Progress in immunoconjugate cancer therapeutics

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Advances in immunoconjugate technology have revitalized the "magic bullet" concept of immunotherapeutics for the treatment of cancer. The growing availability of "human" antibodies, the increased epitope repertoire due to genomics and proteomics efforts, and advances in the means of identification and production of tumor-specific antibodies have greatly increased the potential for cancer therapeutic opportunities. Furthermore, the realization that effector molecule potency must be sufficiently high to be effective at concentrations that might realistically be delivered to the tumor site on an antibody carrier has greatly spurred the fields of medicinal chemistry and radionuclide chelate chemistry to produce such molecules.

Background

Therapeutic immunoconjugates consist of a specifically tumor-targeting antibody covalently linked or chelated to a toxic effector molecule. They can be categorized into three groups defined by the nature of the "effector" molecule; that is, when the effector is an isotope, the conjugate is referred to as a radioimmunoconjugate (RIC); when the effector is a protein toxin, the conjugate is an immunotoxin conjugate (ITC); and when the effector is a small drug, the conjugate is an antibody-drug conjugate or tumor-activated prodrug (TAP) (Blättler et al., 1996).

The initial phases of immunoconjugate chemotherapeutic development proved to be disappointing due to several factors which were not appreciated at first (Blättler and Chari, 2001). It was hoped that the conjugation of clinically useful anticancer drugs to antibodies would result in targeted therapeutics with fewer toxic side effects than with the free drug. However, the pharmacokinetics and pharmacodynamics of the antibody-linked drug mirrored those of the unconjugated antibody, resulting in essentially no efficacy, and toxicities similar to those seen with free drug. Eventually, it was understood that to achieve a therapeutic window with immunoconjugates, the effector molecule had to be cytotoxic in the picomolar range similar to the antigen binding avidities of the antibodies. Thus, many attempts to create conjugates of antibodies to highly toxic bacterial and plant toxins such as pseudomonus exotoxin, diphtheria toxin, ricin, gelonin, saporin, and pokeweed antiviral protein (PAP) were undertaken (Kreitman, 2001). While immunotoxins proved to be highly efficacious in specifically killing tumor cells in vitro and showed antitumor activity in xenograft models, in humans they proved highly immunogenic, inducing neutralizing antibodies targeting both the toxin protein and mouse monoclonal antibody epitopes (Kreitman, 2001). To overcome this difficulty, the next generation of immunoconjugates comprised "humanized" antibodies conjugated to nonimmunogenic radioisotopes, and more recently, small highly cytotoxic drugs.

Antibody improvements

Upon recognition of the clinical limitations of murine antibodies, efforts were undertaken to produce better-tolerated antibodies which would avoid the induction of human anti-mouse antibody (HAMA) response (Reff and Heard, 2001). There are several approaches to making human or human-like antibodies. One focuses on reducing the immunogenicity of murine IgGs by incorporating human residues. Initially, mouse-human IgG chimeras were produced by genetically engineering the mouse variable domains onto human constant regions. The resulting antibodies were approximately 75% human, displaying circulating half-lives approaching those of fully human IgGs. Although chimeric antibodies showed limited immunogenicity in immunosuppressed patients, in other clinical settings, they proved highly immunogenic, preventing repeat dosing (Kreitman et al., 2001). In subsequent generations of humanized antibodies, the number of murine residues was further reduced. In one approach, the murine antibody complementarity-determining regions (CDRs) that form the antigen binding site as well as key framework residues of the variable region are grafted onto a human antibody of similar structure. Several antibodies humanized by CDR-grafting are in the clinic, such as Mylotarg for the treatment of acute myelogenous leukemia (AML) (Sievers et al., 2001) and Herceptin for the treatment of metastatic breast cancer (Pegram et al., 1998) with no evidence of immunogenicity. A related method, termed variable domain resurfacing, uses computer modeling to identify surface residues in the variable region of the murine antibody, which are then replaced by human residues. If the substitution results in a reduction in binding affinity, the residues are "back-mutated" to murine in various combinations to reproduce the high affinity binding of the murine antibody. An antibody that was humanized by variable domain resurfacing is currently undergoing clinical testing as a cytotoxic drug conjugate and shows no evidence of immunogenicity (Tolcher et al., 2003). Another approach to producing human-like antibodies exploits the fact that antibodies raised in cynomolgus monkeys have variable regions virtually indistinguishable from those of human IgGs. Chimeras comprising human constant domains and monkey variable domains have been constructed and are undergoing clinical evaluation.

