

Antibody–Drug Conjugates (ADCs) for Personalized Treatment of Solid Tumors: A Review

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Received: January 5, 2017 / Published online: March 30, 2017
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ABSTRACT

Attaching a cytotoxic “payload” to an antibody to form an antibody–drug conjugate (ADC) provides a mechanism for selective delivery of the cytotoxic agent to cancer cells via the specific binding of the antibody to cancer-selective cell surface molecules. The first ADC to receive marketing authorization was gemtuzumab ozogamicin, which comprises an anti-CD33 antibody conjugated to a highly potent DNA-targeting antibiotic, calicheamicin, approved in 2000 for treating acute myeloid leukemia. It was withdrawn from the US market in 2010 following an unsuccessful confirmatory trial. The development of two classes of highly potent microtubule-disrupting agents, maytansinoids and auristatins, as payloads for ADCs resulted in approval of brentuximab vedotin in 2011 for treating Hodgkin lymphoma and anaplastic large cell lymphoma, and approval of ado-trastuzumab emtansine in 2013 for treating HER2-positive breast cancer. Their success stimulated much research into the ADC approach, with >60 ADCs currently in clinical evaluation, mostly targeting solid tumors. Five

ADCs have advanced into pivotal clinical trials for treating various solid tumors—platinum-resistant ovarian cancer, mesothelioma, triple-negative breast cancer, glioblastoma, and small cell lung cancer. The level of target expression is a key parameter in predicting the likelihood of patient benefit for all these ADCs, as well as for the approved compound, ado-trastuzumab emtansine. The development of a patient selection strategy linked to target expression on the tumor is thus critically important for identifying the population appropriate for receiving treatment.

Keywords: ADC; Antibody–drug conjugate; Auristatin; Calicheamicin; Cancer therapy; Maytansine; Monoclonal antibodies; Payload; Oncology; Targeted drug delivery

INTRODUCTION

Chemotherapy with cytotoxic compounds has been the mainstay of systemic cancer treatment for half a century [1, 2]. Over many years, “optimal” combinations of different cytotoxic agents having different cell-killing mechanisms were developed to maximize the antitumor activity [2]. However, these treatments are generally only partially effective in solid tumors, in part because the maximal achievable doses are limited by systemic toxicity, and as a

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result long-term remissions are rarely seen in patients with metastatic disease. Medicinal chemists and oncologists have long sought ways to increase the delivery of cytotoxic chemicals to cancer cells for increased efficacy, while minimizing exposure of normal healthy tissue [3]. The invention of monoclonal antibodies [4] offered the possibility of exploiting their specific binding properties as a mechanism for the selective delivery of a cytotoxic agent to cancer cells upon chemical conjugation of a cytotoxic effector to a tumor-binding antibody to create an antibody–drug conjugate (ADC). This article is a review based on previously conducted studies and does not involve any new studies of human or animal subjects performed by either of the authors.

THE KEY COMPONENTS OF AN ADC

All three component parts of an ADC, the antibody, the cytotoxic agent, and the linker that joins them, are critical elements in its design. The antibody moiety should be specific for a cell surface target molecule that is selectively expressed on cancer cells, or overexpressed on cancer cells relative to normal cells [5]. The payload of an ADC must be highly cytotoxic so that it can kill tumor cells at the intracellular concentrations achievable following distribution of the ADC into solid tumor tissue, and because only a limited number of payloads can be linked to an antibody molecule (typically an average of 3–4 payloads per antibody) without severely compromising its biophysical and pharmacokinetic properties [5–7]. Indeed, the breakthrough in ADC research that eventually led to the creation of approved and marketed products came with the realization that the cytotoxic agents suitable for ADC approaches need to have potency in the picomolar range to be able to be delivered in sufficient quantity to enough cancer cells to effect a therapeutic benefit [5–7]. The cytotoxic compounds used in approved and marketed ADCs are derivatives of calicheamicin, a class of highly cytotoxic enediyne antibiotics which kill cells by causing DNA double-strand breaks

[5, 8], and derivatives of the potent antimetabolic microtubule-disrupting agents, dolastatin 10 (auristatins) [6, 9] and maytansine [7, 10, 11], the chemical structures of which are shown in Fig. 1a. The two tubulin-acting agents were evaluated in phase I and phase II clinical trials and showed minimal activity at their recommended phase II doses of 2.0 mg/m² (maytansine) and 0.4 mg/m² (dolastatin 10) when each was given once every 3 weeks [12–15]. Gastrointestinal and central neurologic toxicities were dose-limiting for maytansine [12, 13], while myelosuppression was dose-limiting for dolastatin 10 [14, 15].

The third vital component of an ADC is the linker that forms a chemical connection between the payload and the antibody. The linker should be sufficiently stable in circulation to allow the payload to remain attached to the antibody while in circulation as it distributes into tissues (including solid tumor tissue), yet should allow efficient release of an active cell-killing agent once the ADC is taken up into the cancer cells [5]. Linkers can be characterized as either cleavable, where a chemical bond (or bonds) between the payload and the attachment site on the antibody (usually an amino acid) can be cleaved intracellularly [16–18], or as non-cleavable, where the final active metabolite released within the cell includes the payload and all elements of the linker still attached to an amino acid residue of the antibody, typically a lysine or a cysteine residue, following complete proteolytic degradation of the ADC within the lysosome of the cell [19–21].

The design goal for an ADC is to harness the potent tumor cell-killing action of the payload to an antibody, while retaining the favorable *in vivo* pharmacokinetic and biodistribution properties of the immunoglobulin, as well as any intrinsic biologic or immunologic activity it may have [5–7, 21, 22]. Much of the selection of the optimal antibody, the ideal linker–payload chemistry, and the optimal number of payload molecules linked per antibody molecule, are determined empirically, with a focus on maximizing the therapeutic index of the ADC [5, 22]. A summary of the chemistry, biochemistry, and preclinical evaluation leading to the selection of

