Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates

Heather Donaghy

Hanson Wade, 52 Grosvenor Gardens, London, UK

ABSTRACT
Antibody-drug conjugates (ADCs) represent a new class of cancer therapeutics. Their design involves a tumor-specific antibody, a linker and a cytotoxic payload. They were designed to allow specific targeting of highly potent cytotoxic agents to tumor cells whilst sparing normal cells. Frequent toxicities that may be driven by any of the components of an ADC have been reported. There are currently more than 50 ADCs in active clinical development, and a further ~20 that have been discontinued. For this review, the reported toxicities of ADCs were analysed, and the mechanisms for their effects are explored in detail.

Methods to reduce toxicities, including dosing strategies and drug design, are discussed. The toxicities reported for active and discontinued drugs are important to drive the rational design and improve the therapeutic index of ADCs of the future.

ABBREVIATIONS: ADCs, antibody-drug conjugates; FcR, Fc receptors; PBD, pyrrolobenzodiazepines; EGFR, epidermal growth factor receptor; DLT, dose limiting toxicity; q3w, every 3 weeks; Can M, cantuzumab mertansine; Can R, cantuzumab ravtansine; DAR, drug-to-antibody ratio; TDC, THIOMAB drug conjugate; NHL, non-Hodgkin’s lymphoma; CLL, chronic lymphocytic leukemia; MTD, maximum tolerated dose; ORR, overall response rate; ADCC, antibody-dependent cell-mediated cytotoxicity; BV, brentuximab vedotin; PK, pharmacokinetic

Introduction
Antibody-drug conjugates (ADCs) are relative newcomers into oncology treatment. Currently, 3 drugs have been licenced: gemtuzumab ozogamicin (CMA-676, Mylotarg®), which has since been withdrawn in all regions apart from Japan; brentuximab vedotin (BV; SGN-35, Adcetris®) and ado-trastuzumab emtansine (T-DM1, Kadcyla®). However, the field of ADC development is expanding rapidly, with ~55 active clinical compounds, ~20 that have discontinued clinical trials and many more that are showing preclinical promise. ADCs were designed to harness the specificity of antibody targets with the cytotoxicity of chemotherapeutic agents, and should therefore exhibit high specificity and low toxicity. This ideal has been difficult to achieve in practice because ADCs repeatedly induce toxicities that limit the therapeutic window of these compounds or prevent further clinical development. There are many examples of ADCs that have shown early preclinical promise and that entered clinical trials briefly, but have not progressed or have been terminated abruptly. These were terminated for a variety of reasons, including financial and strategic, but there are others that may have failed because of unforeseen or unacceptable toxicities. It is important that the results from these failed compounds are disclosed so that ADC research and development does not continue to repeat mistakes of the past.

In this review, the reported toxicities from 35 active and discontinued compounds were identified and a comprehensive assessment of the role that each component of the ADC has on the toxicities exhibited was carried out. As expected, the payload often drives the toxic effects; however, the target and linker may determine the organ specificity of the toxicity, and this is discussed in detail. Knowledge of the role that each component plays in an ADC will enable development of ADCs with improved therapeutic indices by increasing the efficacy and decreasing the toxicity. Novel techniques for designing ADCs are driving the next generation into the clinic. As the field of ADC design evolves, it will become increasingly important to assess the toxicities of past ADCs in order to design the ADCs of the future.

ADC design
ADCs are composed of 3 well-defined components, the antibody, linker and payload, and these have been comprehensively reviewed by Sievers et al. and Jain et al. An optimal ADC achieving maximum efficacy with minimal toxicity requires the appropriate combination of each of these components.

Antibody
Within the context of toxicities, the antibody needs to bind accurately and efficiently to the antigen on tumor cells whilst avoiding normal cells. It is therefore very important that an antibody binds to a suitable target antigen, one that is preferentially or exclusively expressed on tumor cells. Reasons that an...
antibody may not be suitable include inappropriate or low affinity binding, insufficient antigen expression on tumor cells or a lack of internalization following binding. Some ADCs, however, may not need to be internalized to release toxic payload. As CEACAM5 is not thought to be internalized, the Immunomedics anti-CEACAM5 labetuzumab govitecan (IMMU-130) may release payload after binding of antibody without internalization.4,5 CEACAM5 is also the target of Immunomedics anti-CEACAM5 labetuzumab govitecan, which may not need to be internalized to release toxic payload. As CEACAM5 is not thought to be internalized, the payload may not need to be internalized to release toxic payload. Some ADCs, however, may not need to be internalized to release toxic payload. As CEACAM5 is not thought to be internalized, the payload may not need to be internalized to release toxic payload.

Inappropriate binding may be due to expression of the target antigen on normal cells 6-8 or non-specific binding to Fc receptors (FcR) or lectin receptors, such as the mannose receptor.9,10 Also, antibodies with specific biological effects that bind non-specifically or inappropriately to target antigens on normal cells may induce toxic effects irrespective of the payload. Indeed, MEDI-547, an EphA2-targeting ADC conjugated to MMAE, exhibited toxicities at very low doses, which may suggest an antibody-mediated biological effect rather than delivery of cytotoxic payload.7

## Linker

Linker chemistry is very important in the timely release of the payload from the antibody. Like the antibody, an effective linker will improve the therapeutic index of an ADC by ensuring accurate release of the payload (reviewed in ref.3). Whilst the linker itself does not appear to drive toxicities, the stability of the linker has considerable impact on the toxicities that are then exerted by the payload. The more stable linkers will release their payload in a target-specific manner, inducing more specific toxicities whilst a less stable linker is more likely to undergo non-specific cleavage, resulting in a broader toxicity profile. Increasing the steric hindrance of a linker (e.g., SPDB-DM4 linker payload construct) can enhance the stability of the ADC in circulation, with the aim of reducing non-specific toxicities. Cleavable linkers are mostly cleaved from the payload in endosomes or lysosomal compartments via a variety of mechanisms including acidic degradation (hydrazones); protease cleavage by cathepsin B (dipeptide); thiol-disulfide exchange reactions (disulfide, carbonate). Conversely, non-cleavable linkers (MC and SMCC) require complete lysosomal proteolytic degradation of the antibody, generating a toxic payload with charged lysines or cysteines (Table 1).

