REVIEW

Quantitative Clinical Pharmacology of T-Cell Engaging Bispecifics: Current Perspectives and Opportunities

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T-cell directing/engaging bispecifics (TDBs) enable a powerful mode of action by activating T-cells through the creation of artificial immune synapses. Their pharmacological response involves the dynamic inter-relationships among T-cells, tumor cells, and TDBs. This results in complex and challenging issues in understanding pharmacokinetics, tissue distribution, target engagement, and exposure-response relationship. Dosing strategy plays a crucial role in determining the therapeutic window of TDBs because of the desire to maximize therapeutic efficacy in the context of known mechanism-related adverse events, such as cytokine release syndrome and neurological adverse events. Such adverse events are commonly reported as the most prominent events during the initial treatment cycles and dissipate over time. Therefore, the kinetic characterization of the inter-relationships between exposure/target engagement and safety/efficacy outcomes is crucial in designing the optimal dosing regimen to maximize the benefit/risk of TDB agents. In this review, we discuss the key clinical pharmacological considerations in drug discovery and development for TDBs and provide a summary of TDBs currently in clinical development. We also propose forward-looking perspectives and opportunities to derive insights through quantitative clinical pharmacology approaches.

Advancements in antibody engineering and recent clinical successes have led to enthusiasm for the development of bispecific modalities with the unique ability to bind to two distinct antigens or two different epitopes on the same antigen.1 T-cell directing/engaging bispecific agents (TDBs), in particular, are rapidly becoming an important class of molecules in oncology drug development. These agents enhance recruitment of effector cells (e.g., cytotoxic T-cells) to tumor-associated/specific antigens for targeted cell killing (Figure 1a,b). Like other T-cell engaging therapies, TDBs engage the host’s T-cells thereby driving deep and durable clinical response beyond treatment termination.2 However, unlike chimeric antigen receptor T-cell therapies,3 which take weeks from collection of patients’ T-cells to availability of treatment product, TDBs are available off-the-shelf.

Based on a search with the key word “T-cell bispecific” and its variations in literature and on ClinicalTrials.gov (up until December 2019), we summarized a listing of TDBs currently in clinical development for hematological (Table 1) and solid malignancies (Table 2).1,4 We found 64 TDBs encompassing broad formats ranging from small proteins to full-length immunoglobulin G (IgG) monoclonal antibodies. Information on the developers, tumor targets, molecular formats, disease areas, and clinical trial information for these TDBs is provided.

Clinical efficacy of a TDB is presumed to be driven by the synaptic complex concentration (Figure 1a,b). As such, target engagement depends on its pharmacokinetics (PK), the cellular kinetics and trafficking of T-cells and tumor cells (e.g., B-cell or plasma cells for hematological malignancies), drug-specific parameters (e.g., relative binding affinities to T-cells and tumor cells, intrinsic activity), and system-specific parameters (e.g., target expression levels and turnover). The bispecific binding properties also impact tissue distribution/disposition, which could differ from that of typical therapeutic antibodies.5

A key challenge in the clinical development of TDBs is significant clinical toxicities including cytokine release syndrome (CRS) and neurotoxicity, which are a result of the cascade of immune activation and cytokine release associated with the mechanism of action (MOA) (Figure 1c).6,7 Such toxicity is reversible and time-dependent, and typically most prominent upon first administration and less pronounced with subsequent dosing.8 Although the patient-level risk factors are still being elucidated, aggressive disease and higher tumor burden are suspected to be contributing factors.7 These on-target safety concerns and their unique time-dependency and concentration-dependency represent opportunities to optimize the dosing regimen to maximize the therapeutic window and treatment potential of TDBs. Novel clinical dosing approaches have been implemented in clinical trials that mitigate these acute cytokine-driven toxicities, including various forms of dose fractionation or step-up dosing strategies, pretreatment with target-depleting agents, and administration of corticoid and/or immunosuppressive agents.6,8 Furthermore, the novel pharmacology of TDBs offers unique opportunities to leverage quantitative clinical pharmacology (QCP) approaches to understand the dynamic interplay between the TDB, tumor, and immune system.

