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Improving the Efficacy of Radioimmunotherapy for Non-Hodgkin's Lymphomas

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Abstract

Approximately 66,000 Americans develop non-Hodgkin's lymphomas (NHL) each year. Although the use of unlabeled antibodies such as rituximab has significantly improved survival when combined with standard chemotherapy regimens, two-thirds of lymphoma patients eventually relapse and succumb to their disease. Novel treatments are urgently needed to cure these patients. One strategy involves the use of radiolabeled immunoconjugates that specifically localize radiation delivery to sites of lymphoma while minimizing toxicity to normal tissues. A growing number of studies support the contention that radiolabeled antibody therapy can improve overall survival of lymphoma patients and lead to durable remissions, with probable cures, in many patients. Various approaches for enhancing the effectiveness of radioimmunoconjugates have been studied including: use in newly diagnosed lymphoma patients; combination with chemotherapy or other monoclonal antibodies; use with hematopoietic stem cell transplantation; multi-step pretargeting strategies to further minimize toxicity; and simultaneous targeting of multiple B-cell antigens. This article summarizes the current knowledge supporting the use of radioimmunotherapy, an underutilized but effective treatment modality in Non-Hodgkin's lymphoma patients.

Keywords

Radioimmunotherapy; non-Hodgkin's lymphoma; immunoconjugate; radioisotope; iodine-131 tositumomab; yttrium-90 ibritumomab tiuxetan; pretargeting; antibody

Introduction

Approximately 66,000 Americans develop non-Hodgkin's lymphomas (NHL) each year and approximately one third of them are cured with standard chemoimmunotherapy consisting of rituximab (Rituxan®) and combination chemotherapy regimens. Conversely, two-thirds of the patients with NHL relapse and eventually succumb to their disease despite conventional chemotherapy, radiotherapy, and immunotherapy, emphasizing the urgency for improved treatments for NHL. In recent years, radiolabeled monoclonal antibodies have emerged as a safe and effective, though under-utilized, treatment modality for treatment of B cell lymphomas. 1

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Current Status of Non-myeloablative Radioimmunotherapy

Gerald and Sally DeNardo first established the promise of radioimmunotherapy (RIT) for the treatment of relapsed B-cell lymphomas over 20 years ago.² In a series of pioneering studies using iodine-131 and copper-67-labeled Lym-1 anti-DR antibody, they demonstrated that nearly half of patients treated with radiolabeled antibodies could achieve remission even after failing multiple prior chemotherapy regimens and approximately one-third of patients achieved complete remissions. Unfortunately, despite these early encouraging results, virtually all of the patients relapsed and died by 5 years after treatment³. Most subsequent studies have focused on targeting the CD20 transmembrane antigen which is present on virtually all B-cell lymphomas as well as normal B-lymphocytes. Multiple studies have demonstrated that non-myeloablative doses of either iodine-131 tositumomab (Bexxar®) or yttrium-90 ibritumomab tiuxetan (Zevalin®) induce remissions in 50–80% of patients with either relapsed or refractory indolent lymphomas and 15–50% can achieve complete remissions.^{4–9} Unfortunately, most of these remissions last only 6–15 months, although a minority of patients (15–20%) obtain durable remissions lasting many years.¹⁰ In a randomized study of 143 patients with relapsed follicular lymphoma treated with either rituximab or yttrium-90 ibritumomab tiuxetan, Witzig and others demonstrated that statistically superior overall response rates (ORR) and complete response (CR) rates were achievable with yttrium-90 ibritumomab tiuxetan compared to rituximab (ORR 80% versus 56%; CR 30% versus 16%, $P=0.002$).¹¹ Unfortunately, there was no improvement in the time to progression on the two arms of the study. However, this small randomized trial was not powered to detect such differences. More recently, Dr. Goldenberg and his colleagues have investigated the efficacy of fractionated yttrium-90-DOTA-epratuzumab (anti-CD22) antibody for patients with relapsed B-cell lymphomas.¹² In this study of 54 patients, an ORR of 59% and a CR rate of 43% were observed. Although longer follow-up will be required to determine the durability of these responses, 6 patients remain progression-free with follow-up beyond 1 year and 4 patients remain progression-free with follow-up beyond 2 years.