More recently, various technologies to produce fully human antibodies have been developed. In the phage display approach, the antigen of interest is used to selectively bind phage from highly complex libraries expressing human antibody variable regions (Krebs et al., 2001). Another useful feature of phage antibody library selection, in addition to producing human antibodies, is that the technology does not rely on the immune repertoire of the mouse, and thus may allow access to antibodies that would not be found using traditional hybridoma technology. The disadvantage of antibodies produced by this method is that they are often of low affinity, requiring affinity maturation techniques to improve antigen binding. Affinity maturation is accomplished by generating libraries

Table 1. Immunotoxin conjugates in clinical development

Immunotoxin conjugate	Specificity/drug	Cancer	Company	Development status
BL22 (RFB4 (dsFv)-PE38)	α-CD22 PE38 (ds scFv fusion to PE38)	hairy cell leukemia	NCI	Phase I
SGN10 (BR96 sFv-PE40/BR96-SCIT)	α-Lewis^Y-SCIT (SCA)	breast, colon, prostate, lung	Seattle Genetics/Aventis	Phase I completed
LMB-9 (B3 (ds scFv)-PE38)	α-Lewis^Y-PE38 (ds scFv fusion to PE38)	colorectal, pancreas, esophagus, stomach, breast, NSCLC, GIC, bladder, ovarian	NCI/IVAX	Phase I
LMB-2 (α-Tac(Fv)-PE38)	α-CD25-PE38 (ds scFv fusion to PE38)	hematopoietic malignancies	NCI	Phase I completed
SS1(dsFv)-PE38	α-mesothelin-PE38 (ds scFv fusion to PE38)	mesothelioma, ovarian, squamous cell NSCLC	NeoPharm	Phase I

SCIT = single chain immunotoxin PE40 (40 kDa truncated pseudomonus exotoxin)
PE38 = 38 kDa truncated pseudomonas exotoxin polypeptide
PE40 = 40 kDa truncated pseudomonas exotoxin polypeptide
NSCLC = non-small-cell lung carcinoma
GIC = gastrointestinal cancer
ds scFv = disulfide-stabilized, single-chain, variable domain fragment

consisting of variants of the original antibody in which one of the six CDRs is randomly mutated. The new libraries are then reprobed with antigen to select for higher affinity variants of the original antibody. Although time-consuming, affinity maturation usually identifies antibodies with affinities in the range of those achievable using hybridoma technology. More recently, ribosomal and bacterial surface human antibody display libraries have been developed.

The goal of raising fully human antibodies in mice has been achieved. Transgenic mice in which the murine IgG genes are replaced with the corresponding human genes have been constructed and shown to generate fully human antigen-specific antibodies in response to immunization with antigen. The advantage of this approach is that the animals can be repeatedly immunized with antigen to produce high affinity antibodies.

Immunotoxin conjugates

Although immunotoxin conjugates (ITCs) have historically given poor clinical results efforts continue to improve clinical utility (Kreitman, 2001). The difficulty with immunotoxin conjugates is 2-fold; because the toxin is a protein, in most clinical settings ITCs elicit a human antitoxin response (HATA) thus limiting the efficacy and ability to administer multiple doses. Most immunotoxins tested in the clinic to date are comprised of murine single-chain variable domain fragments (scFv) of IgGs, resulting in the appearance of neutralizing HAMAs also. However, in the case of hematologic malignancies where patients are often severely immunosuppressed, there may be a role for immunotoxin conjugates. For example, patients with hairy cell leukemia present with pancytopenia. Consequently, they respond quite well to an anti-CD22-pseudomonas exotoxin fragment fusion (see BL22 in Table 1). In a phase I clinical trial an 81% overall response rate was observed with 69% complete remission (Kreitman et al., 2001). However, even in this heavily immunosuppressed population a subset fail to respond due to the presence of neutralizing antibodies developed during the course of treatment or more rarely, preexisting antitoxin antibodies (Kreitman et al., 2001). In contrast to patients with hematologic malignancies, patients with solid tumors are generally prohibited from receiving multiple doses of ITC due to the rapid induction of neutralizing antitoxin antibodies (Posey et al., 2002), severely limiting the amount of conjugate that can be delivered to the tumor site. Another drawback to ITCs is their nonspecific toxicity (Kreitman, 2001). This appears to stem from damage to the endothelium, resulting in vascular leak syndrome (VLS). Recombinant, truncated toxins show reduced VLS, having primarily hepatic and renal toxicity (Kreitman, 2001). The hepatic toxicity is likely due to the nonspecific uptake of ITC in Kupffer cells in the liver resulting in the production of TNF-α.

