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Targeted Cancer Therapy: Conferring Specificity to Cytotoxic Drugs

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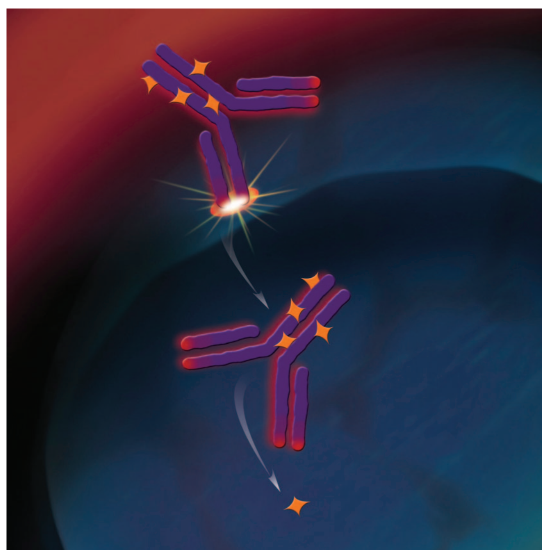
CONSPECTUS

The therapeutic activity of most anticancer drugs in clinical use is limited by their general toxicity to proliferating cells, including some normal cells. Although, chemists continue to develop novel cytotoxic agents with unique mechanisms of action, many of these compounds still lack tumor selectivity and have not been therapeutically useful. Monoclonal antibodies that bind to specific markers on the surface of tumor cells offer an alternative therapy that is tumor specific and thus less toxic. Although highly selective, very few monoclonal antibodies are therapeutically useful since they only display modest cell killing activity. The linkage of monoclonal antibodies to highly cytotoxic drugs can be viewed as a means of (a) conferring higher tumor selectivity to cytotoxic drugs that are too toxic to be used on their own or (b) conferring cell killing power to monoclonal antibodies that are tumor-specific but not sufficiently cytotoxic. This Account provides a brief history of the development of antibody−drug conjugates and shows how the lessons learned from the first generation of conjugates has guided the development of more effective antitumor agents.



The three components of antibody−drug conjugates, that is, the monoclonal anitbody, the cytotoxic drug, and the linker connecting the drug to the antibody, have been methodically studied and optimized. The antimitotic drug maytansine was chosen for use in the targeted delivery approach because of its high *in vitro* potency. Analogues of maytansine bearing a disulfide substituent that allowed linkage to monoclonal antibodies via disulfide bonds were prepared. These analogues retain the high potency of the parent drug. The stability of the disulfide link in antibody−maytansinoid conjugates was varied by introduction of methyl substituents on the carbon atoms geminal to the disulfide link. The optimized disulfide linker was stable in circulation *in vivo*. The circulation half-life of the cytotoxic drug was increased from just a few hours for the unconjugated drug to several days for the conjugate. Upon binding of the conjugate to the tumor cell, internalization and lysosomal processing released the potent cytotoxic agent inside the cell. These conjugates displayed high target-specific cytotoxicity *in vitro*. The antitumor activity of these targeted agents was superior to that of the antibodies alone or the standard anticancer drugs in human tumor xenograft models. Several conjugates from this new class of tumor-targeted anticancer agents are currrently undergoing clinical evaluation. The progress made in the targeted delivery approach and initial clinical results opens the door to the future development of highly potent drugs that were too toxic on their own to be therapeutically useful.

Introduction

The antitumor efficacy of clinically used anticancer drugs is limited by their nonspecific toxicity to proliferating normal cells, resulting in a low therapeutic index and a narrow therapeutic window. Most anticancer drugs have to be used near their maximum tolerated dose (MTD) to achieve a clinically meaningful therapeutic effect. Multidrug

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therapy is a standard modality for the treatment of most cancers.[1] With such intensive chemotherapy, systemic toxicity to the host remains a drawback of cytotoxic drugs in cancer and cures are achieved only in a small set of cancers.

The lack of tumor selectivity of anticancer drugs and the development of multidrug resistance (*mdr*) have given impetus to the development of target-specific agents and new classes of cytotoxic compounds that may be able to overcome *mdr*. One approach that has now been validated by clinical success is the development of small molecules that specifically inhibit kinase enzymes that are believed to play key roles in the development of tumors. Another tumor-selective approach targets specific receptors or other markers that are expressed on the surface of human tumor cells. The targeting molecule can be a monoclonal antibody, a peptide, a vitamin, such as folic acid, a hormone, or a growth factor, such as epidermal growth factor. This Account will be limited to a discussion of the use of monoclonal antibodies for the targeted therapy of cancer.

Monoclonal Antibodies in Cancer

Comparative evaluation of human cancer tissues and normal tissues has identified antigens that are preferentially or exclusively expressed on the surface of cancer cells. Monoclonal antibodies can be generated to recognize and specifically bind to these tumor-associated antigens. They are large proteins with an average molecular weight of about 150 kDa. Upon binding to the tumor cell, a few "functional antibodies" display modest cell-killing activity by themselves. Functional antibodies that are currently approved for the treatment of cancer include Rituxan (rituximab) for B-cell lymphomas, Herceptin (trastuzumab) for breast cancer, Campath (alemtuzumab) for certain leukemias, and Erbitux (cetuximab), Vectibix (panitumumab), and Avastin (bevacizumab) for colorectal cancers. A majority of these antibodies display moderate antitumor activity and are often used only in combination with anticancer drugs. Even in cases where the unconjugated antibody has good activity, the potency is greatly enhanced by conferring additional cell-killing ability by attaching a radioactive isotope to the antibody.