In this paper, we focus our discussion on key drug development considerations from a clinical pharmacology perspective. We also propose forward-looking perspectives and opportunities to derive insights through quantitative clinical pharmacology.
TARGET ENGAGEMENT

Target engagement of TDBs involves the formation of a trimolecular complex consisting of the TDB simultaneously bound to both effector cell and tumor-associated antigens and is presumed to drive the pharmacological effect (i.e., T-cell activation and proliferation, and subsequent tumor killing) through creating an immunological synapse (Figure 1a,b). Unlike traditional therapeutic modalities, which bind to a single target and whose dose/exposure-response (E-R) can typically be described by a nonlinear, Michaelis–Menten binding kinetics, TDBs have a complex E-R relationship that depends on multiple factors. These include drug-specific factors (binding affinity; i.e., $K_d$) for each target and intrinsic activity/potency of the tri-molecule synapse and system-specific factors (target expression, effector:target ratios, effector cell concentration, and potency). Based on stoichiometric principles in a closed system, one expects a bell-shaped E-R curve, where trimolecular complexes increase with TDB concentrations.

Figure 1 Visual schematic of immune synapse, mechanism of action, dose-response relationships for efficacy and safety of T-cell directing bispecifics (TDBs). (a) The TDB target engagement is characterized by the simultaneous binding of the TDB to the tumor-associated antigens, which are expressed on tumor cells and to CD3, which is expressed on T-cells. The trimolecular entity (TDB, T-cells, and target cells) forms an immune synapse, which activates the T-cells. (b) The activated T-cells release cytotoxic granules, such as granzyme B and perforin, leading to tumor cell death. (c) The activated T-cells also release various cytokines, such as TNF-α, INF-gamma, IL-2, and IL-6, which trigger a cascade of immune activation including the activation of macrophages and monocytes and the release of additional cytokines. (d) The therapeutic window of TDBs can be defined by the exposure-response relationships for efficacy (as a result of cytotoxicity) and safety (as a result of systemic cytokine release). Upon repeated dosing of TDBs, the release of proinflammatory cytokines (TNF-α and INF-gamma) from T-cells decreases, and thus the dependency of safety on exposure reduces. This time-dependent and repeat-dose dependent characteristic provides an opportunity to use various dosing strategies (e.g., step-up dosing) and broaden the therapeutic window of TDBs.
Table 1 Summary of TDBs in clinical trials for hematologic malignancies

| T-BsAb | Developer | Tumor target | Format | Disease area | NCT number | Phase |
|--------|-----------|--------------|--------|--------------|------------|-------|
| AMG 420, B1836909 | Boehringer Ingelheim, Amgen | BCMA | BITE | MM | 02514239/03836053 | I/Ib not yet recruiting/recruiting |
| AMG701 | Amgen | BCMA | BITE | MM | 0328708 | I Recruiting |
| CC-93269 | Celgene | BCMA | BITE | MM | 03486067 | I Recruiting |
| JNJ-64007957 | Janssen | BCMA | BsmAb | MM | 03145181/04108195 | I/Ib Recruiting |
| PF-06863135 | Pfizer | BCMA | BsmAb | MM | 03269136 | I Recruiting |
| REGN5458 | Regeneron | BCMA | BITE | MM | 03761108 | I/I Recruiting |
| REGN5459 | Regeneron | BCMA | – | – | 04083534 | I/I Recruiting |
| TNB-383B | Teneobio, Inc. | BCMA | BsmAb | MM | 03933735 | I Recruiting |
| AMG424, Xmab13551 | Amgen | CD38 | BsmAb | MM | 03445663 | I Recruiting |
| GBR1342 | Glenmark Pharmaceuticals | CD38 | BsmAb | MM | 03339111 | I Recruiting |
| RG6160, RO7187797, BFCR4359A | Genentech | FcRH5 | BITE | MM | 03275103 | I Recruiting |
| JNJ-64407564 | Janssen | GPRO5D | BsmAb | MM | 03399799/04108195 | I/Ib Recruiting |
| Anti-CD3 X anti-CD20 bispecific T cells | Barbara Ann Karmanos Cancer Institute | CD20 | – | MM and plasma cell neoplasm | 00938626 | I Completed |
| APVO436 | Aptevo Therapeutics | CD123 | scFv-scFv | AML and MDS | 03647800 | I Completed |
| MGD006/S80880/Flotetuzumab | Macrogenics, city of Hope Medical Center, National Cancer Institute | CD123 | DART | AML, MDS, and CML | 02152956/03739606/04158739 | I Completed |
| JNJ-63709178 | Janssen | CD123 | BsmAb | AML | 02715011 | I/I/Ib not yet recruiting/recruiting |
| SAR40234 | Sanofi | CD123 | IgG1 + 2scFvs | AML, B-ALL, and MDS | 03594955 | I Recruiting |
| Vibeotamab, Xmab14045 | Xencor | CD123 | scFv-Fc (Fab)-fusions | AML, B-ALL, and CML | 02730312 | I Recruiting |
| AMG330 | Amgen | CD33 | BITE | AML | 02520427 | I Recruiting |
| AMV673 | Amgen | CD33 | BITE | AML | 03224819 | I Recruiting |
| AMV564 | Amphivena Therapeutics | CD33 | TandAb | AML and MDS, solid tumors | 03144245/03516591/04128423 | I Recruiting |
| JNJ-67571244 | Janssen | CD33 | BsmAb | AML and MDS | 03915379 | I Recruiting |
| GEM333 | GEMoaB Monoclonals | CD33 | BsmAb | AML | 03516760 | I Recruiting |
| Tepotitamab, MCLA117 | Merus | CLEC12A | BsmAb | AML | 03038230 | I Recruiting |
| AMG427 | Amgen | FLT3 | BITE | AML | 03541369 | I Recruiting |
| MGD011/JNJ-64052781 | Macrogenics, Johnson & Johnson | CD19 | DART | B cell Malignancies | 02743546 | I Recruiting |
| A-319 | Generon | CD19 | BsmAb | ALL and B-ALL | 04056975 | I Recruiting |
| AFM11 | Affimed | CD19 | TandAb | NHL and ALL | 02106091/02848911 | I Recruiting |
| AMG562 | Amgen | CD19 | BITE | Lymphoma | 03571828 | I Recruiting |
| blinatumomab, Blincyto, MT103, MEDI-538, AMG103 | Amgen | CD19 | BITE | ALL and B-ALL | Marketed | I Recruiting |
| GEN3013, DuoBody-CD3XCD20 | Gennab | CD20 | BsmAb | DLBCL, FL, and MCL | 03625037 | I Recruiting |

