Pharm Res (2012) 29:2972–2984
DOI 10.1007/s11095-012-0810-9

EXPERT REVIEW

# Optimising the Delivery of Tubulin Targeting Agents through Antibody Conjugation

Gary D. Stack • John J. Walsh

Received: 2 March 2012 / Accepted: 11 June 2012 / Published online: 10 July 2012


**ABSTRACT** Despite their side effect profile, there currently remains a heavy reliance on traditional cytotoxics and particularly tubulin targeting agents in cancer chemotherapy. To address this concern, significant progress has been made in the selective delivery of drugs to the tumour site. This review will examine the published data in support of the hypothesis that forming antibody conjugates of tubulin targeting agents is an effective approach towards their more effective delivery to the tumour site. Particular emphasis will be placed on the diversity of concepts under investigation, the efficacy of resultant conjugates, evidence of decreased resistance and the side effect profiles of the conjugates.

**KEY WORDS** antibody-drug conjugates · anticancer · resistance · tubulin targeting agents · tumour targeting

**ABBREVIATIONS**

| | |
|---|---|
| ADC | antibody-drug conjugate |
| ALCL | anaplastic large cell lymphoma |
| BMPEO | *bis*-maleimido-trioxyethylene glycol |
| DM | drug maytansinoid |
| EGFR | epidermal growth factor receptor |
| HER2 | human epidermal growth factor receptor 2 |
| IgG | immunoglobulin G |
| mAb | monoclonal antibody |
| MC | maleimidocaproyl |
| MDR-1 | multidrug resistance protein-1 |
| MMAE | monomethyl auristatin E |
| MMAF | monomethyl auristatin F |
| MTD | maximum tolerated dose |
| PAB | *p*-aminobenzyloxy carbonyl |
| PABC | *p*-aminobenzyl carbonyl |
| PEG | polyethylene glycol |
| PSMA | prostate specific membrane antigen |
| Siglec | sialic acid binding Ig-like lectins |
| SMCC | succinimidyl trans-4-(maleimidylmethyl) cyclohexane-1-carboxylate |
| SPDB | *N*-succinimidyl-3-(2-pyridylthio) propionate |
| SPP | *N*-succinimidyl 4-(2-pyridyldithio) pentanoate |
| vc | valine-citrulline |

G. D. Stack • J. J. Walsh (✉)
School of Pharmacy and Pharmaceutical Sciences
Trinity College Dublin
Dublin 2, Ireland
e-mail: jjwalsh@tcd.ie

## INTRODUCTION

Cancer patients with metastases typically present with approximately $10^{12}$ tumour cells and it has been established that greater than 99% of these need to be killed in order to achieve complete remission (1). Despite the emergence of novel treatments for malignancy, conventional chemotherapeutics and tubulin targeting agents in particular remain a cornerstone in many cancer treatment regimens. The major difficulty with these chemotherapeutic agents is that in order to reach that target of 99% cancer cell death, unacceptable toxicities are inevitable. Thus, there remains a significant challenge to develop new therapies that are suitably toxic to cancer cells while leaving normal cells relatively unscathed. The rationale behind the utilisation of antibody conjugation to achieve enhanced delivery of existing cytotoxics to the tumour site is two-fold. Firstly, by masking the toxicity of the drug until it reaches the active site, a lower incidence of side effects is expected. And secondly, by enhancing the specificity of the cytotoxic agent for the desired target cells greater efficacy may be attainable.

In this review the disparate antibody based methodologies utilised to optimise the delivery of tubulin targeting agents to the tumour site which have been published throughout the last ten years will be explored and critically appraised in terms of efficacy, therapeutic window and ability to overcome drug resistance.

## Tubulin Targeting Agents

Microtubules, which are the target of tubulin binding agents, consist of α and β tubulin heterodimers. They are hollow tubes approximately 25 nm in diameter that are involved in various cellular functions, most notably cell movement, intracellular transport and cell division (2). Microtubule dynamics are very tightly regulated both spatially and temporally (3). At the onset of mitosis in particular there are striking changes in the microtubule network in which the disassembly of microtubules is proceeded by the formation of a new network of spindle microtubules (3). Tubulin targeting agents are known to bind to one of three main binding sites on the β-tubulin subunit: the vinca domain, the colcichine binding site and the paclitaxel site (3). Once bound, these agents act as either microtubule-stabilising or microtubule-destabilising agents interfering with mitosis and leading to cell death. One reason that cancer cells are particularly sensitive to these drugs compared with normal cells is that many cancer cells divide much more rapidly than normal cells offering many more opportunities for tubulin binding agents to disrupt mitosis and cause cell death. Hundreds of compounds have been observed to arrest mitosis by interacting with microtubules. It has been shown that they act by potently suppressing microtubule dynamics (4).

Tubulin binding agents are principally natural product or natural product derived small molecule inhibitors that have diverse structural classes and origins. Lead compounds for these agents include substances derived from bacteria, plants, marine sponges and molluscs (2).

Two of the most clinically pertinent classes of tubulin binding agents are the taxanes and the vinca alkaloids. The vinca alkaloids were first identified over 50 years ago while the taxanes where first identified over 40 years ago, but yet both classes of agents still find significant utility in the first line treatment of many solid tumours and haematological malignancies (4,5).

Other tubulin targeting agents that have been evaluated for anticancer activity include the colchinoids and the epothilones (3). In more recent years, tubulin binding agents that display cytotoxicities many orders of magnitude greater than clinically used agents have been developed. New taxoids have been developed that demonstrate approximately 100-fold greater cytotoxicity than the parent compounds (6). The tubulysins isolated from various myxobacterial species are one such class of potent tubulin binding agents and demonstrate $IC_{50}$ values 20- to 1000-fold lower than many clinically used tubulin binding agents (7). The maytansinoids are another such class of compound. The lead molecule of this class, maytansine is an ansa macrolide first isolated from the Ethiopian shrub *Maytenus ovatus* by Kupchan *et al.* (8). Maytansine causes depolymerisation of microtubules and displays almost 100-fold higher cytotoxicity than the vinca alkaloids (9). Synthetic analogues of maytansine referred to as drug maytansinoids (DMs) have subsequently been developed which were found to possess 100- to 1000-fold greater activity than existing cytotoxic agents in the clinic (10,11). DM1 (*N*-methyl-*N*-[3-mercapto-1-oxopropyl]-L-alanine ester of maytansinol) is an example of one such maytansinoid which has been shown to be effective against a wide range of tumour cells with $IC_{50}$ values in the range of 10–100 pM (12).

Dolastatin-10 is another highly potent inhibitor of microtubule assembly and tubulin polymerisation and is a non-competitive inhibitor of the binding of vinblastine to tubulin (6). Synthetic analogues of dolastatin-10 known as auristatins are potent tubulin inhibitors with cytotoxicities 50- to 200-fold greater than the vinca alkaloids (6).

Despite the promise of tubulin binding agents as cancer therapeutic agents, there are a number of significant disadvantages to their use. Paclitaxel for example, while remaining one of the most clinically important agents currently in use, suffers from dose-limiting toxicity and multidrug resistance (5). The action of efflux pumps such as multidrug resistance protein-1 (MDR-1), which actively pumps paclitaxel from resistant cells, is known to cause subtherapeutic intracellular drug concentrations. Resistance is also known to occur due to changes in β-tubulin or apoptotic and mitosis checkpoint proteins (13). In order to overcome these difficulties various attempts have been made to specifically target tumour cells through the molecular conjugation of antibodies to existing tubulin targeting agents. By selectively delivering existing cytotoxic agents to tumour cells the hope is that more potent cytotoxicity to target cells may be achieved while simultaneously reducing systemic toxicity associated with these agents.

## ANTIBODY-DRUG CONJUGATES

In early antibody evaluation, a number of antibodies were produced that bound very specifically to various tumour cell surface antigens. However, in many cases such binding was insufficient to affect tumour growth (6). Two of the most effective monoclonal antibodies for the treatment of cancer currently licensed by the FDA are trastuzumab for breast cancer and cetuximab for colorectal tumours and squamous cell carcinoma of the head and neck, yet both these agents

offer only modest single agent activity (6,14). One way to take advantage of the selectivity of monoclonal antibodies for various cell specific antigens is to utilise molecular conjugation to attach a non-selective cytotoxic agent to a monoclonal antibody selective for tumour specific antigens. Various concepts along this theme have been explored but the most common approach involves the monoclonal antibody acting as a vector to the tumour cell, facilitating internalisation and downstream processing to liberate the cytotoxic effector molecule within the cell (6). This has led some commentators to refer to drug-antibody conjugates as tumour-activated prodrugs (6).

