BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12044

Drug-conjugated antibodies for the treatment of cancer

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Keywords
antibody–drug conjugates, brentuximab vedotin, lorvotuzumab mertansine, maytansine (maytansinoids), SAR3419, trastuzumab emtansine

Received
9 August 2012

Accepted
25 October 2012

Accepted Article Published Online
23 November 2012

Despite considerable effort, application of monoclonal antibody technology has had only modest success in improving treatment outcomes in patients with solid tumours. Enhancing the cancer cell-killing activity of antibodies through conjugation to highly potent cytotoxic 'payloads' to create antibody–drug conjuates (ADCs) offers a strategy for developing anti-cancer drugs of great promise. Early ADCs exhibited side-effect profiles similar to those of 'classical' chemotherapeutic agents and their performance in clinical trials in cancer patients was generally poor. However, the recent clinical development of ADCs that have highly potent tubulin-acting agents as their payloads have profoundly changed the outlook for ADC technology. Twenty-five such ADCs are in clinical development and one, brentuximab vedotin, was approved by the FDA in August, 2011, for the treatment of patients with Hodgkin's lymphoma and patients with anaplastic large cell lymphoma, based on a high rate of durable responses in single arm phase II clinical trials. More recently, a second ADC, trastuzumab emtansine, has shown excellent anti-tumour activity with the presentation of results of a 991-patient randomized phase III trial in patients with HER2-positive metastatic breast cancer. Treatment with this ADC (single agent) resulted in a significantly improved progression-free survival of 9.6 months compared with 6.4 months for lapatinib plus capecitabine in the comparator arm and significantly prolonged overall survival. Besides demonstrating excellent efficacy, these ADCs were remarkably well tolerated. Thus these, and other ADCs in development, promise to achieve the long sought goal of ADC technology, that is, of having compounds with high anti-tumour activity at doses where adverse effects are generally mild.

Introduction

Oncologists viewed monoclonal antibody technology with great optimism when the technology was first developed [1], and then applied it to the generation of antibodies that bound to a variety of tumour-associated antigens [2]. Antibodies offered the promise of targeted elimination of tumour cells without the systemic toxicity associated with chemotherapy. Rituximab, which binds to CD20 expressed by B cells and B cell lymphomas, fulfills this promise [3, 4]. It has excellent single-agent activity and has become the backbone of treatment of non-Hodgkin's lymphoma (NHL). However, over three decades of clinical research with many antibodies to many cancer cell surface targets has resulted in just two targets on solid tumours to which there are antibodies approved for therapy, namely HER2 and EGFR [5, 6]. In general, the immunological mechanisms for cell elimination induced upon antibody binding to cell surfaces have not proven effective against solid tumours without some mechanism for enhanced potency [7].

One approach to enhancing the cell-killing activity of antibodies that bind to cell surface targets on tumour cells is arming them with a cytotoxic effector agent to create compounds known as antibody–drug conjugates (ADCs). The early developments in the field of antibody-mediated delivery of cytotoxic agents to cancer cells were not successful due, in part, to the fact that the potency of the cytotoxic payloads used for the early ADCs was insufficient [8–10]. Recently, with the exciting clinical results now emerging with ADCs employing highly potent cytotoxic agents designed for antibody-mediated delivery, the promise of the ADC field has been reinvigorated [11–13]. In this review, the compounds of this burgeoning field that are in development will be summarized, and the clinical results for the most advanced ADCs (in phase II or III clinical trials), whose chemical structures are shown in Figure 1A to

