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REVIEW ARTICLE

ANTIBODY–MAYTANSINOID CONJUGATES: A NEW STRATEGY FOR THE TREATMENT OF CANCER

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SUMMARY

Early clinical development in the field of targeted delivery of cytotoxic drugs to tumors using antibodies failed to achieve effective, well-tolerated anticancer products. In recent years, several new highly potent cell-killing agents, such as thiol derivatives of the potent antimitotic microtubule agent maytansine, developed by ImmunoGen, are being utilized to make a new generation of antibody–drug conjugates (ADCs). Several antibody–maytansinoid conjugates (AMCs) have shown encouraging efficacy in clinical trials, including trastuzumab-DM1 (T-DM1), which targets HER2$^+$ breast cancer, SAR-3419, which targets CD19 expressed on B-cell malignancies, and IMGN-901, which targets CD56 expressed on both solid tumors (small cell lung cancer and other neuroendocrine tumor types) and hematological tumors, including multiple myeloma. Besides their demonstrated efficacy in phase I and/or phase II clinical studies, these AMCs are well tolerated, with no clinically significant myelosuppression, suggesting that AMC compounds are well suited for evaluation in combination treatment regimens. Maytansinoids are payloads for antibody-mediated delivery that are realizing the promise of ADCs for improved targeted therapy in cancer patients.

INTRODUCTION

Despite nearly 35 years having elapsed since the advent of monoclonal antibody (mAb) technology (1) and its great promise for developing highly specific therapeutic agents, application of antibody technology to cancer treatment has had relatively modest success in yielding approved products. While rituximab and alemtuzumab have excellent single-agent activity in non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL), respectively, with rituximab becoming the mainstay of treatment of indolent NHL, just four "naked" antibodies, trastuzumab, cetuximab, panitumumab and bevacizumab, targeting two overexpressed growth factor receptors (erbB-1 and erbB-2) and a soluble growth factor (VEGF), have been approved for the treatment of solid tumors to date (2). These antibodies have only modest single-agent activity against solid tumors and are generally used to augment the activity of cytotoxic chemotherapy. Furthermore, these treatment regimens are rarely curative for metastatic disease. Thus, new strategies for enhancing the antitumor activity of antibodies are desperately needed if we are to more fully exploit the potential of these exquisitely specific molecules for improving treatment outcomes for patients with cancer.

One of the most promising strategies for increasing the efficacy of antibodies at killing tumor cells is through conjugation to potent cytotoxic agents. Indeed, this notion was the subject of intense research as soon as mAbs specific to tumor-associated cell-surface antigens were readily available (3). The first generation of antibody–cytotoxic agent conjugates were of two types: immunotoxin conjugates (ITs) utilizing toxic proteins such as ricin, diphtheria toxin and *Pseudomonas* exotoxin conjugated to antibodies or engineered antibody fragments, and antibody–drug conjugates (ADCs) utilizing clinically used anticancer drugs such as doxorubicin, methotrexate and vinca alkaloids (3-5). While ITs showed excellent antitumor activity in vitro, their clinical dosing was limited by nonspecific toxicity, especially to vasculature, manifested as capillary leak syndrome (6), and the duration of treatment was often limited by immunogenicity (6, 7). The early ADCs were not successful due in part to the limited potency of the payload (3, 5). Indeed, the limitations imposed on the design of ADCs by the pharmacokinetic and pharmacodynamic properties of antibodies were not fully appreciated at the time (8, 9). Despite solving the immunogenicity problem of antibodies in the early 1990s, as a variety of technologies for generating humanized or fully human antibodies became available (10), a further advance in payload technology was needed.

Analysis of the possible reasons for the failure of these early ADCs led to research to develop cytotoxic payloads that would fulfill two

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basic requirements. First, to take advantage of the long plasma half-life of intact IgG antibodies, the cytotoxin must be nontoxic in conjugated form (i.e., a prodrug concept) at the high plasma concentrations required for sufficient diffusion of the antibody into the tumor tissue, so that dosing is not limited by toxicity in the blood compartment towards hematological tissues and vascular endothelium (3, 5). Secondly, since it became apparent that the peak total amount of antibody deposited in tumor is only approximately 0.01% of the injected dose per gram of tumor (11), limited by its slow diffusion into tumor tissue, the potency of the attached cytotoxic agent once released at the tumor must be 100-1,000-fold greater than that of conventional anticancer drugs (3, 5, 9). These considerations guided the research and development programs at ImmunoGen, leading to the development of maytansinoids as a class of highly potent cytotoxic agents designed to serve as the payload for a new generation of ADCs, which now stand on the threshold of clinical success (8, 9).

