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Current Approaches for ADME Characterization of Antibody-Drug Conjugates: An Industry White Paper

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Running Title

Current Approaches for ADME Characterization of ADCs

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List of abbreviations

mAb, monoclonal antibody; ADC, antibody-drug conjugate; ADA, anti-drug antibody; ADME, absorption, distribution, metabolism, and excretion; C_{max}, maximum concentration; CYP, cytochrome P450; DAR, drug antibody ratio; DDI, drug-drug interaction; FcRn, neonatal Fc receptor; IgG, immunoglobulin G; PD, pharmacodynamics; PK, pharmacokinetics; Tab, total antibody; UGT, UDP-glucuronosyltransferase.

Abstract

An antibody-drug conjugate (ADC) is a unique therapeutic modality composed of a highly potent drug molecule conjugated to a monoclonal antibody (mAb). As the number of ADCs in various stages of nonclinical and clinical development has been increasing, pharmaceutical companies have been exploring diverse approaches to understanding the disposition of ADCs. In order to identify the key ADME issues worth examining when developing an ADC and to find optimal scientifically-based approaches to evaluate ADC ADME, the International Consortium for Innovation and Quality in Pharmaceutical Development launched an "ADC ADME working group" in early 2014. This White Paper contains observations from the working group and provides an initial framework on issues and approaches to consider when evaluating the ADME of ADCs.

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Introduction

Antibody-drug conjugates (ADCs) are novel molecular entities which leverage the specificity of a monoclonal antibody to deliver a potent drug to the intended pharmacological target to achieve the desired therapeutic effect. An ADC is composed of a drug molecule conjugated to a monoclonal antibody (mAb) via a linker (Fig. 1). ADCs are mostly utilized in oncology, where they provide targeted delivery of the cytotoxic drug, and thus broaden its therapeutic margins. The most frequently employed mAbs in the clinic are of the IgG1 isotype (Deslandes, 2014) and the same is true for mAbs used in ADCs. Several classes of drugs are currently being used for ADCs. The most advanced of them are microtubule disrupting agents (auristatins, such as monomethyl auristatin E, MMAE, and monomethyl auristatin F, MMAF, and maytansine derivatives, such as DM1 and DM4) and DNA-damaging agents (such as calicheamicins and duocarmycins) (Adair et al., 2012). The conjugation of these drugs to mAb is typically achieved via the epsilon-amino group of lysines or the thiol residue of cysteines (reduced interchain disulfides or genetically engineered cysteines) on the antibody molecule and a chemical linker. The most frequently used linkers are non-cleavable alkyl linkers such as N-maleimidomethylcyclohexane-1-carboxylate (MCC, used in Kadcyla®), enzymatically cleavable linkers such as the self-immolative para-aminobenzyl (PAB) group attached to a cathepsin-labile valine-citrulline dipeptide (vc, used in ADCETRIS®), and acid-labile hydrazone linkers (AcBut, used in Mylotarg®) (Sapra et al., 2011). Depending on the conjugation chemistry used, different number of drug molecules can be attached to a single mAb. The differences in the drug-antibody ratio (DAR) can affect ADC distribution and pharmacokinetics (Lyon et al. 2015). Each of the individual components of an ADC molecule contributes to the complexity of its disposition, and

overall ADME properties. In order to simplify the terminology, this review refers to the components of an ADC as outlined in Table 1.

The mechanism of action of an intact ADC involves binding of the mAb to its target antigen on a cell surface, followed by internalization via receptor-mediated endocytosis, trafficking from endosomes to lysosomes, and intracellular release of the drug (Fig. 2) (Adair et al., 2012; Alley et al., 2010; Sapra et al., 2011). In addition, ADCs can also be taken up non-specifically via pinocytosis into cells that do not express their target antigen which may contribute to efficacy in the tumor environment or potentially result in adverse effects in normal tissues. The release of the drug from the ADC in the cell could occur via proteolysis of the linker, as with a cleavable linker, or by catabolism of the entire ADC, as with a non-cleavable linker (Adair et al., 2012; Alley et al., 2010; Sapra et al., 2011). Alternatively, internalized ADCs could be recycled back into the circulation via the FcRn-mediated process, a pathway well-characterized for mAb-based therapies (Roopenian et al., 2007). In general, it is desirable that the ADC molecule is stable in circulation and drug is released only in the target tissue. In order to minimize systemic toxicity from released drug, it would need to be rapidly cleared, preferably via several orthogonal pathways (to minimize potential for drug-drug interactions and toxicity).