Currently, there are a limited number of immunotoxin conjugates in clinical development (see Table 1). Recently reported results for SGN-10, a Lewis^Y-targeting scFv fused to a pseudomonas exotoxin fragment, indicate little efficacy in Lewis^Y-positive metastatic carcinoma (Posey et al., 2002). Patients also exhibited gastrointestinal dose-limiting toxicity, modest VLS, and development of HATA. LMB-9, another Lewis^Y-targeting scFv fused to truncated pseudomonas exotoxin similar to SGN10, is also likely to give disappointing clinical trial results. Moreover, an earlier version of LMB-9 comprising the parent antibody, B3, coupled to LysPE38, was reported to directly bind endothelial cells causing antibody-dependent VSL (Kuan et al., 1995). LMB-2, while demonstrating some efficacy in a variety of hematologic malignancies, also elicits neutralizing HATA and HAMA in all treated patients with the exception of 8 patients diagnosed with chronic lymphocytic leukemia (CLL) (Kreitman et al., 2000). A diphtheria toxin fragment targeted by fusion to the cytokine IL-2, denileukin diftitox (Ontak), has been approved for cutaneous T cell lymphoma (CTCL), although 98% of the patients developed HATA by the second treatment dose (Olsen et al., 2001). Nonetheless, a 30% objective response was seen with no difference in immunogenicity between the responder and nonresponder groups. Despite this encouraging clinical response to a cytokine-targeted toxin, it seems unlikely that ITCs targeting solid tumors will demonstrate much clinical efficacy due to the dosing limitations imposed by their inherent immunogenicity.

Radioimmunoconjugates

Like immunotoxin conjugates, radioimmunoconjugates have been under investigation for many years with mixed clinical results (see Blättler et al., 1996, and references therein for review). The β-emitters Yytrium-90 (^{90}Y) and Iodine-131 (^{131}I) are

Table 2. Drug immunoconjugates undergoing preclinical/clinical development

Small drug immunoconjugate	Specificity/drug (antibody)	Cancer	Company	Development status
Mylotarg; gemtuzumab ozogamicin	α-CD33-calicheamycin (humanized by CDR grafting)	AML	Wyeth-Ayrst/ CellTech Group	FDA approval (5/18/00)
cantuzumab mertansine (huC242-DM1/SB-408075)	α-CanAg-DM1 TAP (humanized by resurfacing)	colorectal, pancreatic	ImmunoGen	Phase I completed
BB-10901/huN901-DM1	α-CD56-DM1 TAP (humanized by CDR grafting)	SCLC	British Biotech/ ImmunoGen	in Phase I (UK); Phase I/II (US)
MLN2704 (formerly MLN591-DM1)	α-PSMA-DM1 TAP (DeImmunized)	prostate	Millenium[a]	initiated Phase I (11/21/02)
bivatuzumab mertansine	α-CD44v6-DM1 TAP (humanized)	unspecified	Boehringer Ingelheim[a]	initiated clinical trials (10/15/02)
trastuzumab-DM1/Herceptin-DM1	α-Her2/neu-DM1 TAP (humanized by CDR grafting and framework changes)	breast	Genentech[a]	preclinical development
My9-6-DM1	α-CD33-DM1 TAP (humanized by resurfacing)	AML	ImmunoGen	preclinical development
SGN-15 (BMS-182248/BR96-doxorubicin)	α-Lewis^Y-doxorubicin (chimeric)	breast, colon, prostate, lung	Seattle Genetics/ Aventis	3 Phase II clinical trials; breast completed, will not pursue clinical development
SGN-25 (BR96-auristatin E)	α-Lewis^Y-auristatin E (chimeric)	breast, colon, prostate, lung	Seattle Genetics	preclinical development
SGN-35	α-CD30-auristatinE	hematologic malignancies, lymphomas	Seattle Genetics	preclinical development

