



ELSEVIER

# Antibody–drug conjugates: targeted drug delivery for cancer

Stephen C Alley, Nicole M Okeley and Peter D Senter

The antibody–drug conjugate field has made significant progress recently owing to careful optimization of several parameters, including mAb specificity, drug potency, linker technology, and the stoichiometry and placement of conjugated drugs. The underlying reason for this has been obtained in pre-clinical biodistribution and pharmacokinetics studies showing that targeted delivery leads to high intratumoral free drug concentrations, while non-target tissues are largely spared from chemotherapeutic exposure. Recent developments in the field have led to an increase in the number of ADCs being tested clinically, with 3 in late stage clinical trials: brentuximab vedotin (also referred to as SGN-35) for Hodgkin lymphoma; Trastuzumab-DM1 for breast cancer; and Inotuzumab ozogamicin for non-Hodgkin lymphoma. This review highlights the recent pre-clinical and clinical advances that have been made.

## Address

Seattle Genetics, Inc., 21823 30th Drive SE, Bothell, WA 98021, United States

Corresponding author: Alley, Stephen C ([SAAlley@seagen.com](mailto:SAAlley@seagen.com))

Current Opinion in Chemical Biology 2010, 14:529–537

This review comes from a themed issue on  
Next Generation Therapeutics  
Edited by Michael Gelb and Adrian Whitty

Available online 17th July 2010

1367-5931/\$ – see front matter

© 2010 Elsevier Ltd. All rights reserved.

DOI [10.1016/j.cbpa.2010.06.170](https://doi.org/10.1016/j.cbpa.2010.06.170)

More than 20 monoclonal antibodies (mAbs) have been approved for use in many indications, including cancer [1]. There are multiple mechanisms by which mAbs can exert therapeutic efficacy, but often their activity is not sufficient to produce a lasting benefit. Hence, several strategies have been employed to enhance their activity. Antibody–drug conjugates (ADCs) represent one approach, where the ability to harness mAb specificity and target the delivery of a cytotoxic agent to the tumor may significantly enhance both mAb and drug activities. Conjugation also has an effect on the biodistribution of the drug, sparing normal tissue exposure to the cytotoxic agent and allowing the use of potent agents that would prove too toxic for systemic use. Optimization of a variety of ADC parameters has recently met with considerable success [2–5,6<sup>\*</sup>], and 3 ADCs are now in late-stage clinical trials with about 10 others in earlier stage trials.

## Development of optimized ADCs

### Optimization parameters

Extensive optimization of several ADC parameters has taken place over multiple decades, and clinical development of ADCs has required attention to these details. Table 1 lists these optimization parameters and different solutions for producing pharmacologically active ADCs. There are 3 key components of an ADC: the mAb, the drug, and the linker. Ideally, the mAb will specifically bind to an antigen with substantial expression on tumor cells but limited expression on normal tissues. Specificity allows the utilization of drugs that otherwise would be too toxic for clinical application. Thus, most of the recent work in this field has centered on the use of highly potent cytotoxic agents. This requires the development of linker technologies that provide conditional stability, so that drug release occurs after tumor binding, rather than in circulation. Finally, the method of ADC conjugation, which determines the drug loading stoichiometry and homogeneity, has been shown to play roles not only in pharmacokinetics, but also in activity, potency, and tolerability.

### Antigen binding and drug release

Many different antigens have been evaluated for ADCs [2,4,5,6<sup>\*</sup>]. The vast majority undergo rapid internalization once the ADC binds through a process known as receptor mediated endocytosis, in which antigens localized to clathrin-coated pits or lipid rafts are internalized along with bound antibody [5]. For some antigens, unconjugated mAb and ADC have been observed to internalize with the same rate [7], while for other antigens ADC has been observed to internalize much more efficiently than unconjugated mAb [8], suggesting that internalization processes are variable across antigens and depend on multiple parameters. Once internalized, the ADC is delivered to lysosomes (although there are some exceptions [9]) where effective drug release takes advantage of the catabolic environment found within these organelles. Following release from the lysosome, the drug either binds to its pharmacological target or leaves the cell via active or passive processes [2]. ADCs have demonstrated intracellular accumulation of released drug in antigen positive cells [10,11] but no intracellular accumulation in antigen negative cells [11], highlighting the antigen specificity of drug delivery by ADCs. There have also been some reports that poorly internalized antigens can successfully be targeted with ADCs potentially utilizing drug release strategies that occur in the tumor microenvironment rather than inside cells [12,13]. The requirements for antigen internalization to yield active ADCs is an area of active investigation, and the results may be

Table 1

Key ADC parameters			
Parameter	Description	Leading examples	References
Antigen	Substantial expression on tumor, limited expression on normal tissues	Her2, CD30, CD33	[2,4,5,6*]
Linker	High stability to avoid systemic release	Dipeptides, direct linkage, some hydrazones and disulfides	[17–19]
Drug	High potency since delivery is limited by antigen copy number	Auristatins, maytansines, calicheamicin	[3,14,15]
Drug loading level	Maintain mAb PK by limiting drug loading	2–4 drugs per mAb often optimal	[24]
Conjugation site	Homogeneous drug loading by site-specific conjugation to avoid subpopulations with altered PK	Chemical methods, cysteine point mutants	[21–23]

dependent not only on the linker technology used for drug attachment, but also on the fate of the drug once it is released from the mAb carrier.