ANTIBODY–MAYTANSINOID CONJUGATES (AMCs): ARMING ANTIBODIES WITH A POTENT MAYTANSINOID PAYLOAD

Considerations in the choice of antibody and target

An ideal target would be one expressed at high levels on the surface of tumor cells with minimal to no expression on normal tissue. A mutant version of epidermal growth factor receptor (EGFR, erbB-1) may be one such target (12), but this is rare. Some target antigens may exhibit a quantitative difference in expression between tumor tissue and normal tissue, for example erbB-2/HER2 is overexpressed on 20-25% of breast cancer tissues (13) relative to the amounts expressed rather widely on normal tissues (www.proteinatlas.org). Furthermore, if the payload of an ADC is an antimitotic agent such as a maytansinoid, significant normal tissue expression may be tolerated if such expression is confined to normal tissue having a very low mitotic index. ErbB-2/HER2 may be considered an excellent target for an ADC containing an antimitotic payload by these criteria. In contrast, a target such as CD44v6 may not be suitable because, although highly expressed on some tumors such as squamous head and neck carcinoma, it is also highly expressed on squamous epithelial cells of the skin, in particular on cells with a very high mitotic index (14), which likely leads to skin toxicity defining the dose-limiting toxicity (DLT) for an AMC directed to the antigen (15). Tissue-specific antigens may make good targets for ADCs in cases where the tissue is not vital and/or can readily regenerate from stem cells, for example, B-cell-specific antigens can be used to target NHL (16, 17).

Beyond these quantitative (overexpression on tumor versus normal tissue) and qualitative (the mitotic index of any antigen-positive normal tissue) considerations, other factors determining if a particular target is suitable for an AMC are whether the target is internalized and whether intracellular routing is able to deliver the AMC in sufficient quantity into the appropriate intracellular compartment for efficient release of active maytansinoid (see discussion of mechanism of action). Here, the properties of the antibody itself in driving internalization and/or directing intracellular trafficking may play a significant role in yielding a highly active AMC. Other antibody properties can be screened for –the ability to directly induce apoptosis and/or downregulate growth signals, as well as the ability to engage immune cell killing mechanisms. For such an antibody, creating an AMC from it simply adds another killing mechanism, beyond those intrinsic to the antibody, to the resulting therapeutic candidate molecule (18). In cases where the antibody has little or no intrinsic activity save for specific binding, conjugation with maytansinoids now provides an opportunity to exploit the target where none existed with any naked antibody.

It is not within the scope of this review to discuss antibody isotype, antibody fragments and other engineered antibody types with respect to their utilization in AMCs. Suffice it to say that of the 11 AMCs that entered clinical trials, 10 of them employed intact, humanized or fully human IgG_1 antibodies, and one, BT-062, utilized a chimeric IgG_4 antibody (Table I).

The maytansinoid payload

Maytansine is a potent antimitotic agent causing proliferating cells to arrest in the G_2/M phase of the cell cycle and ultimately to die by apoptosis, while having no effect on nonproliferating cells (19, 20). Maytansine appears to compete with vinca alkaloids for the same binding site on tubulin (21), yet it is significantly more potent, with IC_{50} values in the range of 10-100 pM, depending upon the cell type (22, 23), and it does not cluster with other antitubulin agents in its activity pattern across the NCI 60 panel of human cancer cell lines (24).

In order to link maytansinoids to antibodies, thiol derivatives of maytansine, DM1 and DM4, were synthesized (Fig. 1). The thiol group provides a reactive site on the maytansinoid molecule for ready attachment to antibodies by a variety of linker chemistries, and does not negatively affect the potency of the maytansinoid (22, 23). DM1 and DM4 are now being produced at a scale sufficient for commercialization.

Linker design

The approach taken to conjugate maytansinoids to antibodies is to utilize linkers that form stable amide bonds upon reaction with amino groups of lysine residues of the antibody. Importantly, the *N*-hydroxysuccinimide ester chemistry used to form such amide bonds leaves untouched the inter-chain disulfide bonds of the antibody, bonds that may be important for antibody stability (25). Of the approximately 80 potential lysine residues in an IgG, the actual attachment sites resulting from a controlled linker reaction can be identified by mass spectroscopic peptide mapping techniques. In an AMC with an average of between 3 and 4 maytansinoids per antibody molecule, some 20 modification sites with varying levels of modification can be identified, the pattern of modification providing a fingerprint for assessing product consistency in manufacturing processes (26). The maytansinoid load distribution profile can also be assessed by mass spectroscopy and such profiles are consistent with a binomial distribution. Given an AMC with an average load of about 3.6 maytansinoids per antibody, an assumption of a binomial distribution leads to a calculated value of only 2-3% of antibody without any payload, a value consistent with experimental observations (27). The number of DM1 or DM4 molecules linked per antibody can be optimized empirically for each antibody/target pair; usually, the in vivo activity of AMCs is greatly diminished when fewer than three maytansinoids are linked per antibody, while the upper limit

