Volume 89 | No. 4 | April 2011

# Clinical Pharmacology & Therapeutics

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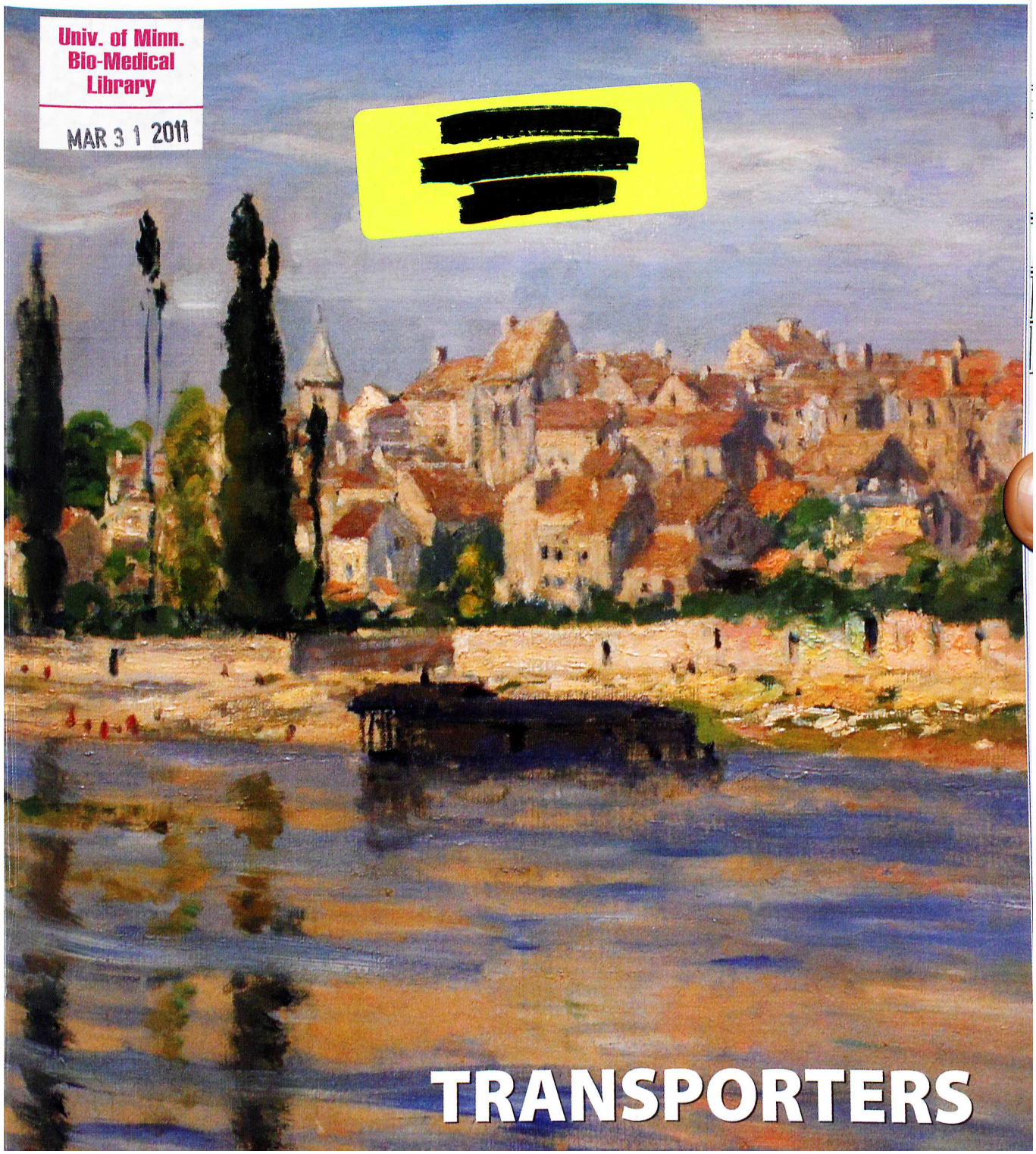

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All business correspondence and inquiries should be addressed to *Clinical Pharmacology & Therapeutics*, Nature Publishing Group, 75 Varick Street, 9th Floor, New York, NY 10013-1917.
Tel: +1 212 726 9672. Fax: +1 646 563 7127.
Publisher: Anna Salt
Production Editor: Chris Robson

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ISSN 0009-9236 EISSN 1532-6535.


*Clinical Pharmacology & Therapeutics* (ISSN 0009-9236) is published monthly by Nature Publishing Group, 75 Varick Street, 9th Floor, New York, NY 10013-1917. Periodicals postage paid at New York, NY and at additional mailing post offices. POSTMASTER: Send address changes to *Clinical Pharmacology & Therapeutics*, Subscription Department, Nature Publishing Group, 75 Varick Street, 9th Floor, New York, NY 10013-1917.
Printed on acid-free paper, effective with Volume 81, Issue 1, 2007.
Printed and bound in the USA by The Sheridan Press, Hanover, PA, USA.

# Immunoconjugates Against Solid Tumors: Mind the Gap

AD Ricart[1]

**The objective of immunoconjugate development is to combine the specificity of immunoglobulins with the efficacy of cytotoxic molecules. This therapeutic approach has been validated in hematologic malignancies; however, several obstacles to achieving efficacy in treating solid tumors have been identified. These include insufficient specificity of targets and poor antibody delivery, most specifically to the tumor core. Heterogeneous antigen expression, imperfect vascular supply, and elevated interstitial fluid pressure have been suggested as the factors responsible for the poor delivery of antibodies. Promising immunoconjugates are in development: immunoconjugates targeting the prostate-specific membrane antigen, trastuzumab-DM1, lorvotuzumab mertansine, and SS1P. Advances in cancer biology and antibody engineering may overcome some of the challenges. New small antibody formats, such as single-chain Fv, Fab, and diabodies, may improve penetration within tumor masses. Nevertheless, the cost of treatment might require justification in terms of demonstrable improvement in quality of life in addition to efficacy; further economic evaluation might be necessary before this approach can replace the current standards of care in clinical practice.**

"Mind the gap" is a warning to train passengers to remind them of the sometimes significant gap between the train door and the station platform.

## REVIEW CRITERIA

The data for this review were obtained by searching PubMed and MEDLINE databases without any date limitation. The search terms included "immunoconjugate," "tumor-targeting agent," "radioimmunotherapy," "antibody-drug conjugate," and "immunotoxin." The abstracts of retrieved citations were reviewed and prioritized by relative content. Full-length articles that were deemed relevant were analyzed before being included in this review, and references were checked for additional material where appropriate. In addition, relevant abstracts (that were not yet reported in the form of full-length articles) were identified using an electronic search of the proceedings of the American Society of Clinical Oncology, the American Association for Cancer Research, the European Society for Medical Oncology, and the San Antonio Breast Cancer Symposia meetings.

## INTRODUCTION

Until recently, drug development in oncology was mostly empirical. The mouse hybridoma technology described by Milstein and Köhler was the instrumental step for the development of monoclonal antibody (mAb) technology.[1] Several anticancer mAbs have been introduced in clinical practice since approval was received for the use of the first such mAb, rituximab, and these are now established as a new component of cancer treatment (**Table 1**).[2] The success of antibody cancer therapy has depended mainly on the ability to generate a desired mAb and the characterization of suitable tumor targets (or suitable targets in the tumor environment), opening an unprecedented opportunity for designer anticancer drugs (**Table 2**). Antibodies are complex molecules and their effects can be ascribed to multiple mechanisms: recruitment of immune cells, activation of complements, sequestration, and cross-linking of targets.[3] There are three principal mechanisms of action of anticancer mAbs: blocking the function of specific molecules, targeting specific cells (generating cytotoxicity in the cells that express the antigen), and functioning as signaling molecules.[4,5] In early clinical trials, mouse mAbs had limited serum stability because of a human antimouse antibody response, rendering repeat dosing ineffective and more toxic.[3,6] But the ability to create more human variants finally made mAbs suitable for use as repeated treatment. Moreover, the task of selecting fully human variable domains was simplified during the 1980s through the isolation of genes encoding human variable regions, their successful expression in *Escherichia coli*, and the introduction of phage-display technology.[5,7–9]

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Received 21 October 2010; accepted 3 January 2011; advance online publication 2 March 2011. doi:10.1038/clpt.2011.8

