Radioimmunotherapy of Relapsed Non-Hodgkin’s Lymphoma with Zevalin, a ⁹⁰Y-labeled Anti-CD20 Monoclonal Antibody

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Abstract

Approximately 55,400 new cases of non-Hodgkin’s lymphoma (NHL) are diagnosed each year, with the overall prevalence of the disease now estimated to be 243,000. Until recently, treatment alternatives for advanced disease included chemotherapy with or without external beam radiation. Based on the results of several clinical trials, the chimeric monoclonal antibody Rituximab has now been approved by the United States Food and Drug Administration as a treatment for patients with relapsed or refractory, low-grade or follicular, B-cell NHL. Several other monoclonal antibodies in conjugated and unconjugated forms have been evaluated in the treatment of NHL. Ibritumomab, the murine counterpart to Rituximab, radiolabeled with ⁹⁰Y (Zevalin), is presently being evaluated in clinical trials. The success of radioimmunotherapy is dependent upon the appropriate choice of antibody, isotope, and chelator-linker. The Ibritumomab antibody targets the CD20 antigen. The antibody is covalently bound to the chelator-linker tiuxetan (MX-DTPA), which tightly chelates the isotope ⁹⁰Y. To date, two Phase I/II Zevalin clinical trials have been completed in patients with low-grade, intermediate-grade, and mantle cell NHL. The overall response rate was 64% in the first trial and 67% in the later phase. Phase II and III trials are ongoing.

Introduction

Approximately 55,400 new cases of NHL³ are diagnosed each year, with the overall prevalence of the disease now estimated to be 243,000. Until recently, treatment alternatives for patients with advanced-stage NHL included only chemotherapy with or without external beam radiation. Unfortunately, relapsed NHL is rarely, if ever, curable, and remissions are less likely and shorter with each subsequent course of therapy. High dose therapy has been developed, in part, to overcome tumor cell resistance, but it is accompanied by heightened toxicity for the patient. Antibody therapy with or without conjugated radionuclides offers an alternative to traditional treatment, with the possibility of decreased toxicity (1).

Monoclonal antibodies have evolved considerably since the years following Köhler and Milstein’s development of hybridomas. Whereas some responses were observed in early clinical studies with murine antibodies, therapy was often limited by the development of HAMA, the relative inability of mouse antibodies to recruit human immune effector mechanisms for tumor killing, and down-regulation of target antigens (2).

To overcome these limitations, antibodies have been genetically engineered to mimic human antibodies more closely. This includes chimeric antibodies composed of murine variable regions and human constant regions, humanized antibodies containing human heavy and light chains with murine complementary determining regions, and PRIMATIZED antibodies containing high levels of homology with their human counterparts (3, 4). Relative to murine antibodies, these antibodies are less immunogenic (5–7). Certain chimeric, humanized, and PRIMATIZED monoclonal antibodies have been reported to have a longer half-life than their murine counterparts and greater interaction with human effector cells (3, 8). In addition to improving on the development of antibodies, therapy has also been enhanced due to the identification of target antigens that are consistently expressed on the cell surface.

Several monoclonal antibodies targeting various lymphocyte antigens have been tested in conjugated and unconjugated forms in patients with NHL including MB-1, a murine antibody against the CD37 antigen (9); Lym-1, a murine anti-HLA-DR antibody (10); LL2, an anti-CD22 antibody targeting B cells (7, 11, 12); RFB4, another anti-CD22 antibody conjugated to deglycosylated ricin A chain (13); anti-B4, a murine antibody against the CD19 antigen (14); Zevalin (IDEC-Y2B8, Ibritu-

³ The abbreviations used are: NHL, non-Hodgkin’s lymphoma; HAMA, human antimouse antibody; HACA, human antimouse antibody; HARA, human antirat antibody; CR, complete response; PR, partial response; DR, duration of response; DTPA, diethylenetriaminepента-acetic acid; ORR, overall response rate; TTP, time to progression; RIT, radioimmunotherapy.
momab tiuxetan), a murine anti-CD20 antibody conjugated to 90Y (15, 16); B1, a murine anti-CD20 antibody labeled with 131I (17, 18); and Rituximab, a chimeric antibody also targeting the CD20 antigen (3, 8, 19).

