FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Title: A Phase I/II Study Evaluating Escalating Doses of ^{90}Y-BC8-DOTA (anti-CD45) Antibody followed by BEAM Chemotherapy and Autologous Stem Cell Transplantation for High-Risk Lymphoid Malignancies

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Investigator Statement:

I have carefully read Protocol 2728 entitled "A Phase I/II Study Evaluating Escalating Doses of ^{90}Y-BC8-DOTA (anti-CD45) Antibody followed by BEAM Chemotherapy and Autologous Stem Cell Transplantation for High-Risk Lymphoid Malignancies" version date 01/04/2017.

I agree to carry out my responsibilities in accordance with the Protocol, applicable laws and regulations (including 21 CFR Part 312), Good Clinical Practice: Consolidated Guidance (ICH-E6), and applicable policies of Fred Hutch.

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1.0   INTRODUCTION

Radioimmunotherapy (RIT) directed against the CD20 surface antigen has emerged as one of the most effective treatment approaches for patients with B-cell non-Hodgkin lymphoma (NHL) [1]. When used as first-line single agent therapy in previously untreated patients, response rates as high as 96% have been reported [2]. In patients who have failed multiple prior therapies, RIT has produced remission rates of 50% to 80% and complete response rates of 25% to 40% [3, 4]. Our group and others have also demonstrated the efficacy of escalated doses of anti-CD20 RIT as part of transplant conditioning regimens, suggesting that outcomes are improved over non-randomized controls [5-9]. Despite these successes, not all patients are cured using these approaches. In addition, patients with lymphomas that do not express the CD20 antigen, such as T-NHL, do not benefit from targeted intensification of therapy directed at CD20. Furthermore, preclinical data suggest that extensive prior exposure to rituximab may partially abrogate the targeting of anti-CD20 radioimmunoconjugates even in patients whose lymphomas do express CD20 [10]. For these reasons, alternative targeting strategies are required in the transplant setting for such circumstances.

CD45, a pan-hematopoeitic antigen, represents an attractive target for RIT based on its lack of shedding or internalization and its reliable expression by the vast majority of lymphoid neoplasms [11-14]. Our group has pioneered the use of anti-CD45 RIT for the treatment of acute myeloid leukemia (AML) demonstrating feasibility, safety, and efficacy [15, 16]. Furthermore, we have recently initiated 2 phase I studies of escalating doses of anti-CD45 RIT followed by autologous stem cell transplantation (ASCT) in patients with relapsed/refractory lymphoma (FHCRC 2238 and FHCRC 2361). The objective of this project is to develop a novel, safe, and effective approach to the addition of anti-CD45 RIT to a standard myeloablative chemotherapy regimen that can be employed prior to ASCT. Ultimately, we aim to improve the outcomes from ASCT for patients with a variety of relapsed or refractory lymphomas.

2.0   BACKGROUND

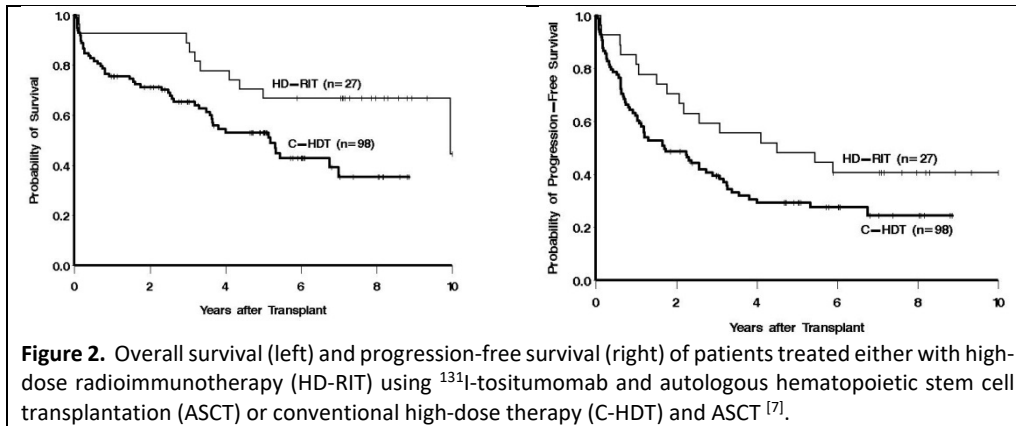**Epidemiology and pathologic characteristics of lymphomas:**
Each year in the United States approximately 66,000 individuals will be newly diagnosed with NHL, resulting in approximately 19,000 deaths annually [17]. The vast majority are B-NHL with the most common histologic entities including diffuse large B-cell (DLBCL), follicular (FL), mantle cell (MCL), small lymphocytic (SLL), and marginal zone lymphoma (MZL) [18, 19]. These lymphomas express many similar surface antigens including CD19, CD20, and CD45. On the other hand, T-cell NHLs are a heterogeneous group of diseases that comprise approximately 10-15% of the lymphoid malignancies and include the histologic subtypes of T-lymphoblastic (TLBL), peripheral T-cell (PTCL), anaplastic large cell (ALCL), NK/T cell NHL (NK/T-NHL), mycosis fungoides/cutaneous T-cell (MF), hepatosplenic gamma-delta lymphoma (HSGDL), and angioimmunoblastic lymphoma (AITL) [19, 20]. In contrast to the B-cell lymphomas, T-cell lymphomas are characterized by considerable antigenic diversity challenging the development of widely applicable targeted therapeutics [20-22]. Finally, Hodgkin lymphoma (HL) is newly diagnosed in about 8,800 individuals each year in the United States [17]. Classical HL is also characterized by a unique immunophenotype with typical expression of CD30, CD15 and absence of CD20 and CD45 [23]. Histologically, the most common variants of HL, however, demonstrate a paucity of malignant Reed-

Sternberg cells, but are extensively infiltrated by T and B-cells expressing typical lymphoid markers, including CD45 (**Figure 1**).

Figure 1: CD45 immunohistochemistry of classical Hodgkin lymphoma. (courtesy of Brent Wood, MD, PhD)
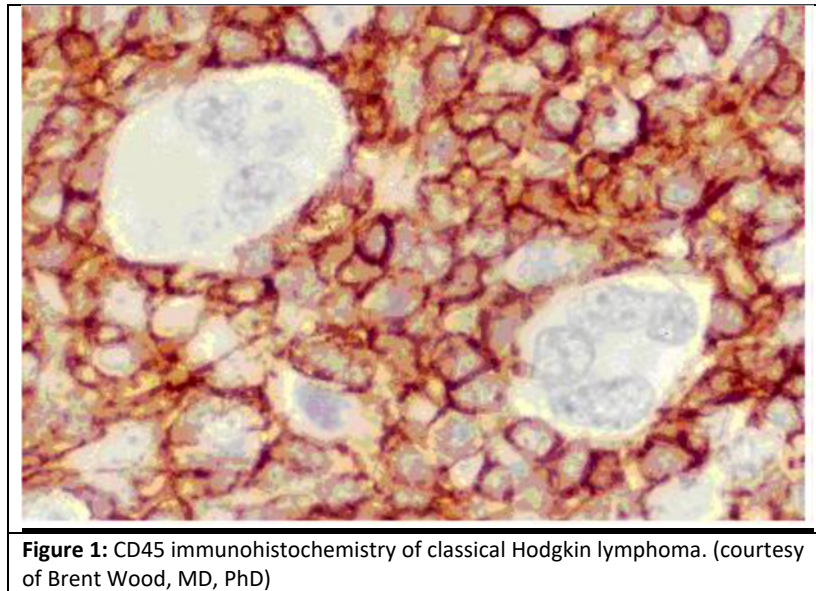
Current Targeted Therapies for B-NHL:

B-cell lymphomas are the first group of malignancies to have benefited from the development of targeted monoclonal antibody (MoAb) therapies. Rituximab, a MoAb that targets the pan-B-cell antigen CD20, has contributed to the first major improvement in the treatment of many B-cell malignancies in decades [24-28]. Despite its activity, most patients that receive rituximab alone achieve only partial responses and remission durations are limited. The addition of radionuclides such as ^{131}I or ^{90}Y to anti-CD20 antibodies exploits the exquisite radiosensitivity of NHLs. This has resulted in improved rates of response and longer remission durations [29, 30]. One approach to further improve outcomes, pioneered by our group and others, involves escalating the dose of CD20 targeted RIT combined with autologous stem cell rescue to support hematopoiesis. We have previously demonstrated that this approach delivers approximately twice the radiation dose to tumor sites as compared to the critical normal organ that received the highest radiation exposure, and ten times more radiation to tumors than is delivered to the whole body. Our data have confirmed that escalating the absorbed dose of RIT correlates with improved progression free survival (PFS) [31]. In phase II clinical trials, we demonstrated that high-dose anti-CD20 RIT yields response rates of >90% in heavily pre-treated B-NHL patients [5]; and, when compared to concurrent non-randomized controls treated with conventional conditioning regimens, RIT-based conditioning yielded improved overall survival (OS) and PFS [6, 7] (**Figure 2**). These early studies also indicated that the antibody protein dose required optimization to improve the ratio of radiation delivered to lymph nodes over the dose delivered to non-target organs [5].