Table I. *Antibody–maytansinoid conjugates taken into development.*

Compound	Developer[a]	Target antigen	Tumor type	Development phase	Number of patients treated[b]	References
T-DM1 (trastuzumab emtansine)	Genentech/Roche	erbB-2/HER2	Metastatic breast cancer	Phase III	274	30, 37-41
IMGN-901 (lorvotuzumab mertansine)	ImmunoGen	CD56	SCLC, MM, Merkel cell carcinoma, neuroendocrine tumors	Phase I & II	142	42-48
SAR-3419	sanofi-aventis	CD19	NHL, other B-cell malignancies	Phase I	38	16, 49
SB-408075 (cantuzumab mertansine)	ImmunoGen/ GlaxoSmithKline	CanAg (glycotope)	Colorectal, gastric, pancreatic	Discontinued[c]	96	28, 32, 50-52
IMGN-242	ImmunoGen	CanAg (glycotope)	Colorectal, gastric, pancreatic	Phase I & II[d]	45	53
IMGN-388	ImmunoGen	α_v-Integrin	Breast, lung, ovarian, melanoma	Phase I	32	54, 55
IMGN-633 (AVE-9633)	ImmunoGen/sanofi-aventis	CD33	AML	Phase I	39	56, 57
Bivatuzumab mertansine	Boehringer Ingelheim	CD44v6	Head & neck carcinoma, breast	Discontinued[c]	62	15, 58-60
BT-062	Biotest AG	CD138	MM	Phase I		61, 62
BIIB-015	Biotest-Idec	Cripto	Breast, colon, other solid tumors	Phase I	N/A	www.clintrials.gov
MLN-2704	Millennium Pharmaceuticals	PSMA	Prostate	Discontinued[c]	84	63-65
SAR-566658	sanofi-aventis	*O*-Linked sialoglycotope of Muc1	Ovarian, breast, other solid tumors	Late preclinical	N/A	66
AMC Compound	Bayer	Undisclosed	Solid tumors	Preclinical	N/A	N/A
AMC Compound	Amgen	Undisclosed	Solid tumors	Preclinical	N/A	N/A
AMC Compound	Amgen	Undisclosed	Cancer indications	Preclinical	N/A	N/A

[a]ImmunoGen, Inc. or ImmunoGen Partner; [b]restricted to data reported prior to December 31, 2009, except for IMGN-388; [c]discontinued after phase I program; [d]phase II in gastric cancer discontinued due to slow enrollment; SCLC, small cell lung cancer; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; AML, acute myeloid leukemia; N/A, not available.

may depend upon the solubility characteristics of the antibody and linker (8). The maytansinoid load for AMCs taken into development to date is in the range of about 3.5-4.0 per antibody (Fig. 1).

Beyond the above considerations of the site of attachment on the antibody and the number of cytotoxin molecules linked, the nature of the linker itself is also important. AMCs in clinical testing today exploit three different linkers (Fig. 1), two different disulfide linkers that are cleavable by thiol–disulfide exchange reactions, although at different rates (8, 20, 28, 29), and one noncleavable thioether link formed by reaction of the thiol of DM1 with a maleimido group of the succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker (30). The choice of linker for a given antibody/target pair is made during preclinical evaluation and can be tailored to suit the biological characteristics of both the target antigen and the tumor cells expressing the antigen, with a goal of maximizing the therapeutic window of the AMC (17, 30). For example, in the case of the antibody trastuzumab, targeting the erbB-2/HER2 antigen on breast cancer, AMCs made with both disulfide (cleavable) and thioether (noncleavable) linkers have similar potency in vitro, while the thioether-linked AMC exhibited slightly greater efficacy in vivo, leading to its selection for clinical development (30). However, for the huC242 antibody targeting the CanAg antigen expressed on colorectal and other gastrointestinal cancers, AMCs made with the noncleavable linker, while active in vitro, displayed very poor antitumor efficacy in vivo, while disulfide-linked conjugates were highly active, with a good therapeutic window in preclinical models (29, 31, 32), and were thus chosen for development (see Table I).

Recently, newer hydrophilic linkers have been developed that may allow linking of a greater number of maytansinoids per antibody, perhaps useful in cases where target antigen expression is low (33, 34), although an AMC containing one of these new linkers has not yet been introduced into clinical testing.

Mechanism of action: intracellular processing of AMCs

Once bound to its target cell-surface antigen, an AMC must be processed to release active maytansinoids able to reach the intracellular target (tubulin). Studies with AMCs having a radiolabel in the maytansinoid moiety have shown that both disulfide-linked conjugates and conjugates using a nondisulfide (noncleavable) linker share a common activation pathway (Fig. 2). The