Approaches to Improve the Efficacy of Radioimmunotherapy

While the results summarized above with standard non-myeloablative doses of radiolabeled antibodies demonstrate the value of this therapeutic approach, it is clear that most patients treated with conventional RIT will relapse and ultimately die of their lymphoma. Therefore, improvements must be sought to provide long-lasting remissions and, eventually, cures for this disease. There are at least five strategies which have recently been utilized to improve the efficacy of RIT for NHL: 1) RIT as part of first-line treatment of newly diagnosed lymphoma patients; 2) combining RIT with chemotherapy or other monoclonal antibodies; 3) high-dose RIT with hematopoietic stem cell transplantation; 4) multi-step pretargeting strategies for improving the efficacy and decreasing the toxicity of RIT; and 5) simultaneous targeting of multiple B-cell antigens. This article serves as a review of the recent clinical and preclinical studies supporting the use of these strategies.

Front-Line Radioimmunotherapy

In recent years there have been six phase II studies conducted in the United States evaluating the potential efficacy of administration of radiolabeled monoclonal antibodies in patients who have not previously been treated with chemotherapy, either using the radiolabeled antibodies by themselves or in combination with induction chemotherapy regimens. Kaminski *et al* treated 76 patients with untreated grade 1 or 2 follicular lymphoma with standard dose iodine-131 tositumomab (Bexxar) as a single agent without chemotherapy and achieved responses in nearly 95% of patients and complete remissions in ~75%. These

remissions were very durable with a time to progression in excess of 5 years and an approximately 90% overall survival (OS) at 5 years.¹³ Press and colleagues in the Southwest Oncology Group studied the use of combined chemo-radioimmunotherapy using six cycles of the CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) regimen followed six to ten weeks later by iodine-131 tositumomab in 84 evaluable patients with previously untreated grade 1–3 follicular lymphoma.¹⁴ An ORR of 98% was observed. After completion of 6 cycles of CHOP chemotherapy, only 44% had achieved a CR. However, the CR rate improved to 74% after a single dose of RIT. While only 18% of informative patients achieved PCR-negativity for the t(14:18) translocation after 6 cycles of CHOP, 84% of evaluable patients had achieved PCR-negativity and molecular remissions at the completion of iodine-131 tositumomab administration. The combination treatment of CHOP followed by iodine-131 tositumomab was extremely well-tolerated in this study. There were substantially more grade 3 and 4 hematopoietic toxicities in patients during the CHOP phase of the treatment than following iodine-131 tositumomab with 46% grade 3 or 4 neutropenia during CHOP compared to only 13% after iodine-131 tositumomab. There was slightly more thrombocytopenia after iodine-131 tositumomab (11% vs. 1%). Red blood cell and platelet transfusions were required in only 2% and 3% of patients respectively following iodine-131 tositumomab on this trial. With a median follow-up of 5.1 years, the 5-year estimate of OS was 87% with a progression-free survival (PFS) of 67%. Comparing the results of this trial, SWOG 9911, to previous Southwest Oncology Group trials using chemotherapy with CHOP alone for similar patients with grade 1–3 follicular lymphoma, there was a 23% improvement both in PFS at 5 years (67% CHOP plus iodine-131 tositumomab vs. 44% CHOP alone) and in 5-year OS (87% with CHOP plus iodine-131 tositumomab vs. 64% CHOP alone). Similar advantageous results were obtained by several groups including: Dr. Leonard and colleagues in a trial of 35 patients treated with three cycles of fludarabine followed eight weeks later by iodine-131 tositumomab¹⁵; Dr. Link and others at the University of Iowa using six cycles of CVP (cyclophosphamide, vincristine, prednisone) followed by iodine-131 tositumomab within 56 days of completion of CVP treatment¹⁶; Dr. Hainsworth and colleagues using four weeks of rituximab alone followed by three cycles R-CHOP (rituximab plus CHOP) then yttrium-90 ibritumomab tiuxetan given five weeks after the last cycle of R-CHOP¹⁷; and by Dr. Jacobs and others with R-CHOP for three cycles followed by yttrium-90 ibritumomab tiuxetan after bone marrow recovery then four weekly rituximab doses one to two weeks after receiving RIT.¹⁸ These studies support the contention that RIT is well-tolerated following chemotherapy. Non-hematologic toxicities are mild and hematologic toxicities are moderate. All six phase II studies demonstrated ORRs between 90–100% with complete CRs of 60–95% and excellent progression-free and overall survivals (Figure 1). These studies also demonstrated that RIT converts many partial remissions to complete remissions and many PCR-positive patients to PCR-negative patients.