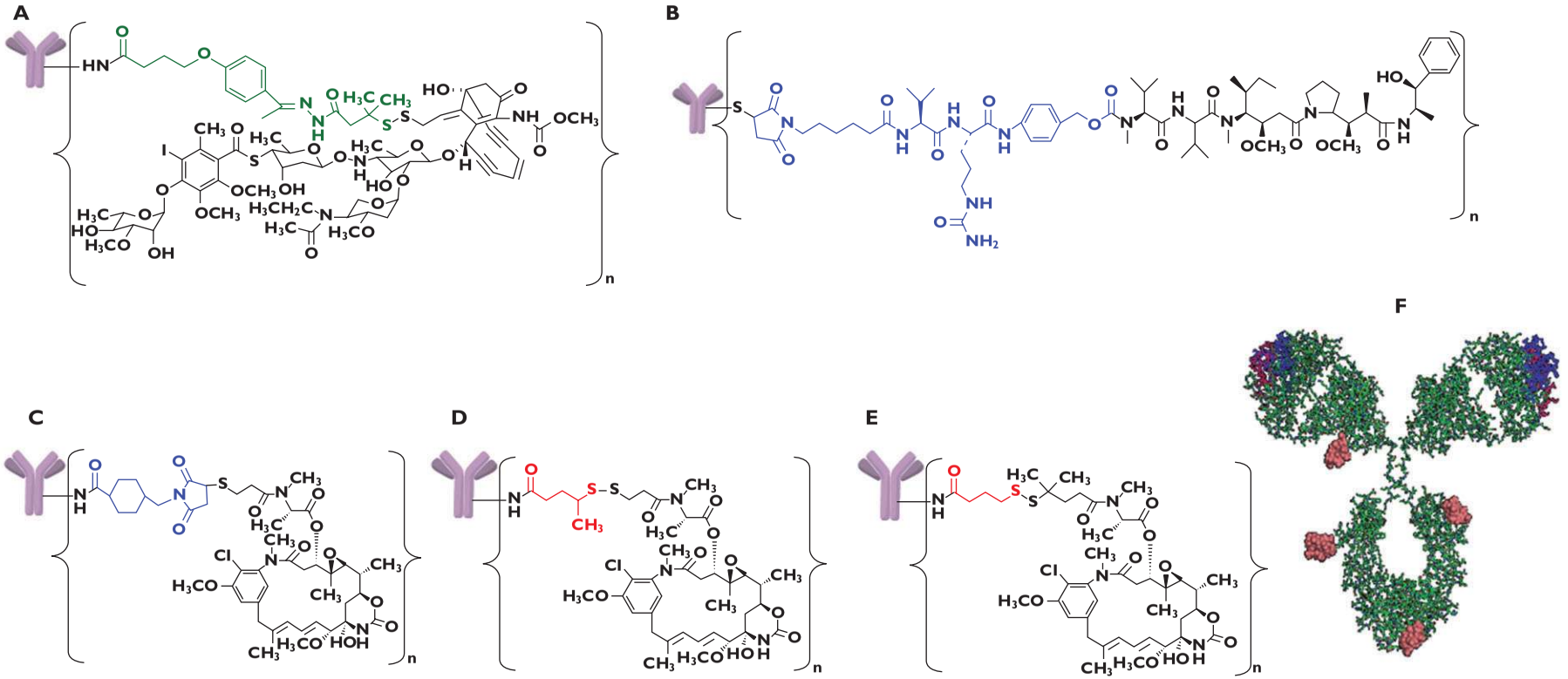

Figure 1

Chemical structures of antibody–drug conjugates. The chemical structures of ADCs described herein are represented in drawings A to E. (A) the structure of the calicheamicin-linker moiety in the conjugates gemtuzumab ozogamicin and inotuzumab ozogamicin, with the linker structure that is released from the active cytotoxic moiety shown in green; (B) the structure of the monomethyl auristatin E-linker moiety in the conjugates brentuximab vedotin and glembatumumab vedotin, with linker structure that is released from the active agent shown in blue; (C) the structure of the maytansinoid-linker moiety in trastuzumab emtansine, with the uncleavable linker structure shown in blue; (D) the structure of the maytansinoid-linker moiety in lorvotuzumab mertansine, with the cleavable linker structure that is released from the cytotoxin shown in red; (E) the structure of the maytansinoid-linker moiety in SAR3419, with the cleavable linker structure that is released from the cytotoxin shown in red; (F) model of an IgG1 antibody linked to four molecules of DM1 via SPP linkage to representative lysine amino acid residues. The residues comprising the heavy chain complementarity-determining regions (CDRs) and the light chain CDRs coloured maroon red and blue, respectively, while DM1 is represented by the salmon-coloured space-filling model of its atoms. For all structures A to E, the average load of cytotoxin molecules per antibody is in the range of 3.5 to 4.0. Conjugates that require lysosomal processing to release the active cytotoxic moiety, either by initial proteolytic cleavage of linker or by proteolytic degradation of the antibody, are shown in blue. Linkers that release active maytansinoid by intracellular reductive cleavage of a disulfide bond are shown in red. The linker moiety used to link calicheamicin is cleaved by hydrolysis at the low pH of endosomes and/or lysosomes, as well as cleavage of the disulfide bond in the intracellular reducing environment. For the chemical names of the linkers used in Figure 1, see the footnote to Table 1

E, will be described in more detail. Figure 1F also shows a model of one such conjugate bearing four molecules of maytansinoid, to illustrate the molecular scale of a 150 kDa IgG antibody conjugated to a cytotoxin-linker moiety of molecular weight about 1 kDa.

ADCs in clinical development

ADCs with potent DNA-acting payloads

The ADCs in clinical evaluation at the time of preparation of this review are listed in Table 1. Four of the 29 listed ADCs utilize DNA-targeting cytotoxic agents as their payload. Two of these compounds, gemtuzumab ozogamicin which targets CD33 expressed by cells of acute myeloid leukemia (AML), and inotuzumab ozogamicin which targets CD22 expressed on malignant B cells, are conjugates of humanized IgG4 antibodies with the highly potent DNA-alkylating agent, the enediyne antibiotic, calicheamicin [14, 15]. The payload is linked *via* an acid-labile hydrazide bond as well as a hindered disulfide bond (see Figure 1A for the chemical structure). Gemtuzumab ozogamicin was approved by the FDA in 2000 under an accelerated approval process for the treatment of relapsed AML in patients older than 60 years based on a response rate of about 30% in a single arm phase II trial in patients given 9 mg m^{-2} (about 0.24 mg kg^{-1}) for two doses given 14 days apart [16, 17]. However, a confirmatory post-approval phase III controlled trial (SWOG S0106) that was begun in 2004 of gemtuzumab ozogamicin combined with daunorubicin and cytosine arabinoside *vs.* the chemotherapy alone, was stopped early due to safety concerns coupled with no improvement in clinical benefit in the combination arm [18]. As a consequence, gemtuzumab ozogamicin was voluntarily withdrawn from the market in 2010 by the sponsor [18, 19]. Subsequent findings in three additional randomized trials suggest that some AML patients may benefit from the addition of ADC to chemotherapy [19–21], but to date, the compound remains off the market and its future development remains uncertain.

Inotuzumab ozogamicin is currently being evaluated in a phase III study in relapsed or refractory aggressive non-Hodgkin's lymphoma (NHL) in combination with rituximab, as well as a number of single agent and combination studies in NHL and acute lymphoblastic leukemia (ALL) (http://clinicaltrials.gov). A phase III study in follicular NHL was terminated due to poor enrolment. A phase I study in non-Hodgkin lymphoma established 1.8 mg m^{-2} (about 0.05 mg kg^{-1}) given every 4 weeks as the maximum tolerated dose (MTD) with thrombocytopenia, neutropenia and leucopenia the most common adverse events [15, 22]. Among the 49 patients who were treated at the MTD in this study, the objective response rate (ORR) was 41%, with rates of 68% and 15% for follicular lymphoma (22 patients) and diffuse large B cell lymphoma (DLBCL, 26 patients), respectively. At the MTD, 63.3% of patients had grade 3 or 4 thrombocytopenia and 34.7% had grade 3 or 4 neutropenia. A total of 24% of all patients in the trial discontinued treatment because of thrombocytopenia. Just one patient experience veno-occlusive disease in the phase I study [22], a toxicity of concern with gemtuzumab ozogamicin [23]. Such hepatic toxicity may be a concern when treatment with the ADC is followed by high dose chemotherapy coupled with autologous stem cell transplant [24]. Inotuzumab ozogamicin has been shown to be active in ALL, with an ORR of 57% (18% complete responses) in a 49 patient phase II trial [25].

An ADC utilizing another DNA agent as its effector moiety is MDX-1203, an anti-CD70 antibody to which is attached a prodrug form of a cytotoxic DNA minor-groove binder, an analogue of CC-1065 (rachelmycin), via a dipeptide linker [26]. This conjugate is being evaluated in a phase I trial in patients with CD70-positive renal cell cancer and CD70-positive NHL [26]. Enrolment has been discontinued (http://clinicaltrials.gov), and reporting of results is eagerly awaited. The fourth ADC in Table 1 utilizing a DNA-acting agent is the CD74-targeting milatuzumab-doxorubicin conjugate in phase I development for multiple myeloma [27].

