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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.[6,9] 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

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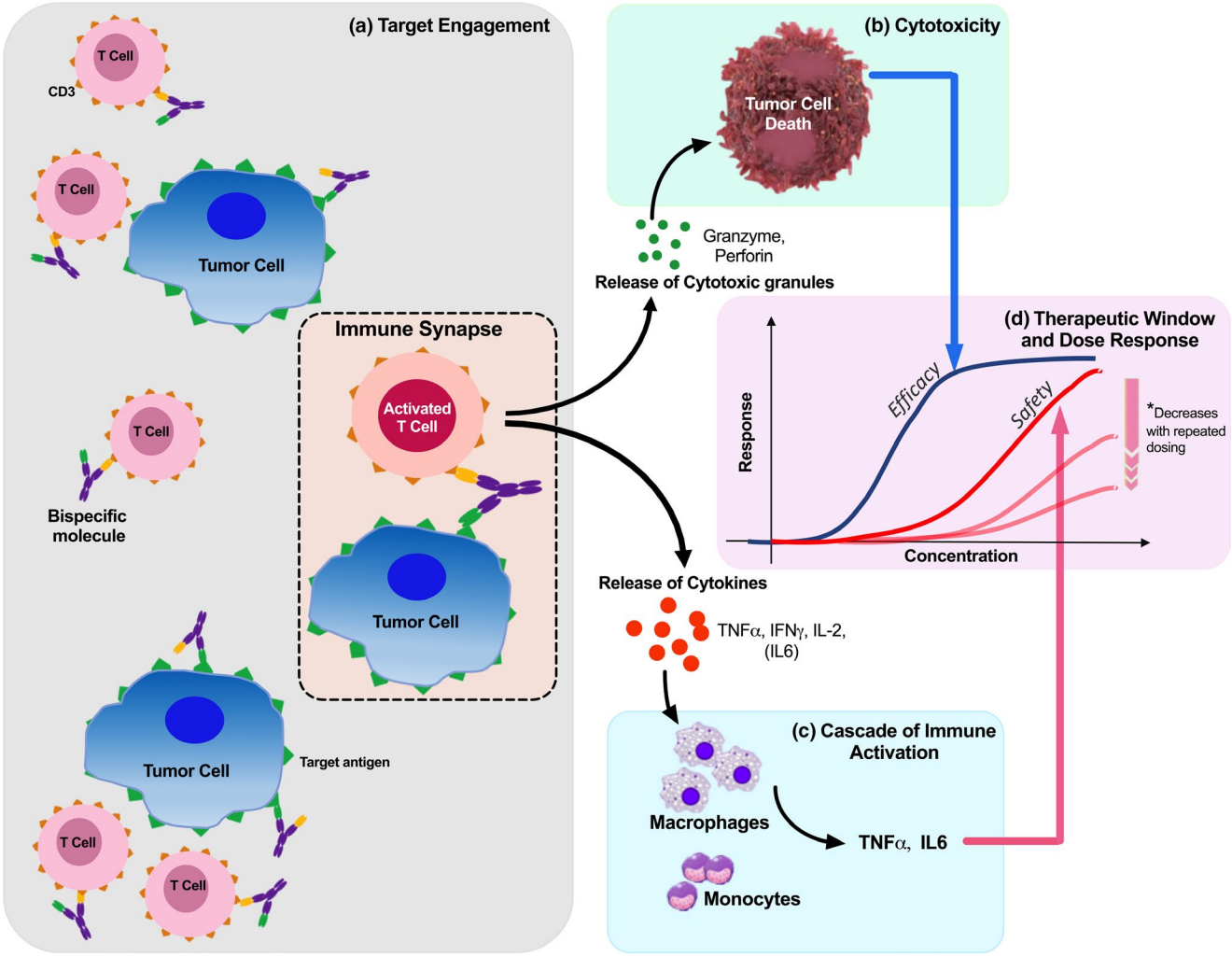

Figure 1 Visual schematic of immune synapse, mechanism of action, dose-response relationships for efficacy and safety of T-cell directing bispecifics (TDBs). (**a**) The TDB target engagement is characterized by the simultaneous binding of the TDB to the tumor-associated antigens, which are expressed 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 TNFα, 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 (TNFα 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.

perspective for TDBs. These include target engagement, preclinical-clinical translation, dose selection, PK, immunogenicity, and exposure-response. In particular, we highlight various QCP examples used to address drug development questions and potential future directions for applications of QCP approaches.