Most recently Morschhauser and colleagues conducted a phase III randomized trial in Europe of yttrium-90 ibritumomab tiuxetan consolidation after first remission in advanced-stage follicular lymphomas. They enrolled 414 patients with newly diagnosed grade 1 or 2 follicular lymphoma who had achieved a complete or partial remission following first-line chemotherapy with CVP, CHOP, fludarabine, chlorambucil or rituximab-chemotherapy combinations.¹⁹ Two hundred and eight patients were consolidated six to twelve weeks after the last dose of chemotherapy with standard-dose yttrium-90 ibritumomab tiuxetan, whereas 206 control patients received no further treatment. The median PFS was 37 months for the 208 patients treated with yttrium-90 ibritumomab tiuxetan versus 13.5 months for those treated with control, demonstrating the marked value of yttrium-90 ibritumomab tiuxetan consolidation ($P \leq 0.0001$) (Figure 2).¹⁹ Toxicity was minimal and largely confined to expected neutropenia, nadiring 5–6 weeks following RIT. The median nadir platelet count was approximately 50,000 and the median nadir neutrophil count was

approximately 1000. These salutary findings have led to the regulatory approval of yttrium-90 ibritumomab tiuxetan (Zevalin®) RIT as first-line consolidation therapy for follicular lymphoma in Europe.