Antibody conjugation is considered to be one of the most promising approaches to increasing the anti-tumour activity of antibodies while simultaneously reducing the systemic toxicity of existing cytotoxics such as tubulin targeting agents (15). The various advantages and disadvantages of antibody-drug conjugates (ADCs) are summarised in Table I.

Despite early promise, the emergence of clinically successful drug-antibody conjugates has been somewhat slow due to the difficulties involved in successfully formulating these agents in presentations that can overcome the pharmacokinetic and pharmacodynamic impediments of these relatively large molecules. Durcy *et al.* (20) framed the characteristics of an ideal antibody-drug conjugate along five distinct headings:

1. *Circulation:* The antibody should be stable in circulation and should not have cytotoxic activity until intracellular release of the active drug.
2. *Antigen binding:* The monoclonal antibody should retain its high binding affinity for its intended target despite molecular conjugation to the cytotoxic agent.
3. *Internalisation:* A sufficient intracellular concentration of the ADC should be obtained to exact cell death. This can be challenging if the antigen is expressed only in limited numbers.
4. *Drug release:* Once internalisation has occurred, release of the cytotoxic agent should be timely and efficient.
5. *Drug action:* The drugs utilised in the ADC should be capable of causing cell death at subnanomolar concentrations.

The three constituent parts of an ADC are the antibody, the cytotoxic drug molecule and the linker connecting them as illustrated schematically in Fig. 1.

It has been suggested that early ADCs failed because of inappropriate drug selection, non-specific antibody selection and use of linkers that were not stable (21). Another difficulty with ADCs is that only a tiny fraction of administered ADC reaches the tumour site (6). Consequently, the cytotoxic agent used needs to be much more potent than currently used clinical agents. Thus, it became apparent that optimisation of each of these three variables was required in order to maximise the potential of ADCs.

## Drug Characteristics

Although antibodies often display high specificity for the tumour, most of them are not potent enough to be clinically efficacious. Imaging studies with radiolabelled antibodies have shown that the peak concentration of antibodies deposited at the tumour site was of the order of 0.01% of the injected dose per gram of tumour at 24 h (22). Therefore the cytotoxicity of the drug in an ADC must be several-fold higher than conventional anti-cancer drugs (6). It has been suggested that the cytotoxicity of the drug used in an ADC must be at the $IC_{50}$ level of 10–100 pM. Since paclitaxel displays an $IC_{50}$ of the order of 10 nM in a number of cell lines it has been suggested that it is not suitable for this approach (23).

The *in vitro* cytotoxicity ($IC_{50}$ values) of the maytansinoids and auristatins are even lower than the concentration of antibodies found to accumulate at the tumour site, making them suitable candidates for ADC synthesis. For this reason

**Table I** Advantages and Disadvantages of Antibody-Drug Conjugates

| Advantages | Disadvantages |
|---|---|
| Targeted binding specific for tumour antigen | Requires that tumour be tested for expression of target antigen |
| Highly potent agents can be selectively delivered to target cells | Molecular target may have some normal tissue expression |
| Wide therapeutic index | Linker may not be stable leading to premature cytotoxic agent release |
| Prolonged circulation half-life | Antibody may not reach target in sufficient concentration to be lethal |
| Stable in circulation | Antigen expression could be heterogeneous especially in solid tumours (17) |
| Decreased adverse effects | ADC must cross the vascular endothelium and circumvent the interstitial pressure within the solid tumour (18) |
| Bystander killing effect of endothelial cells and stromal cells essential to tumour growth | Antibody may itself be immunogenic |
| Reduced MDR-1 mediated resistance compared to systemic administration of the respective drug (16) | Masking of the target antigen with monoclonal antibody devoid of its cytotoxic payload in ADCs with poor stability (19) |

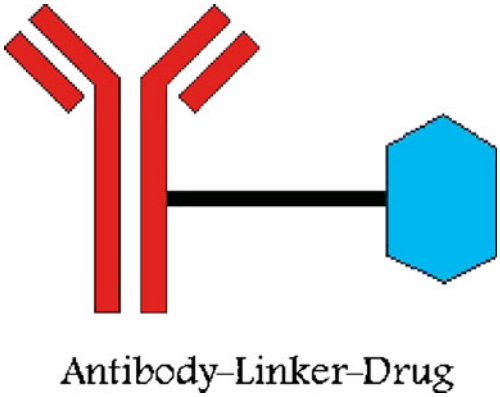


**Fig. 1** Schematic of an antibody-drug conjugate.

the maytansinoid DM1 [$N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine] has become one of the most widely utilised tubulin targeting agents in the synthesis of ADCs (24).

To take advantage of the long plasma half-life of IgG antibodies necessary to achieve maximal deposition at tumour sites the drug must be non-toxic in conjugated form, a requirement which both the maytansinoids and auristatins possess (6). Furthermore, these cytotoxic agents are readily linkable to an appropriate antibody and sufficiently water soluble and stable on storage (10).

Drug loading is another critical factor determining the therapeutic characteristics of an ADC (25). A series of conjugates of the anti-CD30 monoclonal antibody cAC10 linked to either 2, 4 or 8 monomethyl auristatin E (MMAE) molecules were synthesised (25). Although the *in vitro* toxicity of the resultant conjugates was directly dependent on the drug loading, *in vivo* the antitumour activity of the 4 MMAE antibody was comparable with the 8 MMAE containing conjugate while the maximum tolerated dose of the 4 MMAE containing conjugate was found to be double that of the 8 MMAE containing conjugate (25). It has also been demonstrated for other conjugates that the *in vivo* activity drops off significantly when less than three cytotoxic drug molecules per antibody are used while an upper limit is often imposed by the solubility characteristics of the resulting conjugate (12).

## Antibody Characteristics

The cell surface antigen which the antibody recognises is typically a glycoprotein, a carbohydrate, an oncoprotein, a growth factor receptor or a hormone receptor(1). The factors that need be considered in selecting an appropriate antibody target for the synthesis of an ADC include (1):

1. Degree of target expression on tumour cells compared to normal tissues.
2. Affinity of antibody for target.
3. Efficiency of internalisation following antibody binding.
4. Homogeneity of target expression on all target cells.
5. Degree of target shedding into circulation.

Many early monoclonal antibodies investigated as potential drugs lacked specificity. Some of these agents bound to antigens expressed on a wide variety of normal cells that were merely upregulated in various tumours. Cross-reactivity of the antibodies with normal tissues is thus of utmost concern when designing any ADC (1). One such conjugate associated with poor target antigen selection is bivatuzumab mertansine (26). This conjugate consists of the monoclonal antibody bivatuzumab covalently conjugated to the maytansinoid DM1. Bivatuzumab is a humanised monoclonal antibody (BIWA 4) that binds selectively to CD44v6. This cell surface antigen is highly and homogenously expressed on squamous cell carcinoma of the head and neck, oesophagus, lung, cervix and vulva (26). Its expression was also found in some normal tissues such as skin keratinocytes, squamous epithelium of the cervix, epithelium of the cornea and epithelium of the tonsils (26). Tijink *et al.* (26) evaluated this agent in patients with incurable squamous cell carcinoma of the head, neck or oesophagus. In this study, seven patients were administered a total of 23 weekly doses of bivatuzumab mertansine. Following completion of the study, one patient at 100 mg/m$^2$ and one at 120 mg/m$^2$ experienced stable disease during the treatment phase but also suffered severe skin toxicity in the form of desquamation. At the maximum dose level achieved, 140 mg/m$^2$, one patient developed toxic epidermal necrolysis after two infusions and died. It was concluded that expression of CD44v6 was not specific enough for tumour cells to allow the formulation of a safe antibody-based therapy and the development of this agent was discontinued.

Immunogenicity of antibodies is another issue which must be considered. Antibodies that are used in the construction of ADCs need to be humanised to avoid the potential of generating an immune response in the patient upon administration which further complicates the synthesis of these agents (1).

Being large molecular weight biomolecules, antibodies display certain physicochemical properties that are unfavourable to the crossing of biological barriers, thereby somewhat limiting their scope for *in vivo* applications (27). To affect solid tumours, ADCs must cross the vascular endothelium and circumvent the interstitial pressure within the solid tumour (27). Also, expression of target antigens can be heterogeneous throughout the tumour (27). Therefore, the pharmacodynamics of the large immunoglobulin containing ADC may impede its ability to penetrate deep into solid tumours which are often poorly vascularised (1). Smaller, recombinant monoclonal antibody structures such as single chain antibodies and diabodies have been shown to

penetrate into the tumour with greater efficiency than the parent antibodies (28). However, these smaller molecules are more rapidly cleared from the plasma resulting in shorter half-lives (29). In fact overall tumour accumulation of diabodies is found to be more than two-fold less than that achieved for corresponding IgG (15).