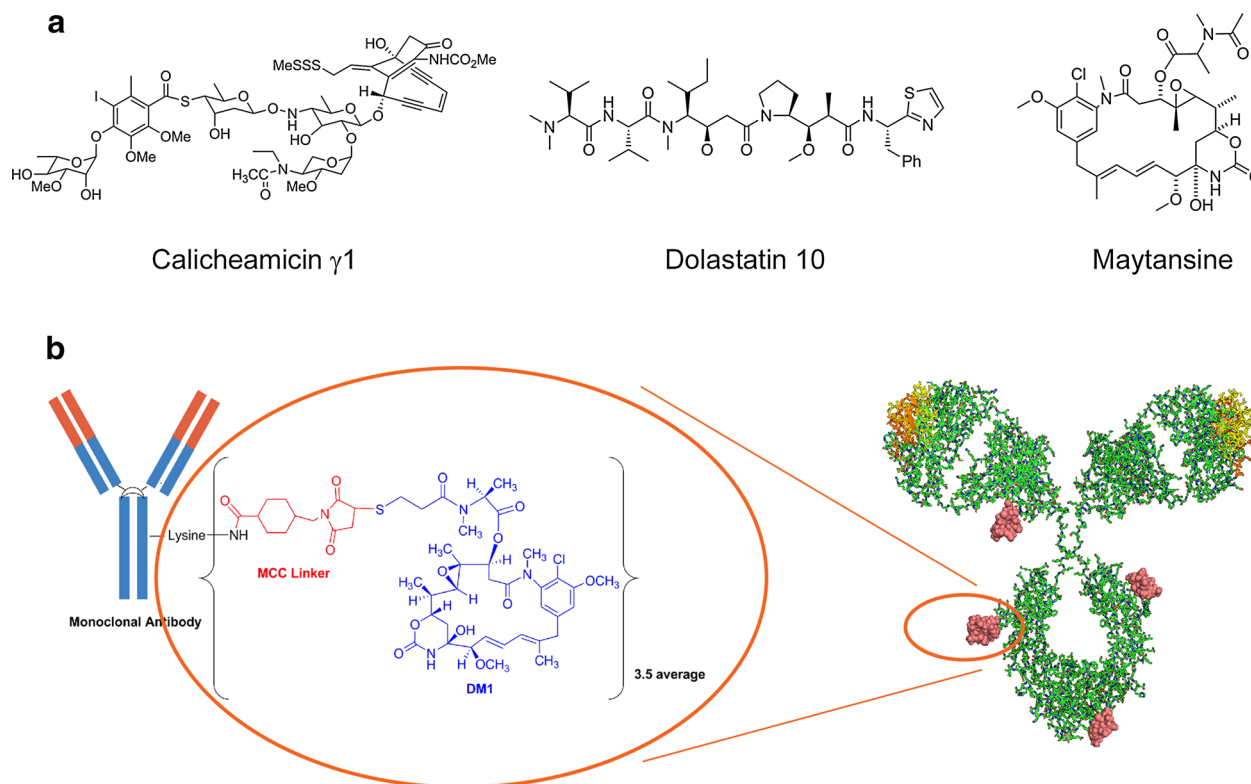


Fig. 1 **a** Chemical structures of the cytotoxic payloads adapted for use in approved and marketed ADCs. **b** Schematic representations of the structure of ado-trastuzumab emtansine (*T-DM1*)

the ADC design that became ado-trastuzumab emtansine (*T-DM1*), the first ADC to receive full approval for a solid tumor indication (vide infra), has been reviewed elsewhere [7], and serves as an exemplar for the work involved in ADC optimization prior to declaring a candidate for clinical evaluation.

THE FIRST ADC APPROVALS WERE FOR TREATING HEMATOLOGIC MALIGNANCIES

The first ADC therapeutics to reach the market were developed in hematologic malignancies, where target antigens are generally well-characterized, lineage-specific cell surface molecules that are uniformly expressed and thought to be more accessible to antibodies circulating in plasma, relative to those present on solid tumors [22]. Such antigens are usually highly

restricted in their distribution, meaning that healthy non-hematopoietic tissue and pluripotent hematopoietic stem cells are not targeted by the ADC. The first ADC to receive marketing approval from the US Food and Drug Administration (FDA) was gemtuzumab ozogamicin (Mylotarg[®]), an ADC created by conjugation of calicheamicin to an anti-CD33 antibody via an acid-labile linkage [23, 24]. CD33 is a marker of normal and malignant cells of the myeloid lineage. Based on a single arm phase II trial, the ADC received accelerated approval in 2000 as a single agent (dosing 9 mg/m² on days 1 and 15) for treating patients with acute myeloid leukemia (AML) in first relapse, and who are at least 60 years old. The overall response rate (ORR) was 26% in these patients, including a subset having incomplete platelet recovery [23, 24], while side effects included delayed hematopoietic recovery and hepatic veno-occlusive disease [24, 25]. Approval was conditioned on execution of randomized clinical trials to confirm

patient benefit [24]. In 2010, the ADC was withdrawn from the US market (although it is still marketed in Japan), following an unsuccessful confirmatory phase III trial which compared the effect of adding a single dose of 6 mg/m² to standard remission induction therapy in patients younger than 60 years [26]. Recently, the results of several other randomized studies, adding gemtuzumab ozogamicin to various induction regimens, utilizing either a single 3 mg/m² dose or fractionated dosing (3 mg/m² on days 1, 4 and 7), to reduce the incidence of hepatic veno-occlusive disease, have suggested improved overall survival (OS) in AML patients whose disease has favorable and intermediate cytogenetic characteristics [27], and have renewed interest in this ADC, and in CD33 as a target [28].

The second ADC to receive marketing approval was brentuximab vedotin (Adcetris[®]), made by conjugation of the potent auristatin tubulin agent, MMAE, to an anti-CD30 antibody via a cleavable valine-citrulline (vc) dipeptide linker [6]. The CD30 antigen, a marker for activated lymphocytes, is highly expressed on the Reed–Sternberg cells of Hodgkin lymphoma (HL) as well as on the malignant cells of anaplastic large cell lymphoma (ALCL). Early phase clinical trials of the “naked” anti-CD30 antibody showed no responses in HL, and, in ALCL patients, only 2 of 41 (5%) had a complete response (CR) [29]. However, arming the antibody with vcMMAE created a highly active compound that received accelerated approval from FDA in August, 2011, for treatment of patients with relapsed HL or systemic ALCL [6]. In the pivotal single arm phase II trials, brentuximab vedotin showed a 75% ORR in patients with relapsed or refractory HL, with a median duration of response of 20.5 months [30], and an 86% ORR (57% CR) in patients with systemic ALCL [31]. The principal dose-limiting toxicity (DLT) was neutropenia, while the main toxicity upon repeated administration was neuropathy [30, 31]. Several phase III trials are in progress to confirm clinical benefit of brentuximab vedotin in randomized studies, both as a single agent and in combination with approved agents (<http://www.clinicaltrials.gov>) [6].

