Targeted Therapy of Cancer: New Prospects for Antibodies and Immunoconjugates

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ABSTRACT Immunotherapy of cancer has been explored for over a century, but it is only in the last decade that various antibody-based products have been introduced into the management of patients with diverse cancers. At present, this is one of the most active areas of clinical research, with eight therapeutic products already approved in oncology. Antibodies against tumor-associated markers have been a part of medical practice in immunohistology and in vitro immunoassays for several decades, have even been used as radioconjugates in diagnostic imaging, and are now becoming increasingly recognized as important biological agents for the detection and treatment of cancer. Molecular engineering has improved the prospects for such antibody-based therapeutics, resulting in different constructs and humanized/human antibodies that can be administered frequently. Consequently, a renewed interest in the development of antibodies conjugated with radionuclides, drugs, and toxins has emerged. We review how antibodies and immunoconjugates have influenced cancer detection and therapy, and also describe promising new developments and challenges for broader applications. (CA Cancer J Clin 2006;56:226–243.) © American Cancer Society, Inc., 2006.

INTRODUCTION

The search for a mechanism to target diseases selectively was first realized when resistance to infectious disease could be transferred from one animal to another through their serum, a process known as passive serotherapy.1 Five years later, in 1895, Hericourt and Richet immunized dogs with a human sarcoma and then transferred the serum to patients.2 This anticipated the “magic bullet” concept of Paul Ehrlich in 1908, that “toxins” could be targeted to cancer and other diseases.3 Another half-century passed before antibodies were identified as the substance in serum responsible for these effects.

Despite being potent immune system instigators for killing infectious agents, clinical research initially focused on immunoconjugates prepared with radionuclides, drugs, or toxins, since unconjugated or “naked” antibodies had little therapeutic benefit in oncology compared with the immunoconjugates. Early immunotherapy trials failed to show substantial responses, but antibodies against carcinoembryonic antigen (CEA) could selectively target and disclose sites of CEA-expressing cancers in patients, and also deliver cytotoxic radioactivity in human colonic cancer xenografts having CEA.7,8 Thereafter, DeNardo, et al.9 reported responses in lymphoma patients to radiolabeled antibodies, and soon others confirmed that radiolabeled antibodies had antitumor activity in non-Hodgkin lymphoma (NHL), but there was also early evidence that the naked antibodies themselves might be effective.10–12 It was during this same period that rituximab (Rituxan, Genentech, and biogen idec), an anti-CD20 IgG, became of interest as a therapeutic for NHL without being radiolabeled.13 The experience and subsequent introduction of rituximab into the treatment of NHL can be credited for the expanded interest in unconjugated antibodies for cancer therapy.

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Antibodies (eg, IgG, which is the most commonly used immunoglobulin form, Figure 1) are unique proteins with dual functionality. All naturally occurring antibodies are multivalent, with IgG having two binding ‘arms.’ Antigen-binding specificity is encoded by three complementarity-determining regions (CDRs), while the Fc-region is responsible for binding to serum proteins (eg, complement) or cells. An antibody itself usually is not responsible for killing target cells, but instead marks the cells that other components or effector cells of the body’s immune system should attack, or it can initiate signaling mechanisms in the targeted cell that leads to the cell’s self-destruction (Figure 2). The former two attack mechanisms are referred to as antibody-dependent complement-mediated cytotoxicity (CMC) and antibody-dependent cellular cytotoxicity (ADCC). ADCC involves the recognition of the antibody by immune cells that engage the antibody-marked cells and either through their direct action, or through the recruitment of other cell types, lead to the tagged-cell’s death. CMC is a process where a cascade of different complement proteins become activated, usually when several IgGs are in close proximity to each other, either with one direct outcome being cell lysis, or one indirect outcome being attracting other immune cells to this location for effector cell function.

Antibodies, when bound to key substances found on the cell surface, also can induce cells to undergo programmed cell death, or apoptosis (Figure 2). For example, if rituximab binds to two CD20 molecules, this triggers signals inside the cell that can induce apoptosis. If rituximab is cross-linked by other antiantibodies, the apoptotic signal is intensified. This cross-linking could also occur when the antibody is bound by another immune cell through its Fc-gamma receptors (FcγR). Other antibodies, such as trastuzumab (anti-HER2/new; Herceptin, Genentech) and cetuximab (anti-epidermal growth factor receptor, EGFR; Erbitux, ImClone Systems and Bristol-Myers Squibb) also have the ability to inhibit cell proliferation. Because cells frequently have alternative pathways for critical functions, interrupting a single signaling pathway alone might not be sufficient to ensure cell death. From this perspective, it is not surprising that antibodies are often best used in combination with chemotherapy and radiation therapy to augment their antitumor effects.