ADCs in clinical development utilizing potent tubulin-acting agents

Serious side effects with gemtuzumab ozogamicin included severe myelosuppression [22] and veno-occlusive disease [23, 24], among others, indicating that this ADC has a side effect profile similar in nature to that of non-targeted chemotherapy regimens. However, during the last decade, the clinical development of ADCs that have highly potent tubulin-acting agents as their payloads has profoundly changed the outlook for ADC therapeutics. Twenty-five of the 29 ADCs listed in Table 1 utilize one of two classes of potent tubulin-binding antimitotic agent, either maytansinoids or auristatins [11, 13]. Both agents bind to the vinca-binding domain of tubulin and have similar cytotoxic potency in the picomolar range [28]. Auristatin conjugates are made by reducing native disulfide bonds within antibodies to generate cysteine sulfhydryl groups for subsequent reaction with maleimido derivatives of auristatins [29, 30], while maytansinoid conjugates are made by attaching thiol derivatives of maytansine to lysine amino groups of antibodies using a crosslinking reagent selected from a portfolio of such agents [11, 31]. To date, ADCs in development utilizing either of these payloads generally have an average of about 3.5 to 4.0 molecules of the cytotoxic agent linked per antibody molecule [9–11], with most antibody molecules having a cytotoxin load between two to six drugs per antibody [11, 29, 31, 32].

The ADCs made with the potent tubulin-binding agents release active payload after internalization and processing within endosomes or lysosomes [30, 33, 34]. There are two designs of auristatin-linker being utilized in

Table 1

ADCs in clinical development*

Antibody–drug conjugate	Target antigen	Linker-cytotoxic compound	Antibody	Tumour type(s)	Developer	Status
Gemtuzumab ozogamicin (Mylotarg®)	CD33 (siglec-3)	Hydrazone, AcBu N-acetyl-γ calicheamicin Calicheamicin	hP67.6 humanized IgG4	Acute myeloid leukemia	Pfizer	US FDA conditional approval 5/2000. Withdrawn 8/2011
Brentuximab vedotin (Adcetris®, SGN35)	CD30	Dipeptide, vc-MMAE	brentuximab chimeric IgG1	Relapsed/refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma	Seattle Genetics Millennium-Takeda	US FDA conditional approval 8/2011. Phase III and Phase I/II combinations
Trastuzumab emtansine (T-DM1)	HER2 (ErbB2)	Non-cleavable thioether SMCC-DM1	trastuzumab humanized IgG1	HER2-positive breast cancer	Genentech- Roche	Phase II and Phase III Biologics License Application filed 08/2012
Inotuzumab ozogamicin (CMC-544)	CD22 (siglec-2)	Hydrazone, AcBu N-acetyl-γ calicheamicin	G5/44 humanized IgG4	B-cell lymphomas	Pfizer	Phase I and Phase I/II Phase III combination
Glembatumumab vedotin (CR011-vcMMAE, CDX-011)	Glycoprotein NMB (osteoactivin)	Dipeptide, vc-MMAE	glembatumumab fully human IgG1	Metastatic breast cancer (MBC) and melanoma	Celldex Therapeutics	Phase II (MBC)
Lorvotuzumab mertansine (IMGN901, huN901-DM1, BB10901)	CD56 (NCAM)	Hindered disulfide SPP-DM1	lorvotuzumab humanized IgG1	SCLC and other CD56-positive solid tumours, multiple myeloma	ImmunoGen	Phase I/II combinations (MM, SCLC)
SAR3419 (huB4-DM4)	CD19	Highly hindered disulfide SPDB-DM4	huB4 humanized IgG1	B cell malignancies (ALL, DLBCL)	Sanofi	Phase II
SGN-75 (h1F6-mcMMAF)	CD70	Non-cleavable mc-MMAF	SGN-70 humanized IgG1	Non-Hodgkin's lymphoma and renal cell carcinoma	Seattle Genetics	Phase I
PSMA ADC	Prostate-specific membrane antigen	Dipeptide, vc-MMAE	anti-PSMA fully human IgG1	Metastatic, hormone-refractory prostate cancer	Progenics Pharmaceuticals	Phase I
BT-062	CD138 (syndecan-1)	Highly hindered disulfide SPDB-DM4	anti-CD138 chimeric IgG4	Multiple myeloma	Biotest	Phase I/II
Anti-AGS-16 ADC (AGS-16M8F)	AGS-16 (ENPP3)	Non-cleavable mc-MMAF	anti-AGS-16 fully human IgG2	Renal cell carcinoma, and liver cancer	Astellas (Agensys)	Phase I
ASG-5ME	SLC44A4 (AGS-5)	Dipeptide, vc-MMAE	anti-ASG-5 fully human IgG2	Pancreatic cancer, and prostate cancer	Astellas (Agensys) Seattle Genetics	Phase I
SAR566658	CA6	Highly hindered disulfide SPDB-DM4	DS6 humanized IgG1	CA6-positive solid tumors	Sanofi	Phase I
BAY 94–9343	Mesothelin	Highly hindered disulfide SPDB-DM4	anti-mesothelin fully human IgG1	Mesothelin-positive solid tumours	Bayer Healthcare	Phase I
IMGN529	CD37	Non-cleavable thioether SMCC-DM1	K7153A humanized IgG1	B cell malignancies	ImmunoGen	Phase 1
IMGN853	FOLR1 (folate receptor alpha)	Highly hindered disulfide Sulfo-SPDB-DM4	M9346A humanized IgG1	Ovarian cancer, NSCL adenocarcinoma	ImmunoGen	Phase 1
ASG-22ME	AGS-22 (nectin-4)	Dipeptide, vc-MMAE	Anti-Nectin fully human IgG	Solid tumours	Astellas (Agensys) Seattle Genetics	Phase I
RG7593	CD22	Dipeptide, vc-MMAE	anti-CD22 human IgG	B cell lymphoma	Genentech-Roche	Phase II
MDX-1203	CD70	Dipeptide (vc), Prodrug of duocarmycin analogue (DNA minor-groove binder and alkylator)	anti-CD70 fully human IgG	Non-Hodgkin's lymphoma and renal cell carcinoma	Bristol-Myers Squibb (Medarex)	Phase I
Milatuzumab-Doxorubicin hLL1-Dox	CD74	Uncleavable thioether SMCC-doxorubicin	IMMU-110 humanized IgG1	Multiple myeloma	Immunomedics	Phase I
RG7596	Not identified	Dipeptide, vc-MMAE	Not identified	Haematologic malignancies	Genentech-Roche	Phase II
RG7450	STEAP1	Dipeptide, vc-MMAE	Anti-STEAP-1	Prostate cancer	Genentech-Roche	Phase I
5 ADCs	Not identified	Dipeptide, vc-MMAE	not identified	Various solid and liquid cancers	Genentech-Roche	Phase I
AMG595	EGFRvIII	Non-cleavable thioether SMCC-DM1	Anti-EGFRvIII Fully human IgG1	Glioblastoma	Amgen	Phase I
1 ADCs	Not identified	Maytansinoid	not identified	Undisclosed cancers	Amgen	Phase 1

*Order of listing of ADCs approximates clinical development stage. 'DM1 and DM4 are thiol-containing maytansinoids [11]; mc, maleimidocaproyl linker; MM multiple myeloma; MMAE, monomethylauristatin E; MMAF, monomethyauristatin F; SCLC, small cell lung cancer; SMCC, 4-(*N*-Maleimidomethyl) cyclohexanecarboxylic acid *N*-hydroxysuccinimide ester; SPDB, *N*-succinimidyl 3-(2-pyridyldithio)butyrate; SPP, *N*-succinimidyl 4-(2-pyridyldithio)pentanoate; sulfo-SPDB, *N*-succinimidyl-4-(2-pyridyldithio)-2-sulfobutanoate; vc, valine-citrulline dipeptide linker.