In order to fully assess the ADME of a novel ADC, one has to characterize the disposition of the intact molecule as well as its components: the target-mediated and catabolic clearance of the mAb; the release, and traditional small molecule distribution, metabolism and excretion of the released drug. This characterization is important during candidate optimization and development.

It can facilitate rational drug design, selection of the appropriate nonclinical models, and prediction of ADME properties in the clinic.

With an increase in the number of ADCs at various stages of nonclinical and clinical development, pharmaceutical companies have been exploring diverse approaches for ADC ADME characterization. In order to identify the key issues worth examining when developing an ADC and find the most optimal experimental systems, the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ consortium) launched an "ADC ADME working group" in early 2014. The IQ consortium is an organization of pharmaceutical and biotechnology companies providing a forum to address issues for the biopharmaceutical industry. This White Paper contains observations from the working group and provides an initial framework on issues and approaches to consider when evaluating the ADME of ADCs. However, there needs to be a continuous re-evaluation of ADME approaches as ADC technology evolves and matures over the next several years.

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Overview of ADC bioanalysis

A typical ADC is a heterogeneous mixture containing multiple drug molecules attached to an antibody at different DARs. Due to their unique composition, heterogeneous nature and because they undergo further dynamic changes in vivo, multiple bioanalytical methods are developed for the characterization of ADCs. These rely on techniques used for biologics and small molecule drugs, but there are also novel methods developed specifically for ADCs such as DAR determination (Hengel, 2014, Xu, 2013). Comprehensive description of the most important ADC analytes and details on the toolbox of bioanalytical techniques have recently been published in special issues of Bioanalysis (Gorovits et al., 2013, Kaur et al., 2013, Gorovits 2015, Myler et al., 2015, Saad et al., 2015, Kumar 2015) thus it is not the intent of this article to discuss them. ADCs could be considered as 'prodrugs' as the small molecule drug has to be released from the ADC to exert its effect. Therefore, measuring the pharmacokinetics (PK) of the ADC and released drug, serves to confirm the mechanism of drug release and helps uncover the ADME pathways important in ADC disposition. Once the clearance mechanisms and the relationship between the different drug-containing products have been established, the ultimate goal is to identify the active species that drive efficacy and toxicity. Due to the limited clinical experience with ADCs, this has not been well delineated yet; therefore multiple analytes are commonly measured (Fig. 1). Typically these are the total antibody (TAb; includes conjugated, partially conjugated, and unconjugated antibody i.e., DAR $\geq$0), ADC (conjugated and partially conjugated antibody i.e., DAR$\geq$1) or antibody-conjugated drug (total drug conjugated to antibody), and unconjugated drug (small molecule drug released from the antibody).

The need to simultaneously optimize multiple components of an ADC (drug, linker, and mAb) represents a challenge which could be assisted by the application of mathematical modeling and simulation (M&S) (Singh et al., 2015). M&S can be employed to identify which of the analyte(s) are critical for establishing exposure-response relationships for both efficacy and safety in order to reduce, on a case-by case basis, the number of bioanalytical methods necessary to characterize exposure in later stage clinical development. In addition, PKPD models incorporating DAR information could describe the relative contributions of the various drug-containing species to efficacy and toxicity, and aid in the optimization of linkers and drugs for future ADCs.

Similar to other biotherapeutics, ADCs can elicit an immune response *in vivo* that may alter their PK, efficacy, or safety. Based on the limited published clinical experience with ADCs thus far, risk assessment and bioanalytical strategies (namely screening, confirmatory and neutralizing assays) followed for traditional mAbs-based therapeutics can be appropriate for ADCs (Carrasco-Triguero, 2015). Since anti-drug antibodies (ADA) can be generated against the mAb, the drug or the linker portion of the ADCs additional characterization might be necessary to determine the specificity of the ADA response. This information may help to understand potential alterations in pharmacokinetics and efficacy or safety of the ADC.