Bevacizumab (Avastin, Genentech) is yet another example of how antibodies can be used therapeutically. This antibody binds to vascular endothelial growth factor (VEGF) that is made by tumor cells to promote vessel formation, thereby preventing it from interacting with endothelial cells to form new blood vessels (Figure 2). Antibodies can also be used to modulate immune response. Antibodies to the cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) stimulate T-cell immune responses by blocking the inhibitory effects of CTLA-4, which can enhance tumor rejection. However, release of this innate inhibitory mechanism can also increase the risk of autoimmunity. Two human anti-CTLA-4 antibodies are currently in early clinical trials (MDX-010, Medarex, and CP-675,206, Pfizer), with evidence that they may have activity in melanoma. There are already a number of antibodies used or being studied as therapeutic agents in cancer as well as autoimmune diseases (eg, alemtuzumab, daclizumab, infliximab, rituximab, epratuzumab). Antibodies also can block molecules associated with cell adhesion, thereby inhibiting tumor metastasis. With such diverse mechanisms of action, there are a number of opportunities for building antibody-based therapeutics.

Antibodies naturally have long serum half-lives. For immunotherapy, this property is helpful because the antibody is maintained in the body fluids, where it can continually interact with its target. For other targeting strategies, most notably with radioconjugates, it can be harmful because the highly radiosensitive bone marrow is continually exposed to radiation, resulting in dose-limiting myelosuppression. The large size of an antibody impacts its ability to move through a tumor mass. A high interstitial pressure inhibits the diffusion of larger molecules within the tumor. Migration within the tumor is also inhibited by a binding-site barrier, a process where the antibody as it is leaving the tumor’s blood vessels binds to the
FIGURE 1  Schematic representation of an IgG molecule, its chemically produced fragments, and several recombinant antibody fragments with their nominal molecular weights. At the bottom, a schematic representation of the process involved in engineering murine MAbs to reduce their immunogenicity is provided. A chimeric antibody splices the VL and VH portions of the murine IgG to a human IgG. A humanized antibody splices only the CDR portions from the murine MAb, along with some of the adjacent “framework” regions to help maintain the conformational structure of the CDRs. A fully human IgG can be isolated from specialized transgenic mice bred to produce human IgG after immunizing with tumor antigen or by a specialized phage display method.
first available antigen, concentrating the antibody in the perivascular space. High-affinity antibodies are less likely to migrate into the tumor bed. Administering higher doses of the antibody can reduce the effect of the binding site barrier and allow the antibody to diffuse more deeply into the tumor bed.

For cytotoxic agents that must be internalized to kill the cell (eg, toxins, cytotoxic drugs), the ability to distribute throughout the tumor is important. Radioconjugates are less affected by this because some radioactive particles can traverse as much as 1.0 cm from where they are deposited (bystander or crossfire effect).

**THERAPY WITH UNCONJUGATED ANTIBODIES**

A renewed interest in the effects of unconjugated antibodies in cancer began in the early 1980s, after murine monoclonal antibodies (MAbs) became available. These initial trials were performed in hematological malignancies, as well as in colorectal cancer and melanoma. As with many innovative treatment approaches that are sometimes introduced before the technology has matured sufficiently to extract maximum benefit, only occasional clinical responses were observed. With insufficient efficacy and the immunogenicity of the foreign murine MAb, most of these studies were terminated. Fortunately, some investigators persevered. An excellent lesson on the tribulations of the development of an antibody product between an academic group and industry is that of alemtuzumab (Campath, Berlex, and Genzyme). Alemtuzumab (anti-CD52) had one of the earliest and protracted developments of an antibody ultimately commercialized. It took over 20 years from the development of the first rat immunoglobulin against CD52, changing the immunoglobulin type, and finally developing a humanized, recombinant form, and involved several commercial firms during this time. Chemotherapy-refractory chronic lymphocytic leukemia was the indication finally approved in 2001.

Due in part to the contributions made by the groups led by Morrison (Columbia and Stan-
ford Universities) and Winter (Cambridge), MAbs now are engineered to remove a significant portion of the murine component of the IgG, substituting human IgG components before entering clinical studies.34–36 Chimeric antibodies essentially splice Vk and Kv regions on the murine antibody to the human IgG, making a molecule that is 75% human and 25% murine IgG, whereas a humanized antibody grafts the CDR regions from a murine MAb, along with some of the surrounding “framework” regions to maintain CDR conformation, onto a human IgG, essentially making a molecule with 5% of its sequence from the parental MAb (Figure 1).37 More recent advances have made available, either by genetic or phage-display methods, the development of fully human MAbs that have now entered clinical trials.38 Such engineered MAbs are postulated to greatly reduce the immunogenicity of antibodies, allowing multiple injections to be given, and the human Fc enhances the interaction with other immune system elements.

Rituximab is perhaps the most prominent example of a highly successful paradigm of antibody therapy. As a chimeric antibody, not only did it have reduced immunogenicity, but its effector function (associated with the Fc-region) was improved. For example, when testing ADCC activity against follicular lymphoma isolated from 43 patients, Weng, et al. reported that only rituximab, not its parent murine anti-CD20 IgG (2B8), had activity in vitro.39 Rituximab was initially approved as a single agent therapy for relapsed or refractory low-grade, follicular B-cell NHL, having an overall response rate of 48% (10% were complete responses, CR) with a median duration of 11.8 months.40,41 Since CD20 is not expressed on precursors B-cells, rituximab induces a depletion of only mature B-cells. Rituximab’s major side effects, which are thought to be associated with the activation of complement pathways, occur during or shortly after its infusion. Other less common side effects include symptoms associated with tumor lysis syndrome, severe mucocutaneous reactions, renal toxicity, cardiac arrhythmias, hypersensitivity reactions, and reactivation of hepatitis B (primarily when used in combination with chemotherapy).42

