Drugs of the Future 2003, 28(9): 905-910

CCC: 0377-8282/2003

Review Article

Recent advances in drug-antibody immunoconjugates for the treatment of cancer

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Abstract

Standard chemotherapy in the treatment of cancer often has a low therapeutic index due to the lack of specificity. Antibody-directed cancer chemotherapy can improve therapeutic indices, with the potential to enhance efficacy and decrease systemic toxicity. Several drug-antibody conjugates have shown impressive antitumor activity in preclinical models and are currently being evaluated in clinical trials. Drug-antibody conjugates may play a substantial role in the future as a single regimen or combination therapy in the treatment of cancer.

Introduction

Traditional chemotherapy is often accompanied by a low therapeutic index due to high systemic toxicity. Antibody-directed therapy has the potential to improve therapeutic efficacy by increasing the intratumoral concentration of drugs and minimizing toxicity through reduction of systemic exposure. However, past experience using antibodies to improve the clinical efficacy of anticancer drugs such as doxorubicin, methotrexate, cisplatin and vinca alkaloids has been disappointing. This could be due to several reasons. First, anticancer drugs used in the clinic generally have low potency and only a limited number of drugs can be loaded onto antibodies, resulting in an insufficient amount of the agent delivered into the tumor. Second, the linker for the conjugate is unstable in serum, resulting in premature release of the drugs before reaching the tumor target; on the other hand, the linker is too stable to be broken down even inside tumor cells (1). Third, murine antibodies were often used in clinical studies, which can induce human anti-mouse host responses, thus preventing prolonged use and multiple dosing. Due to these difficulties, drug-antibody conjugates have not been extensively used in the clinic.

Recent advances in technology are permitting further development in this area. Using humanized or fully human antibodies has largely addressed the immunogenicity issue. Modern protein engineering permits flexible antibody design with appropriate affinity and specificity. In the last few years, remarkable success in the treatment of cancer has been seen in the clinic with several monoclonal antibodies (MAbs) (Table I). The clinical feasibility of drug-antibody conjugates was also clearly demonstrated when gemtuzumab ozogamicin (Mylotarg™) became the first agent of this class to be approved by the FDA for the treatment of leukemia (2). Several other immunoconjugates have also shown impressive activity in animal models and are currently being evaluated in clinical trials (Table II).

Choice of drugs

High potency drugs are preferred over low potency drugs since only a limited amount of an agent can be loaded onto an antibody. This is more obvious when using antibody fragments which have fewer conjugation sites for drugs as compared to whole antibodies. If a drug lacks potency, more antibodies would have to be used to deliver a sufficient amount of the drug which would not only be more expensive but also would saturate antigen binding sites. Although drug carriers such as human serum albumin or liposomes have the potential to

Table I: FDA approved monoclonal antibodies for cancer therapeutics.

Generic name	Trade name	Target	Type	Indication
Rituzimab	Rituxan	CD20	Chimeric	Non-Hodgkin's lymphoma
Trastuzumab	Herceptin	HER-2/neu	CDR-grafted	Breast cancer
Gemtuzumab ozogamicin	Mylotarg	CD33	CDR-grafted	Acute myeloid leukemia
Alemtuzumab	Campath	CD52	CDR-grafted	Chronic lymphocytic leukemia
Ibritumomab tiuxetan	Y-90-Zevalin	CD20	Murine	Non-Hodgkin's lymphoma

Table II: Drug-antibody conjugates for the treatment of cancer.

