

EXPERT OPINION

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Antibody–drug conjugates – a perfect synergy

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Introduction: There is a great unmet need for effective new treatments in cancer, which continues to be a major cause of death. Antibody–drug conjugates (ADCs) are emerging, after a long gestation, as a class of biopharmaceuticals with the potential to address this need by directing highly potent cytotoxic drugs to their point of action. There is increasing interest in ADCs by major pharmaceutical companies and a growing pipeline of candidates for clinical use. This review summarises progress with development of this new class of drugs.

Areas covered: The authors describe separately the antibody and drug elements of ADCs and then examine the technology and consequences of linkage. The work is presented in the light of recent developments in the design, using clinical examples where possible.

Expert opinion: Since their emergence as independent drugs, antibodies and chemotherapy are being brought together in effective synergy. The conjunction is timely: many of the technical challenges in preparing antibodies have been addressed; potent new drugs are available and linker technology is advancing apace. ADCs however are not just a sum of their individual parts. The current challenge is in understanding the holistic nature of this exciting class of drugs that promise a new avenue for cancer treatment. Target selection, the interaction of ADC with tumour and off-tumour targets and the internalisation of ADCs, are critical to the effective maturation of ADC technology. Ongoing recent developments in attachment sites and linker chemistry can provide fine-tuning of drug loading, elements of ADC PK and off-target ADC toxicity.

Keywords: ADC, antibody, antibody–drug conjugate, cancer, chemotherapy, clinical trial, oncology

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1. Introduction

Paul Ehrlich visualised the future of antibodies as ‘magic bullets’ and pioneered the use of chemotherapy. Yet, in over 100 years of research since their birth, these two classes of agents largely have been developed independently. Antibodies, due to their specificity and multi-functionality, have met with remarkable success as cancer drugs and are now market leaders in the field of targeted therapies. However, there is still great clinical need; even when using antibodies in combination with chemotherapy, long-term control of tumours is unusual and the majority of advanced solid tumours are incurable.

Antibodies can be synergistically improved by arming them with a toxic chemical warhead, to create a new class of drugs: antibody–drug conjugates (ADCs) that target drugs, often too powerful to be used on their own. The molecular combination provides an extremely effective means to achieve potent and selective target cell killing. The concept of ADCs has been around for many years [1] but they are now

Article highlights.

- Antibody–drug conjugates (ADCs) are disease-targeted drug constructs that combine the specificity of antibodies (or another type of targeting agent) with the potent toxicity of novel chemical ‘warheads’.
- The discovery and design of new warheads/linkers has allowed ADCs to become one of the most rapidly growing and exciting new classes of anti-cancer therapeutics.
- ADCs are complex drugs. Antibody type, target antigen, linker technology and warhead mechanism/potency must be considered.
- The linker between each antibody and the drug is a unique biochemical entity and reductions in unwanted toxicity are most likely to be achieved by close attention to linking technologies.
- Knowing the requirement for each part is essential but an individual ADC is more than a sum of its individual parts and understanding the holistic nature of each ADC combination is a key factor in achieving success.
- The promising ADC efficacy data generated by pharmaceutical companies and the strong pipeline of potential new agents indicate that the next few years will deliver a number of effective new ADC drugs.

This box summarises key points contained in the article.

rapidly becoming lead agents in the antibody therapeutic field. The reduction to practice for ADCs is timely; recombinant antibody technologies and advanced biological manufacturing have addressed many of the practical issues associated with antibody–drug development, and a plethora of potent drugs and linkers, synthesised specifically for attachment to antibodies, are becoming available. As more ADCs are being examined in preclinical models and clinical trials, the parameters required for success are becoming elucidated. Here the authors review those parameters, with a focus on the means and potential consequences of joining antibodies to drugs and in light of recent advances in translation of ADCs for clinical use in patients with cancer.

2. Composition of ADCs

ADCs are complex entities comprising three components: the targeting element, the drug and a linker (Figure 1). These components are detailed separately below but it should be borne in mind that they are interdependent on one another and on the nature of the target. Thus, the ADC is greater than the sum of its parts and its mode of action is environmentally and biologically determined.

2.1 Targeting element

Although any entity with high affinity for a tumour-associated cell surface antigen (e.g., peptides, vitamins, aptamers) might be considered as the targeting element for an ADC, the most common and established targeting element used, to date, is an antibody. The development of antibodies as independent

drugs has been instrumental in this respect. While the early history of therapeutic antibody development was dominated by the issue of patient immune responses to murine and chimeric antibodies [2], this has largely been addressed [3] by the development of humanised, deimmunised and ‘fully human’ antibodies (the latter derived from phage display or transgenic mice technologies) [4]. Some companies that are developers of ADCs have their own proprietary antibody engineering technologies to produce ‘humanised’ or human antibodies. Thus, ADCs in the current clinical pipeline have been created using antibodies engineered to have reduced, or negligible, immunogenicity (Table 1). Furthermore, the commercial success of antibodies has fuelled an extremely active research field generating new antibodies and knowledge applicable to ADC optimisation.

There are, as yet, insufficient clinical data to direct the choice of targeting agent or even the best antibody format for an optimal ADC because clinically tested ADCs to date have used whole antibodies, mostly of IgG1 isotype, although there are several that are IgG2 or IgG4 (Table 1). Each of these isotypes potentially confers long *in vivo* half-life to the ADC via engagement with the neonatal Fc receptor, FcRn [5,6]. In the case of IgG1, Fc engagement with immune cells or complement components could add additional immune effector functions to the ADC. It remains to be seen, however, whether antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, or Fc-mediated complement-dependent cytotoxicity (CDC) provides significant additional efficacy to an ADC. Conversely, FcR binding could cause internalisation of ADC into innocent FcR-expressing immune cells such as endothelium (FcRn) and reticuloendothelial system monocytes and macrophages (FcγR). It is possible that internal release of active cytotoxic agent from an ADC in these innocent cells might lead to unwanted systemic side effects. Although such effects have not yet been reported, the potential for their existence suggests that design of the non-antigen-binding portion of an antibody is a matter of some note.

Despite the predominant use of IgGs for clinical ADCs, it is not known whether full-sized IgGs are the optimum structure to be used: a long serum half-life may have unwanted consequences if there are any issues of linker instability leading to release of free drug and a potential for non-specific toxicity. Furthermore, it has been noted, from studies with radiolabelled antibodies, that IgGs generally achieve < 0.1% injected dose/g of solid tumour [7] indicating that there is significant room for improvement in overall format of the antibody element to confer optimal pharmacokinetics (PK). Factors affecting antibody penetration and retention in tumour have been reviewed by others [8,9] and recent data indicate that most efficient tumour uptake can be obtained with large proteins such as IgG, which exceed the size limit for renal clearance, or with very small proteins with high binding affinity (long off-rate) [10,11]. Thus, it may be feasible to utilise high affinity antibody fragments or non-antibody scaffolds as favourable carrier platforms for drugs.