Rituximab’s activity is unique among cancer treatments because 40% of the patients retreated with rituximab could again respond with a similar duration.43 Extending the duration of rituximab therapy can improve the response rate, particularly the number of complete responses, and its duration. However, whether given as a maintenance regimen or retreatment at the first sign of progression, the time to chemotherapy was the same.44 With both approaches having equal benefit, retreatment is generally favored because of the higher expense of a maintenance regimen. Despite the success of rituximab as a monotherapy, there are still a number of patients who do not respond to the initial treatment, and over time, many of those who do will relapse. In an attempt to improve outcome, rituximab has been combined with chemotherapy regimens, including CHOP, CVP, and MCP, as front-line treatments, with very promising results in not only follicular B-cell lymphomas, but also in diffuse large B-cell lymphomas.45,46 Indeed, trials examining front-line combinations of rituximab and chemotherapy have already demonstrated improvements in response rates, time to progression, and event-free survival, and while the overall response rates are promising based on current 2- to 3-year follow-up data, more time will be required to fully appreciate its impact.47 Even in chronic lymphocytic leukemia (CLL), where initial testing of rituximab was disappointing, dose intensification and combinations with chemotherapy have provided significant improvements in response.48,49 Early clinical studies combining rituximab with a humanized anti-CD22, epratuzumab (Immunomedics, Inc.) suggested the potential for additional benefit, particularly in patients with diffuse large B-cell lymphomas.50,51 Studies have also assessed the possible role of an anti-CD80 MAb (galiximab, biogen idec) as a monotherapy in NHL,52,53 and clinical trials are in progress testing its combination with rituximab.

Considerable attention has been devoted to understanding the mechanism of action of rituximab, particularly why some B-cell lympho-
mas are affected and why not all patients with follicular lymphomas respond. As mentioned earlier, rituximab has been shown to have CMC, ADCC, and apoptotic activity, with the former two mechanisms believed to have the greatest impact, although there are conflicting views of which of these two pathways contributes the most to the response.14,60–66 Studies in transgenic and other mouse models have supported the importance of the Fc-receptor-mediated mechanism of action for rituximab.67,68 These efforts have contributed in part to a better understanding of the role of various Fc receptors found on a variety of immune effector cells (eg, B-cells, neutrophils, natural killer cells, and monocytes) on (in the case of rituximab) the clearance of B-cells, as well as the plasma half-life of antibodies.69 Not only do the various Fc-receptors influence binding, but the absence of certain carbohydrates on the Fc portion of the IgG can affect both ADCC and CMC activities.70,71 Cartron, et al. found that the expression of the homoygous Fc-gamma RIiA receptor (CD16) 158V genotype correlated with a higher response rate to rituximab, but it did not have an impact on the progression-free survival.72 Weng, et al. found a similar correlation and also noted that the homoygous expression of the Fc-gamma RIiA histidine/histidine genotype correlated independently with a higher response rate, particularly when assessing the response status 6 months from treatment.73 By unraveling the molecular basis for antibody cytotoxicity, not only can more effective antibodies be designed, but it could lead to a more rational approach for combinations to enhance activity, such as the finding that G-CSF up-regulates CD64 (Fc-gamma receptor I), which can enhance the binding of neutrophils and monocytes to B-cells coated with rituximab.74 IL-12 has a similar stimulatory effect in mouse models and more recently has been applied clinically with promising results.74,75 These discoveries are also having an impact on the development of antibodies for treating other cancers.76–80

The approved antibodies listed in Table 1 indicate that immunotherapy is not restricted to hematological malignancies, but includes diverse target antigens and receptors having different biological functions. Trastuzumab is an anti-HER2/neu antibody that has had a major impact on the therapy of breast cancer and is used alone and in combination with drugs.81–83 HER2/neu is overexpressed on a proportion of breast and other cancers, and trastuzumab binds with an extracellular epitope of this target molecule. About 15% of women whose tumors overexpress HER2/neu respond to trastuzumab, but its efficacy is clearly best when used in combination with chemotherapy, where a 25% increase in the median survival (to 29 months) has been reported.81 Further, the addition of this antibody to adjuvant chemotherapy for breast cancer has improved survival markedly.83 Since only a portion of breast cancer patients overexpress HER2/neu and respond to trastuzumab, selection of suitable patients is important. New data are emerging that suggest trastuzumab treatment after adjuvant chemotherapy can have a significant benefit compared with observation, particularly in reducing the rate of distant recurrence.82

As a member of a family of receptor tyrosine kinases, the binding of HER2 by trastuzumab can interrupt intracellular signaling and affect tumor cell growth. Izumi, et al. showed that trastuzumab also has antiangiogenic properties.84 While this may be an important underlying mechanism of action, other evidence suggests that trastuzumab’s activity is principally governed by ADCC.85 However, trastuzumab combined with chemotherapy improves response rates, despite the immunosuppressive activity of the chemotherapy, and trastuzumab’s activity is enhanced when combined with other, nonantibody, Erb inhibitors, such as gefitinib and erlotinib, all of which suggest that its ability to interfere with signaling is important.86 Since HER2 is a member of a family of growth factors known as the neuregulins/hergulin and is expressed in multiple neuronal and non-neuronal tissues in embryos and adult animals, including the heart, it is not surprising that cardiomyopathy has been associated with trastuzumab, particularly when combined with paclitaxel and anthracyclines.87–90