ADCs in current development (Table 1), and both require intracellular proteolysis to release active cytotoxic metabolites within the cell, either for cleavage of the linker to release the aurisatin, or for complete degradation of the antibody to release an auristatin-linker-cysteine moiety as the active species [30, 31]. There are also two different maytansinoids being utilized with four different maytansinoid-linker designs for maytansinoid-conjugates in current development (Table 1). Intracellular release of active maytansinoid is either by complete proteolysis of the antibody moiety to yield an active maytansinoid-linker-lysine metabolite [11, 33, 34], or by reduction of a disulfide bond between the thiol derivative of maytansine and the crosslinker to release a thiol-containing maytansinoid [11, 33–35]. Selection of the linker-payload design with which to arm an antibody to a given target is empiric with an emphasis on *in vivo* assessment in xenograft tumour models to identify the design having the widest therapeutic window in such preclinical models [11, 31, 33–35]. Of the compounds listed in Table 1, the most advanced, brentuximab vedotin and trastuzumab emtansine, are attracting considerable attention for their efficacy and tolerability profile. These ADCs offer the promise of realizing the design goal of the technology, that is, of achieving highly active anti-tumour activity at doses where adverse effects are generally mild.

Brentuximab vedotin Brentuximab vedotin (BV, originally called SGN-35) is an anti-CD30 ADC made utilizing the auristatin MMAE (see Figure 1B), approximately four molecules of which are linked through a peptide linker to free sulfhydryl groups of cysteine residues of the chimeric IgG1 antibody formed by partial reduction of inter-chain disulfide bonds of the immunoglobulin [29, 30, 36]. BV was granted conditional approval under the accelerated-approval process by the FDA in August 2011 for the treatment of two indications, patients with Hodgkin lymphoma (HL) after failure of autologous stem cell transplant or those ineligible for transplant who have failed at least two chemotherapy regimens, and patients with systemic anaplastic large cell lymphoma (ALCL) after failure of multi-agent chemotherapy. Approval for HL was based on a single arm phase II trial in which 102 patients received BV at 1.8 mg kg^{-1} every 3 weeks for up to 16 cycles [37]. Remarkably, tumour reductions were seen in 94% of enrolled patients on this trial. The ORR was 75% with a median progression-free survival for all patients of 5.6 months. Thirty-four percent of patients achieved complete remission (CR) having a median duration of 20.5 months [37]. Approval for ALCL was based on another single arm phase II trial in which 58 patients were also treated at 1.8 mg kg^{-1} every 3 weeks [38]. The ORR was 86% with a median duration of 12.6 months, while 57% of patients achieved a CR with a median duration of 13.2 months [38]. The most common adverse reactions of any grade noted in these trials were peripheral sensory neuropathy (42% of patients), nausea (35%), fatigue (34%), neutropenia (19%) and diarrhoea (18%), seen in $\geq$15% of patients [37]. The neuropathy was consistent with a class effect of tubulin-binding cytotoxic agents, and typically developed after prolonged exposure to BV, with a median onset of grade 2 neuropathy at 27.3 weeks. It was largely reversible and could be managed by dose delays and/or dose reduction [37].

Besides these adverse events, the dose-limiting toxicities (DLTs) observed in the initial phase I clinical evaluation of BV at doses of 2.7 mg kg^{-1} and 3.6 mg kg^{-1} every 3 weeks were febrile neutropenia, thrombocytopenia and hyperglycaemia [39]. A more frequent dosing regimen (weekly) was also evaluated in a phase I study. The results of this trial demonstrated a similar level of activity, with a similar adverse event profile [40]. In these phase I studies, the half-life of intact conjugate was estimated at 4 to 6 days.

Several clinical trials are under way or planned to evaluate further the activity and safety of BV. In particular, a confirmatory phase III trial in HL patients following autologous stem cell transplant is ongoing. In addition, several phase I and phase II trials are under way evaluating BV in combination with chemotherapy for front-line treatment of HL, and as a single agent or as part of a combination regimen for treating HL, and other CD30-positive malignancies [41], in a variety of disease and treatment settings (http://clinicaltrials.gov, and sponsor websites).

Trastuzumab emtansine Trastuzumab emtansine (T-DM1, also called trastuzumab-DM1) combines the humanized IgG1 anti-HER2 antibody, trastuzumab, with the maytansinoid DM1 utilizing an uncleavable cross-linking reagent, SMCC, to couple the thiol of DM1 (about 3.5 DM1 molecules per antibody) to surface amino groups of lysine residues of the antibody via a thioether bond (Figure 1C) [42]. The cytotoxic metabolite released within cancer cells is the DM1-linker-lysine moiety [43, 44]. The particular conjugate design was selected for clinical development based on potent anti-proliferative activity *in vitro* and anti-tumour activity *in vivo* in preclinical models including HER2-overexpressing models resistant to trastuzumab [42]. A phase I clinical trial in patients with HER2-positive metastatic breast cancer (MBC) that had progressed upon treatment with trastuzumab (mean of 24 months on trastuzumab before treatment failure) established an MTD of 3.6 mg kg^{-1}, with the DLT at 4.8 mg kg^{-1} being reversible thrombocytopenia, when T-DM1 was administered once every 3 weeks [45]. Of 15 patients treated at 3.6 mg kg^{-1}, the clinical benefit rate (CBR) was 73% (objective responses plus stable disease of $\geq$6 months), and the confirmed ORR in patients with measurable disease ($n = 9$) was 44%, an exciting signal of activity in a phase I trial. The median duration of treatment for these 15 patients was 238 days [45]. An MTD of 2.4 mg kg^{-1} was established when weekly administration was assessed, equating to double the dose intensity of

every 3 week dosing, and a similar level of activity was observed in this small study [46].

