Perspective

Cancer Research

Whither Radioimmunotherapy: To Be or Not To Be?

Damian J. Green[1,2] and Oliver W. Press[1,2,3]

Abstract

Therapy of cancer with radiolabeled monoclonal antibodies has produced impressive results in preclinical experiments and in clinical trials conducted in radiosensitive malignancies, particularly B-cell lymphomas. Two "first-generation," directly radiolabeled anti-CD20 antibodies, ^{131}iodine-tositumomab and ^{90}yttrium-ibritumomab tiuxetan, were FDA-approved more than a decade ago but have been little utilized because of a variety of medical, financial, and logistic obstacles. Newer technologies employing multistep "pretargeting" methods, particularly those utilizing bispecific antibodies, have greatly enhanced the therapeutic efficacy of radioimmunotherapy and diminished its toxicities. The dramatically improved therapeutic index of bispecific antibody pretargeting appears to be sufficiently compelling to justify human clinical trials and reinvigorate enthusiasm for radioimmunotherapy in the treatment of malignancies, particularly lymphomas. *Cancer Res; 77(9); 2191–6. ©2017 AACR.*

"To be, or not to be, that is the question: Whether 'tis nobler in the mind to suffer the slings and arrows of outrageous fortune, or to take arms against a sea of troubles, And by opposing end them." Hamlet. –William Shakespeare.

Introduction

Impact of monoclonal antibodies on the field of clinical oncology

Antibody therapies have transformed the treatment of cancer in the last 20 years. This transformation has particularly impacted the treatment of B-cell malignancies, where the addition of anti-CD20 antibodies (e.g. rituximab, obinutuzumab, ofatumumab) to conventional chemotherapy has improved overall response rates (ORR), complete response (CR) rates, progression-free survival (PFS), and overall survival (OS) of patients with chronic lymphocytic leukemia (CLL), follicular lymphoma, and diffuse large B-cell lymphomas in both front-line and relapsed settings. The dramatic impact of antibody therapy is not restricted to lymphomas. Trastuzumab has exhibited a potent and salutary impact on the outcome of patients treated for Her2/neu-expressing breast cancer; cetuximab and panitumumab (anti-EGFR antibodies) have improved outcomes for patients with cancer of the head and neck and metastatic colorectal cancer; bevacizumab is effective for metastatic colon cancer and advanced non–small cell lung cancer; and daratumumab (anti-CD38) and elotuzumab (anti-SLAMF7) have demonstrated impressive efficacy in multiple myeloma (1–3). Most impressive are the recent results of immune "checkpoint-inhibiting antibodies," such as ipilimumab (anti-CTLA4), nivolumab (anti-PD-1), and pembrolizumab (anti-PD-1), which are not directly cytotoxic for cancer cells but "release the brakes" on the immune system, allowing cytotoxic T cells to be more effective at recognizing and killing cancer cells. Outstanding results have already been demonstrated with checkpoint inhibiting antibodies even in far advanced refractory solid tumors including melanoma, lung cancer, Hodgkin lymphoma and are under study for a multitude of other malignancies (4–6).

Antibody–Drug Conjugates

Despite the impressive results obtained with unmodified monoclonal antibodies summarized above, single-agent efficacy is generally limited and few patients with cancer are permanently cured with antibody monotherapy. Consequently, investigators have explored the potential utility of augmenting the activity of antibodies by conjugating drugs, toxins, and radionuclides to them to produce more durable remissions. The first successful antibody–drug conjugate (ADC) was gemtuzumab ozogamicin (an anti-CD33 antibody conjugated to calicheamicin), which has significant efficacy in acute myeloid leukemias (AML; ref. 7), particularly those with favorable cytogenetic profiles, including acute promyelocytic leukemia. More recently, brentuximab vedotin (anti-CD30-monomethyl auristatin E) has shown dramatic efficacy in relapsed and refractory Hodgkin lymphoma, with ORR of more than 70% and CR rate of 33%. Patients achieving CR enjoyed 3-year OS rates of 73% and 3-year PFS rates of 58% (8), nor is ADC success restricted to hematologic malignancies. Dramatic results have been obtained with ado-trastuzumab-emtansine (an anti-Her2 antibody conjugated to the microtubule-inhibitory agent DM1), which provides superior PFS (9.6 vs. 6.4 months, $P < 0.001$) and OS (30.9 vs. 25.1 months) compared with treatment with standard therapy (lapatinib plus capecitabine; ref. 9). The ADC field is exploding, with many additional products expected to receive FDA-approval in the next few years.