(Continues)
### Clinical Pharmacology of CD3 Bispecifics

**Table 1 (Continued)**

| T-Ab | Developer | Tumor target | Disease area | Format | Phase | NCT number | TDBs, T-cell targeting |
|------|-----------|--------------|--------------|--------|-------|------------|------------------------|
| mosunetuzumab, RG7828, RO703896 | Genetech | CD20 | NHL and DBCL | BmAb | I/I Recruiting | 02500407/02984022 | BTCT4465A |
| Plamotamab, XmAb13676 | Regeneron | CD20 | FL, CLL, and NHL | BmAb | I/I Recruiting | 02531239/02561021 | REGN1979 |
| glofitamab, RO7082859, RG6026 | Roche | CD20 | B cell lymphoma | BmAb | Terminated | 03533283/03467373/04077723 | FBTA05 |

**TandAb, TDBs, T-cell directing bispecifics.**

**Table 1 (Continued)**

| T-Ab | Developer | Tumor target | Disease area | Format | Phase | NCT number | TDBs, T-cell targeting |
|------|-----------|--------------|--------------|--------|-------|------------|------------------------|
| PBA214 | Xencor | CD20 | NHL and CLL | 21 asymm Fab | I/I Recruiting | 03888105/02842142/04258236 | PBA214 |
| Thomab, Quadrab | Fresenius | CD20 | B cell lymphoma | BmAb | Terminated | 01075598 | Thomab, Quadrab |

- **T-Ab**: T-cell directing bispecific antibody (TDB)
- **Format**: BmAb (bi-specific monoclonal antibody), Fab (antigen-binding fragment)
- **Phase**: I/II Recruiting, Terminated
- **Disease area**: NHL (non-Hodgkin lymphoma), CLL (chronic lymphocytic leukemia), SLL (small lymphocytic lymphoma)
- **Tumor target**: CD20, B-cell maturation antigen (BCMA), CD3, CD19

**Table 1 (Continued)**

| T-Ab | Developer | Tumor target | Disease area | Format | Phase | NCT number | TDBs, T-cell targeting |
|------|-----------|--------------|--------------|--------|-------|------------|------------------------|
| BTCT4466A | BTCT | CD20 | FL, CLL, and NHL | BmAb | I/I Recruiting | 02500407/02984022 | BTCT4466A |
| FBTA05 | Fresenius | CD20 | B cell lymphoma | BmAb | Terminated | 03533283/03467373/04077723 | FBTA05 |

**Table 1 (Continued)**

| T-Ab | Developer | Tumor target | Disease area | Format | Phase | NCT number | TDBs, T-cell targeting |
|------|-----------|--------------|--------------|--------|-------|------------|------------------------|
| PBAT5 | PBA214 | CD20 | NHL and DBCL | BmAb | Terminated | 02531239/02561021 | PBAT5 |
| Thomab, Quadrab | Fresenius | CD20 | B cell lymphoma | BmAb | Terminated | 01075598 | Thomab, Quadrab |

**QCP perspectives and opportunities**

The unique characteristics of target engagement pharmacology discussed above add complexity to molecule and dose optimization in discovery and development. Common dose finding approaches, such as dose escalation to maximal tolerated doses (MTDs) or target-binding saturation, may not be appropriate for TDBs. QCP modeling, which integrates in vitro, in vivo, and available clinical data, can help establish the proof-of-concept and inform the totality of dosing rationale for phase I studies.