Figure 2. Overall survival (left) and progression-free survival (right) of patients treated either with high-dose radioimmunotherapy (HD-RIT) using ^{131}I-tositumomab and autologous hematopoietic stem cell transplantation (ASCT) or conventional high-dose therapy (C-HDT) and ASCT [7].

The efficacy and tolerability of high-dose anti-CD20 RIT and ASCT is further supported by our recent data suggesting that this approach can safely extend potentially curative therapies to adults into their mid-70s (**Table 1**). Collectively, our studies have repeatedly demonstrated the feasibility, safety, and efficacy of delivering high-dose CD20-directed RIT and hematopoietic cell transplantation (HCT) for B-NHL. Our experience and expertise in this area provides the basis for expanding this approach using a novel radioimmunoconjugate and target antigen.
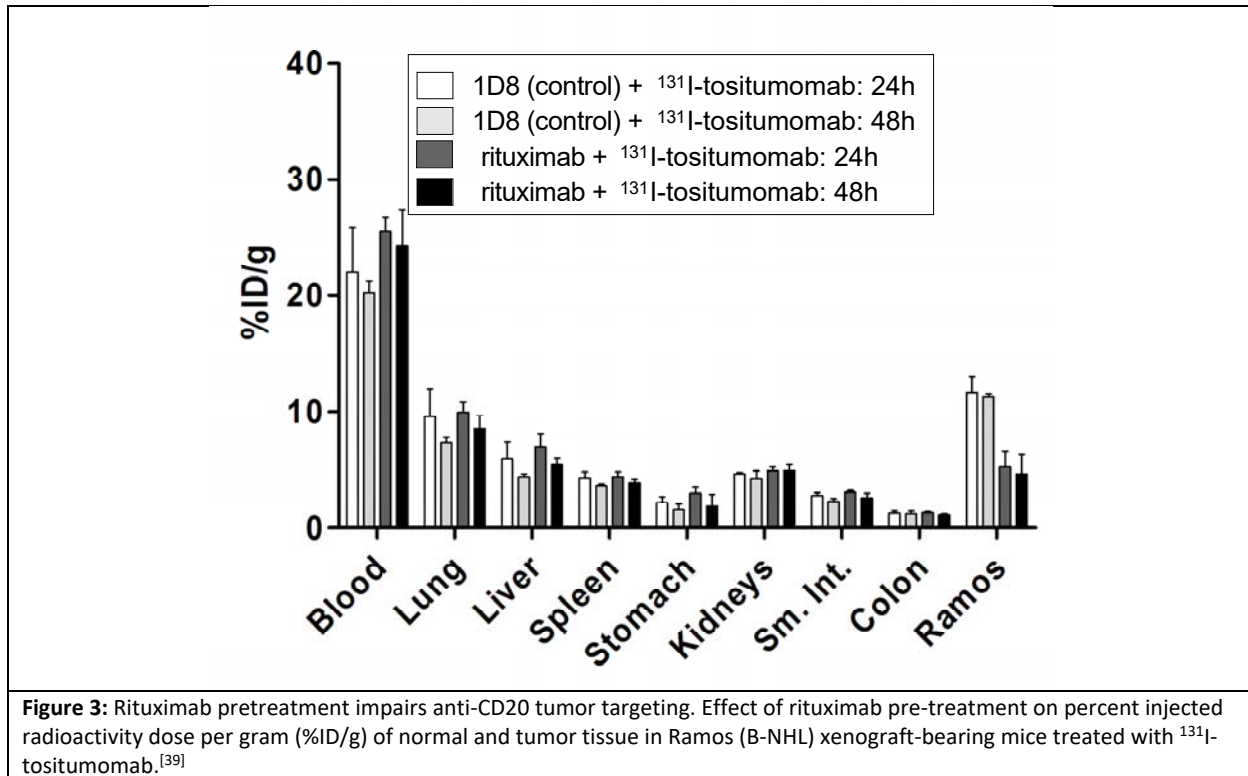
Table 1: All non-hematologic grade 3-5 adverse events for 24 adults ≥60 years of age receiving high-dose (25-27Gy) ^{131}I-tositumomab [32]		
Adverse event	**Grade 3**	**Grade 4**
Cardiovascular	6 (25)	1 (4)
Gastrointestinal	8 (33)	1 (4)
Genitourinary	2 (8)	0 (0)
Hepatic	2 (8)	0 (0)
Infection/Febrile neutropenia	6 (25)	0 (0)
Metabolic/Laboratory	4 (17)	0 (0)
Neurologic	5 (21)	0 (0)
Pain	2 (8)	0 (0)
Pulmonary	2 (8)	0 (0)
Any adverse event	15 (63)	2 (8)
Values are numbers (percentages) of patients. Adverse events were graded according to the National Cancer Institute's Common Toxicity Criteria (NCI CTC), version 2.0 scale. No patients experienced grade 5 (fatal) toxicity.		

Rituximab blocking of CD20 targeted RIT:

Although both standard-dose anti-CD20 RIT and high-dose anti-CD20 RIT and ASCT have shown promise in the treatment of lymphoid malignancies, a new potential limitation of this strategy has arisen since these initial studies. A major theoretical concern of CD20 targeted RIT is the competition for antigen binding sites by circulating rituximab. Rituximab has become a major component of most B-NHL regimens, with the majority of patients receiving multiple doses during the cytoreductive period prior to transplant. Importantly, rituximab is known to persist with measurable levels for over 6 months [33-38] . Our group has demonstrated via *in vitro* and mouse models that circulating rituximab can block the binding of a second anti-CD20 antibody to CD20 (**Figure 3**) [10]. In the clinic, there is no currently available RIT approach to overcome this potential problem other than a protracted rituximab washout period.

Figure 3: Rituximab pretreatment impairs anti-CD20 tumor targeting. Effect of rituximab pre-treatment on percent injected radioactivity dose per gram (%ID/g) of normal and tumor tissue in Ramos (B-NHL) xenograft-bearing mice treated with ^{131}I-tositumomab.[39]

Targeted therapies for T-NHL:

The most recently approved targeted therapy for T-NHL is the antibody-drug conjugate brentuximab vedotin (Adcetris), which was approved for relapsed/refractory ALCL. In a single-agent phase II study, it yielded an 83% objective response rate in patients with ALCL that had recurrent disease after at least one prior therapy [40]. Alemtuzumab (Campath 1H) was the first commercially available MoAb that targeted an antigen found on T-NHL. Alemtuzumab targets CD52, which is expressed on ~50% of T-NHL [41], and can induce responses in 36-55% of patients with relapsed T-NHL or MF [42, 43]. Unfortunately, only 21-36% of patients achieved a complete remission (CR) and the time to treatment failure in responding patients ranged from 6-12 months. A third targeted compound, denileukin diftitox (Ontak®), has been approved for the treatment of MF. Denileukin diftitox is an immunotoxin that targets the IL-2 receptor CD25 and delivers a modified version of diphtheria toxin to CD25-bearing cells. In the pivotal trial of this agent, 30% of patients achieved an objective response including 15% of patients with advanced disease, though the median response duration was 6.9 months [44].

Unlike RIT for B-NHL, these strategies for T-NHL are poorly developed. This situation is attributable in part to the heterogeneity of antigens expressed on these diseases and the potential requirement to develop novel MoAbs for each subtype of similar T-cell malignancies [12]. Limited studies evaluating the use of ^{131}I-anti CD5 antibodies (T101) for the treatment of T-NHL were performed in the early 1980's, and though 5 of 5 pateints had clinical improvement, the remission durations only ranged from 3 weeks to 3 months [45, 46]. Unfortunately, unlike B-NHL, to date there are no FDA-approved anti-T-NHL radioimmunoconjugates. More importantly, most T-NHL RIT programs in development would be limited to a subset of T-lymphoid malignancies due to the varied antigen expression (e.g., CD25, CD30) in these diseases [12, 19]. Thus, an

antigen that is expressed on the majority of T-cells is required to develop a successful, widely applicable RIT-based approach for T-NHL.

Targeted therapies for HL:

Currently, the only FDA approved targeted agent for HL is the antibody-drug conjugate brentuximab vedotin [47]. There are no FDA approved targeted therapies including RIT for HL, though a few groups have pursued such approaches in the past. Polyclonal anti-ferritin antibodies labeled with ^{131}I or ^{90}Y have been studied at nonmyeloablative doses documenting the safety and efficacy of this strategy, though all patients eventually relapsed [48, 49]. Others have studied ^{131}I-anti-CD30 antibody with evidence of disease activity [50]. Although these data are encouraging, directly targeting sparsely distributed Reed-Sternberg cells may have limited the ability to amplify the radiation dose to tumor sites via the 'crossfire' effect.