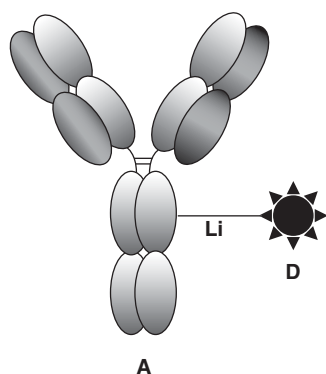


Figure 1. Diagrammatic representation of an ADC. Antibody (A) is attached by a chemical linker (Li) to drug (D).

2.2 Drug

Of the many drugs that have been evaluated for specific targeting [12,13], only a few are consistently used in clinical-stage drug conjugates. The leading candidates are either tubulin inhibitors, which act on dividing cells, or those that target the cellular DNA. There is a relatively small number of companies leading the field in developing these as drugs and prodrugs for ADCs (Table 2).

2.2.1 Tubulin inhibitors

There are two major groups of tubulin inhibitors being developed for ADCs: auristatins and maytansine derivatives. Monomethyl auristatin E (MMAE) and auristatin F are the two forms of auristatins that are in clinical development. MMAE is a synthetic pentapeptide based on the naturally occurring tubulin polymerase inhibitor dolastatin-10 with a 2-amino-1-phenylpropan-1-ol unit at its C-terminus. The MMAE molecule exhibits nanomolar to high sub-nanomolar activity in human tumour cell lines [14]. Auristatin F (with a phenylalanine C-terminus) is at least 100-fold less potent than MMAE as a stand-alone agent as the ionisable phenylalanine carboxyl group presumably hinders cellular absorption. Interestingly, Monomethyl auristatin F (MMAFs) exhibit similar potencies to their MMAE analogues when delivered to cells in an ADC format [15], highlighting the importance of taking a holistic rather than reductionist approach to ADC design.

The other class of tubulin binding drugs employed in ADCs are maytansine derivatives. Maytansine was originally isolated from the bark of the African shrub *Maytenus ovatus* and found to be a potent inhibitor of microtubulin assembly leading to the apoptosis of tumour cells. A series of clinical trials failed to establish a sufficient therapeutic window for maytansine [16] and its picomolar activity *in vitro* suggested a more suitable role as an ADC warhead. Maytansine is a structurally complex polyketide that can be accessed synthetically by derivatizing ansamitocins obtained by fermentation. Maytansine bears a C3 *N*-acyl-*N*-methyl-L-alanyl side chain,

which, although essential for activity, can be modified to carry a disulphide ‘handle’ that can be incorporated in linkers designed to be cleaved by thiols inside target cells.

2.2.2 DNA interacting agents

The other major group of drugs used as ADC warheads are DNA interacting agents. These include calicheamicins, duocarmycins and, more recently, pyrrolobenzodiazepines (PBDs). Calicheamicins are naturally occurring anti-tumour antibiotics found in the soil microorganism *Micromonospora echinospora* ssp. *calichensis*. They associate with the minor groove of DNA and when activated by glutathione undergo a Bergman cyclisation to generate a diradical species which cleaves the DNA backbone. Due to their potency, a number of calicheamicins have undergone preclinical evaluation as stand-alone agents but they have not been progressed due to their delayed toxicity and narrow therapeutic index. However, as for maytansine, their potency recommended them as ADC warheads.

The duocarmycins (including CC-1065) are a family of naturally occurring anti-tumour antibiotics found in *Streptomyces* species. Although CC-1065 is highly potent, these molecules have not been used clinically because of serious delayed hepatotoxicity. Similarly CC-1065 duocarmycin dimer, biselezin was found to induce severe myelosuppression in man. The duocarmycins bind sequence-selectively in the minor groove of DNA forming a covalent linkage to the N3 of adenine. The duocarmycins comprise cyclopropylindole (CPI) tricyclic core attached via an amide bond to a second indole unit. Naturally occurring duocarmycins possess a pyrrole ring (fully aromatic or reduced) fused to the benzo ring of the indole but this has been replaced with a second benzene ring in most ADC warheads. The strained cyclopropyl group allows the formation of the covalent linkage to adenine while rotation of the second indole unit, once in the minor groove can help to activate the molecule. The cyclopropyl group can exist as a ring-open chloromethyl precursor in which case the benzo ring of the indole is in a phenolic, as opposed to cyclohexadienone, form. This allows the duocarmycins to function as a pro-drug if the phenolic form is locked-in by functionalisation of the phenolic hydroxyl group.

The use of methyl substituted cyclopropylbenzoindole (CBI) duocarmycin warheads has also been investigated. These warheads are locked in their cyclopropyl ring-open *seco* forms, by protecting the phenolic hydroxy group as a carbamate. This carbamate forms part of a cathepsin-labile linker, ending in a caproyl maleimide connecting group. Hydrophilic polyethylene glycol (PEG) chains can be incorporated in this linker and *in vivo* studies showed that shorter PEGs were associated with better activity (growth delay at 12 mg/kg) [17]. Alternatively, the CBI unit can be attached to the antibody via the auxiliary indolecarboxamide moiety using cathepsin-labile linkers. In this case, a water-solubilising *N*-methylpiperazino carbamate is used to protect the phenolic group of the CBI moiety [18]. A similar strategy has been

Table 1. Clinical stage ADCs.

