Radioimmunotherapy strategies for non-Hodgkin's lymphomas

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Summary
Radioimmunotherapy offers an exciting new therapeutic modality for patients with relapsed non-Hodgkin's lymphoma; however, considerable debate exists regarding the optimal dose and administration schedule for radioimmunoconjugates. Myelosuppression has been the dose-limiting toxicity of most clinical trials employing radiolabeled antibodies, and this complication has generated both high-dose and low-dose treatment strategies. 'Low-dose' strategies are nonmyeloablative and rely upon repetitive infusions to effectively eradicate tumor masses. Trials incorporating low-dose radioimmunotherapy have documented high response rates, though the durability of these responses remains unclear. The most encouraging nonmyeloablative studies have documented objective responses in 70%-80% of patients, complete responses in 30%-50% of patients, minimal toxicity, and a median response duration of 12 months. In contrast, high-dose trials performed in conjunction with autologous hematopoietic stem cell transplantation have demonstrated objective responses in 95% of patients, complete responses in 85% of patients, with a progression-free survival of 62% and an overall survival of 93% with a median follow-up of two years. Toxicities are considerably higher than those reported with nonmyeloablative regimes, but are modest compared to conventional marrow transplant conditioning regimens incorporating total body irradiation (TBI). Ongoing trials integrating high-dose radioimmunotherapy with high-dose chemotherapy in an autologous transplantation setting are testing the hypothesis that targeted radiotherapy plus chemotherapy will provide increased efficacy and diminished toxicity as compared to nonspecific external beam TBI-containing regimens.

Key words: bone marrow transplantation, immunotherapy, monoclonal antibodies, non-Hodgkin's lymphoma, radioimmunotherapy

Introduction
Patients with relapsed non-Hodgkin's lymphoma are incurable with conventional chemotherapy. Approximately 40%-50% of patients in early first relapse or second remission can be salvaged with bone marrow transplantation following high-dose chemotherapy and radiation, but only 10%-20% of patients transplanted with more advanced disease can be cured [1, 2]. Conventional autologous transplantation is associated with a greater than 50% chance of recurrent lymphoma, in addition to a 3%-15% risk of treatment-related mortality from infection, veno-occlusive disease of the liver, interstitial lung disease, and renal failure [3]. New oncologic strategies are thus needed to improve cure rates for relapsed non-Hodgkin's lymphoma and to diminish treatment-related toxicity.

Monoclonal antibodies appear to offer several therapeutic avenues for patients with relapsed lymphomas. Unmodified monoclonal antibodies provide an approach by which the patient's own immune system might eradicate antibody-coated tumor cells through several different mechanisms. Antibody-coated cells may be lysed by complement fixation or killed by phagocytosis or antibody-dependent cell-mediated cytotoxicity (ADCC). Because B-cell lymphomas express clonal cell surface Ig, idiotypic monoclonal antibodies can be produced to recognize and target cells bearing tumor idiotype [4]. A third approach uses monoclonal antibodies to bind to tumor cell growth factor or cellular receptors such as interleukin-2 and transferrin [5-7]. A fourth approach involves covalent conjugation of chemotherapeutic drugs, toxins, or radionuclides to monoclonal antibodies and delivery of cytotoxic moieties directly to the tumor cells. Therapeutic promise has been demonstrated with each of these approaches, but the most impressive results, so far, have come from studies utilizing radioimmunoconjugates.

