to incorporate angiogenesis, heterogeneous receptor expression, heterogeneous tumor cell sensitivity to payloads, and bystander effects (for payloads that can diffuse to surrounding cells).[16] Using this model, the researchers investigated the effectiveness of co-administration of unconjugated trastuzumab and ADC (for T-DM1 and T-MMAE), as well as bystander killing (for T-MMAE only). Simulations using this model showed both T-DM1 and T-MMAE benefitted from antibody co-administration, including in tumors with intrinsic resistance to the payload. Additionally, whereas co-administration was particularly effective for payloads without bystander effects, such as T-DM1, this benefit is

receptor-expression-dependent, and the antibody carrier dose may even inhibit tumor cell killing at sufficiently low receptor expression levels. These results are consistent with the findings of Singh et al.[14] Model predictions also showed that at clinically tolerable doses, regimens with greater efficacy are more likely to result in resistant cell populations, emphasizing the need to seek alternative cell-killing mechanisms that will increase the durability of the treatment effect.

## Preclinical translation

### A preclinical, mechanism-based pharmacokinetic model of an anti-5T4 MMAF ADC identified key parameters or features associated with drug exposure[33]

The model of anti-5T4 ADC (A1mcMMAF) was described in a 2014 paper in which the authors detailed the development of a mechanism-based PK model to predict tumor concentrations of the ADC and warhead, using experimental data from MDA-MB-435/5T4 and H1975 human tumor xenografts in mice for model building and verification.[33] They conducted a pathway analysis and local sensitivity analysis to determine parameters with the largest effect on the system, and found that payload dissociation and tumor size were key parameters affecting cytotoxic drug exposure in both the plasma and tumor. The authors also noticed that the sensitivity of several key model outputs is dose-dependent. Thus, this model showed the importance of quantification to improve the understanding of the processes driving ADC and warhead disposition, and can be further developed for clinical translation given the appropriate parameters, data, and translational strategy, as discussed in their previous work.[5]

### Using analytical data to model stepwise deconjugation of warheads from the T-DM1 ADC[8]

To better understand the PKs of T-DM1, particularly warhead release and the effects of DAR, Bender et al. developed two modeling approaches using preclinical PK data from rats and cynomolgus monkeys.[8] First, they built a mechanistic PK model of total trastuzumab and DAR concentrations with three compartments – a central and two peripheral compartments. Notably, this is one of the first models of ADC to incorporate stepwise deconjugation of the small molecule drug from the main trastuzumab body, starting from a DAR value of seven all the way to DAR zero (unconjugated trastuzumab). However, this model requires extensive amounts of experimental data, including measurements of T-DM1 at each of the intermediate DAR moieties, in order to identify the rate constants for each step of the deconjugation process. To lower the data burden, they created a reduced three-compartment model, fit to total trastuzumab and T-DM1 concentrations, with the warhead deconjugation represented by a single deconjugation parameter; this reduced model may be useful when data for the individual DAR moieties are not available. Depending on the situation, these two approaches provide more flexibility based on the analytical data available for the ADC.

### A mechanism-based platform model to predict PKs of MMAE-based ADCs using DAR-specific analytes and DAR-dependent clearance[37]

Researchers developed a mechanism-based platform model to predict the PK behavior of MMAE-based ADCs, which can be used as a valuable tool for exploring mechanisms behind ADC disposition for translational predictions.[37] Much like a previous model for T-DM1,[8] this model included DAR-dependent clearance and explicit representation of all DAR species for the ADC, including sequential deconjugation as a higher DAR converts to a lower DAR species. They integrated rodent and cynomolgus monkey PK profiles into a cross-species model, which successfully captured PK profiles of the different analytes – total antibody (including both unconjugated antibody and conjugated antibody), drug-conjugated antibody (antibody with at least one conjugated drug molecule), and/or antibody-conjugated drug (drug that is conjugated to an antibody), simulating administration of both purified ADCs with defined DAR species and ADCs with mixtures of DAR. Additionally, the model predictions for human PKs of an anti-STEAP1-vc-MMAE ADC (DSTP3086S) matched well with the PK measurements from a phase I clinical trial. Thus, they were able to develop this model with ADC disposition mechanisms and apply it to datasets with different payload densities, ADC molecules, animal models, and analyte measurements.