A proof of concept phase II trial was then conducted evaluating T-DM1, used alone, administered at 3.6 mg kg^{-1} every 3 weeks in 112 patients with HER2-positive MBC [47]. All patients had received prior trastuzumab therapy, median duration of 17.6 months (range 1–152 months), as one of their prior systemic anticancer treatments (median of five prior agents for treatment of their MBC). According to independent review, this trial demonstrated an ORR of 25.9%, while 49.1% of patients were assessed as having stable disease for a 75% CBR [47]. T-DM1 was well tolerated, with no dose-limiting cardiotoxicity and 21 patients completed at least 1 year of treatment on study. The most common adverse events were fatigue, nausea and headache (grade 1 or 2). Grade 3 and 4 toxicities were infrequent, the most common being hypokalaemia (8.9%), thrombocytopenia (8.0%) and fatigue (4.5%). These findings were confirmed and extended in a second phase II trial that enrolled 110 patients with HER2-positive MBC who all had previously received a taxane, an anthracycline, capecitabine, lapatinib and trastuzumab, and who had progressed on their last regimen [48]. Importantly, the prior therapies for MBC included two HER2-directed regimens (lapatinib for a median duration of treatment of 6.9 months and trastuzumab for a median duration of treatment of 19.4 months). In these heavily pre-treated patients, the confirmed ORR was 33% with a CBR of 44%, as assessed by independent review, with a median progression-free survival (PFS) of 6.9 months [48]. Among patients with retrospective central confirmation of HER2-positive status ($n = 77$, about 83.5% of those tested), the ORR rose to 40% and the median PFS to 8.0 months. There were no new safety signals, with the most common adverse events being fatigue (62%), nausea (37%) and thrombocytopenia (33%), mostly grade 1 or 2. Indeed, the authors noted that the absence of significant toxicity was noteworthy given the extensive pretreatment of the patients [48].

The combined data from 288 patients who received 3.6 mg kg^{-1} of T-DM1 every 3 weeks in the phase I and II trials were used to determine the pharmacokinetic parameters of T-DM1 [49]. Clearance is best described by a two compartment linear model with a half-life of approximately 4 days and a volume of distribution limited to the plasma volume. In the phase I dose escalation study, the half-life was dose-dependent with faster clearance at or below 1.2 mg kg^{-1}, suggesting that antigen-mediated disposition plays a significant role in T-DM1 clearance [45]. This is consistent with recent data generated utilizing immuno-PET imaging with 89Zr-trastuzumab [50]. The concentration of non-conjugated DM1 was very low, with its maximum concentration having an average value of only about 5 ng ml^{-1}, compared with an average C_{max} for T-DM1 of about 75 to 80 μg ml^{-1} [49], equivalent to about 1.5 μg ml^{-1} of conjugated DM1 [42]. Even this very low amount of free DM1 (about 0.3% of total bound DM1) may have been the consequence of cleavage during *ex vivo* sample handling [51]. The immunogenicity of T-DM1 in these studies was reported to be low, with just 13 of 286 patients (4.5%) testing positive for anti-product antibodies which had no impact on T-DM1 pharmacokinetics [49].

The results of a randomized phase II trial in HER2-positive breast cancer patients previously untreated for metastatic disease show that treatment with T-DM1 provided a significant improvement in PFS, from 9.2 months with standard-of-care trastuzumab plus docetaxel ($n = 70$ patients), to 14.2 months with T-DM1 ($n = 67$ patients), with a similar ORR in the two arms, 58% for trastuzumab plus docetaxel compared with 64% in the T-DM1 arm [52]. The evidence for impressive single agent activity for T-DM1 was coupled with a favourable safety profile in this first line patient population. Adverse events that were ≥grade 3 were reported at about half the rate in the T-DM1 arm (46.4% *vs.* 89.4%), with no new safety signals, and no clinically significant cardiac events [52, 53].

T-DM1 is being evaluated for treatment of MBC in three randomized phase III trials (http://clinicaltrials.gov, and sponsor web sites), in first line treatment of MBC (trial name MARIANNE), after treatment with trastuzumab and a taxane in any setting (trial name EMILIA) and as third line treatment (trial name TH3RESA). The sponsor filed a marketing application with the FDA in August, 2012, based on the results of the 991-patient EMILIA trial, initial data for which were presented at the 2012 annual meeting of the American Society of Clinical Oncology [54]. Treatment with T-DM1 significantly prolonged PFS compared with treatment with lapatinib plus capecitabine in the comparator arm, 9.6 months *vs.* 6.4 months, respectively. Median overall survival was 30.9 months in patients treated on the T-DM1 arm, significantly longer than the 25.1 months achieved by the comparator arm [54]. The ORR was higher with single agent T-DM1 at 43.6% *vs.* 30.8% in the comparator arm. Not only did the efficacy end points favour the T-DM1 arm, fewer T-DM1-treated patients experienced grade 3 or higher adverse events than those treated on the comparator arm (40.8% *vs.* 57%, respectively) and there were no new safety signals in the study [54]. The robust anti-tumour activity and excellent tolerability exhibited by T-DM1 in the phase I, II and III trials suggest that this exciting new agent may change the treatment paradigm for HER2-positive MBC, as well as other HER2-positive cancers [55, 56]. Besides development in breast cancer, a phase II/III clinical trial was recently begun to evaluate the potential for T-DM1 to improve treatment outcomes in patients with HER2-positive gastric cancer. (http://clinicaltrials.gov).

Glembatumumab vedotin Glembatumumab vedotin (GV, CDX-011, previously CR011-vcMMAE) combines an anti-GPNMB fully human IgG2 antibody with the peptide-linked auristatin, vcMMAE [57], the same linker-payload format as used in BV (see Figure 1B). The target, GPNMB, also known as osteoactivin, is a type 1 transmembrane

glycoprotein that is strongly expressed in melanoma and breast cancer [57, 58]. GV is highly active in preclinical xenograft models of melanoma [57]. Two phase I/II trials were conducted in patients with advanced metastatic cancers, one in patients with melanoma [59], and the second in patients with breast cancer [60]. In both studies, the MTD was 1.88 mg kg^{-1} administered once every 3 weeks.