Monoclonal Antibodies as Delivery Vehicles for Cytotoxic Drugs

We have learned that monoclonal antibodies can bind selectively to tumor cells, but binding does not often lead to cytotoxicity. On the other hand, chemotherapeutic drugs have poor selectivity for the tumor; albeit, they have good cell-killing ability. In addition to high selectivity, monoclonal antibodies offer other advantages, such as favorable pharmacokinetics. A humanized antibody has a half-life of several days to weeks in circulation in humans. Also, monoclonal antibodies are nontoxic during circulation and are functional only upon binding to the antigen on the tumor cell. How can these desirable properties of antibodies be exploited for the specific delivery of cell-killing drugs to the desired target? Herein, we discuss the historical development of antibody–drug conjugates and the principles guiding the generation of effective conjugates and their current development status.

Antibody–Drug Conjugates

Principles. Ideally, a conjugate should be designed such that it remains nontoxic in circulation *in vivo* until it reaches its target site. After binding to the target cell, the conjugate is internalized by a process called receptor-mediated endocytosis. The extent of internalization or endocytosis of an antibody depends upon the nature of the cell-surface molecules to which it binds. The first category consists of various receptors that accumulate in coated pits and are quickly internalized. Some of these receptors, such as the transferrin receptor, seem to internalize continuously, irrespective of whether the ligand is bound or not. Others, such as the epidermal growth factor receptor, seem to accelerate in their accumulation in the coated pits upon binding by their natural ligand or with an antibody that mimics such a ligand and subsequently internalize. Other antibodies that do not mimic the ligand may not affect the rate of endocytosis of the receptor. The second category of cell-surface molecules (those that are internalized moderately) are constitutively endocytosed during plasma membrane recycling, and there is no compelling evidence that antibodies bound to them significantly accelerate their internalization. The third category comprises cell-surface molecules that reside permanently on the cell surface and are poorly internalized.

Internalization is followed by scission of the bond between the drug and the carrier antibody molecule to release the fully active drug inside the target cell. A pictorial representation of an antibody–drug conjugate is shown in Figure 1. Typically, on average, four molecules of a drug (depicted as orange spheres) are linked per molecule of antibody, via lysine residues on the antibody. Although the linkage of drug molecules to antibodies is a random phenomenon, with drug attachment through the ε-amino group of any one of the $\sim$80 lysine residues present on an antibody, typically a much fewer number of lysines ($\sim$10) are preferentially accessible for chemical modification. Ideally, the drugs are linked at the Fc or constant region of the antibody, which does not participate in

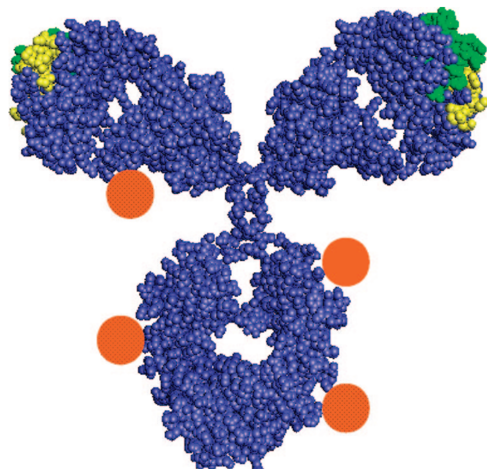

FIGURE 1. Pictorial representation of an antibody–drug conjugate.

binding to the antigen. The antigen-binding complementarity determining region (CDR) loops are shown in yellow and green (Figure 1). Linkage of a larger number of drug molecules is often not possible, because the cytotoxic drugs are usually hydrophobic and poorly soluble in the predominantly aqueous milieu (<5% organic solvent) required to keep the antibody in solution. In addition, the linkage of a large number of drug molecules may adversely alter the pharmacokinetics of the antibody *in vivo* or diminish the binding affinity of the antibody to the antigen on the target cell.

First-Generation Antibody–Drug Conjugates

Design of a Conjugate. In the first-generation antibody–drug conjugates, researchers aimed at enhancing the tumor specificity of clinically used anticancer drugs, such as methotrexate, the *Vinca* alkaloids, and doxorubicin, by linking them to monoclonal antibodies.[2] It was recognized early on that the nature of the linker connecting the monoclonal antibody and drug was important. After internalization into a target cell, a intracellular release mechanism should cleave the linker to release the active drug. Among the linkers used were acid-labile linkers that relied on the acidic pH (~5) of the intracellular compartment, the endosome, and enzymatic-labile linkers that relied on lysosomal enzymes, such as peptidases and esterases, for cleavage.