CD45 as a target antigen:

CD45 is a ~200 kD tyrosine phosphatase expressed on the surface of virtually all cells of hematopoietic origin, with the exception of mature erythrocytes, platelets, and most plasma cells [11, 12]. Most hematologic malignancies including 85-90% of B-NHL and T-NHL also express CD45; however, CD45 is not found on tissues of non-hematopoietic origin [11, 12]. Most data suggest that CD45 is not shed into the bloodstream and is not rapidly internalized upon binding [14].
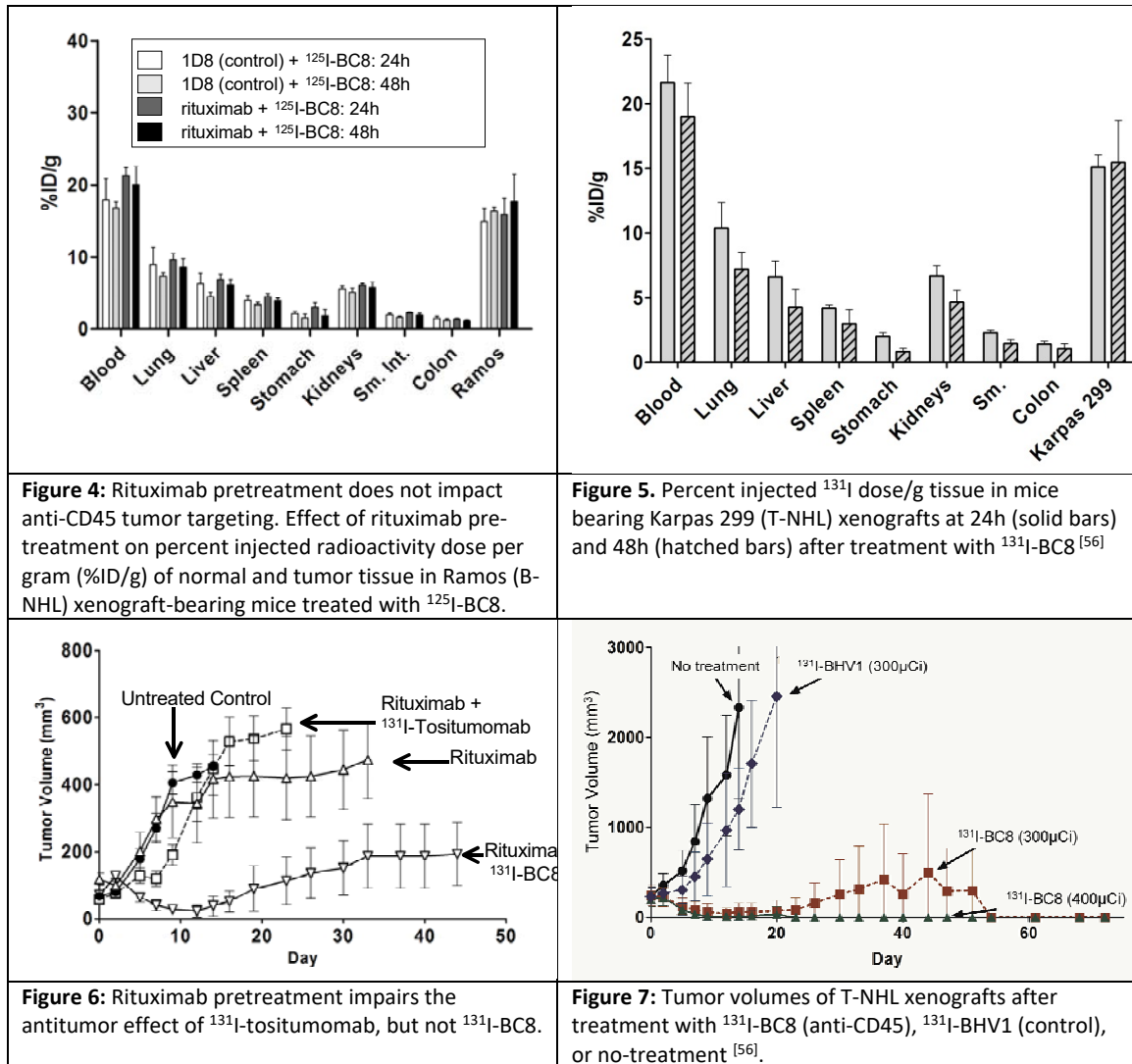
Anti-CD45 RIT for AML:

Work by Drs. Pagel, Matthews, Bernstein, Appelbaum and others in our group has demonstrated that the anti-CD45 antibody BC8 can be utilized to deliver potentially curative doses of radiation to hematolymphoid sites in patients with AML [15, 16, 51]. The initial studies established that up to 10.5 Gy of radiation could be delivered to normal organs with ^{131}I-anti-CD45 therapy could be administered along with 12 Gy of TBI and 100 mg/kg of cyclophosphamide followed by allogeneic HCT. This trial also confirmed that a median of an additional 24 Gy and 50 Gy were delivered to hematolymphoid sites of bone marrow and spleen, respectively [16]. More recently, Dr. Pagel and colleagues have explored further escalating the dose of ^{131}I-BC8 (anti-CD45) therapy in AML to establish the single-agent maximally tolerated dose that can be given prior to allogeneic transplant in combination with established conditioning regimens: a myeloablative preparative regimen including cyclophosphamide and targeted busulfan [15], and a nonmyeloablative preparative regimen using 2 Gy TBI and fludarabine [51, 52]. These studies demonstrated the safety and tolerability of these approaches, both in terms of regimen-related toxicity as well as reliable engraftment following HCT despite the escalated doses of hematolymphoid-targeted radiation. These data as a whole highlight the clinical feasibility of delivering high-dose ^{131}I-anti-CD45 therapy prior to HCT as well as the ability to preferentially target hematolymphoid sites of disease [15, 16, 51, 53].

Preclinical data supporting anti-CD45 RIT in lymphoma:

In vitro data confirm that CD45 is expressed in high copy number on a variety of B-NHL and T-NHL cell lines, exhibits superior cell surface retention as compared to other anti-lymphoma antibodies tested, and cannot be blocked by pretreatment with rituximab [10, 54]. Mouse models corroborated these findings, suggesting that targeting CD45 on B-NHL with BC8 was superior to targeting CD20 and was not impacted
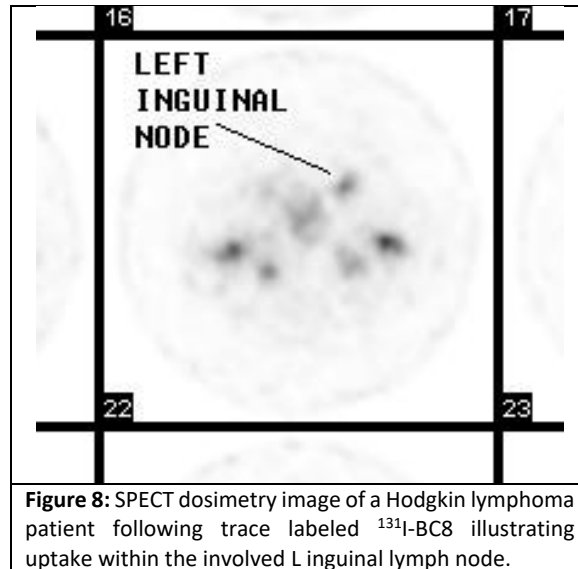
by prior rituximab treatment (**Figure 4**) [39, 55]. Similar results were demonstrated across a variety of T-NHL lines showing preferential targeting to tumor sites using ^{131}I-BC8 (**Figure 5**) [56]. These biodistribution results yielded improved tumor control in animal models (**Figures 6 and 7**).

Figure 4: Rituximab pretreatment does not impact anti-CD45 tumor targeting. Effect of rituximab pre-treatment on percent injected radioactivity dose per gram (%ID/g) of normal and tumor tissue in Ramos (B-NHL) xenograft-bearing mice treated with ^{125}I-BC8.

Figure 5. Percent injected ^{131}I dose/g tissue in mice bearing Karpas 299 (T-NHL) xenografts at 24h (solid bars) and 48h (hatched bars) after treatment with ^{131}I-BC8 [56]

Figure 6: Rituximab pretreatment impairs the antitumor effect of ^{131}I-tositumomab, but not ^{131}I-BC8.

Figure 7: Tumor volumes of T-NHL xenografts after treatment with ^{131}I-BC8 (anti-CD45), ^{131}I-BHV1 (control), or no-treatment [56].