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Disposition mechanisms of an intact ADC

The ADC technology is still in its infancy. Because of the very few ADCs on the market and limited amount of available literature, the biological and chemical processes that drive ADC disposition and thus contribute to efficacy and safety of ADCs are not fully understood. The ADME of an ADC is influenced by all three components of the molecule. The pharmacokinetics (PK) of an ADC is primarily driven by the carrier mAb backbone (Lin, 2012). However, the linker, drug, and DAR also affect stability and PK of the ADC as a whole (Lyon et al, 2015; Senter, 2009; Hamblett et al, 2004). The complex interplay between the ADC components is a topic of intense investigation. For example, in the study conducted by Lyon et al, an anti-CD70 mAb, h1F6, was conjugated to various linkers differing in their hydrophobicity (Lyon et al, 2015). As the hydrophobicity decreased, the clearance of the ADCs decreased along with an increase in the half-life and AUC. Both the distribution and elimination phases were seen to change indicating an overall change in the disposition of the ADCs. The most hydrophilic ADC had a concentration time profile similar to that of the naked mAb, pointing to the importance of optimizing the linker. Another interesting finding was the rapid and increased hepatic uptake of an ADC, especially by the Kupffer cells, as compared to the naked mAb. Uptake processes like these might explain the difference in the *in vivo* disposition of an ADC from that of its parent antibody, and highlight the need to treat ADCs as unique entities.

An ADC can be cleared from circulation by target-mediated uptake followed by degradation in the lysosomal compartment (Fig. 2). In addition, an ADC can be a subject to non-specific uptake via pinocytosis and catabolism by certain cell types in multiple organs including the liver, similar to traditional mAbs. Upon internalization, ADCs can be recycled by FcRn which may result in

prolonged systemic exposure. Additionally, deconjugation of the ADC can result in conversion of the ADC to species with different drug loads as well as unconjugated mAb. The types of *in vitro* and *in vivo* studies that could be used to characterize ADC ADME are discussed below and summarized in Table 2. In general, similar studies would be conducted for ADCs with cleavable vs. non-cleavable linkers, as the fundamental ADME questions that need to be answered are the same.

ADC stability in systemic circulation. Ideally, ADCs should be stable in blood and release the drug only in the target tissue. However, since ADCs remain in circulation for several days following their administration and are continuously exposed to plasma proteases, a gradual release of the drug is possible, depending on the nature of the linker chemistry. Plasma stability of an ADC can potentially be different across species. Understanding the mechanism and extent of drug release in circulation can help develop ADCs with optimal safety/efficacy profiles as it is important to engineer the right balance into the ADC molecule which would allow it to be stable in circulation, but promptly release the drug in the target cells. Instability in plasma might lead to premature release of the drug in circulation and its subsequent distribution to tissues potentially leading to dose limiting toxicities (Saber et al., 2015). Evaluation of *in vitro* stability of an ADC in plasma serves to provide information on linker stability in the systemic circulation in multiple species, as well as on potential released drug-containing products. These studies can be conducted in plasma or serum from human as well as relevant nonclinical species (the incubation is typically conducted at 37ºC at pH7.4 for at least 96 hours at an ADC concentration around the observed or predicted C_{max} in animal species or human). Formation of the released drug, as well as DAR changes, is usually quantified over the study duration. These studies, when conducted

early in the ADC discovery process, can help optimize the combination of the mAb, linker and the drug molecule.

Effect of DAR on ADME properties. Depending on the conjugation chemistry, different numbers of drug molecules can be attached to a single antibody, which is characterized by the DAR representing the average number of drug molecules per antibody molecule. In addition, the DAR of an ADC may change over time *in vivo*. The initial DAR and rate of its' change *in vivo* are important parameters for an ADC, as they may impact the ADC's physicochemical properties, efficacy, safety, and pharmacokinetics. ADCs with high DAR tend to aggregate and have higher clearance than the unconjugated mAb or lower loaded species (Lyon et al., 2015; Senter, 2009; Hamblett et al., 2004). For example, in a study conducted by Hamblett et al, SCID mice were treated with naked mAb, or DAR2, DAR4, or DAR8 ADCs (Hamblett et al., 2004). The results suggested that, while their half-lives were similar, the ADCs with higher DARs had lower exposures (AUC) and greater clearance than the naked antibody or DAR2 ADC. An examination of the concentration time profiles showed a change in the distribution phase of the three ADCs while the terminal phases were parallel; this is reflected in the increasing volume of distribution with increased DAR. While DAR2 ADC had exposure closest to the naked mAb, it was DAR4 ADC which had the best efficacy in the mouse xenograft model, demonstrating that optimizing the DAR for both PK and efficacy was important and not one or the other.