**Table 1 Food and Drug Administration–approved anticancer monoclonal antibodies (mAbs)**

| Antibody | Antigen | Indication | Type of mAb | Mechanism of action | Brand name and manufacturer |
|---|---|---|---|---|---|
| *Naked antibodies* | | | | | |
| Rituximab | CD20 | NHL | Chimeric | ADCC, CDC | Rituxan (Biogen-IDEC and Genentech) |
| Trastuzumab | HER2 | HER2+ breast cancer | Humanized | ADCC, receptor blockade | Herceptin (Genentech) |
| Alemtuzumab | CD52 | B-cell CLL | Humanized | ADCC, CDC | Campath (Genzyme) |
| Bevacizumab | VEGF | Metastatic colon cancer, breast cancer, and NSCLC | Humanized | Ligand blockade | Avastin (Genentech) |
| Cetuximab | EGFR | Metastatic colon and head and neck cancer | Chimeric | Receptor blockade | Erbitux (Imclone Systems) |
| Panitumumab | EGFR | Metastatic colon cancer | Human | Receptor blockade | Vectibix (Amgen) |
| Ofatumumab | CD20 | CLL | Human | ADCC, CDC | Arzerra (GlaxoSmithKline) |
| *Immunoconjugates* | | | | | |
| $^{90}$Y-Ibritumomab tiuxetan | CD20 | Relapsed/refractory NHL | Murine | Radiation (β-emission) | Zevalin (Cell Therapeutics) |
| $^{131}$I-Tositumomab | CD20 | Relapsed/refractory NHL | Murine | Radiation (β- and γ-emissions) | Bexxar (GlaxoSmithKline) |

Gemtuzumab Ozogamicin (Mylotarg, Pfizer), directed against the CD33 antigen present on leukemic myeloblasts in most patients with acute myelogenous leukemia (AML), has been recently withdrawn in the United States because a required postapproval study failed to confirm the drug's clinical benefit.

ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; CLL, chronic lymphoid leukemia; EGFR, epidermal growth factor receptor; NHL, non-Hodgkin's lymphoma; NSCLC, non–small-cell lung cancer; VEGF, vascular endothelial growth factor.

**Table 2 Tumor-associated antigens targeted by immunoconjugates**

| Target | Type of molecule | Expression |
|---|---|---|
| PSMA | Cell-surface glycoprotein | Prostate cancer, vasculature of solid tumors |
| Tenascin-C | Extracellular matrix protein | Glioblastoma multiforme, breast and lung cancer, SCC, and NHL stroma |
| G250 | Membrane-associated carbonic anhydrase (CA IX) | Clear-cell RCC |
| MUC1 | Glycoprotein (mucin) | Ovarian, colorectal, and gastric cancer |
| CanAg | Glycoprotein (mucin) | Pancreatic, colorectal, biliary and gastric cancer, and NSCLC |
| HER2 | Member of the EGFR family | Breast cancer |
| CD56 | Neural cell adhesion molecule | SCLC, Merkel cell carcinoma, neuroblastoma, ovarian cancer, and MM |
| GPNMB | | Melanoma, breast cancer |
| EphA2 | Member of the erythropoietin-producing hepatoma (Eph) family of TK receptors | Breast, prostate, lung and ovarian cancer, and glioblastoma multiforme |
| Integrins | Transmembrane receptors for proteins of the ECM | Solid tumors and blood vessels |
| Cripto | GPI-linked cell-surface glycoprotein | Breast, colon, gastric, pancreatic, lung, ovarian, cervical, and testicular cancer |
| SLC44A4 | Choline transporter-like protein | Pancreatic, prostate, and gastric cancer |
| CD70 | Member of the TNF superfamily | Lymphomas, RCC, and glioblastoma |
| Mesothelin | Glycosylphosphatidylinositol-anchored antigen | Mesothelioma, pancreatic and ovarian cancer, and NSCLC (adenocarcinoma) |
| A33 | Glycoprotein with homology to Ig superfamily | Colorectal cancer |
| CEA | Member of the Ig superfamily | SCLC, colorectal cancer, medullary thyroid carcinoma |

ECM, extracellular matrix; EGFR, epidermal growth factor receptor; Ig, immunoglobulin; MM, multiple myeloma; NHL, non-Hodgkin's lymphomas; NSCLC, non–small-cell lung cancer; PSMA, prostate-specific membrane antigen; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; TK, tyrosine kinase; TNF, tumor necrosis factor.

Humanized mAbs are more effective in inducing antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, and they are less immunogenic.

## FACTORS REGULATING ANTIBODY-TARGETED THERAPY

The efficacy of a particular mAb depends on different variables. These include not only the characteristics of the mAb itself (fine specificity, avidity, and isotype) but also those of the targeted antigen: its function, cell-surface density, presence of secreted isoforms, shedding and/or internalization, normal tissue distribution, and phenotypic expression in the cancer cell population.[4] Antibody-targeted therapy attempts to induce an unprecedented degree of anticancer specificity; however, there are significant obstacles that prevent an ideal targeting of solid tumors (**Table 3**). Overall, the use of intact mAbs is associated with practical limitations because of their pharmacokinetics: their slow

rate of clearance causes significant exposure to normal organs, limits the quantities delivered to tumors, and results in relatively poor diffusion from the vasculature into and through the tumor (**Figure 1**).[10] They are large proteins and are therefore characterized by slower kinetics of distribution as compared to small molecules.[4] Particularly in solid tumors, heterogeneous antigen expression and imperfect vascular supply can limit uniform delivery of antibodies. Impaired clearance of fluid from tumors (due to lack of lymphatic vessels) also leads to increased interstitial pressure within the extracellular matrix.[11] This elevated interstitial pressure in the centers of tumors opposes inward diffusion and induces a net outward gradient from the center of the tumor, thereby slowing the diffusion of immunoglobulin G (IgG) molecules from their extravasation site. Consequently, this gradient within solid tumors differentially inhibits the diffusion of larger molecules in comparison to smaller molecules.[12] It has been observed in experimental studies that tumor penetration seems to be directly related to the size of the antibody molecules, with faster, deeper, more extensive, and more uniform tumor penetration being achieved by single-chain Fvs than by intact IgGs.[13] Complete human antibodies have prolonged half-lives ($t_{1/2}$) owing to their ability to bind to the neonatal Fc receptor. This receptor is expressed on placenta and blood vessel linings and protects serum IgG from degradation. Because Fab and single-chain Fv fragments lack the Fc region, they are not protected by this receptor.[5] There is also a difference in biodistribution kinetics between fragments and whole antibodies, as observed in experimental studies and mathematical models.[14,15] This variation in biodistribution has an impact on the effectiveness of drug delivery to tumors. "Retention" (the percentage of the injected dose found in the tumor throughout a range of time points) is influenced by several factors, including affinity, but is greater for intact IgGs. Higher retention in the tumor would be important for immunoconjugates with "bystander" effect (such as radioimmunoconjugates), whereas rapid clearance from the bloodstream is preferable for peptide cytotoxins. Immunotoxins with longer circulating $t_{1/2}$ lead to increased vascular endothelial injury and more severe "vascular leak syndrome." Rapid elimination from blood would allow repeated and frequent administration, or even short continuous infusions. Hence, the size of the delivery vehicle should be selected on the basis of the mechanism of action of the payload. However, several questions remain unanswered in comparing the use of intact IgGs versus fragments in therapeutic application to solid tumors, because clinical experience is still limited. Antigen shedding can also limit the delivery within the tumor and reduce the clinical activity of mAbs. Given that shedding of membrane proteins is a physiologic process used by cells to modulate the function of surface proteins, soluble antigen in the extracellular fluid of tumors has recently been identified as a significant additional barrier to the activity of mAbs.[16]

**Table 3 Obstacles to achieving efficacy with monoclonal antibody (mAb) therapy**

| |
|---|
| Impaired mAb distribution[a] |
| Limited delivery to tumor sites[a] |
| Insufficient trafficking of effector cells to tumor[a] |
| Antigenic heterogeneity (intratumoral and intertumoral)[a] |
| Shedding and internalization of target antigens[a] |
| Insufficient tumor specificity of target antigens[a] |
| Immunogenicity: human antimouse and antichimeric antibody responses, immune response to peptide cytotoxins |

[a]These obstacles either are not seen or are less critical in hematologic malignancies.