**NONCLINICAL TO CLINICAL TRANSLATION AND FIRST-IN-HUMAN DOSE SELECTION**

For traditional therapeutic modalities, nonclinical toxicology and pharmacology studies support transition to clinical development and selection of first-in-human (FIH) doses using well-described approaches. However, FIH dose selection for TDB can be challenging due to the complexity of the pharmacology with dual binding and the immune-activating MOA. A recent US Food and Drug Administration (FDA) review of CD3 bispecific constructs determined that FIH dose selection using standard approaches based on receptor occupancy, highest nonseverely toxic dose, or no-observed adverse effect level, resulted in doses near or exceeding MTDs in clinics, and hence are not acceptable for these agents.

Conversely, dose selection based on the minimal anticipated biological effect level from sensitive in vitro experiments may be too conservative and result in subtherapeutic doses requiring several escalations to achieve pharmacological/clinical activity. Saber et al. proposed an FIH dose selection corresponding to 10–30%
### Table 2: Summary of TDBs in clinical trials for solid malignancies

| T-BsAb         | Developer                  | Tumor Target | Format          | Disease area                  | NCT number               | Phase            |
|----------------|----------------------------|--------------|-----------------|-------------------------------|--------------------------|------------------|
| AMG160         | Amgen                      | PSMA         | HLE-BiTE        | Prostate cancer               | 03792841                 | I Recruiting     |
| MOR209, APVO414, ES414 | Amgen                      | PSMA         | IgG1 + 2scFvs   | Prostate cancer               | 02262910                 | I Recruiting     |
| AMG160         | Amgen                      | PSMA         | HLE-BiTE        | Prostate cancer (castration resistant) | 03792841 | I Recruiting |
| Pasotuxizumab A212, BAY2010112 | Bayer                     | PSMA         | BiTE            | Prostate cancer               | 01723475                 | I Completed      |
| CC-1           | University Hospital Tuebingen | PSMA         | BsmAb           | Prostate cancer               | 04104607                 | I Recruiting     |
| GBR1302        | Glenmark Pharmaceuticals    | HER2         | BsmAb           | Her2 + cancers                | 02829372/03983395        | I/II Recruiting  |
| M802           | YZYBio                     | HER2         | –               | Breast and gastric cancer     | China                    | I Recruiting     |
| Ertumaxomab    | Fresenius Biotech North America | HER2         | Triomab, Quadroma | Breast cancer               | 01569412/00351858/00522457/00452140 | I Recruiting |
| RG6194, BTRC4017A | Genentech                  | HER2         | BsmAb           | Locally advanced or metastatic HER2-expressing cancers | 03448042 | I Recruiting |
| MGD009, Orilotamab | Macrogenics                | B7-H3        | DART            | NSCLC and melanoma            | 03448042                 | Ia/ib not yet recruiting/recruiting |
| Cislatamab, RG7802, RO6958688, CEA-TCB | Roche                  | CEA          | Crossmab        | NSCLC and other solid tumors  | 03337698/02650713/0234257 | I/II Recruiting  |
| AMG211, MEDI-565 | Amgen                      | CEA          | BTE             | Gastrointestinal adenocarcinoma | 01284231/02291614/02760199 | I completed      |
| AMG757         | Amgen                      | DLL3         | BTE             | SCLC                          | 033199040                | I Recruiting     |
| AMG596         | Amgen                      | EGFRVIII      | BTE             | EGFRVIII + Glioblastoma       | 03296696                 | I Recruiting     |
| A-337          | Generon                    | EpCAM        | BsmAb           | NSCLC                         | China                    | I Recruiting     |
| MT110, AMG110  | Amgen Research (Munich) GmbH | EpCAM        | BTE             | Metastatic colorectal, gastric, and lung cancers | 00635596 | I completed |
| catumaxomab    | Fresenius Biotech and Triom Pharma | EpCAM        | Triomab, Quadroma | Malignant ascites owing to epithelial carcinomas | 16 studies | Withdrawn from the market |
| hu3F8-BsAb     | Memorial Sloan Kettering Cancer Center | GD2         | BsmAb           | Neuroblastoma, osteosarcoma   | 03860207                 | I Recruiting     |
| GD2Bi-aATC     | University of Virginia     | GD2          | –               | Neuroblastoma, osteosarcoma   | 02173093                 | I/II Recruiting  |
| ERY974         | Chugui                      | GPC3         | BsmAb           | Gastric cancer and squamous cell esophageal carcinoma | 02748837 | I Recruiting |
| IMCgp100       | Immunocore Ltd             | gp100        | TCR + scFv      | Skin cancer melanoma, uveal melanoma | 01211262/02570308/03070392/02535078/02898861/01209676 | I/II |
| MGD007         | Macrogenics                | gpA33        | DART            | CRC                           | 03531632/02248805        | (Continues)      |
| T-BsAb | Developer | Tumor Target | Format | Disease area | NCT number | Phase          |
|--------|-----------|--------------|--------|--------------|------------|----------------|
| Activated CIK and CD3-MUC1 | Fuda Cancer Hospital, Guangzhou | MUC1 | – | Solid tumor cancer | 03501056 etc. | II Recruiting |
| REGN4018 | Regeneron | MUC16 | BsmAb | Ovarian, fallopian tube or peritoneal cancers | 03564340 | I Recruiting |
| AMG199 | Amgen | MUC17 | HLE-BITE | MuC17-positive gastric and gastroesophageal junction cancer | 04117958 | I not yet Recruiting |
| PF-06671008 | Pfizer | P-cadherin | DART | Neoplasms | 02659631 | Terminated |
| GEM3PSCA | GEMoAb | PSCA | – | PSMA positive cancer | 03927573 | I Recruiting |
| Tidutamab, XemAb18087 | Xencor | SSTR2 | BsmAb | Neuroendocrine and GIST | 03411915 | I Recruiting |