Radiolabeled Antibodies

Combining monoclonal antibodies with radiation therapy was first studied in hematologic malignancies on the basis of the rationale that these are the most radiosensitive tumors (10).

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington. [2]Department of Medicine, University of Washington, Seattle, Washington. [3]Department of Bioengineering, University of Washington, Seattle, Washington.

Corresponding Author: Damian J. Green, Clinical Research Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., MS: D3-190, Seattle, WA 98109. Phone: 206-667-5398; Fax: 206-667-1874; E-mail: dgreen@fhcrc.org

doi: 10.1158/0008-5472.CAN-16-2523

Indeed, many clinicians believe that radiation therapy remains the single most effective agent for lymphomas. It is not surprising, therefore, that investigators began studies conjugating radionuclides to monoclonal antibodies shortly after the introduction of hybridoma technology in the late 1970s and early 1980s. To employ radioimmunotherapy effectively, several important variables needed to be optimized, including selection of the best cell surface target antigen and targeting antibody. An ideal target antigen for radioimmunotherapy is expressed at a high, uniform density on the surface of all tumor cells, is not expressed on normal cells, is minimally internalized after antibody binding, and is not "shed" into the circulation. Equally important, the cognate antibody of the target should penetrate rapidly into tumor nodules, bind with high avidity to the target antigen, interact minimally with non-malignant tissues, and clear from the blood soon after maximal tumor binding is achieved. Although a "perfect" antigen–antibody pair does not exist, CD20, CD22, and HLA-DR have been effectively targeted on B-cell lymphomas; CD33 and CD45 have shown promise in studies treating AML; and early studies have suggested impressive efficacy targeting CD38 in multiple myeloma (11). Investigators have not reached a universal consensus on the best therapeutic radionuclide for radioimmunotherapy, but ^{131}iodine and ^{90}yttrium have been utilized in the vast majority of studies. These β-particle–emitting radionuclides are readily available, have favorable emission characteristics, and are stably retained on antibodies using simple radiolabeling methods (10). Recently, the increased availability of α-emitter radionuclides, in conjunction with advances in radiochemistry leading to radiolabeling platforms capable of providing critical stability to α-particle–labeled biomolecules (12), has led to promising results using α-emitter radioimmunotherapy in leukemia (13, 14), lymphoma (15), and multiple myeloma (Green and Press, unpublished observations). On the basis of their physical characteristics, α-emitters may be particularly efficacious in minimal residual disease (MRD) settings, where isolated cells and small tumor clusters prevail. MRD elimination prior to stem cell transplant has been associated with improved OS in patients with hematologic malignancies including AML (16) and multiple myeloma (17). In light of these advances, our group and others are exploring a role for α-emitter radioimmunotherapy in a variety of settings.

First-generation radiolabeled antibodies

Early radioimmunotherapy studies attached radionuclides directly to the antibody protein, either using oxidizing agents such as chloramine T or Iodogen reactions for ^{131}iodine or derivatizing the antibody with a chelating agent such as the DOTA macrocycle for ^{111}indium and ^{90}yttrium. Most clinical trials to date have investigated the utility of either tositumomab (anti-CD20) antibody followed by ^{131}I-tositumomab (Bexxar) or rituximab followed by ^{90}Y-ibritumomab tiuxetan (Zevalin) for treatment of B-cell lymphomas. Dozens of published studies using these 2 products showed dramatic efficacy in patients with indolent B-cell lymphomas with ORR of 95% and CR rates of 75% in the front-line setting as a single agent (18) and ORR rates of 60% to 80% and CR rates of 20% to 40% in patients with relapsed or refractory indolent lymphomas (19–22). Many of these responses are durable with median remission durations exceeding 6 years after front-line therapy (18) and with 15% to 20% of relapsed/refractory patients enjoying remissions exceeding 5 years (19–23). Toxicities were mild in all single-agent studies, with reversible myelosuppression being most common. Concern has frequently been expressed about radiation-induced myelodysplasia (MDS) and AML, although the rates observed following radioimmunotherapy are comparable to the incidence of MDS/AML reported with many standard chemotherapeutic agents. (24) Nonhematologic adverse events following Bexxar and Zevalin consisted mainly of grade I/II fatigue, nausea, infusion reactions, and hypothyroidism (with Bexxar). Efficacy appeared similar with either Bexxar or Zevalin; however, the 2 drugs have never been rigorously compared in a randomized setting.