### Using mechanism-based PK-PD models to examine hematological toxicities of ADCs and simulate effects of linker design[6]

Whereas efficacy has been a major consideration in modeling of ADCs, toxicity is a central but less-studied phenomenon, central to translation to use in the clinic. T-DM1 and brentuximab vedotin (SGN-35) are both known

to induce ADC-related thrombocytopenia and neutropenia. To understand these hematological toxicities, using data from literature and mouse xenograft PK and PD studies, researchers built compartmental models (with central and peripheral compartments) with linear elimination and first order payload release.[6] These mechanism-based models were able to accurately reflect the PK profiles and ADC-induced hematological toxicities of both ADCs. They also simulated the effects of the linker design on the associated myelosuppression by changing the payload release rate constant, and by this showed that hematotoxicity may be improved by a fourfold increase in the deconjugation rate of T-DM1, or a 70% decrease in that of SGN-35. This model can serve as a platform for assessing hematological toxicities of ADCs, and shows more generally that toxicity should not be ignored in modeling to focus solely on efficacy.

## Developing a mathematical correlation between in vitro and in vivo ADC efficacy to improve identification of potential ADC candidates[13]

Researchers used data for 19 ADCs to establish an in vitro-in vivo correlation (IVIVC) between the in vitro and in vivo efficacy of those ADCs.[13] They developed a PK-PD model (similar to their previous models[5,7] but less mechanism-based) to characterize in vitro cytotoxicity data from HER2-expressing NCI-N87 cells and used it to calculate the "in vitro tumor static concentration" ($TSC_{in\ vitro}$), a theoretical concentration of continuous ADC exposure at which the number of tumor cells will remain static. For the 19 ADCs tested, the $TSC_{in\ vitro}$ values were found to be between 0.1 and 100 nM. Similarly, the "in vivo tumor static concentration" ($TSC_{in\ vivo}$) was found by incorporating tumor growth inhibition data from murine human tumor xenograft models (also using NCI-N87 cells) into the PK-PD model. The $TSC_{in\ vivo}$ values for the 19 ADCs were approximately in the range of 5–1000 nM. Whereas the models were based on the respective cytotoxicity and tumor xenograft studies and matched the experimental data well, it is difficult to compare the full parameter sets for the models to evaluate the results and in vitro-in vivo relationship. Thus, the TSC values were used as a representative variable for the models' parameter estimates and to look at the correlation between the different ADC parameter sets. Although the average $TSC_{in\ vivo}$ was ~27 times higher than $TSC_{in\ vitro}$, there was a good positive linear correlation between the two, suggesting that $TSC_{in\ vitro}$ is predictive of $TSC_{in\ vivo}$ Thus, this IVIVC can be used to rapidly identify promising early-stage ADC candidates and predict efficacious in vivo ADC concentrations from in vitro data, which can help to optimize the design of these preclinical studies. However, the ADCs tested (which included T-DM1) all had warheads with similar mechanisms of action, so this approach needs to be verified for warheads with differing mechanisms of action.

## Extending the cell-level model to an in vivo systems PK-PD model to predict trastuzumab-vc-MMAE efficacy as a function of intracellular target occupancy[40]

Building upon their previous single cell PK model,[38] Singh et al. developed an in vivo system PK-PD model that similarly predicts T-vc-MMAE efficacy as a function of intracellular target occupancy.[40] This model integrated the previous single-cell PK-PD model with tumor distribution, and was validated using PK and efficacy data from mouse xenograft models with either high-HER2 expressing (NCI-N87) and low-HER2 expressing (GFP-MCF7) tumor cells. The NCI-N87 tumors had higher exposures to total trastuzumab, unconjugated MMAE, and total MMAE compared to the GFP-MCF7, as well as higher tubulin occupancy. However, the plasma PKs of all ADC analytes and prolonged retention of MMAE were similar between both tumor types, and the same set of PD parameters were used. This model was able to capture the in vivo PK data quite well and can serve as the framework for clinical translation of ADCs.