DAR-related ADC aggregation can also potentially change the organ uptake and mechanism of clearance of ADCs, thereby exposing the liver and/or other organs to potentially undesirable high

levels of active drug. Moreover, at higher DARs, a more hydrophilic drug may have less of an impact on the disposition of the ADC than a hydrophobic drug (Lyon et al. 2015).

Also, novel technologies, like masking drug hydrophobicity, can be employed to provide uniform, higher drug loads with improved PK and efficacy (Lyon et al. 2015). Since potentially, the drug can be metabolized while still conjugated to the mAb, those changes can be reflected in the DAR values. In addition, impact of site-specific vs. conventional conjugation should be considered as this can impact the drug release from an ADC (Shen et al., 2012a). The DAR is typically measured by high resolution mass spectrometry (Hengel, 2014, Xu, 2013) which can be applied for both *in vitro* and *in vivo* generated samples.

ADC PK in nonclinical species. Since ADCs, by design, utilize internalization of the ADC-receptor complex as the mechanism for drug's delivery, cross-reactivity of the carrier mAb to the target in nonclinical species would impact the ADC's pharmacokinetics and distribution. The binding affinities of the ADC to the target in multiple species are typically measured during the early stages of drug discovery. If the antibody is not cross reactive to the rodent target, the PK and toxicity may not be reflective of PK or toxicity in a target expressing species. However, it may still provide some information on the non-specific disposition of ADCs and on potential drug-related metabolites (Kamath, 2015). The choice of animal species to evaluate the PK of an ADC incorporating a novel antibody typically follows the same general principles as an unconjugated antibody (ICH S6). In addition, species selection for a novel drug incorporates considerations used for a new chemical entity (e.g., for anticancer products in accordance with ICH S9) on a case-by case basis, based on the mode of action of the drug. In most cases, the

pharmacokinetics of an ADC are characterized at doses low enough to evaluate target mediated clearance and at doses high enough to understand toxicokinetics. TAb, ADC, and released drug are typically quantified over the study duration. ADCs are usually administered by the intravenous (IV) route, thus, obviating the absorption phase. While the TAb and ADC systemic concentration time profiles are those normally observed after IV administration, the profile of the released drug, however, akin to the exposure profile of the active component of a prodrug, typically resembles that of an extravascularly administered compound.

ADC tissue distribution. A general determination of whole body tissue distribution of the ADC can be considered in order to determine distribution between target expressing and non-target expressing tissues. The distribution of an ADC in various tissues and subsequent de-conjugation or catabolism to release the drug impacts its efficacy and safety (Alley, 2009, Boswell, 2011). In addition, information on active drug metabolites in the tissues can also be obtained which may confer additional activity. Tissue distribution studies can be conducted in rodents (rats and/or tumor bearing mice) to evaluate distribution to normal tissues (or tumor). These types of studies are typically conducted with radiolabeled ADCs where the radiolabel could be applied on the drug (usually C-14 or H-3), or simultaneously on both the antibody and drug using a dual-labeled ADC with C-14 and H-3 (Alley 2009). While evaluation of the whole body tissue distribution in rodents using radiolabeled ADC can be considered, this assessment may not always be appropriate due to challenging and expensive synthesis, limitations in sensitivity and resolution of this technique as well as typical lack of cross-reactivity to rodent targets.