Unmodified Monoclonal Antibodies
Treatment of non-Hodgkin's lymphomas with unmodified mouse monoclonal antibodies has demonstrated few toxicities but only modest clinical activity [8]. Studies incorporating mouse monoclonal anti-CD20 antibodies (pan-B-cell reactive antibody) [9] and Camphath series antibodies (pan-lymphoid antibody) [10, 11] have documented only temporary and partial responses. Treatment failure has been felt to be multifactorial, resulting from (1) inactivity of the patient's immune system to recognize and eliminate tumor cells coated with mouse monoclonal antibodies, (2) antigen-negative tumor cell variants, (3) inactivity of monoclonal antibodies to penetrate large tumor masses, (4) antigenic modulation, and (5) the formation of human anti-mouse antibodies (HAMA).
Tumor-specific anti-idiotypic monoclonal antibodies have produced objective responses in 50%-70% of treated patients with relapsed non-Hodgkin's lymphomas [12-14]; however, broad application of this technique has been limited by the logistic difficulties inherent in creating custom anti-idiotypic antibodies for individual patients. This process is labor and time intensive, typically taking a year to produce sufficient antibody for clinical applications. This difficulty has been partially overcome by the recognition of 'public idiotypes', and by the construction of libraries of cross-reactive anti-idiotypic antibodies which can be shared among patients [15]. Furthermore, the existence of high levels of circulating free idiotype protein in many patients and the in vivo selection of idiotype-negative tumor cell variants have proven to be significant obstacles for the clinical implementation of anti-idiotypic antibody therapy. Attempts at preventing the emergence of idiotype-negative variants with concurrent chemotherapeutic have unfortunately been unsuccessful to date [16].

Genetically engineered chimeric monoclonal antibodies containing human Fc constant regions and mouse variable regions have been shown to possess an enhanced ability to fix human complement and to induce antibody-dependent cell-mediated cytolysis with less immunogenicity than murine monoclonal antibodies [17]. Clinical experience with the chimeric anti-CD20 antibody (IDEC-C2B8) in a phase I single-dose, dose-escalation study noted only minor symptoms with antibody infusion, no long-term toxicity, and persistence of antibody bound to tumor cells two weeks after completion of antibody infusion [18]. Tumors responded to C2B8 therapy in 6 of 15 patients (2 partial responses and 4 minor responses). In a subsequent phase II trial, 37 patients received four 375 mg/m² weekly infusions of IDEC-C2B8 [19]. An overall response rate of 50% was noted (3 complete and 14 partial remissions) with a median time to progression of 10.3 months (4.3-20+) for responding patients. Promising results have been reported in preliminary fashion for a combination trial incorporating six infusions of IDEC-C2B8 with six cycles of CHOP in patients with low-grade lymphoma. All 14 patients who have completed therapy so far have had objective responses (11 complete and 3 partial remissions) [20]. All four patients known to be bcl-2 positive by the polymerase chain reaction prior to therapy obtained molecular complete remissions.

Radioimmunotherapy

Many of the limitations of unmodified monoclonal antibodies may be overcome with radioimmunotherapy. Tumor cells are predominantly killed by ionizing radiation emitted by the radioimmunoconjugates, and therefore, this method does not depend on host effector mechanisms, which are often defective in patients with lymphomas. Beta emitters such as iodine-131 and yttrium-90 eliminate tumor cells within 1-5 mm of their deposition [21]. Thus, radionuclide emissions extend over several cell diameters and impinge on antigen-negative cells through crossfire from surrounding antigen-positive tumor cells coated with radiolabeled antibodies. Antibody penetration into large tumor masses is problematic, but may be enhanced by either antibody selection (using F[ab']2, Fab', or Fv fragments) or patient selection ('debunking' patients until they have low tumor burdens prior to therapy). Most investigators believe that noninternalizing surface membrane antigens are optimal targets for radioimmunotherapy with antibodies labeled with iodine-131 using conventional methods susceptible to deiodination since endocytosis of bound antibody results in metabolic degradation and loss of radionuclide from targeted tumor cells. This explanation is probably partially responsible for the success of radioimmunotherapy trials targeting integral surface membrane components such as CD20, which is neither shed into the circulation nor internalized after antibody binding.

