Absorption, Distribution, Metabolism, and Excretion Considerations for the Development of Antibody-Drug Conjugates

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ABSTRACT

Antibody-drug conjugates (ADCs) are a class of therapeutics that are designed to deliver potent small-molecule drugs selectively to cells that express a specific target antigen while limiting systemic exposure to the drug. This is accomplished by conjugating a potent drug onto an antibody-based therapeutic with a linker that is exquisitely stable in plasma. The development of an effective ADC requires optimizing a number of design elements and an extensive understanding of absorption, distribution, metabolism/catabolism, and elimination (ADME) processes for the ADC construct. Furthermore, as ADCs are a combination of an antibody and small-molecule drug, understanding key aspects of the ADME of each individual component is needed. This review aims to provide considerations for the development of ADCs from an ADME point of view.

Introduction

Antibody-drug conjugates (ADCs) are a class of therapeutics that achieves the goal of targeted distribution of potent drugs to desired sites of action while minimizing distribution to non-target cells and tissues (Carter and Senter, 2008). This key design goal is accomplished by leveraging the specificity of an antibody to bind specific proteins on target cells, the potency that can be achieved with small-molecule drugs, and a linker that stabilizes the ADC construct in plasma while allowing for the release of the small molecule or small-molecule derivative upon ADC internalization in target cells (Fig. 1). ADCs represent a modality of therapeutics in which antibodies are empowered with small molecules to produce a more profound effect on target cells. This modality applies to any therapeutic area, and evaluations in oncology have led the current advancements in this technology. Thus, this review will focus primarily on absorption, distribution, metabolism/catabolism, and elimination (ADME) considerations for ADCs in an oncology setting.

The concept of an ADC arguably was put forth by the German physician Paul Ehrlich, who in 1906 postulated the concept of a "magic bullet" to deliver drugs to specific targets (Strebhardt and Ullrich, 2008); the first application of ADCs as "guided missiles" was implemented many decades later (Decarvalho et al., 1964). The first successful clinical application of this concept was fulfilled even later with the advancement of gemtuzumab ozogamicin (GO, Mylotarg; Pfizer/Wyeth Pharmaceuticals, Philadelphia, PA) (Bross et al., 2001), the first ADC to be approved for clinical use. GO was eventually re-approved in 2013. GO is an ADC that consists of a murine monoclonal antibody (mAb) directed to CD33, a carbohydrate antigen expressed on the surface of the human promyelocytic leukemia cell line (HL-60). GO is chemically conjugated to a potent calicheamicin-gamma-1 (calicheamicinOX) derivative that is released after ADC internalization in target cells (Fig. 1). GO was approved for marketing worldwide in 2000 for the treatment of relapsed or refractory acute myeloid leukemia in adults who have not responded to standard chemotherapy. GO was moved from the U.S. market in 2010 after failing to demonstrate adequate clinical benefit to patients enrolled in clinical trials. However, despite this setback, GO represented the clinical validation of the ADC concept and established the preliminary criteria for a clinically effective ADC.

The second-generation ADCs were constructed on the premise that linker stability and higher small-molecule potency are key design elements for an effective ADC. This generation of ADCs is exemplified by brentuximab vedotin (Adcetris; Seattle Genetics, Bothell, WA) and ado-trastuzumab emtansine (T-DM1, Kadcyla; Genentech, South San Francisco, CA), both approved for marketing worldwide. Compared with the activity of the unconjugated antibody (or what is often termed the naked antibody), both brentuximab vedotin and ado-trastuzumab emtansine show remarkable increases in activity while maintaining an acceptable safety profile (Table 1). The next generation of ADCs is expected to further interrogate the biologic and chemical design space for ADCs. Engineering of the antibody to direct conjugation to specific sites, the use of different residues, and the introduction of chemical sites of conjugation have been explored (Junutula et al., 2008; Shen et al., 2012b; Kung Sutherland et al., 2013). The drug-linker technology continues to be advanced by the modification of the chemistry at the site of antibody conjugation to confer stability (Shen et al., 2012a; Lyon et al., 2013), the introduction of chemical entities that mask the physiochemical properties of the drug-linker to improve ADC pharmacokinetics (PK) (Kovtun et al., 2010; Burke et al., 2014), and the introduction of novel linkers (Lyon et al., 2013).