Name	Drug	Target	Indications	Phase
Gemtuzumab	Calicheamicin	CD33	Acute myeloid leukemia	Approved
HuC242-DM1	Mertansine	CanAg	Colorectal, pancreatic cancer	II
HuN901-DM1	Mertansine	CD56	Small cell lung cancer	II
MLN591-DM1	Mertansine	PSMA	Prostate cancer	I
Herceptin-DM1	Mertansine	HER2	Breast cancer	I
Bivatuzumab mertansine	Mertansine	CD44v6	Colorectal, pancreatic cancer	I
SGN-15	Doxorubicin	$Lewis^y$	NSCLC, breast, colorectal, prostate, ovarian cancer	II
SGN-30	Doxorubicin	CD30	HD, ALCL, CTCL, large B-cell lymphoma	I/II
SGN-35	Auristatin E	CD30	HD, ALCL, CTCL, large B-cell lymphoma	I

PSMA = prostate specific membrane antigen; NSCLC = non-small cell lung cancer; HD = Hodgkin's disease; ALCL = anaplastic large cell lymphoma; CTCL = cutaneous T-cell lymphoma

increase the drug-to-antibody ratio, the significantly enlarged molecular size of the conjugate would complicate both the pharmacokinetics and pharmacodynamics of the active anticancer agent.

Calicheamicin was successfully conjugated to anti-CD33 antibody and generated a complete remission rate of 15-20% in patients with relapsed acute myeloid leukemia (2). Like calicheamicin, maytansin, neocarzinostatin, geldanamycin, *etc.* are 100- to 1000-fold more potent than conventional anticancer drugs, an even in subpicomolar ranges these antitumor antibiotics still effectively kill tumor cells. Maytansine has shown promise in clinical studies and a maytansine derivative, DM1, has been attached to humanized antibodies via disulfide bonds which provide stable linkage in the bloodstream but allow release of the fully active drug inside the target cell. Once the DM1 immunoconjugate binds to the tumor target, it is internalized and releases DM1 inside the tumor cell. Released DM1 kills the tumor cells by inhibiting microtubule polymerization. Currently, 2 DM1 immunoconjugates are being evaluated in phase II clinical trials.

HuC242-DM1

HuC242-DM1 consists of CanAg-specific humanized antibody conjugated to DM1. The CanAg antigen is found on many types of cancers including colorectal, pancreatic and gastric cancers, as well as non-small cell lung cancer (NSCLC). In animal studies, HuC242-DM1 completely eliminated human colorectal cancer xenografts in severe combined immune deficiency (SCID) mice with animals remaining tumor free for 200 days. HuC242-DM1 also showed similar results in SCID mice with human pancreatic cancer and NSCLC.

In a phase I trial, 37 patients received 110 courses of HuC242-DM1 at doses ranging from 22-295 mg/m^2. HuC242-DM1 was well tolerated with no hematologic toxicity seen. The dose-limiting toxicity was reversible transaminitis. Gastrointestinal adverse events were common but rarely severe at the highest dose levels. Two patients with chemotherapy-refractory colorectal carcinoma had minor regressions and 4 patients had persistently stable disease for more than 6 courses. A phase II trial is ongoing for the treatment of CanAg-expressing tumors (3, 4).

HuN901-DM1

HuN901-DM1 consists of DM1 conjugated to a humanized MAb N901 targeting CD56. CD56 is a neural cell adhesion molecule that is expressed on the surface of tumor cells of neuroendocrine origin, including small cell lung cancer (SCLC), carcinoid tumors, neuroblastomas and astrocytomas. Dose-dependent antitumor activity of HuN901-DM1 has been demonstrated in 3 different human SCLC tumor xenograft models in mice. As a single agent, HuN901-DM1 completely eradicated human SCLC tumors in these models and was markedly more effective than standard drugs such as cisplatin and etoposide. It also acts synergistically with other cytotoxic drugs in combination therapy. A phase I trial reported that

the maximum tolerated dose for huN901-DM1 was 60 mg/m^2 and the conjugate was well tolerated with no hematologic toxicity observed. A phase I trial also showed preliminary evidence of antitumor activity for the conjugate. HuN901-DM1 is currently in phase II clinical trials for the treatment of SCLC (5).

