

Monoclonal antibody drug conjugates in the treatment of cancer

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Monoclonal antibodies directed to tumor-associated antigens have been chemically conjugated to drugs with different mechanisms of action and different levels of potency. Monoclonal-antibody-directed drug delivery has the potential to both improve efficacy and reduce systemic toxicity. Several immunoconjugates have demonstrated impressive antigen-specific antitumor activity in preclinical models. Phase I trials of a calicheamicin immunoconjugate for treatment of acute myeloid leukemia and a doxorubicin immunoconjugate for treatment of carcinoma have recently been completed.

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Abbreviations

AML acute myeloid leukemia
DOX doxorubicin
MAb monoclonal antibody
MR molar ratio

Introduction

The treatment of cancer is limited by a number of factors including the low therapeutic index of most chemotherapeutic agents, the emergence of drug- and radiation-resistant populations, tumor heterogeneity and the presence of metastatic disease. One of the means to improve the therapeutic index of drugs is by selective or 'targeted' delivery to tumor sites. Tumor-directed therapy has the potential to improve efficacy, by increasing the intratumoral concentration of the targeted agent, and to minimize toxicity by reducing systemic exposure. Monoclonal antibodies (MAbs), MAb fragments, hormones and growth factors have been used to deliver drugs, toxins, radionuclides, enzymes, photosensitizers and cytokines to tumors.

Unfortunately, the clinical efficacy of MAb-directed therapy is frequently limited by expression of the targeted antigen on normal as well as malignant cells. With the exception of MAbs to idiotype domains of lymphocytes, truly tumor-specific MAbs have not been identified; rather, MAbs identify tumor-associated antigens expressed at higher density on malignant cells relative to normal cells. It is therefore necessary to balance the relative selectivity of the MAb with the potency of the agent delivered. Studies in preclinical models with human tumors in immunodeficient mice have demonstrated impressive activity for many of these conjugates. However, it is important to recognize that these models, while useful, frequently over-predict

activity and under-predict toxicity because the antigen targeted is tumor-specific in the mouse but tumor-associated in patients. Nevertheless, several immunoconjugates have shown impressive activity even though the targeted antigen is expressed on normal tissues of immunodeficient animals or normal tissues of immunocompetent animals bearing syngeneic tumors [1,2,3*].

Significant progress has been made for MAb-directed therapies in the treatment of patients who have lymphoma [4,5**]. In addition, the use of the MAb 17-1A following tumor resection has resulted in improved survival of patients with Dukes' C colorectal cancer [6*] and improved response rates were seen in breast cancer patients receiving the anti-HER2 MAb, directed against the HER2 transmembrane tyrosine-kinase receptor coded by the *HER2* gene (also known as *neu* and as *c-erbB-2*), in combination with cisplatin [7]. However, only occasional responses have been reported for MAbs or immunoconjugates used as monotherapy in the treatment of patients with advanced solid tumors. The physical barriers of solid tumors — including elevated interstitial pressure, heterogeneous and reduced functional vasculature and the relatively large distances for MAbs to travel in the tumor interstitium [8*] — contribute to the limited tumor penetration and minimal efficacy seen when MAb-directed therapies are used as single agents in patients with advanced disease.

Several modifications have been used to improve the efficacy of immunoconjugate therapy. Immunogenicity has been reduced by using chimeric [9**] or humanized [5**] MAbs. Attempts to decrease the amount of MAb needed for antitumor activity have included the use of more potent drugs [3*,10] and alternative strategies such as branched linkers [11*] and delivery of liposome-encapsulated drugs [12,13*,14] to increase the quantity of drug delivered per antibody molecule. Improved MAb distribution in solid tumors has been addressed by using pharmacological approaches to improve penetration [15]. Recent studies have demonstrated that directing therapy to antigens expressed on the tumor vasculature (a readily accessible compartment), rather than to tumor-associated antigens of solid tumors, can produce impressive activity in preclinical models [16,17*,18**,19,20]. This review will concentrate on the results of recent clinical trials of MAb–drug immunoconjugates and highlight current strategies to improve the potency, specificity and efficacy of immunoconjugate therapy.

MAB-directed delivery of enediynes

One means to improve immunoconjugate potency and efficacy is to increase the potency of the targeted drugs. Members of the enediyne family of antibiotics are among the

most toxic antitumor compounds described to date. This novel class of agents includes the calicheamicins, neocarzinostatin, esperamicins, dynemicins, kedarcidin and maduropeptin [21]. Although these agents are highly potent *in vitro*, their utility as antitumor drugs has — for the most part — been limited by their low therapeutic index. Antibody-directed delivery provides a potential means to exploit the impressive potency of these compounds while minimizing their systemic toxicity. The use of extremely toxic drugs requires careful MAb selection as even low levels of expression of the targeted antigen by normal cells may lead to significant toxicity. Neocarzinostatin [22–24] and several of the calicheamicins [3•,5•,10,25,26] have been used to produce extremely potent immunoconjugates.