Considerable advancements are continually being made, and many of these advancements revolve around improving the pharmacokinetic and ADME characteristics of ADCs. Thus, it remains important to
characterize the ADME of ADCs to establish their use in humans. Furthermore, in addition to leveraging the attributes that make ADCs promising, ADCs also take on some of the deficiencies associated with its basic components: monoclonal antibodies (mAb) and small molecules. Understanding the ADME of ADC is complex, and it is necessary to understand the ADME of mAb, small molecules, and the ADC construct itself. Each component has unique attributes that require characterization and optimization to develop effective ADCs. A summary of these attributes for ADCs is shown in Table 2 and compared with those of small-molecule drugs and naked mAb.

**Antibody-Drug Conjugate Stability**

The primary mechanism of action of an ADC is the delivery of the conjugated drug to its target cells or tissues. Premature release of the conjugated drug before reaching its intended target may cause unwanted systemic toxicity and reduce the amount of drug delivered to the target tissues. It has been shown that the stability of the ADC construct in the biologic system could affect PK, efficacy, and toxicity. Thus, careful in vitro and in vivo evaluations of ADC stability during lead candidate selection and development are critical.

Drug-linkers, a key determinant of ADC stability, have been a primary focus in ADC technology development. A variety of drug-linkers have been developed and applied in ADC conjugation chemistry. Early linkers such as the hydrazone linker used in GO are more labile with higher drug-to-antibody ratio (DAR) (6 or 8) generally tend to form high molecular weight species (Beckley et al., 2013). With the increased DAR, ADCs may also introduce more hydrophobic chemical groups to the mAb, readily destabilized (Adem et al., 2014; Guo et al., 2014). Conjugation of an antibody to a drug can impact the antibody conformational stability. It has been shown that upon conjugation, ADCs can still maintain similar secondary and tertiary structure as naked mAb; however, when subject to higher temperatures, ADCs are more readily destabilized (Adem et al., 2014; Guo et al., 2014). Conjugation may also introduce more hydrophobic chemical groups to the mAb, resulting in lower colloidal stability and greater aggregation potential of the ADC. These may explain, in part, the observations that ADCs with higher drug-to-antibody ratio (DAR) (6 or 8) generally tend to form high molecular weight species (Beckley et al., 2013).

### TABLE 1

<table>
<thead>
<tr>
<th>Indication</th>
<th>Antibody-Drug Conjugate</th>
<th>Parent mAb</th>
<th>ORR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/R Hodgkin lymphoma</td>
<td>Brentuximab vedotin (anti-CD30)</td>
<td>SGN-30 (anti-CD30)</td>
<td>75</td>
</tr>
<tr>
<td>R/R sALCL</td>
<td>Brentuximab vedotin (anti-CD30)</td>
<td>SGN-30 (anti-CD30)</td>
<td>86</td>
</tr>
<tr>
<td>Metastatic breast cancer</td>
<td>Ado-trastuzumab emtansine (anti-HER2)</td>
<td>Trastuzumab (anti-HER2)</td>
<td>26–64</td>
</tr>
</tbody>
</table>

ORR, objective response rate; R/R, relapsed or refractory; sALCL, systemic anaplastic large cell lymphoma.
TABLE 2