### Drugs and linkers

Early ADCs relied on drugs that were already approved for use as small molecules, such as vinblastine and doxorubicin, and suffered from lack of potency and low clinical activities [4,5]. Substantially more potent drugs that were too toxic to use in an untargeted manner have been more promising as ADCs. These include auristatins (Figure 1), maytansines (Figure 2), and calicheamicins (Figure 3). Auristatins and maytansines both exert their cytotoxic effects by binding to tubulin, causing G2/M cell cycle arrest, and subsequently leading to apoptosis. Monomethylauristatin E, conjugated through a protease-cleavable dipeptide linker (vcMMAE, **1**) [14], and monomethylauristatin F, conjugated directly to mAbs through maleimidocaproic acid (mcMMAF, **3**) [8], are synthetic analogs of dolastatin 10, a natural product originally isolated from the Indian ocean sea hare *Dolabella ariculara*. DM1, conjugated through a disulfide (**5**) or directly through the heterobifunctional succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC) linker (**8**), and DM4, conjugated through a disulfide (**10**), are semi-synthetic analogs of maytansine, a natural product originally isolated from the Ethiopian shrub *Maytenus ovatus* [3]. Calicheamicin, a DNA strand cleaving agent conjugated through an acid-sensitive hydrazone (**13**), is a semi-synthetic analog of a *Micromonospora echinospora* ssp. calichensis fermentation product [15].

There are a variety of catabolic processes that occur to release drug from an ADC. The vcMMAE protease-cleavable drug-linker (**1**) was designed to release MMAE (**2**) in target cells [7], and indeed MMAE was reported as the only intracellular released drug in cancer cell lines (Figure 1) [11]. The directly conjugated mcMMAF drug-linker (**3**) was observed to release cys-mcMMAF (**4**) after ADC catabolism, consistent with proteolytic degradation of the mAb to release the drug (Figure 1) [16]. For

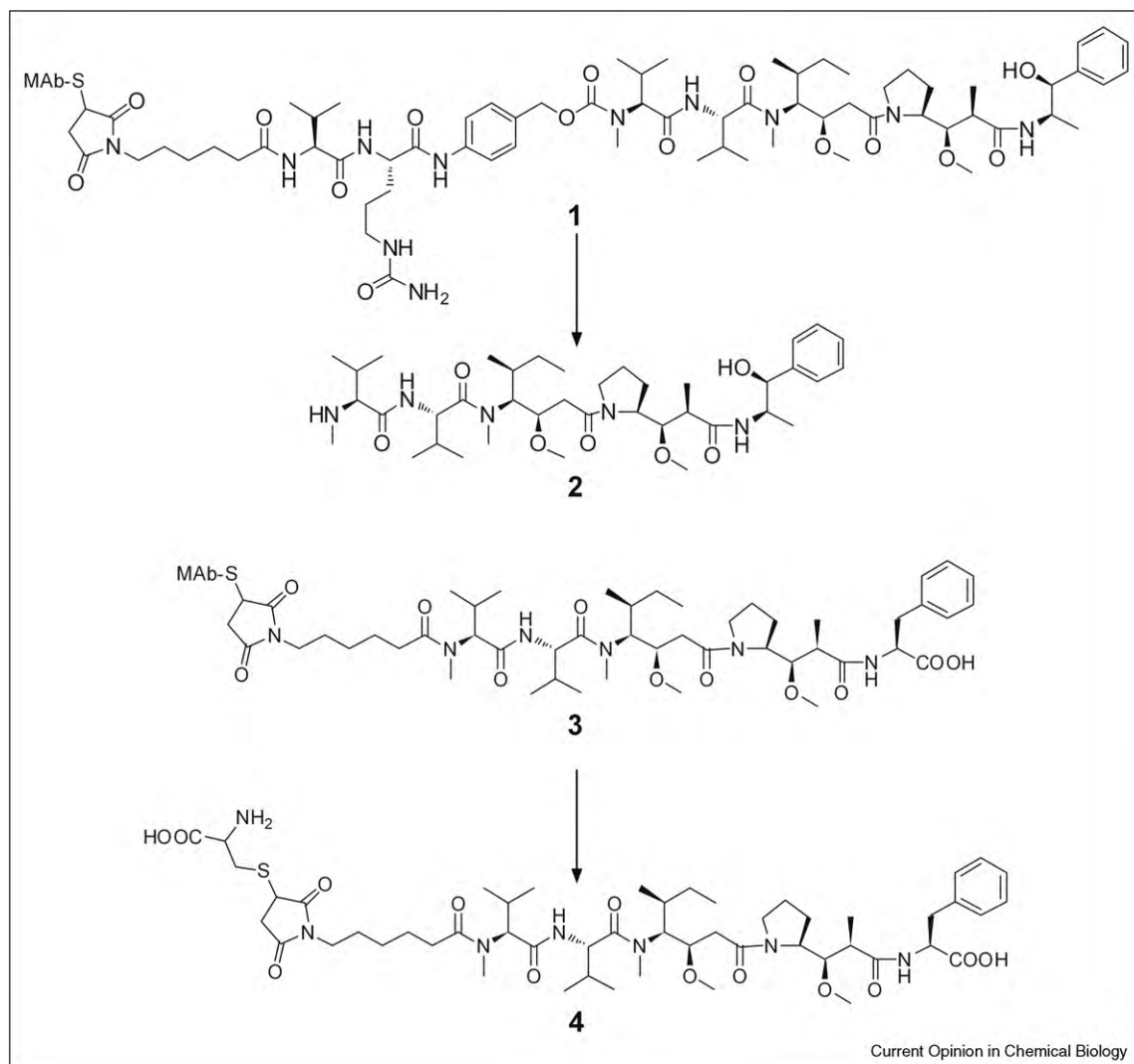
maytansines, directly linked SMCC ADCs and disulfide-linked ADCs both yield  $\epsilon$ -amino lysine-linked drugs (**6**, **9**, **11**). The disulfide-linked drugs can also be reduced to the free thiol and subsequently S-methylated or cysteinylated (**7**, **12**) (Figure 2). These various small molecules have different potencies and cellular permeabilities, presumably because of differences in charged groups on the various catabolites [10]. Not all drug classes may be tolerant of modification with an amino acid, and could be inactive in this form.