Product international non-proprietary Name, synonyms	Antibody type	Linker/linker type	Drug conjugate	Drug mode of action	Target antigen	Tumour type	Application or indication	Approval year or latest clinical phase*	Company/Licensee
Mylotarg [®] Gemtuzumab ozogamicin	Humanised IgG4P	Ac-but acid cleavable linker	Calicheamicin	DNA cleavage	CD33	Haematological tumours	Treatment of patients 60 years and older in first relapse with CD33 ⁺ AML who are not considered candidates for cytotoxic chemotherapy	US 2000 (accelerated approval process) Approval withdrawn June 2010 Several open Phase III and IV studies are in progress	Pfizer/UCB Pharma
Adcetris [™] brentuximab vedotin, SGN-35	Chimeric IgG1	Peptide cleavable Val-Cit linker	MMAE	Tubulin inhibitor	CD30	Haematological tumours	The treatment of patients with Hodgkin's lymphoma after failure of ASCT or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates. The treatment of patients with systemic ALCL after failure of at least one prior multi-agent chemotherapy regimen	US August 2011 (accelerated approval process) Phase III AETHERA study and further Phase II and I studies are in progress	Seattle Genetics/Millennium
Trastuzumab-DM1 trastuzumab emtansine, T-DM1, RG3502	Humanised IgG1	MCC; thioether non-cleavable linker	Maytansinoid DM1	Tubulin inhibitor	CD340 p185HER2	Solid tumours	HER2+ breast cancer and metastatic breast cancer	Phase III (including MARIANNE and EMILIA studies) and several other open studies in progress	Genentech/Roche/Immunogen
CMC-544 inotuzumab ozogamicin	Humanised IgG4P	Ac-but cleavable linker	Calicheamicin	DNA cleavage	CD22	Haematological tumours	Non-Hodgkin's lymphoma	Phase III and several Phase II and I studies in progress	Pfizer/UCB Pharma
CDX-011 glembatumumab vedotin (CR011-vcMMAE)	Human IgG2 (Abgenix Xenomouse transgenic mouse)	Peptide cleavable Val-Cit linker	MMAE	Tubulin inhibitor	GPNMB	Solid tumours	Advanced GPNMB-expressing breast cancer Melanoma	Phase II (EMERGE study)	Celldev/Seattle Genetics
SAR3419	Humanised IgG1 (Immunogen resurfacing)	SPDB; cleavable linker	Maytansinoid DM4	Tubulin inhibitor	CD19	Haematological tumours	Diffuse large B-cell lymphoma (Single agent and separate trial with rituximab), Acute lymphoblastic lymphoma, non-Hodgkin's lymphoma	Phase III completed May 2011 Phase II	Sanofi-aventis/Immunogen
BT-062	Chimeric IgG4	SPDB cleavable linker	DM4	Tubulin inhibitor	CD138 (syndecan-1)	Haematological tumours	Relapsed or refractory multiple myeloma	Phase III	Biotech AG/Immunogen
IMGN901 lorvotuzumab mertansine, BB-10901;	Humanised IgG1 (Immunogen resurfacing)	SPP cleavable linker	DM1	Tubulin inhibitor	CD56	Solid tumours/haematological tumours	Small cell lung cancer; multiple myeloma	Phase III	Immunogen
Milatuzumab-DOX IMMU-110	Humanised IgG1	MCC	Doxorubicin	DNA intercalator	CD74	Haematological tumours	Multiple myeloma	Phase III	Immunomedics, Inc.

* Information on clinical status was obtained from Company websites and/or www.clinicaltrials.gov between 20 December 2011 and 18 March 2012.

Ac-but: 4-(4'-Acetylphenoxy) butanoic acid; AML: Acute myeloid leukaemia; ALCL: Anaplastic large cell lymphoma; ASCT: Autologous stem cell transplant; IND: Investigational New Drug; MC: Maleimidocaproyl; MCC: N-maleimidomethylcyclohexane-1-carboxylate; MMAE: Monomethyl auristatin E; MMAF: Monomethyl auristatin F; n/a: Not available, not disclosed at present; PSMA: Prostate-specific membrane antigen; SPDB: N-succinimidyl 4-(2-pyridylidithio) butyrate; SPP: N-succinimidyl 4-(2-pyridylidithio)-pentanoate; Val-Cit: Valine-citrulline.

Table 1. Clinical stage ADCs (continued).

Product international non-proprietary Name, synonyms	Antibody type	Linker/linker type	Drug conjugate	Drug mode of action	Target antigen	Tumour type	Application or indication	Approval year or latest clinical phase*	Company/Licensee
AGS-16M8F	Human IgG2 (Abgenix Xenomouse transgenic mouse)	MC non-cleavable linker	MMAF	Tubulin inhibitor	Ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3) (AGS-16)	Solid tumours	Renal cell carcinoma	Phase I	Astellas Pharma/Seattle Genetics
AMG 172	n/a	n/a	n/a	Tubulin inhibitor	n/a	Solid tumours	Renal cell adenocarcinoma Clear cell renal carcinoma Clear cell renal cell carcinoma Renal cell carcinoma Glioma	Phase I	Amgen/Immunogen
AMG 595	Human IgG (Abgenix Xenomouse transgenic mouse)	Non-cleavable linker	DM1	Tubulin inhibitor	EGFR variant III	Solid tumours		Phase I	Amgen/Immunogen
ASG-5ME AGS-5ME	Human IgG2 (Abgenix Xenomouse transgenic mouse)	Peptide cleavable Val-Cit linker	MMAE	Tubulin inhibitor	SLC44A4 (AGS-5)	Solid tumours	Pancreatic, prostate cancers	Phase I	Astellas Pharma/Seattle Genetics
ASG-22ME AGS-22ME AGS-22M6E	Human IgG (Abgenix Xenomouse transgenic mouse)	Peptide cleavable Val-Cit linker	MMAE	Tubulin inhibitor	Nectin-4	Solid tumours	Multiple solid tumours to include breast, bladder, lung and pancreatic cancers	Phase I	Astellas Pharma/Seattle Genetics
BAY 94-9343	Fully human IgG1 (Morphosys phage display)	Immunogen 'engineered linker'	DM4	Tubulin inhibitor	Mesothelin	Solid tumours	Advanced solid tumours	Phase I	Bayer-Schering/Immunogen
IMGN529 K7153A-SMCC-DM1	Humanised IgG1 (Immunogen resurfacing)	MCC thioether non-cleavable linker	DM1	Tubulin inhibitor	CD37	Haematological tumours	Non-Hodgkin's lymphoma	Phase I	Immunogen
IMGN853	Humanised antibody	n/a	DM4	Tubulin inhibitor	Folate receptor 1	Solid tumours	Ovarian cancer, non-small cell lung cancer and FOLR1 positive epithelial malignancies	Phase I	Immunogen
MDX-1203	Human IgG (Medarex UltiMab transgenic mouse technology)	Peptide cleavable Val-Cit linker	CC-1065 (rachelmycin)	DNA minor groove alkylator	CD70	Solid tumours	Advanced/recurrent clear cell renal cell carcinoma or relapsed/refractory B-cell non-Hodgkin's lymphoma	Phase I	Bristol Myers-Squibb

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Table 1. Clinical stage ADCs (continued).