Radiolabeled antibodies expose tumor cells to continuous variable, low-dose rate radiation (<0.7 cGy/min) for extended intervals as compared to conventional fractionated external beam radiation (dose rates ~ 100 cGy/min) [22, 23]. Theoretically, continuous radiation delivered by the natural decay of a radionuclide allows less opportunity for tumor cell repair of sublethal damage and possible synchronization of cells into the more radiosensitive G2 phase of the cell cycle [22, 24]. In addition, preferential perivascular deposition of radiolabeled antibodies often obliterates nutritive tumor vessels [22].

Non-Hodgkin's lymphomas appear to be optimally suited for radioimmunotherapeutic approaches. First, B-cell malignancies express a number of well-defined surface antigens for which multiple monoclonal antibodies are available [25, 26]. Second, lymphomas are relatively radiosensitive, and possess a steep dose-response curve for radiation-induced cell killing. Third, radiobiological experiments suggest that lymphoma cells (and other hematopoietic cells) do not have a detectable radiation threshold before cell injury occurs. In contrast, the parenchymal cells of visceral organs (lung, liver, and kidney) possess a threshold for radiation-induced cytotoxicity, suggesting that these cells should be preferentially spared from the toxic effects of the low-dose rate radiation emitted by radiolabeled antibodies [27]. Finally, lymphoma patients have a reduced risk of human anti-mouse antibody formation (10%-33%), presumably due to the inherent immunosuppression associated with the disease [28, 29].

The exquisite radiosensitivity of bone marrow and its tremendous vascularity results in dose-limiting myelosuppression for patients treated with radiolabeled antibodies. Two strategies have emerged to deal with this problem. One approach utilizes repetitive infusions of 'low', nonmyeloablative doses of radiolabeled antibodies, hypothesizing that each treatment should strip away successive layers of tumor cells. Unfortunately, repetitive administration of murine monoclonal antibodies increases the likelihood of HAMA formation and often results in cumulative thrombocytopenia. Proponents of this ap-
proach suggest that HAMA may be diminished with immunosuppressants such as cyclosporine or removed by plasmapheresis [30, 31]. An alternative approach exploits the steep dose-response curve for hematologic malignancies by administering ‘high’, myeloablative doses of radioactivity in conjunction with hematopoietic reconstitution using cryopreserved autologous bone marrow or peripheral blood stem cells, which are reinfused after clearance of circulating radioactivity.

Nonmyeloablative radioimmunotherapy

Many therapeutic trials with nonmyeloablative doses of radiolabeled antilymphoma antibodies have been reported. Varying antigenic targets, antibody doses, isotopes, radioactivity doses, and patient selection criteria make it difficult to compare results and to formulate a consensus regarding optimum treatment strategies. Iodine-131- or yttrium-90-labeled antibodies have provided objective tumor responses when targeting CD20 [32, 33], CD21 [34, 35], CD22 [36], CD37 [37, 38], idiotypic immunoglobulin [39], and HLA class II molecules [40-43]. CD5 has been targeted successfully in cutaneous T-cell lymphomas [30, 44, 45] and chronic lymphocytic leukemia [44, 46]. Overall, nonmyeloablative doses of radiolabeled antibodies have yielded objective partial or complete remissions in approximately 40% of treated patients with response durations varying from 2 to 15 months [reviewed in 47]. The dose-limiting toxicity has been myelosuppression, particularly thrombocytopenia. Transient fever, nausea, and pruritus have also been noted.

A particularly noteworthy phase I study was published by Kaminski from the University of Michigan utilizing anti-B1 (anti-CD20) antibodies in patients with recurrent B-cell non-Hodgkin’s lymphomas [33]. Biodistribution studies were performed with trace-labeled infusions (5 mCi). Patients were pretreated with varying doses of unlabeled antibody to produce the best tumor targeting (highest tumor to whole-body dose ratio) in tracer studies. Theoretically, pretreatment partially saturated easily accessible ‘antigenic sinks’ (such as the spleen) and non-specific binding sites, thus allowing better penetration of subsequently administered radiolabeled antibody to more inaccessible tumor sites (e.g., lymph nodes). Dosimetric calculations determined the amount of radioactivity needed to achieve specific whole-body radiation doses. Of 10 patients, 9 received therapeutic doses (34–66 mCi) of radioimmunotherapy achieving whole-body doses from 25 to 45 cGy. Four patients were treated twice. Partial or complete responses were noted in 6 of 9 patients.