Stability of the drug-linker in circulation is important because the long circulating half-life of the ADC provides exposure for several days post injection. Directly linked ADCs and protease-cleavable linkers are generally more stable in circulation than disulfides and hydrazones [17–19], although the stability of the latter two linkers can be tuned by altering the neighboring chemical structure. For example, substituents on the aromatic group adjacent to the hydrazone in calicheamicin conjugates were observed to significantly affect the rate of calicheamicin release and ADC potency [15]. DM4 has a gem-dimethyl group next to the disulfide, which increases disulfide bond stability compared to DM1 that has a single adjacent methyl group [20].

### Conjugation methods

The method of making ADCs, including the drug loading stoichiometry and resulting conjugation heterogeneity, should maintain the pharmacokinetic properties of the mAb. There are three common methods for conjugating mAbs: alkylation of reduced interchain disulfides, acylation of lysines, and alkylation of genetically engineered cysteines. There are 8 interchain cysteines and up to 100 lysines available for conjugation on IgG1 mAbs, and conjugation to these sites results in heterogeneous mixtures. Cysteine conjugates provide a greater degree of uniformity than lysine-based conjugates [21], while recombinant methods in which cysteines are specifically introduced into the mAb backbone are more uniform still [22,23]. In some instances, it has been observed that the location of the conjugated drug is not as important as the

Figure 1



Structures of auristatin antibody–drug conjugates and catabolic products. ADCs made from vcMMAE (1) are converted to MMAE (2), while ADCs made from mcMMAF (3) are converted to cys-mcMMAF (4).

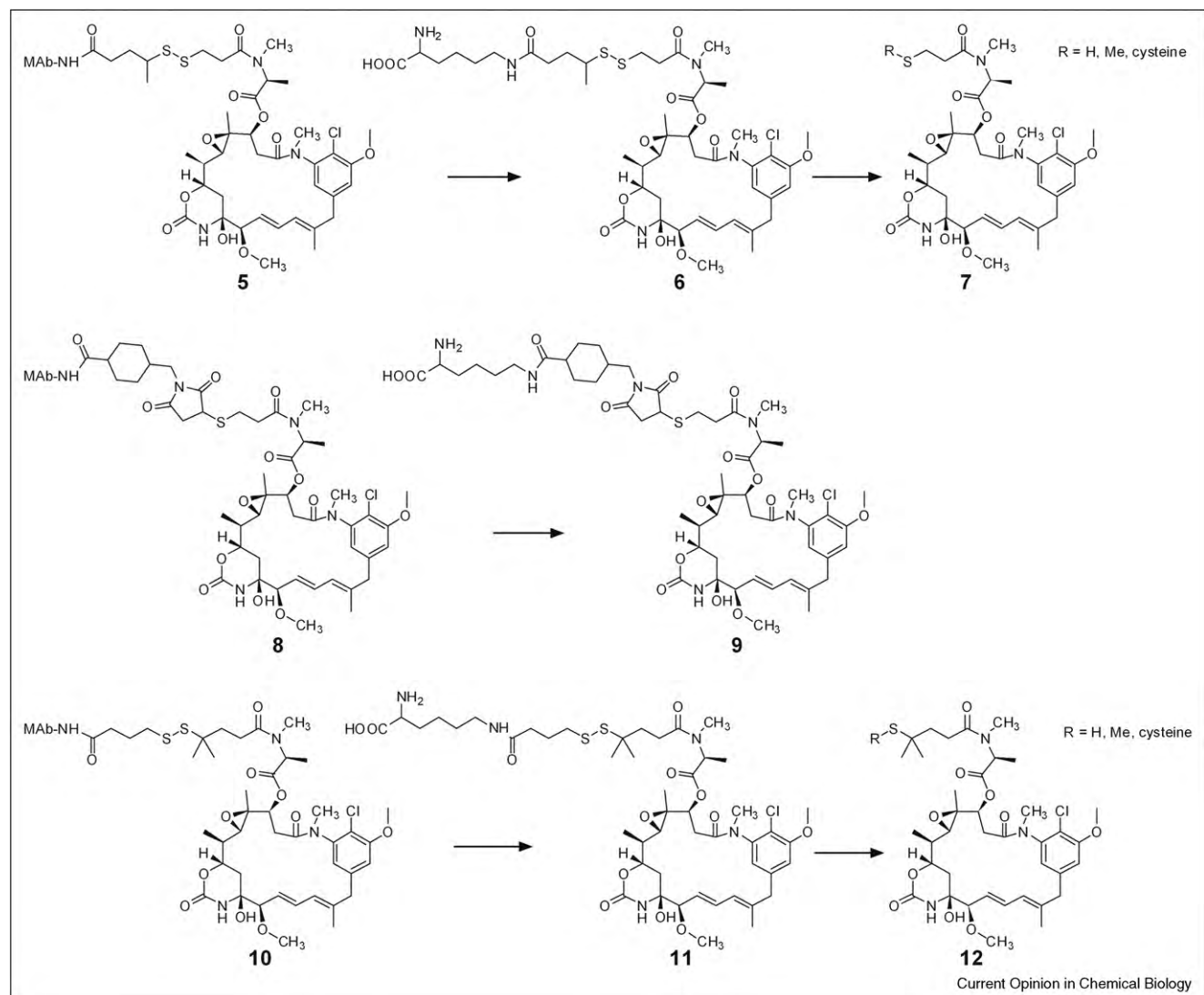
stoichiometry of drug attachment [21,23]. ADCs with two to four drugs per antibody are generally superior to more heavily loaded conjugates that clear very rapidly from the circulation [24]. Using chemical methods for conjugate formation, it has proven difficult to prepare ADCs with only 2 drugs/mAb because a large fraction of the mAb will not be conjugated to any drug. Recombinant methods have been described for this purpose [22,23], and in one case the resulting engineered conjugate had an improved therapeutic window relative to a chemically produced ADC [22]. However the drug loading stoichiometry of the two conjugates were markedly different. The precise roles that conjugation site and stoichiometry play in ADC efficacy and tolerability warrant further investigation.