Product international non-proprietary Name, synonyms	Antibody type	Linker/linker type	Drug conjugate	Drug mode of action	Target antigen	Tumour type	Application or indication	Approval year or latest clinical phase*	Company/Licensee
PSMA ADC	Human IgG (Abgenix Xenomouse transgenic mouse)	Peptide cleavable Val-Cit linker		Tubulin inhibitor	PSMA	Solid tumours	Prostate cancer	Phase I	Progenics/Seattle Genetics
RG7450	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Prostate cancer	Phase I	Genentech/Seattle Genetics
DSTP3086S	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Ovarian cancer	Phase I	Genentech/Seattle Genetics
RG7458	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Ovarian cancer	Phase I	Genentech/Seattle Genetics
DMUC5754A	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Ovarian cancer	Phase I	Genentech/Seattle Genetics
RG7593	Humanised IgG1	Peptide cleavable Val-Cit linker		Tubulin inhibitor	CD22	Haematological tumours	Haematological malignancies	Phase I	Genentech/Seattle Genetics
DCD12980S	n/a	n/a		Tubulin inhibitor	CD79b	Haematological tumours	Haematological malignancies	Phase I	Genentech/Seattle Genetics
RG7596	n/a	n/a		Tubulin inhibitor	CD79b	Haematological tumours	Haematological malignancies	Phase I	Genentech/Seattle Genetics
DCD54501A	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Multiple myeloma	Phase I	Genentech/Seattle Genetics
RG7598	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Multiple myeloma	Phase I	Genentech/Seattle Genetics
RG7599	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Non-small cell lung cancer; ovarian cancer	Phase I	Genentech/Seattle Genetics
DNIB0600A	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Non-small cell lung cancer; ovarian cancer	Phase I	Genentech/Seattle Genetics
RG7600	n/a	n/a		Tubulin inhibitor	Not disclosed	Solid tumours	Pancreatic, ovarian cancers	Phase I	Genentech/Seattle Genetics
SAR566658 huDS6-DM4	Humanised IgG (Immunogen resurfacing)	n/a		Tubulin inhibitor	DS6 (MUC1 analogue)	Solid tumours	DS6-positive and refractory solid tumours	Phase I	Sanofi-aventis/Immunogen
SGN-75	Humanised IgG1	MC non-cleavable linker		Tubulin inhibitor	CD70	Haematological tumours	CD70+ non-Hodgkin's lymphoma or renal cell carcinoma	Phase I	Seattle Genetics
Undisclosed	n/a	n/a		Tubulin inhibitor	n/a	Solid tumours	n/a	IND submitted	Genentech/Seattle Genetics

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Table 2. Major developers of drugs for ADCs.

Developer	Warhead	Mode of action
Seattle Genetics	Auristatins	Tubulin polymerase inhibitor
Immunogen	Maytansines	Tubulin depolymerisation
Pfizer	Calicheamicins	DNA cleavage
Bristol-Myers Squibb	Duocarmycins	DNA minor groove alkylating agent
Syntarga (Synthon)	Duocarmycins	DNA minor groove alkylating agent
Spirogen Ltd.	PBD dimers	DNA minor groove cross-linker
Wilex (Heidelberg Pharma)	α -Amanitin	RNA polymerase II inhibitor

PBD: Pyrrolobenzodiazepine.

pursued with CBI duocarmycins, with comparable results of tumour growth delay at 15 mg/kg [19].

PBDs are a class of naturally occurring anti-tumour antibiotic found, like the duocarmycins, in *Streptomyces*. They were first isolated, structurally elucidated and synthesised by Leimbruber and co-workers in the late 1960s. The molecules bind sequence-selectively in the minor groove of DNA at purine-guanine-purine motifs forming a covalent amination linkage to the N2 of guanine. Joining two PBD units together via a flexible polymethylene tether allows the PBD dimers to cross-link opposing DNA strands producing highly lethal lesions. The non-distortive nature of PBD adducts requires them to be repaired via a homologous recombination repair mechanism, which is frequently impaired in human tumours as opposed to normal tissue. The potency of PBD dimers can be further enhanced by introducing unsaturation about the C2-position of the PBD C-ring and installing substituents that are directed along the floor of the DNA minor groove. Several PBD dimers have exhibited sub-nanomolar/picomolar activity against a range of human tumour cell lines and have demonstrated curative action in human tumour xenograft models. A lead PBD dimer, SG2000, is currently undergoing Phase II clinical [20] trial for the treatment of cisplatin refractory ovarian carcinoma and Phase II leukemia trial is scheduled for later in 2012.

2.2.3 Other drug targets

α -Amanitin, a bicyclic octapeptide RNA polymerase II inhibitor is being investigated as an alternative ADC warhead. Conjugation of α -amanitin to lysines on antibodies, via a stable proprietary linker system, prevents the liver toxicity associated with hepatocyte uptake of free α -amanitin. The α -amanitin-based ADC, Her-DSC-30.0134, where drug is attached to the antibody trastuzumab, with a drug:antibody ratio (DAR) of 4, has exhibited picomolar activity against SKOV33 and SK-Br-3 cell lines. The ADC appears promising as a single 5.7 mg/kg dose of Her-DSC-30.0134 demonstrated curative activity against the herceptin/lapatinib-resistant JIMT-1 xenograft model.

2.3 Antibody–drug linkage

Optimisation of antibody–drug linkage is a pivotal and often understated part of ADC design and development. Sophisticated function is required for the linker itself. Moreover, the chemistry of linkage to the drug and the means of release when the ADC reaches its target cell are all vitally important for success. First, the linker must be capable of attachment to both the targeting element and the drug without affecting the function of either; ideally resulting in a homogeneous ADC in terms of DAR and biophysical properties. Second, it is essential that the linker is stable during storage and in blood during treatment. Third, there must be a strategy for separating the drug from antibody after internalisation in the target cell.

2.3.1 Antibody conjugation site

The antigen binding site of an antibody has been considered as a carrier for derivatized drugs, taking advantage of the high affinity for small haptens that can be achieved in some circumstances [21]. Recombinant DNA technology allows a wide range of antibody-based structural formats and so introducing a number of such binding sites into an ADC is not a major obstacle. More importantly, the drug should not dissociate from the targeting element before it reaches the target cell. Perhaps for these reasons (and historically to obtain high DARs), chemical conjugation has, to date, been the more popular and clinically validated approach.

For chemical conjugation, the linker–drug moieties are generally attached to the targeting antibody either at lysines or thiols. Alternative linking sites may include carbohydrate, glutamine [22] and SNAP-tag [23]. However, as asparagine197-linked carbohydrate is critical to Fc γ R binding (but not to FcRn binding), this has been avoided as an attachment site to Fc. Reactive surface lysine residues are abundant in antibodies (e.g., there are 88 lysines in trastuzumab) [24]. Indeed, lysine has been a common point of conjugation for radioisotope attachment to antibodies using chelating agents. For example, 90Y-ibritumomab tiuxetan (Zevalin) is generated by covalently attaching the chelator, MX-DTPA, to surface lysine residues of the anti-CD20 antibody [25]. Here a low attachment ratio is optimal because conjugate clearance to the liver and spleen and the loss of the positive charge associated with the reactive amine group is minimised [26,27].