Kaminski and colleagues have recently updated their studies and reported on treatment of 47 patients with iodine-131-B1 (anti-CD20) antibody [48]. Serial biodistribution studies conducted with trace-labeled antibody established a dose of 685 mg of unlabeled (‘cold’) anti-B1 antibody to be optimal prior to infusion of 15–20 mg of radiolabeled antibody. Of the 47 evaluable patients with intermediate- or low-grade lymphoma, 32 patients had chemotherapy-resistant disease, 16 had tumor burdens greater than 500 cc, and 11 had a prior bone marrow transplant (BMT). In an escalating-dose format, patients received therapeutic radioimmunotherapy in cohorts of three patients (25–85 cGy whole-body radiation in increments of 10 cGy). The maximum tolerated dose (MTD) for patients who had not previously undergone BMT was determined to be 75 cGy, as defined by prolonged grade 3 and 4 myelosuppression in patients treated at the 85 cGy dose level. Thirty-four of 47 patients achieved an objective response (72%), including 16 complete responses (34%), and 18 partial responses (38%). The median duration of complete responses was greater than 12 months. Acute toxicities (fever, chills, nausea, vomiting, pruritus, urticaria) associated with antibody administration were generally minimal. Low-grade hematologic toxicity was noted in all patients except those who had a history of prior BMT. Despite the temporary disappearance of circulating B cells, no opportunistic infections or decreases in immunoglobulin levels were noted.

High-dose radioimmunotherapy with autologous stem-cell support

Although encouraging results for patients with recurrent B-cell non-Hodgkin’s lymphoma are offered by the nonmyeloablative regimens, theoretical considerations suggest that higher doses of radiation may allow maximal and possibly curative therapeutic benefit from targeted radiotherapy. Our group in Seattle has incorporated high-dose iodine-131-pan-B-cell antibodies with autologous bone marrow rescue in sequential phase I and II studies and are currently accruing patients in a combined modality phase I/II study. The phase I study established the nonhematopoietic dose-limiting radiation toxicity of iodine-131 conjugated to anti-B-cell antibodies [32]. The phase II study determined the response rate, response duration, and resultant toxicities in patients treated at this MTD [49]. The phase I/II study hopes to determine the MTD of iodine-131 anti-B1 antibody which can be given in conjunction with high-dose etoposide and cyclophosphamide as a preparative regimen for autologous bone marrow transplantation.

Phase I trial of I-131 pan-B-cell antibodies

The Seattle phase I trial incorporated iodine-131-labeled anti-B-cell antibodies targeted to CD20 (Bl, 1F5), CD37 (MB1) and idiotypic immunoglobulin antigens in a study of 37 patients with recurrent B-cell non-Hodgkin’s lymphoma [32]. Enrollment criteria included the following: (1) tumor reactivity to designated antigens, (2) normal renal and hepatic function, (3) no active medical problems, (4) no chemotherapy treatment in four weeks, (5) expected survival of at least 30 days, and (6) less than 25% of bone marrow involved with lymphoma. Autologous bone marrow was harvested, purged of B cells with
a cocktail of anti-B-cell antibodies and complement, and cryopreserved for all patients at study entry.