Clinical use of ^{131}I-CD45 in lymphoma:

We have initiated a clinical trial of ^{131}I-BC8 (anti-CD45) in patients with relapsed lymphoma (FHCRC 2238) and have accrued and treated 15 patients as of 6/1/13. Notable results to date include the following: 1. The first use of anti-CD45 RIT for the treatment of lymphoma, 2. The first use of an accelerated ^{131}I-BC8 (anti-CD45) infusion schedule with infusion rates up to 45 mg/hr, 3. Successful use of serial lymph node biopsies and dosimetries without HAMA formation, 4. The first lymph node dose and antigen saturation estimates with anti-CD45 RIT. (**Figure 8** illustrates the ability to image an involved lymph node in a Hodgkin lymphoma patient using trace labeled ^{131}I-BC8.) We have shown the ability to measure differences in biodistributions using intrapatient antibody dose adjustments. We have also demonstrated the safety of this regimen with administration of up to 30 Gy to the liver and 50 Gy to the bone marrow at a protein
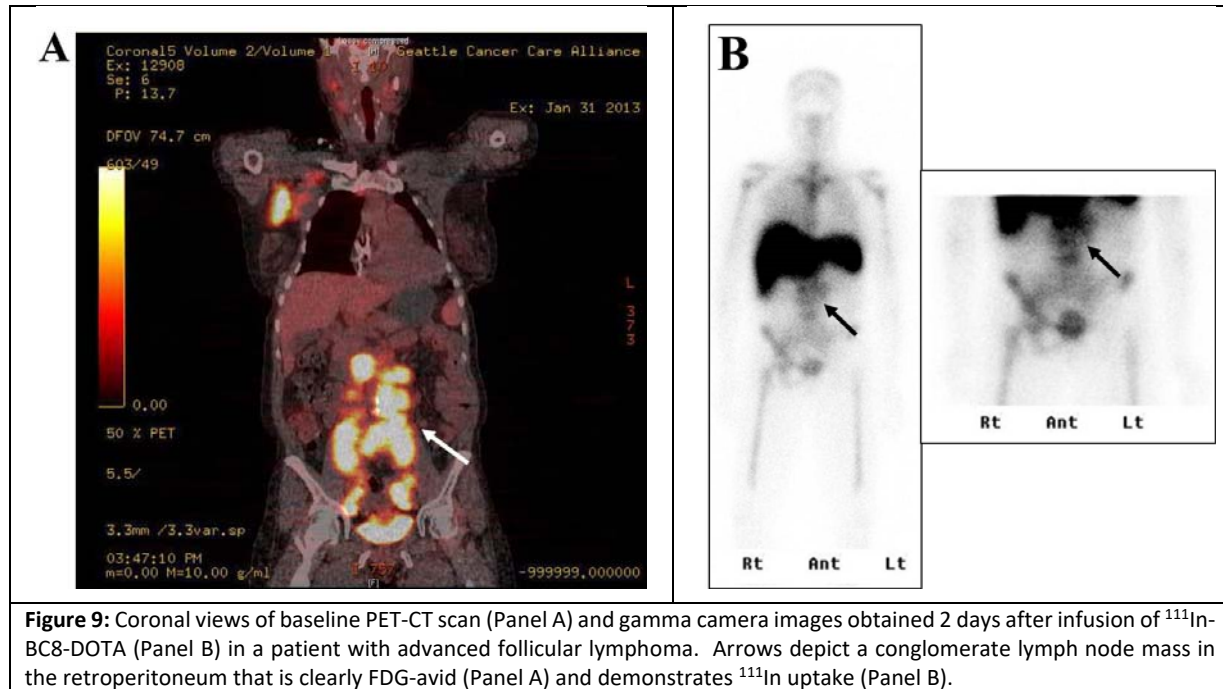
dose of 0.75 mg/kg. Further, we have safely performed tandem myeloablative transplants within 6 weeks of each other, with the second transplant conditioned with BEAM (carmustine [BCNU], etoposide, cytarabine [ara-C], and melphalan). Lastly, of the 15 patients treated as of 6/1/13, 7 are alive and progression-free (5 of whom have HL).

Figure 8: SPECT dosimetry image of a Hodgkin lymphoma patient following trace labeled ^{131}I-BC8 illustrating uptake within the involved L inguinal lymph node.

Clinical use of ^{90}Y-anti-CD45 in lymphoma:

Despite the successes of our ^{131}I-based B-NHL and AML RIT programs, a major limitation of the exportability of this modality has been the reluctance of many other institutions to handle, dispense, and infuse high-doses of gamma emitting radionuclides. This is evidenced, in part, by the number of high-dose anti-CD20 based groups utilizing ^{90}Y and the paucity of those using [131]I [57-60]. Based on the limitations of ^{131}I, we have transitioned to ^{90}Y as our therapeutic radionuclide. However, because ^{90}Y is a pure beta-emitter, it cannot be imaged accurately or conveniently in the patient. We, as others, will assume that the biodistribution of the trace-labeled ^{111}In-antibody represents the biodistribution of ^{90}Y-antibody in the cancer patient [61].

We have started a phase I clinical trial at our center using escalating doses of ^{90}Y-BC8-DOTA as single-agent, myeloablative conditioning prior to ASCT for patients with relapsed/refractory lymphoma (FHCRC 2361). As of 6/1/13, we have safely delivered 12 Gy of radiation to the liver at a protein dose of 0.75 mg/kg, with imaging studies demonstrating good localization of 111In-BC8-DOTA to sites of active disease (**Figure 9**).

Figure 9: Coronal views of baseline PET-CT scan (Panel A) and gamma camera images obtained 2 days after infusion of ^{111}In-BC8-DOTA (Panel B) in a patient with advanced follicular lymphoma. Arrows depict a conglomerate lymph node mass in the retroperitoneum that is clearly FDG-avid (Panel A) and demonstrates ^{111}In uptake (Panel B).

Efforts to Improve Outcomes from ASCT by Adding RIT to Standard Preparative Regimens:

It is recognized that B-NHL that relapses following rituximab-containing induction chemotherapy is increasingly difficult to salvage with standard approaches, including ASCT [62, 63]. One approach that could improve these outcomes is to augment established myeloablative conditioning regimens with RIT. Indeed, anti-CD20 RIT with ^{131}I-tositumomab or ^{90}Y-ibritumomab tiuxetan has been added to a variety of high-dose chemotherapy regimens prior to ASCT [6, 8, 57, 64-69]. Generally, these reports demonstrate little additive toxicity attributable to RIT, considering the often-substantial toxicity seen from the myeloablative chemotherapy backbones to which it was added. However, there have been relatively few randomized controlled trials comparing myeloablative preparative regimens with or without RIT. In one study from Israel, the addition of ^{90}Y-ibritumomab tiuxetan to BEAM for relapsed DLBCL yielded superior 2-year overall survival compared to BEAM alone (91% vs 62%, respectively; P = 0.05) [67]. However, a larger study comparing BEAM plus either rituximab or ^{131}I-tositumomab for relapsed DLBCL showed no difference in overall or progression-free survival between the two arms [65].

These studies primarily used standard doses of ^{131}I-tositumomab or ^{90}Y-ibritumomab tiuxetan. Thus, it is possible that RIT was not sufficiently dose-intensified to achieve maximum efficacy. To this end, Winter and colleagues escalated the dose of radiation delivered to critical organs by ^{90}Y-ibritumomab tiuxetan in combination with BEAM in patients with relapsed/refractory B-NHL [57]. They estimated the maximally tolerated dose (MTD) to be 15 Gy, though they conceded that most of the toxicities observed were similar to those typically seen with BEAM alone (e.g., infections, pulmonary and hepatic toxicity, etc.). They also observed relatively promising clinical outcomes in this cohort of generally high-risk (i.e., heavily pre-treated, non-remission) patients. These authors concluded that dose-escalated RIT in combination with high-dose chemotherapy deserves further investigation, but in the context of careful dosimetry-based RIT administration.

As stated previously, another potential limiting factor in these anti-CD20 RIT approaches to augment standard ASCT is the possibility of a deleterious effect of circulating rituximab and its ability to block the shared CD20 target; none of these studies mentioned above has evaluated this potential issue. With anti-CD45 RIT, there are two clear advantages. First, we avoid any potential blocking by rituximab because of the distinct target antigens. Second, we introduce this option to patients with T-NHL and HL, the former in particular being in substantial need for improved treatments.

Summary:
Despite lymphomas being clinically and antigenically heterogeneous, nearly all types express CD45 surface antigen (either on the tumor cells directly or in the neighboring inflammatory cells). Preclinical models suggest that targeting CD45 can be effective, and initial experience with this approach in patients with lymphoma has demonstrated both safety and potential efficacy. This protocol will evaluate and optimize a regimen using BC8 to target lymphoma and hematolymphoid tissue with radiometals in conjunction with the established myeloablative chemotherapy regimen, BEAM. The eventual goal is to improve cure rates across a spectrum of lymphoma histologies without prohibitively increasing toxicity. The full details of the protocol are described below.