### Pharmacology of ADCs

Ideally, an ADC acts as an inactive prodrug in circulation and only upon internalization into target cells or localization to the tumor is an active drug released. However, only a small amount of the ADC will ever reach the target cells [3,25<sup>••</sup>], highlighting the importance of using a highly potent drug. The remainder will be catabolized in normal tissues, which may cause exposure to the released drug. An optimal drug for ADCs will therefore be one that does not have a long residence time once released in normal tissues, while maintaining a high concentration in the tumor for an extended time.

To evaluate ADC biodistribution, an anti-CD70 mcMMAF ADC with two unique radiolabels was

Figure 2



Structures of maytansine antibody–drug conjugates and catabolic products. DM1 (**5**) and DM4 (**10**) disulfide ADCs are converted to lysine-linked disulfides (**6**, **11**), which are subsequently reduced to the free thiol and then S-methylated or cysteineylated (**7**, **12**). DM1 linked through SMCC (**8**) is converted to the lysine-linked drug (**9**).

prepared to track the mAb (<sup>3</sup>H) and drug (<sup>14</sup>C) simultaneously in mice bearing tumor xenografts. Two radiolabels were used because once the ADC is catabolized and drug released, tracking just the mAb would not be able to determine the biodistribution of the released drug. Antibody-bound drug and released drug were distinguished by precipitating the mAb-bound drug with organic solvent and scintillation counting the supernatant containing the released drug. Both radiolabels quickly accumulated in the tumor, but the mAb peak was 1 d post dose while the drug peak was 2 d post dose. The proportion of released drug relative to conjugated drug increased over time in the tumor. In most normal tissues, both the mAb and drug decreased over time in conjunc-

tion with the serum levels, except in organs involved in hepatobiliary clearance where there was a peak 4 h post dose followed by a rapid decrease (Figure 4a). This difference in tumor versus normal biodistribution and release kinetics yielded a tumor exposure to released drug tens to hundreds of times higher than that of normal tissues [26<sup>\*</sup>].

The size of the ADC delivery vehicle also plays a role in how effectively drug will be delivered to tumors. Diabodies are single-chain variable domain fragments (scFvs) with a linker between the light and heavy chains that promotes dimerization. The biodistributions of vcMMAF-conjugated anti-CD30 mAb and diabody were



experimental observations of the relationship between molecular weight of the delivery agent and the extent of tumor targeting. Calculations in agreement with previous experimental results predicted that the minimum tumor uptake occurred with ~25 kDa vehicles such as scFvs, while maximum tumor uptake should take place with ~150 kDa mAbs and small drugs with very low molecular weights. The long circulating half-life of mAbs more than compensated for a decrease in tumor penetration relative to smaller vehicles [25\*\*]. mAb conjugates may therefore be the optimal vehicle for drug delivery owing to their maximal tumor uptake, as long as normal tissues are minimally exposed to released drug by careful target, drug, and linker selection.

Maytansine ADCs have also been evaluated for their ability to localize to tumors using <sup>3</sup>H labeled drug. Mice with tumor xenografts were dosed with anti-CanAg ADCs and the total amount of <sup>3</sup>H drug (mAb bound and released) in tumor evaluated. A directly linked SMCC-DM1 ADC achieved ~2-fold higher tumor concentrations than disulfide-linked DM1 and DM4 ADCs. Samples were also analyzed for the identity of drug released by ADC catabolism, with the same species observed *in vivo* and *in vitro*. The tumor concentration of ε-amino lysine-linked DM1 was also ~2 fold higher than the sum of the S-methyl and cysteinyl DM1 and DM4 [28].

## ADCs in clinical development

### Approved ADCs

Table 2 lists ADCs currently in the clinic, and recently released clinical data are presented below. Also listed is the ADC Gemtuzumab ozogamicin, an anti-CD33 mAb conjugated to calicheamicin. This ADC was approved in 2000 but has been withdrawn and is no longer commercially available to new patients (<http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm216448.htm>). Gemtuzumab ozogamicin was approved for the treatment of relapsed acute myeloid leukemia in patients older than 60 years based on a response rate of about 30% [29]. The approved dose is 9 mg/m<sup>2</sup>, or about 0.22 mg/kg for a 65 kg adult, a very low dose that reflects the high potency of calicheamicin.

<http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm216448.htm>). Gemtuzumab ozogamicin was approved for the treatment of relapsed acute myeloid leukemia in patients older than 60 years based on a response rate of about 30% [29]. The approved dose is 9 mg/m<sup>2</sup>, or about 0.22 mg/kg for a 65 kg adult, a very low dose that reflects the high potency of calicheamicin.

### Late clinical stage ADCs

ADCs in late stage clinical trials include brentuximab vedotin (SGN-35), an anti-CD30 mAb conjugated to vcMMAE, currently in a pivotal trial for relapsed or refractory Hodgkin lymphoma (HL) following an autologous stem cell transplant, using a dose of 1.8 mg/kg every 3 weeks. The rationale for a pivotal trial was based on data from a Phase I dose escalation trial that included both HL and systemic anaplastic large cell lymphoma (sALCL) patients, whereas those treated with ≥1.2 mg/kg of SGN-35 showed an objective response rate of 54% (15/28), with 9 complete responses. Nearly all (93%) of the patients experienced tumor regression during the course of therapy [30\*\*]. In a second Phase I trial with weekly dosing, patients receiving 0.4–1.4 mg/kg had an objective response rate of 56% (22/39). Among the 5 sALCL patients, 4 had complete responses [31].