Attributes of small-molecule drugs (SMD), antibodies, and ADCs

<table>
<thead>
<tr>
<th>Attribute</th>
<th>SMD</th>
<th>mAb</th>
<th>ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW (Da)</td>
<td>Typically &lt;1 K</td>
<td>~150 K</td>
<td>~150 K</td>
</tr>
<tr>
<td>Dosing route</td>
<td>Typically Oral</td>
<td>Vd close to plasma volume; limited tissue distribution</td>
<td>Intravenous ADC similar to mAb; unconjugated drug similar to SMD</td>
</tr>
<tr>
<td>Distribution</td>
<td>High Vd and well perfused into tissues; potential substrate of transporters</td>
<td>Mostly recycled by body</td>
<td>Long for both ADC and unconjugated drug (days) when dosed as ADC</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Mainly phase I and II metabolism</td>
<td>Catabolism</td>
<td>Both</td>
</tr>
<tr>
<td>Excretion</td>
<td>Mandy biliary and renal excretion</td>
<td>Mostly recycled by body</td>
<td>Both</td>
</tr>
<tr>
<td>Circulation half-life</td>
<td>Typically short (hours)</td>
<td>Long (days)</td>
<td>Long for both ADC and unconjugated drug (days) when dosed as ADC</td>
</tr>
<tr>
<td>PK linearity</td>
<td>Usually linear at low dose and nonlinear at high dose</td>
<td>Usually linear at high dose and nonlinear at low dose</td>
<td>Similar to mAb</td>
</tr>
<tr>
<td>PK analytes</td>
<td>Drug and metabolites</td>
<td>Antibody</td>
<td>ADC or conjugated drug, total antibody, and unconjugated drug</td>
</tr>
<tr>
<td>Analytical methods</td>
<td>Typically LC-MS/MS</td>
<td>Typically ligand binding assays</td>
<td>Both</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>API heterogeneity</td>
<td>Uniform single entity</td>
<td>Generally uniform</td>
<td>Mixture of ADCs with different DAR</td>
</tr>
</tbody>
</table>

API, active pharmaceutic ingredient; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MW, molecular weight; Vd, volume of distribution.

Modifications of the antibody conjugation sites through site-specific mutations can also confer stability to the ADC construct. The conjugation to certain mutated cysteine or non-native amino acid sites appeared to have the potential to be more stable than those using the conventional conjugation sites. Improved efficacy and/or tolerability in animal models has been shown with these site-specific conjugated ADCs (Junutula et al., 2008; Shen et al., 2012b; Jackson et al., 2014).

**Biodistribution**

The biodistribution of the small-molecule drug is fundamentally altered upon conjugation to an antibody. The ability to access tissues that primarily traffic antibodies, enter cells despite poor physiochemical properties, and concentrate in tissues that express a specific target antigen are all enabled through the ADC technology. Thus, understanding and leveraging this process to further expand the distributional therapeutic index is key to developing ADCs. Furthermore, because antibodies are trafficked through biologic mechanisms of catabolism, modulating the distribution to those sites of antibody and ADC degradation becomes an important design parameter. When evaluating the biodistribution of an ADC, the technology used to determine tissue concentrations is a key consideration when interpreting those studies as each modality will provide potentially different but often complementary information. A variety of techniques, including classic studies of assessing radioactivity in tissues after necropsy and dissection (often termed “cut and count” studies), serial imaging studies using technologies such as single photon emission computed tomography or positron emission tomography, or terminal imaging studies using quantitative whole-body autoradiography or other techniques have been used in the assessment of ADC and ADC component biodistribution.

Small molecules typically are widely distributed, as exemplified by a volume of distribution that is appreciably larger than the blood volume of an animal or human. In contrast, antibodies are restricted primarily to plasma and extracellular fluids and are concentrated at tissues that express the target antigen, especially tumors in xenograft mouse studies (Alley et al., 2009; Boswell et al., 2012; Boswell et al., 2013). For an ADC, the pharmacokinetics and biodistribution have been noted to be more like an antibody than a small molecule. Thus, when small molecules are conjugated to antibodies, the biodistribution of the small molecule is fundamentally altered, and the small molecule may be able to reach cells and tissues that could not otherwise be accessible by free diffusion alone. Much insight into the biodistribution of ADCs can be gleaned from the findings with antibodies, but it is also clear that the biodistribution of ADC can be different than that of antibodies.

The biodistribution of an ADC, like antibodies, is dictated by both biophysical and biologic mechanisms. ADC have nanoparticle-like behavior that results in tissue penetration and diffusion that can be understood from a biophysical perspective (Schmidt and Wittrup, 2009). Similar to antibodies, ADCs are thought to be trafficked through the vascular and lymphatic system. In terms of tissue distribution, an appreciable amount of antibody is seen in the liver, lungs, kidneys, and skin (Shah and Betts, 2013; Yip et al., 2014), although the specific concentration in tissues may differ between antibodies and ADCs (Herbertson et al., 2009). In addition, the binding affinity or avidity of the ADC is expected to be important in the penetration of the ADC through target-bearing tissues such as tumors (Rhoden and Wittrup, 2012). Alterations in the binding affinity or the ADC to FcRn, the charge of the ADC, or alteration of the physiochemical properties of the ADC may also affect tissue distribution (Boylan et al., 2010; Boylan et al., 2013; Yip et al., 2014).