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 antigen 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).[10] Based on stoichiometric principles in a closed system, one expects a bell-shaped E-R curve, where trimolecular complexes increase with TDB concentrations

Table 1 Summary of TDBs in clinical trials for hematologic malignancies

T-BsAb	Developer	Tumor target	Format	Disease area	NCT number	Phase
AMG 420, BI 836909	Boehringer Ingelheim, Amgen	BCMA	BiTE	MM	02514239/ 03836053	I/1b 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/II Recruiting
REGN5459	Regeneron	BCMA	–	MM	04083534	I/II 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	03309111	I Recruiting
RG6160, RO7187797, BFCR4359A	Genentech	FcRH5	BiTE	MM	03275103	I Recruiting
JNJ-64407564	Jassen	GPRC5D	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	Jassen	CD123	BsmAb	AML	02715011	I/II/Ib not yet recruiting/ recruiting
SAR440234	Sanofi	CD123	IgG1 + 2scFvs	AML, B-ALL, and MDS	03594955	I Recruiting
Vibecotamab, 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	Jassen	CD33	BsmAb	AML and MDS	03915379	I Recruiting
GEM333	GEMoaB Monoclonals	CD33	BsmAb	AML	03516760	I Recruiting
Tepoditamab, 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	Genmab	CD20	BsmAb	DLBCL, FL and MCL	03625037	I Recruiting

(Continues)

Table 1 (Continued)

T-BsAb	Developer	Tumor target	Format	Disease area	NCT number	Phase
mosunetuzumab, RG7828, RO7030816, BTCT4465A	Genentech	CD20	BsmAb	NHL and DLBCL	03677141/ 03677154/ 03671018/ 02500407	I/II Recruiting
Plamotamab, XmAb13676	Xencor		2:1 asymmetric Fab	NHL and CLL, SLL	02924402	I/II Recruiting
REGN1979	Regeneron	CD20	BsmAb	FL, CLL, and NHL	03888105/ 02651662/ 02290951	I Recruiting
FBTA05	Fresenius	CD20	Triomab, Quadroma	B cell lymphoma	01138579	Terminated
glofitamab, RO7082859, RG6026	Roche	CD20	BsmAb	NHL and DLBCL	03075696/ 03533283/ 03467373/ 04077723	I/Ib Recruiting

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B7-H3, an immune checkpoint from the B7 family; B-ALL, B cell acute lymphoblastic leukemia; BCMA, B cell maturation antigen; BiTE, bispecific T-cell engager; BsmAb, bispecific monoclonal antibody; CEA, carcinoembryonic antigen; CLEC12A, C-type lectin domain family 12 member A; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CRC, colorectal cancer; DART, dual-affinity re-targeting; DLBCL, diffuse large B cell lymphoma; DLL3, delta-like ligand 3; EGFR, epidermal growth factor receptor; EGFRvIII, EGFR variant III; EpCAM, epithelial cell adhesion molecule; Fab, antigen-binding fragment; Fc, fragment crystallizable region; FcRH5, Fc receptor homologue 5 (CD307); FL, follicular lymphoma; FLT3, FMS-like tyrosine kinase 3; GD2, disialoganglioside; GIST, gastrointestinal stromal tumor; Gp100, Glycoprotein 100; gpA33, glycoprotein A33; GPC3, Glypican 3; GPRC5D, G protein-coupled receptor family C group 5 member D; HER2, human epidermal growth factor receptor 2; HLE, half-life extended; IgG1, immunoglobulin G; Mab, monoclonal antibody, MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MUC1, mucin 1; MUC16, mucin 16; MUC17, mucin 17; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; SCLC, small cell lung cancer; scFv, single-chain variable fragment; SLL, small lymphocytic lymphoma; SSTR2, somatostatin receptor 2; TandAb, tandem diabody; TDBs, T-cell directing bispecifics.