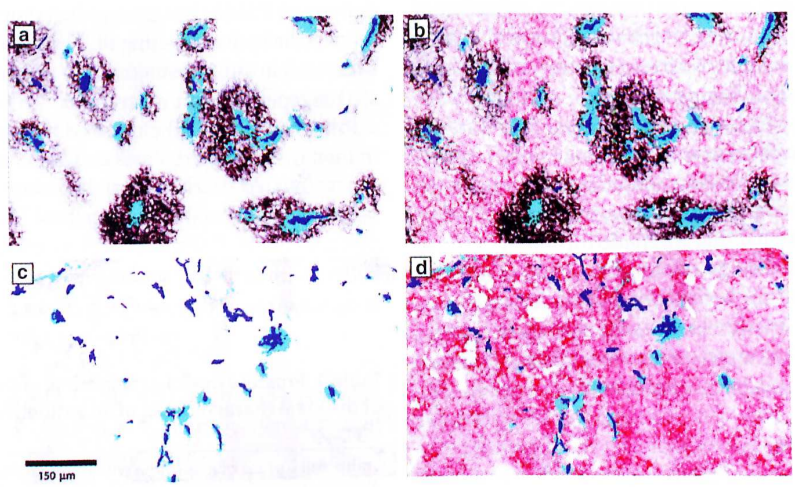


**Figure 1** Direct visualization of heterogeneous extravascular distribution of trastuzumab in human HER2-overexpressing xenografts. Staining for bound trastuzumab and HER2. Tumor cryosections are shown from MDA-435-LCC6HER2-overexpressing xenografts treated with 20 mg/kg trastuzumab for 3 h or left untreated. (**a**) Overlaid images of a treated tumor show bound trastuzumab (black) relative to blood vessels (CD31; dark blue) and the perfusion marker DioC7(3) (cyan). (**b**) Additional staining of the same section for HER2 (red) shows that areas with no bound trastuzumab are overexpressing HER2. An untreated MDA-435-LCC6HER2 tumor shows (**c**) no bound trastuzumab but (**d**) relatively homogeneous HER2 expression. Similarly stained MDA-435-LCC6 vector tumors display no bound trastuzumab or unbound HER2 in treated or untreated tumors (data not shown). Reprinted with permission from Baker, J.H. *et al.*, Direct visualization of heterogeneous extravascular distribution of trastuzumab in human epidermal growth factor receptor type 2 overexpressing xenografts, *Clin. Cancer Res.* 14, 2171–2179 (2008). Copyright ©2008 American Association for Cancer Research.

## ENHANCING ACTIVITY THROUGH THE USE OF IMMUNOCONJUGATES

To date, all mAbs approved for use in the treatment of solid tumors are naked antibodies (**Table 1**). Targeting implies directing antibodies toward cells expressing tumor-associated antigens and is an adaptable approach; antibodies can be engineered to carry moieties (payloads) such as radionuclides, chemotherapeutic agents, toxins, or cytokines. In particular, immunoconjugation can be perceived as a strategy for improving the specificity of cytotoxic drugs or radiation and for enhancing the efficacy of passive immunotherapy, the aim being to integrate the best characteristics of both therapeutic approaches.[3,17] Moreover, this approach does not depend on the host immune system status and is not adversely affected by internalization of mAb-antigen complexes in the case of radioimmunoconjugates. The delivery of the payload can also be increased by several orders of magnitude to target tumor antigens whose expression on the cell membrane is measured in the millions (e.g., HER 2).[17] Nonetheless, conjugation to radionuclides or cytotoxic drugs considerably increases the toxicity profile of mAbs, as exemplified by the comparison of the safety profiles of trastuzumab and trastuzumab-DM1.[18–20]

The requirement for tumor-specific antigen expression is critical for the success of these agents, because sequestration of the cytotoxic payload to nontumor cells might occur even with low antigen expression if the antigen is widely distributed among normal tissues. This process, known as "antigen sink," could manifest as toxicity in an anatomically distant organ and result in lack of antitumor activity. This outcome was graphically illustrated during the clinical development of BR96–doxorubicin conjugate in the mid 1990s. The Lewis$^Y$ antigen, the target antigen for a family of BR96-constructs (BR96 mAb linked to cytotoxic drugs), is expressed with great intensity on a number of carcinomas. Nonetheless, during the phase I clinical studies of BR96–doxorubicin, an unanticipated constellation of symptoms and signs was observed, including intractable nausea, vomiting, and hematemesis, which were dose-limiting.[21] In a subsequent phase II study in patients with metastatic breast cancer, no significant antitumor activity was observed in those receiving BR96–doxorubicin as compared with the control group. Further investigation demonstrated that BR96–doxorubicin binds to Lewis$^Y$ antigen expressed on gastric mucosa cells and that this binding was responsible for both the toxicity and the absence of antitumor activity. Attempts to develop this agent have so far been unsuccessful. The target requirements of a solid tumor antigen are likely to be much more stringent.[17]

### Radioimmunoconjugates

Radioimmunotherapy is an attractive approach as a treatment for lymphomas because the cells in lymphomas are inherently sensitive to radiation. It is also well known that lymphomas metastasize to areas such as the lymph nodes and bone marrow, sites that are readily accessible to circulating mAbs. The two currently available radiolabeled mAbs, yttrium-90-labeled ($^{90}$Y) ibritumomab tiuxetan (Zevalin) and iodine-131-labeled ($^{131}$I) tositumomab (Bexxar), target CD20, the same antigen recognized by rituximab, and show more clinical activity than the naked antibody.[22] They are fully mouse molecules, but this is not a major concern with respect to tositumomab or ibritumomab, which are intended for one-time dosing.[3]

While radioimmunotherapy has shown success in lymphomas, responses in refractory adenocarcinomas have been infrequent. This is attributable, in part, to their relative lack of sensitivity to radiation and the consequent failure to deliver an adequate radiation dose to tumor masses. The physical properties of isotopes (path length, energy of emission, and physical $t_{1/2}$) should be selected on the basis of lesion size and the mAb's properties. For solid tumors, β-emitters would be the optimal choice for lesions that are larger than 2–3 mm, whereas α-emitters might be best suited for treating micrometastasis. $^{90}$Y has a higher β-particle energy and longer range than lutetium-177 ($^{177}$Lu); however, this renders it more toxic.[23] Like $^{131}$I, $^{177}$Lu has a longer $t_{1/2}$ than $^{90}$Y and is therefore more suitable to the pharmacokinetics of mAbs, but its chemistry is similar to that of $^{90}$Y and, when internalized, it is retained by the tumor whereas $^{131}$I can be quickly released. $^{177}$Lu seems to be more effective in treating small lesions.[23]

***Radioimmunoconjugates against PSMA.*** Antigen expression on prostate cancer cells has been studied at length in recent years. Prostate-specific membrane antigen (PSMA) has emerged as one of the most promising targets for mAb-based therapy. PSMA has many of the paramount characteristics of a tumor target antigen, although its functional role is still unclear (**Table 4**).[23] Interestingly, endothelial cells of tumor-associated neovasculature can express PSMA (including carcinoma of the colon, breast, bladder, pancreas, and kidney, and melanoma). The humanized mAb J591, which targets the extracellular domain of PSMA, has emerged as one of the most promising carriers. Selective targeting of $^{111}$In-labeled J591 to tumors has been seen in clinical studies, and the naked mAb is well tolerated in repetitive administration.[24]

Prostate cancer is the most common noncutaneous cancer in men in the United States and is the second leading cause of cancer-related death in men. For more than 60 years, hormonal therapy has been the cornerstone of treatment for advanced prostate cancer. Unfortunately, hormonal therapy is mostly palliative, with little impact on survival; consequently, improved systemic therapies are necessary. Phase I trials of $^{177}$Lu-J591 and

**Table 4 Prostate-specific membrane antigen (PSMA) has many of the ideal characteristics of an antigen for antibody-based therapy**

| |
|---|
| Stable cell surface glycoprotein with minimal shedding or isoform secretion |
| Abundantly expressed in prostate cancer (magnitude of expression) |
| Expression increases with high-grade tumors and hormone-refractory disease (upregulated by androgen deprivation) |
| Highly specific: low expression in normal tissues (most notably small intestine, proximal renal tubule cells, and salivary glands) as compared with tumor |
| Little phenotypic variation in expression in prostate cancer metastases |
| Rapid internalization of the PSMA antibody complex along with any payload carried by the antibody |