[a]ImmunoGen Inc. technology
DM1 = N2′-deacetyl-N2′-(3-mercapto-1-oxopropyl)-maytansine
TAP = tumor activated prodrug
SCLC = small cell lung carcinoma
AML = acute myelogenous leukemia
SCA = single-chain antibody

the most extensively studied isotopes in radioimmunoconjugates due to their radiologic characteristics, ready availability, ease of conjugation, and relatively long half-lives. Despite generally disappointing clinical results thus far, many clinical trials are underway with various radioimmunoconjugates. The most clinically promising results have been obtained in treatment of hematologic cancers due to the radiosensitivity of these tumors and the ability to deliver the requisite dose for tumor eradication. The FDA recently approved the first radioimmunoconjugate, Zevalin (^{90}Y-anti CD20) for treatment of non-Hodgkin's lymphoma (NHL), and Bexxar (^{131}I-anti-CD20) is under review by the FDA (Cheson, 2003). Nonetheless, to achieve robust clinical response, doses often resulting in severe myeloablation must be administered. In treatment of solid tumors, this problem is much more acute. It is generally not possible to deliver therapeutically effective doses to solid tumors without exceeding the maximum tolerated dose (MTD). However, in certain cases where radioimmunoconjugates can be selectively delivered to a specific body cavity where the tumor is located, clinical efficacy may be achieved. Intraperitoneal administration of TheraGyn (^{90}Y-anti-Muc1) is currently undergoing phase III clinical testing. Recently, a Phase II clinical trial was completed with an ^{131}I-labeled anti-tenascin antibody (81C6) in which the RIC was injected directly into the surgically created cranial resection cavity of glioma patients (Reardon et al., 2002). The average absorbed dose at the tumor site, while much greater than generally observed in conventional RIC therapy, was somewhat under the theoretical dose required for the eradication of solid tumors. Nonetheless, some clinical benefit in terms of median survival was observed.

Because α particles, by comparison with β particles, have a much shorter path length as well as a much higher linear energy transfer, they are significantly more selective and potent in killing target cells (McDevitt and Scheinberg, 2002). Because of the short path length, little collateral damage may be inflicted upon nontarget cells, while a single decay of an internalized α-emitter passing through the nucleus can be lethal (Jurcic et al., 2002; Sgouros et al., 1999). In fact, dosimetry comparisons of radioisotopes conjugated to the same antibody showed up to 1000-fold higher absorbed dose ratios in target organs with α-emitters compared with β-emitters (Jurcic et al., 2002; Sgouros et al., 1999). A comparison of the therapeutic efficacies of α-emitter (^{211}At)- and β-emitter (^{131}I)-labeled Mov18 antibody recognizing the folate receptor on OVCAR3 xenografts showed the ^{211}At conjugate to be superior (Andersson et al., 2001). However, the exceedingly short half-life of the α-emitting isotopes with the requisite chemical properties for conjugation, bismuth-212 (^{212}Bi; 61 min), astatine-211 (^{211}At; 7.2 hr), and bismuth-213 (^{213}Bi; 45.6 min) prevented their clinical development (McDevitt et al., 2001). While evidence of preclinical efficacy in the absence of significant toxicity could be obtained (Andersson et al., 2001; Ballangrud et al., 2001; Larsen et al., 1998; McDevitt et al., 2000), as well as patient response in human clinical trials (Jurcic et al., 2002), the short half-life of ^{213}Bi limits delivery to only the most accessible tumor cells, resulting in limited efficacy. However, a means to overcome this problem was recently described (McDevitt et al., 2001) where a long-lived ($t_{1/2}$ = 10 days) α-particle generator, actinium-225 (^{225}Ac) is

attached directly to the targeting antibody. ^{225}Ac, once delivered into the tumor cell, is retained along with its 4 daughter α-emitting radionuclides (McDevitt et al., 2001; McDevitt and Scheinberg, 2002). In several tumor xenograft studies, ^{225}Ac immunoconjugates have been demonstrated to be approximately 1000-fold more potent on a mCi basis than corresponding ^{213}Bi immunoconjugates (McDevitt et al., 2001; McDevitt and Scheinberg, 2002). This can be attributed to both the long half-life of ^{225}Ac and the net 4 daughter α particles produced inside the cell for each internalized ^{225}Ac. It will be interesting to see whether ^{225}Ac immunoconjugates, with their longer half-lives and increased potencies, will be able to penetrate solid tumors. Nonetheless, the practical difficulties in manufacturing and administering radioimmunoconjugates may prevent their widespread use in the clinic.