In the case of trastuzumab–emtansine [28,29] (trastuzumab-DM1, T-DM1, Table 1) TDM-1, antibody lysine side chains are functionalised with the *N*-maleimidomethylcyclohexane-1-carboxylate (MCC) linker. The maleimide group then reacts with a thiol group attached to the maytansinoid warhead. Alternatively, in gemtuzumab ozogamicin (Mylotarg[®], Table 1) and inotuzumab ozogamicin (CMC-544, Table 1) the calicheamicin warhead is attached directly to the antibody lysine via an amide bond. Drug conjugation via surface lysines in T-DM1 results in a DAR of 3.5 but the process results in partial modification of at least 47% of lysine residues in regions of high solvent accessibility and structural

flexibility [30]. This light smear of chemical perturbation over the antibody surface will ensure that any loss of function at a particular conjugation site will not destroy overall ADC functionality. At pH 6 (when lysine side chains are protonated), amine coupling is focused at the α -amino group of the N-terminal residue [31]. This technique has not been described for ADCs, possibly because N-terminal adducts may be too close to the antigen-binding region of IgG.

The use of free thiols for ADC attachment offers possibilities for more controlled and site-specific attachment. Free thiols may be generated on antibodies in a number of ways:

- Modification of surface lysines [32] using:
 - Traut's reagent (2-iminothiolane)
 - *N*-succinimidyl *S*-acetylthioacetate (SATA)
 - *S*-acetylmercaptosuccinic anhydride (SAMSA)
- Reduction [33] of inter-chain disulphides with:
 - dithiothreitol (DTT)
 - 2-aminoethylisothiuronium bromide (AET)
 - tris(2-carboxyethyl)phosphine (TCEP)
- Genetic modifications to:
 - introduce an unpaired cysteine at a specific surface location [33-41]
 - to introduce an unpaired selenocysteine whose reduction is compatible with retention of native disulphide bonds [34]
- Incorporation of unnatural amino acids as sites of drug attachment.

Conjugation to reduced inter-chain disulphides (compared with lysine conjugation) focuses drug attachment on the (H-H) and (H-L) inter-chain disulphides. ADC chemical heterogeneity is thereby reduced, but not eliminated, because the four reducible disulphide bonds (in IgG1) give rise to eight potential free thiols for conjugation. It is possible that this heterogeneity could be reduced further. The H-L disulphide bond is more readily reduced by DTT than the H-H bond [42] and, vice-versa, the reduced H-L disulphide is more readily re-oxidised by 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) [33]. Partial reduction will place drug preferentially on the H-L axis, whereas reduction/reoxidation will focus drug on the H-H axis. However, little difference in maximum tolerated dose (MTD) was noted in the one example where attachment to these two axes was compared [33]. It should be noted that removal of the hinge region inter-chain disulphide bonds from an IgG1 has been reported to greatly impair ADCC, consistent with impaired Fc-Fc γ receptor interactions, and also to reduce CDC [37,43]. Therefore, options for inter-chain disulphide reduction need to be considered carefully.

The anti-tumour efficacy of the anti-CD30 antibody cA10 (brentuximab) conjugated to MMAE with a DAR of

4, was as effective as the ADC with DAR of 8. However, the MTD was doubled and the clearance rate was one-third [44]. Thus, a lower DAR can improve the PK of ADCs, without loss of efficacy, by reducing clearance rate and increasing *in vivo* exposure. Interestingly, the cysteine-coupling location of the drug moiety had little bearing on this pharmacodynamic effect [45]. In recent ADCs, DAR refinement to improve ADC PK has prompted design of novel conjugation technologies to avoid a significant component of naked antibody [35,46].

The question of whether antibody stability is compromised by disulphide reduction remains. Antibodies are likely to differ in this respect. Each of the IgG isotypes is bridged between the heavy and light chains (two S-S bridges per IgG, four cysteines in total), however, the number of cysteines in the hinge region that may contribute to inter-heavy chain bridging varies. IgG1 has 2 inter-heavy chain disulphide bonds, IgG2 has 4, IgG3 has 11 and IgG4 has 2. It is known that loss of the single disulphide bond can destabilise a recombinant Fab [47]. In the absence of such a bond, the affinity of reduced, iodoacetylated heavy and light chains may be dependent on the variable region sequences and their contribution to binding affinity [48]. Thus, there will be a case-by-case variation in the biophysical stability of an ADC based on inter-chain disulphide bond reduction and alkylation. This must be taken into account in antibody selection for ADC construction. Inter-heavy chain disulphide shuffling and its possible effects on antibody function are an additional risk specific to IgG2 [49]. The formation of monovalent half molecules by disulphide bond scrambling in IgG4 is another risk of the disulphide bond reduction strategy of ADC construction [49,50]. However, it is possible that this can be addressed, because a reduced disulphide bond and re-stabilised by alkylation with a bis(thiol)-specific reagent, generating a 3-carbon bridge [49,51].

One of the consequences of drug-linker attachment onto random reactive groups on the surface of an antibody, or by partial reduction of inter-domain thiols is that it can lead to heterogeneity of loading stoichiometry, with some antibody not having any drug loaded at all. There are several examples: in the case of Mylotarg (Table 1) conjugation via surface lysines gives an average DAR of 2 – 3 but approximately 50% of the antibody is loaded with 4 – 6 moles calicheamicin per mole of antibody so 50% of Mylotarg is unconjugated antibody [52]. CMC-544 (Table 1) has a DAR of 5 – 7 mol of calicheamicin dimethyl hydrazide per mol antibody but less than 10% of the antibody is unconjugated [53]. T-DM1 has an average DAR of 3.5 with a heterogeneity of 1 – 7 modified lysine residues/antibody [24]. Conjugation at inter-domain thiols can also lead to heterogeneity of loading. Adcetris[™] (brentuximab vedotin) has a DAR of approximately 4 [54] but each antibody has a drug-linker distribution from 0 to 8 [55].

Further focusing of the position of drug attachment via thiols has been achieved by reducing the number of cysteines that

normally form the inter-domain disulphide bridges, or by genetic insertion of an unpaired cysteine, or introduction of C-terminal selenocysteine. For example, McDonagh *et al.* [45] used Cys-Ser replacement at inter-chain disulphide bonds to generate variants of brentuximab. Cysteines that normally form inter-chain disulphides were reduced from 8 to 4 or 2 and ADCs were generated with near quantitative, homogeneous conjugation at the remaining cysteines. Retention of Fc effector function however, requires the inter-heavy chain disulphide bonds [43], suggesting that FcR-dependent effector functions could be affected by saturation reduction and alkylation at this location.