A second ADC using the calicheamicin payload, inotuzumab ozogamicin, which targets the B cell antigen CD22 has completed a phase III trial (INO-VATE ALL) in relapsed or refractory acute lymphoblastic leukemia [32–34]. The results show that treatment with the ADC produced a higher rate of CR (80.7%) compared to chemotherapy (29.4%), with 23% of the patients alive at 2 years in the ADC-treatment group compared to 10% in the chemotherapy comparator group [34]. Like gemtuzumab ozogamicin, the most concerning non-hematologic toxicities in patients treated with inotuzumab ozogamicin were adverse events in liver, especially veno-occlusive disease [34].

ADCS FOR TREATMENT OF SOLID TUMORS: ADO-TRASTUZUMAB EMTANSINE

The first ADC to receive marketing approval for treatment of a prevalent solid tumor was ado-trastuzumab emtansine (Kadcyla[®]; T-DM1), made by conjugation of the sulfhydryl group of the maytansinoid DM1 to lysine amino groups of the anti-human epidermal growth factor receptor 2 (HER2) antibody, trastuzumab, via reaction with the bifunctional non-cleavable linker, succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) [7, 35, 36]. Figure 1b shows a molecule of T-DM1 (representative of a typical ADC), illustrating the chemical structure of the linker–payload moiety that is attached to lysine residues of the antibody (left panel), and a representation of a molecular model of one antibody molecule (molecular mass 150 kDa) conjugated to four molecules of the maytansinoid DM1 (right panel; molecular weight of linker–payload ~1 kDa). Since there are about 90 lysine amino groups in trastuzumab, and the conjugation reaction with SMCC is carefully controlled to yield a conjugate with an average of 3.5 DM1 molecules per antibody molecule [7], the ADC compound is heterogeneous with respect to loading of the payload and its distribution across the potential sites of reaction on the antibody [37, 38]. Mass spectroscopy shows that about 70–80% of the antibody molecules are

conjugated to between 2 and 5 molecules of DM1, while only about 3% of the antibody remain unconjugated [37, 39, 40]. Peptide mapping methodology shows that up to 70 individual lysine residues are partially modified by linker–payload to an average level of about 4% with a range from 25% to <1% [40]. While the heterogeneity of an ADC presents a challenge for process and analytical development and process control, the challenge can be met by utilizing appropriate development strategies and modern techniques of protein and chemical analysis [7, 37–40].

Phase I and phase II clinical trials in patients with HER2-positive metastatic breast cancer (mBC) established a recommended phase II dose/schedule of 3.6 mg/kg given by intravenous infusion once every 21 days [41–44]. The principal DLT was reversible thrombocytopenia [41, 45], with reversible low-grade increases in hepatic transaminases also observed [41]. Pharmacokinetics were non-linear in the dose range 0.3–4.8 mg/kg as anticipated from preclinical studies and prior clinical experience with trastuzumab, given the normal tissue expression of HER2 [46]. The half-life was approximately 4 days at the 3.6 mg/kg dose level [47]. Plasma levels of free payload were very low [41, 47]. An early signal of antitumor activity (5 confirmed partial responses from 24 enrolled patients; ORR 20.8%) in the phase I study was confirmed in two phase II trials in patients (~110 patients each) who were heavily pre-treated for their HER2-positive mBC, with ORRs of 25.9% [43], and 34.5% [44].

These early clinical studies led to a pivotal phase III trial (“EMILIA”) where patients with mBC who had progressed following treatment with a taxane plus trastuzumab were randomized to receive T-DM1 or the approved second-line treatment of lapatinib (a tyrosine kinase inhibitor which also targets HER2) plus capecitabine [48]. The ORR (43.6% vs. 30.8%; $p < 0.001$), progression-free survival (PFS; 9.6 vs. 6.4 months; hazard ratio 0.65; $p < 0.001$), and OS (30.6 vs. 25.1 months; hazard ratio 0.68; $p < 0.001$), all significantly favored the T-DM1 arm versus the comparator arm of the study [48]. Furthermore, the incidence of adverse events of grade ≥ 3 was less in the T-DM1 arm

(40.8%, with thrombocytopenia and transaminitis most common, consistent with the phase I/II experience) relative to the comparator arm (57.0%, with diarrhea, hand–foot syndrome and vomiting most common). The rate of cardiac adverse events, a concern with HER2-targeted therapy, was low (<2%) in both arms. The “EMILIA” trial established the safety and effectiveness of T-DM1, and, in February, 2013, the FDA granted full approval as a therapy for treating patients with HER2-positive late-stage mBC previously treated with a taxane and trastuzumab. To date, T-DM1 is the only ADC to have received full approval from FDA based on a randomized phase III study in any indication.

A phase III trial (“TH3RESA”) in patients with progressive HER2-positive advanced mBC who had received at least two HER2-directed regimens in the advanced setting, including trastuzumab and lapatinib, and previous taxane therapy in any setting, also favored T-DM1 over the comparator arm (physician’s choice), with improved PFS (6.2 vs. 3.3 months; hazard ratio 0.528; $p < 0.001$) [49], and median OS of 22.7 versus 15.8 months for the comparator arm ($p = 0.0007$) [50]. Of note, patients enrolled in TH3RESA had had a median of 4 prior regimens for treating their advanced disease, with 28–30% of patients receiving >5 such prior treatments [49]. Again, improved efficacy was achieved together with a reduced incidence of grade ≥ 3 adverse events (32% for T-DM1 vs. 43% for the comparator) [49, 50].

A phase III trial of T-DM1 (with and without pertuzumab) versus trastuzumab plus taxane in the first-line treatment of HER2-positive mBC (“MARIANNE”) showed that T-DM1 met the pre-defined non-inferiority criteria, with similar ORR (60–70%) and PFS (~14 months) for all treatment arms [51]. Neither experimental arm showed superior PFS to the trastuzumab plus taxane arm, and addition of pertuzumab to T-DM1 did not improve PFS despite preclinical data that showed synergistic activity for this combination [52]. The duration of response for patients responding to treatment with T-DM1 was ~21 months, while that of patients responding to treatment with trastuzumab plus taxane was 12.5 months. T-DM1 was better

tolerated, with patients having improved (prolonged) health-related quality of life [51].