High-dose Radioimmunotherapy with Stem Cell Transplantation

An alternative approach to improve the outcome of patients with relapsed lymphoma is to utilize myeloablative doses of RIT followed by either autologous or allogeneic stem cell transplantation. This approach is based on observations made at Stanford many years ago by Henry Kaplan indicating that recurrence rates following external beam radiation therapy are a function of the radiation dose delivered.²⁰ In these studies, more than 60% of patients treated with 20 Grays (Gy) experienced in-field recurrences following local radiotherapy, whereas only 6% of patients treated with doses higher than 40 Gy experienced such local recurrences. Our group therefore hypothesized that by replacing conventional total body irradiation, commonly used as a bone marrow transplant conditioning regimen, with targeted high-dose RIT, one would be able to focus a much higher dose of radiation on the tumor and a much lower dose on normal organs, thereby improving the cure rate and decreasing the toxicities (Figure 3). Our group conducted a series of phase I and II studies of myeloablative doses of iodine 131-labeled anti-CD20 and anti-CD37 monoclonal antibodies and demonstrated that 86% of multiply relapsed patients achieved objective remissions and 79% achieved complete remissions with this single agent approach.^{21–23} The median PFS with myeloablative doses of iodine-131 tositumomab was over 5 years, whereas the median PFS from 5 pivotal trials of non-myeloablative iodine-131 tositumomab was less than 1 year. We subsequently combined myeloablative doses of iodine-131 tositumomab with 60 mg/kg of etoposide and 100 mg/kg of cyclophosphamide in a phase I/II-dose escalation study.²⁴ In this trial using individualized patient dosimetry, we demonstrated that the maximally tolerated dose of iodine-131 tositumomab with cyclophosphamide and etoposide was 25 Gy to critical normal organs. The OS and PFS of all treated patients were 83% and 68% respectively. These figures compared favorably to the OS and PFS of a non-randomized control group transplanted after a regimen of external beam total body irradiation, etoposide and cyclophosphamide during the same time period (OS 53%; PFS 36% at 2 years), even after adjusting for confounding factors in a multi-variable analysis.²⁴ Several other groups have emulated this approach and published similarly promising results. Nademanee and colleagues published their experience in 31 patients with B-cell lymphomas treated with high-dose yttrium-90 ibritumomab tiuxetan, etoposide, cyclophosphamide, and autologous stem cell transplantation, and demonstrated an OS of 100% in grade 1 and 2 follicular lymphoma, 93% with follicular large cell lymphoma and diffuse large cell lymphoma and 75% with mantle cell lymphoma at 2 years.²⁵ Dr. Jane Winter in Chicago has recently published similar results using yttrium-90 ibritumomab tiuxetan and BEAM (carmustine, etoposide, cytarabine, melphalan).²⁶ Most recently, Devizzi and colleagues have published results from a study utilizing high-dose yttrium-90 ibritumomab tiuxetan with tandem peripheral blood stem cell re-infusion for autologous transplant.²⁷ The preparatory regimen consisted of 3 cycles of either DHAP (cisplatin, cytarabine, dexamethasone) or CHOP chemotherapy followed by high-dose rituximab plus cyclophosphamide 4–7 g/m², followed by collection of peripheral blood stem cells then rituximab plus high-dose cytarabine 3–4 g/m²/day for 3–6 days. An additional harvest of peripheral blood stem cells was performed after cytarabine treatment if insufficient cells were collected after rituximab-cyclophosphamide or the first harvest was positive for minimal residual disease. Patients were subsequently consolidated with high-dose yttrium-90 ibritumomab tiuxetan at a dose of either 0.8 mCi/kg (13 patients) or 1.2 mCi/kg (17 patients). Patients were given two separate infusions of peripheral blood stem cells following the high-dose RIT: 2 million CD34 cells/kg on day 7 after RIT and ≤ 5 million CD34 cells/kg on day 14. This study included 30 patients with NHL (12 follicular lymphoma, 10 diffuse large cell lymphoma, 3 mantle cell

lymphoma, 5 other), 25 of whom had relapsed or refractory disease, 5 with *de novo* high-risk presentations and 1 with Richter's transformation. After 30 months follow-up, the OS was 87% and the event-free survival was 69%.²⁷ The high-dose RIT was tolerated with moderate cytopenias of short duration. The double stem cell infusion appeared successful in ameliorating both the extent and duration of the cytopenias with half of the patients escaping grade 4 neutropenia or thrombocytopenia. Only 3 of the patients required hospitalization for a median of 2 days, all for febrile neutropenia.

Krishnan also published a phase II study of yttrium-90 ibritumomab tiuxetan.²⁸ Recently, Julie Vose conducted a study with standard-dose iodine-131 tositumomab preceding the BEAM regimen for patients with chemotherapy-sensitive relapsed diffuse large B cell lymphoma which demonstrated approximately 75% survival in 40 patients (unpublished). This latter trial has been translated into a nationwide phase III randomized trial of iodine-131 tositumomab/BEAM versus rituximab/BEAM for patients with relapsed diffuse large B-cell lymphoma through the Clinical Trials Network. This trial has currently accrued approximately 180 of the 225 planned patients.

In conclusion, several independent studies by different investigators have demonstrated that a large fraction of patients with relapsed B cell lymphoma treated with high-dose RIT and stem cell transplantation appear to be permanently cured.