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ADME (mass balance) evaluation. Currently, a human ADME study using radiolabeled material is not recommended for the following reasons. For the cytotoxic/genotoxic drugs typically used in oncology ADCs, dosing of ADCs in healthy volunteers is not appropriate. Therefore, such an evaluation would have to be conducted in cancer patients. Because of the typically long ADC half-life, patients would have to be sequestered for prolonged periods of time (3-4 weeks) with little to no benefit to the patient, which would not be ethical. An ADME study of shorter duration may not be adequate and can result in incomplete mass balance data. In addition, identification of the circulating products of further metabolism of the drug may be challenging due to typically very low concentrations of those products. Therefore, a traditional human ADME study for an ADC is not feasible. An animal (rodent) ADME study using an ADC with radiolabel on the drug may be considered instead (Erikson et al., 2012a). Various matrices such as bile (using bile-duct cannulated rats), urine, and feces can be collected in addition to serum/plasma. This evaluation could help to understand the metabolism and excretion routes of an ADC and released drug (or drug-containing species). However, it should be noted that since most of the ADCs do not cross react with rodent targets, this evaluation would primarily address nonspecific uptake and degradation pathways and may not necessarily represent the disposition of ADC in humans. Also, due to the long half-life of ADCs the study duration would need to be extended in order to achieve good recovery of radioactivity and mass balance.

Novel ADCs with previously characterized drugs. Drugs or linker-drugs that have been previously tested in the clinic can be conjugated to different mAbs to form new ADCs. In these cases, some of the ADME information can be obtained from existing published reports/filings and/or internally garnered unpublished data, and evaluation would focus on generating key data

specific to the novel ADC. Often, a well-studied drug is conjugated to a mAb via a novel linker sequence or using unreported conjugation chemistries (i.e. site-specific relative to conventional cysteine or lysine residue based conjugation chemistry). Therefore, ADME evaluation would address major released drug-containing species, plasma stability of the ADC, major ADC clearance mechanisms, and confirm that projected human PK properties support the intended dose and frequency of administration.

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Release of the drug from an ADC

The drug is intended to be released intracellularly in the target tissue, in most cases via proteolytic cleavage of the linker or catabolism of the entire ADC molecule in lysosomes. However, current ADCs are not completely stable in the circulation and non-specific release of the drug or transfer of the drug to other serum components have been reported (Alley et al., 2008). Understanding the mechanism by which the drug is released from the ADC helps to identify potential pharmacologically active drug-containing products and select the appropriate bioanalytical methods. This evaluation involves identification and quantitation of the major released drug-containing species. In addition, drug might be cleaved by extracellular proteases especially in the proximity of the tumor. Therefore, understanding the cellular permeability of drug-containing products can help understand ADC's pharmacodynamics (PD), and potential bystander effects.

Experimental systems for in vitro assessment of drug release from an ADC. In general, *in vitro* systems that can be utilized for identification of drug-containing species released from an ADC as well as products of their further metabolism are similar to those used for traditional small molecule drug metabolism studies. However, specific experimental conditions might need to be adjusted in order to accommodate unique aspects of an ADC's properties.

Similar to traditional mAbs, tissue distribution of the ADC is low, and the majority of the ADC distributes to the organs where IgG catabolism takes place, with liver playing a prominent role in ADC clearance (Shen et al., 2012b; Boswell et al., 2011). Therefore, one would expect that the majority of ADC catabolism as well as linker and drug metabolism might occur in the liver.

Hepatocytes are the most complete system which contains all relevant microsomal enzymes as well as cytosolic enzymes, such as aldehyde oxidase, peptidases etc. However, due to the lack of target protein expression on hepatocytes, utilizing this system for studying drug release from ADCs is limited. In addition, using hepatocytes for evaluation of the released drug's metabolism may be limited by its permeability. Liver microsomes are a convenient *in vitro* system which contains CYP and UGT enzymes, and is not confounded by the drug's permeability, uptake, or toxicity. However, it lacks the cytosolic and lysosomal enzymes which in many cases are responsible for the release of the drug from the ADC molecule. Therefore, it could be considered a good tool for studying the metabolic pathways of the released drug, but may have limited utility for studying the drug release from ADC molecule.

Since ADCs have been primarily used in the treatment of cancer, cancer cells could potentially be utilized as a system for studying drug release from the ADC (Erickson et al, 2012b). However, selection of the appropriate cell line would depend on the target expression, to facilitate target mediated uptake of ADC by the cells and therefore, it cannot be standardized and used across multiple programs. In general, while cancer cells express some drug metabolizing enzymes, all of those enzymes are found in the liver as well. Moreover, cancer cells have been shown to up-regulate Phase II enzymes and down regulate Phase I enzymes as compared to the liver (Rodríguez-Antona et al., 2002; Zahreddine and Borden 2015).