McDonagh *et al.* noted that other groups had previously attempted to introduce unpaired thiols on antibodies for site-specific conjugation [36,38]. This approach has been pursued since by others [39–41] but developed furthest for ADC conjugation in THIOMAB technology. Here, several locations on the surface of IgG have been defined where cysteine residues might be introduced at the genetic level to allow site-specific attachment of drug without affecting structural integrity [35,46,56]. Attachment at one or more locations has been tested with a number of antibodies with interesting results that highlight the sensitivity of ADCs to conjugation differences. Comparison of three different engineered cysteines on herceptin/trastuzumab has exposed significant differences in the chemical stability of conjugates. Unpaired cysteine was introduced to replace kappa chain valine 205, heavy chain alanine 114 or heavy chain serine 396. Maleimide conjugation at C205 was found to be stable, whereas conjugation at C396 was least stable in serum, with apparent exchange onto other plasma protein free thiols including albumin. The lower stability correlated with increased liver toxicity, presumably by cytotoxin–albumin conjugate uptake. This appears to underline the importance of the reversibility of the maleimide reaction, and the increased susceptibility to reversal *in vivo* in highly solvent-exposed conjugation sites.

An elegant alternative site-specific labelling technology employs incorporation of selenocysteine (Sec) at the C-terminus of an IgG Fc region by introducing a Sec insertion sequence (SECIS) element into the mRNA 3'-untranslated region leading to the stop codon being used as a Sec insertion codon. The nucleophilic selenol group of Sec (pKa 5.2) can then be utilised as a specific attachment location [34]. Another interesting approach to site-specific drug attachment is the incorporation of unnatural amino acids. For example, using Ambrx technology [57], p-acetylphenylalanine has been incorporated into Herceptin Fab at lysine169 and serine202 encoded by an amber codon, facilitating attachment of hydrazine or aminooxy groups to produce a hydrazone or oxime adduct, respectively. However, while much effort is being spent to direct conjugation to specific locations and provide more homogeneous conjugation, it has been shown that the stoichiometry of drug:antibody loading can be more important for efficacy

than the loading positions [42], although the latter may be more relevant for manufacturing and product homogeneity.

2.3.2 Mechanism of conjugation: linker chemistry

Drug warheads can be linked to the antibody by various means. For attachment to antibody thiols, the N-terminus of MMAE has been functionalised with a self-immolative para aminobenzyl (PAB) group which in turn is attached to a cathepsin-labile valine–citrulline dipeptide. The N-terminus of the valine group is coupled to a caproic maleimide group to complete the MMAE–linker molecule. The MMAE–PAB–valine–citrulline caproic maleimide unit comprises a flexible building block for attachment. MMAE has been conjugated in this fashion to brentuximab to produce the recently Food and Drug Administration (FDA)-approved brentuximab vedotin (Adcetris). Alternatively, MMAF-based ADCs are still active, even in the absence of the cleavable valine–citrulline and PAB components of the linker, and the drug can be linked to antibody directly using the caproic maleimide unit (MC-MMAF). This is being used for a number of ADC conjugates (Table 1) and provides useful properties to the ADC (see later below).

As noted earlier, the C3 *N*-acyl-*N*-methyl-L-alanyl side chain of maytansine can be modified to carry a disulphide 'handle', that is, a disulphide that can be incorporated in linkers designed to be cleaved by thiols inside target cells. Addition of alkyl groups adjacent to the disulphide has been shown to increase the half-life of maytansine–ADCs in circulation [58]. Alternatively, maytansines can be linked to antibody by thioether linkages. For example, in T-DM1 the maytansine DM1 is linked to antibody via a bifunctional MCC linker that attaches to lysine residues on the antibody surface. The thiol group in DM1 is then reacted with the maleimide group of the linker to form the thioether bond.

For PBDs, the dimer warheads (e.g., SG2000, Figure 2) have been functionalised with linkers designed to facilitate conjugation to antibodies. For example, linkers have been attached at the C2-anilino substituents (e.g., SG3211, Figure 2) terminating in an antibody-reactive group, for example, maleimide.

2.3.3 Release of drug: linker chemistry

Once the drug is linked to antibody, the next challenge is to ensure active drug release following internalisation into the target cell. This can be achieved using both cleavable and non-cleavable linkers but the mechanisms are different. With cleavable linkers, drug is released from the antibody carrier as a consequence of the linker chemistry and the cell microenvironment. For example, the relatively acidic conditions in the lysosome act to cleave the linker in Mylotarg at a pH sensitive hydrazone. In other cases, there may be a reduction of a disulphide bridge as occurs with the SPP-DM1, SPDB-DM4 linkers (Table 1) due to the relatively high levels of glutathione inside cells compared with serum [13] or, as in the MC-vc-PAB linker in Adcetris, after cleavage of

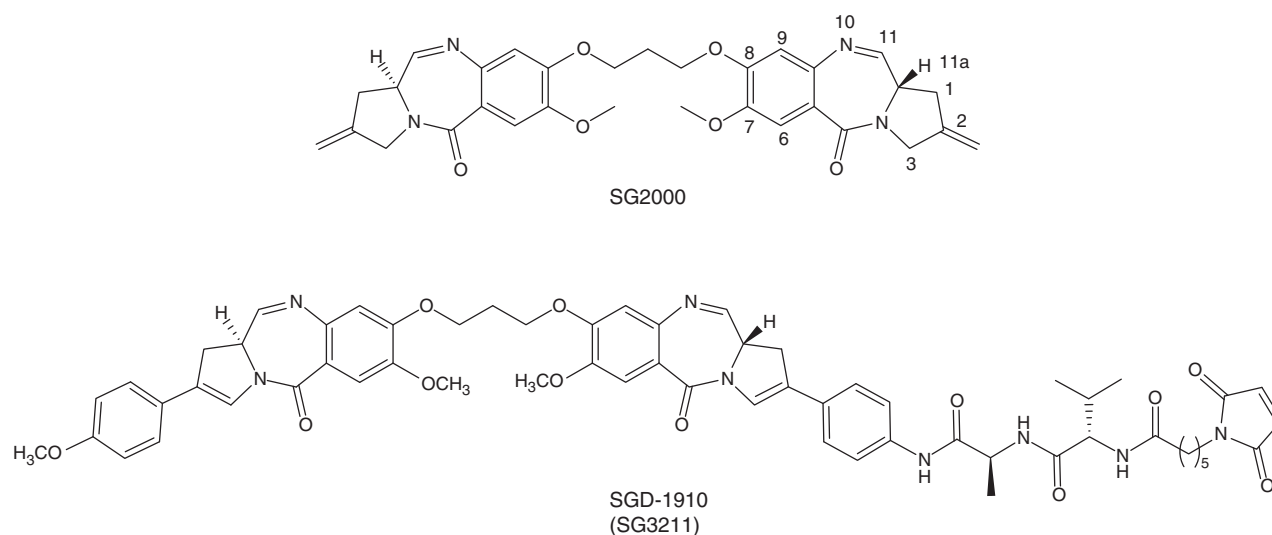


Figure 2. Pyrrolobenzodiazepine warhead SG2000 and C2 pyrrolobenzodiazepine-linker SGD-1910 (SG3211).

the valine-citrulline di-peptide that forms part of the linker. In some cases, there may be two release events: the first acts to detach the drug from the antibody and the second leads to activation of the drug from a prodrug form. In Mylotarg, for example, pH-dependent cleavage of the hydrazone is followed by reduction of a disulphide bridge in the calicheamicin warhead [59]. In the duocarmycin ADC MDX-1203, release occurs by the separate actions of a lysosomal protease then a carboxylesterase [60,61].