Biological Evaluation. *In vitro* evaluation of these first-generation conjugates on target tumor cell lines revealed that, in most cases, these conjugates were only moderately potent and often less active than the parent drug. Target-selective potency was rarely demonstrated *in vitro*. Nevertheless, researchers pursued the evaluation of antitumor activity of these conjugates in human xenograft models in mice. Significant localization of an antibody–methotrexate conjugate at the tumor was demonstrated.[3] Within 3 h after administration, as much as 15% of the injected dose of the conjugate had accumulated per gram of tumor. Researchers were eager to see whether the impressive tumor localization would lead to significant antitumor activity *in vivo*. The therapeutic efficacy of vinblastine[4] and doxorubicin[5] linked to antibodies via acid-labile bonds was shown to be superior to that of the corresponding unconjugated drugs.

Clinical Evaluation. These encouraging preclinical results led to the clinical evaluation of three candidates. A conjugate of the KS1 antibody with the cytotoxic drug methotrexate was evaluated in two different phase I clinical trials in patients with nonsmall cell lung cancer.[6] Immunohistochemical staining of carcinoma samples of the patients, post-treatment, provided convincing evidence of tumor localization of the conjugate. However, no evidence of therapeutic benefit or clinical response was observed in either study. Clinical results with KS1/4-desacetylvinblastine also failed to show a therapeutic benefit of targeted delivery. The antibody–doxorubicin conjugate BR96-Dox underwent evaluation in phase II human clinical trials in gastric adenocarcinoma[7] and metastatic breast cancer.[8] Little or no antitumor activity was noted in these trials.

First-Generation Antibody–Drug Conjugates: Lessons Learned. Several shortcomings of these first-generation conjugates can be identified:

(1) Insufficient potency of the effector molecule: Circulating serum concentrations in patients achieved were not in the therapeutic range.

(2) Limited expression of the antigen: An antibody–drug conjugate has to first bind to specific receptors on the tumor cell surface before being internalized into the cell. Because tumor cells express only a limited number of antigen molecules on the cell surface (typically $<1 \times 10^5$ receptors/cell), the number of molecules of drug that can be delivered by an antibody may not achieve the threshold concentration inside the cell to cause cell death.

(3) Internalization: Internalization mechanisms of antibodies can be inefficient; the actual number of conjugate molecules that are delivered into the cell is often lower than the number of molecules that were bound to the cell surface.

(4) Tumor localization: The localization rate of radiolabeled monoclonal antibodies at the tumor in patients is low (0.003–0.08% of the injected dose/g tumor).[9] In contrast, a much higher accumulation rate (~15–20% injected dose/g tumor) was measured in tumor xenografts in mice.

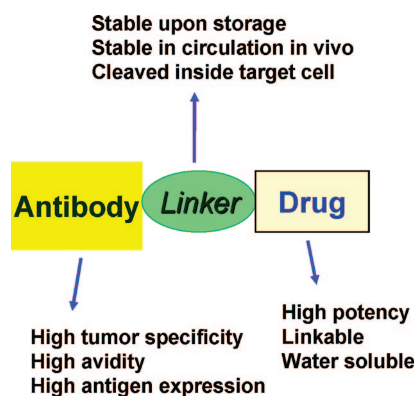

FIGURE 2. Key characteristics of antibody–drug conjugates.

(5) Linker stability: Either the linkers were too stable, resulting in low potency and poor efficacy, or too labile, resulting in poor target specificity and high systemic toxicity.

(6) Immune response: Monoclonal antibodies used in the early conjugates were either of murine origin or partly murine and partly human (chimeric), resulting in an immune response, and the generation of human antimurine antibodies (HAMA) prevented repeat cycles of therapy.

Second-Generation Antibody–Drug Conjugates

We have learned that the success of the targeted delivery approach depends upon three components: (1) the characteristics of the antibody, (2) the potency of the drug, and (3) the method of linkage of the antibody to the drug. The key characteristics needed in these three components to realize the true potential of this approach are shown in Figure 2.

Antibody Selection. The antibody should be carefully selected such that it binds selectively to tumor tissue and has little cross-reactivity with healthy tissues. It should bind to the tumor cells with high avidity (K_D of around 0.1 nM). Preferably, antibodies to antigens with a high expression on the cell surface should be identified. Murine antibodies should be replaced by nonimmunogenic "humanized" forms.

Selection of the Drug. The challenge of the chemists is to find or design new cytotoxic agents that possess the following properties: (1) high potency *in vitro* toward tumor cell lines, with IC_{50} values in the range of 0.01–0.1 nM (i.e., active in the concentration range of antibody binding to tumor cells), (2) a suitable functional group for linkage to an antibody (if a functional group is not already present, the desired substituent has to be introduced at a suitable site to retain potency of the parent drug), (3) reasonable solubility in aqueous solutions to enable the reaction with antibodies, and (4) prolonged stability in aqueous formulations commonly used for antibodies.

The choice of drug could depend upon the sensitivity of the tumor type to drugs with a given mechanism of action. For example, ovarian and breast cancers are sensitive to tubulin agents, while lymphomas are sensitive to DNA-interacting agents. On the basis of these criteria, highly potent drugs with diverse mechanisms of action are now being evaluated in antibody–drug conjugates. The laboratories of the author have developed new effector molecules consisting of the maytansinoids (DM1), which inhibit microtubule assembly, CC-1065 analogues (DC1), which are DNA alkylators, and taxoids (IGT), which stabilize microtubules. These cytotoxic compounds are 100–1000-fold more potent *in vitro* than previously used drugs. The chemistry and use of these drugs, along with that of analogues of calicheamicin (causes DNA double-strand breaks) and auristatins (inhibitors of microtubule assembly), will be described below.