## Quantifying heterogeneous bystander effects in vivo using a systems PK-PD model of trastuzumab-vc-MMAE[42]

Singh et al. also used a joint experimental-computational approach to explore the significance of heterogeneous bystander effects of ADCs in vivo.[42] Using T-vc-MMAE as the model ADC, the researchers conducted mouse tumor xenograft studies (NCI-N87, GFP-MCF7, and co-culture) at varying ADC dosages, measuring plasma and tumor PK, as well as tumor growth inhibition. To account for the different cell populations found in the co-culture tumors, the authors expanded their previous tumor drug distribution model[38] and later integrated it with a PD model where ADC efficacy is driven by intracellular tubulin occupancy. This system's PK-PD model was built upon their previous models and was able to reproduce the results of the experimental data quite well, including the tumor growth profiles for multiple cell lines and dosages. They performed additional simulations to explore alternate dosing

regimens, and much like other simulations previously conducted, found that fractionated dosing may improve overall ADC efficacy and bystander effect by extending intracellular tubulin occupancy. This model provides a platform for quantification of in vivo bystander effects in a heterogeneous tumor.

## Clinical translation

### PK-PD simulations of brentuximab vedotin in cell culture, mice, and humans highlight the importance of ADC and warhead distribution in predicting clinical outcomes[5]

Along with the cellular mechanistic modeling of brentuximab vedotin discussed above,[5] the authors also modeled the PKs of the warhead MMAE and the ADC in a xenograft mouse using a two-compartment model to represent the plasma and tumor, which was integrated with a PD model representing tumor growth to describe the ADC's preclinical efficacy. The PK parameters were obtained from literature-measured values of plasma and tumor PK and ADC concentration-time profiles, whereas PD parameters were derived from tumor growth inhibition data. This preclinical PK-PD model was then translated to a clinical PK-PD model by adjusting model parameters to reflect clinically observed values, using clinical PK data from two different clinical trials. Resulting simulations were compared with clinical trial results, and accurately predicted tumor and plasma warhead concentrations, as well as progression-free survival (PFS) and complete response rates. Through a sensitivity analysis, the authors also identified the drug efflux rate to be an important parameter that is often overlooked. As one of the first ADC models with preclinical-to-clinical translation, this work highlights the importance of ADC and warhead distribution in helping to predict clinical outcomes.

### Comparing and refining PD models of cell growth and killing[7]

The hybrid PD model developed by Haddish-Berhane et al.[7] was used to predict efficacy of T-DM1 in patients based on efficacy in mice. The predicted efficacious dose range was comparable to clinical dosing data, and the same translational strategy was also applied to a novel in-house anti-5T4 ADC (the model for that ADC is described in more detail in the *Cellular Mechanisms* section). Considering the model performance for these two different ADCs, they proposed an improved PD model where the tumor static concentration criterion can be used more generally to predict clinical dosing of ADCs from mouse efficacy data.

### From mouse to human: Clinical translation of a multiscale, mechanism-based PK-PD model of inotuzumab ozogamicin[17]

Inotuzumab ozogamicin is a CD22-targeting antibody linked to N-Ac-γ-calicheamicin DMH molecules for targeting B cell malignancies, such as 'NHL and ALL. For this multiscale, mechanism-based approach,[17] the preclinical model was built with preclinical data, and included ADC disposition and clearance in the plasma and tumor; the cellular-level mechanisms of ADC-Ag binding and warhead release, binding, and efflux; and mouse xenograft tumor growth and inhibition. By integrating human PK profiles, antigen expression levels, tumor volumes, and tumor growth rates, the preclinical model was translated to the clinical scale. This clinical model was able to capture PFS rates observed in clinical studies, and model analysis showed that tumor growth, ADC PK, and warhead efflux to be sensitive parameters and potentially more useful than antigen expression as a predictor of outcome. The model for liquid tumors (ALL) was approximated by eliminating transport to the solid tumor used in NHL. Tumor warhead levels were found to be higher in patients with ALL than patients with NHL, which aligns with the increased accessibility of blood tumors (ALL) compared to solid tumors (NHL). Model simulations also showed that whereas a more conventional dosing regimen works well for NHL, fractionated dosing may provide improved results for ALL. This model can be a useful tool to predict clinical outcomes from preclinical data, and serves as a foundation to build other ADC models used for clinical translation, including many of the other models described.