BR96-doxorubicin

The early clinical experience with BR96-doxorubicin immunoconjugate has been disappointing. BR96-doxorubicin is a chimeric human/mouse MAb linked to 8 doxorubicin molecules. The antibody is directed against the Lewis^Y antigen, which is expressed in 75% of all breast cancers but has limited expression in normal tissues. A randomized phase II study in patients with metastatic breast cancer has demonstrated that BR96-doxorubicin had antitumor activity inferior to doxorubicin alone (6). There was one partial response (7%) in 14 patients receiving the BR96-doxorubicin conjugate, whereas 1 complete response and 3 partial responses (44%) were observed in 9 evaluable patients receiving doxorubicin. Moreover, BR96-doxorubicin was associated with significant gastrointestinal toxicities, including marked serum amylase and lipase elevations, nausea and vomiting with gastritis. These toxicities likely represent the binding of the agent to normal tissues expressing the target antigen, which may have compromised the delivery of the immunoconjugate to the tumor site.

A multicenter phase II study of BR96-doxorubicin conjugate in patients with advanced gastric adenocarcinoma also showed no objective responses, and gastrointestinal toxicity was predominant (7).

However, combination treatment with BR96-doxorubicin (SGN-15) and docetaxel (Taxotere®) was shown to induce objective antitumor responses at well-tolerated doses in many patients. Currently, an ongoing phase II study is examining the efficacy and tolerability of SGN-15 in combination with docetaxel in NSCLC patients and a phase II study is evaluating the efficacy of SGN-15 in combination with gemcitabine (Gemzar®) in patients with advanced ovarian cancer (8).

Preliminary results from the lung cancer study demonstrate that SGN-15 in combination with docetaxel is well tolerated at doses up to 350 mg/m^2. A disease control rate of 57% has been observed in the combination arm as compared to 35% in the docetaxel only arm. Preliminary progression-free survival data indicates a median of 15.3 weeks for patients receiving the combination therapy as compared to a median of 7.4 weeks for patients receiving docetaxel alone.

Another phase II study was conducted to evaluate the safety and estimate the clinical response of the combination of SGN-15 and docetaxel in the setting of metastatic breast cancer. Thirty patients were treated, the majority of whom had failed multiple prior therapies. Final data analysis of the clinical trial demonstrated that the combination therapy was well tolerated with minimal toxicities and evidence of objective antitumor responses.

SGN-30 (anti-CD30-doxorubicin) is a humanized antibody for the treatment of patients with CD30+ hematologic malignancies such as Hodgkin's disease, anaplastic large cell lymphoma and other types of lymphomas. Data from the phase I study demonstrated that SGN-30 is well tolerated and has an apparent half-life of approximately 25 days. Evidence of antitumor activity was observed in 2 patients with Hodgkin's disease and anaplastic large cell lymphoma, respectively, who received the single-dose regimen. SGN-30 is presently in an ongoing phase I/II multiple-dose clinical trial.

Miscellaneous

Several other highly potent antitumor agents have been tested at the preclinical level. Auristatin E is a highly potent antimitotic agent, and its derivative (MMAE) was coupled to various MAbs demonstrating marked effects against Hodgkin's disease (9) and anti-Lewis^Y-expressing carcinoma (10). When conjugated to an antitumor vascular MAb, neocarzinostatin, a member of the enediyne family of antibiotics, improved survival with no side effects in mice bearing Meth-A fibrosarcoma (11). Genistein, a major component of soy, when conjugated to MAb 17.1A showed anticolon cancer activity *in vitro* and *in vivo* (12). Geldanamycin is a highly cytotoxic antibiotic with anti-HER2 activity. Geldanamycin-trastuzumab was found to be superior to trastuzumab alone in inhibiting proliferation of HER2-overexpressing cell lines (13). In addition, the antitumor activities of two other geldanamycin immunoconjugates were compared. *N-tert*-Butyloxycarbonyl-1,3-diaminopropane, a derivative of geldanamycin, showed better efficacy than a 1,4-diaminobutane spacer derivative and both immunoconjugates were more effective than trastuzumab alone (14).