Selection of the Linker. The linker between the antibody and drug has to be designed in a manner that ensures stability during circulation in blood but allows for the rapid release of the cytotoxic drug in its fully active form inside the tumor cells. Furthermore, the conjugate must remain intact during storage in aqueous solution to allow formulations for convenient intravenous administration. Several types of cleavable linkers have been evaluated, notably acid- and peptidase-labile linkers.[6] However, initial studies in the laboratories of the author led to the conclusion that disulfide linkers are a better choice. Disulfide linkers take advantage of three important factors: (1) they are stable at physiological pH; (2) levels of reduced glutathione, an intracellular thiol-containing tripeptide that can cause scission of disulfide bonds and release of the drug inside the cell, is reported to be in the millimolar range in cancer cells;[10] and (3) levels of reduced glutathione in circulation in blood is very low (typically in the micromolar range).

Second-Generation Antibody–Drug Conjugates in Development

Antibody–Maytansinoid Conjugates. Chemistry. Maytansinoids are members of the ansamycin class of natural products. They are potent antimitotic agents that exert their cytotoxic effect by disrupting microtubule assembly. They are about 1000-fold more cytotoxic *in vitro* than clinically used anticancer drugs. Kupchan et al.[11] first isolated the parent drug of this class, maytansine (**1**, Figure 3) from an Ethiopian shrub. The antitumor activity of maytansine was extensively evaluated in human clinical trials,[12] but the results were disappointing because maytansine, although potent *in vitro*, displayed a poor therapeutic window *in vivo*.

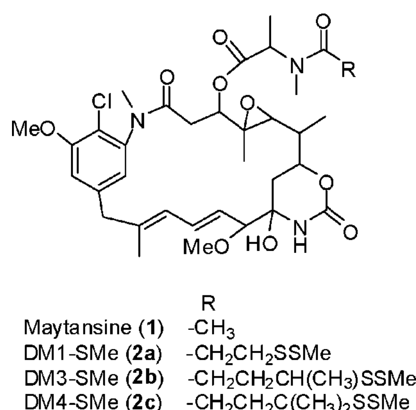

FIGURE 3. Structure of maytansinoids.

The high potency of maytansine made it an attractive candidate for targeted delivery for the selective destruction of tumor cells. The first step was to synthesize analogues of maytansine incorporating a thiol-containing substituent, which could then undergo disulfide exchange with an appropriately modified antibody to give a conjugate. The key requirement was that the nature and position of the substituent would not diminish the potency of the parent molecule. We synthesized several disulfide-containing maytansinoids and selected a lead compound DM1–SMe (**2a**, Figure 3), where the *N*-acetyl group in maytansine was replaced by a methyldithiopropanoyl group.[13] The disulfide bond linking the antibody and the drug can be manipulated to achieve maximal stability during circulation in the blood stream while allowing for efficient cleavage inside the target cell. This is achieved by the introduction of methyl substituents on the carbon atoms geminal to the disulfide link, conferring varying degrees of steric hindrance. We therefore synthesized a set of disulfide-containing maytansinoids with varying chain length and degree of steric hindrance (e.g., DM3–SMe, **2b** and DM4–SMe, **2c**, Figure 3).[14] The disulfide moiety was reduced to give the corresponding thiol-containing maytansinoids for conjugation to antibodies.

Disulfide-linked antibody–maytansinoid conjugates, containing about 3 to 4-linked maytansinoids/antibody molecule, were prepared by modifying lysine residues on the antibody with a bifunctional cross-linking agent to introduce pyridyldithio groups, followed by the reaction of the modified antibody with the thiol-containing maytansinoid. To understand the importance of the disulfide bond, we also linked the maytansinoids to an antibody via a thioether bond that was expected to be "noncleavable".

Maytansinoids have been conjugated to several tumor-specific antibodies, and these conjugates are in various stages of clinical development. The antibody–drug conjugate huC242–DM1 (Cantuzumab mertansine) comprises the maytansinoid