3.0   OBJECTIVES

3.1   Primary Objectives:

1. To estimate the MTD of ^{90}Y-BC8-DOTA (anti-CD45) that can be delivered prior to myeloablative BEAM chemotherapy and ASCT for patients with high-risk B-NHL, T-NHL, and HL.
2. To evaluate the efficacy of ^{90}Y-BC8-DOTA when administered at the estimated MTD prior to BEAM chemotherapy and ASCT for patients with high-risk B-NHL, T-NHL, and HL compared to historical controls treated with BEAM alone.

3.2   Secondary Objectives:

1. To describe the toxicity observed from the addition of ^{90}Y-BC8-DOTA to BEAM.
2. To optimize the protein dose (Ab) to deliver a favorable biodistribution in the majority of patients.
3. To describe response rates and overall survival of patients with high-risk B-NHL, T-NHL, and HL following administration of ^{90}Y-BC8-DOTA plus BEAM prior to ASCT.
4. To describe the impact of rituximab concentrations, B-cell depletion, and disease burden on CD45 targeting.
5. To assess the correlation of lymphoma biomarkers with outcomes.
6. To evaluate the effects of nodal-targeted irradiation by ^{90}Y-BC8-DOTA on immune reconstitution following ASCT.

4.0 STUDY DESIGN

4.1 Description of Study

This is a phase I/II, open label study conducted under an investigational new drug application submitted to the U.S. Food and Drug Administration (FDA) for the radiolabeled anti-CD45 antibody ^{90}Y-BC8-DOTA, here being tested in combination with BEAM chemotherapy. The main portion of the study is a single-arm treatment protocol designed to meet the primary objectives described above.

4.2 Patient Descriptive Factors

Histological subtype by World Health Organization (WHO) classification must be supplied. Patients will be classified as *chemo-responsive* if they achieved at least a partial response with their most recent chemotherapy (e.g. cytoreductive or mobilization chemotherapy). Patients that have never achieved at least a partial response (PR) will be categorized as *primary refractory*. Other patients will be categorized as *chemo-resistant* (i.e. patients that have previously achieved at least a PR, but have not responded to their most recent chemotherapy). Patients' disease stage must be recorded both at the time of diagnosis and the time of treatment. Relevant prognostic factors will be assessed according to the International Prognostic Index, Follicular International Prognostic Index, Mantle Cell International Prognostic Index, T-cell Lymphoma International Prognostic Index, or the International Prognostic Score for Hodgkin Lymphoma [73-77].

4.3 Primary Study Endpoints

1. Dose-limiting toxicities (Grade III/IV Bearman) within 30 days post-transplant.
2. 1-year progression-free survival following ASCT

5.0 PATIENT SELECTION

5.1 Inclusions

1. Patients must have a histologically confirmed diagnosis of B-NHL, T-NHL, or HL. Since CD45+ cells are ubiquitous in all B and T-NHL, but are typically absent in Hodgkin's Lymphoma, only patients with Classical HL must have documented histologic demonstration of CD45+ cells adjacent to the Reed Sternberg cells. Patients must have received at least one prior standard systemic therapy with documented recurrent or refractory disease. Patients with MCL, T-NHL, or other high-risk malignancies may be enrolled/transplanted in CR/PR1.
2. Patients must be 18 years of age or older.
3. Patients must have normal renal function (creatinine <2.0) and normal hepatic function (bilirubin <1.5mg/dL).
4. All patients eligible for therapeutic study must have a minimum of ≥2 x10^6 CD34/kg autologous hematopoietic stem cells harvested and cryopreserved.
5. Patients must have an expected survival of >60 days and must be free of major infection.

5.2. Exclusions

1. Circulating human anti-mouse antibody (HAMA), to be determined before each infusion.
2. Systemic anti-lymphoma therapy given in the previous 30 days before the scheduled therapy dose with the exception of rituximab.
3. Inability to understand or give an informed consent.
4. Lymphoma involving the central nervous system.
5. Other serious medical conditions considered to represent contraindications to ASCT (e.g., abnormally decreased cardiac ejection fraction, DLCO <50% predicted, etc.)
6. Known HIV seropositivity.
7. Pregnancy or breast feeding.
8. Prior autologous or allogeneic bone marrow or stem cell transplant.
9. Prior RT >20 Gy to a critical organ within 1 year of enrollment.
10. SWOG performance status ≥2.0

6.0   DONOR SELECTION

Not applicable. This is a study of autologous transplantation.

7.0   EVALUATION AND COUNSELING OF PATIENT

This protocol should be discussed thoroughly with the subject and family (if appropriate), and all known risks should be described. The autologous stem cell transplant procedure and alternative forms of therapy should be presented as objectively as possible and the risks and hazards of the procedure explained. Consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. A summary of the conference should be dictated for the medical record detailing what was covered.
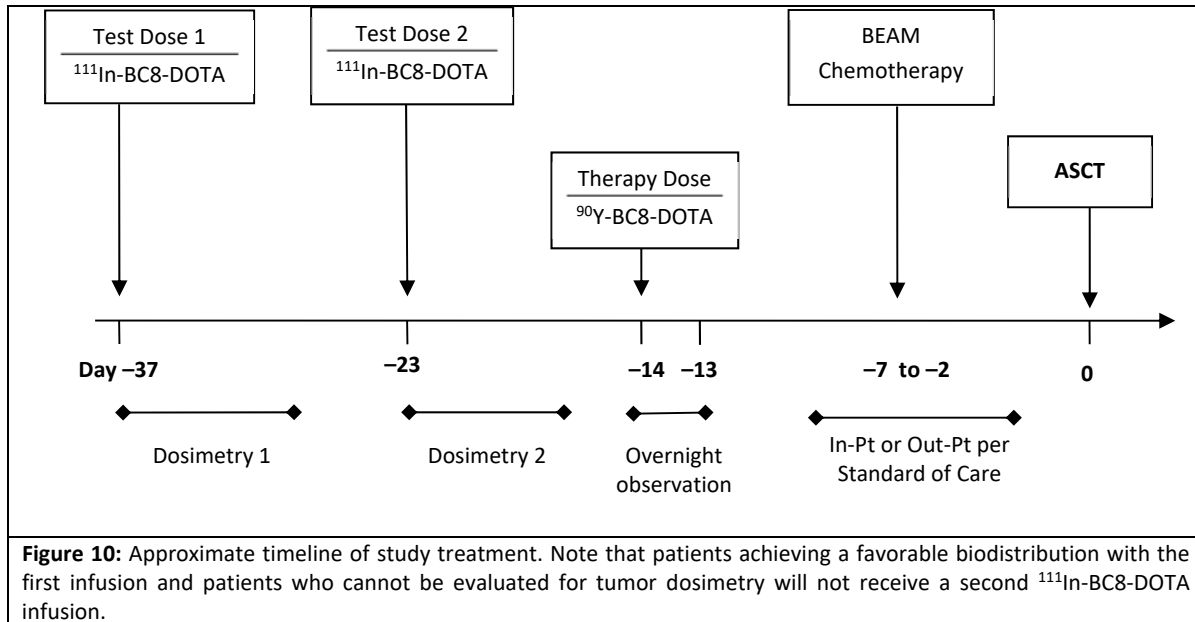
8.0   PROTOCOL REGISTRATION

Patients will be assigned and registered utilizing the institution's standard procedures.

9.0   PLAN OF TREATMENT

9.1   General Outline of Treatment Plan

This trial will assess the safety and feasibility of this regimen and estimate the MTD of the radiation absorbed dose from ^{90}Y-DOTA-BC8 that can be delivered in conjunction with BEAM chemotherapy and autologous stem cell transplantation. **Figure 10** shows the general treatment schema for patients on this study. After informed consent, eligibility confirmation and registration, patients will receive a dosimetric "test" dose of BC8-DOTA trace labeled with ~5-10 mCi ^{111}In for biodistribution studies to allow estimation of the appropriate ^{90}Y dose to be used for therapy. Patients may also be given a second dosimetry infusion

if the first "test" dose shows an unfavorable biodistribution. Each patient may undergo up to two dosimetry infusions, with an interval of no less than 7 days between each dosimetry infusion and HAMA testing repeated before each infusion. Patients will receive a therapy dose of ^{90}Y-BC8-DOTA approximately 7 to 10 days after the final test dose, followed by BEAM chemotherapy approximately 1 week later. ASCT will occur approximately 2 weeks after the ^{90}Y-BC8-DOTA and about 7 days after the start of BEAM.

Figure 10: Approximate timeline of study treatment. Note that patients achieving a favorable biodistribution with the first infusion and patients who cannot be evaluated for tumor dosimetry will not receive a second ^{111}In-BC8-DOTA infusion.

9.2 Peripheral Blood Stem Cell Collection

Peripheral blood stem cell (PBSC) collection is not an integral part of this protocol; however, PBSC will need to be collected by serial leukapheresis per standard practice.