The efficacy results of the MARIANNE trial were disappointing in view of the initial promise of T-DM1 in early phase I and phase II trials [53, 54]. However, the median PFS seen with T-DM1 treatment in MARIANNE (14.1 months; $n = 367$ patients) was similar to that observed in the prior small phase II trial (14.2 months; $n = 67$ patients). A major difference between the phase II and phase III studies is in the median PFS of the control trastuzumab plus taxane arms, reported to be 9.2 months ($n = 70$ patients) and 13.7 months ($n = 365$ patients), respectively [51, 53]. As noted by Perez et al. [51], the latter value is consistent with results of other phase III trials, for example, the CLEOPATRA study of pertuzumab plus trastuzumab plus docetaxel (PFS of comparator arm = 12.4 months, $n = 406$ patients) [55]. In the small phase II study [53], more patients in the control arm were first diagnosed at an earlier stage of disease and had received prior (neo)adjuvant therapy with a taxane (40.0%) versus the T-DM1 arm (32.8%), while in the control arm of MARIANNE, 32.9% of patients had received (neo)adjuvant taxane therapy [51, 56]. One might speculate that more cases of prior (neo)adjuvant therapy in the control arm of the small phase II study [53, 56], might account for its relatively poor PFS (9.2 months) compared with larger studies (12.4 and 13.7 months in phase III studies cited above).

PATIENT SELECTION IN THE TREATMENT OF SOLID TUMORS WITH ADCS

What can we learn from the preclinical and clinical development of T-DM1 with respect to the relationship between target expression on the cancer cells of a solid tumor, and clinical benefit with the ADC? With the caveat that any such learning may be unique to the biology of the HER2 antigen in mBC, there are some observations that may apply generally.

In a phase II trial, mBC patients ($n = 112$) were enrolled on the basis of having had prior treatment with trastuzumab, from which it was

inferred that they were HER2-positive [43]. However, the HER2 status of their disease was retrospectively reassessed on archival primary tumor specimens at a central laboratory (specimens available in 95 cases). While the ORR for the entire population was 25.9%, it was higher (33.8%) in patients confirmed as having HER2-overexpressing mBC ($n = 24$), and only 4.8% in patients whose disease was reassessed as HER2-normal ($n = 21$). There were similar trends in a second phase II study in patients ($n = 110$) who must have had at least two prior HER2-directed regimens [44]; the ORR was 34.5% in the overall population, while in those patients whose HER2 status was confirmed at a central laboratory (in 80 of 95 available specimens), the ORR was 41.3%. The implication of these observations is that, if the phase I trial (ORR 20.8%) had been conducted in all comers with mBC, instead of with patients likely to have HER2-overexpression, the ORR could well have been zero because only about 20% of all mBC patients overexpress HER2, while the majority of cases express normal levels of HER2 (“negative” in the jargon of the test). Thus, patient selection, albeit indirect, may have been a key factor in engendering enthusiasm for continued development of the molecule.

Even within the HER2-positive population, there is likely a range of HER2 expression levels above the minimum level that saturates the read-out of the approved immunohistochemistry test. In confirmed HER2-positive patients, whose levels of HER2 mRNA were assessed by quantitative reverse transcriptase/polymerase chain reaction (qRT-PCR) methodology in a phase II study, those with \geq median values had an ORR of 36% with a median PFS not reached, while those with $<$ median HER2 mRNA levels had an ORR of 28% with a median PFS of 4.2 months [43]. While the numbers are small, similar trends were observed in other phase II trials [44, 54], including first-line treatment settings [53, 56].

Collectively, these observations in clinical studies with T-DM1 are consistent with a simple hypothesis for driving success for ADCs in solid tumors; that is, that the amount of payload delivered to a cancer cell in a tumor is a function of how much ADC can be given to the

patient (i.e., the dose and schedule, within bounds dictated by tolerability), and how much ADC the cell can receive (antigen density). The higher the cell surface density of the target antigen, the more ADC can be taken up and metabolized by the cell, to release the active cytotoxic agent. Furthermore, in the case of HER2, it has been amply demonstrated pre-clinically that the higher the antigen density on a tumor xenograft, the greater the amount of trastuzumab that is retained within the tumor mass relative to a non-binding control antibody [57]. Thus, for an ADC, personalization of therapy can mean selecting only those patients for treatment whose cancers express the target antigen above a threshold level of antigen per cell necessary for anti-tumor activity. However, this criterion is by no means sufficient to predict anti-tumor activity, and further work needs to be done to establish more biomarkers for response to ADC therapeutics beyond simply the presence of the target antigen, for example, markers for sensitivity to the payload, or for aspects of target biology that may affect internalization and intracellular trafficking (may influence rate and extent of payload release from the ADC) [19, 20, 36].

ADCs ADVANCING INTO PIVOTAL TRIALS FOR TREATING SOLID TUMORS

There are >60 ADCs currently in clinical evaluation, the majority of which (72%) utilize a version of the two classes of potent tubulin-binding microtubule-disrupting agents found in the two currently approved ADCs described above [5, 58, 59]. Most ADCs in development are targeting solid tumors and are in early clinical development, a testimony to the relatively recent surge in enthusiasm for the ADC approach following the approvals of brentuximab vedotin and ado-trastuzumab emtansine. Such enthusiasm must be tempered by the discontinuation of development of several compounds, mainly for reasons of insufficient activity at the maximum doses that can be tolerated upon repeat administration [58, 59]. Some of the reasons for the relatively narrow

therapeutic window ascertained from the clinical experience in developing ADCs have been discussed elsewhere [58, 59]. However, several ADCs have advanced into pivotal studies in both solid tumor indications and in hematologic cancers (listed in Table 1). The remainder of this review will focus on the five ADCs currently in pivotal studies for treating solid tumors.

Glembatumumab Vedotin

Glembatumumab vedotin (GV, CDX-011, CR011-vcMMAE) is an ADC comprising a fully human IgG2 anti-glycoprotein nonmetastatic B (gpNMB; osteoactivin) antibody conjugated with vcMMAE (cleavable dipeptide linker). The target membrane glycoprotein is expressed at higher levels in certain cancers, including melanoma, breast cancer, small cell lung cancer (SCLC), glioblastoma and hepatocellular carcinoma, relative to normal tissues [61, 62]. The target is also expressed on tumor stromal cells [62].