$^{90}$Y-J591 have been performed in castration-resistant prostate cancer. In a phase I trial with $^{177}$Lu-J591, an 11% decline rate (≥50%) in prostate-specific antigen was reported, which is a satisfactory result in previously treated patients.[25] Despite this biochemical indication of biological activity, significant tumor regressions were not seen. Another early-phase study, with $^{90}$Y-J591, reported that 2 of 29 patients had objective responses after treatment.[26] Although the two trials had similar patient eligibility criteria, the study populations were not equivalent (e.g., there was a smaller number of patients with measurable lesions in the $^{177}$Lu-J591 trial), and any formal comparison must be avoided. However, we can hypothesize that $^{177}$Lu-J591 may be a better candidate for small-volume lesions (5 mm), whereas $^{90}$Y-J591 may be more effective in larger (≥1 cm) tumors.[23] Bone marrow is the dose-limiting organ in radioimmunotherapy targeting PSMA. Further clinical examination, including research into fractionated dose regimens and combination therapy, is needed in proof-of-concept studies.

***Antitenascin mAb.*** Neuradiab (previously referred to in the literature as antitenascin radiolabeled mAb $^{131}$I-81C6) is a murine mAb conjugated to $^{131}$I and is delivered directly into the surgical resection cavity in a separate procedure after the initial surgery for glioblastoma multiforme. The intention is to deliver a concentrated level of radiation specifically to cancer cells that remain after surgery. The target, tenascin, is a protein that is overexpressed by 99% of all glioblastoma multiforme but is virtually absent from normal brain tissues. Neuradiab was proven to be safe in early phases of development, and phase II data showed a significant increase in overall survival as compared to currently approved therapies.[27] The therapeutic regimen consists of a small dose of the mAb instilled into the surgical cavity, and the external measurements of the resultant absorbed radioactivity are used to calculate the patient-specific amount of neuradiab required to achieve the optimal targeted absorbed dose. This specific dose is then administered into the surgical cavity. Acute reversible neurotoxicity and hematologic toxicity are the most common adverse events. Patient-specific dosing decreases the chances of irreversible neurotoxicity or radionecrosis. A phase III study (the GLASS-ART trial, http://www.glassarttrial.com) comparing neuradiab plus chemoradiotherapy vs. the standard of care alone commenced in 2008, but the sponsor of the study has closed enrollment because of unforeseen delays in patient recruitment.

***Targeting carbonic anhydrase IX.*** G250 is a membrane-associated carbonic anhydrase (CA IX) that is thought to play a role in the regulation of cell proliferation in response to hypoxic conditions and may be involved in carcinogenesis and tumor progression. It is ubiquitously expressed in ~95% of clear-cell renal cell carcinomas, whereas in normal tissue it is restricted to large bile ducts and gastric epithelium. Despite high uptake by tumors and indications of antitumor activity with a $^{131}$I-labeled chimeric mAb (cG250),[28] the utility of radioimmunoconjugates against renal cell carcinoma might be limited by the radiotherapy-resistant nature of this tumor, which also frequently metastasizes to very radiosensitive organs (e.g., lung and liver). Furthermore, new therapeutic options now approved for advanced renal cell carcinoma, including signal transduction and vascular endothelial growth factor (VEGF) receptor inhibitors, are likely to restrict opportunities to use radioimmunoconjugates for this tumor indication.

## Antibody-drug conjugates (ADCs)

Cytotoxic drugs represent a separate class of conjugates. Because nearly all cytotoxic anticancer drugs have dose-limiting toxicity, significant interest has surrounded mAb-based targeting strategies. Usually, the payloads are highly potent cytotoxic agents. Efficient drug delivery to the tumor would minimize drug exposure in normal tissues, increasing the therapeutic index of the cytotoxic drug while potentially minimizing toxicity observed with systemic cytotoxic therapy.[17] The typical example, and the only candidate for regulatory approval, has been gemtuzumab ozogamicin (Mylotarg). It consists of a semisynthetic derivative of calicheamicin (*N*-acetyl-gamma calicheamicin 1,2-dimethyl hydrazine dichloride), a potent enediyne DNA-binding cytotoxic antibiotic, linked to an engineered human IgG4 mAb (hP67.6) directed against the CD33 antigen that is present on leukemic myeloblasts in most patients with acute myelogenous leukemia. Tumor cells exhibiting P-glycoprotein (P-gp)–mediated multidrug resistance may be able to escape the effect of gemtuzumab ozogamicin (calicheamicin is a substrate for the MDR1/P-gp-1 pump). This was suggested by the correlation between clinical response and low levels of dye efflux by leukemic blast cells. Also, *in vitro* drug-induced apoptosis could be increased by P-gp antagonists (e.g., cyclosporine). Elevated levels of P-gp have been found in chemoresistant tumors as well as in drug-sensitive tumors that relapsed after chemotherapy. Together, these findings indicate that immunoconjugate constructs with cytotoxic drugs that are not subject to P-gp efflux would be better candidates for the treatment of solid tumors. Gemtuzumab ozogamicin has recently been withdrawn in the United States because a required postapproval study failed to confirm the drug's clinical benefit (http://media.pfizer.com/files/products/mylotarg_hcp_letter.pdf). This voluntary withdrawal of a US New Drug Application highlights the importance of a rational drug development plan, first confirming clinical benefit with randomized data in the same clinical setting as that of the preceding nonrandomized study (see "Future Directions" below).

Other conjugates of calicheamicin, geldanamycin, and potent tubulin poisons (maytansinoids, auristatins, and taxanes) are undergoing clinical evaluation or are in preclinical development. What all of these drugs have in common is that their cytotoxic potencies are in the picomolar range (**Table 5**).[17,29]

***ADCs.*** IMGN242 (previously known as huC242-DM4) is an advanced, disulfide-bound drug conjugate that comprises the conjugation of approximately four molecules of the potent maytansinoid antimicrotubule agent DM1 to the humanized mAb huC242. The mAb binds specifically to a tumor-associated carbohydrate epitope of CanAg (a novel glycoform of MUC1). This antigen is expressed on gastrointestinal

**Table 5 Selected immunoconjugates against solid tumors**

| Description | Target | Indication | Stage |
|---|---|---|---|
| Trastuzumab-DM1. Humanized mAb conjugated to DM1 | HER2 | HER2+ breast cancer | Phase III |
| R1549. Murine mAb (muHMFg1) conjugated to $^{90}$Y | MUC1 | Ovarian and gastric cancer[a] | Phase II/III |
| IMGN242 (huC242-DM4). Humanized mAb conjugated to DM1 | CanAg | Gastric cancer[a] | Phase II |
| SGN-15. Chimeric BR96 mAb chemically linked to doxorubicin | Lewis$^Y$ | NSCLC[a] | Phase II |
| Pentacea. Humanized mAb bispecific for CEA and DTPA[b] | CEA | SCLC[a] | Phase II |
| CEA-Cide $^{131}$I (labetuzumab). Humanized mAb conjugated to $^{131}$I | CEA | Colorectal cancer[a] | Phase II |
| IMGN901-DM1. Humanized mAb conjugated to DM1 | CD56 | SCLC, Merkel cell carcinoma | Phase I/II |
| CDX-011. Fully human mAb conjugated to MMAE | Glycoprotein NMB | Breast cancer, melanoma | Phase I/II |
| MLN2704. Humanized mAb conjugated to DM1 | PSMA | Prostate cancer[a] | Phase I/II |
| $^{177}$Lu-J591 and $^{90}$Y-J591. Humanized mAb linked to $^{177}$Lu and $^{90}$Y | PSMA | Prostate cancer | Phase I/II |
| PSMA ADC. Fully human mAb conjugated to MMAE | PSMA | Prostate cancer | Phase I |
| SS1P (dsFv)-PE38. A disulfide-stabilized Fv fragment conjugated to truncated *Pseudomonas* exotoxin A | Mesothelin | Pancreatic cancer, NSCLC, and ovarian cancer | Phase I |
| MDX-1203. Fully human mAb conjugated to MGBA | CD70 | Renal cell cancer | Phase I |
| SGN-75. Humanized mAb conjugated to MMAF | CD70 | Renal cell cancer | Phase I |
| MEDI-547. Fully human mAb conjugated to MMAF | EphA2 | Solid tumors | Phase I |
| IMGN388. Fully human mAb conjugated to DM4 | Integrin | Solid tumors | Phase I |
| BIIB015. Humanized mAb conjugated to DM4 | Cripto | Solid tumors | Phase I |
| ASG-5ME. Fully human mAb conjugated to MMAE | SLC44A4 | Pancreatic cancer | Phase I |

DM1, *N*-methyl-*N*-[3-mercapto-1-oxopropyl]-L-alanine ester of maytansinol; DM4, *N*-methyl-*N*-[4-mercapto-4-methyl-1-oxopentyl]-L-alanine ester of maytansinol; DTPA, diethylenetriaminepentaacetic acid; mAb, monoclonal antibody; MGBA, minor groove–binding agent; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin phenylalanine; NSCLC, non-small-cell lung cancer; PE38, *Pseudomonas* exotoxin A; PSMA, prostate-specific membrane antigen; SCLC, small-cell lung cancer.