**Pharmacological Activity**

TDBs are therapeutic proteins that can be administered via iv, sc, and/or intra-tumoral routes. TDBs are also being explored as a step towards better patient convenience (with the short-term and long-term dynamics of the disease) and are given to patients with relapsed or refractory non-Hodgkin's lymphoma. Gen3013 showed promising results in preclinical studies. The development of TDBs is likely to occur as a step towards better patient convenience and is likely to occur as a step towards better patient convenience.

**PK CONSIDERATIONS**

PK considerations are critical in guiding the development of TDBs. Such insights are valuable in predicting the PK behavior of future TDBs. The preclinical-to-clinical translation approach is a valuable tool in enabling the continued assessment of PK properties of TDBs.

**QSP perspectives and opportunities**

Recently, Betts et al. published a quantitative systems pharmacology (QSP) model of the TDB (Table 2). The preclinical-to-clinical translation approach is critical in guiding the development of TDBs.
antibodies.20 Of note, s.c. administration of a TDB could lead to T-cell activation in lymph nodes, thus “first-pass” PK or pharmacodynamic (PD) effects could theoretically be possible and should be investigated. Estimation of absolute bioavailability and absorption rate for presystemic effect on PK or mechanistic modeling efforts (e.g., QSP or physiologically-based PK (PBPK) modeling) could provide further insights in this potential phenomenon.

Distribution
The distribution of TDBs varies and depends on the construct and relative affinities to effector and target cells. Population PK (PopPK) analysis of blinatumomab, a 54 kDa bispecific T-cell engager, consisting of two linked single-chain variable regions, revealed a volume of distribution of 3.40 L, similar to the plasma volume.21 Similarly, PopPK analysis of the full-length IgG-based CD20-CD3 bispecific glofitamab showed a central volume of distribution that approximates plasma volume, suggesting limited tissue distribution in the clinically relevant dose range.15 For TDBs with targets present in the tissue, the volume of distribution can be greater than the plasma volume. For full-length antibodies, extravasation to the tissue interstitial space is primarily driven by convection. For TDBs with an intact Fc region, transcytosis mediated by FcRn can also play a role in distribution.22 The distribution property of TDBs can also be highly dependent on molecule design and relative binding affinities to target tumor cells vs. effector T-cells. Mandikian et al. have shown that a higher binding affinity to CD3 shifts the distribution of HER2-CD3 bispecific antibodies away from tumor to T-cell-rich tissues.26 Distribution of TDBs within the tumor can be a significant source of response heterogeneity and tumor penetration is usually largely reduced in solid tumors.23 These drug-related and disease-related factors are important to consider in order to obtain a deeper understanding of the E-R relationships to inform discovery and development.

