A re-examination of radioimmunotherapy in the treatment of non-Hodgkin lymphoma: prospects for dual-targeted antibody/radioantibody therapy

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Antibody-based therapies, both unconjugated antibodies and radioimmunoconjugates, have had a significant impact on the treatment of non-Hodgkin lymphoma. Single-agent rituximab is an effective therapy, but it is being increasingly used with combination chemotherapy to improve the objective response and its duration. The approved anti-CD20 radioimmunoconjugates (90Y-ibritumomab tiuxetan or 131I-tositumomab) have had encouraging results, with trials now seeking to incorporate a radioimmunoconjugate in various settings. However, new preclinical data raise important questions concerning current radioimmunoconjugate treatment regimens and ways to improve them. In radioimmunoconjugate therapy, nearly 900 mg of the unlabeled anti-CD20 IgG antibody is predosed to the patient before the anti-CD20 antibody conjugated to either 90Y or 131I is given. Combining an unconjugated anti-CD20 antibody therapy with a radioimmunoconjugate binding to a noncompeting antigen might improve responses by allowing optimal uptake of each agent. Preclinical models have indicated that careful consideration should be given to predosing when using competing antibodies, but that consolidation anti-CD20 therapy enhances the efficacy of radioimmunoconjugate therapy. New technologies, such as pretargeted radioimmunotherapy, also hold promise by reducing toxicity without sacrificing efficacy, and consideration should be given to fractionating or giving multiple radioimmunoconjugate treatments. This perspective discusses how these issues could affect current and future clinical trials. (Blood. 2009;113:3891-3895)

Introduction

Targeting cancer with radiolabeled antibodies, first demonstrated by diagnostic imaging1 and subsequently developed into radioimmunotherapy (RAIT), has remained an active field of study for more than 30 years.2 Today, 2 radiolabeled anti-CD20 IgG antibodies, 90Y-ibritumomab tiuxetan (Zevalin; Cell Therapeutics, Seattle, WA; Bayer Schering Healthcare, Berlin, Germany) and 131I-tositumomab (Bexxar; GlaxoSmithKline, Philadelphia, PA), are approved for treatment of patients with follicular and transformed non-Hodgkin lymphoma (NHL) who failed or relapsed from prior therapies, including rituximab and standard chemotherapy.3,4 Although results from ongoing clinical studies support the use of such radioimmunoconjugates in various front-line and salvage treatment settings,5-19 important issues remain regarding how these agents are administered, yet also suggest some potential new treatment paradigms.20

Current radioimmunoconjugate therapy of NHL: development and practice

We believe a major issue is the role and dose of unconjugated anti-CD20 antibody given prior to the radioimmunoconjugate in both products. In the United States, patients first receive 250 mg/m² of rituximab a few hours before receiving 111In-ibritumomab; 2 to 3 days later, an imaging study then establishes a “normal” biodistribution pattern, and then another 250 mg/m² predose of rituximab is given before 90Y-ibritumomab within 1 week of the first dose. In Europe, the 111In imaging study is not required, but patients still receive 2 250 mg/m² doses (approximately 450 mg) of rituximab before the 90Y-ibritumomab, which itself is given with just a few milligrams of the DTPA (diethylene triamine pentaacetic acid) conjugate of the murine anti-CD20 parent antibody, ibritumomab, that was used to engineer the chimeric rituximab antibody. With 131I-tositumomab, a pretreatment dosimetry study is performed to assign a patient-specific radioactivity dose, but before both the pretreatment imaging and the therapy doses, patients receive 450 mg of unlabeled tositumomab. Thus, in each of these treatments, approximately 900 mg of unlabeled antibody is given before the therapeutic anti-CD20 radioimmunoconjugate.