[a]These are not currently under investigation. [b]Pretargeting: mAb followed by $^{131}$I DTPA.

carcinomas, including gastric, pancreatic, and colorectal cancers, as well as in some non-small-cell lung cancers. This mAb seems to compare favorably with an earlier CanAg-targeting compound, cantuzumab mertansine. A phase I trial in patients with carcinomas positive for CanAg indicated 168 mg/m$^2$ as the recommended dose for phase II studies. The principal toxicities were ocular adverse events (corneal deposits, keratitis, blurred vision, and blepharitis), diarrhea, and weakness.[30,31] Clinical development of IMGN242 has been discontinued.

Trastuzumab-DM1 (T-DM1) is the best drug immunoconjugate candidate to date. It consists of a maytansine derivative DM1 attached to Genentech's HER2-binding antibody, trastuzumab. The naked antibody is approved for the treatment of HER2-overexpressing breast cancer (**Table 1**).[20] Preclinical studies indicated that T-DM1 had greater activity as compared with nonconjugated trastuzumab while maintaining selectivity for HER2-overexpressing tumor cells. The mAb with a nonreducible thioether linker (SMCC) was selected for clinical development because of its greater efficacy, improved pharmacokinetics, and lower toxicity as compared with a reducible disulfide linker.[32] The highly tumor-specific expression of the antigen, the magnitude of this expression, its clinical validation as a target, the high stability of the linker, the ability to deplete shed HER2 with naked antibody, and its effectiveness when used with a potent antimicrotubule agent (most breast cancer cells are initially sensitive to antimicrotubule drugs) make this mAb the state-of-the-art immunoconjugate for use against solid tumors.[17] The recommended phase II dose for T-DM1, administered every 3 weeks, was determined to be 3.6 mg/kg (on the basis of phase I study results).[19] A proof-of-concept phase II study (4258 g) of single-agent T-DM1 in patients with previously treated HER2-positive metastatic breast cancer (MBC) reported a confirmed objective response rate (ORR) of 27% as assessed by independent review.[33] A second phase II study that enrolled a homogeneous population of patients with HER2-positive MBC (all patients received prior chemotherapy, trastuzumab and lapatinib) was reported at the San Antonio Breast Cancer Symposium in December 2009.[34] Preliminary data showed a 33% ORR, as assessed by an independent review facility, and a tolerable safety profile at the recommended dose, with no limiting cardiac toxicity. The most frequent adverse events were fatigue, nausea, thrombocytopenia, and elevation of transaminase levels. The impressive ORR observed with the use of T-DM1 in patients with heavily pretreated MBC (including previous treatment with trastuzumab and lapatinib) has spurred the development of this ADC. Clinical trials are now evaluating it in first- and second-line settings.

Preliminary results were recently reported for the first randomized, multicenter, open-label phase II study (TDM4450G) of T-DM1 vs. trastuzumab plus docetaxel in patients with HER2-positive MBC (ESMO 2010).[35] Patients with no prior chemotherapy for metastatic disease were randomized 1:1 to receive T-DM1 at a dose of 3.6 mg/kg intravenously every 3 weeks (T-DM1 arm, 67 patients), or trastuzumab at a dose of 6 mg/kg intravenously

**Table 6 Efficacy and safety of trastuzumab-DM1 (T-DM1) versus trastuzumab plus docetaxel in HER2-positive metastatic breast cancer patients, phase II study TDM4450G**

| | Trastuzumab + docetaxel ($N = 70$) | T-DM1 ($N = 67$) |
|---|---|---|
| *Baseline characteristics* | | |
| ECOG PS 0/1, *N* (%) | 44 (64)/25 (36) | 44 (66)/23 (34) |
| Prior trastuzumab, *N* (%) | 18 (26) | 13 (19) |
| *Efficacy (investigator assessed)* | | |
| Overall response rate, *N* (%) | 29 (41) | 32 (48) |
| 95% Confidence interval | 30.2–53.8 | 35.4–60.3 |
| *Safety* | | |
| Any adverse events (AEs), *N* (%) | 68 (100) | 63 (94) |
| Grade ≥3 AEs, *N* (%) | 51 (75) | 25 (37) |
| Severe AEs, *N* (%) | 15 (22) | 13 (19) |
| AE leading to death, *N* (%) | 0 | 1 (2) |
| *Patient disposition* | | |
| Discontinued treatment, *N* (%) | 25 (36) | 22 (33) |
| Progressive disease, *N* (%) | 19 (27) | 16 (24) |

(8 mg/kg in cycle 1) plus docetaxel at 75 or 100 mg/m$^2$ administered intravenously on day 1 every 3 weeks (T+D arm, 70 patients), until disease progression or unacceptable toxicity. Crossover from the control arm to the T-DM1 arm was allowed after disease progression. Progression-free survival was the primary end point; the secondary end points included ORR, clinical benefit rate, and overall survival. Preliminary safety and response rate data after median follow-up of 5.9 months in the T+D arm and 6.1 months in the T-DM1 arm were reported (**Table 6**). The tumor response and the serious adverse event rates are comparable in the T-DM1 and T+D arms, whereas the incidence of grade 3–4 adverse events was much lower in the T-DM1 arm (37.3%) than in the control arm (75.0%). These results suggest that there is a better therapeutic index for the ADC as compared with standard systemic cytotoxic therapy, although confirmation of this requires waiting for definitive data to mature. It would also be important to measure how the reduced incidence of significant adverse events translates into improvement in the quality of life of these patients. A phase III trial, MARIANNE, is assessing T-DM1 for first-line treatment of HER2-positive MBC. In MARIANNE, T-DM1 given as a single agent and T-DM1 given in combination with pertuzumab (a HER2 dimerization inhibitor also in development) are both compared to naked trastuzumab used in combination with a taxane. Lorvotuzumab mertansine (IMGN901, huN901-DM1, BB-10901) is an ADC that targets the neural cell adhesion molecule CD56, which is expressed in many cancers (**Table 2**). IMGN901 consists of the anti-CD56 mAb, huN901, conjugated via a disulfide bond to DM1. Preliminary antitumor activity has been reported in small-cell lung cancer, CD56-positive small-cell carcinoma, and Merkel cell carcinoma.[36]

Phase I evaluation was completed in 2007 for another immunoconjugate with maytansinoid-1. MLN2704 is designed to deliver the drug directly to PSMA-expressing cells and has been administered safely on a repetitive basis to patients with progressive castration-resistant prostate cancer. The pharmacokinetics of the conjugate was dose proportional, without correlation between clearance and body surface area. Grade 3 toxicity included febrile neutropenia (the only dose-limiting toxicity), reversible elevations of transaminase levels, leukopenia, and lymphopenia. Fatigue, nausea, diarrhea, and peripheral neuropathy were also reported, but these were mild to moderate in intensity. Two (22%) of the nine patients treated at 264 or 343 mg/m$^2$ sustained >50% decrease in prostate-specific antigen levels, and there was measurable tumor regression in the patient who was treated at a dose of 264 mg/m$^2$. Although this immunoconjugate may compare favorably with the naked mAb (J591), new clinical trials have not been started.[37] Finally, two new human IgG1s conjugated to DM4 have entered clinical practice: IMGN388 (an anti-integrin mAb) and BIIB015 (an antibody directed against the cell surface–associated protein Cripto) (http://www.cancer.gov/drugdictionary).