Trastuzumab-DM1, also in late-stage clinical trials, uses the approved anti-Her2 mAb Trastuzumab (Herceptin) directly conjugated to DM1 via SMCC for the treatment of metastatic breast cancer. In a Phase II trial in third line metastatic breast cancer with 3.6 mg/kg Trastuzumab-DM1 dosed every 3 weeks, a 33% objective response rate in 110 patients was observed [32\*\*], demonstrating single agent activity in a population heavily pretreated with agents including Trastuzumab. A Phase III trial investigating Trastuzumab-DM1 versus capecitabine

**Table 2**

#### ADCs in the clinic

Agent	Target	Drug class	Indication	Clinical status
Brentuximab vedotin (SGN-35) [30**,31]	CD30	Auristatin	Hodgkin lymphoma	Phase II (pivotal)
CDX-011 (CRO11-vcMMAE) [34,35]	GPMNB	Auristatin	Melanoma, breast cancer	Phase II
SGN-75 [53]	CD70	Auristatin	NHL, RCC	Phase I
PSMA ADC [54]	PSMA	Auristatin	Prostate cancer	Phase I
MEDI-547 [40]	EphA2	Auristatin	Solid tumors	Phase I
MN immunoconjugate [41]	MN	Auristatin	Cancer	Phase I
Trastuzumab-DM1 [32**]	Her2	Maytansine	Breast cancer	Phase III
IMGN901 [36,37]	CD56	Maytansine	Multiple myeloma, solid tumors	Phase I
IMGN388 [55]	α <sub>v</sub> integrin	Maytansine	Solid tumors	Phase I
SAR3914 [38]	CD19	Maytansine	NHL	Phase I
BT-062 [39,56]	CD138	Maytansine	Multiple myeloma	Phase I
BIIB015 [42]	Cripto	Maytansine	Solid tumors	Phase I
Gemtuzumab ozogamicin [29]	CD33	Calicheamicin	AML	Approved, withdrawn from market
Inotuzumab ozogamicin [33]	CD22	Calicheamicin	NHL	Phase III
MEDX-1203 [43]	CD70	Duocarmycin	NHL, RCC	Phase I

Data gathered from the listed references, clinicaltrials.gov, and sponsor web sites.

and lapatinib in second line metastatic breast cancer and a Phase II trial investigating Trastuzumab-DM1 versus Trastuzumab and docetaxel in front line therapy are currently ongoing.

Inotuzumab ozogamicin, which uses the same calicheamicin drug-linker as Gemtuzumab ozogamicin, is an anti-CD22 ADC for non-Hodgkin lymphoma (NHL) currently in a Phase III trial in combination with Rituximab. In a Phase I/II dose escalation trial, the maximum tolerated dose (MTD) was found to be 1.8 mg/m<sup>2</sup> when dosed every 4 weeks and an expansion cohort was enrolled at this dose. In this expansion cohort, the objective response rate was 88% (14/16) for follicular lymphoma and 71% (10/14) for diffuse large B-cell lymphoma (10/14) [33].

### Early clinical stage ADCs

ADCs in earlier stage clinical trials include CDX-011 (CRO11-vcMMAE), being evaluated in both melanoma and breast cancer, an anti-glycoprotein NMB mAb conjugated to vcMMAE. In Phase I/II studies for both indications, 1.88 mg/kg dosed every 3 weeks was found to be the MTD. In 37 melanoma patients at all dose levels there were 2 partial responses [34], while in 32 breast cancer patients treated at 1.88 mg/kg there were 4 partial responses [35].

Several maytansine ADCs currently in the clinic have had Phase I dose escalation and MTD expansion cohort data reported, including the anti-CD56 IMGN901, a disulfide-linked DM1 ADC for multiple myeloma and solid tumors, the anti-CD19 SAR3419, a disulfide-linked DM4 ADC for NHL, and the anti-CD138 BT-062, a disulfide-linked DM4 ADC for multiple myeloma. Treatment with IMGN901 showed 1 partial response in the first 26 multiple myeloma patients [36] and 5 objective responses in 113 patients with a variety of solid tumors [37]. Objective responses from SAR3419 treatment were obtained in 5 of 27 patients [38], while 2 of 25 patients treated with BT-062 had objective responses [39].

A number of other ADCs have just recently entered clinical trials. SGN-75, an anti-CD70 mAb directly conjugated to the drug-linker mcMMAF [36]. This ADC is being tested in both NHL and renal cell carcinoma (RCC), where CD70 expression has been demonstrated. Additional auristatin ADCs in the clinic target PSMA [37], EphA2 [40], and MN (carbonic anhydrase IX) [41], while additional maytansine ADCs in the clinic target  $\alpha_v$  integrin [40] and cripto [42]. A final ADC recently entering the clinic uses a DNA minor groove binding duocarmycin conjugated to an anti-CD70 mAb [43].

### Pre-clinical ADCs

The drug-linkers being used in ADCs in the clinic have also been evaluated pre-clinically for a variety of targets, including CD19 [43,44], CD20 [44–46], CD79b [44,47],

CD133 [44], BCMA [45], CEACAM6 [46], TMEFF2 [47], PSCA [48], MUC16 [22,49], p97 [9], EphB2 [50], and 5T4 [51]. Many more ADCs such as these can be expected to enter clinical trials soon [52\*].

### Conclusions

Optimization of the parameters influencing ADC activity has led to the development of new agents with promising activities. Recent clinical results have sparked significant additional interest and the number of ADC candidates in clinical development is growing rapidly. Use of highly potent drugs, stable linkers, optimized drug stoichiometry, and appropriate antigen target selection have been crucial in advancing the technology to where it is today. A detailed understanding of ADC pharmacokinetics, catabolism, and biodistribution will facilitate the development of new agents for clinical evaluation.

### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Reichert JM: **Monoclonal antibodies as innovative therapeutics**. *Curr Pharm Biotechnol* 2008, **9**:423-430.
  2. Carter PJ, Senter PD: **Antibody–drug conjugates for cancer therapy**. *Cancer J* 2008, **14**:154-169.
  3. Chari RV: **Targeted cancer therapy: conferring specificity to cytotoxic drugs**. *Acc Chem Res* 2008, **41**:98-107.
  4. Senter PD: **Potent antibody drug conjugates for cancer therapy**. *Curr Opin Chem Biol* 2009, **13**:235-244.
  5. Alley SC, Benjamin D, Law C-L: In *Antibody–Drug Conjugate Therapy*. Edited by An Z. Hoboken, NJ: John Wiley & Sons, Inc.; 2009.
  6. Teicher BA: **Antibody–drug conjugate targets**. *Curr Cancer Drug Targets* 2009, **9**:982-1004.
  - A thorough review of ADC targets being evaluated clinically and pre-clinically.
  7. Sutherland MS, Sanderson RJ, Gordon KA, Andreyka J, Cerveny CG, Yu C, Lewis TS, Meyer DL, Zabinski RF, Doronina SO *et al.*: **Lysosomal trafficking and cysteine protease metabolism confer target-specific cytotoxicity by peptide-linked anti-CD30-auristatin conjugates**. *J Biol Chem* 2006, **281**: 10540-10547.
  8. Law CL, Cerveny CG, Gordon KA, Klussman K, Mixan BJ, Chace DF, Meyer DL, Doronina SO, Siegall CB, Francisco JA *et al.*: **Efficient elimination of B-lineage lymphomas by anti-CD20-auristatin conjugates**. *Clin Cancer Res* 2004, **10**:7842-7851.
  9. Smith LM, Nesterova A, Alley SC, Torgov MY, Carter PJ: **Potent cytotoxicity of an auristatin-containing antibody–drug conjugate targeting melanoma cells expressing melanotransferrin/p97**. *Mol Cancer Ther* 2006, **5**:1474-1482.
  10. Erickson HK, Park PU, Widdison WC, Kovtun YV, Garrett LM, Hoffman K, Lutz RJ, Goldmacher VS, Blattler WA: **Antibody–maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing**. *Cancer Res* 2006, **66**:4426-4433.
  11. Okeley NM: **Intracellular activation of SGN-35, a potent anti-CD30 antibody–drug conjugate**. *Clin Cancer Res* 2010, **16**:888-897.
  12. Dijoseph JF, Dougher MM, Armellino DC, Kalyandrug L, Kunz A, Boghaert ER, Hamann PR, Damle NK: **CD20-specific antibody-targeted chemotherapy of non-Hodgkin's B-cell lymphoma**