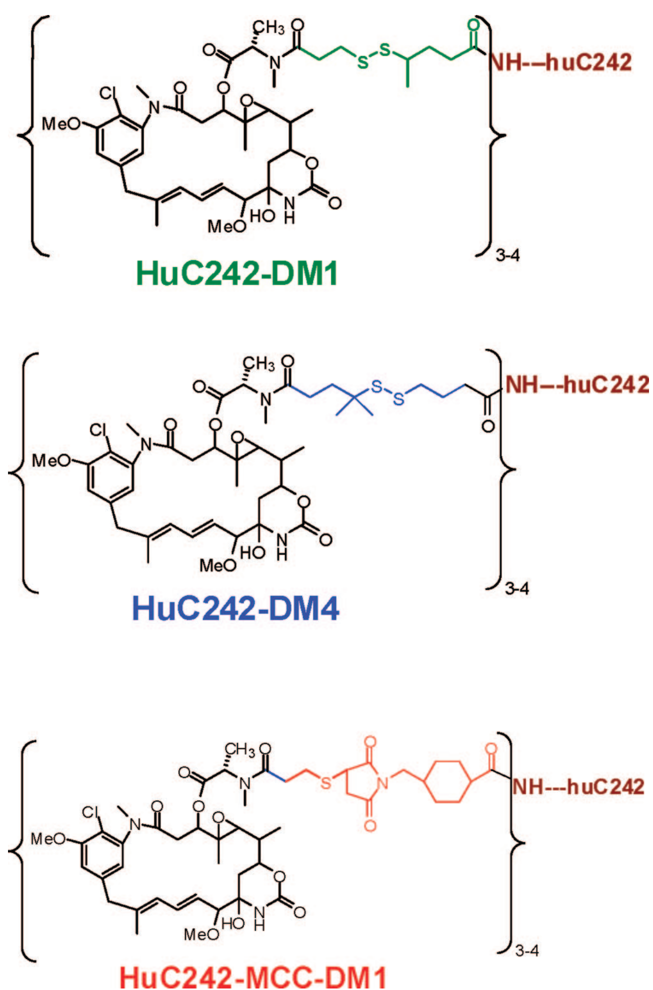

FIGURE 4. Structural representation of antibody–maytansinoid conjugates.

DM1 linked to the humanized monoclonal antibody huC242. The C242 antibody binds with high affinity to the carbohydrate antigen CanAg, which is expressed on the surface of human colorectal, pancreatic, and gastric cancer cells and on some nonsmall cell lung cancers. To evaluate the effect of the linker on stability and antitumor activity, the huC242 antibody was also linked to maytansinoids via sterically hindered disulfide bonds (huC242–DM4) and a thioether link (huC242–MCC–DM1, Figure 4).

Biological Evaluation. *In vitro* cytotoxicity assays on a panel of human tumor cell lines showed that the introduction of a methyldisulfide substituent in maytansine was well-tolerated. DM1–SMe was about 3–10-fold more potent than the parent drug maytansine (**1**), with IC_{50} values ranging from 0.003 to 0.01 nM for DM1SMe (**2a**).[13] Maytansinoids bearing sterically hindered disulfide bonds (DM3–SMe, **2b** and DM4–SMe, **2c**) showed even greater potency than DM1–SMe.[14]

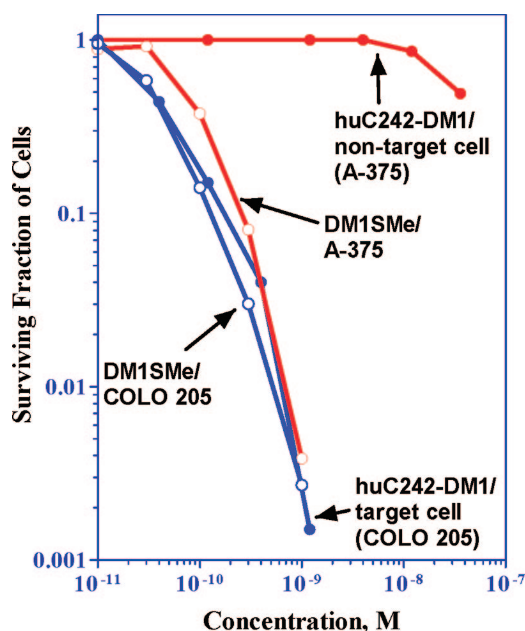

FIGURE 5. *In vitro* potency of unconjugated drug DM1–SMe and huC242–DM1 conjugate toward target COLO 205 and nontarget A-375 cells.

In vitro, huC242–DM1 was effective in killing antigen-expressing COLO 205 cells, with an IC_{50} value of 0.032 nM. The conjugate is about 1000-fold less cytotoxic toward the antigen-negative human melanoma cell line A-375 (Figure 5), demonstrating the antigen specificity of the cytotoxic effect.[15] In contrast, the nonconjugated maytansinonid DM1–SMe is equally cytotoxic toward both cell lines. The maytansinoid conjugate huC242–DM4, bearing a sterically hindered disulfide was about 2-fold more potent than huC242–DM1. Surprisingly, the "noncleavable" thioether-linked huC242 conjugate (huC242–MCC–DM1) was also extremely potent *in vitro* toward target cells. This result prompted us to study the mechanism of processing of antibody conjugates by cells.

Upon internalization by target cells, antibody–maytansinoid conjugates undergo rapid degradation of the antibody component in the lysosome, resulting in the release of the maytansinoid drug attached via the linker to one amino acid (a lysine residue) of the antibody. In the case of a disulfide-linked conjugate, the lysyl-modified maytansinoid undergoes disulfide reduction to release the thiol-containing drug, which then undergoes methylation, presumably catalyzed by a intracellular methyltransferase enzyme to give the highly potent *S*-methylmaytansinoid.[16] This released drug is able to diffuse out of the cell and kill neighboring cells to give a bystander-killing effect.[17] This phenomenon may be important *in vivo* because all cells in a tumor population may not express the antigen but can still be killed. The thioether-linked conjugate also undergoes similar lysosomal degradation, but in this case,