EGFR is also overexpressed in many solid cancers, and when bound by its ligand, cell growth is stimulated. However, when engaged by an EGFR-specific antibody, receptor phos-
phorylation is decreased and cell growth is inhibited. The chimeric antibody against EGFR, cetuximab, also has an effect on neovascularization.\cite{91,92} Cetuximab works best in combination with chemotherapy in colorectal cancer, for which it was initially approved, and with external irradiation in head and neck cancers, which was recently FDA-approved.\cite{17,93} Beside the usual risks associated with antibody infusions, cetuximab causes an acneform rash and other skin reactions in most patients, with 10% of these being severe. There is evidence suggesting that the intensity of the skin rash is associated with its antitumor response and even survival.\cite{94} Other EGFR antibodies, particularly humanized and fully human forms, also are in development, as indicated in Table 2, and may in fact be better tolerated and show evidence of activity without being combined with cytotoxic chemotherapy, which is currently being evaluated in Phase III trials. It is too early to speculate whether they will, in fact, provide any therapeutic advantages over cetuximab.

Bevacizumab targets and blocks vascular endothelial growth factor (VEGF) and VEGF’s binding to its receptor on the vascular endothelium. Since VEGF is released by many cancers to stimulate proliferation of new blood vessels, the combination of bevacizumab and chemotherapy was found to increase objective responses, median time to progression, and survival in patients with metastatic colorectal cancer, compared with chemotherapy alone, but earlier preclinical studies indicated that anti-VEGF antibodies were active alone, as well as in combination with radiation.\cite{22,95,96} It is currently being studied clinically in renal cell, breast, and lung cancers, as well as in a number of other nonhematological and hematological malignancies.\cite{97–99} As might be expected, bevacizumab may cause gastrointestinal perforations and delayed wound healing, as well as hemorrhagic events (primarily seen in small cell lung cancer trials, where bevacizumab is not approved). Arterial thromboembolic events (eg, cerebral infarction, transient ischemic attacks, myocardial infarction, angina) and proteinuria also have been reported.\cite{100}


table

| Generic Name       | Trade name | Agent/Target                  | Cancer Indication       | Approval |
|--------------------|------------|-------------------------------|-------------------------|----------|
| Unconjugated       |            |                               |                         |          |
| Rituximab          | Rituxan    | Chimeric anti-CD20 IgG<sub>1</sub> | B-cell lymphoma         | 1997     |
| Trastuzumab        | Herceptin  | Humanized anti-HER2 IgG<sub>4</sub> | Breast                  | 1998     |
| Alemtuzumab        | Campath-1H | Humanized anti-CD52           | CLL†                    | 2001     |
| Cetuximab          | Erbitux    | Chimeric anti-EGFR            | Colorectal              | 2004     |
|                    |            |                               | Head/neck               | 2006     |
| Bevacizumab        | Avastin    | Chimeric anti-VEGF            | Colorectal              | 2004     |
| Radioconjugates    |            |                               |                         |          |
| Satumomab pendetide| OncoScint* | <sup>111</sup>In-murine anti-TAG-72 IgG<sub>1</sub> | Colorectal, ovarian     | 1992     |
| Nofetumomab merpentan| Verluma* | <sup>90</sup>Y-c-murine anti-EGP-1 Fab' | SCLC†                   | 1996     |
| Acrutumomab        | CEA-Scan*  | <sup>90</sup>Y-c-murine anti-CEA Fab' | Colorectal              | 1996     |
| Capromab pendetide | ProstaScint| <sup>111</sup>In-murine anti-PSMA | Prostate                | 1996     |
| Ibritumomab tixetan| Zevalin    | <sup>90</sup>Y-murine anti-CD20 IgG<sub>3</sub> + rituximab | B-cell lymphoma         | 2002     |
| Tositumomab        | Bexar      | <sup>131</sup>I-murine anti-CD20 IgG<sub>2</sub> + unlabeled tositumomab | B-cell lymphoma         | 2003     |
| Drug conjugates     |            |                               |                         |          |
| Gemtuzumab ozogamicin | Mylotarg | Humanized anti-CD33 IgG<sub>2</sub> conjugated to colicheamicin | AML§        | 2000     |

*No longer commercially available.
†CLL = chronic lymphocytic leukemia.
‡SCLC = small cell lung cancer.
§AML = acute myelogenous leukemia.