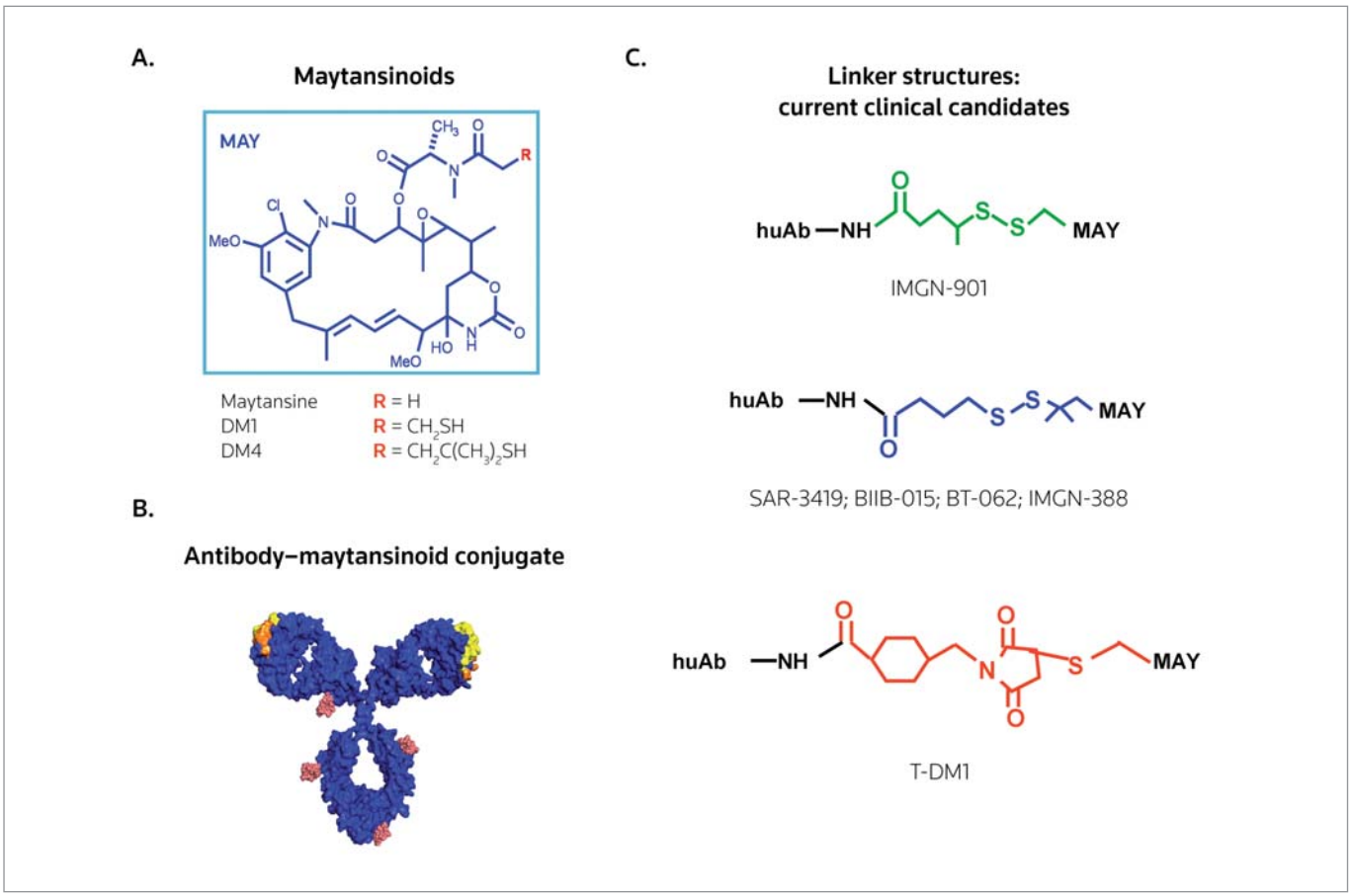

Figure 1. Antibody–maytansinoid conjugates: structure of payload and linker. (**A**) Chemical structure of maytansine and its thiol derivatives, DM1 and DM4; (**B**) space-filling model of an IgG1 antibody (blue) linked to four molecules of DM1 (salmon pink). The complementary-determining regions of the antibody heavy and light chains are orange and yellow, respectively; (**C**) linker structures between antibody and maytansinoid for six antibody–maytansinoid conjugates currently in clinical evaluation. MAY, maytansinoid; huAb, antibody.

antigen–AMC complexes are internalized into endosomes, which then fuse with lysosomes, leading to complete proteolysis of the antibody moiety of the AMC to its constituent amino acids, thus releasing the maytansinoid still linked to a residual lysine amino acid (20, 31). The maytansinoid-linker-lysine, is the only metabolite measurable in the case of the noncleavable thioether link formed by the reaction of SMCC and DM1 (20, 30). The analogous active compound is generated from disulfide-linked AMCs, although these maytansinoid-linker-lysine moieties are further processed in the cytoplasm via thiol–disulfide exchange reactions to yield the thiol-containing maytansinoids DM1 and DM4 (20, 29). Ultimately, *S*-methyl derivatives of DM1 and DM4 can be formed via *S*-methylation by an unknown intracellular methyltransferase (20). All of these maytansinoids produced by intracellular metabolism of the AMCs can bind tubulin and thus induce cell cycle arrest, and ultimately cell death (27). In the case of the neutral lipophilic maytansinoid thiols or their *S*-methyl derivatives generated from disulfide-linked AMCs, these compounds can enter neighboring cells by diffusion across cell membranes (Fig. 2), and allow the possibility for bystander cytotoxicity contributing to potent antitumor efficacy in vivo (29).

The finding that the initial (or only) product of intracellular processing is the maytansinoid-linker-lysine moiety opens up the possibility of changing the properties of the intracellularly active metabolite by changing the properties of the linker. The new hydrophilic linkers mentioned above have been found to increase the potency of AMCs towards cells that are multidrug-resistant because of increased expression of efflux pumps (33, 34). Neutral maytansinoids are subject to efflux via multidrug resistance protein MDR1 (35), and this process may be inhibited by making the intracellularly released compound more hydrophilic (33). However, the advantages of such linkers for increased retention of the cytotoxic payload within the targeted cell may be offset by reducing the potential for bystander killing in contributing to tumor eradication (29). Again, for any given target antigen, the optimal combination of antibody, linker and maytansinoid effector for maximal therapeutic window is assessed empirically during preclinical optimization of the AMC design.