In the linker class described as ‘uncleavable’, separation of antibody and drug occurs by degradation of the antibody protein in the lysosome leaving the drug attached to the conjugated amino acid. For example, lysine in the case of T-DM1; and cysteine in the case of the MC-MMAF-based ADCs such as SGN75 and AGS-16M8F.

The more recent PBD ADCs can be formatted in either cleavable or uncleavable linker formats. Linkers at the N10 position and located on C2-anilino substituents must be fully cleavable in order to liberate an active PBD, while the aromatic tether can accommodate both cleavable and non-cleavable linkers.

The nature of the linker will influence the potential for extracellular drug release from ADC leading to off-target toxicity. While extracellular drug release prior to antibody localisation is clearly undesirable, drug release following localisation and internalisation into the target cell can lead to desirable bystander effects. In some cases, the drug, once separated from the antibody, is thought to be released from the targeted cell by active efflux mechanisms via p-glycoprotein. The subsequent killing of neighbouring cells is believed to be a factor in the pharmacodynamics and efficacy of many drugs being considered as ADC warheads [62]. In other situations, the chemical nature of the drug itself, or its metabolites, may allow drugs to diffuse from the targeted cell after release from the antibody

carrier. This is believed to be a factor in DM1 release when conjugated to a disulphide-containing SPDB linker [63]. In other cases, the nature of the drug and the cleavage mechanism means the drug cannot be cleaved outside the cell or cannot escape from the cell (e.g., MC-linked MMAF and MCC-linked DM1). Where extracellular dissociation or drug efflux does occur, this could lead to drug uptake by adjacent tumour and non-tumour cells as well as leakage back into circulation. Thus, there is a range of considerations to be addressed when choosing appropriate drug-linker combinations and the choice will be affected by the nature of target antigen and tumour (see below).

3. Drug targets

Key issues in selecting antigen targets for ADC efficacy are: i) appropriate antigen density on the tumour cell surface and lack of biologically available target on crucial tissue elsewhere, providing desired specificity; ii) appropriate rate of internalisation after antibody binding followed by mechanisms of trafficking that promote active drug release from the antibody; iii) ability to address tumour heterogeneity by presence throughout the tumour or on areas of most concern in terms of malignant progression. The issue of antigen heterogeneity, coupled with the factors involved in solid tumour penetration, suggests that lymphoid tumours will be more tractable for treatment with ADCs (e.g., Mylotarg, Adcetris, CMC-544) but several solid tumour targets are also being assessed (Table 1) [64,65]. If ADCs are to be successful, there is a need to identify further useful tumour targets with the features mentioned above [66].

A common feature of cell surface targets for therapeutic antibodies in oncology is that they are not strictly tumour-specific. Targets may be expressed on cells in other tissues, which may

lead to unwanted effects. A search for tumour-specific targets continues but an alternative approach to greater tumour specificity may be made by targeting two different antigens on the tumour cell using bispecific targeting platforms. Together with ADCs, bispecific antibodies are undergoing a renaissance and there are now a range of new formats. Many bispecifics are being developed for immune effector cell recruitment via CD3 or CD16 specificity but there is an opportunity to use molecules with specificity for two tumour-associated antigens and provide superior targeting specificity while reducing off-target effects. Depending on the targets there may also be additional benefits in blocking ligand binding at two targets on the tumour cell. Whatever format of targeting element is used, the need for a high specificity index is essential.

4. Manufacture

ADCs are relatively complicated products which require careful assembly. In some cases, preparation of antibody and drug–linker and the final conjugation are done at different sites by specialist companies. Production of antibody at large scale and at reasonable cost of goods can now be achieved [67]. It might be expected that the major pharmaceutical companies that are now interested in antibodies as products and that have begun to enter into supply deals with the ADC drug providers would have facilities for the assembly and formulation of ADCs.

5. Commercial development of clinical pipeline

Over the last 18 months (to March 2012), there have been a number of significant developments with ADCs. In 2010, Mylotarg [68–70], the first ADC product approved, was voluntarily withdrawn from the market. Mylotarg was approved in the USA by the FDA in 2000 under the accelerated approval procedure for the treatment of patients 60 years and older in first relapse with CD33⁺ acute myeloid leukaemia (AML) who are not considered candidates for cytotoxic chemotherapy [71]. Statements by Mylotarg's developer Pfizer [72,73] noted *'The approval of single agent Mylotarg in the U.S. was granted under FDA's accelerated approval regulations based on overall response rate in three non-comparative studies and required submission of additional data to confirm clinical benefit. The required post-approval study (SWOG S0106) [74] combining chemotherapy and Mylotarg did not demonstrate improved survival compared with chemotherapy alone in patients with previously untreated AML'*. Very recently, however, a conference report [75,76] has identified that Mylotarg may be beneficial in new studies. Pfizer has noted [77] that it is discussing, with FDA, future courses of action for Mylotarg.

In 2010, Genentech submitted a Biologics Licence Application (BLA) to the US FDA for trastuzumab-DM1 for treatment of patients with advanced HER2-positive breast cancer who have previously received multiple HER2-targeted

medicines and chemotherapies [78]. However, the FDA issued a 'Refuse to File' letter for accelerated approval for the company's T-DM1 ADC [78]. Genentech noted [79] that in its review of the BLA, the FDA stated the T-DM1 trials did not meet the standard for accelerated approval because all available treatment choices approved for metastatic breast cancer, regardless of HER2 status, had not been exhausted in the study population. Genentech are now performing additional studies with T-DM1 and have noted results from a Phase II study in people with previously untreated HER2-positive metastatic breast cancer, as well as noting other Phase III studies. Most recently, good initial results for the EMELIA Phase III study have been reported [80]. This international randomised open-label Phase III study, comparing T-DM1 alone with lapatinib plus Xeloda® (capecitabine), showed significant improvement in progression-free survival. Genentech plan to submit a BLA for T-DM1 in 2012 based on the EMELIA [81] study.

