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

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Abstract

Antibody-based therapies, both unconjugated antibodies and radioimmunotherapy, 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 (\(^{90}\)Y-ibritumomab tiuxetan or \(^{131}\)I-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 radioconjugate therapy, nearly 900 mg of the unlabeled anti-CD20 IgG antibody is pre-dosed to the patient before the anti-CD20 antibody conjugated to either \(^{90}\)yttrium or \(^{131}\)iodine is given. Combining an unconjugated anti-CD20 antibody therapy with a radioimmunoconjugate binding to a non-competing antigen might improve responses by allowing optimal uptake of each agent. Preclinical models have indicated that careful consideration should be given to pre-dosing 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 impact current and future clinical trials.
Targeting cancer with radiolabeled antibodies, first demonstrated by diagnostic imaging\textsuperscript{1} and subsequently developed into radioimmunotherapy (RAIT), has remained an active field of study for more than 30 years.\textsuperscript{2} Today, two radiolabeled anti-CD20 IgG antibodies, $^{90}$Y-ibrutinomab tiuxetan (Zevalin\textsuperscript{®}; Cell Therapeutics, Seattle, WA; Bayer Schering Healthcare, Berlin, Germany) and $^{131}$I-tositumomab (Bexxar\textsuperscript{®}; GlaxoSmithKline, Philadelphia, PA), are approved for treatment of patients with follicular and transformed NHL who failed or relapsed from prior therapies, including rituximab and standard chemotherapy.\textsuperscript{3,4} Although results from ongoing clinical studies support the use of such radioimmunoconjugates in various front-line and salvage treatment settings,\textsuperscript{5-19} important issues remain regarding how these agents are administered, yet also suggest some potential new treatment paradigms.\textsuperscript{20}

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/m\textsuperscript{2} of rituximab a few hours before receiving $^{111}$In-ibrutinomab, and then 2-3 days later, an imaging study establishes a “normal” biodistribution pattern, and then another 250-mg/m\textsuperscript{2} pre-dose of rituximab is given before $^{90}$Y-ibrutinomab within 1 week of the first dose. In Europe, the $^{111}$In-imaging study is not required, but patients still receive two 250-mg/m\textsuperscript{2} doses (~450 mg) of rituximab before the $^{90}$Y-ibrutinomab, which itself is given with just a few mg of the DTPA (diethylene triamine pentaacetic acid) conjugate of the murine anti-CD20 parent antibody, ibrutinomab, that was used to engineer the chimeric rituximab antibody. With $^{131}$I-tositumomab, a pre-therapy dosimetry study is performed to assign a patient-specific radioactivity dose, but before both the pre-therapy imaging and the therapy doses, patients
receive 450 mg of unlabeled tositumomab. Thus, in each of these treatments, ~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 (e.g., <10 mg) can deliver the maximum radiation tolerated by these treatments. However, clinical studies using anti-HLA-DR and CD37 radioantibodies found considerable uptake in the spleen and other organs. Like CD20, these antigens are expressed on normal and malignant cells, often at similar levels, and depending on the number of normal B cells (e.g., 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 impact the distribution of the radionuclide antibody to all tumor sites. By performing 3 successive pre-therapy 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 reduced, and tumors often better visualized with higher doses. Press et al. reported 2.5 mg/kg as the optimal targeting dose for the 131I-anti-B1 antibody (i.e., 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. In a separate study with 131I-anti-B1, Kaminski et al. reported tumor visualization of all known lesions >2 cm using just a tracer dose. A pre-dose of 135 mg of unlabeled anti-B1 improved the tumor/whole-body radiation-absorbed dose ratio in 2 of 5 patients compared to omission of the pre-dose, with no differences found in the other 3 patients. Of greater interest was the finding that 2 patients receiving a 685-mg pre-dose before 131I-anti-B1 imaging exhibited objective remissions before the therapeutic 131I-anti-B1 was administered, making them unassessable for dosimetry, but in a follow-up
report, the 685-mg pre-dose appeared to be favored.\textsuperscript{27} Wahl et al. later reported studies that compared no pre-dose to 95- and 450-mg pre-dosing of tositumomab, and showed that the 95-mg pre-dose significantly slowed blood clearance as compared to no pre-dose.\textsuperscript{28} Despite only a marginal difference in blood clearance rates at the 2 pre-dose levels, the 450-mg pre-dose was selected for subsequent Phase II/III trials,\textsuperscript{3} because this enhanced the biodistribution compared to the lower dose in patients with bulky disease and enlarged spleens.

Initial clinical experiences with \textsuperscript{90}\textsuperscript{Y}-ibritumomab tiuxetan also demonstrated rapid blood clearance when only 2 mg of the \textsuperscript{111}In-labeled murine 2B8 anti-CD20 IgG1 was administered. In contrast, when patients received a 1.0-mg/kg pre-dose 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.\textsuperscript{29} At a pre-dose level of 2.5 mg/kg, 92\% of known sites were disclosed in 4 patients, as compared to 56\% of known sites in 14 patients given the lower pre-dose, but there was no direct comparison of targeting at the 2 pre-dose levels or of the 2.5 mg/kg pre-dose vs. no pre-dose. One patient with splenic lesions was reported to have tumor nodules that were better visualized without a pre-dose 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/m\textsuperscript{2} and 250-mg/m\textsuperscript{2} pre-dosing in cohorts of 3 patients also reported the desired changes in normal tissue distribution with no apparent reduction in tumor uptake.\textsuperscript{30} A pre-dose of 250 mg/m\textsuperscript{2} was selected, because the higher dose of rituximab was considered a potential boost to the anti-tumor activity of the radioimmunoconjugate.
Preclinical studies in mice bearing human B-cell lymphoma xenografts found better responses with the unlabeled anti-B1 compared to $^{131}$I-anti-B1, which supported the notion that the addition of unlabeled antibody to the radioconjugate would contribute to the response. Subsequent findings have indicated that an anti-CD20 IgG pre-dose radiosensitizes the cells, thereby providing some added benefit to the combination treatment. Parenthetically, Kapadia et al. found that lower doses of rituximab radiosensitize cells, while higher doses actually appeared to be protective.

