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EXPERT REVIEW

An Introduction to the Regulatory and Nonclinical Aspects of the Nonclinical Development of Antibody Drug Conjugates

Janice A. Lansita[1] • John M. Burke[2] • Joshua F. Apgar[2] • Barbara Mounho-Zamora[3]

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ABSTRACT Antibody drug conjugates (ADCs) are promising therapies currently in development for oncology with unique and challenging regulatory and scientific considerations. While there are currently no regulatory guidelines specific for the nonclinical development of ADCs, there are harmonized international guidelines (e.g., ICHS6(R1), ICHM3(R2), ICHS9) that apply to ADCs and provide a framework for their complex development with issues that apply to both small and large molecules. The regulatory and scientific perspectives on ADCs are evolving due to both the advances in ADC technology and a better understanding of the safety and efficacy of ADCs in clinical development. This paper introduces the key scientific and regulatory aspects of the nonclinical development of ADCs, discusses important regulatory and scientific issues in the nonclinical to clinical dose translation of ADCs, and introduces new concepts in the areas of pharmacokinetic/pharmacodynamic (PK/PD) modeling and simulation.

KEY WORDS antibody drug conjugate · nonclinical development · regulatory, toxicology

✉ Barbara Mounho-Zamora
bmounho-zamora@toxstrategies.com

[1] ToxStrategies, Inc., Baltimore, MD, USA

[2] Applied BioMath, LLC., Winchester, MA, USA

[3] ToxStrategies, Inc., 70 SW Century Dr., Suite 100-409 Bend, OR 97702, USA

ABBREVIATIONS

ADC	Antibody-drug conjugate
ADME	Absorption, distribution, metabolism and excretion
ASCT	Autologous stem cell transplant
BW	Body weight
BSA	Body surface area
CNS	Central nervous system
DAR	Drug-to-antibody ratio
DM1	Mertansine; N2′-deacetyl-N2′-(3-Mercapto-1-oxopropyl)-Maytansine
DM4	N2′-deacetyl-n2′-(4-Mercapto-4-Methyl-1-oxopentyl)-6-Methylmaytansine
EMA	European Medicines Agency
FcRn	Neonatal Fc receptor
FDA	US Food and Drug Administration
FIH	First-in-human
HER2	Human epidermal growth factor receptor 2
hERG	Human Ether-à-go-go-Related Gene
HNSTD	Highest non-severely toxic dose
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
mAb	Monoclonal antibodies
MMAE	Monomethyl auristatin E
MMAF	Desmethyl-auristatin F
MOA	Mechanism of action
MTD	Maximum tolerated dose
PBD	Pyrrolobenzodiazepine
PD	Pharmacodynamics
PK	Pharmacokinetics
POC	Proof-of-concept
PNS	Peripheral nervous system

SMCC	Succinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate
STD10	Severely toxic dose in 10% of rodents
TDC	ThioMAb drug conjugate
TK	Toxicokinetics

INTRODUCTION TO ADCS

Antibody-drug conjugates (ADCs) are monoclonal antibodies (mAbs) conjugated to small molecule drugs by a chemical linker (Fig. 1). Generally, these small molecule drugs are highly potent cytotoxic agents also known as "payloads" or "warheads." The payloads currently used in ADCs are typically potent genotoxic agents that target rapidly dividing cells and inhibit DNA synthesis, cell division, or damage DNA directly, such as analogs of calicheamicin, duocarmycin, auristatins, and maytansinoids [1]. The number of payloads conjugated to a mAb (N) is known as the drug-to-antibody ratio (DAR). To date, ADCs have mainly been developed for oncology indications. By combining antibodies and small molecules into one drug, they are designed to deliver the cytotoxic drug directly to the tumor cell, thus improving the therapeutic index by reducing the toxicity and improving efficacy of the payload compared to the unconjugated cytotoxic drug alone. To be both safe and effective, the ADC should 1) be stable until reaching the tumor, 2) be internalized into the tumor cell and 3) contain a small molecule drug or payload that is released at sufficient concentrations to kill the tumor cell. The ADC dose needed to kill the tumor cell will depend on the potency of the cytotoxic agent, the DAR, and the amount of payload released as free drug at the tumor site. An optimal DAR has been defined as generally 2-4 payload molecules per mAb based on experience with trastuzumab emtansine [2, 3]. An exciting area that is rapidly developing for ADCs is new linker chemistries such as site-specific conjugation, non-natural amino acids and aldehyde tagging. By further controlling the release of the payload specifically at the site of the tumor, better efficacy and safety may be achieved for ADCs. In summary, ADCs are promising therapeutic candidates for the treatment of cancer as they have the potential to be effective and potent therapies that provide an improved therapeutic window in comparison to either the naked antibody or unconjugated cytotoxic payload alone.