## Payload

The payloads utilized in ADCs are highly potent cytotoxic drugs, exerting their effects on critical cellular processes required for survival (Table 1). Most compounds in current clinical testing utilize either maytansine derivatives (DM1/DM4) or auristatins (MMAE/MMAF), which are both microtubule inhibitors. These typically induce apoptosis in cells undergoing mitosis by causing cell cycle arrest at G2/M. More recent work shows that microtubule inhibitors may also disrupt non-dividing cells in interphase.11 These findings provide explanation of how the microtubule inhibitors are cytotoxic to slowly replicating or non-dividing tumor cells.

Other classes of cytotoxic drugs used in ADCs include enediyynes (calicheamicin), duocarmycin derivatives, pyrrolobenzodiazepines (PBDS) and indolobenzodiazepines, all of which target the minor groove of DNA, and quinoline alkaloids (SN-38), which inhibit topoisomerase 1. The majority of payloads utilized in ADCs are highly potent, often cytotoxic in the picomolar range, which is a requirement because only a very small amount (<1%) of the injected dose of antibody localizes to the tumors.9 However, it is this potency that drives the toxicity of ADCs, resulting in the majority of toxicities being characterized by the class of payload.

## Other components to consider

The conjugation process of ADCs results in a heterogeneous population of constructs with multiple drug-antibody-ratios. Indeed, the first ADC to receive FDA approval, gemtuzumab ozogamicin, had an average DAR of 2–3, but nearly half the antibodies had no drugs attached.12,13 The variations in the number of cytotoxic molecules conjugated to each antibody can have significant effects on the disposition of an ADC,14 with implications for toxicity.

The conjugation site is an area of intense scrutiny currently, with increased emphasis on this as a means to control the specific pharmacokinetic (PK) profile. Engineering of ADCs to contain specific numbers of payload molecules per antibody at known sites results in a homogeneous population of ADCs, which may result in a better therapeutic window (increased efficacy vs. decreased toxicity). Techniques for site-specific conjugation of ADCs currently under investigation include the use of engineered cysteines, unnatural amino acids and the addition

### Table 1. Antibody-drug conjugate linker payload combinations.

| Payload Class       | Payload       | Linker     | Cleavable/non-cleavable | Nature of bond | Active metabolite |
|---------------------|---------------|------------|-------------------------|----------------|------------------|
| Microtubule inhibitor | DM1           | SPP        | Cleavable               | Disulfide      | DM1              |
|                     | DM1           | SMCC       | Non-cleavable           | Thiether       | Lys-SMCC-DM1     |
|                     | DM4           | SPPDB      | Cleavable               | Disulfide      | DM4, 5-methyl DM4|
|                     | DM4           | Sulfo-SPPDB| Cleavable               | Disulfide      | DM4, 5-methyl DM4|
|                     | MMAF          | MC         | Non-cleavable           | Thiether       | Cysteine-mc-MMAF |
|                     | MMAE          | VC         | Cleavable               | Dipeptide      | MMAE             |
| DNA synthesis inhibitor | Calicheamicin | Acetyl butyrate | Cleavable | Hydrazide      | Calicheamicin    |
|                     | Doxorubicin   | —          | Cleavable               | Hydrazide      | Doxorubicin      |
|                     | Duocarmycin derivative | VC | Cleavable               | Dipeptide      | Duocarmycin      |
|                     | PBD           | VC         | Cleavable               | Dipeptide      | PBD              |
| Topo-isomerase inhibitor | SN-38       | CL2A       | Cleavable               | Carbonate      | SN-38            |

Abbreviations: SPP, N-succinimidyl 4-(2-pyridyldithio) pentanoate; SMCC, succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; SPPDB, N-succinimidyl 4-(2-pyridyldithio) pentanoate; MMAF, monomethyl auristatin F; MC, maleimidocaproyl; MMAE, monomethyl auristatin E; VC, valine-citrulline; PBD, pyrrolobenzodiazepine
of a selenocysteine, glutamine or aldehyde tag. Addition of an engineered cysteine site to yield engineered thio (THIOMAB) antibody conjugates results in ADCs with approximately 2 drugs per antibody. There are several ADCs utilizing this technology in preclinical testing.

The only ADCs in the clinic to have disclosed the use of site-specific conjugation techniques are SGN-CD33A and SGN-CD70A, which use engineered cysteines to site-specifically conjugate 2 PBD dimers per antibody.

As ADCs are evolving it is becoming clear that no single component of the ADC will drive the efficacy or indeed toxicity. Rather, each has a role to play, and the biology of the tumor and target antigen may also influence the activity of an ADC. Improving the therapeutic index of an ADC requires optimization of each component in combination with the others to generate the best ADC for specific disease indications.

**Mechanisms for toxicities**

ADC toxicities can be mediated via any of the components of the drug. Low level expression of a target antigen on normal cells may result in specific toxicities whilst early cleavage of the linker, releasing free drug, may produce more widespread toxicities. Other ways that an ADC may induce toxicity is through Fc and mannose receptor binding. The majority of ADC toxicity is thought to be derived from the payload. Normal, rapidly dividing cells are at risk of toxicity from microtubule inhibitors as they exert their cytotoxic effects on rapidly proliferating cells. Normal cells that are commonly affected by standard chemotherapy and by ADCs include cells lining the digestive tract, causing gastrointestinal symptoms; cells in the hair follicles, causing hair loss; and myeloid cells, causing myelosuppression. Some key toxicities are found with different payloads (Table 2). In particular, MMAE induces peripheral neuropathy and neutropenia; MMAF is associated with thrombocytopenia and ocular toxicities; DM1 causes gastrointestinal effects as well as thrombocytopenia and neutropenia, depending on the linker and consequent metabolites; ocular toxicity is the most common adverse event with DM4-conjugated ADCs; calicheamicin causes thrombocytopenia and hepatic dysfunction; and early indications from SN-38 conjugated drugs suggests neutropenia as a frequent toxicity.

How and why ADCs exert these toxicities on different cell types is of considerable interest when trying to minimize toxicity for future drug development, and some of the known factors are discussed below.

**Thrombocytopenia**

Thrombocytopenia may arise from either enhanced destruction or decreased production of platelets. It manifests as increased bruising and bleeding (gums and nose bleeds), and in severe cases can cause mucosal hemorrhage. Thrombocytopenia induced by ADCs is thought to be due to inhibition of megakaryocyte differentiation and apoptosis of megakaryocyte progenitors. It is a key toxicity of calicheamicin-utilizing ADCs, and of potent tubulin-acting agents that use non-cleavable linkers. Toxicity is mediated, in the case of MMAF- and DM1-conjugated ADCs, by the toxic metabolites cys-mc-MMAF or lys-SMCC-DM1, respectively. Thrombocytopenia (all grades) was reported in 32%, 26% and 11% of patients treated with the MMAF ADCs AGS-16M8F, SGN-75, and ABT-414, respectively. In the SGN-75 trial, there was no cumulative effect with repeated cycles. In the case of SGN-CD19A, the incidence of grade 3 thrombocytopenia is about 10%.