Both preclinical and clinical data clearly supported the value of administering a pre-dose of the unlabeled anti-CD20 IgG to improve tumor targeting and extend the residence time of the radioimmunoconjugates in the blood, and certainly by using this approach, the radiolabeled agents were able to improve the objective response rate as compared to their corresponding unlabeled antibody. 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 mg of a radioimmunoconjugate would have its optimal uptake in a tumor when administered after 900 mg of unlabeled antibody. While 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 $^{131}$I-tositumomab to lymphoma cell lines. Furthermore, tumor uptake of $^{131}$I-tositumomab was reduced by 55%, and tumor responses were reduced significantly in nude mice bearing lymphoma xenografts when given a pre-dose of 0.4 mg of rituximab (e.g., ~1.6 mg/kg human equivalent dose based on FDA recommended conversion factor), compared to tumor-bearing mice treated with $^{131}$I-tositumomab alone.
Corroborating results have been obtained subsequently using a humanized anti-CD20 IgG, veltuzumab, against Burkitt-lymphoma-bearing nude mice, where a pre-dose of 1.0 mg (~4.0 mg/kg human equivalent) of veltuzumab given 1 day or even 1 h before $^{111}$In-veltuzumab reduced tumor uptake by 40%. When the pre-dose was reduced to 0.25 mg given 1 day or 1 h before $^{111}$In-veltuzumab, tumor uptake was reduced by only 20%. Progression of tumors in mice pre-treated with 0.25 or 1.0 mg veltuzumab was somewhat more rapid than those given only $^{90}$Y-veltuzumab (0.05 mg), but time to progression to 2.5 cm$^3$ was not significantly different. Thus, in both reports, tumor uptake was decreased by cold antibody pre-doses, 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.

**Pre-dosing and dual-targeted immunotherapy/radioimmunotherapy**

Gopal et al. showed that rituximab would not interfere with the binding and therapeutic activity of an $^{131}$I-labeled anti-CD45 IgG antibody, but emphasized that an anti-CD45 radioimmunoconjugate would be restricted to a myeloablative setting due the broad expression of CD45 on hematopoietic cells, suggesting other targets might be considered in non-transplant settings. Mattes et al. examined RAIT using $^{90}$Y-epratuzumab (humanized anti-CD22) combined with unlabeled veltuzumab anti-CD20 IgG therapy, which began one day before $^{90}$Y-epratuzumab (1.0 mg), followed by three additional 0.5-mg weekly doses of veltuzumab. Biodistribution studies showed no interference in tumor targeting of the radioimmunoconjugate and, more importantly, 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 non-competing RAIT and unlabeled antibody therapy (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 $^{90}$Y-veltuzumab/veltuzumab combination. Animals given $^{90}$Y-veltuzumab followed 1 week later with veltuzumab consolidation therapy exhibited markedly improved responses (with complete ablations) compared to $^{90}$Y-veltuzumab or veltuzumab alone. Interestingly, when this same antibody therapy regimen was initiated one 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 pre-dose of 0.25 mg (human equivalent of 1.0 mg/kg) given 1 day before the $^{90}$Y-veltuzumab and then followed one week later by the full veltuzumab therapy regimen resulted in all animals achieving durable complete responses.

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 vs. tumor binding. Nevertheless, these studies raise questions about the optimal pre-dose 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 pre-dosing practice is optimal for all patients, particularly those who have been splenectomized 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 ~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 pre-dose 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 \textsuperscript{131}I-rituximab therapy regimen 4 weeks after completing a full 4-week rituximab therapy, finding that the clearance of the \textsuperscript{131}I-rituximab was slowed with a reduced pre-dose of 100 mg/m\textsuperscript{2}\textsuperscript{.41} Although they compared clearance of a trace dose of \textsuperscript{131}I-rituximab in 5 patients prior to the rituximab induction to a tracer dose after rituximab induction, all the post-induction patients were first pre-dosed, and how the
induction dosing might impact 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.

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 pre-dosing,
it was found that weekly-fractions of total doses $>$20 mCi/m$^2$ in 29 relapsed NHL patients with
different subtypes resulted in 72.4% objective responses (55.2% CR/CRu); all eleven follicular
cell lymphoma patients responded at 2 x 20 mCi/m$^2$ (10/11 CR/CRu).$^{42}$ 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.$^{43}$ 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 non-targeted radiation contributes to the therapeutic responses induced by RAIT, questioning whether pre-treatment 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. $^{177}$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 anti-tumor responses very significantly, while reducing hematological 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 under-utilized in the USA. Indeed, the prospects for radioimmunotherapy are increasing as it becomes further integrated into multi-modality 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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