Elimination
TDBs are metabolized by the same catabolic pathways as endogenous proteins and are eliminated by nonspecific Fc receptor-mediated catabolism and/or TMDD. The clearance for TDBs is governed by their structure/molecular weight and factors impacting TMDD, such binding properties (affinity/avidity), target levels, circulating endogenous or exogenous targets, and turnover rates for soluble and/or membrane bound receptors. Therefore, dose-dependent and time-dependent PK is possible for TDBs. For example, PopPK of mosunetuzumab and REGN1979 have been characterized preclinically and/or clinically with a time-varying clearance, consistent with traditional anti-CD20 antibodies (e.g., rituximab and obinutuzumab), to represent target (B-cell) binding and associated target modulation with treatment.15,21 Higher TDB clearance may be anticipated for agents with higher CD3 affinity, as illustrated with the CLL-1/CD3 bispecific antibody.23 In general, PK covariate investigations should consider impacts of disease status (e.g., tumor burden and cachexia) and/or circulating competing agents on bispecific clearance. The relevance of nonlinear pathways also depends on the clinical dose/regimen and may not be universal for TDBs. For glofitamab, linear clearance alone was sufficient to describe its disposition, although this is potentially due to its unique dosing approach, which relies on single dose obinutuzumab (Gazyva) pretreatment (Gpt) for safety mitigation.13 The PK of blinatumomab on the other hand, was described by a linear one-compartment PK model. The interpatient variability for clearance was high (coefficient of variance of ~ 60%) with a multimodal distribution described by a mixture model.21 The small size of blinatumomab makes it susceptible to rapid catabolism and high clearance, resulting in a short half-life of ~ 2 hours and necessitating continuous i.v. infusion.21 Current research looks into further improving the PK properties of these fragment-based bispecific engagers by fusing with human serum albumin or the Fc part of an IgG molecule. For full-length bispecific antibodies, the clearance is typically reduced owing to an intact Fc region enabling FcRn-mediated recycling.12,13,20 However, the clinical half-lives can plausibly vary depending on the extent of TMDD for different molecule designs and target biology. For example, in patients with relapsed/refractory non-Hodgkin lymphoma, mosunetuzumabs have reported an apparent half-life of 6–11 days, whereas REGN1979 has a half-life of 2–3 days (increased to > 2 weeks at steady-state), although both are full-length antibodies targeting CD20 and CD3 antigens.26,27 The clinical relevance of prolonged half-lives for the efficacy and durability of immune-stimulatory (agonist-type) agents remains to be characterized. However, the enhanced half-lives for full-length antibodies have afforded these agents with convenient dose schedules of every 1–2 weeks for REGN1979 or every 3 weeks for mosunetuzumab and glofitamab, in contrast to the continuous infusion required for bispecific T-cell engager, such as blinatumomab.

QCP perspectives and opportunities
QCP approaches can be adopted to better understand PK characteristics in the physiological context to enhance the understanding and prediction of PK of TDB in humans. Investigation of nonclinical (e.g., mouse xenograft and cynomolgus monkey) or clinical biodistribution (e.g., novel imaging techniques using radiolabeled material) and elimination coupled with QCP approaches can further delineate distribution and elimination of TDBs. In one recent analysis, in vivo drug uptake in tumor tissues was predicted for immunocytokine bispecific (CEA-IL2v) using a PK/PD model that incorporates the expansion of target cells and associated TMDD, coupled with tumor imaging data collected in patients with cancer.28 PBPK modeling could be another valuable approach in describing the biodistribution and elimination of TDBs as a function of relative binding affinities within the physiological context of tissue-specific transport/elimination pathways. Several researchers have described the tissue distribution of T-cells using a PBPK framework in the mouse for ex vivo stimulated T-cells or nontransduced chimeric antigen receptor T-cells.29,30 These models can serve as good starting points toward building a full PBPK model for TDBs by incorporating the bispecific binding properties to T-cells and target cells. Similar to what was described for small molecules and traditional antibodies,
PBPK modeling can be similarly exploited to develop TDBs and quantitatively characterize its disposition in circulation and tissues, including at the sites of action. Furthermore, PBPK modeling adds valuable insights into clinical development questions, such as PK in special populations and drug interaction risks through TDB-induced cytokine elevation, as done for blinatumomab.