ANTIBODY–MAYTANSINOID CONJUGATES IN CLINICAL DEVELOPMENT

A total of 11 AMCs have been introduced into development and are listed in Table I. Eight are currently active in clinical evaluation from

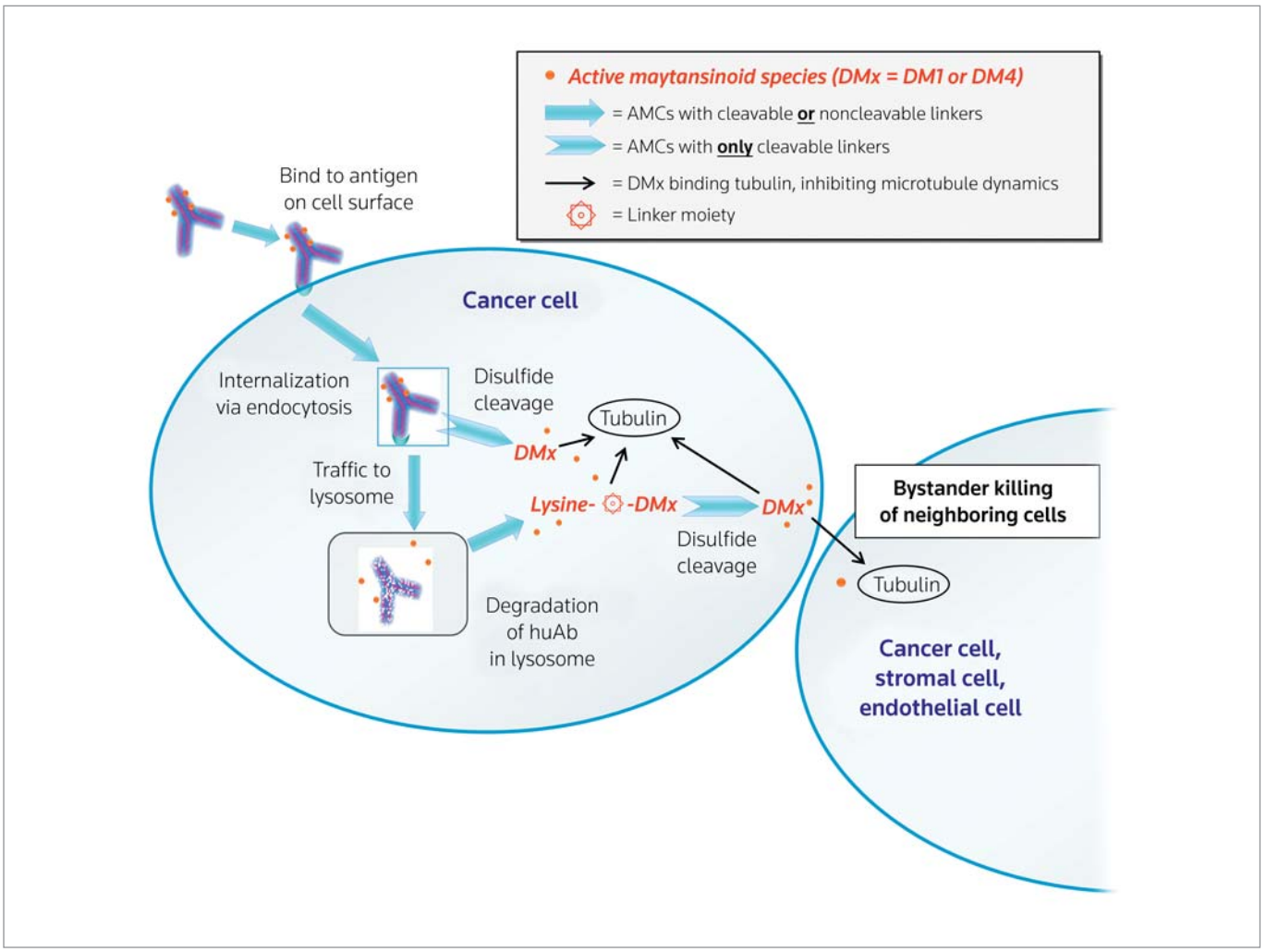

Figure 2. Mechanism of action of antibody–maytansinoid conjugates. The scheme is based on Erickson et al. (20) and Kovtun et al. (29). AMCs, antibody–maytansinoid conjugates; huAb, antibody.

phase I to phase III trials, with several other AMCs in preclinical development. Overall, data have been presented on more than 700 patients in published reports covering 10 of the compounds listed in Table I, including 21 phase I and phase II clinical trials. In these studies, human antibody responses to AMCs were reported to be low or undetectable, and no alterations of pharmacokinetic properties that could be attributed to anti-product antibodies were noted in any study to date. Thus, maytansinoid conjugation to nonimmunogenic humanized antibodies does not appreciably alter the low likelihood of their inducing an immune response in cancer patients. Four of the compounds, SAR-3419, SB-408075, IMGN-242 and IMGN-633 (AVE-9633), utilized resurfacing technology as the method of humanization (10), and no anti-AMC immune responses were detected in the 218 patients treated to date with these AMCs.