Modification of the structure of the monoclonal antibodies used in ADCs has also been attempted. An engineered thio-trastuzumab-DM1 conjugate has been developed (30) which possesses two free cysteine residues to facilitate the attachment of two molecules of DM1 to each thio-trastuzumab molecule *via* a non-reducible *bis*-maleimido-trioxyethylene glycol (BMPEO) linker. Trastuzumab is a recombinant human anti-epidermal growth factor receptor 2 (HER2) monoclonal antibody. HER2 is a receptor tyrosine kinase found to be overexpressed on the surface of breast cancer cells in ~30% of newly diagnosed patients and is found to be associated with a poor prognosis. Furthermore, 60–70% of breast cancer cell bone metastases overexpress HER2 (31). The conjugate retained similar *in vitro* cell proliferation inhibitory activity and HER2 binding properties to the conventional trastuzumab-succinimidyl trans-4-(maleimidylmethyl) cyclohexane-1-carboxylate-DM1 (trastuzumab-SMCC-DM1). Furthermore, the conjugate showed improved efficacy over the conventional trastuzumab-DM1 at DM1 equivalent doses and retained efficacy at equivalent antibody doses. The thio-trastuzumab conjugate also displayed a greater than two-fold reduction in toxicity in a safety study in rats compared with the conventional ADC. It is thought that this was due to the lower drug loading per antibody and the improved stability of the linker unit in circulation (30).

Previous experiments utilising these thio-mAbs led to the development of thio-anti-MUC16-maleimidocaproyl-valine-citrulline-*p*-aminobenzyl-carbonate-MMAE (thio-anti-MUC16-MC-vc-PABC-MMAE) (32). This conjugate contained a derivatised humanised anti-MUC16 monoclonal antibody engineered to contain two free thiol groups to which MMAE was conjugated *via* a MC-vc-PABC linker. The conjugate synthesised was found to be as efficacious as a conventional conjugate in a MUC16 ovarian cancer xenograft model in mice. Moreover, it was tolerated at higher doses in rats and cynomolgus monkeys than the same conjugate prepared by conventional approaches suggesting the potential of this strategy in the synthesis of more therapeutically favourable ADCs (32).

## Linker Unit Selection

For an antibody-drug conjugate to be effective the chemotherapeutic agent should remain non-toxic as part of the conjugate while in circulation but should be readily liberated on internalisation into the target cell (19). Thus, the choice of linker is of paramount importance in the synthesis of an ADC with a favourable therapeutic index. Several strategies have been employed to maximise the stability of ADCs in circulation while allowing for selective release of the tubulin binding agent at the desired target. Acid labile hydrazone linkers were among the early approaches to be considered however, in more recent times conjugates containing disulfide linkages, peptide based linkages and thioester linker units have found favour due to their greater stability in circulation. The advantages and disadvantages of each of these methodologies and others will thus be discussed in turn together with examples of how these technologies have been utilised to facilitate the selective delivery of the parent ADC to the tumour site.

### *Acid-Labile Linkers*

Early linkers evaluated include various acid-labile hydrazone linkers that utilised the acidic environment of endosomes for cleavage (19). Linkages such as those containing hydrazones have relatively short half-lives that can be significantly less than the expected circulating half-life of the ADC. This can present toxicity issues especially in the case of high potency cytotoxics such as MMAE (19).

Drugs linked to monoclonal antibodies *via* acid-labile linkers have been found to undergo approximately 50% drug release within 2 days upon incubation in human serum (19). Peptide and disulfide containing linkers were subsequently adjudged to be superior to these acid sensitive linkers due to the selectivity of cleavage of these linker types for the tumour intracellular environment (33).

### *Disulfide-Containing Linkers*

Disulfide containing linkers are very commonly employed in the synthesis of ADCs due to their stability at physiological pH (10). While this linker unit is thermodynamically very stable, it is kinetically labile in the presence of sulfhydryl containing functional groups (24). For example, as the concentration of glutathione, a sulfhydryl containing molecule, is 1000-fold higher within cells than in the circulation preferential intracellular drug release of conjugates containing this linker unit has been achieved (10,24).

The relative strength of the disulfide bond in various maytansinoid-antibody conjugates can be manipulated to achieve maximum stability in circulation while allowing for efficient cleavage inside the target cell (24). This is achieved by the introduction of methyl substituents on the carbon atoms geminal to the disulfide link conferring varying degrees of steric hindrance (24). Those conjugates where the methyl groups were located on the maytansinoid side of the disulfide bond were found to have superior activity *in vivo* than those with the methyl groups located on the antibody

side of the disulfide bridge despite the fact that both conjugates have identical stability in terms of the fate of the disulfide bridge (12).

The humanised monoclonal antibody, huC242, has been used in a series of experiments to synthesise conjugates with maytansinoids in order to explore the effect of the linker group on the activity of resultant conjugates (34). This antibody binds to the CanAg antigen expressed on colorectal, pancreatic and certain non-small cell lung cancer cells (34). Conjugates of huC242 and the maytansinoids DM4 and DM1 containing a cleavable disulfide linkage huC242-*N*-succinimidyl-3-(2-pyridylthio) propionate-DM4 (huC242-SPDB-DM4) (Fig. 2) and a non-cleavable thioether linkage (huC242-SMCC-DM1) (Fig. 3) were synthesised and evaluated *in vitro* and *in vivo* (34). It was found that both conjugates displayed comparable activity *in vitro* though huC242-SPDB-DM4 was significantly more active in multiple xenograft tumour models. It was concluded that both conjugates are efficiently degraded by lysosomes to release the free maytansinoid molecule together with a number of cytotoxic metabolites. It has been suggested that the enhanced activity of the disulfide containing conjugate is related to the formation of the potent metabolite, S-methyl-DM4, following lysosomal reduction of the disulfide bond. Lopus *et al.* showed that such maytansine derivatives are themselves potent microtubule poisons, interacting with microtubules as efficiently as, or more efficiently than, the parent molecules (35).

In further experiments conducted by Kovtun *et al.* (36,37), it was observed that huC242-DM1 conjugates with non-cleavable linkers such as SMCC (Fig. 3) had similar *in vitro* activity to disulfide containing *N*-succinimidyl 4-(2-pyridyldithio) pentanoate (SPP) linked conjugates (Fig. 4) but were not as active *in vivo*. Considerable evidence suggests that a bystander killing of neighbouring cells is responsible for this disparity (36). It was found that conjugates coupled *via* a reducible disulfide linker unit were capable of this effect while similarly potent conjugates linked *via* a non-reducible thioester bond were not. It has been suggested that this mechanism may overcome various barriers to the even

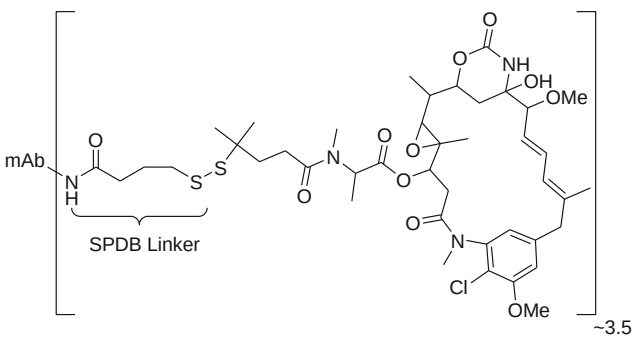


**Fig. 2** mAb- SPDB-DM4.

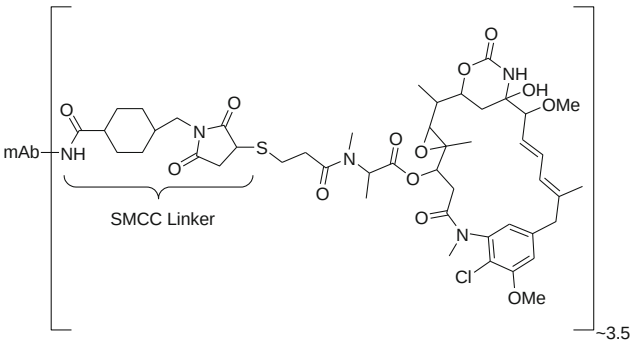


**Fig. 3** mAb-SMCC-DM1.

distribution of maytansinoid effector molecules to all cells within a tumour. However, it should be noted that in the case of a number of other maytansinoid-antibody conjugates such as trastuzumab linked to DM1, it was the SMCC linked conjugate which was the most effective (12) (Fig. 3).