***Auristatins antibody conjugates.*** Auristatins exert their cytotoxic effects by binding to tubulin, causing cell cycle arrest at the G2/M phase, and leading to apoptosis. They are synthetic analogs of dolastatin 10, a natural product originally isolated from the Indian Ocean sea hare, *Dolabella auricularia*. CDX-011 is an immunoconjugate of CR011, an IgG2 directed to glycoprotein NMB, linked to monomethyl auristatin E. CDX-011 was selected because of its significant dose-dependent antitumor activity against melanoma tumor xenografts. The phase I studies determined that the maximum tolerated dose of CDX-011 was 1.88 mg/kg administered every 3 weeks and the dose-limiting toxicity was skin rash/desquamation; preliminary antitumor activity was observed in patients with advanced melanoma and breast cancer.[38] The maximum tolerated dose for the treatment of patients with breast cancer was also 1.88 mg/kg every 3 weeks. A phase II study is evaluating CDX-011 in cancer subsets that significantly express glycoprotein NMB. Another monomethyl auristatin E immunoconjugate, directed against PSMA, is being tested in castration-resistant prostate cancer.

MEDI-547 is a human IgG1 anti-EphA2 mAb, with MMAF as the payload, that showed substantial activity against EphA2-expressing tumors in mouse xenograft tumor models.[39] ASG-5ME is the newest auristatin immunoconjugate to be used in clinical practice. It is an ADC composed of a fully human mAb attached to monomethyl auristatin E via an enzyme-cleavable linker. The antibody is directed to SLC44A4, a novel target that is upregulated in a number of epithelial tumors including pancreatic cancer. Two anti-CD70 ADCs are currently in phase I evaluation. SGN-75 is an antibody (h1F6) conjugated to the auristatin derivative MMAF, with activity against human renal cell carcinoma cells grown orthotopically in nude mice.[40] Because mature dendritic cells and activated T and B lymphocytes express CD70, it would be pertinent to monitor the potential effects on immunity.

***Novel ADCs of other cytotoxic agents.*** MDX-1203 is also an anti-CD70 ADC, consisting of a mAb covalently linked to a prodrug form of a DNA minor groove–binding agent (duocarmycin).

### Immunotoxins

Improvements over the past few years, using modern protein engineering and potent toxins from bacteria and plants, have produced several new candidates. The intention to use toxins as payloads is very attractive because of (i) the theoretical lack of cross-resistance with cytotoxic agents and (ii) the fact that the rate of proliferation is not a major determinant of activity (cell cycle phase-nonspecific agents). Some toxins perform better against particular classes of target cells. However, one additional obstacle to the successful treatment of solid tumors for this class is the immune response to the toxin component, which may limit the treatment exposure. Again, the archetypal immunotoxin is an immunoconjugate against leukemia—in this case, a rare type of leukemia known as hairy-cell leukemia. This disease is resistant to purine analogs including cladribine, and is associated with a poor prognosis. Classic or variant hairy cells are virtually always strongly positive for CD22, an adhesion molecule expressed exclusively on B cells. To target CD22-expressing cells, a recombinant immunotoxin, RFB4(dsFv)-PE38 (BL22), was designed that contains the variable domain (Fv) of the anti-CD22 mAb RFB4. The Fv is fused to a truncated *Pseudomonas* exotoxin A, known as PE38, which contains domains responsible for cell death but lacks the domain necessary for cell binding.[41,42] In clinical studies, this immunotoxin was administered by intravenous infusion on alternate days for a total of three doses. Remarkable activity was observed, with serious but completely reversible hemolytic-uremic syndrome developing in a few patients, mainly during subsequent cycles of treatment.[43] Other common toxic effects included transient hypoalbuminemia, elevations of transaminase levels, fatigue, and edema. One immunotoxin, denileukin diftitox, has been approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma, but it is an engineered protein combining interleukin-2 and diphtheria toxin. This approval shows that a peptide cytotoxin is an effective payload in relapsed/refractory disease. In a randomized study in patients who had received a median of two previous therapies (range 0–6), denileukin diftitox showed a statistically significant improvement in ORR and progression-free survival as compared to placebo.

An immunotoxin against mesothelin (SS1P: anti-mesothelin dsFv-PE38) was recently tested in a phase I study. Mesothelin is a promising candidate because of its limited expression in normal tissues and high expression in some solid tumors (**Table 2**). SS1P was administered on alternate days for either three or six doses to 34 patients with mesothelin-expressing solid tumors (mesothelioma, ovarian, and pancreatic cancer). The initial cohort received six doses of SS1P and the maximum tolerated dose was 18 μg/kg/dose. Dose-limiting toxicity included grade 3 urticaria ($N = 1$) and grade 3 vascular leak syndrome ($N = 2$). To enable further SS1P dose escalation, 17 patients were treated on the three-dose schedule. The dose-limiting toxicity, grade 3 pleuritis, was seen in both of the patients treated with 60 μg/kg and in one of nine patients who received 45 μg/kg. At the maximum tolerated dose of 45 μg/kg, the mean $C_{max}$ of SS1P was 483 ng/ml. Indications of clinical activity were noted in some heavily pretreated patients.[44] Phase II evaluation of SS1P for mesothelin-expressing malignancies is under consideration, and current clinical trials are evaluating it in combination with chemotherapy (http://clinicaltrials.gov). The synergy observed in preclinical studies with paclitaxel provides a strong rationale for combination of SS1P with conventional chemotherapy.[16] Preliminary evidence suggests that the synergy would be caused by a paclitaxel-induced fall in the levels of shed antigens.

### Decreasing the toxicity of immunoconjugates

Because current immunoconjugates are still limited by toxicity, including high and persistent localization of circulating immunoconjugates in the liver, modifications to reduce systemic effects are being investigated. For radioimmunoconjugates, one approach is to create linkers that are cleavable by hepatic lysosomal proteases in order to liberate the conjugate and accelerate the clearance from the liver.[3,45,46] For ADCs and immunotoxins, the stability of the immunoconjugate within the plasma, with subsequent selective release of the payload within the cell cytoplasm, is crucial.[17] The most widely used linkers for ADCs include peptide, acid labile, ester, and disulfide, but new uncleavable linkers are beginning to be used (e.g., maleimidocaproyl linker in SGN-75).[29,40] The drug conjugation site can modulate not only the stability but also the biological activity of an ADC. Several promising new ADCs, comprising potent payloads attached to intact mAb or to antibody fragments through optimized linker technology, are showing striking levels of activity in preclinical models, and some are entering clinical trials. Moderate success in improving the tumor-to-liver and tumor-to-kidney radiation dose ratios has also been achieved in preclinical studies.[46,47]

### Antibody-cytokine fusion proteins (immunocytokines) and pretargeted antibody conjugates

Another approach to enhancing the effector functions of antibodies is to engineer bispecific antibodies. These comprise two specificities: one for the cell to be eliminated and one for receptors on effector cells such as cytotoxic T cells.[5] Several interleukin-2 immunocytokines have been tested clinically. Interferons have also been genetically fused to the heavy chain of antibodies. Alternatively, mAb-prodrug conjugates can be targeted to tumors, where they are activated by endogenously expressed enzymes or by external stimuli such as light.[3,29]

## FUTURE DIRECTIONS: CONSTRUCTS WITH mAb FRAGMENTS AND ENRICHED CLINICAL DESIGNS

Antibody fragments, including single-chain Fvs, diabodies, triabodies, and nanobodies (which are one-tenth of the size of a mAb), combine the advantages of both small molecules and mAbs, resulting in lower costs, improved efficacy, flexible formatting, low toxicity, and the potential for alternative delivery routes.[48] New immunoconjugates can be produced by means of this novel genetic engineering. Moreover, penetration within the tumor mass and residence time in the bloodstream or target zone can also be optimized with antibody

fragments. Rapid targeting/rapid clearing fragments are suited for conjugation with peptide cytotoxins and for imaging using positron-emission tomography. Also, nonrecombinant chemical conjugation of peptides onto antibodies is an emerging approach.