Choice of targets

HER family

The HER family is composed of 4 transmembrane receptors and their ligands: 1) HER1 (EGFR, erbB-1, or variant EGFVIIIr) and its ligands EGF, TGF-α, amphiregulin, β-cellulin, HB-EGF and epiregulin; 2) HER2 (erbB-2), which has no known ligand; 3) HER3 (erbB-3) and its ligand heregulin; and 4) HER4 (erbB-4) and its ligands β-cellulin, HB-EGF and heregulin. Ligand binding leads to receptor hetero- or homodimerization, which, in turn, signals autophosphorylation of the intracellular tyrosine kinase. Tyrosine kinase phosphorylation results in activation of a series of downstream effectors that increase angiogenesis, cell-cycle progression and cell motility, and that decrease apoptosis. Overactivity of this pathway occurs in many advanced cancers, including breast, lung, prostate, colon and head and neck cancers (15).

Trastuzumab (Herceptin®), a MAb inhibiting HER2 activity, is FDA approved for the treatment of metastatic breast cancer. Cetuximab (Erbitux™, IMC-C225, C225) is a chimeric MAb that binds to the extracellular domain of the EGFR, thus preventing signal transduction and tyrosine kinase autophosphorylation. Initial results of an ongoing phase II trial of cetuximab in combination with docetaxel in 20 patients with chemotherapy-refractory NSCLC show clinical activity (4 with partial responses and 6 with stable disease) and good tolerability (16). These 2 MAbs are good candidates for delivering chemotherapeutics because of the possibility of a synergistic effect between the signaling antibody and the chemotherapeutics (17).

Given the fact that taxanes are considered a major therapeutic option for the treatment of many cancers with EGFR overexpression, it is logical to combine EGFR inhibitors with taxane. Combination therapy has the potential to increase the efficacy of both agents and to overcome EGFR-mediated taxane resistance. An animal study has been conducted using an immunoconjugate composed of C225 and a C-10 methyldisulfanylpropanoyl taxoid in human squamous cancers. In this *in vivo* study, the conjugate completely inhibited EGFR-expressing tumor growth in SCID mice (18). Enhanced anticancer activity was also observed for paclitaxel-C225 (19). Furthermore, paclitaxel-trastuzumab linked with a bifunctional linker (A-Z-CINN) was shown to be more effective in killing tumor cells than equivalent concentrations of coadministered trastuzumab and paclitaxel (20).

Tumor vascular antigen

Even though the drug-antibody immunoconjugate has achieved some success in the treatment of hematological malignancy, in general, it has generated less favorable results for solid tumors. The main reason is the poor accessibility of the solid tumors to the immunoconjugate. Poor accessibility is caused by several factors such as elevated interstitial pressure, heterogeneous antigen expression, poor blood circulation in large tumors and the relatively long distances required for the antibody to travel in the tumor interstitium (21).

To overcome poor tumor accessibility, use of an anti-tumor vascular antibody would be a rational approach because tumor vascular antigens are much easier to access than tumor surface antigens. The effectiveness of this approach was recently shown in clinical studies reported at the 39th Annual Meeting of the American Society of Clinical Oncology. For example, bevacizumab (Avastin™) is a humanized MAb against vascular endothelial growth factor (VEGF), a protein that plays a critical role in tumor angiogenesis and maintenance of established tumor blood vessels. A randomized phase III clinical trial showed that patients with newly diagnosed metastatic colon cancer who received bevacizumab together with the chemotherapy combination known as IFL had substantially longer overall survival rates than patients who received IFL alone.

Wakai *et al.* attempted to target tumor vascular antigen with a MAb (TES-23) conjugated with neocarzinostatin (NCS). TES-23 is directed against rat KMT-17 fibrosarcoma-derived endothelial cells. The immunoconjugate was able to induce tumor hemorrhagic necrosis and showed marked antitumor effects against rat KMT-17 fibrosarcoma. In addition, mice bearing Meth-A fibrosarcoma treated with the immunoconjugate showed improved survival with no side effects (11).