The calicheamicins produce double-stranded breaks in DNA. Calicheamicin conjugates — in which a hydrazide of calicheamicin γ^1_1 was linked to oxidized sugars on the internalizing anti-polyepithelial-mucin MAb CT-M-01 — produced potent antigen-specific activity against subcutaneous breast-tumor xenografts in athymic mice [10].

A Phase I study of CMA-676, an immunoconjugate of calicheamicin γ^1_1 conjugated to a humanized (human IgG₄) anti-CD33 MAb (hP67.6), has recently been completed [5•]. The CD33 target antigen is expressed on acute myeloid leukemia (AML) and maturing hematopoietic cells but not on normal stem cells. Forty patients with refractory or relapsed AML were treated intravenously with 0.25–9.0 mg/m² of CMA-676. Toxicity was primarily hematologic; however neither the hematologic nor nonhematologic side effects was considered dose-limiting. Fever and chills occurred in 80% of patients and were the most common nonhematologic side effect. Leukemic cells were eliminated from the blood and marrow of 20% of treated patients. At the 9 mg/m² dose level, >75% saturation of CD33 sites was seen on peripheral-blood blast cells. Clinical responses were seen at dose levels of 1–9 mg/m². Responses were seen only in patients whose peripheral blast cell demonstrated $\geq 75\%$ saturation of CD33 and had low efflux of 3,3'-diethyloxycarbocyanine iodide, an assay that determines functional efflux mediated through MDR1- and non-MDR1-dependent mechanisms. The efflux data suggest that intracellular delivery of the calicheamicin by the hP67.6 MAb did not overcome multidrug resistance. Data from this Phase I trial in patients with advanced AML are encouraging and support evaluation of CMA-676 in a setting of newly diagnosed or minimal-residual disease.

Calicheamicin θ^1_1 , a more potent analog of calicheamicin γ^1_1 , was conjugated to an anti-ganglioside-GD₂ MAb (14G2a) and showed impressive antitumor activity when used to treat experimental liver metastases in syngeneic immunocompetent mice [3•]. Dose-dependent activity was observed against a neuroblastoma line heterogeneous for antigen expression. The conjugate of 14G2a with calicheamicin θ^1_1 was both more efficacious and less toxic

than unconjugated calicheamicin θ^1_1 or mixtures of 14G2a and calicheamicin θ^1_1 , indicating effective antibody-directed targeting. The use of a syngeneic tumor model heterogeneous for antigen expression more closely approximates the clinical situation and provides an important model system for evaluating immunoconjugate efficacy.

MAb-directed delivery of anthracyclines

The anthracycline family of antitumor antibiotics, most notably doxorubicin (DOX) and daunorubicin, has been used extensively for drug targeting applications [27]. The immunoconjugate BR96–DOX [1,28] was evaluated in Phase I [9•] and II [29•] clinical trials. BR96–DOX (which is chimeric with human IgG₁) binds a Le^y-related, tumor-associated antigen expressed on most human carcinomas [30] and on normal cells of the gastrointestinal tract of humans, dogs and rats [1]. BR96–DOX induced cures of human lung, breast and colon carcinomas in athymic mice and rats [1,27,31] and syngeneic colon tumors in immunocompetent rats [2].

Although cures were seen in multiple preclinical models, only tumor stabilization and a small number of partial regressions were seen in a Phase I trial of patients with advanced disease. A therapeutically relevant anticonjugate response was not observed and there were no significant hematologic or cardiac toxicities. The dose-limiting toxicity was acute gastrointestinal toxicity with dose-related nausea, vomiting and gastritis [9•].

A randomized Phase II trial was performed in patients with metastatic breast carcinoma [29•]. Patients received 700 mg/m² of BR96–DOX (20 mg/m² DOX) or 60 mg/m² of DOX every three weeks. There was one partial response (in a patient with hepatic metastases) in the fourteen patients receiving BR96–DOX and one complete and three partial responses in the nine patients receiving DOX. Interestingly, two of the four patients who crossed over to the BR96–DOX arm of the trial after persistent stable disease during DOX treatment achieved partial regression of hepatic metastases following BR96–DOX therapy.

Localization of BR96 and DOX was seen in tumor biopsies of patients receiving BR96–DOX, indicating that BR96 successfully delivered DOX to tumors [9•]. These data, taken together with the low clinical response rates, suggest that the dose that could be safely administered every three weeks was insufficient to maintain the intratumoral concentration of DOX required to achieve regression. Preclinical studies in antigen-expressing rats indicate that administering BR96–DOX in combination with cytotoxic drugs or at a low dose for an extended duration can substantially reduce both the dose per injection and the cumulative dose needed to cure established experimental tumors (PA Trail, unpublished data). It is likely that BR96–DOX, like most antibody therapies, will be most effective in minimal-disease settings.