In preclinical studies, the monoclonal antibody, nBT062 was conjugated to DM1 and DM4 using different linker technologies. nBT062-SMCC-DM1, nBT062-SPP-DM1 and nBT062-SPDB-DM4 were then evaluated *in vitro* and *in vivo* against multiple myeloma cell lines (38). It was found that nBT062-SPDB-DM4 was the most efficacious of the conjugates *in vivo*, demonstrating target dependent antitumour activity. Bystander killing in the presence of CD138-positive cells observed *in vitro* was hypothesised to be particularly important for BT062 activity *in vivo* causing the death of tumour cells that heterogeneously express CD138 and disrupting the tumour microenvironment (39). The structure of nBT062-SPDB-DM4, designated BT062 consists of a murine/human IgG4 chimeric form of B-B4 (nBT062) targeting CD138 conjugated to DM4 *via* a disulfide linkage (Fig. 2). Phase I clinical trials are currently underway evaluating the safety, tolerability, pharmacokinetics and efficacy of BT062 in patients with CD138-positive multiple myeloma (39). BT062 is also at an advanced stage of preclinical development for the treatment of a number of CD138-positive solid tumours (40).

Polson *et al.* (41) systematically examined potential antigenic targets and drug linker combinations in an attempt to develop the most clinically relevant ADC for the treatment of non-Hodgkin's lymphoma. The antibodies used targeted the antigens CD19, CD20, CD21, CD22, CD72, CD79b, and CD180, all of which are found to be highly expressed on non-Hodgkin's lymphoma cells. The disulfide linker containing maytansinoid derivative SPP-DM1 (Fig. 4) and the non-cleavable thioether derivative SMCC-DM1 (Fig. 3) were variously conjugated to the different antibodies. Similarly, the cathepsin B substrate containing MC-vc-PABC-MMAE and the non-cleavable MC-MMAE were also conjugated to the various antibodies. It was found that the ADCs with cleavable linkers mediated efficacy *via* all these

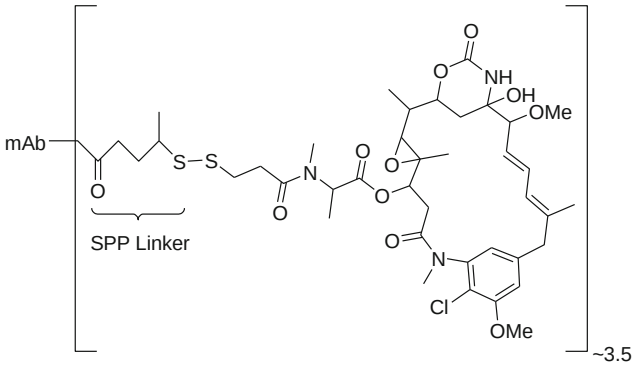


**Fig. 4** huC242-SPP-DM1.

targets whereas the ADCs with non-cleavable linkers were only effective when conjugated with anti-CD22 and anti-CD79b. It was also found that those conjugates containing uncleavable linkers showed reduced toxicity *in vivo*. It was suggested that the reason for the broad applicability of the cleavable disulfide linker containing ADCs is that extracellular cleavage to release the cytotoxic agent occurs following antigen binding and it is the free drug that crosses the cell membrane to exert its effect passively, whereas internalisation and intracellular metabolism is necessary to enable release of the cytotoxic agent in the case of ADCs with a non-cleavable linker.

### *Peptide Linker Units*

Ma *et al.* (42) synthesised a conjugate by combining a prostate specific membrane antigen (PSMA) monoclonal antibody and MMAE *via* a valine-citrulline linkage (Fig. 6). This linkage was designed to maintain serum stability while maximising intracellular drug release by human cathepsin B (42). Cathepsin B is a lysosomal exocarboxydipeptidase which is often found to be upregulated in tumour cells particularly during tumour progression (43). *In vitro*, the conjugate potently eliminated all PSMA-positive cell lines with $IC_{50}$ values ranging from 65 to 210 pM whereas these concentrations had no effect on PSMA-negative cells. In fact, the conjugates demonstrated nearly 1000-fold selectivity compared with an isotype control conjugate. *In vivo* efficacy was shown in a mouse xenograft

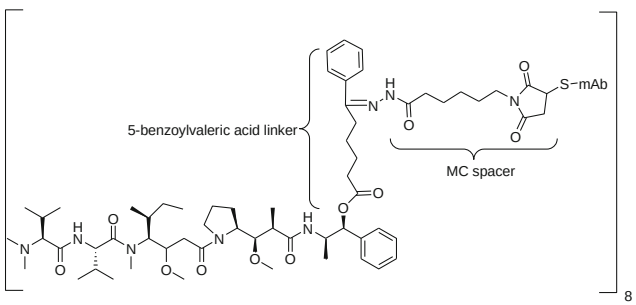


**Fig. 5** mAb-AEBV.

model of androgen independent prostate cancer cell line, C4-2. Each of the two regimens tested were effective in increasing median survival and decreasing serum PSMA in a dose dependent fashion. Overall, a 40% cure rate was observed in animals with established tumours following treatment with a 6 mg/kg regimen. Furthermore, no apparent toxicity was observed with this regimen which may permit the use of even more intense regimens resulting in further improvements in antitumour activity (42).

Auristatin E and the related compound MMAE were conjugated to the chimeric monoclonal antibodies cBR96 (specific for the Lewis Y antigen on carcinomas) and cAC10 (specific to CD30 on haematological malignancies) (44) using three distinct linker methodologies. CD30 has limited expression in normal tissues but is widely expressed in Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma and cutaneous T-cell lymphoma (45). Firstly, conjugates containing the 5-benzoylvaleric acid ester of auristatin E were synthesised (Fig. 5). Secondly, conjugates consisting of the dipeptide valine-citrulline attached to MMAE *via* the self-immolative *p*-aminobenzyl carbonyl (PABC) spacer unit were synthesised. Finally, conjugates consisting of the dipeptide lysine-phenylalanine attached to MMAE *via* the same *p*-aminobenzyl carbonyl spacer were prepared (Fig. 6). All three conjugates also contained a maleimidocaproyl (MC) spacer group. It was found that the peptide linker containing conjugates were much more stable in buffers and plasma than the hydrazone linker conjugates. The mAb-val-cit-MMAE conjugates also demonstrated greater *in vitro* specificity and lower *in vivo* toxicity than corresponding hydrazone containing conjugates. In tumour xenograft models, the peptide linked conjugates induced tumour regression and cures with therapeutic indices as high as 60-fold (i.e. 1/60th of the maximum tolerated dose of cAC10-Val-Cit-MMAE was shown to achieve therapeutic efficacy in a CD30 positive anaplastic large cell lymphoma (ALCL) tumour model).

### *Alternative Linker Units*

In an attempt to further increase the therapeutic window of cAC10-maleimidocaproyl-valine-citrulline-*p*-aminobenzyloxycarbonyl-monomethyl auristatin F conjugate (cAC10-MC-vc-PABC-MMAF), a series of alternate linker units were investigated (46). One conjugate containing a noncleavable

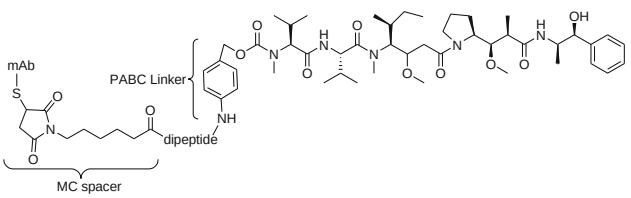


**Fig. 6** mAb-Val-Cit-MMAE and mAb-Phe-Lys-MMAE (where dipeptide = valine-citrulline and phenylalanine and lysine respectively).

maleimidocaproyl linker unit (Fig. 7) was shown to have similar activity *in vitro* and *in vivo* but could be administered at 3-fold higher doses than cAC10-MC-vc-PABC-MMAF.

Novel linker mechanisms have also been investigated in ADCs with a view to evading multidrug resistance mechanisms such as the overexpression of the transporter MDR-1. In one such study conducted by Kovtun *et al.* (47) a DM1-antibody conjugate using a maleimidyl-based hydrophilic linker, $PEG_4Mal$, was shown to overcome MDR-1 mediated multidrug resistance *in vitro* and eradicate human MDR-1 xenograft tumours *in vivo*. Following uptake into cells, it was found that conjugates with the $PEG_4Mal$ linker (Fig. 8) were transformed to cytotoxic conjugates that were better retained by MDR-1 expressing cells than metabolites of a similar conjugates with a SMCC linker (Fig. 3).