Thrombocytopenia is more widespread for the calicheamicin-containing ADCs than those containing MMAF. In a Phase 2 trial of inotuzumab ozogamicin in non-Hodgkin’s lymphoma (NHL) patients, 77% of patients experienced thrombocytopenia, which was ≥ grade 3 in 53% and caused treatment discontinuation in 22% of patients. The recovery was variable, but was reduced to grade 1 or was resolved by 3 months post treatment in most patients. Meanwhile, nearly all (99%) the acute myeloid leukemia (AML) patients treated with gemtuzumab ozogamicin experienced ≥ grade 3 thrombocytopenia, and 15% of patients experienced ≥ grade 3 bleeding. Early results from the first-in human study of PF06647263 show 33% of patients experiencing thrombocytopenia.

Furthermore, thrombocytopenia has been reported for the DM1 conjugates ado-trastuzumab emtansine and lorvotuzumab mertansine. Interestingly, the incidence of thrombocytopenia following treatment with ado-trastuzumab emtansine was higher in Asians than Caucasians (≥ grade 3 in 45% and 12%, respectively). Trastuzumab and ado-trastuzumab emtansine have been shown to bind to FcγRIIIa on megakaryocyte progenitors, but only ado-trastuzumab emtansine affected platelet production, indicating that the thrombocytopenia induced by this ADC was due to DM1 or its metabolite lys-SMCC-DM1. In contrast, thrombocytopenia is not particularly clinically significant of the tubulin inhibitors that use cleavable linkers, such as vc-PAB-MMAE or disulfide-linked DM4 conjugates.

**Neutropenia**

Disruption of microtubule function during mitosis in the bone marrow results in neutropenia. The reduced neutrophil counts increase the incidence of infections, including febrile neutropenia and sepsis. This is a consistent toxicity in ADCs utilizing MMAE. It is thought to be due to the instability of the valine-citrulline cleavable linker in the plasma or due to the rapid clearance of higher drug-loaded species present in these conjugates, resulting in systemic release of free drug and thus a broader spectrum of toxicities. The actively dividing hematopoietic cells are preferentially affected by MMAE, resulting in neutropenia that is largely reversible with neutrophil numbers improving between treatment cycles. Neutropenia has been reported for BV, pinatuzumab vedotin, polatuzumab vedotin, glembatumumab vedotin and PSMA-ADC (Table 2).

In a target-dependent toxicity, the CD33-specific ADCs gemtuzumab ozogamicin and SGN-33A are also known to induce neutropenia through binding to CD33 on the surface of myeloid progenitor cells, whilst no myelosuppression was observed for AVE9633.
| Drug                      | Target/Linker/ Payload | MTD (Q3W dosing unless otherwise stated) | DLT                  | Key toxicities | Other toxicities | Reference |
|---------------------------|------------------------|----------------------------------------|---------------------|----------------|-----------------|-----------|
| ABT-414                   | EGFR/MC/MMAF           | 1.25 mg/kg (Q2W)                       | Corneal deposits    | O              |                 | 21        |
| AGS-16C3F                 | ENPP3/MC/MMAF          | 1.8 mg/kg                              | Thrombocytopenia, ocular toxicity | O              | T               | 19        |
| SGN-7S, Vosetuzumab mafodotin | CD70/MC/MMAF          | 3 mg/kg                                | Thrombocytopenia, ocular toxicity, nausea | O              | T               | 20        |
| MEDI-547                  | EphA2/MC/MMAF          | 0.08 mg/kg                             | Hemorrhage, epistaxis | G              |                 | 7         |
| SGN-CD19A                 | CD19/MC/MMAF           | 5 mg/kg                                | Keratopathy        | O              |                 | 22        |
| BAY79-4620                | CA-9/VC/MMAE           | 2.3 mg/kg                              | Pancreatitis, cardiac arrest | G              |                 | 77        |
| ASG-516S                  | SLC44A4/VC/MMAE        | 1.2 mg/kg                              | G Hemorrhage        | N              |                 | 7         |
| Glembatumumab vedotin, CDX-011 | gpNMVB/VC/MMAE      | 1.88 mg/kg                             | Worsening peripheral neuropathy | G              | N               | 79        |
| MLN0264                   | GCC/VC/MMAE            | 1.8 mg/kg                              | Neutropenia         | G              | N               | 80        |
| PSMA ADC                  | PSMA/VC/MMAE           | 2.3 mg/kg                              | Neutropenia, elevated liver function tests | N              | P               | 38,40     |
| Brentuximab vedotin, Adcetris | CD30/VC/MMAE          | 1.8 mg/kg                              | Thrombocytopenia, hyperglycemia, febrile neutropenia | G              | N               | 41        |
| Polatuzumab vedotin, RG7593 | CD22/VC/MMAE          | 2.4 mg/kg                              | Neutropenia         | N              | P               | 83,84     |
| Polatuzumab vedotin, RG7596 | CD79b/VC/MMAE         | 2.4 mg/kg (1.0 mg/kg for CLL)          | Neutropenia, fungal infection | G              | N               | 41        |
| DEDN6526A, RG7636         | ETBR/unknown/MMAE      | 2.4 mg/kg                              | Infusion reaction, transaminitis, elevated ALT, AST | G              |                 | 85        |
| DMOT4039A                 | MSLN/unknown/MMAE      | 2.4 mg/kg                              | Hyperglycemia, hypophosphatemia | G              |                 | 86        |
| RG7458, DMUC5754A         | MUC16/unknown/MMAE     | 2.4 mg/kg                              | Neutropenia, elevated uric acid | G              | N               | 87        |
| PF-06647263               | EFNA4/AB/Calicheamicin |Continuing                              | Neutropenia         | T              | G               | 27        |
| Inotuzumab ozogamicin, CMC-544 | CD22/AB/Calicheamicin | 1.8 mg/m£ (Q4W)                       | Thrombocytopenia, neutropenia | T              | G               | 27        |
| Gemtuzumab ozogamicin, Myotarg, CMA-676 | CD23/AB/Calicheamicin | 9 mg/m£ (Q3 W)                        | Prolonged neutropenia | T              | N               | 26        |
| Bivatuzumab mertansine, B1W-1 | CD44v6/SPP/DM1        | 300 mg/m£                             | Liver enzymes, vomiting, skin toxicity | T              | G               | 88        |
| Trastuzumab emtansine, Kadcyla, T-DM1 | HER2/SMCC/DM1       | 3.6 mg/kg                             | Thrombocytopenia    | T              | G               | 88        |
| MLN2704                   | PSMA/SPD/DM1           | > 343 mg/m£                            | Febrile neutropenia | G              | P               | 89        |
| Cantuzumab mertansine     | CanAg/SP/DM1           | 235 mg/m£                             | Transaminase elevations | G              |                 | 54        |
| IMGN901, Lorvotuzumab mertansine | CD56/SP/DM1         | 112 mg/m£                             | Fatigue, acute reversible renal failure | T              | G               | 90        |
| IMGN242, Cantuzumab ravstansine | CanAg/SP/DM4        | 168 mg/m£                             | Decreased visual acuity, corneal deposits, keratitis | O              |                 | 32        |
| IMGN388                   | CD51/SPDB/DM4          | 130 mg/m£                             | Headache and confusion | G              |                 | 91        |
| IMGN853, Mivetuximab soravstansine | FOLR1/sulfo-SPD/DM4 | 6 mg/kg                               | Punctate keratitis, blurred vision | O              |                 | 33        |
| SAR3419, Colutuzumab ravstansine | CD19/SPDB/DM4       | 160 mg/m£                             | Ocular toxicity     | O              |                 | 34        |
| AVE9633                   | CD33/SPD/DM4           | > 260 mg/m£                            | Keratitis, liver toxicities | O              |                 | 30        |
| BT-062, Indatuximab ravstansine | CD138/SPD/DM4       | 160 mg/m£                             | Mucositis, neutropenia, skin toxicity, increased aminotransferases | G              | N               | 92,93     |
| IMMU-130, Labetuzumab govitecan | CECAMS/CL2A/CL28-SN-38 | 8–10 mg/kg                           | Typhilitis, neutropenia, nausea/vomiting | G              | N               | 61        |
| IMMU-132, Sacituzumab govitecan | TROP-2/CL2A/CL28-SN-38 | 8–10 mg/kg                           | N                  |                 | 70,71     |
| SGN-15, BMS-18248         | Lewis Y antigen        | 700 mg/m£                             | Pancreatitis, hemorhagic gastritis | G              |                 | 94        |
| MDX-1203, BMS93661        | CD70/Vc/duramycin derivative | > 15 mg/kg                           | Hypersensitivity | G              |                 | 95        |
| SGN-CD33A                 | CD33/VC/PRB           | 40 µg/kg                               | Hypocellular marrow | G              | N               | 76        |