AMCs are generally well tolerated, with maximum tolerated doses (MTDs) in the range of about 3.4 mg/kg (126 mg/m^2) to about 6.3 mg/kg (235 mg/m^2) for dose intensity over 3 weeks, with the most common dosing schedules being a single dose every 3 weeks or weekly dosing (references in Table I). These dose ranges are similar to those used for naked antibody therapy – important if the antibody moiety of the AMC contributes to the overall antitumor activity via intrinsic activities of the antibody itself. In general, there is no one dose-limiting toxicity (DLT) that defines the MTDs of the different AMCs in clinical testing, indicating that the antibody component and its target antigen play a role in defining the DLT. Most noteworthy is the lack of clinically significant myelosuppression across all the AMCs, a finding that should allow for the ready combination of AMCs with standard chemotherapy regimens in the target diseases. Furthermore, severe gastrointestinal toxicity, dose-limiting in clinical studies with maytansine itself (36), is not reported to be a clinically significant toxicity for AMCs, suggesting that the linkers utilized in AMCs have good stability in patients.

While several AMCs show very promising antitumor activity in early-stage clinical trials (the three most advanced compounds being discussed in detail in the following section), as in other fields, not all agents entering the clinic continue to advance. For some com-

pounds, commercial/economic considerations result in product reprioritization or discontinuation. Other factors that come into play include target-related toxicity, which may change the risk–benefit calculation; bivatuzumab mertansine, despite clear evidence of antitumor activity in phase I studies, was discontinued for this reason (see references in Table I). Another emerging theme from the clinical experience to date summarized in Table I may be that one should not expect antibody-mediated delivery of a cytotoxic payload to confer activity to a cytotoxic drug if the tumor type is not sensitive to drugs of that class. For example, agents acting on tubulin (vinca alkaloids, taxanes) have not demonstrated any significant activity in colorectal cancer, and thus, the only modest signals of activity obtained with cantuzumab mertansine and IMGN-242 in phase I studies in largely colorectal cancer patient populations (see references in Table I) may not be surprising given that maytansinoids are also natural product-derived tubulin-targeting agents. Breast cancer and lymphoma, cancers where tubulin-targeting agents are used in conventional chemotherapy regimens, appear to be sensitive to AMCs in phase I studies, as described in more detail below. As more experience is gained in clinical studies and more understanding is developed about factors critical for the success of AMC compounds, future developments in AMC technology may help overcome some of the mechanisms of resistance, for example, the utilization of novel linker chemistries mentioned above (33).

ANTIBODY–MAYTANSINOID CONJUGATES DEMONSTRATE BENEFIT IN THE TREATMENT OF SOLID AND LIQUID TUMORS

T-DM1 (trastuzumab emtansine)

T-DM1, or trastuzumab emtansine (nonproprietary name for the antibody covalently linked to the DM1-SMCC linker adduct), combines the anti-HER2 antibody trastuzumab (Herceptin®) with DM1 via a noncleavable thioether link (see Fig. 1). The AMC was selected for clinical development based on potent antiproliferative activity in vitro and antitumor activity in vivo in preclinical models, including HER2-overexpressing models resistant to trastuzumab (30). A phase I clinical trial in patients with metastatic breast cancer (MBC) that had progressed upon treatment with trastuzumab (mean of 24 months on trastuzumab before treatment failure) established an MTD of 3.6 mg/kg, with DLT at 4.8 mg/kg being reversible thrombocytopenia when given as a single infusion every 3 weeks (37). Of note, this toxicity has not been reported to be clinically significant with any of the other eight AMCs for which an MTD was established (Table I). Of 15 patients treated at 3.6 mg/kg given every 3 weeks in the phase I trial, the median progression-free survival (PFS) was 9.8 months and the clinical benefit rate (CBR) was 53% (objective responses plus stable disease of $\geq$ 6 months), an exciting signal of activity in a phase I trial. The objective response rate (ORR) in patients with measurable disease (n = 9) was 44%. An MTD of 2.4 mg/kg was established in the same study for weekly administration (double the dose intensity of every-3-week dosing), and a similar level of activity was observed (41).

A proof-of-concept phase II trial was then performed to evaluate single-agent T-DM1 given as 3.6 mg/kg every 3 weeks in 112 patients with MBC who had received prior trastuzumab therapy for a median duration of 17.8 months (range: 1-152 months). According to independent review, this trial demonstrated an ORR of 23.9% and an overall CBR of 35.8% when including a stable disease maintained for at least 6 months (38). These patients had been treated with a median number of three prior chemotherapeutic agents in addition to previous treatment with trastuzumab. Twenty-six patients (23.2%) were still receiving T-DM1 at the date of data cutoff for this report. T-DM1 was well tolerated, with no dose-limiting cardiotoxicity. The most common adverse events were fatigue and nausea (grade 1 or 2). Grade 3 and 4 toxicities were infrequent, the most common being hypokalemia (8%), thrombocytopenia (7.2%) and fatigue (4.5%).