Even with relatively hydrophilic cytotoxic agents such as DM1 and DM4 conjugated to antibodies *via* the somewhat hydrophobic linkers SPDB and SMCC, only conjugates with 4-5 drug molecules per antibody could be synthesised. When conjugates with higher maytansinoid loads were attempted the resultant conjugates tended to aggregate and precipitate (48). In an attempt to increase the drug antibody ratio without causing aggregation or affecting the binding affinity of the resultant conjugate, a number of different hydrophilic linkers containing either a negatively charged sulfonate group or a hydrophilic, uncharged PEG group were synthesised (48). An important advantage of the conjugates containing these more hydrophilic linkers is their potential to overcome multidrug resistance. Incorporation of the sulfonate group both in cleavable and non-cleavable linkers was found to be associated with enhanced activity of B38.1-DM1 conjugates against MDR cell lines. B38.1-DM1 conjugates consisting of a $PEG_4$ linker were also observed to display enhanced activity *in vitro* against MDR cell lines. Incorporation of the sulfonate group or the $PEG_4$ group into non-reducible linkers in DM1 and DM4 conjugates of huC242 also resulted in approximately 100-fold greater cytotoxicity against both COLO 205 and COLO 205/MDR cells. *In vivo*, the B38.1-DM1 and B38.1-DM4 conjugates prepared with sulfonate or PEG containing linkers demonstrated enhanced antitumour activity against the COLO205MDR xenograft model in mice.

A β-glucuronic acid based linker was also utilised for the synthesis of a number of maytansinoid conjugates in which

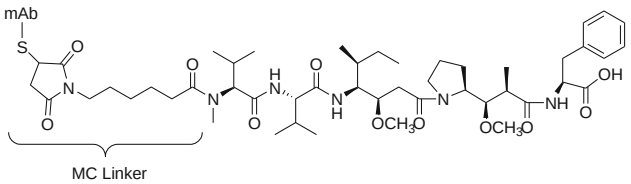


**Fig. 7** mAb–mcMMAF.

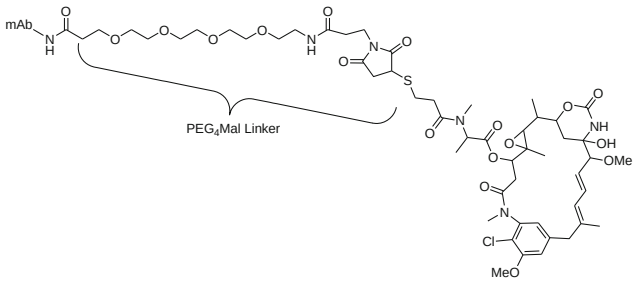


**Fig. 8** mAb–$PEG_4Mal$–DM1.

hydrolysis by the lysosomal enzyme β-glucuronidase releases the active drug. MMAE and MMAF were conjugated to the monoclonal antibodies c1F6 (anti-CD70) and cAC10 (anti-CD30) *via* a novel glucuronic acid linker (Fig. 9) (49). It has been shown that β-glucuronidase is abundantly present within lysosomes and is overexpressed in some tumour types while extracellular enzyme activity is low (50). The resulting conjugates were found to be highly stable in plasma, well tolerated at high doses and efficacious both *in vitro* and *in vivo*.

Trastuzumab was chemically linked to paclitaxel *via* a novel A-Z-CINN linker resulting in an ADC with the potential for light accelerated release of paclitaxel at the tumour site (51). *In vivo*, in HER2 positive BT-474 mammary tumour cells in mice, the conjugate showed enhanced antitumour activity following 5 min light exposure (light from a halogen bulb delivered through a fiber optic probe) adjacent to the tumour and was also found to cause more rapid and extensive reduction in tumour volume than a 10-fold higher concentration of free paclitaxel and free trastuzumab administered to the mice (51).

## Antibody-Drug Conjugates in Preclinical Development

The novel taxoid SB-T-12136 was variously linked to three murine monoclonal antibodies against the human epidermal growth factor receptor (EGFR) (23,52). The antibodies KS-61, KS-77 and KS-78 were linked to SB-T-12136 *via* a 4-

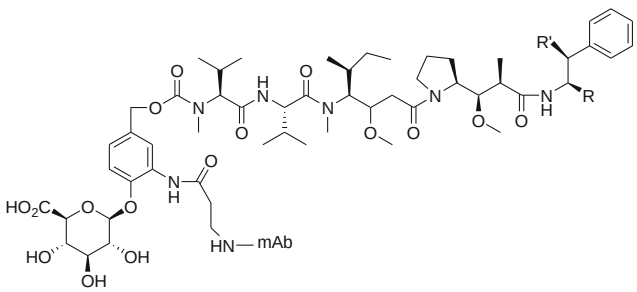


**Fig. 9** MMAE/MMAF-glucuronic acid linker-antibody conjugate (where R = Me and R′ = OH for MMAE while R = COOH and R′ = H for MMAF).

thiopentanoyl linker (Fig. 10). These conjugates were shown to possess remarkable target-specific antitumour activity *in vivo* against EGFR-expressing A431 tumour xenografts in mice resulting in complete inhibition of tumour growth without any noticeable toxicity.

Anti-CD22-SMCC-DM1 is another ADC in preclinical development. It consists of DM1 conjugated to a humanised antibody that targets CD22, a siglec (sialic acid binding Ig-like lectins) family lectin expressed predominantly on mature B-cells (53). It was found that most non-Hodgkin's lymphoma patients express CD22 and this conjugate was shown to inhibit the proliferation of several non-Hodgkin's lymphoma cell lines *in vitro* and also induced complete regression of tumours in xenograft mouse models. It was well tolerated by cynomolgus monkeys and significantly decreased circulating B-cells as well as follicle size and germinal centre formation in lymphoid organs. It has been suggested that the low levels of expression of CD22 should not limit the scope of this conjugate as some cell lines with very low levels of CD22 expression were sensitive to these agents (53).

Chimeric anti-CD20 antibody, rituximab, has already found use in the clinic in the treatment of certain B-cell lymphomas, most notably Hodgkin's lymphoma (54). The specific binding of rituximab and the anti-CD20 antibody IF5 for CD20 positive B-lymphoma cells both offer the potential for the synthesis of an effective ADC (54). Conjugates of these antibodies linked to MMAE *via* a valine-citrulline linker were synthesised by Law *et al.* (54). These conjugates were shown to be selectively toxic to CD20 positive B-cell lymphoma cell lines *in vitro* with $IC_{50}$ values of between 50 ng/ml and 1 μg/ml. Furthermore, rituximab-vc-MMAE showed antitumour efficacy in human lymphoma CD20 positive tumour xenograft models in mice at doses where rituximab was ineffective.

While CD20 immunotherapy adequately depletes mature B-cells, it does not deplete pre-B cells or immature B cells, some B cell subpopulations, plasma cells or their malignant counterparts (55). Because CD19 is expressed earlier in B cell development it offers the potential to target lymphoblastic leukaemias and lymphomas (55). Therefore, a humanised anti-CD19 antibody (hBU12) was conjugated to MMAE *via* a valine-citrulline linker to yield hBu12-vc-MMAE (56). This conjugate was shown to exhibit potent cell killing against rituximab sensitive and resistant non-Hodgkin's lymphoma cell lines *in vitro*. Furthermore, high rates of durable tumour growth suppression were observed in mice implanted with these tumours. Further examples of ADCs containing tubulin targeting agents currently in preclinical development are shown in Table II.

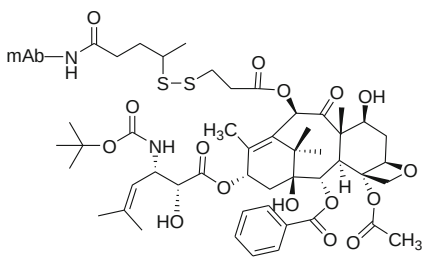


**Fig. 10** SB-T-12136-mAb conjugates.

## Antibody-Drug Conjugates Undergoing Clinical Evaluation

Brentuximab vedotin is now approved for the treatment of CD30-positive hematologic malignancies (61) while trastuzumab emtansine is in phase III for HER2-positive breast cancer (21). A number of other promising ADCs are undergoing phase I/II evaluation at present (Table III). Of these, C242-DM1 was among the first maytansinoid-antibody conjugates synthesised. C242 recognises CanAg (MUC1), a mucin type glycoprotein expressed on the surface of human colorectal cancer cells. It was shown to be effective in mice xenograft models with complete tumour regression observed (62). This agent subsequently progressed to clinical evaluation as cantuzumab mertansine (huC242-DM1) in which the antibody was humanised. Cantuzumab mertansine consists of up to four molecules of mertansine conjugated to each antibody. The drug showed considerable tumour localisation without inducing severe haematological toxicity in patients with pancreatic and colorectal cancers (63).

Another agent that has shown great potential in preclinical testing is huC242-DM4 (12). Treatment of mice with a single dose of huC242-DM4 resulted in complete regression of tumour xenografts at a dose well below the maximum tolerated dose while equivalent doses of free maytansinoid and a DM4 conjugate with a non-binding antibody had no effect on tumour growth in this model (12). *In vivo* distribution experiments in mice have shown that 1000-fold greater accumulation of maytansinoid at the tumour site can be achieved compared with non-conjugated maytansinoids after 24–28 h. This conjugate contains a disulfide linkage and has an increased stability under physiological conditions. It was found to have a half-life of 7.7 d in mice *versus* ~2 d for cantuzumab mertansine, and is now undergoing phase I clinical evaluation (6).