Circulating small molecule or related analytes are expected in blood after degradation of an ADC in tissues. Thus, it is also important to understand small-molecule distribution. Small molecule drugs are expected to be widely distributed. As an example, monomethyl auristatin E (MMAE) concentrations have been shown to be higher in a number of tissues compared with plasma concentrations. In rats, MMAE was rapidly and widely distributed. Tissues with levels of MMAE that are 10-fold higher than in plasma (area-under-the-curve ratio) include the anterior pituitary gland (111), bone marrow (52), posterior pituitary gland (48), thyroid (41), small intestine (40), thymus (39), spleen (36), lung (33), lymph node (31), cecum (27), uveal tract of the eye (25), large intestine (21), salivary gland (21), choroid plexus (20), kidney cortex (20), kidney medulla (19), adrenal gland (18), heart (17), brown adipose tissue (14), Harderian gland (14), urinary bladder (13), stomach (12), liver (12), and pancreas (12). MMAE levels in the brain and spinal cord were below the limit of quantitation. The half-life of MMAE in the thymus (3 days), pituitary glands (0.7–1.0 day), and eye uveal tract (24 days) is longer than the half-life in plasma (0.7 day). (Seattle Genetics, 2014). These results are consistent with those of another quantitative whole-body autoradiography study with a valine-citrulline–MMAE ADC (Pastuskovas et al., 2005). However, one particular point to bear in mind is that the
distribution of a small-molecule drug after administration of that same small-molecule drug is determined, in part, by the physicochemical properties of that small-molecule drug. Thus, small-molecule drugs that are highly hydrophilic may not access cells or tissues as well as highly lipophilic small-molecule drugs. Once conjugated to an antibody as an ADC, the ADC uses alternative mechanisms to enter cells, so small-molecule drug biodistribution studies may not be representative of the tissue distribution of the release small-molecule drug from an ADC. In addition, some tumors are considered to be highly heterogeneous with variable target-antigen expression, so highly membrane-permeable small-molecule drugs could be advantageous in conferring a pharmacologic effect on neighboring cells that do not express or poorly express the target antigen (Okeley et al., 2010).

In addition to free diffusion, small molecules can be substrates of uptake or efflux transporters. In turn, those transporters may increase or reduce the intracellular concentration of a small-molecule drug in tumors and/or normal tissues (Chu et al., 2013). Strategies have been developed to reduce the liabilities associated with upregulation of P-glycoprotein and other potential transporters in tumor cells (Kotun et al., 2010; Kung Sutherland et al., 2013) This may affect the small molecule PK (Han et al., 2013a) as well as the antitumor effect (Naito et al., 2000; Jager et al., 2011). Both MMAE and mertansine (DM1), two lead drugs used in ADCs, have been implicated as a substrate of P-glycoprotein (Seattle Genetics, 2011; CDER, 2013).

Catabolism/Metabolism

The mAb portion of an ADC is expected to be catabolized through proteolytic degradation by tumors and other tissues into amino acids and recycled into other proteins. Many of the factors that can impact mAb catabolism, such as FcRn-mediated recycling, Fc-gamma interactions, and receptor-mediated endocytosis, may also affect ADCs and result in altered PK. Based on preclinical studies, antigen expression and receptor/cell density should also be important factors in the disposition of an ADC. This section will focus on the factors unique to ADCs that should be considered during ADC development.

Perhaps more importantly, conjugation itself may modulate the catabolism of ADCs. It has been shown that ADCs in general have shorter terminal half-lives compared with those of their respective unconjugated parent mAb, and this stability is linker dependent. For example, the average serum half-life of brentuximab vedotin is 4 to 6 days in patients, shorter than that of the unconjugated mAb SGN-30, which had a terminal half-life of 1 to 3 weeks (Bartlett et al., 2008; Younes et al., 2010). The number of conjugates on an ADC may also affect ADC catabolism, with a higher DAR leading to a shorter serum half-life and lower exposure. Hamblett et al. (2004) have shown that for a valine-citrulline–MMAE ADC in mice, the clearance of purified eight-drug load ADC was 3-fold faster than that of a four-drug load ADC. The increased clearance and reduced exposure of ADCs with a higher DAR may explain, despite the greater drug load per antibody, the comparable in vivo antitumor activity of those ADCs with lower DAR. In addition, the type of drug linkers may also play a role in the circulation stability of an ADC. When trastuzumab was conjugated with DM1 using either a thioether or disulfide linker, the resulting ADCs had different plasma clearance. The disulfide-linked T-SPP-DM1 ADC was cleared approximately 2-fold faster in mice than the thioether-linked trastuzumab emtansine (T-DM1) ADC (Erickson et al., 2012). Because the clearance of the respective total antibodies is comparable, the difference in ADC clearance is due to the different linkers used for conjugation. To maximize the ADC exposure for a given target at a given dose, these and other design factors should be taken into consideration for the lead selection of an ADC construct.