As explained above, several developmental challenges have been identified that could potentially limit this therapeutic approach in solid tumors. The biotechnology available today provides the means for tailoring a mAb against a selected tumor type. The biology and the standard therapeutic options for that particular cancer must be taken into account in the design of the immunoconjugate construct. Reciprocally, the selection of patients and the treatment setting should be guided by the technical features of the immunoconjugate and the characteristics of the target antigen. In early phases of development, it would be important to test the optimal stability of the immunoconjugate within the plasma through the pharmacokinetic parameters of the intact ADC, the naked mAb, and the free cytotoxic component. Additionally, from a pharmacokinetic perspective, the possibility of substantial interpatient variability should be considered. Differential clearance, due to tumor load and/or high levels of plasma antigen, could be the cause of this variability, and could subdivide patients into two subsets.[31,49] Dynamic tumor imaging could be used to evaluate the targeting ability of the immunoconjugate as well as to predict and monitor therapeutic outcome (**Figure 2**). The traditional phase I design (modified Fibonacci) has limitations: it sometimes exposes too many patients to subtherapeutic doses, takes a long time to complete, and provides very limited information about interpatient variability and cumulative toxicity. There is no compelling basis for this approach (except that experience has shown it to be safe). New designs address these problems and will be useful in first-in-human evaluations of immunoconjugates. Accelerated titration designs appear to be effective in reducing the number of patients who are undertreated, speeding completion, and providing more information. Continual reassessment methods use a dose-toxicity model to guide the escalation, with a Bayesian statistical approach. However, these designs have not yet been widely implemented, mostly because they are logistically complex, requiring continuous attention from investigators and immediate updates of the patients' toxicity so as to make decisions in real time.

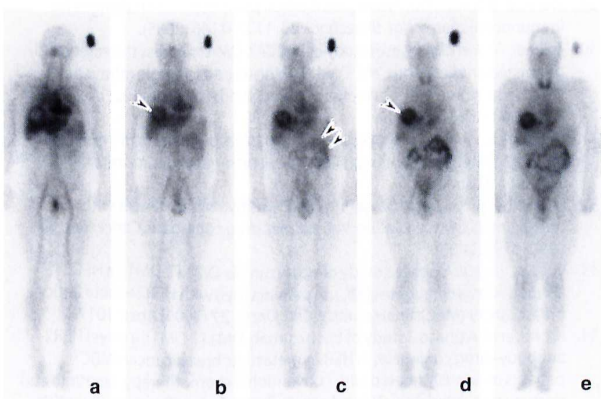


**Figure 2** Anterior whole-body γ-camera images after the infusion of $^{131}$I-huA33 in a patient with advanced colorectal carcinoma (dose level, 30 mCi/m$^2$) (**a**) day 0, (**b**) day 1, (**c**) day 2, and (**d**) day 5 post–scout infusion, and (e) day 6 post-therapy infusion. A standard for quantitation of $^{131}$I-huA33 uptake is present adjacent to the left shoulder. (**a**) Initial (day 0) images show blood pool appearance only, with a metastatic lesion in the liver demonstrating an initial hypovascular appearance. (**b**) Excellent targeting of the metastatic lesion in the liver by an anti-A33 monoclonal antibody (mAb) (arrow), as early as day 1, and (**c,d**) increasing rapidly with time. (**d**) Some central necrosis in the tumor is also evident (arrow). Gradual bowel uptake (double arrow) of $^{131}$I-huA33 is also seen. (**e**) The post-therapy image shows biodistribution and tumor uptake of $^{131}$I-huA33 identical to those seen after the scout dose. Reprinted with permission from Chong, G. *et al.* Phase I trial of $^{131}$I-huA33 in patients with advanced colorectal carcinoma, *Clin. Cancer Res.* 11, 4818–4826 (2005). Copyright ©2005 American Association for Cancer Research.

Proof of concept and noteworthy antitumor activity may be most efficiently demonstrated in nonrandomized studies, limiting enrollment to patients whose tumors significantly express the specific target. Once predefined clinical activity in one particular setting is achieved, randomized evaluations can be performed.[50] To appreciate the full potential of immunoconjugates, different strategies should be implemented in clinical trials to reduce tumor shedding, to optimize biodistribution (previously using naked antibodies or chemotherapy), and to improve dosimetry for radioimmunoconjugates.

Phase II studies are designed to determine whether a new agent shows sufficient activity to warrant further development; patient benefit is usually evaluated in phase III. Given the low rate of success in developing therapeutic compounds, it is vital to efficiently screen out inactive drugs in phase II development. Although the resulting sample size is inadequate to provide a precise estimate of activity, the most important factor influencing phase III success is the quality of the preliminary data. Indeed, phase II is the crucial phase in the development of oncology therapeutics, given the high cost of development, the large number of experimental drugs in the queue, and the need for new therapies and efficient use of patient resources. Some phase II single-arm study designs that could be considered in the development of immunoconjugates are the window-of-opportunity design (for chemosensitive tumors) and adaptive designs.

## CONCLUSION

The discovery of more specific cancer antigens, in addition to major advances in mAb technology, has resulted in the invention of rationally designed immunoconjugates. Acceptable toxicity and preliminary activity in patients, sometimes with excellent targeting of known sites of metastases, warrant further investigation of this therapeutic approach. This might translate into new therapeutic opportunities against solid tumors in the near future, including personalized therapy. Because immunoconjugates appear to predominantly target malignant cells, it is expected that they will produce less toxicity at clinically effective doses as compared with nonspecific anticancer therapies. Therefore, immunoconjugation is a clear attempt to increase the therapeutic index of cytotoxic agents. However, just as "mind the gap"

is a warning to train passengers, the early and recent failures of ADCs should remind us of the still significant gap between proof of concept and regulatory approval in therapies for solid tumors. Different paradigms must be adopted in the clinical development process of these conjugates: enriched clinical designs with the level of target expression as a predictive biomarker (the bottom-up approach, as with naked trastuzumab), imaging to help early defining of the targeting characteristics of the mAb, and adaptive designs in phase II development.

Rapid advances in antibody engineering suggest that clinical testing will expand and will include antibody fragments. Nevertheless, the potential cost of administration of mAbs will undoubtedly require an evaluation of the improvement in the quality of life of cancer patients. This approach will allow optimal therapy in clinical practice.

**ACKNOWLEDGMENTS**

The author is grateful to colleagues who have contributed to the field of immunoconjugates and to the patients who have participated in the clinical trials. Apologies are extended to those whose work has not been cited because of lack of space. The author thanks Katherine Liau for her critical reading of the article and helpful suggestions.

All products discussed in this article, except yttrium-90-labeled (90Y) ibritumomab tiuxetan (Zevalin) and iodine-131-labeled (131I) tositumomab (Bexxar), are not labeled for the uses under discussion and are still investigational.

This paper was presented, in part, at the 110th Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, 18–21 March 2009, National Harbor, MD.

**CONFLICT OF INTEREST**

A.D.R. is employed by Pfizer.