CD37 also represents an attractive target for an antibody-maytansinoid ADC due to its high expression in B-cell malignancies such as non-Hodgkin's lymphoma and chronic lymphocytic leukaemia and its limited expression in normal tissues (64). Furthermore, anti-CD37 antibodies have been shown to possess intrinsic anti-tumour activity and therefore offer the potential for the synthesis of a dual acting ADC (64). The humanised anti-CD37 monoclonal antibody K7153A was thus conjugated to DM1 using either the hindered disulfide

**Table II** Antibody-Drug Conjugates in Preclinical Development

| Antibody-drug conjugate | Target antigen | Cytotoxic agent | Linker used | Anticancer activity/Stage of development | References |
|---|---|---|---|---|---|
| Anti-CD79b-SMCC-DM1 | Anti-CD79b | DM1 | SMCC | *In vitro* activity against non-Hodgkin's lymphoma cell lines<br>Effective *in vivo* in follicular, mantle cell and Burkitt's lymphoma | (57) |
| Anti-CD79b-MC-MMAF | Anti-CD79b | DM1 | MC | *In vitro* activity against non-Hodgkin's lymphoma cell lines<br>Effective *in vivo* in follicular, mantle cell and Burkitt's lymphoma | (57) |
| Anti-CD79b-vc-MMAE | Anti-CD79b thiomab | MMAE | Vc | *In vitro* and *in vivo* activity against non-Hodgkin's lymphoma cell lines | (58) |
| Anti-huCD79b (SN8)-MCC-DM1 | Humanised anti-CD79b antibody | DM1 | SMCC | Caused complete regression of Burkitt's, follicular, and mantle cell lymphoma xenograft tumours | (59) |
| IMGN853 | Anti-folate antibody M9346A | DM4 | sulfo-SPDM | Selective cytotoxicity *in vitro* and *in vivo* for FR+ cells, efficacy in MDR-1-positve tumours | (60) |

containing SPP linker or the non-cleavable SMCC linker. It was found that the K17153A-SMCC-DM1 (designated IMGN529) was more active against lymphoma cells both *in vitro* and *in vivo* against SU-DHL-4 subcutaneous xenograft tumours. This agent is currently undergoing phase I clinical trials in patients with relapsed or refractory non-Hodgkin's lymphoma (65).

The conjugate MEDI-547 (1C1-mcMMAF) consists of a fully human monoclonal antibody against murine and human EphA2 (1C1) attached to MMAF *via* a stable maleimidocaproyl linker (Fig. 7). The EphA2 receptor is a subtype of the erythropoietin producing hepatoma receptor that is overexpressed in tumours of the breast, prostate, lung, colon and in glioblastoma multiforme (66). It has also been reported that the EphA2 receptor is upregulated in the neovasculature of tumours, which may contribute to the activity associated with agents targeting this receptor (67). *In vivo* experiments in mouse orthotopic xenograft models resulted in 86% to 88% growth inhibition in the orthotopic Hec-1A and Ishikawa models following treatment with this agent. The mice treated with this ADC also had a lower incidence of metastasis than controls and this effect was observed without overt signs of toxicity (68). This agent was subsequently evaluated in phase I clinical trials however the outcome of these studies have yet to be reported.

**Table III** Antibody-Drug Conjugates Under Clinical Investigation

| Antibody-drug Conjugate | Target; Indications | Clinical Stage | References |
|---|---|---|---|
| Brentuximab vedotin (SGN-35; Brentuximab-MC-VC-MMAE) | CD30; hematologic malignancies, Hodgkin's lymphoma | FDA approved | (71) |
| Trastuzumab emtansine (Trastuzumab-SMCC-DM1) | HER2-positive breast cancer | Phase III | (21) |
| Lorvotuzumab mertansine (IMGN901; HuN901-SPP-DM1) | CD56; Merkel cell cancer, small cell lung cancer, multiple myeloma ovarian cancer | Phase II | (10) |
| Glembatumumab vedotin (CDX-011; CDX-011-MC-VC-MMAE) | Glycoprotein NMB; melanoma, breast cancer | Phase II | (21) |
| SAR3419 (huB4-SPDB-DM4) | CD19; B cell lymphoma | Phase I and II | (73,74) |
| IMGN388 (Antibody-SPDB-DM4) | Integrin; antivascular/solid tumours | Phase I | (17) |
| BIIB-015 (Antibody-SPDB-DM4) | Cripto; solid tumours | Phase I | (17) |
| BT-062 (Anti-CD138-SPDB-DM4) | CD138; multiple myeloma | Phase I and II | (39) |
| BAY 79-4620 (3ee9-MMAE) | CAIX (MN); solid tumours | Phase I | (17) |
| MEDI-547 (1C1-MC-MMAF) | EphA2; ovarian cancer, solid tumours | Phase I | (75) |
| SGN-75 (SGN-70-MC-VC-MMAF) | CD70; renal cell carcinoma, non-Hodgkin's lymphoma | Phase I | (76) |
| SAR566658 (huDS6-DM4) | CA6; ovarian, cervical, breast cancers | Phase I | (17) |
| PSMA-ADC (anti-PSMA-MMAE) | PSMA; prostate cancer | Phase I | (77) |
| MLN2704 (MLN591-DM1) | PSMA; prostate cancer | Phase II | (6) |
| IMGN529 (Antibody-SMCC-DM1) | Anti-CD37 | Phase I | (65) |
| AVE9633 (Antibody-DM4) | Anti-CD33 antibody (huMy9); acute myeloid leukaemia | Phase I | (10) |

Trastuzumab emtansine is currently undergoing a number of phase III clinical trials in patients with HER-positive metastatic breast cancer (21). It consists of the anti-HER2 monoclonal antibody trastuzumab conjugated to an average of 3.5 molecules of the maytansinoid DM1 *via* a nonreducible SMCC thioether linkage (Fig. 3). It has been shown that trastuzumab emtansine retains the mechanism of action of unconjugated trastuzumab and is active against lapatinib resistant cell lines and tumours (69). It was also effective in patients for whom trastuzumab alone was found to be inadequate (12). Trastuzumab emtansine's systemic side effects are significantly minimised due to its targeted delivery to HER2 positive cells. Two phase II studies comparing trastuzumab-DM1 with trastuzumab plus docetaxel showed improved tolerability and at least equivalent efficacy. Two phase III randomised trials are currently underway for refractory and HER2-positive breast cancer (70).

In August 2011, brentuximab vedotin (cAC10-vc-MMAE, SGN-35) was approved by the FDA for the treatment of patients with Hodgkin's lymphoma and systemic ALCL (71). To date, the only other ADC to have made it to the clinic is Wyeth's Mylotarg® (gentuzumab ozogamicin) which received FDA approval in 2000 for the treatment of acute myeloid leukaemia. However, it was voluntarily withdrawn after follow up studies raised concern about its safety and clinical efficacy (21). Brentuximab vedotin is a conjugate of the chimeric monoclonal antibody cAC10 directed against CD30 covalently coupled to MMAE. The valine-citrulline peptide linker unit connecting the two components is subject to cleavage by lysosomal enzymes following internalisation into target cells (Fig. 6). The tumour necrosis factor receptor family member CD30 is highly expressed on the cell surfaces of Hodgkin's disease, ALCL and a subset of non-Hodgkin's lymphomas but has only limited expression on healthy tissues. Brentuximab vedotin potently interferes with the growth of CD30-positive haematological tumours including Hodgkin's lymphoma and ALCL (72). It has been suggested that the *in vivo* efficacy of brentuximab vedotin might be mediated, in part, by the diffusion of free MMAE from the target cells resulting in a bystander effect that kills the normal supporting cells in close proximity to malignant cells (61).

## CONCLUSIONS

A vast array of delivery strategies are currently being evaluated for the more selective targeting of tubulin binding agents to the tumour site. Encouragingly, many of these methodologies are resulting in agents with improved tumour targeting, enhanced pharmacokinetic characteristics and greater efficacy. The licensing of brentuximab vedotin in particular marks significant progress towards the synthesis of targeted conjugate molecules and away from non-specific small molecule tubulin inhibitors with their associated toxicities. Many of the obstacles associated with early ADCs have been overcome and new generation ADCs are demonstrating greater tumour specificity and enhanced potency. Lessons have undoubtedly been learned from the failed development of bivatuzumab mertansine and much greater care has been taken in selecting antigen targets with limited physiological expression. Optimisation of the cytotoxic payload has resulted in a high level of utilisation of the tubulin targeting auristatins and maytansinoids due to their high potency and ease of conjugation. In particular, the selection of more appropriate linker groups has facilitated the synthesis of conjugates which are more stable in circulation, more selectively cytotoxic for their intended targets and ultimately more efficacious. However, it should be clear from the vast array of linker methodologies used that different malignancies and different ADCs benefit from different linker units and it is still necessary to evaluate the most appropriate linker for each specific therapeutic indication. The sheer number of ADCs at an advanced stage of clinical evaluation suggests that we are on the cusp of a revolution in the immunological treatment of malignancy. Therefore, it would appear that antibody conjugation is certainly a viable methodology for the synthesis of tubulin targeting agents with improved tumour selectivity, lower incidences of resistance and side effects and superior therapeutic efficacy than currently used clinical agents.