Abbreviations: SPP, N-succinimidyl 4-(2-pyridyldithio) pentanoate; SMCC, succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; SPDB, N-succinimidyl 4-(2-pyridyldithio) pentanoate; MMAF, monomethyl auristatin F; MC, maleimidocaproyl; MMAE, monomethyl auristatin E; VC, valine-citrulline; PBD, pyrrolobenzodiazepine; O, ocular toxicity; T, thrombocytopenia; G, gastro-intestinal events including nausea, vomiting and diarrhea; N, neutropenia; P, peripheral neuropathy.
Ocular toxicity

Ocular toxicities have been reported in a range of ADCs and for a variety of targets (Table 2). A recent comprehensive review of the ocular toxicities reported for ADCs has highlighted the variety of ocular events that are associated with ADCs, with blurred vision, keratitis, dry eye and micropigment epithelial damage being the most commonly reported. There is a clear payload association, with ocular toxicity typically induced by ADCs that include DM4 and MMAF. Both tend to utilize a stable linker: a stable cleavable disulfide link in the case of SPDB-DM4, and an uncleavable link in the case of mc-MMAF. The SPDB-DM4 ultimately releases neutral diffusible metabolites (DM4 and S-methyl-DM4), which have the potential for bystander killing of neighboring cells, while the mc-MMAF produces cysteine-mcmMMAF as the charged active metabolite within the cell, which does not have bystander effects. It is unclear why the eye is particularly sensitive to toxicities with these payloads, but, for MMAF-conjugated ADC, the toxicity may be related to accumulation of the drug within cells. Conversely, ocular events are rarely described for ADCs utilizing MMAE.

Ocular toxicities have been reported as a dose-limiting toxicity (DLT) for ABT-414 (corneal deposits); AGS-16C3F; cantuzumab ravtansine (decreased visual acuity, corneal deposits and keratitis); mirvetuximab soravtansine (punctate keratitis and blurred vision); and coltuximab ravtansine. The incidence of ocular toxicities for SGN-75 had a median time to onset of 44 days, following multiple doses of SGN-75. For this trial, the incidence of ocular events was 57% (≥ grade 3, 21%), and resulted in treatment discontinuation in 15% of patients. Toxicities included dry eye (30%), corneal epitheliotropism (15%), blurred vision (11%) and keratitis (9%), and were reversible with a median time to resolutions of 79 days.

Prophylactic steroid eye drops have been reported to be used successfully to reduce the incidence of ocular events for ABT-414 and SGN-CD19A, whilst dose modification of the DM4-conjugated drugs SAR3419 and IMGN853 has been used to successfully reduce the incidence and severity of ocular toxicity (see section below on dose modifications to minimize toxicity) and the addition of a sulfo group to the SPDB linker has been used in IMGN853 as a means to reduce ocular toxicity (patent number US2012028228A1).

Many of the critical signalling molecules that drive cancer growth are expressed in ocular tissues, in particular, epidermal growth factor receptor (EGFR) is expressed in corneal basal epithelial cells and signalling through this receptor is important in many ocular functions (e.g., sebaceous glands, hair follicles conjunctiva, capillaries). ADCs targeting EGFR might be particularly susceptible to ocular toxicities; however, 2 ADCs in clinical development (AMG-595 and ABT-414) are designed to limit binding to wild-type EGFR, and thus reduce ocular toxicities. AMG-595 binds specifically to a mutant form of EGFR, EGFRvIII expressed by some glioblastoma multiforme patients, and should not bind EGFR wildtype, which may prevent ocular events. ABT-806, the antibody used in the ADC ABT-414, binds to an epitope of EGFR that is only exposed on activated and tumor-specific EGFR without binding wild-type EGFR on normal tissues. ABT-414 is conjugated to MMAF via a mc linker, whilst AMG-595 utilises DM1 with an SMCC linker. Initial results for ABT-414 show that ocular disturbances are indeed significant toxicities for this compound, with 89% of patients reporting ocular events, which appears more than has been reported for other MMAF-conjugated ADCs (see above), but it is difficult to conclude definitively because of differences in reporting and the small number of results available. The toxicities for AMG-595 have not yet been reported. Head-to-head comparison of these 2 compounds in clinical testing will determine whether targeting this mutation will reduce the toxicities, particularly ocular ones seen with EGFR wild-type ADCs.