**IMMUNOGENICITY**
As TDBs represent therapeutic proteins, there exists a potential for immunogenicity. All the clinical examples highlighted above (blinatumomab, mosunetuzumab, and glofitamab) deplete antibody producing B-cells as part of their MOA, and, therefore, limited immunogenicity (< 2% for blinatumomab and none reported for mosunetuzumab or glofitamab) is observed. However, for TDBs targeting other antigens or with more complex formats, immunogenicity could arise and hamper PK/PD, safety, and/or efficacy. Because TDBs bind two targets, domain characterization should be conducted to identify the arm to which arising antibodies bind. This may provide insight into sources of toxicity, impairment of PK or PD, or efficacy. For example, antibodies arising to arms binding to target antigens could prevent binding to intended targets and impair efficacy, or theoretically provide crosslinking to activate arms engaging effector cells, leading to systemic toxicity.

**QCP perspectives and opportunities**
Integrated assessment of PK-PD-ADA response can add useful insights to inform dosing strategies. Campagne et al. developed an integrated translational PK/PD model for anti-CD3/CD123 bispecific antibody, flotetuzumab, which accounts for TMDD on the disposition of flotetuzumab by peripheral CD3 + T-cell activation and expansion, target dynamics, complex formation, as well as the loss of drug due to ADA development. Such integrated models are useful to put into context the potential relevance and risk of immunogenicity and can be translated across different species and/or disease/biological contexts.

**E-R CHARACTERIZATION AND CLINICAL DOSING IMPLICATIONS**

**E-R for efficacy**
The unique target engagement of TDBs leading to formation of trimolecular synapse can complicate E-R relationship for efficacy characterization. However, observed clinical data to-date suggests increases in efficacy with increasing dose/exposure and, in some cases, toward a plateau. Blinatumomab E-R analyses have revealed a positive relationship between steady-state concentrations and complete responses (CRs). Recent analyses by Dufner et al. also suggested durable remission and better median overall survival at the clinical MTD of 60 µg/m² per day compared with lower dose levels. A novel exposure metric, clinical CD20 receptor occupancy (RO%), was derived using mass action principles (i.e., TDB concentrations and in vitro CD20 binding affinities) and used to investigate E-R relationships for mosunetuzumab and glofitamab. This approach also accounts for competition for CD20 receptor binding from individual patient anti-CD20 antibody concentrations in circulation from either prior therapies or from Gpt as they bind to the same target epitopes. E-R analyses reveal significant and positive relationships between average CD20 RO% and complete responders toward a plateau in response to treatment, and with clinically meaningful efficacy observed at ≤ 1% CD20 RO% for both agents. These recent examples add to the increasing pool of knowledge in understanding the clinical dose/E-R relationships for emerging TDBs and experience in utilizing QCP analyses to derive clinical dosing regimens.

**E-R for safety**
Similar to traditional antibody therapy, clinical safety following TDB therapy largely depends on the target pharmacology. CRS is the most prevalent side effect, with IL-6 as a key mediator. Safety characterization reveals dose-dependent and time-dependent CRS occurring primarily upon initial treatment, which subsequently dissipates due to target depletion and/or immune desensitization post-treatment. This temporal pattern associated with CRS offers an opportunity to dissociate the drivers for safety from efficacy to broaden the therapeutic window of TDBs. Specifically, through QSP and E-R modeling of IL-6 and CRS events, implementation of step-up dosing, in which small but pharmacologically active doses associated with low CRS risk, are initially administered to reduce circulating target cells and/or invoke immune desensitization. Thereafter, high therapeutic doses are administered to achieve efficacy within the plateau of response; thus, enabling a QCP informed dosing approach for TDBs (Figure 1d). This has been successfully applied to mosunetuzumab to mitigate CRS, as evidenced by no apparent E-R relationship for CRS across a wide therapeutic dose range. Additional safety-mitigation approaches, such as the unique Gpt approach, have been applied for glofitamab, in which target B-cells are depleted by single dose obinutuzumab prior to administration of glofitamab. Notably, limited cytokine-mediated neurotoxicity has been observed for both mosunetuzumab and glofitamab, supporting the utility of these novel safety approaches. A potential combination of both safety-mitigation approaches could further yield beneficial effects and is being investigated for glofitamab. Collectively, through careful understanding of target pharmacology and with use of QSP and E-R modeling, QCP approaches could enable favorable safety profiles for novel TDBs. For TDBs in development for targets expressed in both tumor and healthy tissues, on-target off-tumor toxicities can play an important role in determining the therapeutic window. Model-based insights on E-R relationship across high-expressing vs. low-expressing tissues can provide critical insights into the dosing/regimen strategy.