At least 7 phase II studies and 2 phase III studies have tested radioimmunotherapy in newly diagnosed patients receiving front-line therapy, either alone or as consolidation following chemotherapy (18, 25–33). These studies have all demonstrated outstanding efficacy with ORR of 90% to 100% and CR rates of 60% to 100%. More importantly, the remissions induced have been very durable with the median remission durations exceeding 6 years in all studies with sufficient follow-up (25, 33). The efficacy of this strategy was validated in a phase III randomized trial of ^{90}Y-ibritumomab tiuxetan consolidation after first remission in advanced-stage follicular lymphomas. In this study of 414 patients with newly diagnosed lymphoma who had achieved a complete or partial remission following first-line chemotherapy; the median PFS was 37 months for patients treated with ^{90}Y-ibritumomab tiuxetan compared with only 13.5 months for those who did not receive consolidative radioimmunotherapy ($P \leq 0.0001$). These salutary findings led to the regulatory approval of ^{90}Y-ibritumomab tiuxetan (Zevalin) radioimmunotherapy as first-line consolidation in both Europe and the United States. A subsequent phase III intergroup study compared front-line CHOP chemotherapy for 6 cycles followed by ^{131}I-tositumomab consolidation with CHOP chemotherapy plus 6 doses of rituximab. The trial enrolled 554 patients with previously untreated, advanced-stage follicular lymphoma (33–35). Although the initial report of this study showed no differences in either PFS or OS between the 2 treatment arms, follow-up has demonstrated a significant improvement in 10-year PFS for patients treated with CHOP + radioimmunotherapy (given without any rituximab; 57%) compared with patients treated on the CHOP/rituximab arm (42%; $P = 0.01$; ref. 34). There were no statistically significant differences in 10-year OS of patients treated on the 2 arms of this study, nor were there significant differences in the rates of myelodysplasia, secondary malignancies, or grade III–V adverse events.

FDA approval of radiolabeled antibodies

In February 2002, Zevalin became the first radiolabeled antibody to receive FDA approval. Its initial indication was the treatment of relapsed or refractory low-grade, follicular B-cell non–Hodgkin lymphoma (NHL), including patients with rituximab-refractory follicular NHL. The label was expanded in 2014 to include the use of Zevalin for consolidation of patients following initial treatment with front-line chemotherapy. Bexxar was approved in 2003 for treatment of relapsed, refractory, and transformed indolent lymphomas. However, to the surprise and disappointment of most radioimmunotherapy investigators, neither of the 2 approved RIT products found broad application in clinical practice. In February 2014, GlaxoSmithKline discontinued the manufacture and sale of Bexxar

due to poor sales. Zevalin remains available in the United States, but sales are poor.

The reasons for the commercial failures of Bexxar and Zevalin remain controversial. One survey of physicians indicated a major impediment to the sales of radioimmunotherapy reagents was the fact that these reagents could not be administered in the offices or infusion rooms of practicing hematologists and oncologists but required referral to Nuclear Medicine and Radiation Oncology consultants (36). These referrals often involved extensive "phone tag," communication gaps, and frustration on the parts of the referring hematologist–oncologist, the treating Nuclear Medicine or Radiation Oncology physician, and the patient. Reimbursement concerns and phobias concerning radiation therapy also probably played roles, particularly in the context of exaggerated concerns about the risks of MDS/AML. The simultaneous emergence of several competing therapies targeting B-cell malignancies, which could be easily administered by practicing oncologists (bendamustine) or could be conveniently dosed by oral administration (ibrutinib, idelalisib), undoubtedly also contributed significantly to limited integration of Bexxar and Zevalin into clinical practice. To overcome these formidable obstacles will require enhancements to radioimmunotherapy that produce outcomes far superior to those achievable by other options in the marketplace. "Pretargeted" radioimmunotherapy (PRIT) is an approach that might achieve this goal.

PRIT

PRIT employs multistep delivery of reagents to dissociate the slow distribution phase of the large antibody molecule from the administration of the therapeutic radionuclide (37–40). Tumor-reactive antibodies are administered in a nonradioactive form, allowing them to accumulate in tumor sites without subjecting the body to nonspecific irradiation from circulating radiolabeled antibodies (10). After a delay of 24 to 48 hours to allow maximal accretion of antibody in the tumor, a small molecular weight, radioactive reagent with high affinity for the tumor-reactive antibody is delivered. This second reagent is very small and rapidly penetrates tumors where the radioactive ligand is trapped by the pretargeted antibody. Unbound molecules of the radioactive reagent are rapidly cleared from the blood in the urine or bile, often facilitated by the use of a "clearing agent" injected immediately before the radiolabeled moiety. This intervention eliminates excess unbound antibody from the blood and avoids it from combining with the radiolabeled ligand outside the tumor environment (40). Several PRIT technologies have been developed including the use of antibody–streptavidin (SA) conjugates or fusion proteins used together with a dendrimeric *N*-acetylgalactosamine–containing clearing agent, followed by ^{90}Y-DOTA biotin (41). While this has been the most popular and widely employed PRIT approach, it has been criticized because of the immunogenicity of streptavidin and the potential for "blocking" of streptavidin-binding sites by endogenous circulating biotin, which might compete with binding to ^{90}Y-DOTA-biotin.