These findings were confirmed and extended in a second phase II trial that enrolled 110 patients with MBC who had all received prior exposure to a taxane, an anthracycline, capecitabine, lapatinib and trastuzumab, and who had progressed on their last regimen. Importantly, prior therapies for metastatic disease included two HER2-directed regimens (lapatinib for a median duration of 6-9 months and trastuzumab for a median duration of 14.4 months). In these heavily pretreated patients, the confirmed ORR was 32.7%, with a CBR of 44.5%, as assessed by independent review (39). The median follow-up was only 8.3 months and 40 patients were still on study at the time of the report. The HER2 status of patients was retrospectively assessed for 91 patients at a central laboratory; 76 (83.5%) were confirmed to be HER2-positive, and in these patients the ORR rose to 39.5% and the CBR increased to 52.6%. There were no new safety signals in this study. On the basis of these strong phase II data in advanced MBC, Genentech/Roche intend to apply in 2010 for marketing approval in the U.S. (Roche, April 15, 2010, press release and conference call).

A population pharmacokinetic model was developed for T-DM1 using the combined data from the 274 treated patients in the phase I and two phase II trials (40). The data for T-DM1 were best described by a two-compartment linear model, with a half-life of 4.5 days. In the phase I study, the half-life was dose-dependent, suggesting that antigen-mediated clearance plays a significant role in T-DM1 clearance (37), in agreement with recent data generated utilizing immuno-PET imaging with 89Zr-trastuzumab (67). The concentration of nonconjugated DM1 was very low, its maximum concentration being only 0.007% of that of T-DM1 (40). The immunogenicity of T-DM1 in these studies was reported to be low and had no impact on T-DM1 pharmacokinetics. T-DM1 is also being studied as a single agent in a phase III trial in a second-line setting versus lapatinib plus capecitabine, as a single agent in a phase II trial in a first-line setting versus trastuzumab plus docetaxel, as well as in a variety of combination trials with docetaxel, pertuzumab and a phosphatidylinositol 3-kinase (PI3K) inhibitor, GDC-0941 (http://clinicaltrials.gov). A phase III trial for first-line treatment of MBC is planned to start in mid-2010. The robust antitumor activity and excellent tolerability exhibited by T-DM1 suggest that this exciting new agent may change the treatment paradigm for $HER2^+$ metastatic breast cancer, with great promise for improved outcomes for patients.

IMGN-901 (lorvotuzumab mertansine)

IMGN-901, or lorvotuzumab mertansine (nonproprietary name for the antibody covalently linked to the DM1-SPP linker adduct), targets the CD56 antigen, also known as neural cell adhesion molecule 1, or NCAM-1 (6, 68). It comprises a humanized version of the N901

antibody (69) conjugated to DM1 via a hindered disulfide linker (Fig. 1). CD56 is expressed on a variety of cancer cells of hematopoietic and neuroendocrine origin, including multiple myeloma (MM) and certain leukemias and lymphomas (47), small cell lung cancer (SCLC) (6, 68), ovarian cancer (70), carcinoid tumors and neuroblastoma (68). Its normal tissue expression is restricted to natural killer (NK) cells and a subset of T cells in the hematopoietic compartment, as well as in nerve tissue and heart muscle, where it plays a role in cell–cell adhesion (68). IMGN-901 has exhibited potent antitumor activity in a variety of preclinical xenograft models in these disease indications (47, 70) and is being studied in both solid and hematopoietic tumors in clinical trials (42-48).

In MM, where 70-80% of patients have disease expressing CD56 (47), a phase I dose-escalation trial established 112 mg/m^2 (~ 3.0 mg/kg) as the MTD when IMGN-901 was administered weekly for 2 consecutive weeks every 3 weeks (46). A phase I trial in CD56$^+$ solid tumors established 75 mg/m^2 (~ 2.0 mg/kg) as the MTD when it was administered daily for 3 consecutive days every 3 weeks (45). The dose intensities between these two trials in hematological and solid tumors were remarkably consistent (~ 6 mg/kg over 3 weeks). The rationale for the daily x 3 dosing schedule was based on pharmacokinetic data obtained in an earlier trial in CD56$^+$ solid tumors evaluating a schedule of weekly x 4 every 6 weeks (42). The half-life of IMGN-901 was only about 21 h at doses ≥ 60 mg/m^2, relatively short for an antibody-based therapy and likely due to the normal tissue antigen sink of CD56 expressed on NK cells (42). The most common side effects were grade 1 or 2 headache, fatigue and neuropathy. DLTs were grade 3 fatigue in two of six patients dosed at 140 mg/m^2 in the MM trial, and grade 3 toxicities of myalgia (one patient) and headache and back and shoulder pain (one patient) seen in two of two patients dosed at 94 mg/m^2 given daily x 3 every 3 weeks (45, 46) in the solid tumor trial. Earlier findings of dose-limiting headache having onset within about 8 h and largely resolved by about 48 h in the weekly x 4 every 6 weeks study in CD56$^+$ solid tumors (42) were not seen in later studies that utilized routine low-dose steroid prophylaxis prior to treatment (43, 45, 46). There were no clinically significant changes in hematological parameters and no evidence for myelosuppression.

Encouraging antitumor activity was reported in the phase I study in MM. Of the 26 heavily pretreated patients (mean of 6 prior therapies), 8 patients stayed on treatment with IMGN-901 for longer than their last round of treatment with approved therapies, perhaps because IMGN-901 works by a different mechanism of action than approved drugs used to treat MM. For example, 1 patient whose prior treatment regimen duration was only 49 days before disease progression achieved a partial response (PR) and received IMGN-901 for more than a year, and was still on study at the time of the report (46). Another patient who had received 10 prior chemotherapy regimens and whose tenth regimen was only 8 days in duration sustained an objective minimal response and was treated with IMGN-901 for 154 days before disease progression. Two other patients had objective minimal responses and another 11 patients had stable disease. Based on preclinical data reporting synergism between lenalidomide and IMGN-901 (48), a combination trial has recently opened to study this combination in CD56$^+$ multiple myeloma patients (http://clinicaltrials.gov).