**IMMUNOCONJUGATES**

Antibodies also function as carriers of cytotoxic substances, such as radioisotopes, drugs, and toxins (Figure 3). In NHL, anti-CD20 radioconjugates have superior antitumor activity compared with their unconjugated antibody counterparts, but there is increased, albeit manageable, hematological toxicity.\cite{101,102} These findings are
FIGURE 3 Immunoconjugates are formed primarily by chemical reactions. Radioconjugates can be formed by coupling radiiodine to tyrosine residues, or by binding chelates to lysine residues, which are then used to bind a variety of radio-metals, such as $^{90}$Y. Cysteine residues are also useful for coupling radionuclides, particularly $^{99m}$Tc and rhenium, but cysteine is also used for conjugation of drugs and toxins, which can also be coupled to lysine residues. In addition, the carbohydrates found on IgG can be modified to allow the coupling of chelates or drugs. Drugs have also been coupled to an intermediate carrier that allows for a higher number of drugs to be bound to the antibody. Toxin conjugates usually need to be modified to remove their innate cell binding properties, with the biologically active portion then coupled to the antibody or used as a portion of a recombinant fusion protein.

TABLE 2 Selected Unconjugated Antibody Therapeutics in Advanced Clinical Testing

| Generic Name | Agent/Target | Cancer |
|--------------|--------------|--------|
| Apolizumab   | Human anti-HLA-DR | CLL*, SLL** |
| Chimeric 14.18 | Chimeric anti-ganglioside (GD2) | Neuroblastoma |
| Epratuzumab  | Humanized anti-CD22 | NHL§ |
| Galiliximab  | Humanized anti-CD80 | NHL§ |
| HuMax-CD4    | Fully human anti-CD4 | CTCL‡ |
| Lumiliximab  | Humanized anti-CD23 (Fc-epsilon RII) | CLL* |
| MDX-010      | Anti-CTLA-4 | Melanoma |
| Matuzumab    | Humanized anti-EGFR | CRC‡ |
| Orgegovomab  | Murine anti-CA-125 | Ovarian |
| Panitumumab  | Human anti-EGFR | NSCLC¶, CRC‡, renal |
| Pertuzumab   | Humanized anti-HER-2 | Breast, prostate, ovarian |
| Rencarex    | Chimeric anti-G250 | Kidney |
| Vitaxin      | Humanized anti-αvβ3 integrin | Melanoma, prostate |

*CLL = chronic lymphocytic leukemia.
†CRC = colorectal cancer.
‡CTCL = cutaneous T-cell lymphoma.
§NHL = non-Hodgkin lymphoma.
¶NSCLC = non-small cell lung cancer.
**SLL = small lymphocytic lymphomas.
strong incentives to continue the pursuit of immunoconjugates for cancer therapy.

**Radionuclides**

Radionuclides were the first group of immunoconjugates to be examined. Table 3 lists some of the more commonly used radionuclides conjugated to antibodies for cancer treatment. Because the radioactivity can be detected easily by external scintigraphy, it is also noteworthy to mention the additional application of radionuclide labeling for imaging. The demonstration of cancer targeting with a radionuclide conjugated to CEA resulted in the development of radionuclide antibodies for cancer imaging. Since then, 99mTc- and 111In-radioconjugates have been commonly used for this application, but with the advent of positron-emission tomography (PET), investigators are now beginning to take advantage of this technologically superior imaging system by radiolabeling tumor-associated antibodies with positron-emitters.

Whereas external beam radiation delivers a focused beam of high dose rate radiation for short bursts that are divided over several weeks and is designed to treat local disease, radioimmunotherapy (RAIT) is typically given as an intravenous injection, thereby allowing radioactivity to be delivered to tumors throughout the body. Tumor uptake of a radionuclide IgG occurs gradually, taking 1 to 2 days before peak uptake occurs. Peak uptake is typically <0.01% of the total injected dose per gram tumor, but the radioactivity deposited in the tumor can be detected several weeks later. Because of its kinetics, the radiation-absorbed dose delivered by RAIT occurs at a much lower dose rate than external beam irradiation, but is continually present for a period of time defined by the physical half-life of the radionuclide and the biological half-life of the antibody residing in the tumor. This continuous, low dose rate radiation exposure can be as effective as intermittent, high dose rate radiation.

When it comes to choices of radionuclides for therapy, tumor size is the primary consideration. Medium-energy beta-emitters, such as 131I (0.5 MeV) and 177Lu (0.8 MeV), can traverse 1.0 mm, while high-energy beta-emitters, such as 90Y or 186Re (2.1 MeV), can penetrate up to 11 mm, making it possible for beta-emitters to kill across several hundred cells, referred to earlier as a bystander or crossfire effect. This is considered a significant attribute for radioconjugates compared with other immunoconjugates, since they can be therapeutically active even if heterogeneous antigen expression, tumor architecture, or other factors impede targeting of every cell. Although higher energy beta-emitters have the potential of killing cells across a longer path-length, the absorbed fraction is higher for the lower energy beta-emitters (ie, probability of hitting the nuclear DNA), making them efficient killers. Alpha-emitters, such as 213Bi and 211At, traverse only a few cell diameters, but an alpha particle is also a far more efficient (energetic) killer than even a low-energy beta particle, requiring fewer “hits” to damage cellular processes. Low-energy electrons, such as are produced by Auger emitters (125I, 67Ga, or 111In, for example) have to be in close contact, preferably inside a cell or in the nucleus, to exert a cytotoxic effect. As one might expect, beta-emitters are most likely best applied in situations where the tumors are ≥0.5 cm in diameter; otherwise a substantial portion of the energy from the radioactive decay will be absorbed in the surrounding normal tissue. The alpha and low-energy electron emitters are best applied when the disease burden is smaller, more localized, or where there may be single or small clusters of cells (eg, leukemia, malignant ascites).