Several novel conjugation strategies have been developed to improve the potency of anthracycline conjugates. An enzymatic coupling procedure that attaches DOX to galactose residues of an anti-carcinoembryonic-antigen MAb demonstrated antigen-specific activity *in vitro* and improved both the potency and efficacy (relative to unconjugated DOX) against tumors transplanted onto the chorioallantoic membrane of embryonated chicken eggs [32]. However, as conjugates were applied to tumor-containing discs on the chorioallantoic membrane, the relative utility of this method awaits demonstration of distal site activity in a more rigorous antitumor model that requires MAb-directed delivery.

The potency of immunoconjugates can be improved by increasing the quantity of drug delivered per MAb molecule. In the case of DOX immunoconjugates, significant losses in affinity and antigen-specific cytotoxicity were seen when ≥ 10 molecules of DOX were directly conjugated per MAb [33]. The use of branched linkers, in which each linker to the MAb carries two DOX molecules, resulted in an increase in the drug : MAb molar ratio (MR) from 8:1 to 16:1. This increase in the MR was accompanied by an increase in antigen-specific potency *in vitro* [11•] and a two-fold decrease in the amount of MAb required to achieve partial regression of subcutaneous tumors in preclinical models. The use of water-soluble polymeric carriers [34] has also been attempted to increase conjugate MRs.

Immunoliposomes

The encapsulation of drugs in MAb-targeted liposomes can be used to selectively increase the concentration of drug delivered to antigen-expressing cells [12,13•,14,35]. The pharmacokinetics and clearance of liposomes were improved by incorporating lipid derivatives of polyethylene glycol (PEG) into liposome formulations [36,37]. These sterically stabilized liposomes enhance accumulation in tumours [38]. Importantly, immunoliposomes utilizing internalizing MABs — such as anti-HER-2 [39] or anti-CD19 [13•] — can be used to selectively deliver high concentrations of drug into the cytoplasm of antigen-expressing cells.

Targeting the tumor vasculature

The progressive growth and metastasis of tumors requires the formation of new blood vessels (angiogenesis) from the pre-existing vasculature [40]. Immunoconjugates directed against antigens differentially expressed on tumor endothelium offer several potential advantages over targeting tumor-associated antigens expressed on cells of solid tumors. Directing therapy to the accessible vascular compartment reduces the impact of the physical barriers of solid tumors, such as heterogeneous blood flow and elevated interstitial pressure, which restrict the penetration and distribution of MABs through the tumor parenchyma [8•]. Endothelial cells are highly regulated, genetically stable cells that are less likely to develop the classical drug resistance observed in tumor cells [40]. In

addition, as angiogenesis is required for tumor progression, therapies directed against the tumor vasculature should have broad-spectrum activity. Several recent studies have demonstrated that targeting the tumor vasculature with MABs [16,17•,20], growth factor ligands [41,42] or peptides that bind α_v integrins [18••] can produce impressive antitumor activity. Identification of appropriate target antigens that are expressed on the tumor vasculature, but not on cells of normal vessels, is an area of ongoing interest. Potential antigens for vascular targeting include VEGFR-2 (vascular endothelial growth factor receptor 2), endoglin, endosialin, aminopeptidase A [16,19] and VEGF complexed with its receptor [43]. Screening of phage display libraries identified several peptides that selectively localized in the tumor vasculature. These peptides were conjugated to DOX and shown to have impressive antitumor activity that was associated with damage to the tumor vasculature [18••]. The *in vivo* screening of phage peptide libraries is an interesting approach to identify novel molecules expressed on angiogenic blood vessels.

Conclusions and future directions

Although immunoconjugates are not currently established chemotherapeutic agents, several of them have demonstrated evidence of biologic activity in patients with advanced disease [5••,9••,29•]. The current objectives are aimed at improving the efficacy and therapeutic index of immunoconjugates by optimizing selectivity and potency. The development of MAB therapies directed against the tumor vasculature is an area of considerable interest and various research approaches to identify antigens and conjugation strategies with appropriate selectivity are being pursued.

The promise offered by MAB-based therapies has begun to be realized with the approval of an anti-CD20 MAB for treatment of non-Hodgkin's lymphoma and an anti-HER2 MAB for treatment of metastatic breast carcinoma. The calicheamicin conjugate CMA-676 [5••] has shown encouraging data in a Phase I trial of patients with refractory AML. Although immunoconjugates may be active as single agents, it is likely that their major role — especially in treatment of solid tumors — will be in combination-chemotherapy regimens or minimal-disease settings. In addition to research efforts directed at improving immunoconjugate constructs, clinical studies to define optimal therapeutic strategies are underway and will further clarify the role of immunoconjugates as anticancer agents.

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