IMGN-901 also shows encouraging activity in CD56$^+$ solid tumors. A recent report (45) showed one durable complete response (CR) (≥ 4 years in remission), one durable PR and one case of clinically meaningful stable disease in three of six patients with metastatic Merkel cell carcinoma (mMCC). While numbers are small, these are remarkable findings in this rare, aggressive small cell cancer of the skin –the median survival of mMCC is only about 7 months (71). The observations in mMCC are supported by encouraging findings with IMGN-901 when used to treat SCLC, a cancer similar in both cell morphology and clinical course to mMCC. Two partial responses (1 unconfirmed) and 15 patients with clinically meaningful stable disease were noted from the 68 patients with SCLC from among the 113 total patients treated in the two phase I solid tumor trials (43-45). A CBR of 50% for mMCC and 25% for SCLC in phase I trials in aggressive cancers with dismal outcomes supports further investigation of IMGN-901 for the treatment of CD56$^+$ tumors, and the acceptable tolerability profile, in particular the lack of clinically significant myelosuppression, warrants investigation in combination trials.

SAR-3419 (huB4-DM4)

Conjugation of DM4 to the humanized anti-CD19 antibody huB4 (10, 69, 72, 73) creates an AMC containing a highly hindered disulfide linker for the treatment of NHL and other B-cell malignancies. SAR-3419 shows superior antitumor activity to rituximab in preclinical xenograft models of NHL (16, 72) and has entered phase I clinical trials (16). The first report of preliminary results from a trial of single-dose administration every 3 weeks for 6 cycles showed that the MTD was 160 mg/m^2 (~4.3 mg/kg), a dose level now being used to treat an expanded cohort of 15 patients. The DLT at doses > 200 mg/m^2 was reversible toxicity to the cornea that did not preclude continued dosing (at 208 mg/m^2), albeit with dose delays of 1-2 weeks, with no other clinically significant grade 3 or 4 toxicities reported. As with other AMCs, there was no clinically significant myelosuppression (N = 38 patients), suggesting that SAR-3419 may be readily combined with conventional chemotherapy regimens. The half-life of SAR-3419 in these patients was 4-6 days across all doses of the phase I trial (49).

Of the 27 patients evaluable for response at the time of reporting, tumor shrinkage was reported in more than half of the patients, with 5 objective responses. Notably, 7 of 14 rituximab-refractory patients showed tumor shrinkage, with 1 objective response. These early results from a phase I trial demonstrated promising activity and tolerability, especially considering the wide dose range (10-270 mg/m^2), the heavy pretreatment of these patients (24% had prior stem cell transplant), and the mixed histology of those enrolled (49). A second study with a weekly dosing schedule is under way, with first results expected to be reported in 2010.

CONCLUSIONS

Recent reports have demonstrated that AMCs have very promising antitumor activity in a variety of solid (MBC, SCLC, mMCC) and hematological tumors (MM, NHL). The compounds are well tolerated, with MTDs in a similar dose range to those used for "naked" antibodies in the clinic, with dose intensities in the range of 3.4-6.3 mg/kg over 3 weeks using various schedules. Of note, the DLTs tend to vary somewhat from AMC to AMC. While factors such as the dis-

ease setting and the different prior therapies used may impact the nature of the toxicity seen in phase I trials with different AMCs, the clinical findings also suggest that the target of the antibody moiety may play a role in the toxicity profile of particular AMCs. This observation also speaks to the stability of the linkers used in the AMCs: if they were not stable, one could expect to see consistent maytansinoid-related DLTs across all studies (see reference 36 for a summary of the clinical toxicity profile of maytansine). Also noteworthy is that the three most advanced AMCs each utilized a different linker-maytansinoid design (Fig. 1). Each design proved optimal for the particular antibody/target antigen pair, the empirical selection in preclinical studies being influenced by the properties of the target (e.g., antigen density, internalization rate, processing efficiency, biology of target cancer). These three AMCs also include one compound utilizing an antibody that has intrinsic naked antibody activity (trastuzumab in T-DM1) and two compounds utilizing antibodies that lack significant antitumor activity until conjugated (16, 47).

Of the three AMCs that are furthest advanced in clinical testing, T-DM1 has advanced most rapidly, from first-in-human dosing to phase III clinical trials in about 4 years. Its development was greatly aided by the fact that so much was already known about the HER2 target, in particular with regard to patient selection, thanks to the prior development of trastuzumab itself. For other AMCs to novel targets such knowledge will need to be developed during clinical trials. It is exciting to report that, after nearly 30 years of research, the emerging clinical data with several compounds suggest that AMCs promise to make a real difference in the lives of patients with cancer in the very near future, providing active antitumor agents that lack the severe toxicities associated with chemotherapy.

DISCLOSURES

The author is an employee of ImmunoGen, Inc., the developer of the maytansoid conjugate technology.

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