Lysosomal preparations represent another potential *in vitro* system. While lysosomes can be used to study the release of the drug from the ADC, as they mimic ADC degradation in the cell, it is an artificial system which does not contain drug-metabolizing enzymes such as CYPs or UGTs,

and therefore, it cannot be used for metabolism studies of the drug itself. In addition, uptake of the ADC into the lysosomes might be limited, which may hamper the stability assessment.

The liver S9 fraction contains all major drug metabolizing enzymes, does not rely on the permeability of the drug, is transporter independent, and is less susceptible to cytotoxic agents. In addition, the S9 fraction can be used at either pH 7.4 (to study metabolism of the drug) or acidified to mimic the pH of the lysosomal environment which is the site of degradation of an ADC. Therefore, this system can be used for studying drug release and profiling of drug-containing species of both intact ADC and drug.

In general, it is recommended that understanding of the linker and drug chemical structures and potential reactions that they can undergo, be taken into consideration when selecting the *in vitro* test system and the most straightforward (or simplest) system is used.

DDI potential. Based on the information from the limited number of ADCs in the clinic, their potential for DDI is typically considered to be low. However, since First in Human clinical studies with ADCs are typically conducted in patients who also take multiple concomitant medications, it is useful to assess the DDI risk of the released drug at the pre-clinical stage based on its ADME characteristics. The drug released from an ADC can be eliminated unchanged or metabolized by enzymes such as the CYP system (Fig. 2). Direct renal or biliary elimination could potentially be a significant component of the overall drug's clearance. Drug-drug interactions are quite common for small molecule drugs mainly due to inhibition or induction of drug metabolizing enzymes and transporters. In most cases, systemic concentrations of the released drug are extremely low, and, therefore, the risk of the ADC being a DDI perpetrator can

be considered minimal (Han, 2014). However, one might expect the liver to receive higher concentrations of the drug, as part of non-specific catabolic clearance of an ADC, than those inferred from drug's systemic concentration. Nevertheless, in a clinical DDI study, ADCETRIS® (Brentuximab Vedotin), a vc-MMAE ADC, did not affect the PK of midazolam (CYP3A substrate) (ADCETRIS® Drug Label). Investigation of a novel drug as an enzyme inhibitor or inducer should be conducted in accordance with the most current version of FDA guidelines (FDA Draft Guidance for Industry, 2012) and EMA guidelines (EMA Guideline on Investigation of Drug Interactions, 2015).

In general, the probability of a released drug to be a DDI victim exists and impact can be high due to the fact that these cytotoxic drugs typically have narrow therapeutic margin. Therefore, inhibition of their clearance might lead to an increase in drug exposure in tissues and circulation, which could result in toxicities. When coadministered with rifampicin (CYP3A inducer) and ketoconazole (CYP3A inhibitor) no changes in PK of ADCETRIS® were observed. However, exposure of released MMAE was reduced by ~46 % and increased by ~34 % by coadministration of rifampicin and ketoconazole, respectively (ADCETRIS® Drug Label). Also, while no formal DDI studies have been conducted with Kadcyla®, a DM1-containing ADC, its label contains a caution that coadministration with strong CYP3A4 inhibitors should be avoided due to the potential for an increase in DM1 exposure and toxicity (Kadcyla® Drug Label). As most of the patients will also take a number of concomitant medications, a DDI risk assessment for the ADC including *in vitro* evaluation of enzyme interactions (in particular reaction phenotyping for CYP metabolism) for drug and potential major circulating drug metabolites need to be performed

during development to determine if formal clinical studies should be conducted. Studies to assess transporter-mediated DDI may be valuable at later stages of the development.

Considerations for the released drug. Potentially, upon release the drug can undergo further metabolism, which can affect the observed toxicity and pharmacology of the ADC. Understanding the mechanism and identification of the metabolites may provide insight into drug-related species to monitor in subsequent animal and human studies. However, systemic concentrations of these species are generally low and, therefore, there may be no need, or insufficient assay sensitivity may not allow detecting them. *In vivo* samples obtained from high dose toxicity animals might be the best place to look for such products. If deemed necessary, based on *in vitro* or animal *in vivo* data, identification of circulating products of further metabolism of the drug may be performed in patients using unlabeled ADC.