- using calicheamicin-conjugated rituximab. *Cancer Immunol Immunother* 2007, **56**:1107-1117.
13. Polson AG, Calamine-Fenaux J, Chan P, Chang W, Christensen E, Clark S, de Sauvage FJ, Eaton D, Elkins K, Elliott JM *et al.*: **Antibody-drug conjugates for the treatment of non-Hodgkin's lymphoma: target and linker-drug selection.** *Cancer Res* 2009, **69**:2358-2364.
  14. Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, Chace DF, DeBlanc RL, Gearing RP, Bovee TD, Siegall CB *et al.*: **Development of potent monoclonal antibody auristatin conjugates for cancer therapy.** *Nat Biotechnol* 2003, **21**:778-784.
  15. Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, Hallett W, Tsou HR, Upeslacijs J, Shochat D *et al.*: **Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia.** *Bioconjug Chem* 2002, **13**:47-58.
  16. Doronina SO, Mendelsohn BA, Bovee TD, Cerveny CG, Alley SC, Meyer DL, Oflazoglu E, Toki BE, Sanderson RJ, Zabinski RF *et al.*: **Enhanced activity of monomethylauristatin F through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity.** *Bioconjug Chem* 2006, **17**:114-124.
  17. Alley SC, Benjamin DR, Jeffrey SC, Okeley NM, Meyer DL, Sanderson RJ, Senter PD: **Contribution of linker stability to the activities of anticancer immunoconjugates.** *Bioconjug Chem* 2008, **19**:759-765.
  18. Sanderson RJ, Hering MA, James SF, Sun MM, Doronina SO, Siadak AW, Senter PD, Wahl AF: **In vivo drug-linker stability of an anti-CD30 dipeptide-linked auristatin immunoconjugate.** *Clin Cancer Res* 2005, **11**:843-852.
  19. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, Blattler WA, Lambert JM, Chari RV, Lutz RJ *et al.*: **Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate.** *Cancer Res* 2008, **68**:9280-9290.
  20. Widdison WC, Wilhelm SD, Cavanagh EE, Whiteman KR, Leece BA, Kovtun Y, Goldmacher VS, Xie H, Steeves RM, Lutz RJ *et al.*: **Semisynthetic maytansine analogues for the targeted treatment of cancer.** *J Med Chem* 2006, **49**:4392-4408.
  21. Sun MM, Beam KS, Cerveny CG, Hamblett KJ, Blackmore RS, Torgov MY, Handley FG, Ihle NC, Senter PD, Alley SC: **Reduction-alkylation strategies for the modification of specific monoclonal antibody disulfides.** *Bioconjug Chem* 2005, **16**:1282-1290.
  22. Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, Chen Y, Simpson M, Tsai SP, Dennis MS *et al.*: **Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index.** *Nat Biotechnol* 2008, **26**:925-932.
  23. McDonagh CF, Turcott E, Westendorf L, Webster JB, Alley SC, Kim K, Andreyka J, Stone I, Hamblett KJ, Francisco JA *et al.*: **Engineered antibody-drug conjugates with defined sites and stoichiometries of drug attachment.** *Protein Eng Des Sel* 2006, **19**:299-307.
  24. Hamblett KJ, Senter PD, Chace DF, Sun MM, Lenox J, Cerveny CG, Kissler KM, Bernhardt SX, Kopcha AK, Zabinski RF *et al.*: **Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate.** *Clin Cancer Res* 2004, **10**:7063-7070.
  25. Schmidt MM, Wittrup KD: **A modeling analysis of the effects of molecular size and binding affinity on tumor targeting.** *Mol Cancer Ther* 2009, **8**:2861-2871.
- An excellent mathematical model of tumor uptake of targeting agents based on size and affinity.
26. Alley SC, Zhang X, Okeley NM, Anderson M, Law CL, Senter PD, Benjamin DR: **The pharmacologic basis for antibody-auristatin conjugate activity.** *J Pharmacol Exp Ther* 2009, **330**:932-938.
- Evaluation of biodistribution of ADCs in a mouse xenograft model using two radiolabels for mAb and drug simultaneously.
27. Kim KM, McDonagh CF, Westendorf L, Brown LL, Sussman D, Feist T, Lyon R, Alley SC, Okeley NM, Zhang X *et al.*: **Anti-CD30 diabody-drug conjugates with potent antitumor activity.** *Mol Cancer Ther* 2008, **7**:2486-2497.
  28. Erickson HK, Widdison WC, Mayo MF, Whiteman K, Audette C, Wilhelm SD, Singh R: **Tumor delivery and in vivo processing of disulfide-linked and thioether-linked antibody-maytansinoid conjugates.** *Bioconjug Chem* 2009.
  29. Larson RA, Boogaerts M, Estey E, Karanes C, Stadtmauer EA, Sievers EL, Mineur P, Bennett JM, Berger MS, Eten CB *et al.*: **Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin).** *Leukemia* 2002, **16**:1627-1636.
  30. Younes A, Forero-Torres A, Bartlett NL, Leonard JP, Lynch C, Kennedy DA, Sievers EL: **Multiple complete responses in a Phase 1 dose-escalation study of the antibody-drug conjugate SGN-35 in patients with relapsed or refractory CD30-positive lymphomas.** *Blood (ASH Annual Meeting Abstracts)*, vol 112. 2008:1006.
- Clinical data with a single agent ADC demonstrating objective responses in heavily pretreated Hodgkin lymphoma patients.
31. Fanale M, Bartlett NL, Forero-Torres A, Rosenblatt JD, Horning SJ, Franklin AR, Lynch C, Sievers EL, Kennedy DA: **The antibody-drug conjugate brentuximab vedotin (SGN-35) induced multiple objective responses in patients with relapsed or refractory CD30-positive lymphomas in a phase 1 weekly dosing study.** *Blood (ASH Annual Meeting Abstracts)*. 2009. . abstract 2731.
  32. Krop I, LoRusso P, Miller KD, Modi S, Yardley D, Rodriguez G, Zheng M, Amler L, Rugo H: **A phase II study of Trastuzumab-DM1 (T-DM1), a novel HER2 antibody-drug conjugate, in patients with HER2+ metastatic breast cancer (MBC) who were previously treated with an anthracycline, a taxane, capecitabine, lipatinib, and trastuzumab.** *32nd Annual San Antonio Breast Cancer Symposium; San Antonio, TX: 2009. . abstract 710.*
- Clinical data with a single agent ADC demonstrating objective responses in heavily pretreated metastatic breast cancer patients.
33. Fayad L, Patel H, Verhoef G, Smith MR, Johnson PWM, Czuczman MS, Coiffier B, Hess G, Gine E, Advani A *et al.*: **Safety and clinical activity of the anti-CD22 immunoconjugate inotuzumab ozogamicin (CMC-544) in combination with rituximab in follicular lymphoma or diffuse large B-cell lymphoma: preliminary report of a phase 1/2 study.** *Blood (ASH Annual Meeting Abstracts)*. 2008. . abstract 266.
  34. Hwu P, Sznol M, Pavlick A, Kluger H, Kim KB, Boasberg P, Sanders D, Simantov R, Crowley E, Hamid O: **A phase I/II study of CR011-vcMMAE, an antibody-drug conjugate (ADC) targeting glycoprotein NMB (GPNMB) in patients (pts) with advanced melanoma.** *J Clin Oncol* 2009, **27(15S)** abstract 9032.
  35. Burris H, Saleh M, Bendell J, Hart L, Rose AAN, Dong Z, Siegel PM, Crane MF, Donovan D, Crowley E *et al.*: **A phase I/II study of CRO11-vcMMAE (CDX-011), an antibody-drug conjugate, in patients with locally advanced or metastatic breast cancer.** *32nd Annual San Antonio Breast Cancer Symposium; San Antonio, TX: 2009. . abstract 6096.*
  36. Chanan-Khan A, Wolf J, Gharibo M, Jagannath S, Munshi N, Anderson KC, DePaolo D, Lee K, Miller KC, Guild R *et al.*: **Phase 1 Study of IMG901, used as monotherapy, in patients with heavily pre-treated CD56-positive multiple myeloma. A preliminary safety and efficacy analysis.** *Blood (ASH Annual Meeting Abstracts)*. 2009. . abstract 2283.
  37. Fossella F, Woll PJ, Lorigan P, Tolcher A, O'Brien M, O'Keefe J, Zildjian S, Qin A, O'Leary J, Villalona-Calero M: **Investigation of IMG901 in CD56+ solid tumors: results from a phase I/II trial (study 001) and a phase I trial (study 002).** *13th World Conference on Lung Cancer*. 2009.
  38. Younes A, Gordon L, Kim S, Romaguera J, Copeland AR, de Castro Fariel S, Kwak L, Fayad L, Hagemester F, Fanale M *et al.*: **Phase 1 multi-dose escalation study of the anti-CD19 maytansinoid immunoconjugate SAR3419 administered by IV infusion every 3 weeks to patients with relapsed/refractory B-cell NHL.** *Blood (ASH Annual Meeting Abstracts)*. 2009. . abstract 585.
  39. Chanan-Khan A, Jagannath S, Heffner T, Avigan D, Lee K, Lutz RJ, Haeder T, Ruehle M, Uherek C, Wartenberg-Demand A *et al.*: **Phase 1 Study of BT062 given as repeated single dose once every 3 weeks in patients with relapsed or relapsed/refractory multiple myeloma.** *Blood (ASH Annual Meeting Abstracts)*. 2009. . abstract 1862.