9.3 Detailed Investigational Treatment Plan: Biodistribution Studies

9.3.1 ^{111}In-DOTA-BC8 Administration

In order to determine the mCi ^{90}Y-DOTA-BC8 Ab required to deliver the desired dose of radiation, each patient will undergo biodistribution studies prior to receiving a therapy dose. In the biodistribution step, patients will receive a trace-labeled infusion of ^{111}In-DOTA-BC8. Gamma camera images and an optional lymph node biopsy (if accessible) will be obtained over the next several days to ascertain radiolabeled antibody biokinetics. As described in section 9.3.1.1, a second dosimetry infusion may be utilized for antibody dose optimization. One to two weeks after the biodistribution infusions, patients will receive a therapy infusion of ^{90}Y-DOTA-BC8.

9.3.1.1 Antibody Dose Optimization

A secondary objective of the protocol is to optimize the protein (antibody) dose to achieve a favorable biodistribution in the majority of patients. Biodistribution is considered favorable when evaluable tumor sites receive higher doses of radiation exposure (cGy/mCi) than any critical normal organ (e.g., lung, liver, kidney).

Briefly, each patient will receive an initial infusion at the designated dose level (see **Table 2**) labeled with ~5-10 mCi [111]In. After a minimum of two patients receive initial infusions at dose level 1, the initial dose level for each subsequent patient will be determined as described under Section 16 (Statistical Considerations). If the subsequent dosimetry analysis shows a favorable biodistribution as described above, the patient will not undergo additional dosimetry testing with ^{111}In-BC8-DOTA, and the patient's ^{90}Y-BC8-DOTA therapy dose will be administered at the same protein dose used for the test dose. If the dosimetry shows an unfavorable biodistribution, the patient will repeat the dosimetry infusion and analysis at the next higher protein dose level. There will be a maximum of two dosimetry infusions per patient, with an interval of no less than 7 days between infusions, and HAMA testing repeated before each dose. If neither ^{111}In-BC8-DOTA dose results in a favorable biodistribution, the patient's therapy dose will be calculated based on the more favorable of the two. Patients who are for any reason not evaluable for tumor dosimetry will receive a single dosimetry infusion and will be treated at that same antibody protein dose level.

Table 2: Antibody dose escalation to optimize biodistribution.	
Dose level	BC8-DOTA dose (mg/kg ideal body weight*)
1	0.75
2	1.0
3	1.25
4	1.5
5	1.75
6	2.0
7	2.25
8	2.5
* ideal body weight will be used to determine dose for patients at or above ideal body weight; actual weight will be used for patients below ideal body weight.	

9.3.1.2 ^{111}In-DOTA-BC8 dose and infusion rate

The infusion schema will be as follows with PI or designee (MD or Research Nurse) discretion to further reduce rate or pause infusion as needed to insure individual patient safety:
- Radiolabeled antibody will be diluted to approximately 25 ml
- Antibody will be initially infused intravenously at a rate of 7.5 mg/hr

- If no toxicities have occurred within the first 60 minutes, the rate may be increased in 7.5 mg/hr increments every 30 minutes until completion of the infusion. If toxicities occur at any point during infusion, refer to **Table 3** for recommended interventions and rate adjustment as needed.
- If ≥ grade 3 allergic toxicity is encountered and does not resolve, or other types of toxicity ≥ grade 4 occur, the infusion must be terminated and the patient taken off protocol.
- Hepatic and/or renal toxicity will be determined by laboratory samples obtained at the end of infusion and the following day. If a patient experiences grade 3 hepatic or renal toxicity after the biodistribution dose, determination of whether or not the patient can proceed to the therapy dose will be made by the P.I. (or designee) and the IND Sponsor in consultation with the attending physician, and will depend in part upon the rate of recovery from the laboratory abnormalities. A patient experiencing grade 4 hepatic or renal toxicity after the biodistribution dose will not receive the therapy dose, and will be taken off this protocol and treated on an alternate protocol or standard of care regimen.

9.3.1.3 Premedications

- acetaminophen 650 mg PO
- diphenhydramine 25-50 mg IV ; may be given in divided doses if desired (e.g., 35 mg followed approximately one hour after start of radiolabeled infusion by remaining 15 mg)
- ondansetron 8mg IV (For therapy infusion repeat every 8 hours (+/- 15 minutes) for first 24 hours from premedication time at start of infusion)
- hydrocortisone 100 mg IV (repeated every 2 hours (+/- 15 minutes) until the completion of the infusion.)
- granisetron 1 mg PO or IV; if patient does not tolerate ondansetron OR
- prochlorperazine 10 mg PO or IV; if patient does not tolerate ondansetron
- ½ NS (with or without D5) 200 ml/hr throughout radiolabeled antibody infusion. For the therapy infusion, the IV fluids are to start 2-4 hrs prior to the antibody infusion and to continue at least 2-4 hrs after the antibody infusion is complete.

9.3.1.4 Vital Signs

Vital signs will be obtained prior to infusion and monitored approximately every 30 minutes (+/-5 minutes) for the first 2 hours and prior to each infusion rate escalation or if not escalating dose at least hourly (+/- 15 minutes) until the infusion is complete or more often if clinically indicated.

9.3.1.5 Management of Toxicities

Planned management of infusion reactions by scheduled and PRN medications may include:
- Fever: acetaminophen 650 mg (or 15 mg/kg) PO pre therapy and every 4 hours PRN
- Rigors: meperidine 25-50 mg IV (or 0.5–1 mg/kg) every 2-4 hours PRN
- Pruritis: diphenhydramine 25-50 mg (or 1 mg/kg) PO or IV every 2-4 hours PRN

- Nausea: lorazepam 0.5-2 mg (0.05 mg/kg) IV every 4 hours PRN; diphenhydramine 25-50 mg PO or IV every 4 hours PRN; prochlorperazine 5-10 mg IV/PO every 4 hours PRN; ondansetron 8mg IV/PO then every 8 hours PRN
- Cough, chest or throat tightness, wheezing: diphenhydramine and hydrocortisone may be repeated as above; albuterol by nebulizer 2.5–5 mg up to every 1-2 hours PRN
- Low blood pressure: up to 500 ml Normal Saline Bolus given IV over 30 min. May repeat x 1 PRN.
- Hypoxemia: Supplemental oxygen PRN to keep Oxygen saturation within normal limits
- Anaphylaxis: Cessation of antibody infusion and alert the Code Team (if in an outpatient department) or if inpatient the Rapid Response Team or patient's inpatient team for additional orders.

Table 3 - Infusion-related hypersensitivity reaction management

Symptom Severity according to NCI CTCAE v. 4.0	Symptom Examples that could occur during antibody infusion	Intervention Recommendation	Rate Adjustment
Mild Grade 1 - Mild transient reaction; infusion interruption not indicated; intervention not indicated	Fever without rigors, flushing or mild rash, mild nausea without vomiting, mild headache	Close monitoring, Continuation of radiolabeled antibody infusion	N/A
Moderate "Grade 2 – Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids; prophylactic medications indicated for ≤ 24 hrs	Rigors, more severe rash, dyspnea, chest tightness, hypoxemia, tachycardia, hypotension, severe nausea with or without vomiting, diarrhea	Administration of PRN medications as appropriate for symptom(s) along with close direct monitoring; pause of infusion in most cases	If infusion is paused, when symptoms are under control (near baseline), infusion will be restarted at one rate lower than when paused (or at 7.5 mg/hr if not increased from the beginning rate.) The rate will be kept at this lower infusion rate for a minimum of 1 hour prior to resuming the rate escalation schema (adding 7.5 mg/hr each ½ hour until infusion is complete or further reactions occur.) *At the discretion of the PI or designee the rate can be kept at the lower rate, reduced further and/or increased at a slower rate than suggested by the original schema.
Severe Grade 3 - Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms after initial improvement ; hospitalization required for clinical sequelae	Symptomatic hypotension; symptomatic bronchospasm; intractable vomiting; generalized urticaria/angioedema,	Pause infusion, Give additional PRN medications as appropriate to symptomology; If reaction does not resolve, or progresses in spite of these measures, then infusion will be terminated and patient taken off study	If symptoms resolve, and patient returns to baseline or close to baseline, and after consultation with the PI, it is decided to resume the infusion, the rate adjustment listed under "Moderate – Grade 2" will be followed.
Life Threatening Grade 4 – Urgent intervention indicated	Ongoing severe hypotention; ongoing symptomatic bronchospasm or throat swelling; or other life-threatening symptoms	Infusion will be terminated and patient taken off study.	N/A

9.3.2 Quantitative Imaging

Due to patient variability, the most objective way to achieve consistent target doses of radiation exposure in human trials of radioimmunotherapy is by first performing gamma camera imaging using trace infusions

of radionuclide to assess pharmacokinetics and biodistribution [61]. Yttrium-90 (^{90}Y), a pure beta emitting radiometal, will be used as the therapeutic radionuclide in this study. ^{90}Y cannot be used for imaging due to the lack of discrete gamma emissions in the quantitative imaging window. Therefore, ^{111}In (~5-10 mCi), which is a gamma emitter, will be used for imaging purposes.