Information on plasma protein binding and permeability of the drug can be used for understanding ADC's off-target toxicity due to the released drug's distribution into cells/tissues by active uptake or passive diffusion, rather than for understanding the ADC's pharmacological activity as it is driven by the drug released inside the target cells.

For novel drugs, a pharmacokinetic study in rodents following an intravenous administration of the unlabeled unconjugated drug should be conducted. The dose is typically selected based on the total conjugated drug load at the ADC dose which is expected to be below the maximum tolerated dose. *In vivo* metabolite scouting can be included in the study design. Collection of

urine and bile can also be incorporated into this evaluation; however, sensitivity may limit the utility of these data.

Conclusions

ADME characterization for an ADC is a complex process as it needs to take into account both the mAb and small molecule components of this modality. While no standard “one size fits all” approach can be applied to all ADCs, the current review outlines the advantages and disadvantages of the currently used experimental systems and strategies, and provides guidance that should help investigators to develop successful novel ADCs with desirable ADME properties. Since ADC technology is still evolving, there needs to be a continuous re-evaluation of ADME approaches as it matures over the next several years.

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Figure legends

Figure 1. Diagram of a typical ADC. An ADC consists of a mAb conjugated to a drug via a linker. Different number of drug molecules can be attached to a single mAb, which results in ADCs with different DARs.

Figure 2. Disposition of a typical ADC. An ADC can be delivered inside the cell either by antigen receptor-mediated internalization or by nonspecific endocytosis followed by the drug release in the lysosomal compartment through linker proteolysis or whole ADC catabolism. The free drug can bind to its intracellular target, or be released from the cell into extracellular space or systemic circulation. In addition, in case of a cell containing drug metabolizing enzymes, the free drug can be metabolized and metabolites can be secreted (along with the unchanged drug) into systemic circulation or bile (in case of hepatocytes).

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TABLE 1. Terminology used in the current review

Definition
Drug – also referred to as "payload", "warhead", or "toxin" - Compound which exerts the intended pharmacological effect (e.g. tubulin binding) of an ADC. In the case of a cleavable linker, intact drug is released from the ADC; In the case of a non-cleavable linker, released from the ADC drug contains the linker and an amino acid fragment
mAb – antibody portion of the ADC
Linker – Chemical bridge which links the drug to the mAb
ADC - Whole antibody-drug conjugate molecule which contains the drug conjugated to the mAb via a linker.

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TABLE 2: Types of *in vitro* and *in vivo* studies for characterization of ADC ADME

Molecule	ADME data
*ADC**	*In vitro* stability in plasma or serum from animals and humans.
*ADC**	PK in pharmacology and toxicology species
*ADC***	Animal (rodent) ADME: PK, excretion, and metabolism
ADC	Identification of circulating metabolites formed from the released drug in patients
Drug	Rodent PK
Drug	Plasma protein binding across species
Drug	*In vitro* characterization of metabolites formed from the released drug (safety species and human)
Drug	Reaction phenotyping
Drug	Passive/active (uptake or efflux) transport (as substrate)
Drug	CYP inhibition and induction

* Analytes that could be measured as appropriate include Tab, ADC, unconjugated drug

** This evaluation is recommended to be conducted with an ADC bearing a radiolabel on the drug

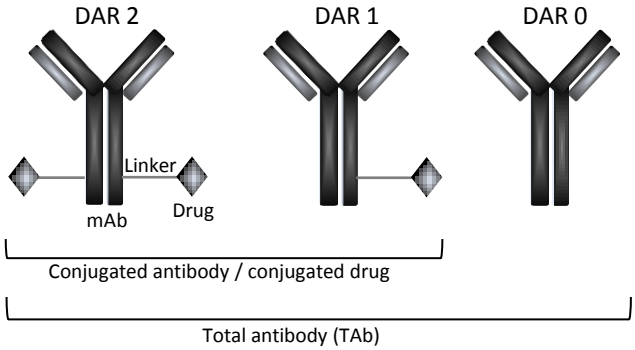
DAR 2
DAR 1
DAR 0
Linker
Drug
mAb
Conjugated antibody / conjugated drug
Total antibody (TAb)

Figure 1