To avoid the limitations imposed by SA-biotin PRIT, investigators have developed several alternative approaches. Foremost among these alternatives is the use of bispecific monoclonal antibody technologies. Goldenberg and colleagues developed bivalent haptens that permit cooperative binding, linking 2 bispecific antibodies together on the tumor surface using a bivalent histamine–succinyl–glycine hapten as a bridge (42). Another innovative approach utilizes molecularly engineered "dimerization and docking domains" containing self-assembling protein kinase A motifs with engineered cysteine residues (43, 44). A third approach (45) employs molecularly engineered bispecific antibodies incorporating complementary reactive groups in the antibody-binding pocket, which bind covalently and irreversibly to radiolabeled electrophilic ligands (46, 47). Wittrup perfected a versatile, modular (IgG-scFv) bispecific antibody format with an IgG portion specific for tumor and a high-affinity scFv specific for DOTA-yttrium generated by yeast surface display. Our own studies (48) have utilized a modification of the Wittrup's approach employing 2 scFv domains rather than an IgG–scFv construct (49). Finally, Hnatowich has developed a unique methodology employing complementary hybridization of phosphorodiamidate morpholino oligomers that rely on Watson–Crick pairing to capture radiolabeled ligands. (50) Although each of these "second-generation" PRIT methods has been shown to be superior to "first-generation" radioimmunotherapy with directly radiolabeled antibodies, until the publication of our comparative studies in *Cancer Research* (48), no head-to-head comparisons had been conducted to discern which of the PRIT approaches was most promising for clinical development.

Head-to-head comparison of SA-PRIT and bispecific antibody PRIT

To facilitate selection of the most promising PRIT construct for future clinical trials, we performed a comparative analysis of the biodistribution and therapeutic efficacy of the 2 most popular PRIT strategies, namely, SA-biotin and bispecific antibody PRIT. We engineered a bispecific fusion protein consisting of scFv targeting human CD20 on one end and Yttrium-DOTA on the other end (Fig. 1). The fusion protein traps the radiolabeled ligand (^{90}Y-DOTA) using a very high-affinity anti-Y-DOTA scFv (C825) generated by Wittrup using yeast surface display (51). Head-to-head comparative biodistribution experiments comparing SA-biotin and bispecific Ab (2H7-Fc-C825) PRIT in mice bearing human lymphoma xenograft tumors demonstrated tumor targeting by the bispecific construct (2H7-Fc-C825) that was virtually identical to SA-biotin PRIT after 24 hours (8.37% $\pm$ 1.21% ID/g vs. 8.19% $\pm$ 1.02% ID/g, respectively). However, residual radioactivity in the blood and normal organs was consistently higher following SA-biotin PRIT than after bispecific Ab PRIT (2.09% $\pm$ 0.46% ID/g vs. 0.59% $\pm$ 0.09% for the blood and 2.07% $\pm$ 0.35% ID/g vs. 0.55% $\pm$ 0.07% for the kidneys, respectively). Consequently, tumor-to-normal ratios were superior for bispecific Ab PRIT with 2H7-Fc-C825. Therapy studies performed in mice bearing either Ramos or Granta subcutaneous lymphoma xenografts demonstrated that bispecific 2H7-Fc-C825 PRIT was very effective and significantly less myelosuppressive than SA-biotin PRIT. All animals (100%) receiving optimal doses of bispecific 2H7-Fc-C825 fusion protein (2.8 nmol), followed by ^{90}Y-DOTA-biotin (1,000 μCi), were alive and cured after 150 days, whereas tumor-bearing control groups demonstrated rapidly progressive tumor growth with 0% survival beyond 25 days ($P < 0.001$). In addition to reduced immunogenicity and the absence of endogenous biotin interference, these findings suggest that bispecific PRIT is preferred for future clinical trials because of a slightly superior biodistribution profile, less myelosuppression, and superior efficacy.