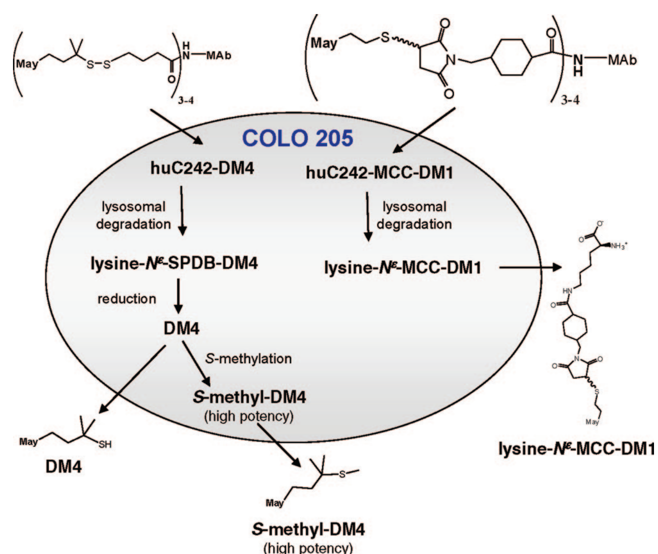

FIGURE 6. Processing of antibody–maytansinoid conjugates by cells.

the released drug remains in the lysyl-modified form, which is presumably still able to interact with tubulin, resulting in cell death. However, because this form of drug is charged, it is not able to diffuse into neighboring cells and, hence, a bystander effect is not observed (Figure 6).

The premise of the antibody– drug conjugate approach is that linking of a small drug molecule to a large antibody molecule would confer the drug with the favorable pharmacokinetic properties of the antibody. A comparison of the clearance from circulation *in vivo* of free maytansine and the huC242–DM1 conjugate showed that this was indeed the case. The terminal half-life of maytansine was extended from 2.1 to 44 h by conversion into a conjugate. Similarly, the area under the curve (AUC) of the conjugated drug was increased as much as 60-fold over that of the free drug.[18]

Do the increased tumor selectivity and longer circulation half-life of the conjugated drug result in greater antitumor activity? The antitumor efficacy of C242–DM1 was compared to that of unconjugated maytansine and to 5-fluorouracil (5-FU)/leukovorin and irinotecan (CPT-11), the most commonly used anticancer drugs for the treatment of colorectal cancer. In a subcutaneous human colon tumor (HT-29) xenograft model, unconjugated maytansine had little antitumor activity even at its MTD. Treatment with 5-FU/leukovorin or irinotecan at their respective MTDs resulted in modest delays in tumor growth. In contrast, C242–DM1 caused complete eradication of the tumor (Figure 7).[15] Importantly, at curative doses, C242–DM1 was nontoxic to the animals. Thus, targeted delivery using an antibody greatly improves the therapeutic window of the maytansinoid. This approach opens the

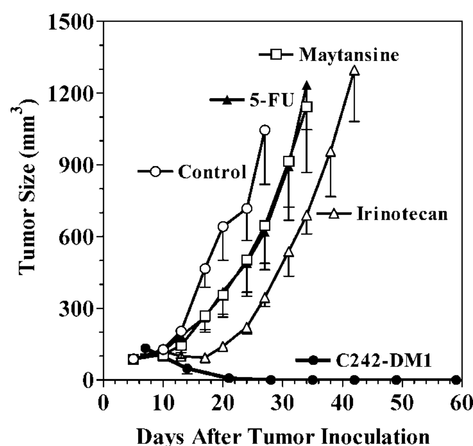

FIGURE 7. *In vivo* antitumor activity of the C242–DM1 conjugate.

door to the therapeutic use of highly potent compounds that were previously too toxic to be useful.

The *in vivo* stability of the antibody–maytansinoid link and the antitumor activity of two disulfide-linked huC242–maytansinoid conjugates (huC242–DM1 and huC242–DM4) and a thioether-linked conjugate (huC242–MCC–DM1) were compared in mice. The *in vivo* stability of the thioether-linked conjugate was the greatest, with a half-life of 134 h. The conjugate bearing a sterically hindered disulfide huC242–DM4 showed good stability ($T_{1/2}$ = 102 h), while huC242–DM1 had a $T_{1/2}$ of 47 h (Figure 8a). Upon treatment at low, noncurative doses, the conjugate with intermediate stability, huC242–DM4, showed the highest efficacy in a human COLO 205 xenograft model in mice, suggesting a fine balance between the linker stability and antitumor activity (Figure 8b).

Maytansinoids linked to monoclonal antibodies to various targets are currently being evaluated. In the laboratories of the author, DM1 was linked to the humanized antibody huN901, which binds to the CD56 antigen expressed on small cell lung cancers (SCLCs), neuroblastomas, and multiple myeloma. In a human SCLC xenograft model in mice, treatment with huN901–DM1 resulted in cures at nontoxic doses, while a mixture of the unconjugated DM1 drug and the huN901 antibody had no effect on tumor growth. huN901–DM1 also showed good activity in animal models of multiple myeloma.[19]

Similar results were reported In human prostate tumor xenografts in mice, where the maytansinoid conjugate J591–DM1 (MLN2704) targeting prostate cancer was extremely efficacious and caused tumor growth delays lasting 100 days.[20] Preclinical results from efforts to augment the antitumor activity of trastuzumab (Herceptin), a monoclonal antibody that is approved for the treatment of breast cancer, have also been reported.[21] In experimental tumor models, the maytansinoid