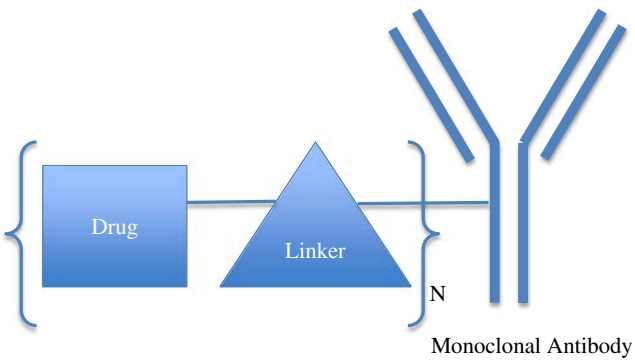

Fig. 1 Antibody drug conjugate composition.

Monoclonal antibody therapies are targeted towards unique epitopes with high affinity and specificity to proteins (*e.g.*, cell surface receptor or ligand). Thus, it is the antibody portion of the ADC that drives the specificity to tumor antigens. For the anti-cancer ADCs, the tumor antigens are generally expressed at a higher density on the cell surface of tumor cells relative to normal tissues. Monoclonal antibodies have a long serum or plasma half-life, which appears to be attributed to the interaction of the Fc portion of the mAb with the neonatal Fc receptor (FcRn) expressed on various cell types such as endothelium and epithelium [4]. The mAb is "recycled" by being internalized into a cell endosome by the FcRn without being degraded into amino acids, and then released back into the circulation [5, 6]. Ideally, after a mAb is conjugated to the payload, it should retain its original properties of having a long serum or plasma half-life, high affinity, and high specificity to its protein target expressed on tumor cells (with no or limited binding to normal tissues). There are numerous ADCs currently in clinical development for oncology with various solid tumor and hematologic targets being explored [7, 8].

The linker is not a simple connection between the antibody and payload but is actually a complex, multifunctional component designed not only to stably connect the payload to the antibody, but also to control release of the payload from the antibody once the ADC is internalized into the tumor cell by receptor-mediated endocytosis. Defining the optimal balance of plasma stability and drug release to the tumor cell is a complex challenge in the development of ADCs. Ideally, the linker should be stable during circulation in the bloodstream, yet labile once internalized into tumor cells so the active payload can be released. Once the ADC is internalized into the tumor cell by lysosomes/endosomes, for ADCs with acid-labile linkers, the linker may be degraded due to the acidic pH of the lysosome/endosome while for ADCs with non-cleavable linkers, the mAb portion would be degraded by lysosomes, with both mechanisms ultimately leading to the release of the cytotoxic payload. Following degradation of the ADC in the lysosome, the cytotoxic payload is released to kill the tumor cell. Linkers are classified into two general classes, cleavable and non-cleavable, and have been designed to be acid labile (*e.g.*, hydrazone), reducible (*e.g.*, disulfide), enzymatically cleavable (*e.g.*, peptide or dipeptide) or non-cleavable (*e.g.*, thioether) [9]. Thus, the linker chemistry can be designed depending

on the desired properties of the ADC. Many ADC linkers are covalently bound to reactive primary thiol groups of cysteine and primary amino groups of lysine. The DAR and sites of conjugation can be controlled to modify the properties of the ADC, and several manufacturers are utilizing various technologies to specifically engineer the linker and payload to pre-determined sites. Such technologies include engineered cysteines (*e.g.*, ThioMAb Drug Conjugates (TDCs)), different peptides, aldehydes, and non-natural amino acids [10, 11]. For example, the number and location of cysteines for TDCs can be selected to enhance solubility, serum stability of the linker, and retention of antigen binding without compromising the yields of the ADC [12, 13].