The circulating unconjugated drug after ADC administration, on the contrary, has metabolic properties of small-molecule compounds (Table 3). Once released from the ADC, the small-molecule drug may be metabolized by cytochrome P450 (P450) enzymes and subject to potential drug-drug interactions from P450 inhibitors or inducers. Dependent on the type of drug-linkers, one or more active small-molecule drugs may be released by an ADC in vivo. After administration of ado-trastuzumab emtansine in patients, the DM1-containing catabolites MCC-DM1, Lys-MCC-DM1, and DM1 were identified in plasma with the observed maximum concentrations in the low to middle nanomolar range (Krop et al., 2010; Burris et al., 2011; CDER, 2013). In vitro studies suggest that Lys-MCC-DM1 is metabolically stable and is not an inhibitor of P450 enzymes, whereas DM1 may be metabolized by CYP2D6, CYP3A4, and CYP3A5 and is also a time-dependent inhibitor of CYP3A (Davis et al., 2012). The metabolism of other maytansinoid-containing catabolites released from ADCs with different drug-linkers were also studied and reviewed (Sun et al., 2011; Davis et al., 2012; Erickson and Lambert, 2012). After administration of brentuximab vedotin in patients, MMAE was the only released drug identified in plasma with peak concentrations in the low nanomolar range (CDER, 2011; Han et al., 2013a).

In vitro data indicate that MMAE is both a substrate and inhibitor of CYP3A. MMAE-related metabolites after brentuximab vedotin administration in patients were identified in feces and urine at very low levels, detectable only in highly concentrated samples (CDER, 2011).

The unconjugated small-molecule drugs are potent cytotoxins, and any changes in their exposure due to P450 enzymes-mediated drug-drug interactions (DDIs) may potentially impact the safety in patients, thus in vitro and in vivo evaluations of the metabolism of these molecules should be considered in ADC drug development. On the other hand, the DDI potential of the unconjugated small-molecule drugs as an inhibitor appear to be small as the inhibitory K_i values estimated from the in vitro assays are typically in the micromolar range (Davis et al., 2012; Han et al., 2013a), several log orders greater than those observed circulating drug levels. However, due to the

<table>
<thead>
<tr>
<th>Attribute</th>
<th>MMAE</th>
<th>DM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic turnover</td>
<td>Minimal</td>
<td>Extensive</td>
</tr>
<tr>
<td>P450 involvement</td>
<td>CYP3A4/5</td>
<td>CYP3A4/5, 2D6</td>
</tr>
<tr>
<td>Inhibition potential</td>
<td>I_50 ~ 10 \mu M for CYP3A (tested up to 100 \mu M)</td>
<td>I_50 ~ 0.02 \mu M for 3A4 and 0.07 \mu M for 2D6 (tested up to 50 \mu M), also C08 and 3A5</td>
</tr>
<tr>
<td>Induction potential</td>
<td>NA (tested up to 10 \mu M)</td>
<td>NA (tested up to 1 \mu M)</td>
</tr>
<tr>
<td>P-glycoprotein</td>
<td>Substrate, but not inhibitor</td>
<td>Substrate, but not inhibitor</td>
</tr>
<tr>
<td>Route of excretion</td>
<td>Primarily bile/feces</td>
<td>Primarily bile/feces</td>
</tr>
</tbody>
</table>

NA, not applicable.
mechanism of ADC internalization and intracellular release of the drug, it is possible that the intracellular drug levels in the liver could be higher than those in plasma. Careful characterization of the unconjugated drug is necessary before making decisions in the clinical evaluation of their DDI potentials.