In the phase I/II study conducted in patients with melanoma [59], a total of 117 patients were treated with a variety of doses and schedules. Antitumour activity was reported, with five objective responses (14%) noted in the phase II expansion cohort in patients treated at the MTD on the 3 week schedule ($n = 36$ patients). Treatment-related severe adverse events (grade 3 or 4) occurring in >10% of patients treated at 1.88 mg kg^{-1} every 3 weeks (a total of 43 patients) were rash (26% of patients) and neutropenia (14% of patients). The most frequent treatment-related adverse events of any grade at this dose schedule included rash (70%) fatigue (65%), alopecia (63%), pruritis (63%), diarrhoea (47%) and neuropathy (35%). There was evidence of increased activity with more frequent dosing (weekly or 2 weekly doses every 3 weeks), but this was accompanied by increased toxicity, notably the incidence of grade 3 or 4 neuropathy increased from 5% at the MTD on the every 3 week schedule ($n = 43$ patients) to 27% at the MTD (1 mg kg^{-1}) of weekly dosing ($n = 15$ patients), which led to the selection of 1.88 mg kg^{-1} every 3 weeks dose schedule for the phase II expansion [59]. Dose-dependent pharmacokinetics were observed, the half-life of the conjugate increasing with increasing dose on the once every 3 week schedule, from 16 h up to 38 h during dose escalation, providing evidence of a saturable target antigen-mediated disposition [59].

The finding that GPNMB was expressed on the tumour and/or stroma of > 40% of breast cancer samples, and that high expression in triple negative breast cancers was associated with increased risk of recurrence of disease [58], provided the rationale for the second phase I/II study conducted in 42 breast cancer patients [60]. Only two patients (6%) had confirmed partial responses from 34 patients who received the MTD dose of 1.88 mg kg^{-1} every 3 weeks, although patients were not pre-selected for GPNMB expression in this study. Immunohistochemical analysis done on tumour samples obtained during the study showed significant GPNMB expression on stroma and/or tumour cells in nine of 14 cases examined (64%). The two patients with a partial response (PR) were both GPNMB-positive, and continued on treatment for 27 and 54+ weeks [60]. The most frequent treatment-related adverse events of any grade across all doses, 1.0 mg kg^{-1} ($n = 3$), 1.34 mg kg^{-1} ($n = 5$) and 1.88 mg kg^{-1} ($n = 34$), included fatigue (50%), rash (48%), nausea (40%), neuropathy (38%), alopecia (33%), neutropenia (31%) and vomiting (31%). Of these, the only severe adverse events (grade 3 or 4) occurring in >10% of patients were neutropenia (21% of patients). On the basis of these findings, a randomized phase II trial in patients with advanced, GPNMB-expressing, heavily pre-treated breast cancer is ongoing (http://clinicaltrials.gov), comparing treatment with single agent GV *vs.* single agent chemotherapy (investigators' choice).

Lorvotuzumab mertansine Lorvotuzumab mertansine (LM, also known as IMGN901, and previously as BB-10901) comprises a humanized IgG1 version of the N901 antibody, lorvotuzumab [61, 62], conjugated at lysine residues to an average of about 3.7 DM1 molecules per antibody molecule *via* the SPP cross-linker that forms a hindered disulfide bond with the DM1 (Figure 1D) [32]. The antibody targets the CD56 antigen, also called neuronal cell adhesion molecule, or NCAM [61, 63], which is expressed on a variety of cancers of haematopoietic and neuroendocrine origin, including multiple myeloma (MM) and certain leukemias and lymphomas [64, 65], small cell lung cancer (SCLC) [66], ovarian cancer [67], carcinoid tumours and neuroblastoma [66]. LM has exhibited potent anti-tumour activity in a variety of preclinical xenograft models in these disease indications [64, 67, 68]. LM is being studied in both solid and haematopoietic tumours in clinical trials.

A phase I trial in CD56-positive solid tumours established 75 mg m^{-2} (~2.0 mg kg^{-1}) as the MTD, when LM was administered daily for 3 consecutive days every 3 weeks, and established 60 mg m^{-2} as the recommended phase II dose on this schedule [69, 70]. In MM, where 70–80% of patients have disease expressing CD56 [64], a phase I dose escalation trial established 112 mg m^{-2} (~3.0 mg kg^{-1}) as the MTD when LM was administered weekly for 2 consecutive weeks on a 3 week cycle [71]. The half-life of LM was only about 1 to 1.5 days at doses ≥60 mg m^{-2} across all the phase I studies. This relatively short half-life is likely due to natural killer cells which express CD56 [61] serving as an antigen sink [72]. The DLTs in patients dosed at 140 mg m^{-2} in the MM trial were grade 3 fatigue in two of six patients and grade 3 acute renal failure in one of these patients [71]. Grade 3 toxicities of myalgia (one patient) and headache and back and shoulder pain (one patient) in two of two patients dosed at 94 mg m^{-2} given daily for 3 days on a 3 week cycle were the DLTs in the solid tumour trial [69, 70]. In the first clinical trial conducted with LM, assessing the compound given weekly for 4 weeks on a 6 week cycle in CD56-positive solid tumours, found dose-limiting headache at doses ≥60 mg m^{-2}, having onset within about 8 h and largely resolved by about 48 h [72, 73]. Dose-limiting headache was not seen in later studies once routine low dose steroid prophylaxis was utilized prior to treatment [69–71]. The most common side effects were grade 1 or grade 2 headache, fatigue, nausea and neuropathy, each seen in about 30% of patients across all the phase I studies (193 total patients reported). The incidence of grade 3 peripheral neuropathy across the above three studies was only 2.5%, with no reported grade 4 events [69–74]. There were no clinically significant changes in haematologic

parameters with no evidence for clinically significant myelosuppression.

Encouraging evidence for antitumour activity was reported in the above three clinical studies of single agent LM. In the 37 patients with MM who were treated with LM at doses ranging from 40 mg m^{-2} to 140 mg m^{-2} (25 patients treated at ≥112 mg m^{-2}), the overall CBR was 41%, with three objective PRs, three objective minimal responses and 15 patients with stable disease for ≥3 months [71, 75]. In the two trials conducted in patients with CD56-positive solid tumours, there were two PRs (one unconfirmed) and 15 cases of clinically meaningful stable disease noted from the 68 patients with SCLC included in the 113 patients evaluated at the time of data cut-off of the most recent report [69, 70, 73, 74]. From among 12 patients with Merkel cell carcinoma (MCC) from among the 45 evaluable patients on the trial evaluating the daily times three dosing schedule [70], there were two durable complete responses (2+ years), and three patients with clinically meaningful stable disease (4 to 7+ cycles of treatment), notable observations in this rare, aggressive small cell cancer of the skin [76]. The findings of activity in MCC support the observations of activity in SCLC since these aggressive cancers are similar in cell morphology, in their near uniform expression of CD56, and in the dismal outcome of their clinical course [70, 74, 76, 77].