The biodistribution of trace-labeled ^{111}In-DOTA-BC8 will be determined in a manner similar to that used in our prior ^{131}I–BC8 studies. Before receiving the imaging test dose of ^{111}In-DOTA-BC8, patients will have the volumes of normal organs (lungs, kidneys, liver, spleen) measured by CT scans. Patients will have at least three to four sets of gamma camera images performed. Beginning on the day of infusion, one set immediately after the ^{111}In-DOTA-BC8 infusion, followed by up to 3 additional images over 7 days

Data obtained from gamma camera scans following the trace-labeled infusions of ^{111}In-DOTA-BC8 will be used to evaluate the ^{111}In biodistribution and to estimate the amount (mCi) of ^{90}Y necessary to deliver the prescribed target amount of therapeutic ^{90}Y-DOTA-BC8. Images will be inspected for general biodistribution of activity in the body, especially liver, kidney, bone marrow and spleen. Radiation absorbed doses (cGy per mCi ^{90}Y administered) will be calculated for all tissues and organs (with special attention to bone marrow, liver, lung, and kidney) according to methods recommended by the Medical Internal Radiation Dose (MIRD) Committee of The Society of Nuclear Medicine using OLINDA-EXM software as described in Appendix 3. These calculations will be based on the organ activities determined by quantitative serial gamma-camera imaging using the biodistribution data obtained with tracer ^{111}In-DOTA-BC8 for all major source organs showing activity above background. Results in absorbed dose per unit administered activity of ^{90}Y will be reported for all 25 target organs listed in OLINDA and for the whole body.

9.3.3 Antibody Samples

If lymph node biopsy is done as described in section 9.3.4, aliquots of the injectate will be taken prior to infusion to be used as quantitative standards for assessment of counts in the lymph node biopsy.

9.3.4 Lymph Node Biopsy

Core lymph node biopsies may be obtained approximately 48 hours following the antibody infusion in patients with tumors in locations amenable to biopsy in order to estimate the absorbed radiation dose. Tissue sections will be weighed and counted via gamma counter for ^{111}In content. The percentage injected dose (%ID) of labeled BC8 antibody per gram of lymph node tissue will be calculated using a quantitative standard of the injectate to adjust for decay. Morphologic analysis of the core specimens will be performed by SCCA pathology. When adequate tissue is available, single cell suspensions will be produced and flow cytometry analysis will be performed to document the presence of bound antibody and % saturation of CD45 sites. If biopsy specimens are deemed inadequate, repeat biopsy (excisional when possible) will be performed at a second site within 24 hours.

Patients may undergo selective SPECT/CT imaging up to 4 times after the end of antibody infusion for purposes of tumor dosimetry calculation based on imaging. In patients whose initial tumor size is greater than 2.0 cm in greatest diameter, but at sites not amenable to biopsy, combined SPECT/CT imaging will

be used to calculate tumor dosimetry when possible. Tumor dosimetry may also be calculated for patients in whom biopsy is available.

9.3.5 Dosimetry Analysis and Outcomes

Based on the results of the imaging studies, the radiation absorbed doses to critical normal organs will be calculated using OLINDA dosimetry [78]. The radioactivity amount calculated to deliver a desired dose to the critical non-target organ receiving the highest radiation absorbed dose per mCi of administered activity will be established for each patient. In addition, the dose to tumor will be determined from gamma camera measurements for tumors that can be imaged and by direct counting of biopsied lesion(s) if done.

Patients <u>not</u> achieving favorable biodistributions with the initial infusion will undergo a second dosimetry infusion (no sooner than 7 days after the prior infusion, to allow clearance of the antibody), imaging, and biopsies at the next higher antibody dose level (**Table 2**) and will again be assessed to determine if a favorable biodistribution has been achieved. In contrast, patients determined to have favorable biodistributions (tumor sites receiving higher doses of radiation exposure per mCi of administered radioactivity than critical normal organs) with the <u>initial</u> infusion will not require a second infusion. Patients who are not evaluable for tumor dosimetry will not receive a second ^{111}In-BC8-DOTA infusion and will be treated at their initial antibody protein dose level.

9.4 Detailed Investigational Treatment Plan: ^{90}Y-DOTA-BC8 Therapy

All patients who remain HAMA negative will proceed to the therapeutic infusion of ^{90}Y-BC8-DOTA. See Section 9.3.1 for infusion administration specifics. The antibody (protein) dose for the therapeutic infusion will be the dose at which the patient achieved the most favorable biodistribution. Patients who do not achieve a favorable biodistribution will be treated at the more favorable of the two protein doses given, or at the same dose as the single biodistribution dose (in patients ineligible for tumor dosimetry). In the first phase of this study, the ^{90}Y dose will be determined by the dose escalation schema outlined in Section 19 (Statistical Considerations) and in **Table 4.** Once a sufficient number of patients have enrolled to establish the MTD, enrollment will continue in a second cohort where the radiation dose administered will be at the MTD (see Section 19 Statistical Considerations for further details).

Table 4: ^{90}Y-BC8-DOTA dose escalation as measured by estimated radiation dose to the normal organ receiving highest dose.

Dose level	Dose Administered (Gy)
1	10
2	12
3	14
4	16
5	18
6	20
7	22
8	24
9	26

Table 4: ^{90}Y-BC8-DOTA dose escalation as measured by estimated radiation dose to the normal organ receiving highest dose.	
Dose level	Dose Administered (Gy)
10	28
11	30
12	32
13	34
14	36
15	38
16	40
17	42

10.0 NON-INVESTIGATIONAL DRUGS, STEM CELL OR BONE MARROW ADMINISTRATION

10.1 BEAM Chemotherapy

Approximately 7 days after the ^{90}Y-BC8-DOTA infusion, BEAM chemotherapy will be initiated according to Seattle Cancer Care Alliance (SCCA)'s standard pharmacy guidelines. In general, BEAM will be given as per **Table 5**, but doses of individual agents may be adjusted at the discretion of the attending physician. *If at any time the SCCA develops Standard Practice Guidelines for BEAM, then this protocol's administration schedule and dosage will change to concur with the SCCA Guidelines.

1. BCNU (Carmustine) will be administered on day -7. Dose: 300 mg/m^2 IV in D5W 250 ml over 3 hours (one day only)
2. Etoposide (VP-16, Vepesid) will be administered on days -6, -5, -4 & -3. Dose: 100 mg/m^2 IV in D5W per Pharmacy standard over 2 hours BID x 4 days (or 200 mg/m^2 total daily dose x 4 days)
3. Ara-C (Cytarabine) will be administered on days -6, -5, -4 & -3. Dose: 100 mg/m^2 IV in D5W 250 ml over 4 hours BID x 4 days (or 200 mg/m^2 total daily dose x 4 days)
4. Melphalan will be administered on day -2. Dose: 140 mg/m2 IV in NS 250 ml over 30 minutes (one day only)

Table 5: Timing of BEAM Treatment	Day							
	-7	-6	-5	-4	-3	-2	-1	0
BCNU (300 mg/m^2 IV x 1 d)	X							
Etoposide (100 mg/m^2 IV BID x 4 days) (or 200 mg/m^2 total daily dose x 4 days)		X	X	X	X			
Ara-C (100 mg/m^2 IV BID x 4 days) (or 200 mg/m^2 total daily dose x 4 days)		X	X	X	X			
Melphalan (140 mg/m^2 IV x 1 day)						X		
Day of REST (*optional)							X	
Autologous Stem Cell Infusion								X

NOTE: The dates outlined in **Table 5** are to be used as a general timeframe. The precise days of initiation for BEAM relative to the date of stem cell infusion can be adjusted +/- 1 day if necessary to accommodate clinic/hospital scheduling.

10.2 Stem Cell Infusion

PBSC will be thawed and infused per standard practice approximately 14 days after the therapy dose. At day 14, a maximum of 0.005 cGy/mCi would be imparted to the marrow space (Fisher et al, unpublished

data). Prior data have suggested that marrow absorbed doses up to 5 cGy from ^{90}Y allow for prompt engraftment [8]. *Stem cells should not be infused within 24 hours of melphalan infusion if a day of rest is not taken (**Table 5**).

10.3   Adjunctive Therapy

Adjunctive therapy will follow standard practice guidelines.

11.0   EVALUATIONS

11.1   Pre-transplant evaluations

Patients will undergo a pre-transplant work-up as per standard practice for patients undergoing autologous transplantation, including standard clinical staging assessments for lymphoma (e.g., CT scans of chest, abdomen, and pelvis, and neck at the discretion of treating MD or investigator; PET-CT within 56 days prior to treatment is strongly recommended; bone marrow biopsy if indicated; etc.), and standard clinical workup for transplant including pulmonary function testing and assessment of cardiac ejection fraction (usually by echocardiogram or MUGA scan, performed commonly per standard practice but not required in all instances). In addition, the following assessments will be performed:

1.   Testing for presence of HAMA
2.   Serum rituximab level will be measured at baseline (prior to any study-related treatment) to corroborate laboratory findings that CD45 targeting is not impacted by the presence of rituximab (see Figures 4 and 6 above).
4.   Measurement of organ volumes (obtained from above-noted imaging studies used for pre-transplant staging)

11.2   Post-transplant evaluations

Patients will routinely be followed within the general institutional guidelines and based on the patient's clinical condition. A summary of the common follow-up guidelines used when patients are followed on an outpatient basis are summarized in **Table 6** below. All hospitalized patients have daily clinical assessments. Initial response assessments will be conducted approximately 1 month post-transplant as described below.