The primary concern for using radionuclide-labeled IgG is that it remains in the blood for an extended period of time, which continually exposes the highly sensitive red marrow to radiation, resulting in dose-limiting myelosuppression. Smaller forms of the antibodies, such as a F(ab′)2 or Fab’, and more recently, molecularly engineered antibody subfragments (Figure 1) with more favorable pharmacokinetic properties, are removed more rapidly from the blood, thereby improving tumor/blood ratios. There have been reports of improved therapeutic responses using smaller-sized antibodies, but these smaller entities frequently are cleared from the blood by renal filtration, and as a result, many radionuclides (eg, radiometals) become trapped in a higher concentration in the kidneys than in the
As a consequence of their more rapid blood clearance, the fraction of the injected activity delivered to the tumor is lower with an antibody fragment than with an IgG.

Multistep pretargeting methods, such as those using bispecific antibodies, represent a promising method for imaging and therapy (Figure 4). In this strategy, the bispecific antibody has one arm that binds to the tumor antigen while the second binds to a hapten that is typically incorporated in a small peptide that can be radiolabeled. The unlabeled bispecific antibody is first given time to circulate and bind to the tumor, and once it has cleared from the blood, the radiolabeled peptide is given. The small sized radiolabeled peptide escapes from the vasculature very rapidly, where it can bind to the other arm of the bispecific antibody on the tumor. Within minutes, the rest of the peptide clears from the blood, leaving behind only the peptide that localizes to the bispecific antibody bound to the tumor. This method has been shown in preclinical testing to improve tumor/blood ratios by as much as 40-fold, with tumor uptake increased by as much as 10-fold compared with a directly-radiolabeled antibody fragment. This same method can increase the total radiation dose to tumors by 1.5-fold and increase the dose rate by 3-fold, resulting in improved antitumor responses. Advances in molecular engineering have greatly enhanced the ability to provide uniform and highly novel pretargeting agents. Other pretargeting approaches have been studied, each showing improved tumor/blood ratios, as well as improving therapy when compared with directly-radiolabeled antibodies. Dosimetry data from a pilot clinical study with 90Y-biotin pretargeted by a new recombinant streptavidin-anti-TAG-72 antibody are promising, and in other indications, such as medullary thyroid cancer and glioma, encouraging therapeutic results using pretargeting methods have been reported.

Two anti-CD20 IgG-radioconjugates are currently FDA-approved for the treatment of indolent and transformed forms of NHL, 90Y-ibritumomab tiuxetan (Zevalin, biogen idec) and 131I-tositumomab (Glaxo SmithKline). Both of these treatments improve the objective response rate compared with the unlabeled anti-CD20 antibody used to deliver the radionuclide. Initially, there was some concern that while objective response rates were significantly improved, the pivotal trial performed with 90Y-ibritumomab tiuxetan did not show a statistical improvement in the duration of the response compared with its unlabeled antibody (ie, rituximab). However, continued follow up has shown the complete responses have been more durable. Durable responses have also been reported with 131I-tositumomab, and importantly, there is evidence that when used as a front-line therapy, it is better tolerated and may improve responses compared with standard chemotherapy. Clinical studies are also beginning to evaluate the use of 90Y-ibritumomab tiuxetan as a front-line treatment and are showing these treatments do not preclude patients from receiving additional cytotoxic therapies. Although more randomized clinical trials (RCT) and long-term follow up to assess the risk for late

| Radionuclide | Emission | Half-life | Range | Approximate # Cell Diameters* |
|--------------|----------|----------|-------|-------------------------------|
| 131Iodine    | β        | 8.0 d    | 0.08–2.3 mm | 10 to 230 |
| 90Yttrium    | β        | 64.1 h   | 4.0–11.3 mm | 400 to 1100 |
| 177Lutetium  | β        | 6.7 d    | 0.04–1.8 mm | 4 to 180 |
| 186Rhenium   | β        | 17.0 h   | 1.9–10.4 mm | 200 to 1000 |
| 67Copper     | β        | 61.9 d   | 0.05–2.1 mm | 5 to 210 |
| 211Astatine  | α        | 7.2 h    | 60 µm   | 6 |
| 212Bismuth   | α        | 46 min   | 84 µm   | 8 |
| 125Iodine    | Auger    | 60.5 d   | <100 nm | (1) |
| 111Indium    | Auger    | 3.0 d    | <100 nm | (1) |

*Assuming a tumor cell is 10 µm in diameter.
toxicities (eg, myelodysplasia) are needed, it is impressive that a single treatment with a radiolabeled antibody with fewer side effects than the chemotherapy that is given over several months can provide such a significant benefit.133 New efforts are underway to explore the use of these agents in other clinical indications, and new radioconjugates are being examined in lymphoma and leukemia.112,134–138