Drug immunoconjugates

Promising immunoconjugates currently under clinical investigation consist of antibodies conjugated to small highly cytotoxic drugs. Early clinical trials with antibodies conjugated to clinically useful drugs such as antifolates, vinca alkaloids, and approved anthracyclines such as doxorubicin were disappointing due to lack of potency (Chari, 1998). Subsequently, it was hypothesized that only drugs with potencies approaching 10^{-11} M would be useful in immunoconjugates because a targeting antibody might be capable of depositing this amount of drug at a solid tumor site (Blättler and Chari, 2001). Because dosimetry studies with radioimmunoconjugates have demonstrated that only 0.001 to 0.01 percent of the injected dose per gram of solid tumor is actually deposited at the tumor (Sedlacek et al., 1992), the drug must be stable enough to be cytotoxic only to antigen-positive cells. There are a limited number of cytotoxic drugs that fulfill this potency and stability requirement (Blättler and Chari, 2001). Among these are (1) inhibitors of tubulin polymerization exemplified by the maytansinoids, dolastatins, auristatin, and cryptophycin; (2) DNA alkylating agents like CC-1065 analogs and duocarmycin; and (3) the enediyene antibiotics such as calicheamicin and esperamicin which catalyze DNA double-strand breaks. More recently, extremely potent taxoid drugs which inhibit microtubule depolymerization have been developed (Ojima et al., 2002). Only a subset of the available highly potent cytotoxic drugs have been linked to antibodies and shown to retain potency (Chari et al., 1995; Liu et al., 1996; Ojima et al., 2002; Senter et al., 2002; Sievers and Linenberger, 2001).

The only antibody-drug conjugate approved by the FDA to date is gemtuzumab ozogamicin (Mylotarg) (Table 2) for the treatment of patients with CD33-positive AML in first relapse who are 60 year of age or older and who are not considered candidates for cytotoxic chemotherapy (Bross et al., 2001). It is comprised of a humanized antibody recognizing the CD33 antigen attached via a bifunctional hydrazine linker to the highly potent cytotoxic drug, calicheamicin (Sievers and Linenberger, 2001). Of the patient population treated with gemtuzumab ozogamicin, 30% achieve remission (Sievers et al., 2001). Of concern is the high incidence of severe myelosuppression in patients treated with gemtuzumab ozogamicin, which correlates with the expression of CD33 on normal myeloid and megakaryocytic precursors. However, CD33 is absent from pluripotent hematopoietic stem cells allowing regeneration the full repertoire of CD33-positive hematopoietic cells. Some patients also exhibit hepatic toxicity with veno-occlusive disease-like symptoms, possibly due to targeting of CD33-positive hepatic Kupffer cells (Sievers and Linenberger, 2001). However, calicheamicin alone is known to cause hepatotoxicity in preclinical models (Bross et al., 2001), suggesting that the toxicity may be nonspecific rather than targeted by the antibody.

Antibody-drug conjugates have also been named tumor-activated prodrugs (TAPs), since it could be demonstrated that conjugates of the cytotoxic agent to antibody rendered it noncytotoxic to cells devoid of the target antigen. TAP therapeutic antibody conjugates (Blättler and Chari, 2001) are currently under clinical investigation (see Table 2). These conjugates comprise humanized antibodies linked via a disulfide linker to the highly potent ($IC_{50} \sim 10^{-11}$ M) maytansine derivative, DM1. Because the linker is stable in the blood, the conjugate is nontoxic until it reaches the tumor site. Specific antigen binding and internalization result in release of the drug and potent killing of tumor cells. In SCID xenograft studies, with mice bearing large subcutaneous or disseminated tumors, TAP conjugates routinely effect cures at doses well below the MTD under conditions where conventional therapeutics have little tumor growth inhibitory effect (Blättler and Chari, 2001; Liu et al., 1996; Ross et al., 2002; Schwall et al., 2001).

Three phase I trials have been conducted with cantuzumab mertansine for the treatment of patients with CanAg-positive malignancies (Helft et al., 2001; Rowinsky et al., 2002; Tolcher et al., 2003). CanAg is a tumor-specific carbohydrate epitope found on the Muc1 mucin of most colorectal and pancreatic tumors as well as a large proportion of non-small-cell lung, gastric, uterine, and bladder cancers (Tolcher et al., 2003). The cantuzumab mertansine conjugate was well-tolerated with the dose-limiting toxicity found to be reversible transaminitis (elevated liver enzymes). No evidence of human anti-human antibodies (HAHA) or human anti-DM1 antibodies (HADA) was observed. The absence of immunogenicity allowed repeated dosing of patients. In fact, one patient with diffuse peritoneal carcinomatosis and chronic ascites obtained a complete resolution of ascites after 5 weeks of treatment, and was subsequently treated repeatedly with no evidence of disease progression. Several other biological responses were observed, including partial tumor regressions, stabilization of disease, and reduction in circulating carcinoembryonic antigen (CEA) levels.