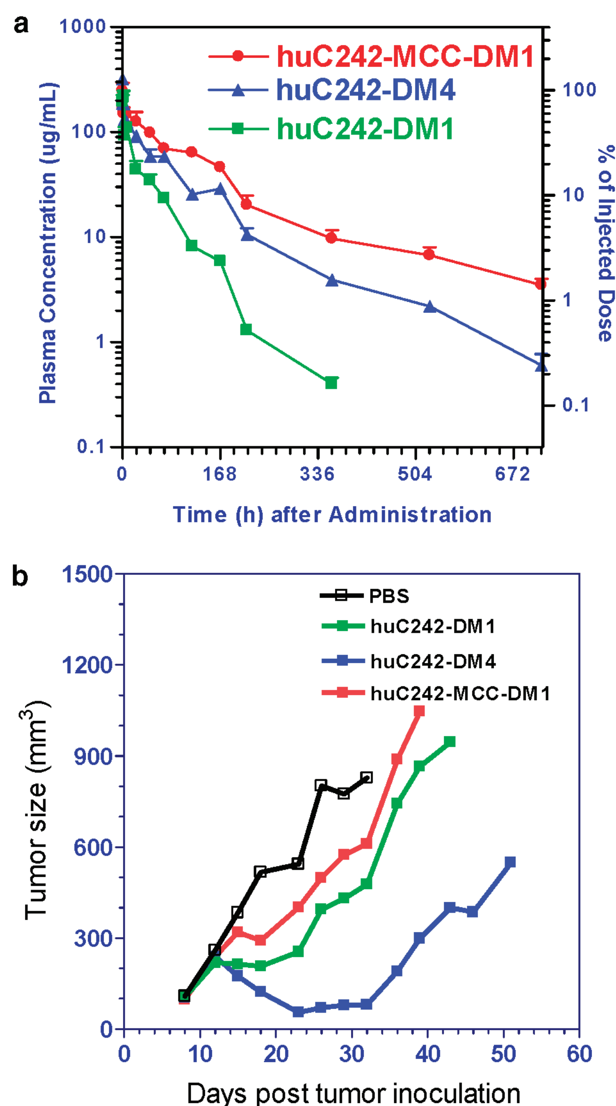

FIGURE 8. (a) Effect of the linker on *in vivo* stability. (b) Effect of the linker on *in vivo* antitumor activity.

conjugate trastuzumab–DM1 exhibited greater antitumor activity than the unconjugated antibody, supporting further investigation in a clinical setting.

Clinical Evaluation. Conjugates containing DM1 are in various stages of clinical evaluation. The first such conjugate, Cantuzumab mertansine (huC242–DM1), has recently completed phase I evaluation in cancer patients.[22] From these trials, the investigators concluded that the conjugate was well-tolerated. Clinical trials with unconjugated maytansine had previously shown severe side effects in the form of neutropenia, gastrointestinal toxicity, and peripheral neuropathy.[12] These side effects were absent or mild in clinical trials with the antibody–maytansinoid conjugate. As expected, the terminal elimination half-life of the conjugate was long (average of 41 h). Patients did not elicit immune responses to either the antibody or the maytansinoid. The investigators reported pre-

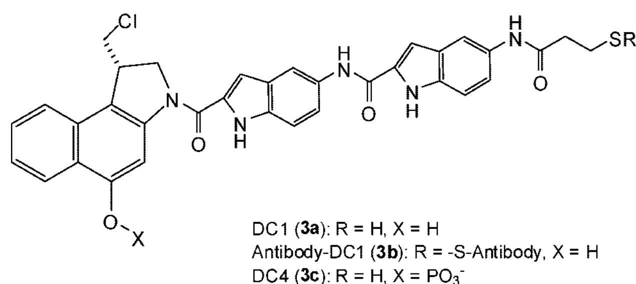

FIGURE 9. Structures of DC drugs.

liminary evidence of tumor localization of the maytansinoid conjugate and also encouraging signs of antitumor activity. However, on the basis of the superior activity and greater stability of huC242–DM4 *in vivo*, this conjugate has replaced huC242–DM1 in human clinical trials. Data from these early clinical trials provide strong evidence that this targeted delivery approach may be able to overcome many of the shortcomings associated with the therapy of unconjugated drugs or the first-generation antibody–drug conjugates.

Human clinical trials with other maytansinoid conjugates are ongoing. These include huN901–DM1 for the treatment of small cell lung cancer and multiple myeloma, AVE9633 (huMY9-6–DM4) for the treatment of acute myeloid leukemia, and Trastuzumab–DM1 for breast cancer.

Antibody–CC-1065 Analogue Conjugates

CC-1065 is a highly cytotoxic bacterial natural product that is 1000-fold more cytotoxic than other DNA-interacting agents, such as doxorubicin and *cis*-platin. CC-1065 binds in a sequence-selective manner to the minor groove of DNA, followed by alkylation of adenine bases on the DNA.[23] Human clinical trials[24] with adozelesin, a highly potent synthetic analogue, failed to show a therapeutic benefit. However, the high *in vitro* potency of adozelesin and its unique mechanism of action made it an ideal candidate for tumor-specific delivery. We synthesized a disulfide-containing analogue of adozelesin, called DC1 (**3a**, Figure 9). DC1 was extremely potent and killed tumor cells with an IC_{50} value of 0.02 nM.