Peripheral neuropathy

Numbness and tingling in the extremities that spreads to cause shooting pains and muscle weakness are characteristics of peripheral neuropathy. This is primarily seen with microtubule inhibitor drugs, such as the auristatins and maytansinoid derivatives (Table 2), as well as with taxanes and vinca alkaloids. Whilst these drugs are normally active on highly proliferating cells, adult neurones do not actively divide, but microtubules play an important role in the survival and function of neurones by mediating the active transport of proteins from the cell body to distal synapses. Peripheral neuropathy is thought to occur because of disruption of microtubule function. It is particularly associated with MMAE conjugates with a protease-cleavable linker (e.g., valine-citrulline). The cleavable linker is less stable than other linkers, allowing systemic release of free drug.

Peripheral neuropathy is widely reported in patients treated with BV, affecting up to 50% of patients, but is less frequent for polatuzumab vedotin and glembatumumab vedotin, affecting 9% and 4% of patients, respectively. For BV, the incidence of peripheral neuropathy was cumulative, with a median time to onset of any grade peripheral neuropathy of more than 12 weeks. The time to onset for grade 2 and 3 neuropathy was 27 and 38 weeks, respectively. Peripheral neuropathy resulted in dose reduction in 10% of patients, dose delay in 13% and drug discontinuation in 9% patients. The majority of patients had resolution or improvements to their peripheral neuropathy; however, complete resolution was noted in only 50% patients.

Skin toxicity

The EGFR signalling pathway and receptor tyrosine kinases are important in normal functioning of epithelial cells. Therefore, ADCs that target these pathways are likely to exhibit higher incidence of skin toxicity, for example, bivatuzumab mertansine, which induced very severe skin toxicities due to expression of CD44v6 on normal keratinocytes. This ADC targets CD44v6, which is expressed in a variety of solid tumors, and was in clinical testing for head and neck squamous cell carcinoma and metastatic breast cancer. Preclinical testing in cynomolgus monkeys indicated reversible skin toxicities due to expression of CD44v6 on normal keratinocytes. Based on preclinical data, mild to moderate skin toxicities were expected in the clinic, and were experienced in the majority of patients; however, one patient
in the dose escalation developed grade 4 epidermal necrolysis at 140 mg/m² (below the MTD determined in a parallel study) and subsequently died.6 In this instance, the human expression of CD44V6 on keratinocytes resulted in very effective on-target cytotoxicity by bivatuzumab mertansine, but the target was inappropriate, resulting in unacceptable toxicities. This highlighted the requirement to thoroughly assess normal expression of a target antigen prior to the introduction to the clinic.

**Toxicity to endothelial cells**

MEDI-547, a potential candidate for treatment of solid tumors, targeted EphA2, a member of the receptor tyrosine kinase family. The antibody was cross reactive with EphA2 of various species in vitro, including rats, mice and cynomolgus monkeys. The first-in-man study started at a dose of 0.08 mg/kg, which was ten times lower than the highest non-severely toxic dose predicted by rat and cynomolgus monkeys. In humans, however, this dose exceeded the maximum tolerated dose, with excessive bleeding, hemorrhage and epistaxis noted in the patients treated. This unexpected toxicity was thought to be due to the antibody component of the ADC, rather than the auristatin payload.7 This suggests that there are instances when the level of toxicity predicted from animal studies is not the level observed in human patients, and highlights the requirement for continued development of suitable animal studies, which may more accurately predict human responses.

**Gastro-intestinal**

Most patients experience gastro-intestinal toxicities following chemotherapy treatment. Nausea and vomiting can affect more than 90% of patients, particularly those treated with cisplatin, cyclophosphamide or dacarbazine, and 20% of patients still experience symptoms even in the presence of prophylactic anti-emetics.49 Most ADCs also induce gastro-intestinal toxicity, but they are mostly grade 1 or 2 in severity. Gastro-intestinal toxicities, including nausea, vomiting, diarrhea and constipation, are most frequently reported for MMAE-, calicheamicin- and DM1-conjugated ADCs (Table 2). The mechanisms by which ADCs induce gastro-intestinal toxicity have not been explored, but may result from the non-specific effects of microtubule inhibitors on rapidly proliferating cells within the gastro-intestinal tract. There are a variety of mechanisms by which chemotherapy is thought to induce toxicity (discussed in ref. 50), but the mechanisms by which ADCs induce this toxicity have not been determined.

Of note, about 30% of patients treated with irinotecan monotherapy experience diarrhea, whilst early indications from the SN-38-conjugated ADCs suggest they have significantly lower rates of diarrhea than irinotecan (Table 3), possibly indicating lower distribution of the ADC in the gastro-intestinal tract than the naked small molecule cytotoxic.

**Hepatic disturbances including veno-occlusive disease**

Veno-occlusive disease occurs when toxic injury to the liver sinusoids causes sloughing of endothelial cells. These then embolize to hepatic venules and cause fibrosis of venules, leading to hepatic congestion and a failure to remove toxins or other waste products. It has been reported as a toxicity for inotuzumab ozogamicin51 and gemtuzumab ozogamicin. Other compounds that have reported elevated hepatic transaminases include cantuzumab mertansine, AVE9633 and bivatuzumab mertansine. It has been proposed that this effect is mediated via uptake of ADCs by mannose receptor expressed on hepatic sinusoidal cells.

**Preclinical toxicities**

Many of the antibodies in clinical and preclinical stage ADCs are not cross-reactive with mice or rats, preventing assessment of “on-target” toxicities and only allowing non-specific, antigen-independent effects in these species. Murine assessment of toxicities may be informative for less stable ADCs, but as more specific, stable ADCs start to enter the clinic, a better understanding of animal toxicities will be needed, and better preclinical models may be required.