Multi-step Pretargeting

Despite the dramatic successes of high-dose RIT combined with autologous stem cell transplantation using conventional directly radiolabeled monoclonal antibodies, there are still several problems with this approach. It is quite toxic, requiring prolonged hospitalization in most cases. It is associated with significant morbidities including 3–5% transplant related mortality and recurrence in some patients. Several studies have indicated that the efficacy of RIT can be enhanced and the tumor to normal organ ratios of radioactivity dramatically improved by multi-step pretargeting methods.^{29–35} These multi-step methods generally involve initial administration of the targeting monoclonal antibody conjugated to a non-radioactive adapter molecule. It generally takes 24–48 hours for the large antibody molecule to localize optimally in tumor sites. Following this localization, a small molecular weight radiolabeled ligand is administered which rapidly penetrates the tumor site and binds tightly to the adapter molecule. Any excess radiolabeled ligand is rapidly excreted in the urine. This approach can dramatically improve the tumor to normal organ ratios of absorbed radioactivity (Figure 5).³⁶ We have been investigating this approach using antibody-streptavidin chemical conjugates and fusion proteins, which target the CD20, CD22, HLA-DR and CD45 antigens. In mouse xenograft models of both leukemias and lymphomas, we have been able to cure 100% of animals using optimal doses, which are minimally toxic. Conversely in these models, we were unable to cure any animals using conventional yttrium-90 or iodine-131 directly radiolabeled monoclonal antibodies. Similarly favorable results have been obtained by Drs. Goldenberg, Sharkey, and colleagues, who have investigated a bivalent hapten that permits cooperative binding, thereby linking two bi-specific antibodies on the tumor cell surface using the bivalent hapten as a bridge. Their "affinity enhancement system" employs Fab fragments of anti-tumor antibodies and Fab fragments of anti-hapten antibodies. Spontaneous cyclization of the bivalent hapten resulting from two molecules of bispecific F(ab')₂ binding to two antigen molecules on the tumor cell surface stabilizes the radioligand on the cell surface by cooperative binding. This approach has yielded impressive results in both imaging and therapeutic applications. More recently this group has engineered a novel platform technology termed the "dock and lock" method which uses a natural binding between the regulatory sub-units of cAMP-dependent protein kinase and the anchoring domains of A kinase anchor proteins for quantitative and

site-specific coupling of different biological modular sub-units for diverse medical applications, including pretargeted radioimmunotherapy.³⁵ They have generated bi-specific trivalent binding complexes composed of three stably linked Fab fragments capable of selective delivery of radiotracers to human cancer xenografts resulting in rapid significantly improved cancer targeting and imaging providing tumor to blood ratios from 66 ± 5 at 1 hr and 395 ± 26 at 24 hrs.^{33, 35, 37}

Despite these promising preclinical results, several aspects of the pretargeting strategy must be carefully considered to further refine this therapy. These include optimization of antibody or peptide conjugate dose, identification of the ideal time interval between antibody administration and subsequent radiolabelled ligand delivery, reduction of reagent immunogenicity and selection of the appropriate radioisotope. Each of these variables must be tailored to the clinical situation. The increased cost and complexity of administering pretargeted RIT compared to directly-labeled RIT may also be disadvantageous. Nevertheless, the potential for pretargeted RIT to deliver higher doses of radioactivity to tumor while minimizing toxicity to normal tissue cannot be overlooked and provides strong incentive to pursue this treatment modality.

Targeting Multiple Different Antigens

We and others have investigated the utility of targeting several different tumor antigens simultaneously. In a series of studies investigating simultaneous targeting of CD20, CD22, and HLA-DR in lymphoma xenograft models, we discovered that the radioimmunoconjugate yielding the best tumor uptake and tumor to normal organ ratios of radioactivity varied depending on the target antigen expressed on the cell line employed, with anti-CD20 and anti-DR yielding more promising results overall than anti-CD22.^{31, 38, 39} An alternative approach is being proposed by Sharkey and colleagues, who suggest combining an unconjugated anti-CD20 antibody with a radioimmunoconjugate targeting a non-competing antigen such as CD22. Pre-clinical models with this approach have indicated that careful consideration must be given to pre-dosing when using competing antibodies, but that consolidation using anti-CD20 therapy enhances the efficacy of radioimmunoconjugate therapy.⁴⁰