Radioimmunoconjugates are intended to be prepared at high specific activity to maximize the radiation delivered. Thus, a relatively small amount of protein (eg, < 10 mg) can deliver the maximum radiation tolerated by these treatments. However, clinical studies using anti–HLA-DR and anti-CD37 radioantibodies found considerable uptake in the spleen and other organs.21-24 Like CD20, these antigens are expressed on normal and malignant cells, often at similar levels, and depending on the number of normal B cells (eg, splenomegaly), the radioimmunoconjugate will confront a considerable antigen sink that competes for the conjugate’s binding to tumor sites. In addition, excessive tumor burden also can negatively affect the distribution of the radiolabeled antibody to all tumor sites. By performing 3 successive pretherapy imaging studies in the same patient with increasing amounts of the MB-1 anti-CD37 IgG or the murine anti-B1 anti-CD20 IgG (later designated tositumomab), it was found that blood clearance was slowed, splenic uptake was reduced, and tumors were often better visualized with higher doses.24,25 Press et al reported 2.5 mg/kg as the optimal targeting dose for the 131I–anti-B1 antibody (ie, the
protein dose that assured a higher uptake of radioactivity in tumor sites than in the liver, lungs, or kidneys), yielding “favorable dosimetry” in 56% of the patients.25 In a separate study with 131I–anti-B1, Kaminski et al reported tumor visualization of all known lesions greater than 2 cm using just a tracer dose.26 A predose of 135 mg of unlabeled anti-B1 improved the tumor/whole-body radiation–absorbed dose ratio in 2 of 5 patients compared with omission of the predose, with no differences found in the other 3 patients. Of greater interest was the finding that 2 patients who received a 685 mg predose with just a diagnostic amount of 131I–anti-B1 for imaging exhibited objective remissions before the therapeutic 131I–anti-B1 was administered while these responses made them unassessable for dosimetry; in a follow-up report, the 685 mg predose appeared to be favored.27 Wahl et al later reported studies that compared no predose to 95 mg and 450 mg predosing of tositumomab, and showed that the 95 mg predose significantly slowed blood clearance compared with no predose.28 Despite only a marginal difference in blood clearance rates at the 2 predose levels, the 450 mg predose was selected for subsequent phase 2/3 trials,3 because this enhanced the biodistribution compared with the lower dose in patients with bulky disease and enlarged spleens.

Initial clinical experiences with 90Y–britunomab tuxetan also demonstrated rapid blood clearance when only 2 mg of the 111In-labeled murine 2B8 anti-CD20 IgG1 was administered. In contrast, when patients received a 1.0 mg/kg predose of unlabeled antibody, blood clearance was slowed, splenic uptake was reduced nearly 4-fold, and whole-body scans revealed better tumor visualization in 6 of 10 patients.29 At a predose level of 2.5 mg/kg, 92% of known sites were disclosed in 4 patients, compared with 56% of known sites in 14 patients given the lower predose, but there was no direct comparison of targeting at the 2 predose levels or of the 2.5 mg/kg predose versus no predose. One patient with splenic lesions was reported to have tumor nodules that were better visualized without a predose of 1.0 mg/kg, illustrating some variability. There was no apparent impact on the quantitative amount of the radioconjugate delivered to the tumor, but tumor uptake was highly variable. Later studies using chimeric rituximab in place of the parental murine 2B8 antibody that examined 100 mg/m2 and 250 mg/m2 predosing in cohorts of 3 patients also reported the desired changes in normal tissue distribution with no apparent reduction in tumor uptake.30 A predose of 250 mg/m2 was selected because the higher dose of rituximab was considered a potential boost to the antitumor activity of the radioimmunoconjugate.

Preclinical studies in mice bearing human B-cell lymphoma xenografts found better responses with the unlabeled anti-B1 compared with 131I–anti-B1, which supported the notion that the addition of unlabeled antibody to the radioconjugate would contribute to the response.31 Subsequent findings have indicated that an anti-CD20 IgG predose radiosensitizes the cells, thereby providing some added benefit to the combination treatment.32–35 Parenthetically, Kapadia et al found that lower doses of rituximab radiosensitize cells, while higher doses actually appeared to be protective.34

Both preclinical and clinical data clearly supported the value of administering a predose of the unlabeled anti-CD20 IgG to improve tumor targeting and extend the residence time of the radioimmunoconjugates in the blood; certainly, by using this approach, the radiolabeled agents were able to improve the objective response rate compared with their corresponding unlabeled antibody.4,36 However, if the specific targeting of radiation improves the response in this setting, might further improvements occur if more radiation were delivered? In our view, it seems unlikely that a few milligrams of a radioimmunoconjugate would have its optimal uptake in a tumor when administered after 900 mg of unlabeled antibody. Although the CD20-antigen sink may be substantial, this amount of unlabeled antibody can easily compete with the radioimmunoconjugate and compromise the selective localization of radiation to the tumor. Gopal et al showed recently that enough rituximab was present in patient sera 4 weeks after treatment to interfere with the binding of 131I-tositumomab to lymphoma cell lines.37 Furthermore, tumor uptake of 131I-tositumomab was reduced by 55%, and tumor responses were reduced significantly in nude mice bearing lymphoma xenografts when a predose of 0.4 mg rituximab (eg, approximately 1.6 mg/kg human equivalent dose based on US Food and Drug Administration [FDA]–recommended conversion factor5), compared with tumor-bearing mice treated with 131I-tositumomab alone.38 Corroborating results have been obtained subsequently using a humanized anti-CD20 IgG, veltuzumab, against Burkitt lymphoma–bearing nude mice, where a predose of 1.0 mg (approximately 4.0 mg/kg human equivalent) of veltuzumab given 1 day or even 1 hour before 111In-veltuzumab reduced tumor uptake by 40%.39 When the predose was reduced to 0.25 mg given 1 day or 1 hour before 111In-veltuzumab, tumor uptake was reduced by only 20%. Progression of tumors in mice pretreated with 0.25 or 1.0 mg veltuzumab was somewhat more rapid than those given only 90Y-veltuzumab (0.05 mg), but time to progression to 2.5 cm3 was not significantly different.39 Thus, in both reports, tumor uptake was decreased by cold antibody predoses, potentially compromising the therapeutic efficacy of the radioimmunoconjugates. Fortunately, there are alternative strategies to derive the maximum therapeutic benefit by combining the radioimmunoconjugate with a different unlabeled antibody.