The best information on tolerability/toxicity studies may be obtained from non-human primate models, specifically cynomolgus monkeys, although these have not proven to be an accurate guide in predicting the toxicities in humans, e.g., MEDI-547, bivatuzumab mertansine (described above). The starting dose for the first Phase 1 clinical study of ado-trastuzumab emtansine was determined by studies of cynomolgus monkeys. These toxicology studies found microscopic axonal degeneration of sciatic nerves, which suggested peripheral neuropathy would be the dose limiting toxicity (DLT).55 Low level HER2 expression on human and cynomolgus glial cells and peripheral nerve spindle cells was anticipated to drive this toxicity. However, in patients, the DLT that was observed was thrombocytopenia, which was minimal in the animal models. This is thought to be an antigen-independent effect, as both ado-trastuzumab emtansine and an ADC made with an irrelevant antibody and the same linker-payload (SMCC-DM1) were subsequently shown to impair megakaryocyte maturation in vitro.

**Toxicities of ADCs vs. standard treatments**

The efficacy and tolerability of standard chemotherapeutic regimens are both dose-dependent, i.e., better efficacy may be achieved with higher doses, but the risk of toxicity also increases. Due to their targeted nature, the tolerability of ADCs may not be related to the clinical outcome. It is, therefore, important to ensure that the toxicities for ADCs are less debilitating than those of standard treatments. There are many trials currently underway that compare currently approved drug regimens with ADCs, but the majority of these have yet to report results. As these studies mature, it will be important to compare the toxicities of ADCs with standard treatment. Currently, it is possible to compare toxicities of ado-trastuzumab emtansine, inotuzumab ozogamicin and glenbatumumab vedotin to a comparator arm. The comparator arms may contain drugs with the same or different mechanism of action to that of the ADCs. Like many of the ADCs, taxanes (e.g., docetaxel) and vincus alkaloids (e.g., vincristine)
are microtubule inhibitors and thus have similar class effect toxicities. Other classes of chemotherapeutic agents, such as the tyrosine kinase inhibitors (e.g., labatinib), nucleoside analogues (e.g., gemcitabine) and alkylating agents (e.g., bendamustine) each have their own key toxicities, which may be more or less debilitating than the investigational ADC. Ado-trastuzumab emtansine induced more thrombocytopenia and elevated AST, but lower neutropenia and diarrhoea than other regimens including trastuzumab plus docetaxel or labatinib plus capecitabine (Table 4).

Inotuzumab ozogamicin (anti-CD22-calicheamicin ADC) reported higher incidence of veno-occlusive disease than intensive chemotherapy (15% vs. 1%, respectively) in patients with acute lymphoblastic leukemia (ALL) on the INO-VATE ALL study. This trial is showing significantly better responses than the comparator arm for patients with ALL. In a Phase 3 trial of relapsed/refractory NHL patients, inotuzumab ozogamicin was associated with higher incidence of thrombocytopenia, but lower neutropenia than the comparator arm of bendamustine or gemcitabine. This study was terminated for futility in these largely refractory NHL patients.

In the Emerge trial, heavily pretreated breast cancer patients were treated with glembatumumab vedotin or the investigator’s choice, which consisted of eribulin (n=15), ixabepilone (n=7), gemcitabine (n=5), vinorelbine (n=5), doxorubicin (n=3) or albumin-bound paclitaxel (n=2). Patients treated with glematumumab vedotin experienced less thrombocytopenia (4%) and elevation of AST (8%) than the investigator’s choice (15%, 44% and 27%, respectively). Conversely, incidence of rash was 47% in glematumumab vedotin treated compared to 2% in the comparator arm and the rate of peripheral neuropathy was nearly double in the glematumumab vedotin arm than the investigator’s choice (23% compared to 12%).

In a combination study of BV for frontline treatment of Hodgkin’s lymphoma, patients were given a standard regimen containing doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) plus BV or a modified regimen of doxorubicin, vinblastine, and dacarbazine (AVD) plus BV. Patients receiving BV plus ABVD experienced unacceptable pulmonary toxicity effects (44% of patients), which was a higher rate than that generally seen for ABVD alone. Bleomycin is known for its adverse effects on the lung, and addition of BV to the bleomycin containing ABVD regimen enhanced this effect. Removing bleomycin from the BV arm removed the pulmonary effects and did not reduce the number of complete responses.

### Table 3. Grade 3/4 toxicities reported for SN-38 ADCs & irinotecan.

| Dosing regimen | Irinotecan monotherapy | Labetuzumab govitecan | Sacituzumab govitecan |
|----------------|------------------------|-----------------------|----------------------|
|                | once weekly 125 mg/m²   | once weekly 10 mg/kg or twice weekly 6 mg/kg | days 1 and 8 of 21 day cycle 10 mg/kg |
| Neutropenia    | 26%                    | 10%                   | 24%                  |
| Febrile neutropenia | 3%                  | 2%                    | 6%                   |
| Leukopenia     | 31%                    | 2%                    | 3%                   |
| Diarrhea       | 28%                    | 3%                    | 2%                   |
| Anemia         | 7%                     | 3%                    | 6%                   |

### Table 4. Reported toxicities for ado-trastuzumab emtansine (T-DM1) compared to other treatment options in three randomized trials.

|                | Th3resa (NCT01419197) 96 | NCT00679341 97 | EMLIJA (NCT00829166) 98 |
|----------------|--------------------------|----------------|------------------------|
| T-DM1          | Investigator’s Choice    | T-DM1          | Trastuzumab + docetaxel | T-DM1          | Lapatinib + capecitabine |
| Thrombocytopenia | 15%                     | 28%            | 28%                   | 28%            | 3%                      |
| Neutropenia    | 5%                       | 16%            | 6%                    | 6%             | 9%                      |
| Leukopenia     | <1%                      | 10%            | 26%                   | 10%            | 27%                     |
| Diarrhea       | 10%                      | 16%            | 46%                   | 23%            | 8%                      |
| Elevated AST   | 8%                       | 44%            | 6%                    | 22%            | 9%                      |
| Febrile neutropenia | <1%                   | 0%             | 14%                   | 10%            | 8%                      |
| Anemia         | 9%                       | 13%            | 27%                   | 10%            | 8%                      |
| Dyspnea        | 10%                      | 15%            | 27%                   | 15%            | 27%                     |
| Arthralgia     |                          | 23%            | 30%                   | 23%            | 30%                     |
| Cough          |                          | 26%            | 21%                   | 19%            | 29%                     |
| Vomiting       |                          | 25%            | 26%                   | 19%            | 29%                     |
| Increased ALT  |                          | 26%            | 6%                    | 17%            | 9%                      |
| Abdominal pain |                          | 6%             | 13%                   | 6%             | 13%                     |
| Peripheral edema |                      | 10%            | 44%                   | 10%            | 44%                     |
| Pyrexia        |                          | 41%            | 23%                   | 41%            | 23%                     |
| Headache       |                          | 41%            | 18%                   | 41%            | 18%                     |
| Back pain      |                          | 28%            | 32%                   | 28%            | 32%                     |
| Epistaxis      |                          | 28%            | 9%                    | 28%            | 9%                      |
| Pneumonia      |                          | 9%             | 2%                    | 9%             | 2%                      |
| Alopecia       |                          | 4%             | 6%                    | 4%             | 6%                      |
| Palmar – plantar erythrodysesthia |                 |                | 1.20%                 | 58%            |
| Mucosal inflammation |                  |                | 6.70%                 | 19%            |