**Elimination**

Impairment of a route of elimination may result in unwanted accumulation of ADC or unconjugated drug. To understand the potential liability associated with impaired elimination, consideration must be given to the elimination of both ADC and unconjugated drug during preclinical and clinical evaluations of an ADC.

The elimination of an ADC after intravenous administration is typically through two processes. First, the ADC is catabolized through proteolytic degradation by the tumor and other tissues into amino acids and is recycled into other proteins. Second, complete loss of a conjugated drug due to deconjugation processes results in the formation of naked mAb or mAb with a partial drug-linker, which may not be measured in a ligand-binding assay as an ADC. In the case of ado-trastuzumab emtansine, an integrated population PK analysis suggests that deconjugation accounts for slightly more than half of ADC clearance while catabolism accounts for the remainder of ADC clearance (Lu et al., 2013). The clearance of naked mAb appears to be similar to the catabolism-mediated clearance of ADC. That, together with the formation of naked mAb from ADC, may explain the longer terminal half-life of the total antibody (TAb). One area in ADC development is developing more stable drug-linkers to minimize the deconjugation processes and improve ADC PK and antitumor activities, as discussed in more detail in the section on ADC stability. Based on population PK analysis, patient body weight was identified as a statistically significant covariate for the clearance of both brentuximab vedotin and ado-trastuzumab emtansine. Dosing based on body weight is considered appropriate for both ADCs.

The elimination of the unconjugated drug after ADC administration has also been evaluated in both preclinical and clinical studies. Based on preclinical data, the major elimination pathway of DM1-containing catabolites in rats after ado-trastuzumab emtansine administration is the fecal/biliary route. About 80% of radioactivity was recovered in the feces, and 50% was recovered in the bile (Shen et al., 2012a). A clinical excretion study of brentuximab vedotin showed that over a 1-week period the primary excretion route of MMAE was via feces, which accounted for approximately 72% of the recovered MMAE, with the remaining MMAE recovered in urine (Han et al., 2013a). These data suggest that for unconjugated DM1 and MMAE the liver is the major elimination organ and that ADCs conjugated to these drug species should be considered for the evaluation of the relationship of hepatic function and drug exposure during clinical development.

**Bioanalytical Considerations**

The current generation of ADCs are a heterogeneous mixture of defined biologic-chemical entities, as evidenced by the DAR distribution (Senter, 2009). Each individual species of ADC is pharmacologically active, with potentially distinct pharmacokinetics and intrinsic pharmacology and toxicity. Current technologies to measure antibodies and small molecules are limited in the extent they can be used to characterize ADCs in biologic matrices (Gorovits et al., 2013; Lin et al., 2013), but advancements have recently been made to measure intact ADCs in biologic matrices (Hengel et al., 2014). Selecting the analytes to measure to understand the pharmacodynamic and toxic effects of ADCs is a scientific and technical challenge. Among these analytes, most drug developers measure TAb to monitor the total antibody–driven activity, ADC or conjugated small molecule to monitor the ADC-driven activity, and released small-molecule drug to monitor the unconjugated drug activity. Exploratory evaluations may be needed if multiple species are released from the ADC, such as in the case of T-DM1 (Shen et al., 2012a) and maleimide transfer for brentuximab vedotin (CDER, 2011). As with other protein-based therapeutics, antitherapeutic antibodies are also routinely monitored.

Strategies are in place to control conjugation further to result in an ADC with a uniform DAR (Junutula et al., 2008; Kung Sutherland et al., 2013), but it is likely that the species in circulation will include lower DAR species. Although it would be ideal to monitor each individual ADC species of a specific DAR, as DAR can alter PK (Hamblett et al., 2004), this is currently a technical challenge (Hengel et al., 2014). Thus, the most common approach is to monitor all ADC species, all antibody species (TAb), and the unconjugated small molecule. An alternative approach is to monitor conjugated small molecules as a surrogate for ADC. Various analytical approaches have been developed and are discussed at length in other publications (Kaur, 2013; Lin et al., 2013).

**Clinical Pharmacokinetics**

Characterizing the PK of ADCs requires the measurement of multiple species that have fundamentally different ADME properties. Thus, to understand the pharmacologic and toxic effects of an ADC and related analytes, a comprehensive analysis is required.