Linker design

Conjugate design can affect serum stability, tissue biodistribution, tumor uptake, therapeutic efficacy and toxicity of an immunoconjugate. Therefore, good understanding and careful selection of these parameters will lead to the rational design of an immunoconjugate thus optimizing its clinical outcome.

Inappropriate linker usage was responsible for the failure of several clinical trials (21). Hamann *et al.* compared the effects of hydrazone and an amide linker on an antibody-calicheamicin conjugate. The hydrazone linker is formed by attachment of a hydrazide derivative to the oxidized carbohydrates that occur naturally on antibodies. The amide linker, however, is formed by reacting a calicheamicin derivative containing an activated ester with the lysines of antibodies. The hydrazone linker is acid-labile and capable of releasing the active drug by hydrolysis in lysozomes. However, in this case, the amide linker was too stable and could not release the drug by hydrolysis (22).

Recently, a new protease-sensitive dipeptide linker (valine-citrulline) was introduced and showed tumor-selective activity. The immunoconjugate, consisting of MMAE, a derivative of auristatin E to MAb cAC10 (anti-CD30) with a dipeptide linker, has exhibited marked anti-Hodgkin's disease activity in a SCID mouse xenograft model. This linker is very stable; although only 2% of the drug was released following a 10-day incubation period in human plasma, it was readily cleaved by lysosomal proteases following receptor-mediated internalization (9). Doronina *et al.* compared the properties of immunoconjugates with different linkers. The linkers used for conjugation included protease-sensitive dipeptide and an acid-labile hydrazone. The peptide-linked MAb-valine-citrulline-MMAE and MAb-phenylalanine-lysine-MMAE conjugates were much more stable in plasma than the conjugates of MAb and the hydrazone of 5-benzoylvaleric acid-AE ester (AEVB). As a result, the MAb-valine-citrulline-MMAE conjugates exhibited greater antitumor activity both *in vitro* and *in vivo* as compared to the corresponding hydrazone conjugates (10).

A novel linker consisting of poly(ethylene glycol) (PEG) and a dipeptide was also tested for conjugation of adriamycin to the MAb, NL-1. Initially, adriamycin-conjugated PEG linkers were prepared with different amino

acid compositions, alanyl-valine (Ala-Val), alanyl-proline (Ala-Pro) and glycyl-proline (Gly-Pro) sequences, to confirm selective digestion with model enzymes. Adriamycin was released from the linkers by the model endoproteases, thermolysin and proline endopeptidase, with different efficacy. When conjugates were prepared using these adriamycin-bound linkers, conjugates did not lose binding affinity or specificity for common acute lymphoblastic leukemia antigen (CALLA) expressed on the Daudi cell surfaces as the target of NL-1 antibody (23).

Conclusions

Clinical studies using drug-antibody immunoconjugates have revealed crucial issues that must be addressed to ensure efficacy, such as drug potency, linker design, antibody internalization, drug-to-antibody ratio, tumor antigen accessibility, *etc*. Improvements in clinical efficacy with reduced toxicity have already been established for a number of drug-antibody immunoconjugates. In the future, the ability to maximize the potential of these new agents in the clinical setting will be a challenge. Identification of the subset group of patients who will most benefit from the drug can be the key to success of drug-antibody immunoconjugates in the future. For example, trastuzumab is only effective in patients who overexpress HER-2/*neu*. Some failed investigational drugs such as gefitinib were shown to be extremely effective in a small subset of patients, suggesting that better patient selection could have led to success. In the current stage, it is likely that treatment standards will move towards using a combination of traditional chemotherapeutic agents in addition to one or more molecularly targeted agents like immunoconjugates. These new immunoconjugates may also be used following traditional therapy to eliminate minimal residual disease. Overall, the drug-antibody is a complex system that imposes additional demands on the pharmaceutical industry in terms of manufacturing, quality control and regulation. However, the benefits will outweigh the efforts given the recent technological advances and the potential use of drug-antibody conjugates in future anticancer regimens.

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