Reported adverse events affecting >5% of patients.
trial is currently underway that compares BV plus AVD with ABVD alone.\textsuperscript{59}

SN-38 is the active metabolite of irinotecan, enabling a direct comparison of the toxicities of ADCs utilizing SN-38 payloads (labetuzumab govitecan and sacituzumab gofitavec) with those of irinotecan monotherapy. The most significant difference is a reduction in diarrhea experienced by patients administered the ADCs compared to monotherapy (Table 3).\textsuperscript{60,61}

Although small, this group of studies indicates that the toxicities of the ADCs show some restricted distribution, rather than overall cytotoxicity seen with the comparator arms. However, it is still clear that, in the ADCs that have been developed to date, the main toxicities are driven by the payload metabolites, indicating a non-specific nature to the ADCs. The next generation of ADCs, with better targeted antibodies, more stable linkers, and with attention paid to the chemical nature of the final toxic metabolite, will hopefully show fewer payload specific toxicities. Instead, the toxicities may be more organ or cell-type specific, which can be predicted and, if necessary, managed with prophylactic care.

**Dose modifications to minimize toxicity**

The assessment of PK is important in optimizing the efficacy of a drug whilst minimizing the toxicity. IMGN853 and SAR3419 both had narrow therapeutic windows, but effective dose modifications have reduced the high peak plasma concentrations and minimized toxicities. IMGN853 (mirvetuximab soravtansine) is a folate receptor alpha (FR\alpha)-targeting ADC conjugated to DM4 via a sulfo-SPDB linker, and is currently in a Phase 1 clinical study for patients with FR\alpha+ platinum resistant ovarian cancer. Early testing with this drug, using total body weight to determine dose, revealed high levels of reversible ocular toxicity that was associated with high early exposure levels of mirvetuximab soravtansine.\textsuperscript{62} Clinical benefit was seen at lower doses with a lower incidence of ocular toxicity. Dose modification, based on adjusted ideal body weight calculations, reduced the variability of plasma concentrations and kept them below the threshold for ocular toxicity. As a result of this dose modification, the Phase 1 dose expansion cohort is continuing with early indications of clinical benefit with 40% overall response rate (ORR) in heavily pre-treated FR\alpha+ platinum resistant ovarian cancer.\textsuperscript{63}

Another drug that has undergone dose modification to limit the toxicity whilst maximizing the anti-tumor efficacy is SAR3419 (coltuximab ravtansine). This is a CD19-targeting ADC that utilizes the DM4/SPDB payload/linker combination. Initial Phase 1 studies of dosing once every 3 weeks (q3w) gave an ORR of 22%, and attempts to increase this were made by increasing the frequency of dosing whilst reducing the dose. Weekly administration of coltuximab ravtansine resulted in accumulation of the drug, due to a long half-life of 7 days and slow clearance, which in turn caused the development of late onset (at week 7 or 8) grade 3 peripheral neurotoxicities. The plasma concentration was maintained at a plateau level by reducing the dosing to once every 2 weeks after an initial phase of once weekly dosing for 4 weeks. This optimal dosing schedule reduced the incidence of nervous system and ocular toxicities compared to weekly dosing, and improved the ORR to 33% compared to q3w dosing.\textsuperscript{64}

Gemtuzumab ozogamicin was approved for CD33-positive AML patients in first relapse who were not candidates for other therapies. The recommended dose was 2 infusions of 9 mg/m\textsuperscript{2} gemtuzumab ozogamicin, given at least 2 weeks apart. Key toxicities experienced by patients given this dose of drug included thrombocytopenia (99%) and neutropenia (97%), which was often prolonged. Hepatic events were common, with 23% of patients having grade 3 or 4 hyperbilirubinemia and 17% patients had grade 3 or 4 elevated alanine transaminase or aspartate transaminase levels.\textsuperscript{26} There was also a high incidence of veno-occlusive disorder, particularly in patients who received hematological stem cell transplantation following treatment with gemtuzumab ozogamicin.\textsuperscript{26} More recently, fractionated dosing of gemtuzumab ozogamicin has been investigated so that patients receive the 9 mg/m\textsuperscript{2} divided over 3 doses of 3 mg/m\textsuperscript{2} on days 1, 4 and 7.\textsuperscript{65} This dose modification resulted in lower incidence of thrombocytopenia and fewer abnormal liver function indicators.\textsuperscript{65} Analysis of the PK of gemtuzumab ozogamicin, and of inotuzumab ozogamicin, has shown that the toxicities are driven by peak plasma concentrations, whilst the efficacy is driven by the AUC.\textsuperscript{66} Reducing the dose will thus reduce the peak plasma concentration and reduce toxicities, whilst more frequent dosing will allow for longer exposure to the drug, to maintain the efficacy.\textsuperscript{66}

Overall, study results suggest that the therapeutic window of ADCs can be increased by dose modifications to optimize the plasma concentration, and thereby enhance clinical benefit, whilst limiting the toxicity. Good monitoring of PK profiles in patients beyond Phase 1 will continue to be important in managing toxicities that are observed as trials are extended.