The payloads that are currently being evaluated for use in ADCs are generally highly potent anti-cancer therapeutics where proof-of-concept or efficacy has been established for the treatment of cancer (Table I). Overall, these payloads inhibit microtubule assembly or directly damage DNA. Ideal characteristics of cytotoxic drugs used as payloads for ADCs include plasma stability, high potency, and a well-characterized mechanism of action. The selection of the payload is based on the tumor type. For example some payloads work best on actively proliferating tumors (*e.g.*, tubulin polymerization inhibitors such as auristatin and maytansine) while DNA damaging agents are effective against both proliferating and non-proliferating tumors (*e.g.*, doxorubicin and calicheamicin).

Currently marketed ADCs in the United States (US) include Kadcyla® (ado-trastuzumab emtansine), which was approved in 2013, and Adcetris® (brentuximab vedotin), which was approved in 2011. Kadcyla is an anti-HER2-targeted antibody and microtubule inhibitor conjugate indicated (as a single agent) for the treatment of patients with HER2-positive, metastatic breast cancer [14]. Kadcyla contains a thioether linker, succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC), conjugated to mertansine (DM1), a maytansine derivative cytotoxic drug. Adcetris is an anti-CD30 antibody conjugated to the antimitotic agent monomethyl auristatin E (MMAE) by a protease-cleavable citruline-valine (val-cit) dipeptide linker [15]. Adcetris is indicated for the treatment of Hodgkin lymphoma (after failure of autologous stem cell transplant [ASCT] or after failing two prior multi agent chemotherapy regimens in patients who are not ASCT candidates), and for systemic anaplastic large cell lymphoma after failure of at least one prior multi agent chemotherapy regimen. Finally, Mylotarg (gemtuzumab ozogamicin), an anti-CD33 targeted ADC conjugated to calicheamicin using a hydrazone linker, was approved in the US in 2000. Mylotarg, however, was voluntarily withdrawn from the market in 2010 due to increased patient deaths in a clinical trial that did not demonstrate adequate benefit over conventional cancer therapies [16].

The nonclinical target organs of toxicity for both Kadcyla and Adcetris appear to be related to the anti-mitotic activity of the cytotoxic payloads targeting rapidly dividing cells. For Kadcyla, the nonclinical findings associated with the DM1 payload included clastogenicity, elevations in hepatic transaminases, neuropathy and thrombocytopenia. For Adcetris, the nonclinical toxicities associated with the MMAE payload included clastogenicity, myelotoxicity, bone marrow hypocellularity and lymphoid depletion, hematotoxicity (reduced leukocytes, erythrocytes, reticulocytes, neutropenia), hepatotoxicity, embryo-fetal lethality/teratogenicity (rats) and testicular toxicity (based on repeat dose studies). In summary, based on the approved ADCs Kadcyla and Adcetris, the observed nonclinical toxicities appear to be mainly attributed to the small molecule cytotoxic payload. Although the field of ADCs is rapidly changing, historical experience with the approved ADCs is important to consider when determining which nonclinical studies are important for characterizing the toxicity profiles of ADCs in clinical development.