On successive weeks, patients were infused with trace iodine-131-labeled (5–10 mCi) monoclonal antibody at doses of 0.5, 2.5, and 10 mg/kg to determine the antibody dose delivering the most favorable biodistribution. Data from gamma camera imaging and tumor biopsy specimens were used to estimate radiation doses absorbed by tumor sites and critical normal organs (lung, liver, kidney). Patients demonstrating favorable biodistribution studies (tumor sites received more radiation than critical normal organs) were eligible to receive a therapeutic infusion of high-dose radiolabeled B-cell antibodies using the antibody (Bl, 1F5, MB1, or anti-idiotypic antibody) and dose (0.5, 2.5, or 10 mg/kg) found to be optimal in the trace-labeled biodistribution studies. Therapeutic infusions were administered in a dose-escalation format in cohorts of three patients targeted to deliver 1000, 1500, 1700, 2400, 2700, and 3100 cGy to the normal organ receiving the most radiation. Radiation isolation was maintained until a patient’s body activity fell below 5 mR/hr at one meter. Autologous bone marrow was reinfused if the neutrophil count was less than 200/mm³ on two consecutive days with an aplastic marrow.

A majority of patients achieved a favorable biodistribution at antibody doses of 2.5 mg/kg of anti-B1 or 10 mg/kg of anti-MB-1. Massive splenomegaly adversely affected biodistribution with only 2 of 16 patients with splenomegaly meeting criteria for therapeutic infusions as compared with 17 of 22 patients with normal size spleens and five of five patients with prior splenectomies. Positive tumor imaging was documented in 24 patients. Nineteen patients with favorable biodistribution received infusions of 234 to 777 mCi iodine-131 antibodies at doses between 58 and 1168 mg. Tumor sites were estimated to receive between 1000 cGy and 9200 cGy. Severe myelosuppression occurred in all patients, with 15 requiring reinfusion of autologous marrow. Successful engraftment occurred in all patients. Platelet and leukocyte nadirs occurred between 10 to 14 days at the two highest therapeutic doses, and after 3 to 4 weeks at the two lowest doses. Neutrophil recovery occurred at a median of 22 ± 9 days after marrow infusion, whereas platelet recovery was more variable (median 20 ± 27 days). Three patients experienced a delay in platelet recovery of greater than 100 days.

Mild to moderate nausea occurred in 80% of patients, fever in 73%, and asymptomatic thyroid-stimulating hormone elevations in 42%. Mucositis was rare and mild. Two of four patients treated at the two highest doses had cardiopulmonary complications which defined the nonhematopoietic, dose-limiting toxicity. One patient treated with 2700 cGy developed reversible, diffuse interstitial pneumonitis and congestive cardiomyopathy two months after treatment. The only patient treated at 3100 cGy developed hypotensive syncope necessitating transient dopamine administration. The MTD was thus determined to be ≤2700 cGy.

Tumor responses were impressive. Sixteen of 19 patients achieved a complete response, and 2 patients achieved a partial response for an objective response of 95%. Nine patients remain in continuous remission from 15 to 72 months. Median remission duration exceeds 21 months.

Six major observations emerged from this study [32]. First, high-dose iodine-131 anti-B-cell antibodies can be successfully administered with minimal toxicity to patients with relapsed non-Hodgkin’s lymphoma when followed by autologous stem-cell rescue. Second, radioimmunotherapy with iodine-131 anti-B-cell antibodies is limited to doses delivering ≤2700 cGy to normal organs. Third, the optimal dose for anti-B1 (anti-CD20) is 2.5 mg/kg, whereas 10 mg/kg appears optimal for anti-MB-1 (CD37). Theoretically, larger doses of anti-CD37 antibodies are required because they are internalized and cross-react with T cells and platelets. Fourth, favorable biodistributions are more likely to occur in patients without splenomegaly and tumor burdens less than 500 cc. Fifth, patients with favorable biodistributions have an 84% rate of complete remission and an 11% rate of partial remission. Sixth, the median duration of remission exceeds 21 months.