Based on these promising signals of clinical activity in these difficult to treat cancers, and on the preclinical results reporting improved antitumour activity of LM in combination with chemotherapeutic regimens [68, 78–80], and coupled with the acceptable tolerability profile of LM, in particular the lack of clinically meaningful myelosuppression [69–75], clinical studies of LM in combination with lenalidomide and low dose dexamethasone in MM [75, 81], and in combination with carboplatin and etoposide in SCLC (http://clinicaltrials.gov), have been initiated. The early experience reported with the LM plus lenalidomide/dexamethasone combination in a single arm study demonstrated encouraging activity for this regimen [75, 81]. LM is being assessed in combination with carboplatin/etoposide for first line SCLC (extensive-stage disease) in a phase II trial which started in March 2012 (http://clinicaltrials.gov), using an LM dose of 112 mg m^{-2} (two weekly doses on a 3 week cycle) established in an initial dose escalation phase [82]. Of 33 patients dosed in the phase I portion of the study, 13 were SCLC patients and six of these (46.2%) had an objective response, including two of seven patients who were platinum resistant/refractory [82]. In the phase II assessment, previously untreated SCLC patients will be randomized 2:1 to receive either (i) up to six cycles of carboplatin/etoposide plus LM followed by maintenance LM or (ii) up to six cycles of the standard-of-care chemotherapy doublet only.

SAR3419 (huB4-DM4) SAR3419 is an ADC comprising a humanized monoclonal IgG1 antibody (huB4) attached to 3.5 to 4 molecules of the maytansinoid DM4 through reaction of an optimized cleavable linker with lysine amino groups of the antibody (Figure 1), to form a hindered disulfide bond between the SPDB linker and DM4 [83–85]. SAR3419 shows superior anti-tumour activity to rituximab in preclinical xenograft models for NHL [86, 87] and is now in phase II clinical evaluation.

The preliminary results from the first phase I trial of single dose administration every 3 weeks for up to six cycles found the MTD to be 160 mg m^{-2} (~4.3 mg kg^{-1}), a dose level subsequently used to treat an expanded cohort of 20 patients [88, 89]. Of the 35 response-evaluable patients at the time of initial reporting of the study, tumour shrinkage was reported in more than half the patients (74%), with six objective responses [88]. Notably, seven of 15 patients with rituximab refractory disease showed tumour shrinkage, with one objective response. Tumour shrinkage was seen in a variety of lymphoma subtypes including DLBCL, follicular lymphoma and marginal zone lymphoma [89]. The DLT at doses >200 mg m^{-2} was reversible toxicity to the cornea that did not preclude continued dosing with dose delays of 1–2 weeks at 208 mg m^{-2}, with no other clinically significant grade 3 or 4 toxicities reported [88, 89]. As with other maytansinoid ADCs, there was no clinically significant myelosuppression ($n = 38$ patients), suggesting that SAR3419 may be readily combined with conventional chemotherapy regimens. The half-life of SAR3419 in these patients appeared to be 4–6 days across all doses of the phase I trial [89]. These early results demonstrated promising activity and tolerability, especially considering the wide dose range (10 mg m^{-2}–270 mg m^{-2}), the heavy pre-treatment of these patients (24% had prior stem cell transplant), and the mixed histology of those enrolled in the phase I trial.

A second study with a schedule of weekly dosing for 8 weeks, with the possibility of a further four cycles if sought, established an MTD of 55 mg m^{-2} (about 1.5 mg kg^{-1} $week^{-1}$) [90]. The dose intensity reached was similar to that of the 3 week dosing schedule. A total of 44 patients received doses ranging from 10 mg m^{-2} to 70 mg m^{-2} per week, with 21 patients treated at the MTD. Of 38 patients receiving doses of ≥20 mg m^{-2} per week, 12 (32%) achieved an objective response including six with CR/CR unconfirmed. Responses were seen in a variety of lymphoma sub-types (follicular lymphoma, DLBCL and Mantle cell lymphoma). SAR3419 was well tolerated on this dosing schedule, with a median number of doses delivered per patient of eight overall, and a median relative dose intensity of 0.96 at the MTD, with the investigators commenting on the noteworthy lack of clinically significant myelosuppression [90]. While reversible ocular toxicity was noted on this dosing regimen, it was with a late onset (mainly post cycle 7 or 8) and the incidence and severity of the observations was markedly reduced relative to the 3 week schedule [88, 90]. The half-life for the plasma clearance of SAR3419 after the last administered dose was about 8 days, consistent with

observations of plasma accumulation of SAR3419 on the weekly schedule [90, 91]. A second cohort of patients ($n = 25$) was enrolled, utilizing a modified schedule consisting of four weekly doses of 55 mg m^{-2} followed by four bi-weekly doses based on pharmacokinetic simulations and pharmacodynamic observations, to evaluate an approach to reduce even further the incidence of the reversible corneal toxicity [90, 91]. Preliminary reports suggest this approach was successful. The signals of clinical efficacy were maintained with a 28% ORR (seven of 25 patients), 33% in heavily pre-treated DLBCL patients (three of nine patients on the study), yet only one reversible grade 1 corneal event was noted in the 25 patients [91]. The sponsor has initiated three phase II trials since September 2011, one in ALL and two in DLBCL. One of the latter studies is a combination trial with rituximab (http://clinicaltrials.gov).

ADCs in early Phase I clinical development utilizing potent tubulin-acting agents Besides the two compounds described above, there are thirteen other auristatin conjugates that are in early phase I clinical trials (Table 1). SGN-75, a conjugate of a humanized anti-CD70 monoclonal antibody with mcMMAF is currently in a phase I clinical trial for relapsed or refractory non-Hodgkin lymphoma or metastatic renal cell carcinoma [92, 93]. The linker is uncleavable and the cytotoxic metabolite released within cancer cells is the cytotoxin-linker-cysteine moiety, the cysteine being the amino acid residue of the antibody to which the auristatin was attached [13, 29, 92]. Preliminary efficacy signals reported include a PR in a Mantle cell lymphoma patient among 16 NHL patients on study, and two PRs out of 21 evaluable renal cell cancer patients on study (86, and sponsor web site), an encouraging signal of activity early in a phase I study. The MTD was 3 mg kg^{-1} given every 3 weeks and the terminal half-life was estimated to be 6 to 10 days. Adverse events $\geq$grade 3 that were seen in >2 patients included thrombocytopenia, dyspnoea and fatigue, while the most common adverse events (any grade) were fatigue (23%), nausea (30%), dry eye (23%) and thrombocytopenia (23%) among the 41 patients treated [93]. A conjugate of a fully human anti-prostate specific membrane antigen (PSMA) monoclonal antibody with MMAE [94] is in a phase I trial in patients with taxane refractory, metastatic, castration resistant prostate cancer [95]. A preliminary report about the phase I trial described cohorts of patients (40 total subjects) who received doses of 0.4 mg kg^{-1} to 2.8 mg kg^{-1} [95]. While the MTD was not reached, and dose escalation continues, there were early suggestions of antitumour activity as reflected in declines in serum prostate specific antigen levels. Preliminary phase I clinical results were also reported for two other auristatin-ADCs, AGS-16M8F, an antiENPP3 antibody conjugated with mcMMAF [96] and ASG-5ME, and anti-SLC44A4 antibody conjugated with vcMMAE [97].