**Predosing and dual-targeted immunotherapy/radioimmunotherapy**

Gopal et al showed that rituximab would not interfere with the binding and therapeutic activity of an 131I-labeled anti-CD45 IgG antibody, but emphasized that an anti-CD45 radioimmunoconjugate would be restricted to a myeloablative setting due to the broad expression of CD45 on hematopoietic cells, suggesting other targets might be considered in nontransplantation settings.37 Mattes et al examined RAIT using 90Y-epratuzumab (humanized anti-CD22) combined with unlabeled veltuzumab anti-CD20 IgG therapy, which began 1 day before 90Y-epratuzumab (1.0 mg), followed by 3 additional 0.5 mg weekly doses of veltuzumab.30 Biodistribution studies showed no interference in tumor targeting of the radioimmunoconjugate and, more important, the therapeutic response in the Burkitt xenograft model was significantly enhanced, converting short-term partial and complete responses with the radioimmunoconjugate alone to long-term, complete responses for 80% of the established xenografts. These studies illustrate how a noncompeting RAIT and unlabeled antibody (dual-targeted) treatment regimen can be more effective than either agent alone. Subsequent testing found that unlabeled anti-CD20 therapy could be started 7 days after the anti-CD22 radioimmunoconjugate treatment and also improve therapeutic responses (R.M.S. and M. Jules Mattes, unpublished data, August 15, 2008). This concept was then examined in the same xenograft model using 90Y-veltuzumab/veltuzumab combination. Animals given 90Y-veltuzumab followed 1 week later with veltuzumab consolidation therapy exhibited markedly improved responses (with
complete ablations) compared with $^{90}$Y-veltuzumab or veltuzumab alone.\textsuperscript{39} Interestingly, when this same antibody therapy regimen was initiated 1 day before $^{90}$Y-veltuzumab treatment, the animals no longer benefited from the additional antibody dosing, exhibiting a similar time to progression as the group receiving $^{90}$Y-veltuzumab alone. However, a reduced predose of 0.25\ mg (human equivalent of 1.0\ mg/kg) given 1 day before the $^{90}$Y-veltuzumab and then followed 1 week later by the full veltuzumab therapy regimen resulted in all animals achieving durable complete responses.\textsuperscript{39}

Since anti–human CD20 antibodies do not cross-react with murine B cells, we are unable to simulate the same antigen sink that anti-CD20 antibodies confront clinically to determine the dynamics of sink versus tumor binding. Nevertheless, these studies raise questions about the optimal predose that should be administered, particularly with regard to future trials that seek to incorporate radioimmunoconjugate therapy into other treatment regimens containing rituximab. These data question whether the current predosing practice is optimal for all patients, particularly those who have undergone splenectomy or have a small tumor burden. The clear intent in the initial clinical experience was to enhance the radioimmunoconjugate response by injecting large amounts of unlabeled antibody, because high doses of the unlabeled antibody were therapeutically active on their own.\textsuperscript{27} Thus, despite evidence that smaller doses of unlabeled antibody, in the amount of approximately 1.0 to 1.5\ mg/kg, had the same desired effect of increasing the radioimmunoconjugate’s circulating half-life, reducing splenic uptake, and improving tumor visualization in most patients, it was concluded that therapeutically, “more is better.” Our results actually support this premise, but place the emphasis on selecting conditions that first enhance the ability of the radioimmunoconjugate to localize to the tumor. In a situation where the antibody being used for RAIT has therapeutic activity on its own, it is desirable to integrate the unlabeled antibody treatment at its full biologically active dose with RAIT in a sequence that does not impair binding of the radioimmunoconjugate. However, when the radioimmunoconjugate and the unlabeled antibody bind competitively to the same antigen, minimizing a predose needed to reduce the antigen sink is important, but once the radioimmunoconjugate has localized, consolidation therapy with an unlabeled antibody could be beneficial. Selecting antibodies that will not compete for the same target antigen greatly simplifies this approach. In addition, an effective unlabeled antibody also could be given as part of an induction therapy, which should reduce the antigen sink if the 2 antigens reside on the same cells, as well as activating possible signaling pathways or other mechanisms that might enhance the RAIT effect. Interestingly, Illidge et al initiated a repeated $^{131}$I-rituximab therapy regimen 4 weeks after completing a full 4-week rituximab therapy, finding that the clearance of the $^{131}$I-rituximab was slowed with a reduced predose of 100\ mg/m$^2$.\textsuperscript{31} Although they compared clearance of a trace dose of $^{131}$I-rituximab in 5 patients prior to the rituximab induction to a tracer dose after rituximab induction, all the postinduction patients were first predosed, and how the induction dosing might affect the trace $^{131}$I-rituximab was not addressed. Importantly, this trial did report major and durable responses.