Conclusion

Radiolabeled antibodies are effective and safe for treatment of patients with B-cell lymphomas in multiple clinical settings. Innovative new approaches promise to increase the durability of remissions achieved with radiolabeled antibody therapy. Incorporation of RIT into front-line treatment along with standard induction combination chemotherapy is extremely promising, yielding very high complete remission rates and long remission durations. It is hopeful that some of these patients will be permanently cured. Particularly promising are new multi-step pretargeting methods which have shown dramatic results in animal models and merit investigation in clinical trials in the near future.

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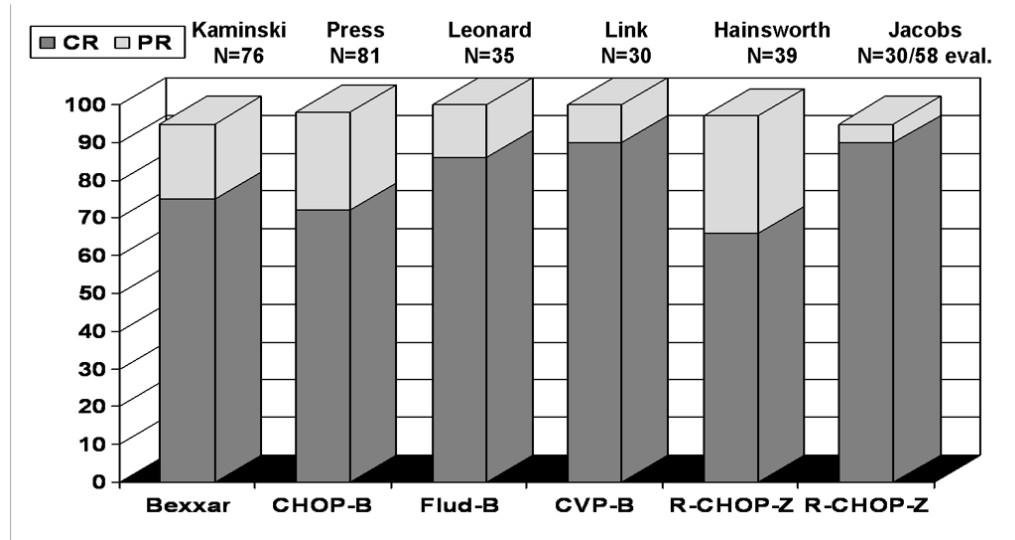
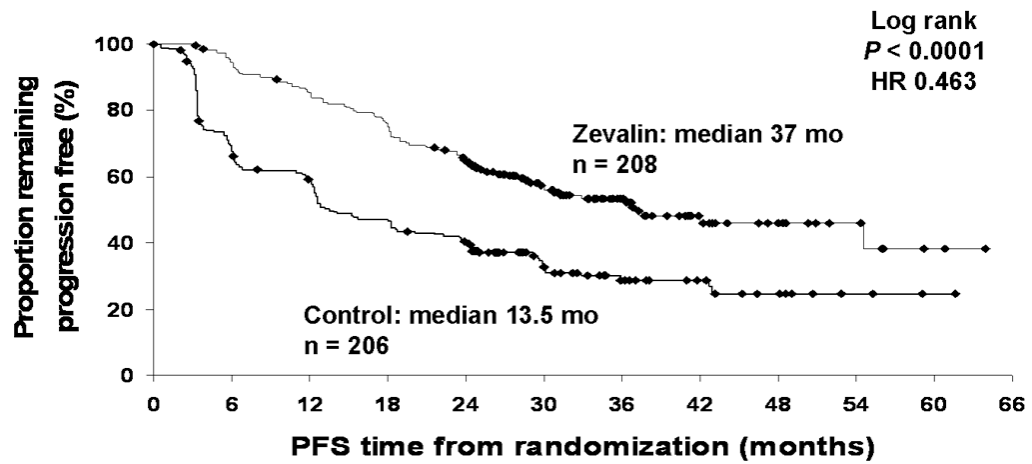


Figure 1. Studies using radioimmunotherapy with or without chemotherapy for previously untreated lymphoma patients (references 13–18). Abbreviations used: N, number of patients; CR, complete response; PR, partial response; B, Bexxar/iodine-131 tositumomab; Z, Zevalin/yttrium-90 ibritumomab tiuxetan.