## ACKNOWLEDGMENTS AND DISCLOSURES

The authors would like to acknowledge funding from the Irish Council for Science Engineering and Technology (IRCSET) and Enterprise Ireland.

## REFERENCES

1. Chari RV. Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy. Adv Drug Deliv Rev. 1998;31(1–2):89–104.
2. Kavallaris M. Microtubules and resistance to tubulin-binding agents. Nat Rev Cancer. 2010;10(3):194–204.
3. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer. 2004;4(4):253–65.
4. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat Rev Drug Discov. 2010;9(10):790–803.
5. Kavallaris M, Verrills NM, Hill BT. Anticancer therapy with novel tubulin-interacting drugs. Drug Resist Updat. 2001;4(6):392–401.
6. Lambert JM. Drug-conjugated monoclonal antibodies for the treatment of cancer. Curr Opin Pharmacol. 2005;5(5):543–9.
7. Vlahov IR, Wang Y, Kleindl PJ, Leamon CP. Design and regioselective synthesis of a new generation of targeted chemotherapeutics. Part II: Folic acid conjugates of tubulysins and their hydrazides. Bioorg Med Chem Lett. 2008;18(16):4558–61.
8. Kupchan SM, Komoda Y, Court WA, Thomas GJ, Smith RM, Karim A, *et al.* Tumor inhibitors. LXXIII. Maytansine, a novel

antileukemic ansa macrolide from Maytenus ovatus. J Am Chem Soc. 1972;94(4):1354–6.
9. Oroudjev E, Lopus M, Wilson L, Audette C, Provenzano C, Erickson H, *et al.* Maytansinoid-antibody conjugates induce mitotic arrest by suppressing microtubule dynamic instability. Mol Cancer Ther. 2010;9(10):2700–13.
10. Lopus M. Antibody-DM1 conjugates as cancer therapeutics. Cancer Lett. 2011;307(2):113–8.
11. Chari RVJ, Martell BA, Gross JL, Cook SB, Shah SA, Blättler WA, *et al.* Immunoconjugates containing novel maytansinoids: promising anticancer drugs. Cancer Res. 1992;52(1):127–31.
12. Lambert JM. Antibody-maytansinoid conjugates as anticancer therapeutics: Proving their benefit. Hum Antibodies. 2007;16(1–2):5–8.
13. Yusuf RZ, Duan Z, Lamendola DE, Penson RT, Seiden MV. Paclitaxel resistance: molecular mechanisms and pharmacologic manipulation. Curr Cancer Drug Targets. 2003;3(1):1–19.
14. Argiris A, Duffy AG, Kummar S, Simone NL, Arai Y, Kim SW, *et al.* Early tumor progression associated with enhanced EGFR signaling with bortezomib, cetuximab, and radiotherapy for head and neck cancer. Clin Cancer Res. 2011;17(17):5755–64.
15. Kim KM, McDonagh CF, Westendorf L, Brown LL, Sussman D, Feist T, *et al.* Anti-CD30 diabody-drug conjugates with potent antitumor activity. Mol Cancer Ther. 2008;7(8):2486–97.
16. Guillemard V, Uri Saragovi H. Prodrug chemotherapeutics bypass p-glycoprotein resistance and kill tumors *in vivo* with high efficacy and target-dependent selectivity. Oncogene. 2004;23(20):3613–21.
17. Teicher BA, Chari RVJ. Antibody conjugate therapeutics: challenges and potential. Clin Cancer Res. 2011;17(20):6389–97.
18. Christiansen J, Rajasekaran AK. Biological impediments to monoclonal antibody-based cancer immunotherapy. Mol Cancer Ther. 2004;3(11):1493–501.
19. Francisco JA, Cerveny CG, Meyer DL, Mixan BJ, Klussman K, Chace DF, *et al.* cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood. 2003;102(4):1458–65.
20. Ducry L, Stump B. Antibody−drug conjugates: linking cytotoxic payloads to monoclonal antibodies. Bioconjug Chem. 2009;21(1):5–13.
21. Webb S. Pharma interest surges in antibody drug conjugates. Nat Biotechnol. 2011;29(4):297–8.
22. Goldenberg DM. Monoclonal antibodies in cancer detection and therapy. Am J Med. 1993;94(3):297–312.
23. Wu X, Ojima I. Tumor specific novel taxoid-monoclonal antibody conjugates. Curr Med Chem. 2004;11(4):429–38.
24. Widdison WC, Wilhelm SD, Cavanagh EE, Whiteman KR, Leece BA, Kovtun Y, *et al.* Semisynthetic maytansine analogues for the targeted treatment of cancer. J Med Chem. 2006;49(14):4392–408.
25. Hamblett KJ, Senter PD, Chace DF, Sun MM, Lenox J, Cerveny CG, *et al.* Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. Clin Cancer Res. 2004;10(20):7063–70.
26. Tijink BM, Buter J, de Bree R, Giaccone G, Lang MS, Staab A, *et al.* A phase I dose escalation study with anti-CD44v6 bivatuzumab mertansine in patients with incurable squamous cell carcinoma of the head and neck or esophagus. Clin Cancer Res. 2006;12(20 Pt 1):6064–72.
27. Garanger E, Boturyn D, Dumy P. Tumor targeting with RGD peptide ligands-design of new molecular conjugates for imaging and therapy of cancers. Anticancer Agents Med Chem. 2007;7(5):552–8.
28. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res. 1992;52(12):3402–8.
29. Adams GP, Schier R, Marshall K, Wolf EJ, McCall AM, Marks JD, *et al.* Increased affinity leads to improved selective tumor delivery of single-chain Fv antibodies. Cancer Res. 1998;58(3):485–90.
30. Junutula JR, Flagella KM, Graham RA, Parsons KL, Ha E, Raab H, *et al.* Engineered thio-trastuzumab-DM1 conjugate with an improved therapeutic index to target human epidermal growth factor receptor 2–positive breast cancer. Clin Cancer Res. 2010;16(19):4769–78.
31. Braun S, Schlimok G, Heumos I, Schaller G, Riethdorf L, Riethmuller G, *et al.* ErbB2 overexpression on occult metastatic cells in bone marrow predicts poor clinical outcome of stage I-III breast cancer patients. Cancer Res. 2001;61(5):1890–5.
32. Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, *et al.* Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index. Nat Biotech. 2008;26(8):925–32.
33. Schrama D, Reisfeld RA, Becker JC. Antibody targeted drugs as cancer therapeutics. Nat Rev Drug Discov. 2006;5(2):147–59.
34. Erickson HK, Park PU, Widdison WC, Kovtun YV, Garrett LM, Hoffman K, *et al.* Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. Cancer Res. 2006;66(8):4426–33.
35. Lopus M, Oroudjev E, Wilson L, Wilhelm S, Widdison W, Chari R, *et al.* Maytansine and cellular metabolites of antibody-maytansinoid conjugates strongly suppress microtubule dynamics by binding to microtubules. Mol Cancer Ther. 2010;9(10):2689–99.
36. Kovtun YV, Audette CA, Ye Y, Xie H, Ruberti MF, Phinney SJ, *et al.* Antibody-drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen. Cancer Res. 2006;66(6):3214–21.
37. Kovtun YV, Goldmacher VS. Cell killing by antibody–drug conjugates. Cancer Lett. 2007;255(2):232–40.
38. Ikeda H, Hideshima T, Fulciniti M, Lutz RJ, Yasui H, Okawa Y, *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(12):4028–37.
39. Lutz RJ, Whiteman KR. Antibody-maytansinoid conjugates for the treatment of myeloma. Mabs. 2009;1(6):548–51.
40. Beck A, Senter P, Chari R. World antibody drug conjugate Summit Europe: February 21-23, 2011; Frankfurt, Germany. Mabs. 2011;3(4):331–7.
41. Polson AG, Calemine-Fenaux J, Chan P, Chang W, Christensen E, Clark S, *et al.* Antibody-drug conjugates for the treatment of non-Hodgkin's lymphoma: target and linker-drug selection. Cancer Res. 2009;69(6):2358–64.
42. Ma DS, Hopf CE, Malewicz AD, Donovan GP, Senter PD, Goeckeler WF, *et al.* Potent antitumor activity of an auristatin-conjugated, fully human monoclonal antibody to prostate-specific membrane antigen. Clin Cancer Res. 2006;12(8):2591–6.
43. Podgorski I, Sloane BF. Cathepsin B and its role(s) in cancer progression. Biochem Soc Symp. 2003;70:263–76.
44. Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, Chace DF, *et al.* Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol. 2003;21(7):778.
45. Wahl AF, Klussman K, Thompson JD, Chen JH, Francisco LV, Risdon G, *et al.* The anti-CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation *in vitro* and affects antitumor activity in models of Hodgkin's disease. Cancer Res. 2002;62(13):3736–42.
46. Doronina SO, Mendelsohn BA, Bovee TD, Cerveny CG, Alley SC, Meyer DL, *et al.* Enhanced activity of monomethylauristatin f through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity. Bioconjug Chem. 2005;17(1):114–24.
47. Kovtun YV, Audette CA, Mayo MF, Jones GE, Doherty H, Maloney EK, *et al.* Antibody-maytansinoid conjugates designed to bypass multidrug resistance. Cancer Res. 2010;70(6):2528–37.