1. Köhler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
2. Blattman, J.N. & Greenberg, P.D. Cancer immunotherapy: a treatment for the masses. *Science* **305**, 200–205 (2004).
3. Lin, M.Z., Teitell, M.A. & Schiller, G.J. The evolution of antibodies into versatile tumor-targeting agents. *Clin. Cancer Res.* **11**, 129–138 (2005).
4. Breedveld, F.C. Therapeutic monoclonal antibodies. *Lancet* **355**, 735–740 (2000).
5. Brekke, O.H. & Sandlie, I. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. *Nat. Rev. Drug Discov.* **2**, 52–62 (2003).
6. Goodman, G.E., Beaumier, P., Hellström, I., Fernyhough, B. & Hellström, K.E. Pilot trial of murine monoclonal antibodies in patients with advanced melanoma. *J. Clin. Oncol.* **3**, 340–352 (1985).
7. Better, M., Chang, C.P., Robinson, R.R. & Horwitz, A.H. Escherichia coli secretion of an active chimeric antibody fragment. *Science* **240**, 1041–1043 (1988).
8. McCafferty, J., Griffiths, A.D., Winter, G. & Chiswell, D.J. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* **348**, 552–554 (1990).
9. Skerra, A. & Plückthun, A. Assembly of a functional immunoglobulin Fv fragment in Escherichia coli. *Science* **240**, 1038–1041 (1988).
10. Batra, S.K., Jain, M., Wittel, U.A., Chauhan, S.C. & Colcher, D. Pharmacokinetics and biodistribution of genetically engineered antibodies. *Curr. Opin. Biotechnol.* **13**, 603–608 (2002).
11. Jain, R.K. Barriers to drug delivery in solid tumors. *Sci. Am.* **271**, 58–65 (1994).
12. Jain, R.K. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. *Cancer Res.* **50**, 814s–819s (1990).
13. Yokota, T., Milenic, D.E., Whitlow, M. & Schlom, J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. *Cancer Res.* **52**, 3402–3408 (1992).
14. Schmidt, M.M. & Wittrup, K.D. A modeling analysis of the effects of molecular size and binding affinity on tumor targeting. *Mol. Cancer Ther.* **8**, 2861–2871 (2009).
15. Kim, K.M. *et al.* Anti-CD30 diabody-drug conjugates with potent antitumor activity. *Mol. Cancer Ther.* **7**, 2486–2497 (2008).
16. Zhang, Y. & Pastan, I. High shed antigen levels within tumors: an additional barrier to immunoconjugate therapy. *Clin. Cancer Res.* **14**, 7981–7986 (2008).
17. Ricart, A.D. & Tolcher, A.W. Technology insight: cytotoxic drug immunoconjugates for cancer therapy. *Nat. Clin. Pract. Oncol.* **4**, 245–255 (2007).
18. Baselga, J. *et al.* Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. *J. Clin. Oncol.* **23**, 2162–2171 (2005).
19. Krop, I.E. *et al.* Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. *J. Clin. Oncol.* **28**, 2698–2704 (2010).
20. Hudis, C.A. Trastuzumab–mechanism of action and use in clinical practice. *N. Engl. J. Med.* **357**, 39–51 (2007).
21. Saleh, M.N. *et al.* Phase I trial of the anti-Lewis Y drug immunoconjugate BR96-doxorubicin in patients with lewis Y-expressing epithelial tumors. *J. Clin. Oncol.* **18**, 2282–2292 (2000).
22. Connors, J.M. Radioimmunotherapy–hot new treatment for lymphoma. *N. Engl. J. Med.* **352**, 496–498 (2005).
23. Scott, A.M. Radioimmunotherapy of prostate cancer: does tumor size matter? *J. Clin. Oncol.* **23**, 4567–4569 (2005).
24. Milowsky, M.I. *et al.* Vascular targeted therapy with anti-prostate-specific membrane antigen monoclonal antibody J591 in advanced solid tumors. *J. Clin. Oncol.* **25**, 540–547 (2007).
25. Bander, N.H., Milowsky, M.I., Nanus, D.M., Kostakoglu, L., Vallabhajosula, S. & Goldsmith, S.J. Phase I trial of 177lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *J. Clin. Oncol.* **23**, 4591–4601 (2005).
26. Milowsky, M.I., Nanus, D.M., Kostakoglu, L., Vallabhajosula, S., Goldsmith, S.J. & Bander, N.H. Phase I trial of yttrium-90-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. *J. Clin. Oncol.* **22**, 2522–2531 (2004).
27. Reardon, D.A. *et al.* Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J. Clin. Oncol.* **24**, 115–122 (2006).
28. Bleumer, I. *et al.* A phase II trial of chimeric monoclonal antibody G250 for advanced renal cell carcinoma patients. *Br. J. Cancer* **90**, 985–990 (2004).
29. Wu, A.M. & Senter, P.D. Arming antibodies: prospects and challenges for immunoconjugates. *Nat. Biotechnol.* **23**, 1137–1146 (2005).
30. Tolcher, A.W. *et al.* A phase I study of huC242-DM4 to assess the safety and pharmacokinetics of huC242-DM4 administered as a single intravenous infusion once every three weeks to subjects with solid tumors. *Eur. J. Cancer* (suppl. 4) (2006) Abstr 212.
31. Qin, A. *et al.* The pharmacokinetics and pharmacodynamics of IMGN242 (huC242-DM4) in patients with CanAg-expressing solid tumors. *J. Clin. Oncol.* **26** (2008) Abstr 3066.
32. Lewis Phillips, G.D. *et al.* Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res.* **68**, 9280–9290 (2008).
33. Vogel, C.L. *et al.* A phase II study of trastuzumab-DM1 (T-DM1), a HER2 antibody-drug conjugate (ADC), in patients (pts) with HER2+ metastatic breast cancer (MBC): final results. *J. Clin. Oncol.* **27** (2009) Abstr 1017.
34. Krop, I. *et al.* A phase 2 study of trastuzumab-DM1 (T-DM1), a novel HER2 antibody–drug conjugate, in HER2+ metastatic breast cancer (MBC) patients previously treated with conventional chemotherapy, lapatinib and trastuzumab. 32nd Annual San Antonio Breast Cancer Symposium (2009) Abstr 710.
35. Perez, E.A. *et al.* Efficacy and safety of trastuzumab-DM1 versus trastuzumab plus docetaxel in HER2-positive metastatic breast cáncer patients with no prior chemotherapy for metastatic disease: preliminary results of a randomized, multicenter, open-label phase 2 study (TDM4450G). *Ann. Oncol.* **21**, vii2 (2010) Abstr LBA3.
36. Woll, P.J. *et al.* Clinical experience of IMGN901 (BB-10901, huN901-DM1) in patients with Merkel Cell Carcinoma (MCC). AACR-NCI-EORTC International Conference; Molecular Targets and Cancer Therapeutics (2009) Abstr 237.
37. Galsky, M.D. *et al.* Phase I trial of the prostate-specific membrane antigen-directed immunoconjugate MLN2704 in patients with progressive metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* **26**, 2147–2154 (2008).
38. Hwu, P. *et al.* A phase I/II study of CR011-vcMMAE, an antibody-drug conjugate (ADC) targeting glycoprotein NMB (GPNMB) in patients (pts) with advanced melanoma. *J. Clin. Oncol.* **27**, (2009) Abstr 9032.
39. Jackson, D. *et al.* A human antibody-drug conjugate targeting EphA2 inhibits tumor growth in vivo. *Cancer Res.* **68**, 9367–9374 (2008).

40. Oflazoglu, E. *et al.* Potent anticarcinoma activity of the humanized anti-CD70 antibody h1F6 conjugated to the tubulin inhibitor auristatin via an uncleavable linker. *Clin. Cancer Res.* **14**, 6171–6180 (2008).
41. Kreitman, R.J. *et al.* Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N. Engl. J. Med.* **345**, 241–247 (2001).
42. Kreitman, R.J. *et al.* Phase II trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with hairy cell leukemia. *J. Clin. Oncol.* **27**, 2983–2990 (2009).
43. Kreitman, R.J. *et al.* Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. *J. Clin. Oncol.* **23**, 6719–6729 (2005).
44. Hassan, R. *et al.* Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. *Clin. Cancer Res.* **13**, 5144–5149 (2007).
45. Akizawa, H. & Arano, Y. Altering pharmacokinetics of radiolabeled antibodies by the interposition of metabolizable linkages. Metabolizable linkers and pharmacokinetics of monoclonal antibodies. *Q. J. Nucl. Med.* **46**, 206–223 (2002).
46. DeNardo, G.L. *et al.* Preclinical evaluation of cathepsin-degradable peptide linkers for radioimmunoconjugates. *Clin. Cancer Res.* **9**, 3865S–3872S (2003).
47. Kukis, D.L., Novak-Hofer, I. & DeNardo, S.J. Cleavable linkers to enhance selectivity of antibody-targeted therapy of cancer. *Cancer Biother. Radiopharm.* **16**, 457–467 (2001).
48. Nelson, A.L. & Reichert, J.M. Development trends for therapeutic antibody fragments. *Nat. Biotechnol.* **27**, 331–337 (2009).
49. Bruno, R., Washington, C.B., Lu, J.F., Lieberman, G., Banken, L. & Klein, P. Population pharmacokinetics of trastuzumab in patients with HER2+ metastatic breast cancer. *Cancer Chemother. Pharmacol.* **56**, 361–369 (2005).
50. Rowinsky, E.K. Curtailing the high rate of late-stage attrition of investigational therapeutics against unprecedented targets in patients with lung and other malignancies. *Clin. Cancer Res.* **10**, 4220s–4226s (2004).