*Median observation period was 3.5 years.

Figure 2.

Median progression-free survival of patients with advanced grade 1 or 2 follicular lymphoma treated with Zevalin/yttrium-90 ibritumomab tiuxetan consolidation after first remission (thin line) compared to controls (thick line) [reproduced with permission from Morschhauser *et al.*, *J Clin Oncol* 2008; 26:5160.]¹⁹

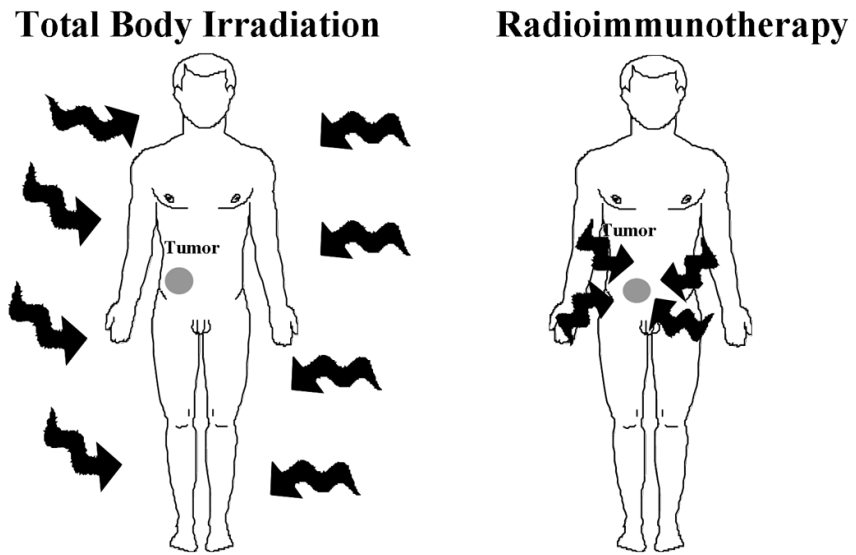


Figure 3. Schematic illustration demonstrating targeted exposure of tumor tissue versus normal tissue using radioimmunotherapy compared to conventional total body irradiation as conditioning prior to hematopoietic stem cell transplant.