Recently, Seattle Genetics' Adcetris [54,82,83] gained accelerated approval for two indications: the treatment of patients with Hodgkin's lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates [55], and the treatment of patients with systemic anaplastic large cell lymphoma (ALCL) after failure of at least one prior multi-agent chemotherapy regimen [84]. The approval was based on a study in Hodgkin's lymphoma of 102 patients that showed a 75% overall response (OR) rate (34% complete remission (CR); 40% partial remission (PR)) and a 58 patient study in ALCL showing an 87% OR (57% CR; 30% PR) [85]. As with Mylotarg, further studies will be needed to obtain full approval. Adcetris comprises a chimeric antibody and there have been indications of transient immune reaction to the antibody element [54]. Whether this becomes an issue for Adcetris remains to be seen. Several other recombinant antibodies stimulate some level of immune reaction with no obvious severe impediment to function [86,87].

Following along behind these product candidates there is now a relatively long clinical pipeline of other ADCs (Table 1) of which Pfizer's CMC-544 [70,88] is now in Phase III studies [89]. This list is rapidly enlarging with companies such as Genentech [90] and Seattle Genetics [91] noting substantial preclinical products and collaborations. It is expected that several more candidates will enter clinical development during 2012 and the near future, although as with most areas of pharmaceutical development some candidates will not be continued (e.g., recent notices of discontinuation of clinical development of the ADCs IMGN388 [92] and BAY79-4620 [93]).

In addition to drug development progress, there have been a number of business activities indicating general interest in this product group. Recently, Heidelberg Pharma and Syntarga, both developing new drugs and linkers, have been acquired by Willex [94] and Synthon [95], respectively. Spirogen

Ltd. recently announced a collaboration with Genentech on the discovery and development of ADCs using Spirogen's PBD drugs and associated linker technology [96]. A single Spirogen PBD has recently been positively reported by Seattle Genetics as a potent anti-CD70 ADC in models of renal cell carcinoma [97]. Both Seattle Genetics and Immunogen have announced further collaborations, providing large Pharma with access to their technologies. Recently, Seattle Genetics and Oxford Biotherapeutics entered an agreement [98] to jointly discover novel ADCs. Under the collaboration, Oxford Biotherapeutics is to generate panels of monoclonal antibodies against novel tumour-specific antigens identified using its Oxford Genome Anatomy Project (OGAP[®]) database which will then be screened for activity using Seattle Genetics' ADC technology. The OGAP proteomic database includes information on over 7500 cancer membrane proteins.

6. Expert opinion

After decades of independent development, antibodies and small chemical toxins are being successfully conjugated to create a new class of targeted anti-cancer missiles. The concept of synergistically combining the specificity of antibodies with the lethality of ultra-toxic chemicals is not new but a key development is that the utility of ADCs has now been exemplified in the clinic. Clinical feasibility, combined with the discovery and design of new warheads and linkers, has fuelled the research field leading to many promising new ADC combinations. Despite the market withdrawal of Mylotarg in 2010, the principle of highly potent armed antibodies for tumour therapy has been well made and has opened up the antibody arena for a new harvest of effective therapeutic modalities.

The ultimate goal must be successful treatments for a broad patient group, either as personalised medicines, based on individual tumour expression of the ADC target antigen or by identification of tumour-associated targets with broad patient group distribution. Target selection is a key factor in achieving these goals. Targets should not be down-regulated during therapy and, for ADCs, a further limitation in antigen selection is the requirement for internalisation; a feature of some, but by no means all plasma membrane targets.

Knowledge of how an individual ADC behaves *in vivo* is critical for success. Information on how the ADC accesses and interacts with the target antigen on the tumour, how it internalises, how soluble target interacts and competes and how off-target binding and non-tumour ADC uptake translates into off-target toxicity is vitally important. Antibody selection is crucial here. While technical developments in antibody technology in the last 20 years have enabled generation of high affinity, highly selective, humanised antibodies, identifying the most appropriate target for these antibodies when used as ADCs remains a challenge. Nonetheless, clinically proven and internalising antibodies, such as trastuzumab, provide an excellent starting point for research in this area.

New ADC candidates are being rapidly generated as more novel ultra-cytotoxic drugs are emerging along with superior methods of attaching the drug to the targeting agent in order to optimise drug antibody ratios, improve product homogeneity and reduce undesired dissociation from the antibody prior to targeting. The linker between each antibody and the drug is a unique biochemical entity. Understanding how this interface will impact on ADC biology and utility is fundamental for success. Significant reductions in non-specific or off-target toxicity are most likely to be achieved by close attention to these linking technologies. Therefore, it is possible that the next advances will be made by utilising recent knowledge on the chemistry of conjugation and linker design. The avoidance of linker cleavage until the ADC has entered the acidic endosome is now a possibility. However, the reversibility of the maleimide reaction suggests that further scope for toxicity reduction may be found in selection of the protein-reactive moiety on the linker. Recent research into the effect of drug linkage sites on linker stability and the effect of drug attachment stoichiometry on PK of the ADC, shows that basic chemical studies of the ADC and the understanding gleaned from them has an important translational impact on therapeutic success.

The growing body of preclinical and clinical data for these new products will guide further design to improve specificity and reduce off-target effects. Improvements could be achieved by better initial targeting, improved linker design to avoid detachment of drug prior to targeting, better retention of drug within the targeted cell and an increased understanding of the catabolic routes of ADCs, which may involve further consideration of FcR interactions.

In the short to medium term, it is expected to see more ADC candidates entering clinical trials and increased adoption of this class of products among the major pharmaceutical companies, whose long experience in drug design and development can lead to new generations of drug and linker design. The current late-stage candidates should provide further product approvals in the next 2 – 3 years with the earlier-stage clinical pipeline providing several further products in the ensuing years, further validating the approach. Late-stage clinical trials for some of these products will take several years but while there may be a period of consolidation as the pipeline develops and new product formats are tested in the clinic, the potential returns for the industry in this area of therapy should provide the impetus for continued development. The significant level of ADC development combined with the strong pipeline of drug candidates and growing engagement by large Pharma give promise that the next few years will deliver a number of exciting new approved ADCs to treat a range of cancers.

Declaration of interest

JR Adair is a consultant at Ithaka Life Sciences, which provides consultancy services to Spirogen Ltd. PW Howard is a

founding scientists and head of targeted therapeutics at Spirogen Ltd., which is involved in research and development of pyrrolbenzodiazepines and related antibody–drug conjugates. DG Williams is an employee of Spirogen Ltd. JA Hartley is a founder scientist of Spirogen Ltd., has an equity interest in the company and is paid as a consultant. In addition, this author has received grants from Cancer research UK, the

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