As discussed earlier, the PK of ADCs is typically characterized by three analytes: the ADC, which accounts for the conjugated mAb; the TAb, which consists of both conjugated and unconjugated mAb; and the unconjugated drug in plasma or serum. The ADC and TAb PK profiles are similar to other mAb-based therapeutics, which typically reach a maximum plasma concentration \( \left( C_{\text{max}} \right) \) at the end of intravenous infusion and decline multieXponentially with terminal half-lives in the range of days. The TAb concentrations in serum or plasma are higher than those of the ADC and appear to decline more slowly. The distribution volumes of ADC and TAb are slightly greater than the plasma volume. The unconjugated drug in blood usually displays formation-limited kinetics and has an apparent terminal half-life similar to or slightly shorter than that of the ADCs. The molar concentrations of the unconjugated drugs are often 100- to 1000-fold lower than those of the ADCs (Younes et al., 2010; Girish et al., 2012). Shown in Fig. 2 are the typical PK profiles of the three analytes.

At the approved clinical dose levels, both brentuximab vedotin and ado-trastuzumab emtansine ADCs appear to reach serum \( C_{\text{max}} \) at close to the end of infusion and decline multieXponentially before the next dose. The terminal half-lives were 4 to 6 days and about 4 days, respectively, and no or minimal accumulation was observed when dosed once every three weeks. Consistent with their half-life estimates. The plasma unconjugated DM1 levels after ado-trastuzumab emtansine administration were generally below the limit of quantification, and the TAb was close to the end of infusion (Girish et al., 2012; CDER, 2013). Plasma unconjugated MMAE had a delayed time to reach the maximum plasma concentration \( T_{\text{max}} \) of 2 to 3 days compared with that of DM1 (CDER, 2011). The PK sampling schedule should consider the PK profiles of both the antibody and small molecule when evaluating ADCs with different drug-linkers.

During concomitant use of other medicines or as part of a multiagent combination therapy, ADCs carry a potential risk of clinical drug-drug interactions. Both therapeutic protein and classic small-molecule DDIs must be considered because ADCs are a protein therapeutic that release an active small-molecule drug (Huang et al., 2010; Evers et al., 2013).
The unconjugated drugs are potent cytotoxins and are often the substrate or inhibitor of P450 enzymes. In the case of brentuximab vedotin, a dedicated clinical DDI study was conducted to evaluate the effect of a strong CYP3A inhibitor, ketoconazole, and an inducer, rifampin, on MMAE exposure and the effect of MMAE on the exposure of a CYP3A substrate, midazolam (Han et al., 2013a). Brentuximab vedotin did not affect midazolam exposures, and the ADC exposures were unaffected by terminal half-lives in the range of days. TAb concentrations are generally higher than those of the ADC with slower decline. The unconjugated drug in blood usually displays formation-limited kinetics, and its concentration molar units are often 2 to 3 log order lower than those of ADCs.

Fig. 2. Typical PK profiles of ADC, TAb, and unconjugated drug. The serum or plasma PK of ADCs is typically characterized by three analytes: 1) ADC, which accounts for the conjugated mAb; 2) TAb, which consists of both conjugated and unconjugated mAb; and 3) unconjugated drug. The ADC and TAb concentrations typically reach Cmax at the end of intravenous infusion and decline multieexponentially, with terminal half-lives in the range of days. TAb concentrations are generally higher than those of the ADC with slower decline. The unconjugated drug in blood usually displays formation-limited kinetics, and its concentration molar units are often 2 to 3 log order lower than those of ADCs.

Conclusion

ADCs are a clinically effective therapeutic modality to specifically deliver small molecules to target cells while minimizing the systemic exposure to the small molecules. The ADME of ADCs is complex and requires careful consideration of the ADC construct, small-molecule, and antibody ADME. In particular, characterizing and understanding the plasma stability, biodistribution, metabolism/catabolism, and mechanism of elimination are important for leveraging the promise of ADCs. New technologies to further improve the therapeutic window of ADCs—such as site-specific conjugation and the use of hydrophilic chemical groups to mask the hydrophobic nature of the drug-linker—are currently under development and hold promise to be part of the next generation of ADCs.

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Wrote or contributed to the writing of the manuscript:  Han, Zhao.
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