The application of RAIT to other tumors is considerably more challenging. The higher radioresistance of solid tumors most certainly is the primary reason why RAIT has not been as successful for these tumors, since the targeting of a variety of solid tumors is as good, if not better, than that seen in lymphoma. Despite efforts to increase the administered radiation dose by using bone marrow or peripheral stem cell support, and even by combining high-dose radioimmunotherapy with chemotherapy, clinically significant antitumor responses in solid tumors remain elusive.108 A Phase III trial in lung cancer has indicated some success in advanced disease, but for the most part, as first emphasized in animal model testing, RAIT is more likely to succeed when the disease burden is minimal or when used as an adjuvant treatment.139–141 Early clinical studies appear to corroborate these preclinical findings, at least in colorectal cancer, where RAIT post salvage resection of colorectal liver metastases indicated a doubling of the survival time compared with historical or contemporaneous controls.142 Additionally, clinical studies are applying radiolabeled antibodies for intracompartmental treatments, such as intracranial and intraperitoneal therapies, where it may be possible to increase the accessibility and amount of antibody targeted to tumors in these regional areas.143–145 Preclinical studies have shown that nontherapeutic doses of chemotherapy can enhance the effects of RAIT, while other studies have shown that relatively small doses of radiolabeled antibodies can enhance the therapeutic activity of a standard chemotherapy regimen.146–151 The reduced hematological toxicity associated with pretargeting approaches should allow radioconjugates to be combined more readily with cytotoxic drugs.152,153 In addition, combinations with unconjugated antibodies, such as cetuximab that can enhance the tumor’s radiosensitivity, may be another option for treating EGFR-positive tumors.154 Thus, while challenges remain for antibody-targeted radionuclides in solid tumors, preclinical and initial clinical studies are encouraging.

**Drug Immunoconjugates**

In the late 1950s, Mathé, et al. linked methotrexate to the globulin fraction of a hamster antiserum directed against the mouse
leukemia L1210 cell line to protect mice from subsequent inoculation with L1210 cells, providing the first evidence that antibodies could be used to target drugs. As with radioconjugates, clinical success was first achieved in a hematological malignancy, with the FDA approving in 2000, gemtuzumab ozogamicin (Mylotarg; Wyeth Ayerst) for the treatment of relapsed acute myelocytic leukemia in adults (≥60 years of age). Gemtuzumab ozogamicin is a conjugate of a humanized anti-CD33 IgG linked to colicheamicin, a potent antitumor agent isolated from a bacterium. The prospects of using it as a front-line treatment and expanding its indications to include pediatric cancer patients, and in combination with chemotherapy, are under evaluation. Aside from the standard precautions for side effects associated with its infusion, other primary side effects include complications associated with severe hematological toxicity, mucositis, as well as hepatotoxicity (hyperbilirubinemia, elevated ALT, AST, and bilirubin).

Conjugation of a drug to an antibody alters the drug’s pharmacodynamics, essentially “detoxifying” it, and this has allowed drugs that otherwise would be too toxic for human use alone (ie, ultratoxic drugs) to be tested as antibody-drug conjugates. Current clinical trials with drug conjugates almost exclusively use drugs that are far more potent than most chemotherapeutic agents, and other highly potent agents also are under development. The union of a biologic (antibody) and a drug (a chemical) must be made chemically, with the conjugate retaining the binding activity of the antibody, as well as the biological activity of the drug (Figure 4). Drugs may be coupled directly to an antibody or to inert carriers, such as dextrans or amino acid polymers, which have been used to increase the drug-substitution level of the conjugate. Responses are dose-dependent, and therefore, optimizing the drug-antibody substitution level will improve the chances for success. However, a careful balance between maximizing the drug payload and maintaining favorable pharmacokinetic and biodistribution properties must be achieved.

Leukemias are a particularly attractive target for immunoconjugate therapy since the individual cells are readily available in the bloodstream and marrow. Drugs must get inside the cell to be active, and therefore, a target that is actively internalized would be more important than the target’s relative abundance. For example, MAbs against CD74, which is found in low density on B-cells, monocytes, lymphomas, myelomas, and certain carcinomas, have been reported to be highly efficient carriers for drugs, toxins, and radionuclides because CD74 is readily recycled. However, gemtuzumab ozogamicin is active even in CD33-negative cell lines because these cells are highly endocytic, and therefore, the conjugate can be internalized without specifically binding to the cell. When internalized, the drug must be liberated from the antibody to regain its activity. Separation of the drug from the antibody generally occurs in the lysosomes. Ineffective trafficking and drug separation inside the cell can have a profound impact on the potency of the conjugate. Often, drugs are coupled to antibody using linkages that can only be cleaved in the acidic milieu of the lysosomes. There were hopes that antibody-drug conjugates might overcome drug resistance by bypassing the P-glycoprotein mechanism for extruding drugs. Unfortunately, this has not been realized, but one study has suggested that this might be possible under certain circumstances.

Pretargeting approaches also have been applied to drugs. Most often referred to as ADEPT (antibody-directed enzyme prodrug therapy), this strategy first targets an antibody-enzyme conjugate to the tumor. Once the conjugate is sufficiently cleared from the blood, a prodrug, which is not biologically active, is given. The prodrug is converted to an active form and released from the enzyme-conjugate. Enzymatic conversion of the prodrug continues, resulting in locally increased levels of the active drug. The ADEPT method has been tested extensively in preclinical models, as well as in early Phase I clinical studies, which initially identified the immunogenicity and clearance of the antibody-
enzyme conjugate as obstacles, but preclinical studies suggest that these problems may be overcome in the near future.178–180

While there are still a number of challenges to be met, new agents are being developed that will likely lead to expanded clinical evaluation of drug immunoconjugates.