Other prospects

As efforts today begin to integrate $^{90}$Y– or $^{131}$I–anti-CD20 radioimmunoconjugates into new treatment regimens and settings, it is important to reconsider how to best configure the radioimmunoconjugate within a treatment program that relies on optimization methods proposed more than 20 years ago. These preclinical data offer an alternative approach that could benefit patients with follicular lymphoma, perhaps replacing the more toxic chemotherapy regimens with an anti-CD20 treatment to first reduce the normal B-cell sink, shrink the tumor burden, and even potentially sensitize the tumor to an anti-CD22 radioimmunoconjugate (or RAIT directed at another B-cell antigen target), which could then be followed by another consolidation regimen of anti-CD20 IgG therapy. Aggressive NHL might also benefit from an anti-CD22 radioimmunoconjugate therapy following rituximab plus CHOP (cyclophosphamide/doxorubicin/vincristine/prednisone).

Whether RAIT should be administered as a single dose or fractionated, as practiced with external beam irradiation, is also a topic of consideration. In a trial evaluating doses of $^{90}$Y-epratuzumab (humanized anti-CD22 IgG) fractionated over 2 or 3 weeks without any predosing, it was found that weekly fractions of total doses greater than 740\ MBq/m$^2$ (20\ mCi/m$^2$) in 29 relapsed patients with NHL with different subtypes resulted in 72.4\% objective responses (55.2\% complete response [CR]/CR unconfirmed [CRu]); all 11 patients with follicular cell lymphoma responded at $2 \times 740$\ MBq/m$^2$ ($2 \times 20$\ mCi/m$^2$; 10 of 11\ CR/CRu).\textsuperscript{42} The single maximal tolerated dose of this agent did not show such encouraging responses, but did demonstrate that prior evidence of targeting with the $^{111}$In-epratuzumab did not predict response.\textsuperscript{43} More recent studies with $^{90}$Y-ibritumomab tiuxetan also showed no correlation between prior targeting and tumor response, providing considerable data that question the utility of the diagnostic $^{111}$In imaging study for this agent.\textsuperscript{44,45} Overall, these findings suggest that nontargeted radiation contributes to the therapeutic responses induced by RAIT, questioning whether pretreatment antibody targeting is necessary to qualify patients.

Selection of the most appropriate radionuclide also is a topic of discussion. For example, Morschhauser et al recently reported encouraging results from a randomized study, where consolidation $^{90}$Y-ibritumomab therapy significantly improved progression-free survival (36.5 vs 13.3 months for control arm) in patients who had achieved a partial or complete response after receiving a front-line induction therapy.\textsuperscript{46} While acknowledging the additive value of the current RAIT regimen, it is appropriate to ask whether a $^{90}$Y therapeutic, with its long-range beta emission that is better suited for bulky disease, should be used in patients who have already achieved a complete remission.\textsuperscript{177}Lu might be substituted for a $^{90}$Y-based radioconjugate, and provide more favorable, shorter-range energy emissions in the setting of minimal or occult disease.

Further, methods to improve selective radionuclide localization by separating the targeting antibody from the radioimmunoconjugate (pretargeting) also improve RAIT.\textsuperscript{39,47-51} Pretargeting has been shown to enhance antitumor responses very significantly, while reducing hematologic toxicity, suggesting that this method might be better tolerated with chemotherapy regimens that are now being added to RAIT. This method also benefits from the incorporation of an anti-CD20 IgG consolidation therapy.\textsuperscript{39}

These considerations do not detract from the currently approved method of RAIT, which is still underused in the United States. Indeed, the prospects for radioimmunotherapy are increasing as it
becomes further integrated into multimodality treatment paradigms. Included with these should be combinations of dual-targeting antibodies, comprising both unlabeled and radiolabeled antibodies against different antigen targets.

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