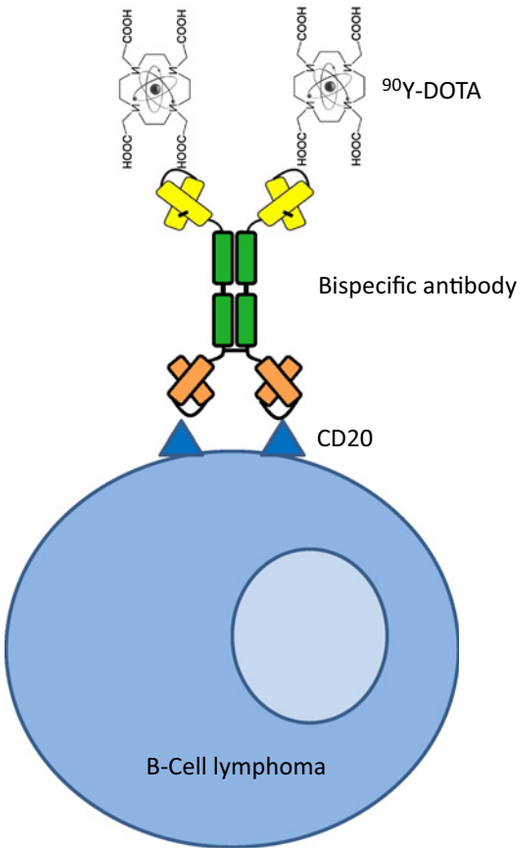

Figure 1.
Bispecific antibody PRIT. A bispecific antibody construct was engineered that spontaneously dimerizes after expression by CHO-DG44 cells. One single-chain antibody fragment binds with high affinity to the CD20 antigen on B-cell malignancies, whereas the other single-chain antibody fragment binds with exceptionally high affinity to an ^{90}yttrium-DOTA hapten.

Implications and Future Directions

Despite impressive efficacy and safety profiles that led to FDA approval of 2 radioimmunoconjugates for the treatment of B-cell lymphoma (^{131}I-tositumomab and ^{90}Y-ibritumumab tiuxitan), these agents have rarely been incorporated into clinical care. Radioimmunotherapy-targeting CD20 remains in the National Comprehensive Cancer Network Guidelines as a first-line therapy for elderly or infirm patients with follicular lymphoma and as a recommended approach to consolidation or second-line therapy for follicular NHL. Nonetheless, overall utilization remains low, and radioimmunotherapy is administered disproportionately within the confines of academic centers (36). Limited use is likely a consequence of multiple factors, which include the availability and ease of administration associated with other novel targeting agents and concerns about radiation toxicity, particularly to the bone marrow. Concerns regarding reimbursement to community oncologists cannot be trivialized; however, the absolute cost of radioimmunotherapy for consolidation is lower than the cost of maintenance rituximab [$46,000; and $54,000 to $72,000 (12–16 courses)], respectively (52). Radioimmunotherapy may also offer a quality-of-life advantage to patients because administration involves a single-patient infusion visit as compared with frequent infusions during rituximab maintenance.

Innovations that improve targeting, diminish toxicity, and highlight the unique favorable attributes associated with radioimmunotherapy may help overcome a history of limited adoption. We have recently shown that parallel bispecific antibodies targeting CD38 on myeloma cells or CD45 on AML cells work as well as the CD20 antibodies published in *Cancer Research* (ref. 15; and Green and Press, unpublished data). We are currently scaling up the production of the bispecific antibodies for human clinical trials. Future studies will also investigate potential synergy of PRIT with small-molecule inhibitors (e.g., ibrutinib, navitoclax, idelalisib), radiosensitizing agents PARP inhibitors (53), bortezomib (54), and a role for bispecific PRIT in stem cell transplantation. Ultimately, superior efficacy will provide the most compelling argument for the adoption of PRIT, and well-designed clinical trials, convincingly recapitulating the impressive responses seen with bispecific antibody delivery systems in preclinical models, will be essential to reinvigorate interest in this field.

Disclosure of Potential Conflicts of Interest

O.W. Press has ownership Interest (including patents) in Emergent Biosolutions and is a consultant/advisory board member for Roche. No potential conflicts of interest were disclosed by the other author.

Grant Support

This work was supported by grants from the U.S. NIH NCIK08 CA151682 (D.J. Green), NCI R01 CA076287 (O.W. Press), NCIR01 CA136639 (O.W. Press), NCIR01 CA154897 (O.W. Press), NCIP01 CA044991 (O.W. Press), NCIR21 CA155911 (D.J. Green), NCIR01 CA109663 (O.W. Press), Multiple Myeloma Opportunities for Research and Education (MMORE), The Quest for Truth Foundation, and The Brotherton Family Fund and by the David and Patricia Giuliani Family Foundation.

Received September 13, 2016; revised October 26, 2016; accepted January 20, 2017; published OnlineFirst April 20, 2017.

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