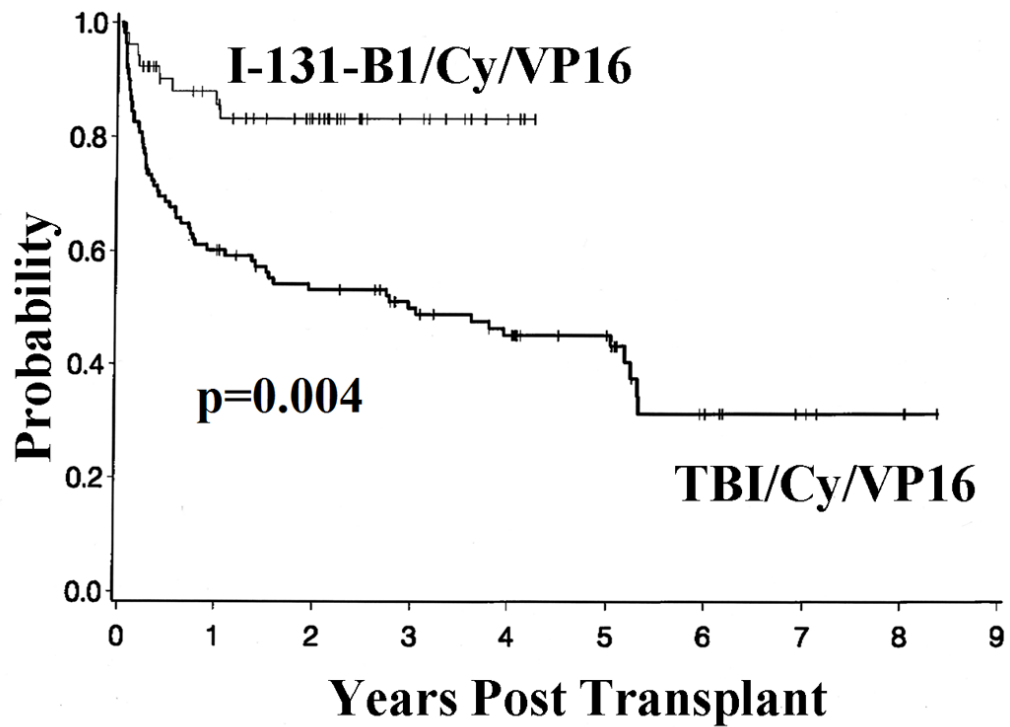


Figure 4. Comparison of overall survival in patients treated with myeloablative iodine-131 tositimomab, cyclophosphamide, and etoposide (I-131-B1/Cy/VP-16) followed by autologous transplant versus non-randomized controls conditioned with total body irradiation, cyclophosphamide and etoposide (TBI/Cy/VP-16) [reproduced with permission from Press *et al.*, *Blood* 2000;16:2938.]²⁴

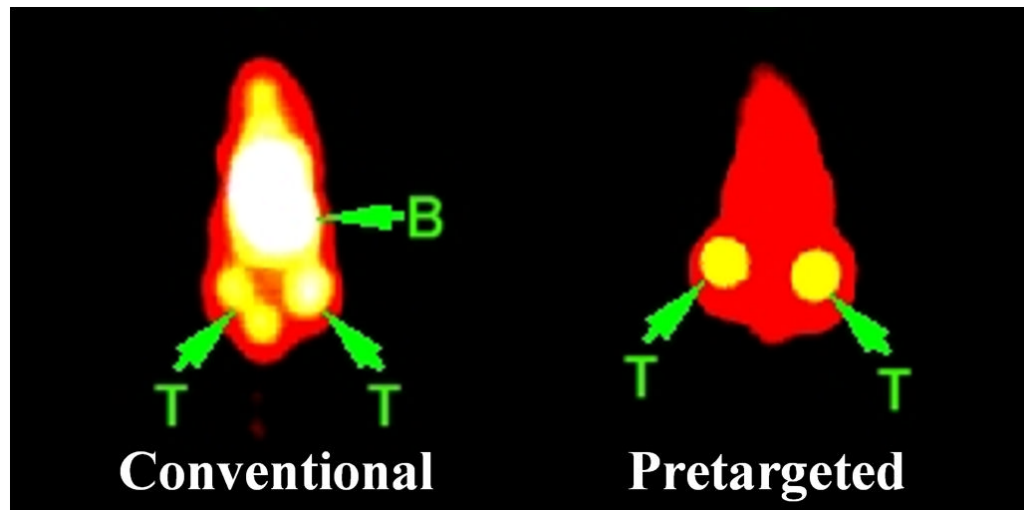


Figure 5.

Improved tumor to normal organ ratio of absorbed radioactivity with the pretargeting approach compared to conventional radioimmunotherapy. Gamma images taken of mice bearing bilateral lymphoma xenografts 24 hours after treatment with either directly labeled $^{111}\text{-indium}$ 1F5 (anti-CD20) antibody (conventional) or unlabeled 1F5 antibody-streptavidin conjugate followed by $^{111}\text{-Indium}$ DOTA-biotin (pretargeted). Abbreviations: T, tumor; B, blood pool. [reproduced with permission from Subbiah *et al.*, *J Nuc Med* 2003;44:440.]³⁶