The chimeric antibody Rituximab and the murine IDEC-2B8 (Ibritumomab) antibody bind to the CD20 antigen that is present on B cells in approximately 93% of patients with B-cell NHL (20). The M, 35,000 phosphoprotein, CD20, is an appealing target for antibody therapy because it is not found on precursors to B cells, plasma cells, or other nonlymphoid normal tissues (20, 21). Therefore, after antibody depletion of B cells, only a minority of patients have significant decreases (>50%) in quantitative serum immunoglobulin (22), and a normal B-cell population can be reconstituted from stem cells and pre-pro-(or pro) B cells. The CD20 antigen plays a role in cell cycle initiation and differentiation. Interference with antigen expression through antibody binding results in inhibition of cell proliferation. The antigen does not shed from the surface of CD20+ cells and does not appear to be internalized and subsequently down-regulated (23, 24). In vitro studies have demonstrated that Rituximab binds human complement and lyses lymphoid B-cell lines through complement-dependent cytotoxicity (3). Additionally, Rituximab has significant activity in assays for antibody-dependent cellular cytotoxicity, can directly induce apoptosis (8), and can sensitize chemoresistant cells to the cytotoxic effects of doxorubicin, cisplatin, and etoposide (25).

The IgG1c chimeric monoclonal antibody Rituximab has been approved by the United States Food and Drug Administration as a treatment for patients with relapsed or refractory, low-grade or follicular, B-cell NHL and by the European Union and other countries as a treatment for stage III/IV follicular NHL (20). The M, 35,000 phosphoprotein, CD20, is an appealing target for antibody therapy because it is not found on precursors to B cells, plasma cells, or other nonlymphoid normal tissues (20, 21). Therefore, after antibody depletion of B cells, only a minority of patients have significant decreases (>50%) in quantitative serum immunoglobulin (22), and a normal B-cell population can be reconstituted from stem cells and pre-pro-(or pro) B cells. The CD20 antigen plays a role in cell cycle initiation and differentiation. Interference with antigen expression through antibody binding results in inhibition of cell proliferation. The antigen does not shed from the surface of CD20+ cells and does not appear to be internalized and subsequently down-regulated (23, 24). In vitro studies have demonstrated that Rituximab binds human complement and lyses lymphoid B-cell lines through complement-dependent cytotoxicity (3). Additionally, Rituximab has significant activity in assays for antibody-dependent cellular cytotoxicity, can directly induce apoptosis (8), and can sensitize chemoresistant cells to the cytotoxic effects of doxorubicin, cisplatin, and etoposide (25).

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Rationale for Development of Zevalin

Unconjugated antibodies, such as Rituximab, bind targeted cells and recruit immune mechanisms or directly induce apoptosis to destroy the malignancy one cell at a time. The addition of a conjugated radioisotope could potentially enhance efficacy, particularly in bulky and poorly vascularized tumors, because radiation can target more distant unbound cells.

Several issues are important in developing an optimal radioimmunotherapeutic regimen. The appropriate combination of isotope and antibody determines the safety and efficacy of treatment: the isotope must possess suitable physical properties; and the antibody must successfully target a desired antigen. To ensure the safety of a regimen, dosimetry assessments have usually been performed before administering the therapeutic radiolabeled antibody to estimate the absorbed radiation dose to normal organs.

Antibody Selection. Appropriate antibodies must be specific for a designated antigen and must also penetrate the tumor successfully. Studies comparing whole immunoglobulin and antibody fragments suggest that fragments may penetrate tumors more effectively, but this advantage is offset by their high rate of clearance (27, 28). Antibodies that are directly cytotoxic through either apoptotic or effector mechanisms can augment the efficacy of the isotope/antibody complex. Finally, if the antibody elicits an immune reaction such as a HAMA/HACA/HARA, theoretically, it could interfere with therapeutic activity by rapidly clearing antibody from circulation and could potentially increase toxicity.

Isotope Selection. The radioisotope used for therapeutic purposes must have high energy emissions to deliver a cytotoxic dose to tumor cells, whereas imaging agents must have emissions that penetrate body tissue and can be detected by imaging instruments. Iodine-131 was one of the first isotopes used for RIT because physicians had experience with this isotope in the treatment of thyroid disease. In several recent studies, 90Y has been favored for radioimmunoconjugation (15, 16, 29, 30).