**QCP perspectives and opportunities**
A semimechanistic PK/PD model was developed by Chen et al. to characterize in vivo cytokine profiles upon administration of TDBs after repeated dosing. In this model, the production of IL-6 was induced by synaptic complex formation, and a time-variant negative feedback loop was incorporated to capture the attenuation of cytokine peaks
following repeated doses. In most of the models mentioned in this review, T-cell dynamics was restricted to a single compartment without explicit representation of trafficking. Hosseini et al. introduced a QSP model that explicitly includes blood and lymphoid tissues, and trafficking of CD8 + T lymphocytes and target cells between these tissues; uses in vivo preclinical and clinical PK/PD data for model calibration and validation; and describes both safety (cytokines) and efficacy (target cell depletion) aspects of treatments with TDBs. Notably, the key factors for the successful application of QSP modeling, in this case, to inform the clinical development of mosunetuzumab, included: (1) the ability to establish the preclinical-to-clinical translation of the dynamics of immune cells (i.e., T and B cells) and IL-6 response, (2) the availability of a surrogate PD biomarker of IL-6 for inferences of clinical safety, and (3) the ability to foster a healthy learn-and-confirm cycle by incorporating key elements of model-informed dosing hypothesis in the design of phase I clinical dose finding. This approach was used to inform the step-up clinical dosing strategy used for mosunetuzumab and is being investigated for glofitamab. Recently, Jiang et al. developed an integrated PBPK-PD model to describe the cytokine release profile and target cell depletion of blinatumomab in various patient populations following different dosing regimens. Integrated PBPK-PD models illustrate the complex interaction between the TDB and its dual targets and can be envisaged to link its predicted target-site concentrations to outcomes and to understand response heterogeneity. Taken together, these integrated modeling approaches add multidimensional insights on the target engagement pharmacology and its relevance to clinical efficacy and safety. Although progress has been made to understand the drivers for efficacy and safety for TDBs, there remains knowledge gaps in terms of the optimal dosing regimen (i.e., frequency, duration, and dose levels/sequence) to induce efficacy in a durable and tolerable fashion. Further clinical data from alternative dosing regimens or routes of administration (e.g., subcutaneous) could shed further insights in the quest to maximize the therapeutic benefits of TDBs.

**SUMMARY AND CONCLUDING REMARKS**

TDBs represent exciting new approaches for cancer treatment. Their unique MOA, disposition properties, and the

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**Figure 2** Quantitative clinical pharmacology (QCP) approaches to inform preclinical and clinical drug development decisions. Depending on the context and the nature of drug development questions, different QCP approaches can be useful. As T-cell directing bispecifics (TDBs) move in the pipeline from early research to late stage clinical development, questions generally go from more mechanistic to more descriptive in nature. Mechanistic PK/PD and QSP modeling are useful to gain mechanistic insights and inform early dose selection. PBPK modeling can be used to understand tissue-specific PK and PD and for assessment of drug-interaction risks. Population PK/PD modeling can help with understanding the key PK characteristics and population-level covariates. Exposure-response modeling can help inform the relevant exposure drivers and covariates for safety and efficacy characterization. PBPK, physiologically-based pharmacokinetic; PD, pharmacodynamics; PK, pharmacokinetics; QSP, quantitative systems pharmacology.
diversity in structural formats open up great opportunities to leverage QCP approaches to integrate multidimensional data across molecules to promote learnings at a platform level. As summarized in Figure 2, various QCP approaches have been successfully leveraged to inform drug development questions at varying stages. It is an exciting time marked by the expanding use of new quantitative methodologies in drug development, such as machine learning, to gain insights across large datasets. The vision of model-informed drug development is that integration of models becomes routine in drug development. What remains critical is the acute ability to anticipate and define the “key questions,” which can only become meaningful through cross-functional conversations and collaborations. Furthermore, concerted efforts between regulators and drug developers can play a critical role in facilitating the use of QCP approaches to enhance the efficiency of drug development and to help design drugs with a better benefit/risk profile. The recent Model-Informed Drug Development regulatory initiative offers the opportunity for our QCP community (sponsor and regulatory) to utilize the outlined perspectives and opportunities to optimize the development of these complex agents.

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Conflict of Interest. At the time of writing the manuscript, the authors were all employees of Roche/Genentech and also own stock/stock options. Peter N. Morcos is currently an employee of Bayer AG (Whippany, NJ, USA) and was employed by Bayer AG at the time of manuscript submission.

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