Phase I/II clinical trials have begun with BB-10901 (huN901-DM1), which targets CD56 expressed on SCLC and tumors of neuroendocrine origin. Results from the first Phase I trial indicate that huN901-DM1 toxicity is modest, and preliminary evidence of antitumor activity was observed (Fosella et al., 2002). Clinical trials with DM1 conjugates targeting prostate-specific membrane antigen (PSMA) for the treatment of prostate cancer and CD44v6-expressing solid tumors have also been initiated recently.

In addition to the four TAPs in clinical trials, others are at the research and preclinical development stage (Table 2). Trastuzumab-DM1 is a DM1 conjugate to the Herceptin antibody used to treat metastatic breast cancer. Trastuzmab-DM1 is able to eradicate tumors in models where Herceptin only slows growth (Schwall et al., 2001). Furthermore, in an aggressive tumor model in which Herceptin has no activity, trastuzumab-DM1 shrinks tumors by more than 90%. My9-6-DM1 is a DM1 conjugate to a humanized antibody targeting CD33,

which is expressed on leukemic blasts of greater than 80% of AML patients. My9-6-DM1 treatment of SCID mice bearing established subcutaneous HL-60 or THP-1 xenografts resulted in complete eradication of the tumor at doses well below the MTD (Lutz et al., 2002).

Other antibody-drug conjugates under development are BR96-doxorubicin and BR96-auristatin E, both targeting Lewis^Y-expressing solid tumors and anti-CD30-auristatin E (see Table 2). BR96-doxorubicin has previously been shown to have marginal efficacy in clinical trials (Ajani et al., 2000; Saleh et al., 2000; Tolcher, 2000; Tolcher et al., 1999), while displaying severe upper GI toxicity. The lack of efficacy is likely due to the minimal potency of doxorubicin (IC_{50} $\sim 10^{-8}$ M). The GI toxicity may be explained by conjugate binding to Lewis^Y expressed on normal GI tract epithelium. The normal tissue binding may also contribute to the lack of efficacy by acting as an antigen sink, thus compromising the conjugate delivery to tumor tissue. While auristatin E, a small molecule with activity and potency similar to maytansine (Senter et al., 2002), may be a superior immunoconjugate effector molecule compared with doxorubicin, it is not clear that any Lewis^Y-targeting antibodies will be useful for conjugate chemotherapeutics due to the GI tract crossreactivity.

The availability of highly potent drugs with alternate mechanisms of cytotoxicity may provide an opportunity for tailoring treatment of a malignancy with the most active drug for that disease target, thus optimizing the therapeutic efficacy. Considerable effort is underway to develop additional cytotoxic drugs with stabilities and potencies suitable for conjugate development (Blättler and Chari, 2001) to complement the currently available arsenal. CC-1065, a DNA alkylating agent, has 1000-fold more cytoxic activity than other DNA targeting drugs such as doxorubicin or etoposide. CC-1065-like derivatives containing thiols have been synthesized and conjugated to anti-B4 antibody via a disulfide linkage (Chari et al., 1995). The resulting conjugate, anti-B4-DC1, proved highly potent both in vitro and in curing SCID mice bearing a highly aggressive disseminated lymphoma xenograft. The clinically useful taxoids, paclitaxel and docetaxel, do not have sufficient potency to be useful in immunoconjugates (Blättler and Chari, 2001; Ojima et al., 2002). However, considerable progress has been made in the synthesis of second-generation taxoids with much greater potency (Kingston et al., 1998; Ojima et al., 2002). A potent taxoid derivative conjugated to an anti-EGFR antibody via a disulfide linker has been shown to be active in a xenograft model, completely eradicating all histopathological evidence of tumor cells (Ojima et al., 2002). It will be interesting to see whether highly potent drugs suitable for conjugation can be developed for additional targets such as DNA synthesis, metabolic pathways, and antiapoptotic pathways.

Because of their potency, lack of immunogenicity in humans, ease of synthesis, chemical stability, and clinical convenience, small drug immunoconjugates represent the most promising direction for future immunoconjugate cancer therapy.

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