**Toxin Immunoconjugates**

Except for denileukin diftitox (Ligand Pharmaceuticals), which is a modified diphtheria toxin coupled to interleukin-2 for the treatment of cutaneous T-cell lymphoma, no other immunotoxins have been approved by the FDA; however, there have been a number of clinical trials with a variety of toxins conjugated to antibodies.181–183

Toxins are truly ultratoxic agents, requiring relatively few copies to kill the cell, but they face the same delivery issues as a drug conjugate. Immunotoxins have been produced primarily from toxins that are ribosomal inactivating proteins, interfering with the reading of mRNA and thereby disrupting protein synthesis.182 Most are natural proteins derived from plants, bacteria, or fungi, but RNases isolated from vertebrates are also being examined.184 Since toxins have their own means for binding to cells, the cell-binding portion must be separated from the active portion of the toxin to improve targeting specificity (Figure 4).185 As proteins, toxins are amenable to recombinant production as antibody- (or other biological targeting substance, such as interleukin-2) toxin fusion proteins.182,183 However, toxins are foreign proteins, and therefore the formation of neutralizing antibodies is a concern for repeated use. The possible exception is RNase, which may be less immunogenic.186

Therapy of B-cell lymphoma using ricin A-chain conjugates prepared chemically with deglycosylated ricin A-chain and either an anti-CD19 or an anti-CD22 murine IgG was limited by the development of vascular leak syndrome (consisting of edema, tachycardia, dyspnea, weakness, and myalgia).187–189 Recent insights into the molecular structure of the active ricin A-chain have revealed a motif that is responsible for binding to endothelial cells, which could be an important determinant in the development of dose-limiting vascular leak syndrome.190

A recombinant anti-CD22 x *Pseudomonas* exotoxin has been highly effective in patients with hairy cell leukemia, while not being as active in NHL CLL.191 In hairy cell leukemia, clinical benefit (86% CR rate with a median duration of 36 months) was observed after a single cycle of conjugate treatment at a dose level of 40 µg/kg every other day x 3, with the most common toxicities being hypoalbuminemia, transaminase elevations, fatigue, and edema; a reversible hemolytic uremic syndrome requiring plasmapheresis also was observed in several patients. This conjugate’s activity in hairy cell leukemia and with manageable toxicity is an exciting new development for immunotoxin conjugates.

Similar to the experience with other immunoconjugates, solid tumors remain a formidable challenge for therapy with immunotoxins. An immunotoxin prepared as a recombinant *Pseudomonas* exotoxin x anti-Lewis-Y antibody (BR96) was tested in 46 patients with Lewis-Y-positive tumors, with no objective responses reported.192 The dose of this conjugate was limited by gastrointestinal toxicity, likely because BR96 is cross-reactive with normal gastrointestinal epithelium.193

**ECONOMIC CONSIDERATIONS**

One lesson learned from this review is that the new biological agents, particularly the unconjugated MAb, are more effective when used in combination with other therapeutic agents, including perhaps other antibodies. Since not all patients are responsive, presumably because of differences in the receptors being targeted, molecular testing will become part of the paradigm of biological therapy to choose drugs on an individual patient basis.

But these considerations can have staggering financial implications. If the average monthly price is $4,800 for bevacizumab and $12,000 for cetuximab, combinations of these together with drugs in colorectal cancer treatment can...
range between $11,000 and $27,000 monthly, along with pharmacy and dispensing costs. Since these can be prescribed over several months, the costs can challenge the healthcare system and third-party payers, as cautioned recently by Wittes.194

CONCLUSIONS

Antibodies and immunoconjugates are gaining a significant and expanding role in the therapy of cancer. Because patients generally tolerate antibody treatments with minimal side effects, compared with many other cancer treatment modalities, immunoconjugation with antibodies represents an exciting opportunity for combining with standard modalities, such as chemotherapy, as well as combinations between diverse biological agents, including antibody combinations in NHL therapy and possibly cetuximab + bevacizumab (with chemotherapy) in metastatic colorectal cancer.195 As we learn more about how cancer and other diseased cells control their proliferation and spread, undoubtedly unconjugated antibodies will be used to disrupt these functions by targeting important sites or regulators of cell proliferation, metabolism, adhesion, migration, spread, and other properties of malignancy. The use of antibodies to target radionuclides, drugs, and toxins is expanding as the next generation of MAb-based products for cancer therapy. At least in the case of targeted radionuclides, clinical studies have shown that these immunoconjugates are more effective than immunoconjugation with the antibody alone, which highlights the enhanced efficacy achieved when a cytotoxic agent is targeted by an antibody that is also active.

This review has summarized the strides made over the past 25 years for developing new, selective, therapeutic strategies based on the evolution of various antibody forms and an identification of new cellular targets. Molecular biology has been at the basis of developing this new generation of antigen-binding molecules. As new target molecules and receptors on tumor cells are identified in the future, the experiences gained with the use of current immunoconjugates will enable a more rapid translation to clinical evaluation and use when next-generation antibodies and immunoconjugates are developed.

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