Regulatory Guidelines for the Nonclinical Safety Assessment of ADCs

Nonclinical toxicology studies are required for the development of therapeutic medicines, including ADCs. However, currently there are no harmonized guidelines specifically for the nonclinical safety assessment of ADCs. Both the European Medicines Agency (EMA) and US Food and Drug Administration (FDA) consider existing guidelines adequate for the regulation and nonclinical safety assessment of ADCs [17]. Because ADCs are comprised of both a mAb and a small molecule payload, guidelines for both biological products and small molecule drugs apply to ADCs, and therefore, should be consulted in determining the appropriate toxicology studies to be conducted for the clinical development of an ADC. Several nonclinical regulatory guidelines are relevant to ADCs (Table II). These include ICH S6(R1) (2011) for the nonclinical development of biologics; ICH M3(R2) (2009) for the timing, duration and types of nonclinical studies needed; ICH S3A (1994) for the evaluation of toxicokinetics; ICH S2(R1) (2011) for genotoxicity testing; and ICH S7A (2000)/ICH S7B (2005) for the evaluation of safety pharmacology [18–22]. Currently, the majority of ADCs are being developed for hematologic and solid tumor indications, and therefore, ICH S9 (2009) should also be consulted for guidance on the nonclinical safety assessment of oncology ADCs [23]. Important considerations in the development of the nonclinical safety assessment of biological and small molecule therapeutics apply to ADCs, and include selection of pharmacologically relevant species, anticipating the target organs of concern, and the selection of doses used in the nonclinical toxicology studies. However, a scientific and regulatory consideration that is unique to ADCs is the development of multiple bioanalytical

Table I List of Selected Payloads and Mechanisms of Cytotoxicity

Payload	Mechanism of Cytotoxicity
Auristatin *e.g.*, Monomethyl auristatin E (MMAE); desmethyl-auristatin F, (MMAF)	Tubulin polymerase inhibitor
Maytansine; maytansinoids (*e.g.*, DM1, DM4)	Tubulin depolymerisation
Calicheamicin	Superoxide formation and double-strand DNA breaks
Doxorubicin	DNA intercalator
Camptothecin	Topoisomerase I inhibitor
Duocarmycins	DNA minor groove alkylator
Pyrrolobenzodiazepine (PBD) dimers	DNA minor groove cross-linker
α-Amanitin	Inhibitor of RNA polymerase II and III
De-Immunized Bougainin	Protein synthesis arrest by the deadenylation of ribosomal RNA

assays to measure the total drug conjugate, total antibody, free drug, and anti-drug antibodies (ADA). The development of these bioanalytical assays can be complex, time-consuming, and resource-intensive. Finally, as discussed in the following section, similar to other biological and small molecule therapeutics, the nonclinical development plan for ADCs should be case-by-case and based on current scientific and regulatory considerations.

Nonclinical Studies

The nonclinical studies needed to adequately evaluate the toxicity of an ADC will depend on the specific characteristics of the antibody target, linker, and small molecule payload. The design of the nonclinical plan should be tailored specifically to the ADC, and key principles that should be considered include: 1) determination of the pharmacologically relevant species, which is usually dependent on the cross-reactivity of the antibody portion of the ADC to the target antigen; 2) the pharmacokinetics (PK) and pharmacodynamics (PD) of the antibody and payload components of the ADC; and 3) the toxicity profile of the ADC.

Table II ICH Regulatory Guidelines Relevant to the Nonclinical Safety Assessment of ADCs

Regulatory Guidelines
Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use S2(R1) (2011)
Note for Guidance on Toxicokinetics: the Assessment of Systemic Exposure in Toxicity Studies S3A (1994)
Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals S6(R1) (2011)
Safety Pharmacology Studies for Human Pharmaceuticals S7A (2000)
The Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals S7B (2005)
Nonclinical Evaluation for Anticancer Pharmaceuticals S9 (2009)
Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2) (2009)

To support first-in-human (FIH) dosing, generally the types of nonclinical studies needed would include PK, nonclinical proof-of-concept or pharmacology studies, safety pharmacology studies, general toxicity studies and genotoxicity studies (Table III). For therapeutic drugs being developed for the treatment of patients with advanced malignancies, the nonclinical studies may be abbreviated based on the overall risk *vs.* benefit profile of the ADC for advanced cancer patients with a severe and life-threatening illness with limited life-spans. Based on current regulatory guidance for patients with advanced cancer, generally reproductive toxicity studies are not required for clinical development but are required for submission in the marketing application, while carcinogenicity studies are not warranted to support marketing [23]. ICH S9 (2009) states that embryo-fetal development studies are not essential for pharmaceuticals that are genotoxic and target rapidly dividing cells (*e.g.*, crypt cells, bone marrow) or belong to a class that has been well characterized as causing developmental toxicity. Fertility and pre-postnatal development toxicology studies are also not warranted to support the development of drugs in patients with advanced cancer. Additionally, immunotoxicity evaluations are generally not needed when immune suppression is a predictable outcome based on the