There are also six more ADCs in clinical evaluation utilizing the maytansinoid 'payload', besides the three compounds in phase II and III trials described above (Table 1). BT-062 is an anti-CD138 chimeric IgG4 antibody conjugated to the maytansinoid DM4 through reaction with the cleavable SPDB linker to form a highly hindered disulfide bond with the DM4 [98, 99]. A phase I trial resulted in determining an MTD of 160 mg m^{-2} (~4.3 mg kg^{-1}) when dosing once every 3 weeks in MM patients [100]. Among 13 evaluable patients treated at the MTD from among 32 total patients in the trial, there was one PR, one objective minor response of at least 1.5 years duration and five patients with stable disease for at least 105 days, for an overall CBR of about 50%. Adverse event signals relating to the expression of CD138 on epithelial tissues were noted, especially at the maximum administered dose of 200 mg m^{-2}, and included mucositis, stomatitis and hand/foot syndrome. Adverse events at the MTD were generally mild to moderate and the safety profile was considered favourable [100, 101]. A phase I/IIa study was recently initiated to characterize further tolerability and anti-MM activity in a more frequent dosing regimen, dosing weekly for 3 weeks on a 4 week cycle (http://clinicaltrials.gov). Thus far, BT062 is well tolerated up to 120 mg m^{-2} on this schedule [101]. Other maytansinoid ADCs that are in early phase I clinical evaluation, and which utilize the SPDB linker for conjugation of DM4, include SAR566658, a conjugate with the anti-CA6 antigen, a tumour-associated glycotope of Muc-1 that is expressed on ovarian, breast, cervical, lung and pancreatic cancers [102], and BAY 94–9343, a conjugate of an anti-mesothelin antibody that targets mesothelioma, ovarian and pancreatic cancers [103]. In early 2012, phase I evaluation of IMGN529, an anti-CD37 humanized IgG1 antibody conjugated to DM1 via the uncleavable SMCC linker [104, 105], was initiated in patients with NHL, and recently, a phase I trial was begun with IMGN853, an anti-FOLR1 humanized IgG1 antibody conjugated to DM4 using a novel hydrophilic disulfide linker [106, 107].

In the next 12–24 months, with many ADCs in early phase I clinical trials (see Table 1), there will likely be a wealth of new clinical results which will provide valuable insights into the design of the next generation of ADCs. For example, the field may learn what targets make good targets for ADCs made with potent tubulin-acting agents, and understand what are the best linker-payload designs for an optimal ADC directed to certain targets or for certain indications. However, some general safety trends may already be noted from the clinical development so far reported. ADCs to two unrelated targets made with vcMMAE show neutropenia as a DLT at doses near 2 mg kg^{-1} given once every 3 weeks [30, 59], while maytansinoid ADCs made with a variety of linker formats generally show little or no clinically significant myelosuppression [11]. ADCs made with uncleavable links with both the auristatin and maytansinoid payloads, where the active metabolite is a payload-linker-amino acid moiety [31, 33], may show thrombocytopenia as a side effect which may

be dose-limiting [45, 93, 96, 108, 109]. This side effect has not been clinically significant with ADC designs that release final active metabolites that lack hydrophilic charges groups that may be more readily effluxed from cells. One can expect that as more clinical information for more of the compounds listed in Table 1 becomes available, oncologists will learn how best to design and develop ADCs to maximize the impact of these agents in treating disease.

Conclusions

With the recent approval in 2011 of brentuximab vedotin (Adcetris®), and the extensive phase III programme being undertaken to develop trastuzumab emtansine which led to an application for marketing approval by the sponsor in August, 2012, it has become apparent that ADC technologies utilizing highly potent tubulin-acting agents are able to generate highly active, well-tolerated, anticancer agents that fulfill the long-awaited promise of the field. Active compounds can be generated against targets expressed on haematologic tumours (for example, brentuximab vedotin and SAR3419) as well as against targets expressed on solid tumours (for example, trastuzumab emtansine, lorvotuzumab mertansine), and they can be generated from antibodies such as trastuzumab that have some intrinsic antitumour activity, as well as from antibodies such as brentuximab and lorvotuzumab that have demonstrated no preclinical or clinical activity as 'naked' antibodies. The clinical experience gained over the last decade also suggests that these ADCs, conjugates of cytotoxic payloads to human/humanized antibodies, are generally not more immunogenic than 'naked' human/humanized antibodies in cancer patients.

Of the ADCs that target a solid tumour, trastuzumab emtansine has been the compound that has advanced most rapidly, from first-in-human dosing to phase III clinical trials in about 4 years [9, 54]. Its development was greatly aided by the fact that so much was already known about the HER2 target, in particular with regard to patient selection, thanks to the prior development of trastuzumab itself. For other ADCs to novel targets, such knowledge will need to be developed during clinical trials, as for example, in the evaluation of antitumour activity of glembatumumab vedotin in breast cancer patients confirmed to express GPNMB on their tumour [60]. Development of a companion diagnostic test similar in nature to the immunohistochemical test for HER2 on breast cancer biopsies is likely essential for development of an ADC to a heterogeneous target such as GPNMB.

It is exciting to report that, after nearly 30 years of research, the emerging clinical data with several compounds suggest that ADCs promise to make a real difference to the lives of patients with cancer. As more compounds advance, one can envisage a future where patients are treated with active anti-tumour agents, among them ADCs, that lack the severe toxicities associated with chemotherapy.

Competing Interests

The author has completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declares JML had support from ImmunoGen, Inc., the company that developed the maytansinoid-ADC technology, for the submitted work, written as part of his duties as an employee of ImmunoGen, Inc., JML has been an employee of ImmunoGen, Inc. since 1987 to present and several compounds utilizing ImmunoGen's technology are described in this manuscript and JML has no other relationships or activities that could appear to have influenced the submitted work.

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