A phase I/II trial of GV in patients ($n = 117$) with advanced melanoma established 1.88 mg/kg administered once every 3 weeks as the recommended phase II dose [61]. At this dose and schedule, there were 4 confirmed PRs (10%) in patients assessed for efficacy ($n = 40$). Treatment-related adverse events of grade ≥ 3 included neutropenia (19%) and, upon repeat dosing, neuropathy (7%) similar to observations with other vcMMAE-containing ADCs [6, 63], as well as with dolastatin 10 [64]. However, for GV, the most common grade ≥ 3 adverse event was rash (30%), and there was a high incidence of alopecia (65%), and skin toxicity defined the DLT in the dose-expansion phase of the study [61]. Since skin toxicity is generally not observed for other vcMMAE-ADCs, such toxicities are likely target-directed via antibody binding to gpNMB expressed in normal epithelial tissue. Dose-dependent pharmacokinetics was observed during dose-escalation, the half-life of the ADC increasing from 16 to 38 h, providing evidence of saturable target-mediated disposition [63, 65].

The expression of gpNMB in breast cancer led to a randomized phase II study (“EMERGE”)

Table 1 ADCs marketed and/or in pivotal clinical development

Antibody–drug conjugate	Target antigen	Linker-cytotoxic compound	Antibody	Tumor type(s)	Developer	Development status
A. Marketed antibody–drug conjugates						
Mylotarg® (Gemtuzumab ozogamicin)	CD33 (siglec-3)	Cleavable hydrazone, AcBu <i>N</i> -acetyl- γ calicheamicin	Gemtuzumab hP67.6 humanized IgG4	Acute myeloid leukemia (AML)	Pfizer	US FDA conditional approval 5/2000. Withdrawn 8/2011 Marketed in Japan
Adcetris® (Brentuximab vedotin; SGN35)	CD30	Cleavable dipeptide, vc-MMAE (auristatin)	Brentuximab Chimeric IgG1	Relapsed/refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma	Seattle Genetics Millennium-Takeda	US FDA conditional approval 8/2011 Multiple Phase 1–4 combination and monotherapy trials in CD30-positive indications
B. Antibody–drug conjugates in pivotal clinical development						
Kadcyla® (Ado-trastuzumab emtansine; T-DM1)	Human epidermal growth factor receptor 2 (HER2; ErbB2)	Non-cleavable thioether SMCC-DM1 (maytansinoid)	Trastuzumab Humanized IgG1	HER2-positive breast cancer	Genentech-Roche (ImmunoGen ADC technology)	US FDA full approval 2/2013 Multiple Phase 1–4 combination and monotherapy trials in HER2-positive indications
Inotuzumab ozogamicin (CMC-544)	CD22 (siglec-2)	Cleavable hydrazone, AcBu <i>N</i> -acetyl- γ calicheamicin	Inotuzumab G5/44 Humanized IgG4	B-cell acute lymphoblastic leukemia (B-ALL) & other B cell malignancies	Pfizer	Phase 3 (B-ALL) Multiple Phase 1–3 combination and monotherapy trials in B cell indications

Table 1 continued

Antibody–drug conjugate	Target antigen	Linker-cytotoxic compound	Antibody	Tumor type(s)	Developer	Development status
Glembatumumab vedotin (CR011-vcMMAE; CDX-011)	Glycoprotein NMB (osteostatin)	Cleavable dipeptide, vc-MMAE (auristatin)	Glembatumumab Fully human IgG1	Metastatic breast cancer (MBC) and melanoma	Celldx Therapeutics (Seattle Genetics ADC technology)	Pivotal randomized phase 2 (triple negative breast cancer) Ongoing evaluation in combination
Anetumab ravtansine (BAY 94-9343)	Mesothelin	Cleavable highly hindered disulfide SPDB-DM4 (maytansinoid)	Anetumab Anti-mesothelin fully human ^a IgG1	Mesothelin-positive solid tumors	Bayer Healthcare (ImmunoGen ADC technology)	Pivotal randomized phase 2 (mesothelioma) Phase 1 and 2 monotherapy and combination in mesothelin-positive tumors
Mirvetuximab soravtansine (IMGN853)	FOLR1 (folate receptor alpha; FR α)	Cleavable charged highly hindered disulfide Sulfo-SPDB-DM4 (maytansinoid)	Mirvetuximab ^b M9346A Humanized IgG1 (by variable domain resurfacing method)	Ovarian cancer, endometrial cancer, lung adeno-carcinoma	ImmunoGen	Phase 3 trial (FR α -positive, platinum-resistant epithelial ovarian cancer) Ongoing phase 2 evaluation in combination
Depatuxizumab mafodotin (ABT-414)	Epidermal growth factor receptor (EGFR)	Uncleavable maleimido-caproyl mcMMAF (auristatin)	Depatuxizumab ^b (ABT-806) Humanized IgG1	EGFR-overexpressing solid tumors, glioblastoma	Abbvie (Seattle Genetics ADC technology)	Phase 2b/3 trial in combination with temozolomide (glioblastoma) Other phase 1 and 2 trials ongoing

Table 1 continued

Antibody–drug conjugate	Target antigen	Linker-cytotoxic compound	Antibody	Tumor type(s)	Developer	Development status
Rovalpituzumab tesirine (SC16LD6.5; Rova-T)	Delta-like protein 3 (DLL3)	Cleavable dipeptide (valine-alanine) Pyrolobenzodiazepine (PBD) dimer	Rovalpituzumab (SC16) Humanized IgG1	Small cell lung cancer (SCLC)	Stemcentrx (now Abbvie) (Spirogen ADC technology)	Pivotal phase 2, single arm (3rd or later line for SCLC) Phase 2 trial in 1st-line combination
Vadastuximab talirine (SGN-CD33A)	CD33	Cleavable dipeptide (valine-alanine) Pyrolobenzodiazepine (PBD) dimer	Vadastuximab ^b Humanized IgG1	Acute myeloid leukemia (AML)	Seattle Genetics (Spirogen ADC technology)	Phase 3 in combination with azacitidine or decitabine (older patients with newly diagnosed AML)

Compounds in bold are directed towards solid tumor indications

MMAE monomethylauristatin E, *MMAF* monomethylauristatin F, *vc* valine-citrulline dipeptide linker, *mc* maleimidocaproyl linker, DM1 and DM4 are thiol-containing maytansinoids [5], *SPDB N*-succinimidyl 3-(2-pyridylthio)butanoate, *SMCC* 4-(*N*-maleimidomethyl) cyclohexanecarboxylic acid *N*-hydroxy-succinimide ester, *suif*/*o*-*SPDB N*-succinimidyl-4-(2-pyridylthio)-2-sulfobutanoate