at low concentrations, reach a maximal number and optimum concentration range, and decrease at very high TDB concentrations, which favor the formation of TDB-target or TDB-T-cell complexes.[11] The *in vivo* significance of a bell-shaped E-R has not been established and should be considered in the context of the biological system where the levels of TDB, target cells, and T-cells are dynamically changing. Given the agonistic nature of the MOA, clinical efficacy could theoretically be achieved without the need for higher concentration ranges. Clinical data for novel full-length IgG-based CD20-CD3 TDB, mosunetuzumab and glofitamab, indicates that clinical efficacy is seen at doses as low as 1.2 mg or less and associated with < 1% CD20 target engagement level (see section Exposure-Response).[12,13]

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. Jiang *et al*. presented a target cell-biologics-effector cell complex-based cell killing model using *in vitro* data to represent affinity-dependent binding of bispecific antibodies to CD3 and target receptors, expression levels of CD3 and target receptors, and concentrations of effector and target cells.[10] Model-based predictions were extrapolated to *in vivo* settings and predicted the clinical efficacious doses of blinatumomab.[10] Such approaches provide insights into the MOA and are useful for investigating the interplay between drug-specific effect and the biological context of different diseases (e.g., target expression and distribution) and patient immune status (e.g., variability in T-cell activity). The applications of these models can also be extended to inform discovery efforts, including molecule optimization and candidate selection.

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.[14] 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 Completed
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
Ertumaxomab	Fresenius Biotech North America	HER2	Triomab, Quadroma	Breast cancer	01569412/ 00351858/ 00522457/ 00452140	II Terminated
RG6194, BTRC4017A	Genentech	HER2	BsmAb	Locally advanced or metastatic HER2-expressing cancers	03448042	I Recruiting
MGD009, Orlotamab	Macrogenics	B7-H3	DART	NSCLC and melanoma	03448042	Ia/Ib not yet recruiting/ recruiting
Cibisatamab, RG7802, RO6958688, CEA-TCB	Roche	CEA	Crossmab	NSCLC and other solid tumors	03337698/ 02650713/ 02324257	I/Ib/II Recruiting
AMG211, MEDI-565	Amgen	CEA	BiTE	Gastrointestinal adenocarcinoma	01284231/ 02291614/ 02760199	I completed
AMG757	Amgen	DLL3	BiTE	SCLC	033199040	I Recruiting
AMG596	Amgen	EGFRvIII	BiTE	ECFRvIII + Glioblastoma	03296696	I Recruiting
A-337	Generon	EpCAM	BsmAb	NSCLC	China	I
MT110, AMG110	Amgen Research (Munich) GmbH	EpCAM	BiTE	Metastatic colorectal, gastric, and lung cancers	00635596	I completed
catumaxomab	Fresenius Biotech and Trion 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	Chugi	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/ 02889861/01209676	I/II
MGD007	Macrogenics	gpA33	DART	CRC	03531632/02248805	

(Continues)

Table 2 (Continued)

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	04117958	I not yet Recruiting
PF-06671008	Pfizer	P-cadherin	DART	Neoplasms	02659631	Terminated
GEM3PSCA	GEMoaB Monoclonals GmbH	PSCA	–	PSCA positive cancer	03927573	I Recruiting
Tidutamab, XmAb18087	Xencor	SSTR2	BsmAb	Neuroendocrine and GIST	03411915	I Recruiting

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B7-H3, an immune checkpoint from the B7 family; B-ALL, B cell acute lymphoblastic leukemia; BCMA, B cell maturation antigen; BiTE, bispecific T-cell engager; BsmAb, bispecific monoclonal antibody; CEA, carcinoembryonic antigen; CLEC12A, C-type lectin domain family 12 member A; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CRC, colorectal cancer; DART, dual-affinity re-targeting; DLBCL, diffuse large B cell lymphoma; DLL3, delta-like ligand 3; EGFR, epidermal growth factor receptor; EGFRvIII, EGFR variant III; EpCAM, epithelial cell adhesion molecule; Fab, antigen-binding fragment; Fc, fragment crystallizable region; FcRH5, Fc receptor homologue 5 (CD307); FL, follicular lymphoma; FLT3, FMS-like tyrosine kinase 3; GD2, disialoganglioside; GIST, gastrointestinal stromal tumor; Gp100, Glycoprotein 100; gpA33, glycoprotein A33; GPC3, Glypican 3; GPRC5D, G protein-coupled receptor family C group 5 member D; HER2, human epidermal growth factor receptor 2; HLE, half-life extended; IgG1, Immunoglobulin G; Mab, monoclonal antibody, MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MUC1, mucin 1; MUC16, mucin 16; MUC17, mucin 17; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; SCLC, small cell lung cancer; scFv, single-chain variable fragment; SLL, small lymphocytic lymphoma; SSTR2, somatostatin receptor 2; TandAb, tandem diabody; TCR, T-cell receptor; TDBs, T-cell directing bispecifics.