The 90Y radionuclide has several theoretical advantages over 131I. As a pure β-emitting, high-energy isotope, 90Y can deliver more energy to the tumor than 131I (2.3 MeV versus 0.6 MeV of energy for 131I). Additionally, 90Y has a longer β energy path length than 131I (X90 = 5 mm for 90Y versus X90 = 1 mm for 131I), which allows tumor cells in the range (100–200 cell diameters) of the β emissions to be killed without direct binding of the antibody, a potential advantage for patients with bulky or poorly vascularized tumors. The shorter half-life of 90Y (2.7 days for 90Y versus 8 days for 131I) approximates the biological half-life of the radiolabeled antibody, thereby limiting the toxicity to healthy tissue. Because 90Y is a pure β emitter, normal organs are spared γ radiation, and patients can be treated on an outpatient basis. In contrast, the high-energy γ emissions from 131I (0.36 MeV) increase the absorbed radiation dose to the whole body and necessitate significant patient restrictions or hospitalization with extensive shielding to protect the hospital staff. Furthermore, 131I has a propensity to dehalogenate in the blood and at tumor sites, resulting in 131I accumulation in the thyroid, posing a risk of hypothyroidism. Prophylactic potassium iodide therapy before and after dosing decreases thyroid exposure, although protection is incomplete (17, 31, 32).

Studies comparing the dosimetry of 90Y and 131I radioconjugates concluded that 90Y radioimmunoconjugates result in a more favorable therapeutic index and more effectively deliver radioactivity to tumors than does 131I. Juweid et al. (30) compared the dosimetry of 131I- and 90Y-labeled LL2 (anti-CD22, an internalizing antigen) using the γ emitter 111In as a surrogate for 90Y. The estimated mean absorbed radiation dose to tumors > 3 cm in diameter was approximately 10 times greater with 90Y than with 131I (21.5 cGy/mCi with 90Y versus 2.4 cGy/mCi with 131I). The tumor/organ absorbed radiation dose
ratios were higher with $^{90}$Y than with $^{131}$I, except in the liver, where the ratios were statistically similar (33). Studies using a tumor xenograft model concluded that even with noninternalizing monoclonal antibodies, there is a more favorable dosimetry and therapeutic index with $^{90}$Y-labeled antibodies than with $^{131}$I-labeled antibodies (34, 35). More recently, this finding has also been observed in preliminary clinical studies using $^{111}$In-/ $^{90}$Y-labeled MN-14 anti-carcinoembryonic antigen monoclonal antibody (36).

Rao and Howell (37) proposed a new internal radionuclide biological effectiveness scoring model based on time-dose fractionation and compared antibodies labeled with $^{90}$Y or $^{131}$I administered at a dose of 300 cGy. Using this theoretical model, they projected the tumor:nontumor ratio for $^{90}$Y at approximately 50% higher for $^{90}$Y versus $^{131}$I, whereas the total body doses were comparable. A higher initial tumor dose rate for $^{90}$Y than for $^{131}$I contributed to the more favorable tumor:nontumor ratio for $^{90}$Y. Additionally, the ratio of dose rates (tumor dose rate relative to the body dose rate at peak tumor uptake) was approximately 50% greater for $^{90}$Y versus $^{131}$I. Ultimately, the activity required to achieve the same biological effect was 7.8 GBq for $^{90}$Y versus 25 GBq for $^{131}$I, and the specific activity required to achieve the same effect was 6.2 times greater for $^{131}$I than for $^{90}$Y.

Unlike $^{131}$I, $^{90}$Y cannot be used for imaging and dosimetry. Indium-111 has a similar physical half-life to $^{90}$Y and has therefore been used as an imaging agent before RIT with $^{90}$Y (38-41). Imaging with $^{111}$In produces higher resolution scans than $^{131}$I because gamma cameras are manufactured to detect energies in the range of those associated with $^{111}$In (approximately 140 keV), and not the higher levels emitted by $^{131}$I (80% at approximately 364 keV). In addition, with $^{111}$In, patients are exposed to lower level γ emissions than with $^{131}$I, thereby limiting toxicity to normal organs.

**Chelator-Linker Selection.** The method of linking antibodies with radionuclides is a key factor in determining the effectiveness and toxicity of therapy. Chelates are used to link radioactive metals such as $^{90}$Y to antibodies because direct metal incorporation into antibodies is not feasible. $^{131}$I links directly to the antibody and does not require a chelate. For both isotopes, the antibody-isotope complex must be stable to successfully transport the radiouclide to the tumor site. With $^{131}$I, dehalogenation can occur, causing the release of iodine. Similarly, if a chelate is used that binds the antibody poorly, free metal may be released and migrate to bone. In addition to providing a secure linkage, the chelator-linker must not interfere with antibody specificity or increase immunogenicity.