The antibody conjugate Anti-B4–DC1, which binds to the CD19 antigen expressed in B-cell lymphoma was cytotoxic for the target Namalwa cell line, with an IC_{50} value of 0.02 nM.[25] Unlike the unconjugated DC1 drug, Anti-B4–DC1 showed high selectivity, being at least 1000-fold less cytotoxic to the nontarget MOLT-4 cells.[25] In human tumor xenograft models, Anti-B4–DC1 was shown to be far superior to clinically used anticancer drugs in the treatment of lymphoma. Despite its high potency, DC1 conjugates were not further developed because of the poor solubility of DC1 in aqueous buffers, making conjugation reactions inefficient. A solution to this problem was found, and DC1 was converted into the water-soluble phosphate prodrug DC4 (**3c**), which is now amenable to conjugation reactions with antibodies in aqueous buffers. Phosphate prodrugs (e.g., etoposide phosphate) are known to be rapidly converted into the active drug in circulation *in vivo* by the action of phosphatises.[26]

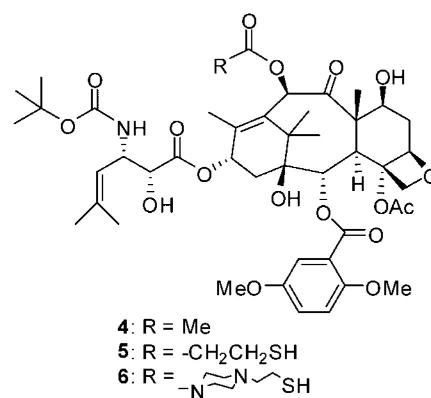

FIGURE 10. Structures of potent taxoids.

Antibody–Taxane Conjugates

Paclitaxel and its semisynthetic analogue docetaxel are two of the most active agents in the treatment of cancer. Paclitaxel and docetaxel are not sufficiently potent for use in antibody conjugates. Recently, we have synthesized a series of highly potent taxoids. A lead taxoid (**4**, Figure 10) was shown to be 100-fold more potent than paclitaxel *in vitro* against taxane-sensitive and multidrug-resistant cell lines. We have introduced disulfide-containing substituents that enable linkage to antibodies while maintaining extraordinarily high cytotoxicity.[27–29] Representative examples of these taxoids (**5** and **6**) are shown in Figure 10. Promising initial results from the evaluation of conjugates of some of these potent taxoids has been reported.[30]

Antibody–Calicheamicin Conjugates

Calicheamicins are antitumor antibiotics that bind to the minor groove of DNA and produce site-specific double-strand DNA breaks, causing cell death. Calicheamicins are potent at sub-picomolar concentrations *in vitro*, but their low therapeutic index precluded further development. To link them to antibodies via acid-labile bonds, a hydrazide functionality has been introduced into calicheamicin γ_1. A structural representation of an antibody–calicheamicin conjugate is shown in Figure 11.

In tumor xenograft models, antibody–calicheamicin conjugates displayed good antitumor efficacy, resulting in complete tumor regressions.[31] The most advanced of these

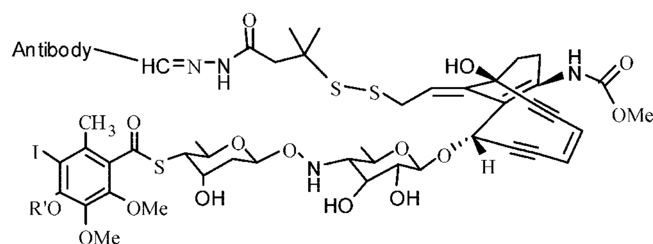

FIGURE 11. Structural representation of the antibody–calicheamicin conjugate.

conjugates, anti-CD33–calicheamicin, (gemtuzumab ozogamicin or Mylotarg), has been approved for the treatment of acute myeloid leukemia (AML).[32] Recently, the development of a calicheamicin conjugate of an antibody directed against the CD22 antigen expressed in B-cell lymphomas has been reported.[33]

Antibody–Auristatin Conjugates

Auristatins are synthetic analogues of the potent marine cyclic pentapeptides, the dolastatins, originally isolated from the sea hare *Dolabella auricularia*.[34] The dolastatins are highly cytotoxic compounds that share a common mechanism of action with maytansine and cause cell death by inhibiting tubulin polymerization. Human clinical trials with dolastatins were disappointing because the high systemic toxicity of the drug dampened the hope of any therapeutic benefit. The synthetic analogues Auristatin E and F have been linked via a peptidase-labile linker to monoclonal antibodies. Therapeutic efficacy, leading to tumor regressions and cures in human tumor models in mice have been reported.[35,36] Further evaluation of these conjugates is ongoing.

Conclusions

The new generation of antibody–drug conjugates has incorporated drugs that are considerably more potent than standard anticancer agents. The most advanced of these agents is the humanized anti-CD33 antibody–calicheamicin conjugate Mylotarg, which has been approved for the treatment of AML. The early clinical results and the approval of Mylotarg opens the door to the future development of antibody conjugates of highly potent drugs that are too toxic to be useful by themselves. On the basis of the impressive efficacy data in animal tumor models, several more of these targeted agents are being evaluated in the clinic. In addition, this approach makes it possible to enhance the potency of unconjugated antibodies that display little or no antitumor activity on their own.

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BIOGRAPHICAL INFORMATION

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FOOTNOTES

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