^a MorphoSys phage technology

^b Although these antibodies were humanized, changes in naming methodology at INN resulted in the ‘ximab’ suffix of chimeric antibodies [60]

^c Trials prospectively designated as pivotal studies that were open for enrollment on or before December 31, 2016

comparing GV to investigator's choice (IC) of single agent chemotherapy (2:1 randomization) in patients ($n = 124$) with refractory mBC (patients with a median of four prior lines of cytotoxic therapy for advanced/metastatic disease) selected for the expression of target on at least 5% of tumor epithelial cells or stromal cells [62]. The toxicity findings in patients receiving GV were similar to those described above in a melanoma patient population, with rash, fatigue, nausea, neutropenia, alopecia and peripheral neuropathy being most common (>20% of patients). The most common adverse event of grade ≥ 3 was neutropenia (22%) in this patient population. The confirmed ORR was 6% (5/83) for GV versus 7% (3/41) for IC. Retrospective analysis focusing on patients expressing gpNMB on $\geq 25\%$ of tumor epithelial cells showed a confirmed ORR of 13% (3/23) for the GV arm versus 9% (1/11) for IC, with a suggestion of greater activity for GV in patients with triple-negative breast cancer (TNBC) relative to IC [62]. Although there was just one confirmed partial response (PR) in the 10 patients with TNBC (from 28 TNBC patients treated with GV) that met the $\geq 25\%$ cut-off for gpNMB expression, an additional 3 patients had responses at a single time point [62]. GV is currently in a pivotal phase II trial "METRIC" (NCT01997333) in patients with metastatic TNBC where $\geq 25\%$ of tumor cells express gpNMB by an immunohistochemistry screen (expected to be $\sim 40\%$ of all TNBC patients), randomized 2:1 to receive either GV or capecitabine [66].

Anetumab Ravtansine

Anetumab raptansine (BAY 94-9343) consists of a fully human anti-mesothelin antibody conjugated to the maytansinoid DM4 via a cleavable disulfide linker [67]. Mesothelin is highly expressed in certain tumors, including 100% of cases of mesothelioma, the majority of cases of ovarian and pancreatic adenocarcinomas, and a high proportion of cases of non-small cell lung cancer, gastric cancer and TNBC [see references cited in 67]. Normal tissue expression is limited to cells lining the pleura, pericardium and peritoneum. The ADC was shown to have

strong activity in xenograft models of pancreatic and ovarian cancers, as well as in models of mesothelioma, with the degree of activity generally correlating with the level of mesothelin expression [67].

A phase I study in patients with ovarian cancer or with mesothelioma established 6.5 mg/kg given intravenously once every 3 weeks as the recommended phase II dose. A total of 45 patients were treated during dose-escalation (0.15 to 7.5 mg/kg), and 32 patients were treated in an expansion cohort at 6.5 mg/kg [68]. A further 71 patients were treated with the ADC at 1.8 or 2.2 mg/kg in expansion cohorts evaluating weekly dosing. The DLTs at 7.5 mg/kg were peripheral neuropathy and reversible corneal toxicity (keratitis, blurred vision). At the 6.5 mg/kg dose ($n = 38$ subjects), peripheral sensory neuropathy (37%), and reversible corneal epitheliopathy (50%) were mostly grade 1 or 2, with only a low incidence of these toxicities at grades ≥ 3 (3% and 8%, respectively). Fatigue, nausea, diarrhea, anorexia and vomiting, mostly grade 1 or grade 2, were also common (37–63%). Dose-proportional pharmacokinetics were observed in the dose-ranging studies, suggesting that target-mediated clearance by normal tissue was negligible; the half-life of the ADC was about 6 days [68], similar to that of other ADCs with the same linker-payload design [69].

The ORR at the 6.5 mg/kg dose level was 18% (7 responses in 38 subjects), with 2 PRs in ovarian cancer patients (10%), and, notably, 5 PRs in patients with mesothelioma (31%), all of which were in patients for whom anetumab raptansine was second-line treatment ($n = 10$; ORR 50%) [70]. The responses in mesothelioma were remarkably durable, with 4 of 5 responses continuing for >500 days [68]. Based on these results, a randomized phase II pivotal trial was initiated in December 2015, investigating 6.5 mg/kg of anetumab raptansine given every 3 weeks, in comparison to vinorelbine (2:1 randomization), as second-line therapy for treating metastatic pleural mesothelioma [71]. In January 2017, this study completed enrollment (<http://www.clinicaltrials.gov>). The inclusion criteria included the requirement to exceed a predetermined threshold value for

mesothelin expression as assessed by an immunohistochemistry screening assay.

Mirvetuximab Soravtansine

Mirvetuximab soravtansine (IMGN853) is a folate receptor alpha (FR α)-targeting antibody–maytansinoid conjugate utilizing a charged, cleavable disulfide linker for conjugating DM4 to the humanized anti-FR α antibody [72]. FR α is expressed at high levels in the majority of cases of epithelial ovarian cancer, and is also expressed in many cases of endometrial cancer and lung adenocarcinoma [72, and references therein]. Most normal tissues do not express FR α (transport of folate into cells is thought to be mediated by other folate-binding proteins such as reduced folate carrier), making it a promising target for an ADC approach [72]. All three ADC components, the choice of antibody, linker and maytansinoid, were optimized in generating mirvetuximab soravtansine, and the final lead candidate showed potent anti-cancer activity in several xenograft models with good correlation between the degree of activity and FR α expression levels [72].

A phase I dose-finding clinical study determined 6.0 mg/kg adjusted ideal body weight (AIBW) given once every 3 weeks as the recommended phase II dose [73]. In the 44 patients treated on an every 21 days schedule at doses ranging from 0.15 to 7.0 mg/kg (total body weight), the most common adverse events were diarrhea, fatigue, vision blurred and nausea, mostly grade 1 and 2, seen in 20–40% of subjects. As with anetumab ravtansine, reversible corneal ocular events, vision blurred and punctate keratitis, noted in 2 of 5 patients treated at the 7.0 mg/kg dose level, defined the DLT. Reversible corneal toxicity is a finding commonly noted for ADCs having a disulfide-linked DM4 (maytansinoid) or a non-cleavable linker–MMAF (auristatin) as the linker–payload moiety [68, 69, 73–76], and reversible corneal adverse events were one of the DLTs for another protein-bound microtubule-disrupting agent, nanoparticle albumin-bound paclitaxel (Abraxane[®]) [77].