Table III List of Nonclinical Studies to Support FIH Trials of an ADC for Oncology

Nonclinical Study	ADC or Payload
Single-dose toxicity	ADC
Single-dose toxicity	Payload (if no existing data)
Repeat-dose toxicity (including safety pharmacology endpoints)	ADC
Tissue cross-reactivity	ADC
Subchronic and chronic toxicity (for advanced cancer indications a maximum study duration of 3 months is required)	ADC
Genotoxicity (Ames and *in vivo* micronucleus)	Payload

pharmacology of the drug (*e.g.*, anti-proliferative or anti-mitotic effects on bone marrow progenitor cells of the immune system) [24]. Finally, for novel payloads to be used in patients with advanced cancer, genotoxicity studies are not necessary to support clinical trials, but should be conducted to support marketing [23]. Many of the payloads of ADCs are already known to be genotoxic and are well characterized with respect to developmental toxicity.

Nonclinical toxicology studies with ADCs should be conducted in two pharmacologically relevant species (1 rodent and 1 non-rodent), if feasible. Generally, the mAb component of the ADC drives the selection of the pharmacologically relevant species because mAbs are highly specific for their target. Several mAbs have limited cross-reactivity to animal species used for toxicology studies, and often are pharmacologically active only in non-human primates. Thus, in many cases, toxicity studies for mAbs are conducted only in non-human primates. Determination of a pharmacologically relevant species can include various methods, including an evaluation of sequence homology of the protein target across human and different animal species and demonstrating that the binding affinity and pharmacological activity (using *in vitro* and/or *in vivo* assays) of the ADC to the human and animal target are similar. The expression of the target antigen can drive the distribution, clearance and toxicity of the ADC; therefore, using pharmacologically relevant animal species for nonclinical testing is critical for the nonclinical safety assessment of the ADC.

Consistent with the regulatory guidelines for small molecule and biological therapeutics, characterization of the PK of the ADC is a critical component of the nonclinical development program (*e.g.*, ICH S6(R1), ICH S9, ICH M3(R2), ICH S3A). The PK data are important for selecting appropriate doses for the pivotal toxicology studies and determining exposure or potential dose–response relationships for clinical dosing. Attributes of an ADC that may impact the dose–response relationship include the stability of the linker, the stability of the small molecule conjugate, the DAR, and the ADA response. Recent publications have shown that the degree of conjugation can impact the PK and tissue distribution of an ADC [25, 26]. Since ADCs consist of a mixture of biological and small molecule components, multiple bioanalytical methods are needed for the PK analysis of ADCs [27]. Current best practice as described by Roberts *et al.* (2013) and Alley *et al.* (2013) recommend that the conjugate (measured as the antibody-conjugated drug or conjugated cytotoxic), total antibody, unconjugated drug/cytotoxic and the ADA response be measured to adequately determine the overall PK, exposure-response relationships, and data interpretation [28, 29]. Since payloads can be released by different mechanisms, special considerations may be needed for evaluation of the payload plus linker or payload plus partial linker depending on the mechanism of release. For example if the linker is non-cleavable (and the complete degradation of the mAb by lysosomes is needed to liberate the payload), depending on the metabolism of the payload and linker, the toxicokinetics (TK) of the payload plus linker may need to be evaluated instead of the payload alone. Because the method development and validation of multiple assays for Good Laboratory Practices (GLP) studies can be resource-intensive (*i.e.*, time, cost and personnel), assay development should be planned and considered early in nonclinical development.

Similar to the bioanalytical assays for PK, development of the ADA assay(s) can be challenging and resource intensive; however, the samples for ADA testing can be archived and analyzed later (after the completion of the toxicology study) if the data are needed to aid in the interpretation of the toxicology study [18]. The absorption, distribution, metabolism and excretion (ADME) properties of the small molecule payload of the ADC may need to be characterized if existing data are not available and especially if the ADC is not stable. In addition, if the ADC is not stable and the small molecule drug is widely distributed, the ADME data may be informative for interpretation of the toxicity data.