Some of the first chelators evaluated for use with radiolabeling and RIT include the polyaminocarboxylic acids EDTA and DTPA. Derivatives of these chelator-linkers were developed to increase stability. In preclinical studies, Kozak et al. (42) demonstrated that the chelating agents Ca-DTPA, 1B-EDTA, and 2B-DTPA were not appropriate for immunotherapy because they prematurely released the radiometal, resulting in bone deposition of the radioisotope. However, other derivatives of DTPA, including the chelator-linker tiuxetan (MX-DTPA), did not compromise antibody specificity, did not alter the metabolism of antibody conjugates, and did not result in a measurable release of yttrium from the antibody.

The five carboxyl groups on tiuxetan strongly chelate $^{90}$Y. In addition, tiuxetan has a higher affinity for both the $^{111}$In and $^{90}$Y radionuclides relative to DTPA because the additional methyl group on its backbone strengthens the chelate (42-45). Tiuxetan reacts rapidly and efficiently with antibodies to form a rugged urea-type bond and does not interfere with antibody binding to the target antigen. In mice, the chelator-linker exhibited excellent in vivo retention of yttrium, with no demonstrable loss of radioisotope over 4 days (42). A comparison of the tiuxetan derivative with other chelates containing DTPA or its cyclic anhydride derivative demonstrated that tiuxetan yielded $^{90}$Y-labeled antibody conjugates with increased tumor:nontumor $^{90}$Y ratios and resulted in both decreased bone uptake and greater in vivo chelate retention of $^{90}$Y (42, 46).

In vitro studies with tiuxetan used as a chelate for Chinese hamster ovary-expressed Zevalin demonstrated minimal release of $^{90}$Y from the antibody after incubation in human serum. Furthermore, no evidence of antibody degradation due to radioysis was observed. Using this chelate, the radiolabeled Zevalin antibody retained >70% binding to CD20+ cells. Preclinical studies in mice confirmed in vitro stability, demonstrating <1.5% injected dose/gram in bone after 3 days. This DTPA derivative has been used successfully as a chelator-linker in several lymphoma studies using $^{90}$Y- and $^{111}$In-labeled antibodies (47).

**Zevalin Therapy**

Ibritumomab is a murine IgG1κ monoclonal antibody. The antibody is chelated via the chelator-linker, tiuxetan, to $^{90}$Y for therapy or to $^{111}$In for imaging. The radioisotopes $^{111}$In and $^{90}$Y are obtained from Nycomed-Amersham. Like its recently approved, unlabeled, chimeric counterpart, Rituximab, Ibritumomab targets the CD20 antigen on B cells (3).

To date, two Phase I/II Zevalin clinical trials have been completed. In an early Phase I/II study, 14 patients with relapsed or refractory, low-grade or intermediate-grade NHL were treated with single doses of Zevalin ranging from a low dose of 20 mCi to a myeloablative dose of 50 mCi (15). Three patients received two treatments, with a cumulative dose of 60–70 mCi. Bone marrow harvest was required for all patients. Before scanning with IDEC-In2B8 and treatment with $^{90}$Y-labeled Zevalin, patients received an infusion of the unlabeled, murine anti-CD20 antibody Ibritumomab to improve the biodistribution of the radiolabeled antibody.

The ORR was 64%, with a median TTP in the nine responders of 9.3 months (range, 5.5–13.4 months). Adverse reactions were mainly hematological. Peripheral stem cell reinfusion due to the anticipated, prolonged marrow suppression occurred in three patients (two who received 50 mCi of $^{90}$Y-labeled Zevalin, and one who received a cumulative dose of 60 mCi).

Overall, hematological toxicity was manageable and transient, with severe myelosuppression occurring at doses > 40 mCi (>0.6 mCi/kg) of $^{90}$Y-labeled Zevalin. Hematological toxicity correlated best with mCi of $^{90}$Y administered per kilogram of body weight as compared to the total mCi or mCi/m². Therefore, later studies calculated the dose based on mCi/kg. By 6 months after treatment, circulating CD20+ B-cell levels had
returned to normal. At 2 months posttreatment, one patient developed HAMA, with the titer decreasing over time.

A later Phase I/II study was performed in 58 patients to determine the optimal nonmyeloablative dose of 90Y-labeled Zevalin in relapsed or refractory patients with low-grade, intermediate-grade, or mantle cell NHL (16, 29, 48). In this study, Rituximab, the chimeric anti-CD20 antibody, was substituted for murine Ibritumomab as the unlabeled antibody administered before treatment with 90Y-labeled Zevalin.