48. Zhao RY, Wilhelm SD, Audette C, Jones G, Leece BA, Lazar AC, *et al.* Synthesis and evaluation of hydrophilic linkers for antibody–maytansinoid conjugates. J Med Chem. 2011;54(10):3606–23.
49. Jeffrey SC, Andreyka JB, Bernhardt SX, Kissler KM, Kline T, Lenox JS, *et al.* Development and properties of β-glucuronide linkers for monoclonal antibody−drug conjugates. Bioconjug Chem. 2006;17(3):831–40.
50. Albin N, Massaad L, Toussaint C, Mathieu MC, Morizet J, Parise O, *et al.* Main drug-metabolizing enzyme systems in human breast tumors and peritumoral tissues. Cancer Res. 1993;53(15):3541–6.
51. Gilbert CW, McGowan EB, Seery GB, Black KS, Pegram MD. Targeted prodrug treatment of HER-2-positive breast tumor cells using trastuzumab and paclitaxel linked by A-Z-CINN™ Linker. J Exp Ther Oncol. 2003;3(1):27–35.
52. Ojima I, Geng X, Wu X, Qu C, Borella CP, Xie H, *et al.* Tumor-specific novel taxoid-monoclonal antibody conjugates. J Med Chem. 2002;45(26):5620–3.
53. Polson AG, Williams M, Gray AM, Fuji RN, Poon KA, McBride J, *et al.* Anti-CD22-MCC-DM1: an antibody-drug conjugate with a stable linker for the treatment of non-Hodgkin's lymphoma. Leukemia. 2010;24(9):1566–73.
54. Law CL, Cerveny CG, Gordon KA, Klussman K, Mixan BJ, Chace DF, *et al.* Efficient elimination of B-lineage lymphomas by anti-CD20-auristatin conjugates. Clin Cancer Res. 2004;10(23):7842–51.
55. Yazawa N, Hamaguchi Y, Poe JC, Tedder TF. Immunotherapy using unconjugated CD19 monoclonal antibodies in animal models for B lymphocyte malignancies and autoimmune disease. Proc Natl Acad Sci USA. 2005;102(42):15178–83.
56. Gerber HP, Kung-Sutherland M, Stone I, Morris-Tilden C, Miyamoto J, McCormick R, *et al.* Potent antitumor activity of the anti-CD19 auristatin antibody drug conjugate hBU12-vcMMAE against rituximab-sensitive and -resistant lymphomas. Blood. 2009;113(18):4352–61.
57. Polson AG, Yu SF, Elkins K, Zheng B, Clark S, Ingle GS, *et al.* Antibody-drug conjugates targeted to CD79 for the treatment of non-Hodgkin lymphoma. Blood. 2007;110(2):616–23.
58. Dornan D, Bennett F, Chen Y, Dennis M, Eaton D, Elkins K, *et al.* Therapeutic potential of an anti-CD79b antibody-drug conjugate, anti-CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma. Blood. 2009;114(13):2721–9.
59. Zheng B, Fuji RN, Elkins K, Yu S-F, Fuh FK, Chuh J, *et al. In vivo* effects of targeting CD79b with antibodies and antibody-drug conjugates. Mol Cancer Ther. 2009;8(10):2937–46.
60. Ab O, Bartle LM, Rui L, Coccia J, Johnson HA, Whiteman KR, *et al.* Abstract 4576: IMGN853, an anti-Folate Receptor I antibody-maytansinoid conjugate for targeted cancer therapy. Cancer Res. 2011;71(8 Supplement):4576.
61. Katz J, Janik JE, Younes A. Brentuximab vedotin (SGN-35). Clin Cancer Res. 2011;17(20):6428–36.
62. Liu C, Tadayoni BM, Bourret LA, Mattocks KM, Derr SM, Widdison WC, *et al.* Eradication of large colon tumor xenografts by targeted delivery of maytansinoids. Proc Natl Acad Sci USA. 1996;93(16):8618–23.
63. Tolcher AW, Ochoa L, Hammond LA, Patnaik A, Edwards T, Takimoto C, *et al.* Cantuzumab mertansine, a maytansinoid immunoconjugate directed to the CanAg Antigen: a Phase I, pharmacokinetic, and biologic correlative study. J Clin Oncol. 2003;21(2):211–22.
64. Robak T, Robak P, Smolewski P. TRU-016, a humanized anti-CD37 IgG fusion protein for the potential treatment of B-cell malignancies. Curr Opin Investig Drugs. 2009;10 (12):1383–90.
65. Park PU, Yi Y, Li M, Chicklas S, Lai KC, Mayo MF, *et al.* Abstract 2830: antibody and linker selection for the anti-CD37 antibody-maytansinoid conjugate IMGN529 for the treatment of B-cell malignancies. Cancer Res. 2011;71(8 Supplement):2830.
66. Ireton RC, Chen J. EphA2 receptor tyrosine kinase as a promising target for cancer therapeutics. Curr Cancer Drug Targets. 2005;5 (3):149–57.
67. Ogawa K, Pasqualini R, Lindberg RA, Kain R, Freeman AL, Pasquale EB. The ephrin-A1 ligand and its receptor, EphA2, are expressed during tumor neovascularization. Oncogene. 2000;19 (52):6043.
68. Lee J-W, Stone RL, Lee SJ, Nam EJ, Roh J-W, Nick AM, *et al.* EphA2 targeted chemotherapy using an antibody drug conjugate in endometrial carcinoma. Clin Cancer Res. 2010;16(9):2562–70.
69. Junttila T, Li G, Parsons K, Phillips G, Sliwkowski M. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. Breast Cancer Res Treat. 2011;128(2):347–56.
70. Mathew J, Perez EA. Trastuzumab emtansine in human epidermal growth factor receptor 2-positive breast cancer: a review. Curr Opin Oncol. 2011;23(6):594–600.
71. Younes A, Yasothan U, Kirkpatrick P. Brentuximab vedotin. Nat Rev Drug Discov. 2012;11(1):19–20.
72. Oflazoglu E, Kissler KM, Sievers EL, Grewal IS, Gerber H-P. Combination of the anti-CD30-auristatin-E antibody-drug conjugate (SGN-35) with chemotherapy improves antitumour activity in Hodgkin lymphoma. Br J Haematol. 2008;142(1):69–73.
73. Blanc V, Bousseau A, Caron A, Carrez C, Lutz RJ, Lambert JM. SAR3419: an anti-CD19-Maytansinoid Immunoconjugate for the treatment of B-cell malignancies. Clin Cancer Res. 2011;17 (20):6448–58.
74. Sanofi-Aventis. An Open Label Non-Randomized Phase 2 Study Evaluating SAR3419, an Anti-CD19 Antibody - Maytansine Conjugate, Administered as Single Agent by Intravenous Infusion to Patients With Relapsed or Refractory CD19+ Diffuse Large B-Cell Lymphoma. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 12 May 02]. Available from: http://www.clinicaltrials.gov/ct2/show/NCT01472887?term=SAR3419&rank=1 NLM Identifier: NCT01472887].
75. MedImmune LLC. Study of MEDI-547 to Evaluate the Safety, Tolerability, and Biologic Activity of IV Administration in Subjects With Relapsed or Refractory Solid Tumors. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 12 Feb 23]. Available from: http://clinicaltrials.gov/ct2/show/NCT00796055?intr=%22MEDI-547%22&rank=1 NLM Identifier: NCT00796055.
76. Oflazoglu E, Stone IJ, Gordon K, Wood CG, Repasky EA, Grewal IS, *et al.* 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 (19):6171–80.
77. Ricart AD. Immunoconjugates against solid tumors: mind the gap. Clin Pharmacol Ther. 2011;89(4):513–23.