pharmacological activity as a function of TDB concentration in sensitive assays as an acceptable approach.[14] Toxicology studies for therapeutic proteins generally include safety and PK assessment in nonhuman primates (e.g., cynomolgus monkeys ("cyno")). Cyno PK could be scaled to project human PK through allometric scaling for clearance and volume parameters.[15] In scenarios where the TDB may be subject to target-mediated drug disposition (TMDD; see section PK Considerations), human PK predictions should account for differences in target expression, binding affinity, and/or other intrinsic differences between cyno and human. Notably, antidrug antibodies (ADAs) which oftentimes emerge in cynos may not directly translate to humans.

QCP perspectives and opportunities

Recently, Betts *et al*. published a quantitative systems pharmacology (QSP) model to characterize the *in vivo* efficacy of P-cadherin/CD3 bispecific construct in tumor-bearing mice.[11,16] The model is a combination of mechanistic (e.g., binding of drug to CD3 and target antigen) and empirical (e.g., T-cell proliferation) components to capture PK and T-cell profiles in circulation. It integrates PK of TDB and its binding to shed target antigen and circulating T-cells, its distribution to tumor, and the formation of a trimolecular complex to T-cells and target expressing tumor cells to drive antitumor efficacy. The QSP model was then translated from mouse to human to support the clinical starting dose of P-cadherin/CD3 bispecific construct using the minimal anticipated biological effect level approach.[11,14] Such models integrate the *in vitro* and *in vivo* pharmacology and provide additional insights into a reasonable starting dose over PK-based approaches alone. The preclinical-to-clinical translation remains an active area of research. A clinical sampling plan to allow for biomarker data collection (e.g., T-cell activation, cytokine kinetics, and target cell dynamics) to capture the short-term and long-term dynamics can be valuable to enable the continued assessment of translational assumptions and predictability across TDB molecules. Such insights are critical in guiding further optimized approach to predict the FIH dose selection of future TDB molecules.

PK CONSIDERATIONS

Absorption

TDBs are therapeutic proteins largely administered via i.v. routes. However, s.c. routes are also being explored as a step toward better patient convenience (with the short treatment administration) and better safety (with the enhanced safety profile due to the reduced absorption rate).[17] Preliminary phase I clinical data from epcoritamab (GEN3013; DuoBody - CD3/CD20) following s.c. administration showed that the maximum plasma concentration (C_{max}) occurred during 2–4 days after dosing and that GEN3013 is well-tolerated with early signs of clinical activity in patients with relapsed or refractory non-Hodgkin's lymphoma.[18,19] Empirically, a first order absorption process from the lymphatic system following s.c. administration is likely to occur, as ascribed for traditional monoclonal

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.[13] 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.[5] 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.[15,24] Higher TDB clearance may be anticipated for agents with higher CD3 affinity, as illustrated with the CLL-1/CD3 bispecific antibody.[25] 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.[31]

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.[12,13,32] 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,[33] 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.[11] However, observed clinical data to-date suggests increases in efficacy with increasing dose/exposure and, in some cases, toward a plateau.[12,13,34] Blinatumomab E-R analyses have revealed a positive relationship between steady-state concentrations and complete responses (CRs).[21] Recent analyses by Dufner *et al*. also suggested durable remission and better median overall survival at the clinical MTD of 60 μg/m^2 per day compared with lower dose levels.[2] 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.[12,13] 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.[12,13] 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.[12,13] 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.[12,13,21] 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.[8,12] 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.[35] 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.[12] 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.[13] Notably, limited cytokine-mediated neurotoxicity has been observed for both mosunetuzumab and glofitamab, supporting the utility of these novel safety approaches.[12,13] A potential combination of both safety-mitigation approaches could further yield beneficial effects and is being investigated for glofitamab.[13] 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.[36] 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.[35] 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.[12,13,35] 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.[37] 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.[37,38] 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