**Modifications to drug to decrease toxicity**

Agensys, Inc., an affiliate of Astellas Pharma Inc., have developed AGS-16M8F and AGS-16C3F for the treatment of renal cell carcinoma. These are fully human IgG2 antibodies conjugated to MMAF via a mc linker. AGS-16C3F was generated in Chinese hamster ovary cells to allow increased production compared to the hybridoma-derived AGS-16M8F. In preclinical testing they were shown to have similar PK and toxicological profiles,\textsuperscript{67} but the Phase 1 studies do show some differences. In the initial Phase 1 study, using AGS-16M8F, no MTD was reached at 4.8 mg/kg, but there were significant ocular toxicities, resulting in discontinuation of treatment in 3/8 subjects. However, 4.8 mg/kg exceeded the MTD for AGS-16C3F, resulting in de-escalation to find the MTD of 1.8 mg/kg. Side effects reported for AGS-16C3F were fatigue and thrombocytopenia. AGS-16C3F is continuing in clinical trials because disease control has been observed. The incidence of thrombocytopenia in patients given either drug is similar, because disease control has been observed. The incidence of thrombocytopenia in patients given either drug is similar, because disease control has been observed. The differences in the toxicities for these 2 compounds is surprising as preclinical results found similar antibody binding, cytokotoxicity and tumor size reductions for both AGS-16M8F and AGS-16C3F.\textsuperscript{67} It is unclear why the compounds have different MTDs, but it may be due to differences in protein glycosylation.\textsuperscript{68} The differences
suggest a role for the manufacturing process as well as each of the components of an ADC in driving toxicities. This is an area that warrants further investigation.

Cantuzumab mertansine (Can M) and cantuzumab ravtansine (Can R) are two ADCs that target CanAg, which is overexpressed on tumors of the colon and pancreas. They differ in the payload and linker combinations. Whilst Can M has a relatively labile disulfide link formed between the SPP linker and DM1, Can R has a more hindered, relatively stable disulfide link formed by conjugation of DM4 to SPDB. These two drugs had different toxicity profiles; elevated transaminases in the case of Can M54 and ocular toxicities with Can R.32 The incidence of elevated hepatic transaminases was greatest in patients with hepatic metastases, suggesting a bystander effect on normal hepatocytes. Neither compound has progressed further than Phase 1, possibly due to their minimal anti-tumor effects.32,54

**Different toxicities in different disease types with the same drug**

During the dose escalation of polatuzumab vedotin, DLTs were observed in patients with CLL at much lower doses than NHL. The MTD for NHL was defined as 2.4 mg/kg, but 2 of 5 CLL patients treated with 1.8 mg/kg experienced DLTs of grade 4 neutropenia and grade 4 fungal infection. This resulted in a MTD for CLL of 1.0 mg/kg, below the levels required for clinical benefit.41 Analysis of the PK profile revealed lower exposure and faster clearance in patients with CLL compared to NHL, consistent with target-mediated clearance due to higher numbers of circulating B cells in CLL.41

In a study of 43 melanoma patients treated with glembatumumab vedotin (CDX-011), the incidence of rash as an adverse event was 74%, and in 30% of patients this was of grade 3 or higher severity, affecting more than 50% of the body surface area,69 whilst the incidence for breast cancer patients was 47%, with only 4% ≥ grade 3.42 A correlation was noted between incidence of rash and improved ORR and progression-free survival for melanoma patients, and improved overall survival for breast cancer patients. Melanoma patients also experienced higher levels of pruritus (63%) and alopecia (65%) than breast cancer patients (21% and 25%, respectively).42,69 The incidence of hematological adverse events was similar in the 2 patient groups, with ~30% experiencing neutropenia (of which 20% was ≥ grade 3) and 5% experiencing thrombocytopenia.42,69

Early reports from sacituzumab govetican (IMMU-132) suggest that there may be differences in the incidence of neutropenia in different patient groups, with 30% of triple negative breast cancer patients, 24% of gastrointestinal cancer patients and 18% of lung cancer patients reporting neutropenia.70-72 It is perhaps too early to conclude that these are true differential toxicities due to the small numbers of patients, and additional monitoring is warranted as more patients in each disease group are enrolled.

**The future of ADCs, preclinical evidence**

Reducing the DAR heterogeneity of an ADC may result in a better clinical profile, both in efficacy and toxicity. The majority of ADCs currently in clinical testing are a heterogeneous mixture of compounds with different DARs, generally ranging from 0–8 drugs per antibody. Multiple variants of ADCs may cause a broad spectrum of PK values, which may limit the therapeutic window.

Novel techniques for designing ADCs that enable the production of compounds with specific numbers of drugs per antibody will potentially produce ADCs with better therapeutic indices. For example, studies on a range of conventionally conjugated CD30-MMAE ADCs with DARs of either 2, 4, or 8 show that DAR 8 was cleared more rapidly with a lower therapeutic index than DAR 4 or 2 in murine studies.14 Site-specific conjugation73 or improved linker design74 may improve therapeutic index of ADCs by reducing the hydrophobicity of higher DAR ADCs, and thus increasing the exposure of the drug in vivo.

There are several potential ways in which to site-specifically conjugate ADCs, including substituting amino acids with a cysteine (THIOMAB) or non-natural amino acids and enzymatic conjugation using bacterial transglutaminases.74 Using THIOMAB technology, a MUC16 ADC was generated by substitution of heavy chain alanine 114 with cysteine, producing a THIOMAB drug conjugate (TDC) with an average DAR of 1.6 compared to 3.5 for the original anti-MUC16 ADC. A murine xenograft model demonstrated that the TDC had superior in vivo efficacy and tolerance, with lower liver and bone marrow toxicities than the original MUC16 ADC.15 cAC10 (the same antibody as in BV) was conjugated to MMAE using the bacterial transglutaminase method to produce a homogeneous ADC with DAR 4. In rats, this new ADC showed better tumor uptake than BV and lower non-targeted uptake in liver and spleen, which allowed for a higher maximum tolerated dose.75

The first publicly disclosed site-specific ADC in clinical development is SGN-33A, a CD33-targeting ADC that has been stably linked to the PBD dimer using site-specific engineered cysteines. This generates an ADC with 2 PBD dimers per antibody. Initial toxicities that have been reported include neutropenia (Table 2).76 SGN-70A also utilizes this technology, but toxicities have yet to reported.

**Conclusions**

This review of the toxicities that have been reported for ADCs has shown that, for the majority of compounds, the toxicities are driven by the payload and the nature of the final metabolite. There are some specific examples of target-mediated toxicities. Understanding the role that each of the different parts of an ADC plays in the toxicity of a drug (aside from that needed for efficacy) will help in the design of candidates with better safety profiles. Where available, comparison with other treatment options were discussed. The compounds that have progressed to Phase 3 testing (BV, inotuzumab ozogamicin, ado-trastuzumab emtansine, and glembatumumab vedotin) do indeed have fewer toxicities than those of standard treatments. However, the majority of ADCs are in Phase 1 clinical testing, and the toxicities that some of these report are not manageable and will likely result in their failure to progress. As the field expands to include more rationally designed ADCs with specific DARs, it
will be important to monitor the toxicities, to ensure that the improved targeting of drugs will improve the therapeutic index.

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