This Phase I/II study took place at seven sites and comprised three groups of patients. The group 1 segment was designed to determine the optimal dose of Rituximab (100 or 250 mg/m²) to be used as unlabeled antibody before IDEC-In2B8 and 90Y-labeled Zevalin administration; the group 2 segment was designed to determine the optimal dose of 90Y-labeled Zevalin (0.2, 0.3, or 0.4 mCi/kg); and the group 3 segment was designed to further evaluate safety and efficacy of the optimal Rituximab/Zevalin doses. Dosimetry calculations to determine the safety of treatment were performed at the study site.

Dosimetry studies were performed using 111In-labeled Zevalin 1 week before treatment to estimate the absorbed radiation dose to normal organs and bone marrow from 90Y-labeled Zevalin treatment. After injection of 5 mCi of IDEC-In2B8, the organ 111In activity was measured by region analysis at each imaging time using the geometric mean technique and converted to 90Y using the 90Y decay factor. The residence time was calculated from the area under the curve for each organ. Estimated absorbed radiation doses of 90Y-labeled Zevalin in normal organs and bone marrow were calculated using the MIRDOS3 program.

Patients in this study had a median of two (range, one to seven) prior chemotherapy regimens; 20% were refractory to prior chemotherapy. Most patients (59%) had bulky disease (masses ≥ 5 cm).

In all patients, normal organ- and bone marrow-absorbed radiation doses were less than the protocol-defined upper limit of acceptable (2000 and 300 cGy, respectively). The absorbed radiation dose was highest in the spleen (often involved with NHL); the median dose was 27.5 cGy/mCi (1.37-99.51 cGy/mCi) in 56 patients; the projected spleen dose was >2000 cGy (2448 cGy) in a single patient with a gastric maligoma underlying the spleen. Of the remaining major organs, the median estimated 90Y-absorbed radiation dose in 56 patients was 13.7 cGy/mCi (range, 3.21-38.48 cGy/mCi) in the liver, 9.8 cGy/mCi (range, 3.92-13.80 cGy/mCi) in the lungs, and 1.5 cGy/mCi (range, 0.24-1.84 cGy/mCi) in the kidney. Predicted absorbed tumor radiation dose in 18 tumors from nine selected patients was 1712 cGy (range, 575-6710 cGy). The median T1/2 for blood and plasma 90Y was 28 h (range, 14-36 h). The median area under the curve for blood and plasma 90Y was 24 and 22 µg h/ml (range, 4-48 µg h/ml), respectively.

In this study, the intent-to-treat ORR was 67% (25% CR and 41% PR) with a 95% confidence interval of 54–80% in 51 patients with low-grade, intermediate-grade, or mantle cell NHL treated at 0.2, 0.3, or 0.4 mCi/kg. In 34 patients with low-grade NHL, the ORR in all three dose groups was 82% (73% CR and 66% PR), and the ORR was 43% (6 of 14) intermediate-grade patients (29% CR and 14% PR). This trial required all lymph nodes to regress to ≤1.0 × 1.0 cm. When these data were reanalyzed using less stringent criteria (<1.5 × 1.5 or ≤ 2.0 × 2.0 cm), the CR rates were 31% or 41%, respectively (49). None of the three patients with mantle cell disease responded. Responses were seen in patients with bulky disease (41%) and in patients with splenomegaly (50%).

Estimates of median TTP in responders and DR projected by Kaplan-Meier analysis are 12.7 months (95% confidence interval, 11.3–19.0) and 11.6 months (95% confidence interval, 10.2–17.8), respectively. The mean change in lesion size was –97% in patients with a CR, –86% in patients achieving a PR, and –37% in patients with stable disease.

Adverse events were primarily hematological, transient, and reversible. Three patients required hospitalization for infection during the 1-year period posttreatment, and all recovered with appropriate therapy. The median nadir for the patients treated at 0.4 mCi/kg was 9.9 g/dl for hemoglobin, 1,100/mm³ for absolute granulocyte count, and 50,000/mm³ for platelets. For patients whose nadirs fell below 10 g/dl hemoglobin, 1,000/mm³ granulocytes, and 50,000/mm³ platelets, median time to recovery to these values was 9.5, 10.5, and 14 days, respectively. Mean serum immunoglobulin levels for all 51 patients remained within the normal range throughout the 1-year observation period. One patient (2%) developed a HAMA and HACA posttreatment.

In summary, Zevalin RIT in conjunction with the unlabeled chimeric anti-CD20 antibody, Rituximab, is a novel short-course treatment administered on an outpatient basis. Responses have occurred in patients with bulky disease and in patients who are refractory to chemotherapy. Results of ongoing Phase III trials will further clarify the role of Zevalin in the treatment of relapsed or refractory NHL.

References


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*Clin Cancer Res* 1999;5:3281s-3286s.

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