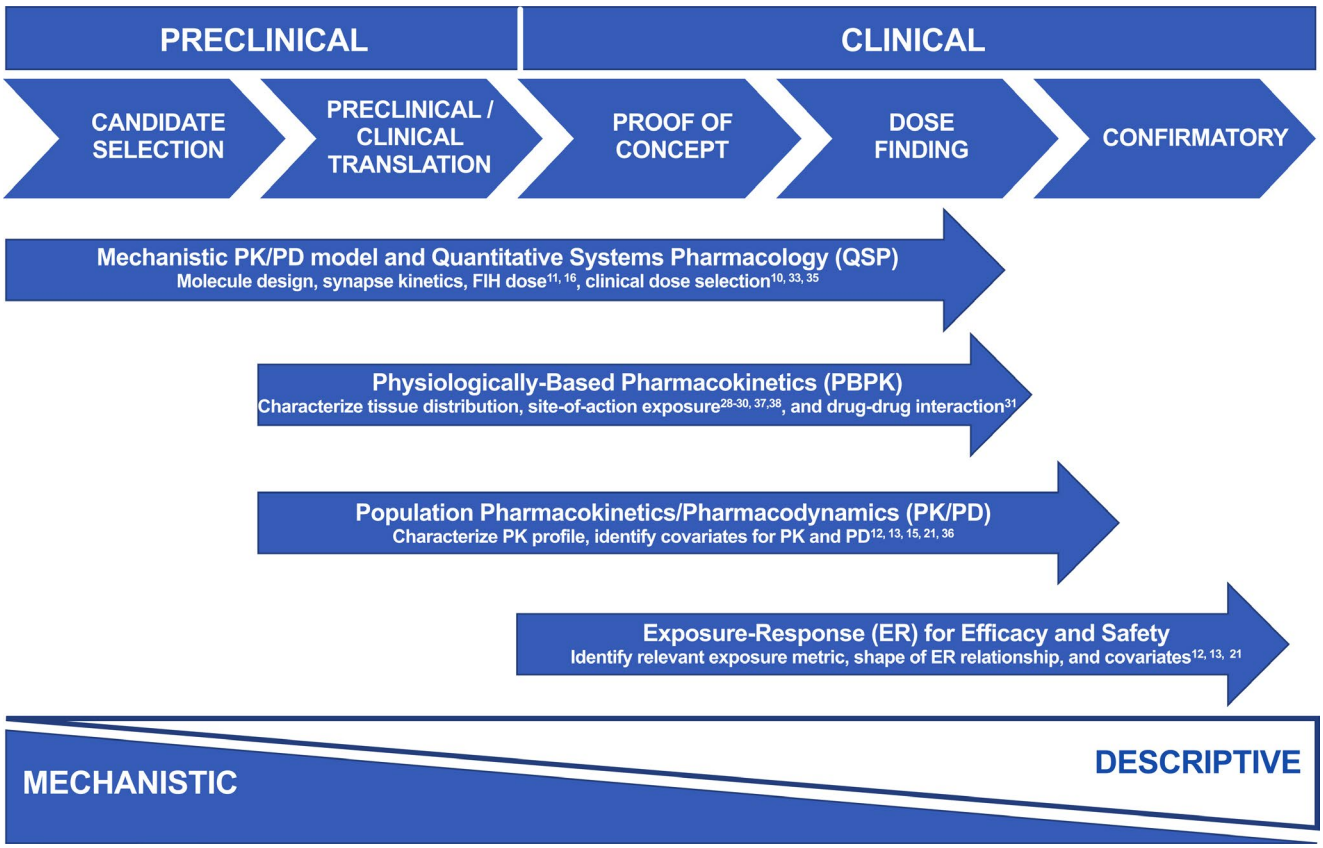

Figure 2 Quantitative clinical pharmacology (QCP) approaches to inform preclinical and clinical drug development decisions. Depending on the context and the nature of drug development questions, different QCP approaches can be useful. As T-cell directing bispecifics (TDBs) move in the pipeline from early 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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