### Applying preclinical to clinical translation of PK-PD models of T-DM1 to simulate clinical trials and potential dosing regimens[11]

Singh and Shah developed a general ADC PK-PD modeling and simulation strategy to address translation issues, including differences between preclinical and clinical tumors, by using human-specific parameters. This strategy has been applied to inotuzumab ozogamicin, as described previously.[17] Using this same approach along with their previous preclinical tumor drug disposition model,[9] the researchers conducted a similar case study using T-DM1, using tumor growth inhibition data from various mouse models to derive the efficacy parameters for the model.[11]

Combined with predicted human PK parameters (estimated via allometric scaling of monkey PK parameters) and clinically observed breast cancer tumor volume and growth parameters, a translated PK-PD model of T-DM1 was developed and used to simulate clinical trials to predict PFS and objective response rates (ORRs). The model worked well, and the predicted outcomes were comparable to those from three separate clinical trials. Model predictions suggested that increasing the clinically approved dose would only provide a limited improvement in ORR, a fractionated dosing regimen may provide a more substantial improvement in efficacy, which is consistent with earlier findings on this topic.[17] The authors hypothesized that this improved response resulted from the additional time for accumulation of the warhead in the tumor with the fractionated regimen, allowing more time for the cell killing effects to take place.

## DISCUSSION

Each of the models discussed above has areas of strength focusing on unique aspects of ADC biology and pharmacology. Together, they provide a solid foundation for computational modeling of ADCs. The complexity of the mechanisms included in the models increases as successive modeling papers built upon each other, with additional mechanistic detail, spatial effects, tumor heterogeneity, and bystander effects among the components explored in increasing detail. Some key collective insights include the importance of ADC and warhead distribution at the cellular and tumor scales to understanding overall ADC performance, the methods for preclinical to clinical translation using in vitro and in vivo data, and the variations in efficacy for novel dosing methods (such as carrier doses and fractionated dosing) depending on factors, such as antigen expression.

Although much progress has been made in QSP modeling of ADCs, there continues to be opportunities for further development in each of these areas and others, such as greater mechanistic detail at the intracellular level that can provide a more complete picture of the biological phenomena at work, deeper study into the effects of tumor heterogeneity, the full extent of bystander killing and healthy tissue sinks in humans, and modeling of ADC toxicity. Although this will require additional experimental data and collaboration, incorporating these features will increase our knowledge of the systems, processes, and mechanisms governing ADCs, leading to improved rational ADC design and patient treatment outcomes.

More recent models generally have an increasing level of mechanistic detail due to availability of more detailed bioanalytical data, particularly on the intracellular level and for interaction between the warhead and the site of action. For instance, the role of physiological pH can be taken into account in the model parameters, as some warheads can become more or less active at differing pH levels, such as the open versus closed lactone forms for camptothecins.[46] Additionally, more mechanistic detail can be included in the warhead influx and efflux kinetic processes at the tumor cell membrane. In particular, active transport is difficult to measure and thus is often overlooked in current models; in the future, specific drug transporters, such as P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP) could be incorporated for relevant cell lines. Furthermore, any potential impact of drug–drug interactions on tumor cell penetration (via bystander activity) can also be considered. Bystander killing has been explored in several of the aforementioned models, denoting its importance to ADC efficacy and toxicity. As more detailed experimental measurements become available, more detailed mechanistic models can be developed to provide a more complete and robust representation of the system.