Observations of anti-tumor activity during dose-escalation led to investigation of mirvetuximab soravtansine at 6.0 mg/kg AIBW given every 3 weeks in a cohort of patients ($n = 46$) with platinum-resistant epithelial ovarian cancer that was FR α -positive, defined as expression with at least 2+ intensity (scale 0–3+) on $\geq 25\%$ of tumor cells by an immunohistochemistry assay [78]. There were no grade ≥ 3 ocular toxicities noted, and the incidence and severity of reversible grade 1 and 2 ocular events decreased with the introduction of improved management procedures including the use of preservative-free lubricating eye drops [78]. The confirmed ORR was 26% (1 CR and 11 PRs) in this heavily pretreated patient population (up to five prior systemic treatment regimens). Notably, the confirmed ORR was 44% in the patients ($n = 16$) with 1–3 prior lines of therapy, and whose FR α expression was at least 2+ on $\geq 50\%$ of tumor cells [78], data that have defined this as the patient population for a pivotal phase III study “FORWARD I” (NCT02631876) which opened in December, 2016 [79]. Approximately 60% of epithelial ovarian cancer patients meet the FR α inclusion criteria based on this threshold [78, 80].

Depatuxizumab Mafodotin

Depatuxizumab mafodotin (ABT-414) is an anti-epidermal growth factor receptor (EGFR) antibody–auristatin conjugate wherein the charged auristatin MMAF is conjugated to the antibody via an uncleavable linker [81]. The ADC targets a domain on the activated form of EGFR that is only present at high levels on tumors whose growth is driven by overexpression of EGFR. Thus, the antibody binds well to EGFR-overexpressing cancer cells with little or no binding of the antibody to normal tissues, as demonstrated in phase I clinical studies with the “naked” antibody, ABT-806, showing that, even dosing at 24 mg/kg, it lacks the skin toxicity characteristic of EGFR inhibition induced by cetuximab [82]. Dose-proportional pharmacokinetics observed during dose-escalation also suggested minimal target-mediated clearance by normal tissue [82]. Binding of the antibody to

the overexpressed receptor on tumor cells inhibits EGFR signaling, and conjugation to MMAF does not interfere with this activity of the “naked” antibody [81]. Thus, ABT-414 is an ADC designed with a concept similar to that of T-DM1 [7, 35], wherein the antibody itself has anti-tumor activity which is augmented by conjugation to a payload.

A phase I clinical trial in patients with recurrent glioblastoma established 1.25 mg/kg of ABT-414 given once every 2 weeks as the maximum tolerated dose (MTD), both as monotherapy and in combination with monthly temozolomide [83]. Evaluation of ABT-414 monotherapy at the MTD in an expanded cohort of 60 patients with EGFR-amplified glioblastoma (EGFR amplification occurs in approximately 50% of glioblastoma cases) led to a recommendation that the phase II dose be reduced to 1.0 mg/kg every 2 weeks for further development owing to a high incidence of ocular (corneal) toxicities (92% all grades; 32% grade ≥ 3) at the 1.25 mg/kg dose level [75]. The ocular side effects were reversible in 4–6 weeks to up to 6 months, depending on the severity of the symptoms [75]. In 56 patients evaluated for efficacy using the Response Assessment in Neuro-Oncology criteria, 3 (5.4%) achieved a partial response, while 24 (43%) had stable disease [75]. The 6-month PFS estimate was 25.3%. ABT-414 is now being evaluated in a randomized placebo-controlled phase IIb/III study (NCT02573324), in combination with concurrent chemo-radiation and adjuvant temozolomide in patients with newly diagnosed glioblastoma having EGFR amplification (“Intelligence1”), which was initiated in September, 2015, as well as in a randomized trial in EGFR-amplified recurrent glioblastoma (NCT02343406).

Rovalpituzumab Tesirine

The newest ADC for a solid tumor indication that has transitioned into a pivotal clinical study, is rovalpituzumab tesirine (Rova-T), an ADC comprising a humanized anti-delta-like protein 3 (DLL3) antibody conjugated to a pyrrolobenzodiazepine (PBD) dimer via a

protease-cleavable valine-alanine dipeptide-containing linker [84]. DLL3 is expressed on the surface of certain tumor cells, including SCLC and large cell neuroendocrine cancer, but is absent from normal adult tissue. It is also thought to be expressed on tumor progenitor cells and cancer stem cells. The ADC induces durable tumor regressions in a variety of xenograft models of patient-derived tumors, its efficacy correlating with DLL3 expression [84].

Rovalpituzumab tesirine was evaluated in a phase I trial wherein a total of 74 SCLC patients received doses ranging from 0.05 to 0.8 mg/kg given once every 3 weeks or every 6 weeks [85]. Immunohistochemistry assessment of DLL3 expression (biopsy samples available for 48/74 patients) indicated that the target was detectable on at least 1% of tumor cells in 88% (42/48) of the cases, while 67% (32/48) showed at least 50% of tumor cells staining positive. The most common grade ≥ 3 toxicities (all patients) were serosal effusions (11%), thrombocytopenia (12%) and skin toxicities (8%), while lower grades of these toxicities were also common (all grades: 35, 20 and 49%, respectively). The recommended phase II dose was 0.3 mg/kg given twice with a 6-week interval between dosing [85]. Among 56 evaluable patients treated at either 0.2 mg/kg every 3 weeks, or 0.4 mg/kg every 6 weeks, there were 9 responses (16%) as confirmed by independent central review. Eight of these responses occurred in the 26 patients whose DLL3 expression was detected on $\geq 50\%$ of tumor cells (the other case was a patient whose DLL3 level could not be assessed) for a 31% confirmed ORR, consistent with a hypothesis that the level of expression of the DLL3 target on tumor cells correlates with the degree of anti-tumor activity for an ADC directed towards this target [85]. These data have led to a single arm pivotal phase II clinical study of rovalpituzumab tesirine in third-line and later treatment of subjects with relapsed or refractory DLL3-expressing SCLC (NCT02674568). Despite the data suggesting that most/all responders expressed the target antigen on $\geq 50\%$ of tumor cells, the inclusion criteria for this phase II trial has a cut-off for DLL3 expression on tumor cells of only $>1\%$ (<http://www.clinicaltrials.gov>).