40. Lee JW, Han HD, Shahzad MM, Kim SW, Mangala LS, Nick AM, Lu C, Langley RR, Schmandt R, Kim HS *et al.*: **EphA2 immunoconjugate as molecularly targeted chemotherapy for ovarian carcinoma.** *J Natl Cancer Inst* 2009, **101**: 1193-1205.
41. Al-Ahmadie HA, Alden D, Qin LX, Olgac S, Fine SW, Gopalan A, Russo P, Motzer RJ, Reuter VE, Tickoo SK: **Carbonic anhydrase IX expression in clear cell renal cell carcinoma: an immunohistochemical study comparing 2 antibodies.** *Am J Surg Pathol* 2008, **32**:377-382.
42. Gong YP, Yarrow PM, Carmalt HL, Kwun SY, Kennedy CW, Lin BP, Xing PX, Gillett DJ: **Overexpression of Cripto and its prognostic significance in breast cancer: a study with long-term survival.** *Eur J Surg Oncol* 2007, **33**:438-443.
43. Zhang Q, Derwin D, Sufi B, Chen L, Guerlavais V, Green L, Passmore D, Sung J, Rangan V, Dai R *et al.*: **The critical role of cleavable linkers for efficacy and low toxicity with minor groove binding alkylating agent (MGBA) based antibody–drug conjugates.** *American Association of Cancer Research 100th Annual Meeting*. 2009. . abstract 1722.
44. Smith LM, Nesterova A, Ryan MC, Duniho S, Jonas M, Anderson M, Zabinski RF, Sutherland MK, Gerber HP, Van Orden KL *et al.*: **CD133/prominin-1 is a potential therapeutic target for antibody–drug conjugates in hepatocellular and gastric cancers.** *Br J Cancer* 2008, **99**:100-109.
45. Ryan MC, Hering M, Peckham D, McDonagh CF, Brown L, Kim KM, Meyer DL, Zabinski RF, Grewal IS, Carter PJ: **Antibody targeting of B-cell maturation antigen on malignant plasma cells.** *Mol Cancer Ther* 2007, **6**:3009-3018.
46. Strickland LA, Ross J, Williams S, Ross S, Romero M, Spencer S, Erickson R, Sutcliffe J, Verbeke C, Polakis P *et al.*: **Preclinical evaluation of carcinoembryonic cell adhesion molecule (CEACAM) 6 as potential therapy target for pancreatic adenocarcinoma.** *J Pathol* 2009, **218**:380-390.
47. Afar DE, Bhaskar V, Ibsen E, Breinberg D, Henshall SM, Kench JG, Drobnjak M, Powers R, Wong M, Evangelista F *et al.*: **Preclinical validation of anti-TMEFF2-auristatin E-conjugated antibodies in the treatment of prostate cancer.** *Mol Cancer Ther* 2004, **3**:921-932.
48. Ross S, Spencer SD, Holcomb I, Tan C, Hongo J, Devaux B, Rangell L, Keller GA, Schow P, Steeves RM *et al.*: **Prostate stem cell antigen as therapy target: tissue expression and in vivo efficacy of an immunoconjugate.** *Cancer Res* 2002, **62**:2546-2553.
49. Chen Y, Clark S, Wong T, Dennis MS, Luis E, Zhong F, Bheddah S, Koeppen H, Gogineni A, Ross S *et al.*: **Armed antibodies targeting the mucin repeats of the ovarian cancer antigen. MUC16, are highly efficacious in animal tumor models.** *Cancer Res* 2007, **67**:4924-4932.
50. Mao W, Luis E, Ross S, Silva J, Tan C, Crowley C, Chui C, Franz G, Senter P, Koeppen H *et al.*: **EphB2 as a therapeutic antibody drug target for the treatment of colorectal cancer.** *Cancer Res* 2004, **64**:781-788.
51. Boghaert ER, Sridharan L, Khandke KM, Armellino D, Ryan MG, Myers K, Harrop R, Kunz A, Hamann PR, Marquette K *et al.*: **The oncofetal protein, 5T4, is a suitable target for antibody-guided anti-cancer chemotherapy with calicheamicin.** *Int J Oncol* 2008, **32**:221-234.
52. Ducry L, Stump B: **Antibody–drug conjugates: linking cytotoxic payloads to monoclonal antibodies.** *Bioconjug Chem* 2010, **21**:5-13.
- A review focused on the various linkers used in ADCs and their impact on stability and drug release.
53. Oflazoglu E, Stone IJ, Gordon K, Wood CG, Repasky EA, Grewal IS, Law CL, Gerber HP: **Potent anticarcinoma activity of the humanized anti-CD70 antibody h1F6 conjugated to the tubulin inhibitor auristatin via an uncleavable linker.** *Clin Cancer Res* 2008, **14**:6171-6180.
54. Ma D, Hopf CE, Malewicz AD, Donovan GP, Senter PD, Goeckeler WF, Maddon PJ, Olson WC: **Potent antitumor activity of an auristatin-conjugated, fully human monoclonal antibody to prostate-specific membrane antigen.** *Clin Cancer Res* 2006, **12**:2591-2596.
55. Chen Q, Millar HJ, McCabe FL, Manning CD, Steeves R, Lai K, Kellogg B, Lutz RJ, Trikha M, Nakada MT *et al.*: **Alphav integrin-targeted immunoconjugates regress established human tumors in xenograft models.** *Clin Cancer Res* 2007, **13**:3689-3695.
56. Ikeda H, Hideshima T, Fulciniti M, Lutz RJ, Yasui H, Okawa Y, Kiziltepe T, Vallet S, Pozzi S, Santo L *et al.*: **The monoclonal antibody nBT062 conjugated to cytotoxic Maytansinoids has selective cytotoxicity against CD138-positive multiple myeloma cells in vitro and in vivo.** *Clin Cancer Res* 2009, **15**:4028-4037.