Nonclinical proof-of-concept (POC) pharmacology studies should be conducted to determine an appropriate clinical dose range and dose schedule. For oncology indications, POC studies are conducted in anti-tumor animal models. These POC data can be used in addition to the toxicity data to determine a clinical dose range that brackets efficacy while attempting to reduce the potential risk of serious adverse toxicity [23, 28].

Safety pharmacology evaluations for ADCs can generally be conducted in the context of the general toxicology studies by evaluating endpoints to assess the core battery of potential cardiovascular, respiratory and central nervous system/peripheral nervous system (CNS/PNS) toxicity(ies) as described in ICH S6(R1) [18]. Stand-alone safety pharmacology studies are generally not needed for the small molecule and/or the linker alone unless there is a specific cause for concern. Additionally, Roberts *et al.* (2013) recommend that an *in vitro* hERG study be conducted if free concentrations of a novel cytotoxic are measured in the nonclinical toxicity studies [22, 30].

As described above, the relevant regulatory guidance documents for ADCs recommend that the general toxicology studies should be conducted in two pharmacologically relevant species, one rodent and one non-rodent, if feasible [18, 19]. One important consideration that should be addressed early in development of an ADC is the extent of toxicology studies required - that is, is the evaluation of the nonclinical toxicity of the ADC alone sufficient, or does the toxicity profile of each component of the ADC (*e.g.*, linker, and small molecule payload) need to be assessed individually. The extent and type of toxicology studies necessary for an ADC will depend on whether safety data already exist for the antibody, linker and/or payload. Many ADCs

utilize mAbs that are approved/marketed and therefore, the nonclinical and clinical toxicity profile of the mAb has already been characterized. Similarly, if safety data already exist for the linker and payload, then toxicity studies on the linker alone or the payload alone may not be necessary. For example, the payload may already have an existing nonclinical and clinical safety database (*e.g.*, DM1, the maytansinoid payload of Kadcyla), and therefore, these data can be referenced rather than repeating toxicology studies of the payload. However, if the cytotoxic drug (payload) is novel, toxicity studies with the payload alone should also be conducted to characterize the toxicity profile of the unconjugated small molecule payload. Similarly, studies with the linker alone or the linker plus payload may be considered if the linker is novel. However, in Saber and Leighton (2015), findings from a retrospective analysis of 20 ADCs support that the payload drives the toxicity of ADCs with comparable toxicity observed for the linker plus payload *vs.* the payload alone [31]. These data imply that the linker has a small contribution to the toxicity of an ADC compared with the payload [31]. For genotoxic payloads targeting rapidly dividing cells, the repeat-dose toxicity study of the payload alone can be done in one species, which is generally the rat [23]. Additionally, the toxicity data for the small molecule payload alone may help determine which toxicities are attributable to the ADC *vs.* the unconjugated drug.

The extent of the safety studies for the ADC components (*i.e.*, the antibody, payload and linker) is an issue that is currently being evaluated by the ICH as described in a Final Concept Paper S9: Q&As on Nonclinical Evaluation for Anticancer Pharmaceuticals [32]; therefore, for current regulatory guidance the proposed nonclinical package should be discussed with regulatory authorities to ensure the planned studies are adequate. The study duration and dosing schedule (single-dose *vs.* repeat-dose) of general toxicology studies to support development of the ADC will depend on the anticipated PK, pharmacology, the clinical indication(s), the clinical trial duration and dose schedule. Additionally, the anticipated toxicities and endpoints will depend upon the mechanism of action and pharmacology of the drug. For example, because cytotoxic payloads arrest cells in mitosis, mitotic figures would be an anticipated observation that would confirm the mechanism of action of the ADC.