THE PLACE OF ADCS IN THE TREATMENT OF SOLID TUMORS

The common theme that emerges from the clinical experience obtained during development of the one marketed ADC and the five more now in pivotal clinical trials for the treatment of solid tumors is the importance of patient selection for the level of target expression on tumor cells. The development of T-DM1 was greatly aided by the fact that trastuzumab itself was already a marketed therapeutic agent for which there was already a marketed diagnostic test to define those breast cancer patients (~20% of the total) whose cancer overexpressed HER2 and who were thus most likely to benefit from treatment with T-DM1 [7]. In the case of the five ADCs in pivotal trials for mesothelioma, FR α -overexpressing platinum-resistant ovarian cancer, gpNMB-positive TNBC, EGFR-amplified glioblastoma and DLL3-expressing SCLC, the relationship between anti-tumor activity and the level of target expression in the tumor required investigation during clinical evaluation of phase I and phase II trials. All five ADCs utilize a diagnostic test (generally an immunohistochemistry test) in their respective pivotal clinical trials in order to select the target-positive patient population most likely to benefit from treatment with the ADC, by virtue of the expression of the antibody target above a pre-defined threshold level identified during early clinical development.

ADCs that have shown promising activity in solid tumors in early phase clinical trials, but that have not yet advanced into pivotal trials, also show most benefit in patients whose tumors express the highest levels of target antigen; for example, lifatuzumab vedotin which targets NaPi2b [86], and SAR566658 which targets the CA6 antigen of mucin-1 [87]. It is likely that a prototype diagnostic test will be a necessary accompaniment to virtually all ADCs in development for targeting solid tumors. In the future, it may be that non-clinical studies in patient-derived tumor xenograft models will better identify the threshold levels of target expression for favorable anti-tumor

activity, and accelerate the development of appropriate calibration of a diagnostic test to measure such antigen levels, prior to initiation of clinical trials [88].

While the development pathway for three of the five ADCs in pivotal studies for treating solid tumors (Table 1) is to evaluate them as monotherapy versus a chemotherapy comparator arm—similar to the development of T-DM1 [48]—all three compounds are also being studied in combination with a variety of chemotherapeutic and biologic agents, as appropriate for the particular disease (<http://www.clinicaltrials.gov>). The relatively benign side-effect profiles for many ADCs (relative to cytotoxic chemotherapy) suggest that they may be well suited to combine with other agents with the goal of further improvement in treatment outcomes for cancer patients, ultimately in first-line treatment settings. For example, mirvetuximab soravtansine is being evaluated in combination with either carboplatin, pegylated liposomal doxorubicin, or bevacizumab, in a multi-arm trial called FORWARD II (NCT02606305) [89].

In contrast, the initial strategy for the development of ABT-414 is in combination with chemo-radiation in the first-line treatment of glioblastoma, a disease for which the current standard of care has a poor outcome [90]. The development strategy for rovalpituzumab tesirine, on the other hand, is to seek an initial accelerated approval based on response rate in a single arm monotherapy trial in third-line or later treatment for SCLC, a setting for which there are no good treatment options available [91].

The recent findings of Zippelius et al. [92–94] suggest that ADCs, especially those made with the potent microtubule-disrupting agents as the payload, may be combined with immune checkpoint inhibitors such as anti-PD1 antibodies (pembrolizumab, nivolumab) or anti-PD-L1 antibodies (atezolizumab) for enhanced and sustained anti-tumor effect. Not only do such ADCs induce immunogenic cell death, but the ADC-mediated tumor accumulation of a potent microtubule agent appears to activate intra-tumor dendritic cells, inducing uptake of antigens and the migration of

antigen-loaded dendritic cells to lymph nodes where they can trigger activation of T-cells which may be directed towards tumor-associated antigens [92–95]. Pre-clinically, Müller et al. have demonstrated that T-DM1 renders HER2-positive breast cancer highly susceptible to checkpoint blockade [94]. This research has stimulated interest in clinical evaluation of this potential, with ongoing clinical trials of T-DM1 in combination with pembrolizumab (anti-PD-1) and with atezolizumab (anti-PD-L1) (<http://www.clinicaltrials.gov>), the recent addition of a combination of mirvetuximab soravtansine with pembrolizumab as an arm of the FORWARD II clinical study of this maytansinoid-ADC in combination regimens [89], and with the addition of an anti-PD1-containing arm to a combination trial of GV in treatment of melanoma (NCT02302339).

CONCLUSIONS

In 2013, ado-trastuzumab emtansine (T-DM1) became the first ADC approved for the treatment of a prevalent solid tumor (HER2-positive breast cancer). This success [7], after decades of disappointment in developing immunoconjugates as therapeutic agents [96], has sparked a flurry of research and development with over 80 ADCs being taken into clinical evaluation. There are now 5 more ADCs in pivotal clinical development for treating solid tumors (Table 1). The level of target expression is a key parameter in predicting the likelihood of patient benefit for these ADCs, and the co-development of a diagnostic test to assess the level of cell target antigen is important for selecting the patient population appropriate for receiving treatment. There is clearly much yet to learn about the optimal application of ADCs in the treatment of cancers, especially in establishing the best combination modalities for treating different cancers, including the role of the emerging immune-oncology therapies. Nonetheless, the opportunity for improved cancer therapy incorporating ADCs into treatment paradigms

offer exciting possibilities for better outcomes for cancer patients.

ACKNOWLEDGEMENTS

We wish to thank Cecilia Bennett (independent editor) and Richard Bates (ImmunoGen Inc.) for skillful editing of this manuscript. No funding or sponsorship was received for writing this review, or for publication of this article. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for the manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

Disclosures. John M. Lambert is an employee of, and has equity ownership in, ImmunoGen, Inc., of Waltham, MA, USA, the developer of the maytansinoid ADC platform technology utilized in T-DM1, anetumab ravtansine and mirvetuximab soravtansine. Charles Q. Morris is a former employee of, and has equity ownership in, ImmunoGen.

Compliance with Ethics Guidelines. This article is a review based on previously conducted studies and does not involve any new studies of human or animal subjects performed by either of the authors.

Data Availability. All data discussed in this review article are drawn from published sources and cited as appropriate in the text.

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