The importance of the immune system in cancer is well known.[47] These interactions have been explored in QSP models for other immuno-oncology therapies.[48] However, this has not yet been incorporated into QSP models of ADCs thus far. Integrating ADC models with existing immune system models may help to investigate immune system effects on ADCs and vice versa.[49,50]

Although ADCs can look extremely promising in preclinical experiments, one of the most challenging aspects of ADC development is the lack of understanding of the underlying differences between humans and animal models, which can cause ADCs to fail in the clinical phase despite earlier success in preclinical studies, leading to wasted time and resources. In most cases, mouse xenograft data has been used for preclinical in vivo modeling, although some models incorporate data from multiple species.[37] Some models also used IVIVC metrics as a method to assist in predicting drug performance earlier in the drug development process.[13] Further work can be done to explore the interspecies differences that need to be accounted for during preclinical to clinical translation to better predict the clinical efficacy of early-stage ADCs.

Failure of ADCs in the clinic often results from the inability to reach the efficacious dose prior to the onset of dose limiting toxicities (DLTs). However, most QSP modeling efforts for ADCs thus far have generally been restricted to efficacy modeling; the lack of toxicity modeling for ADCs is currently a gap in the field. Developing QSP models focused on understanding ADC toxicity will be crucial to minimizing toxic side effects and expanding the therapeutic window.

Due to availability of data and interest, most published QSP models for ADCs thus far are developed for approved

ADCs, with T-DM1 being the most well-studied, along with other trastuzumab-based ADCs or those with tubulin inhibitors, such as MMAE. Therefore, although the specific drugs focused on in these models may be different, the findings and methodologies can still be applied to the decision-making process for future ADCs undergoing the drug development process. Moving forward, researchers can incorporate QSP modeling for ADCs in earlier stages of the drug development process, which can allow for added insights earlier on in the discovery and design process (e.g., when evaluating in vitro efficacy and toxicity of an ADC). Predictive models can help us simulate clinical outcomes with preclinical data. This cannot only help researchers to identify key mechanisms and processes, but also avoid potential pitfalls to steer the direction of ADC development earlier in the process, from informing the design of the ADC itself, to proposing dosing regimens that enable improved efficacy or less toxicity. Similarly, building models for ADCs that have failed in clinical trials can help us gain a better understanding of why an ADC did not perform as expected.

QSP models are valuable in saving time, effort, and resources during the drug development process. This can include narrowing down therapeutic candidates during the discovery phase, predicting clinical efficacy from preclinical data to focus on the likely best candidates, or simulating many different dosing regimens to identify optimal strategies during clinical development. The ability to run simulations in silico allows researchers to test scenarios that may be impractical, expensive, or infeasible to perform experimentally. Compared to traditional PK-PD modeling, QSP models contain more mechanistic detail and therefore enable nuanced insights into the underlying biology that cannot be gained through PK-PD modeling alone. Complex molecules, like ADCs that have multiple design levers, and key contextual considerations that are critical to the ADC's performance (e.g., tumor heterogeneity, bystander killing, target expression, etc.), require detailed mechanistic modeling to accurately quantify the processes involved and facilitate translation to human settings where data is difficult to generate. Investments in such QSP models enable a much deeper understanding of the ADC's interactions and the resulting efficacy and toxicity, leading to more informed decision making and improved therapy design.

## CONCLUSION

System pharmacology models of ADCs have evolved greatly in recent years, from empirical and semimechanistic PK-PD models, towards more complex, more integrated, and more mechanism-based models. Modeling efforts from both academic and industry groups have helped to quantify and provide insights into the ADC mechanisms and observed phenomena, by simulating the effect of key ADC design parameters, characterizing PK and biodistribution characteristics, quantifying bystander killing, and simulating novel dosing regimens. Future models that account for factors such as immune response may further improve in their ability to predict efficacy and toxicity of ADCs. Moving forward, these models will continue to be very important tools to support design of ADCs, enable preclinical to clinical translation, facilitate faster development, and ultimately develop safer and more effective ADCs.

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## CONFLICT OF INTEREST

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