Although genotoxicity studies are not needed for mAbs and large molecules as they are not anticipated to interact directly with DNA [18], genotoxicity studies may be needed to evaluate the small molecule payload and/or the linker if these components of the ADC were not previously tested for genotoxicity. Genotoxicity testing of novel cytotoxic payloads for patients with advanced cancer should be evaluated prior to registration/marketing [23]. The need for evaluating the linker will depend on various factors including the stability of the ADC, the cleavage mechanism of the linker and the reactivity of the linker (*i.e.*, the likelihood for the linker to interact with DNA and cause chromosomal aberrations and/or mutations). Generally, the genotoxicity studies conducted would include the Ames and *in vivo* micronucleus assays [20].

Tissue cross-reactivity (TCR) studies of the ADC should be done in at least human tissues as required for mAbs [18]. The purpose of TCR studies is to evaluate both the anticipated and unanticipated binding of an ADC to human tissues. These data can be informative when unanticipated toxicities are seen in the nonclinical or clinical studies. Although TCR studies can be done using the antibody alone, conjugation with the linker and small molecule may impact the binding of the ADC to the protein target. Therefore, TCR studies using the intact ADC are generally recommended.

Challenges of Determining the FIH Dose for ADCs

All of the nonclinical toxicity data are integrated to determine a reasonably safe starting dose for the FIH trial. In general, the determination of the FIH clinical dose is based on the pivotal nonclinical toxicology studies. For oncology products, ICH S9 recommends that the FIH clinical dose is either $1/10^{th}$ the severely toxic dose in 10% (STD 10) of rodents or $1/6^{th}$ the highest non-severely toxic dose (HNSTD) for nonrodents. The HNSTD is defined as the highest dose that does not result in "evidence of lethality, life-threatening toxicities or irreversible findings." [23].

Saber and Leighton (2015) recently performed an analysis of 20 ADCs (2 approved products and 18 products in clinical development) comparing different algorithms for setting the FIH dose that included: $1/10^{th}$ the STD10 (using body weight [BW] or body surface area [BSA] for scaling between animals and humans), $1/6^{th}$ the HNSTD (using BW or BSA), $1/10^{th}$ the HNSTD (using BW or BSA), and $1/10^{th}$ the NOAEL (using BW and BSA) [31]. The authors concluded that for a specific small molecule payload, when the linker, DAR, and frequency of dose administration remain the same, the clinical maximum tolerated dose (MTD) is similar regardless of the antibody target or linker used. For example, for ADCs containing valine-citrulline (vc)-MMAE as the linker-payload, the MTD ranged from 1.8 to 2.4 mg/kg based on 8 different products in clinical development. Similarly, for ADCs containing DM4, the clinical MTDs ranged from 4.1 to 4.5 mg/kg based on a smaller dataset of 3 different products in clinical development. Interestingly, this consistency was not seen in the HNSTD values from the nonclinical toxicology studies but that could be due to a number of reasons (*e.g.*, variable dose frequency, large multiple between dose levels, no pre-specified definition of dose-limiting toxicity for nonclinical studies). However, consistency in HNSTDs was observed for vc-MMAE ADCs dosed once every 3 weeks for 4 doses (HNSTD range of 5–6 mg/kg) and DM4 ADCs following a single-dose (HNSTD 10–15 mg/kg). Importantly, Saber

and Leighton (2015) conclude that safe starting doses were derived using: $1/6^{th}$ the HNSTD in nonrodents or $1/10^{th}$ the STD10 in rodents based on BSA scaling. Other acceptable algorithms included $1/10^{th}$ the HNSTD in nonrodents using BSA scaling and $1/10^{th}$ the NOAEL in rodent or monkeys using BW scaling. Unsafe methods for deriving the FIH dose included: $1/6^{th}$ the HNSTD, $1/10^{th}$ the HNSTD, and $1/10^{th}$ the STD10 using BW scaling [31]. Although these algorithms can be safely applied to known payloads and linkers, it is unclear whether these algorithms would consistently hold true for novel ADCs with novel payloads, linkers, DARs, and/or targets. Therefore, PK/PD models that integrate all aspects of the nonclinical data (*e.g.*, biology, pharmacology, toxicology, *etc.*) and are more predictive of nonclinical to clinical dose translation are clearly needed.