Phase II trial of I-131 anti-B1 antibody

The Seattle phase II trial was then initiated to determine the response rate, response duration, and resultant toxicities in patients treated at the MTD [49]. Twenty-five patients with relapsed B-cell lymphoma were entered. Twenty-one of the 22 patients with favorable biodistributions received therapeutic infusions of iodine-131 anti-B1 (anti-CD20) calculated to deliver 2500–2700 cGy to normal organs. All treated patients received autologous hematopoietic stem-cell (purged bone marrow or peripheral blood stem-cell) reinfusion. Complete response was documented in 17 of 21 patients (results updated since original publication [49]). One patient achieved a partial
response, and one patient achieved a minor response. Overall survival and progression-free survival were 95% and 81%, respectively, for a median follow-up period of 12 months.

Toxicity was moderate, compared with conventional transplant conditioning regimens. Nausea occurred in 71%, mild mucositis in 24%, partial alopecia in 20%, and elevated thyroid-stimulating hormone in 30%. Neutrophil and platelet count recovery were similar to those encountered in the phase I study. Patients who received peripheral blood stem cells noted an abbreviated period of neutropenia (13–17 days) and thrombocytopenia (4–18 days). Seven minor and three serious infections occurred. All infections resolved with antibiotic therapy except in the case of one patient who died of septic shock secondary to gram negative bacteremia associated with an inflamed hemorrhoid.

Biodistribution studies confirmed earlier phase I findings with favorable distributions in patients without splenomegaly and tumor burdens less than 500 cc. All 3 patients with large tumor burdens (1259–3610 cc) who initially failed to meet biodistribution criteria subsequently achieved favorable biodistributions after cytoreductive chemotherapy reduced their tumor burdens (440–660 cc). Sixteen of 18 patients (89%) with normal size spleens achieved favorable biodistributions compared to 3 of 7 patients (43%) with splenomegaly.

Overall, our phase I and II studies have revealed a progression-free survival of 62% and an overall survival of 93% with a median follow-up of two years [32, 49]. The median duration of continued complete remission is greater than 24 months. Toxicities are modest compared to conventional TBI-containing conditioning regimens used for bone marrow transplantation. Overall and complete response rates exceed conventional salvage chemotherapy regimens and are at least equivalent to those reported for conventional bone marrow transplantation.

**Phase I/II trial of I-131 anti-B1 antibody and chemotherapy**

Following completion of the phase I and II studies, a phase I/II study was initiated to determine the maximum tolerated dose of iodine-131 anti-B1 antibody that can be given in conjunction with high-dose etoposide and cyclophosphamide as a preparative regimen for autologous bone marrow transplantation. Cohorts of four patients are treated on an escalating dose format for iodine-131 anti-B1 antibody (20, 23, 25, 27 Gy), etoposide (0 or 60 mg/kg), and cyclophosphamide (100 mg/kg). Sixteen patients have been entered on this trial so far. One patient died of disseminated *Varicella zoster* two months after radioimmunotherapy. One patient developed grade 3 veno-occlusive disease of the liver, one patient developed grade 3 mucositis, and one patient had mild, reversible interstitial pneumonitis. Fifteen of 16 patients remain free of tumor progression after a follow-up period of 1 to 17 months. Additional patient accrual and follow-up will be necessary to assess the toxicities and efficacies of this combined chemoimmunotherapeutic approach.

**Conclusion**

Radioimmunotherapy of relapsed non-Hodgkin's lymphomas with iodine-131-labeled anti-CD20 monoclonal antibodies has produced high response rates with modest toxicity in patients failing conventional chemotherapy. However, the optimal dose and treatment schedule for radiolabeled antibodies remains contentious. Our current approach is to stratify patients according to age and their desire for a curative attempt. For patients under the age of 60 desiring a potentially curative treatment approach, we administer myeloablative doses of radiolabeled antibodies in conjunction with autologous hematopoietic stem-cell transplantation. Patients with significant coexistent medical illnesses or patients not interested in assuming the risks associated with autologous stem-cell transplantation are offered nonmyeloablative protocols. Toxicity is felt to be minimal, although responses may be less durable.

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