PK/PD Modeling and Simulation Supporting Translation of ADCs

Traditional PK/PD modeling techniques have inherent limitations for providing accurate FIH dose predictions for biological products, particularly for ADCs, which have both antibody and small molecule components [33]. Traditional PK/PD models fit model parameters to data collected in an *in vivo* experiment and then translate those parameters to a new species through linear allometric scaling. As these are fit values, they do not represent the biophysical properties of ADCs. As a result, it is extremely difficult to validate these parameters preclinically using other sources of information such as nonclinical safety and mechanism of toxicity data. Moreover, the linear allometric scaling does not capture the species differences that affect ADCs such as affinity and avidity for the antibody target, receptor numbers, endocytosis rates, and linker cleavage rates. However, it is possible to measure many of these parameters through direct *in vitro* and *in vivo* experiments.

Recently, novel PK/PD modeling has advanced to address the nonlinear mechanisms for biological products, including ADCs. These new PK/PD models can be partly or wholly based on biophysics, which is a key area that has been largely ignored in traditional PK/PD models. New semi-mechanistic PK/PD models have been developed specifically for ADCs. For example, Shah *et al.* (2012) developed a PK/PD model for an anti-CD30 ADC, which integrated multiple data sets (*in vitro* functional data, *in vivo* mouse PK data, and *in vivo* mouse xenograft PK/PD data) and demonstrated good agreement with the human clinical PK/PD data [34]. However, Shah *et al.* (2012) relied upon traditional retrospective approaches and parameter estimates (*e.g.*, volumes, and clearance rates) to determine unknown parameters rather than incorporating key biophysical considerations into the model [34].

Systems pharmacology can also address the limitations of traditional PK/PD modeling by integrating all of the relevant nonclinical data (*e.g.*, *in vitro*, *ex vivo*, *in vivo*) and existing clinical data with the known mechanism of action (MOA) of the drug and the disease pathogenesis [35]. Thus, systems pharmacology models act as a central repository of data and hypotheses. Setting the model parameters to match known physiological values (*e.g.*, actual blood volume) enables the full integration of these data to provide more accurate predictions for FIH doses. This approach of applying biophysical translation and systems pharmacology rather than traditional allometric scaling allows numerical testing of hypothetical dose levels and dose schedules prior to entering clinical trials. In fact, the FDA recently used a systems pharmacology approach to analyze the proposed dose regimen of Natpara®, a parathyroid hormone indicated to control hypocalcemia in patients with hypoparathyroidism [36]. Using this modeling, the FDA concluded that while a once daily dose regimen was adequate to control serum calcium levels, it did not control urinary hypercalciuria. A systems pharmacology model could be applied prospectively, and in the case of highly potent ADCs, this approach could be used to integrate the safety concerns for the mAb, the payload and MOA. Such integration would ideally lead to better predictions of safe and efficacious dose regimens in clinical trials.

CONCLUSIONS

Antibody drug conjugates are a promising class of large and small molecule therapeutic products that build upon the targeted specificity of mAbs and the potency of small molecule cytotoxic drugs. While there are no current regulatory guidelines specifically for the nonclinical safety assessment of ADCs, the nonclinical program should refer to relevant regulatory guidelines (*e.g.*, ICH M3(R2), ICH S6(R1), *etc.*) and use a case-by-case evaluation to fully assess the PK/PD, safety pharmacology, and toxicology of the ADC. As previously described, the toxicity of Kadcyla and Adcetris appear to be mainly attributed to the small molecule cytotoxic payload. While the toxicity of ADCs appears to be driven by the payload, the PK/PD and distribution are modulated by both the mAb and the payload. Therefore, the selection of the FIH dose for the initial clinical trial should critically evaluate the anticipated PK/PD and toxicity of the ADC and consider PK/PD modeling and systems pharmacology approaches for dose translation. As more experience with ADCs is gained and the technology moves forward for all ADC components including the mAb, linker, and payload, the regulatory requirements for nonclinical development will continue to evolve and may in fact become more streamlined as the PK/PD and toxicity profile of ADCs are better understood.

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