Table 6: General Post-Transplant Outpatient Monitoring Guidelines		
Assessment	Interval	
	When ANC<500 or PLT<20	When ANC≥500 and PLT≥20K
CBC	Daily	Weekly
Electrolytes	Weekly	Weekly
Liver Function Tests	Weekly	Weekly
Clinical assessment	Weekly	Weekly

11.3   Measurement of Response (assessed > 1 month post therapy)

Response will be interpreted by investigator review of radiographic findings along with MD assessed physical findings and bone marrow or blood reports and will follow the revised lymphoma response criteria established by an international working group [79].

11.4   Long-term evaluations (Appendix 2)

After the primary toxicity endpoint is reached for each patient (30 day toxicity) patients will be followed at 3, 6 and 12 months and then annually for disease progression and survival. In addition, all non-hematologic toxicities of ≥grade 3 (NCI CTCAE) will continue to be recorded through day 100 after transplant. Patients with progressive disease will only be followed for survival and development of myelodysplasia or secondary malignancies.

12.0   DEFINITIONS

Measurable Disease: Bidimensionally measurable lesions with clearly defined margins by physical examination (e.g. adenopathy), plain x-ray with one diameter 0.5 cm or greater, or CT/MRI (both diameters must be greater that the distance between the cuts of the imaging study).

Evaluable Disease: Unidimensional measurable lesions, masses with margins not clearly defined, lesion with both diameters less than 0.5 cm, or lesions on scans with both diameters smaller than the distance between cuts.

Non-Evaluable Disease: Pleural effusions, ascites, or disease documented only by indirect evidence (e.g. by lab values).

13.0   TOXICITIES AND COMPLICATIONS

13.1 ^{111}In/^{90}Y-DOTA-BC8

Two separate types of toxicities are anticipated with the use of radiolabeled DOTA-BC8: acute toxicities associated with monoclonal antibody infusion, and longer term toxicities associated with radiation dose.

13.1.1   Acute toxicity associated with DOTA-BC8 infusion

Although unlikely, serious allergic reactions (eg, anaphylaxis) may occur at any time during the administration of monoclonal antibodies (mAbs), including BC8-DOTA. Infusion related reactions have been observed with administration of other mAbs, and include headache, fever, chills, facial flushing, erythema, pruritus, rash, myalgia, throat or chest tightness, dyspnea, nausea, vomiting, diarrhea, abdominal discomfort, diaphoresis, hypertension, lightheadedness, hypotension, palpitations.

Subsequent to the infusion the patient may additionally experience joint pain and/or swelling, and hepatic or renal toxicities.

13.1.2 Toxicity associated with radiolabeled DOTA-BC8

The radiation risk associated with the administration of ~5-10 mCi of ^{111}In is estimated to be low. The radioisotope is widely used in clinical imaging applications at this dose without reports of myelosuppression or other clinical complications. ^{111}In is used as a tracer to follow the biodistribution of the antibody and to estimate the radiation absorbed dose for ^{90}Y based on the assumption that both isotopes behave similarly when labeled to the BC8 antibody. An adverse event of grade 4 hypotension has been reported as probably related to ^{111}In administration in one patient with B Cell lymphoma. The patient required 2 days of hospitalization until all side effects were resolved.

The major toxicity associated with the use of ^{90}Y-labeled antibodies is myelosuppression. This effect is partly due to the loss of free ^{90}Y metal ion that accumulates in bone and irradiates the bone marrow. The degree of myelosuppression is a function of residence time in the blood, chelate stability and the injected dose of ^{90}Y. We expect this effect to be amplified by the hematolymphoid targeting of BC8 (Anti-CD45) and expect myeloablation will occur at high dose levels, requiring reconstitution by ASCT, as planned in this study.

13.1.3 Radiation Toxicity

Severe (grade 4) bone marrow suppression is expected during the course of ^{90}Y dose escalation. It is anticipated that bone marrow suppression will occur 7-14 days after therapeutic antibody infusions. Other organs that may receive significant radiation doses and thus experience toxicity include the liver, lungs, gastrointestinal tract, kidneys and thyroid gland. Late effects of radiation may include hypothyroidism, pulmonary fibrosis, cataracts, growth retardation, sterility and carcinogenesis.

In previous studies using high doses of ^{131}I-BC8 in an allogeneic transplant setting for leukemia and myelodysplastic syndrome, significant pulmonary toxicities that were considered at least possibly related to the study drug occurred within the first 100 days after transplant in 7 of the first 74 patients treated (9%). These events included Grade 3-4 dyspnea and hypoxia, bronchiolitis obliterans organizing pneumonia (BOOP), and pneumonitis/pulmonary infiltrates. In the same patient population, 16 of 34 (47%) patients surviving more than 100 days after transplant experienced delayed pulmonary events during follow-up. Most of these were mild (e.g., grade 1-2 cough), but 4 patients showed radiographic changes suggestive of pneumonitis and/or fibrosis.

Though the rates of lung injury are expected to be significantly lower in this study of autologous transplantation, lung damage resulting from radiation exposure could limit further treatment options for participants whose disease is not cured or relapses following study treatment. This could include ineligibility for other transplant regimens and for this reason patients with chemotherapy-sensitive DLBCL who are curable with standard transplant regimens are ineligible for this study.

13.2   BEAM Toxicity

13.2.1   BCNU (Carmustine – a nitrosourea derivative/alkylating agent)

Adverse Effects: The most serious and frequent adverse effect is delayed hematologic toxicity, which is cumulative and usually occurs weeks after administration. Nausea and vomiting occur frequently after IV administration.   Pulmonary toxicity, which can be rapidly progressive and fatal, is characterized by pulmonary infiltrates and hypoxia. Most of the cases have occurred in patients receiving total doses exceeding 1400 mg/m^2, although pulmonary fibrosis has occurred with lower total doses. Hepatotoxicity (reported in up to 26% of patients) is generally mild and reversible. Progressive azotemia and renal failure have occurred in patients who have received large cumulative doses, and occasionally in patients after lower doses.

13.2.2   Etoposide (VP-16, Vepesid) – a simi-synthetic podophyllotoxin)

Adverse Effects: Reversible myelotoxicity has been uniformly observed to be the major toxicity of Etoposide and represents the only clinically significant side effect. Transient, modest nausea, usually without vomiting, is common. Occasional alopecia is reported, as well as occasional hypotension, anaphylaxis or fever.

13.2.3 Ara-C (Cytarabine – a synthetic pyrimidine nucleoside and pyrimidine antagonist antimetabolite)

Adverse Effects: The major adverse event is myelosuppression. Nausea and vomiting may occur more frequently in patients receiving rapid IV infusion of the drug. Other reported adverse effects include fever, rash, alopecia, skin ulceration, conjunctivitis, chest pain, urinary retention, renal dysfunction, dizziness, somnolence, neuritis or neurotoxicity, and reactions at the injection site including pain, inflammation, thrombophlebitis, or cellulitis. A cytarabine syndrome manifested as fever, myalgia, bone pain, maculopapular rash, conjunctivitis, malaise and occasionally chest pain has been reported. If symptoms of the syndrome require treatment, administration of corticosteroids should be considered.

13.2.4   Melphalan – (L-phenylalanine mustard, an alkylating agent)

Adverse Effects: The most common side effect is bone marrow suppression. Gastrointestinal disturbances such as nausea, vomiting, diarrhea and oral ulceration occur infrequently.   Other reported adverse reactions include pulmonary gibrosis and interstitial pneumonitis, skin hypersensitivity, vasculitis, alopecia, hemolytic anemia and allergic reaction.

13.3 Other Expected Toxicities

It is expected that patients will frequently require admission during the first 30 days post-transplant for expected transplant-related toxicities, often due to neutropenic fever, infections, or mucositis causing severe pain and/or interfering with food and fluid intake.

14.0 PROTOCOL ENROLLMENT AND SPECIAL CONSIDERATIONS

A. All patients will require placement of a double lumen central venous catheter prior to the therapeutic infusion; the biodistribution infusion also must be infused through a central venous catheter, but alternative devices (e.g., peripherally-inserted central catheter [PICC] or port) are permitted.

B. All patients receiving therapeutic amounts of ^{90}Y-labeled antibodies will be treated and housed overnight in single rooms at the University of Washington.

C. All blood and tissue samples containing radioisotopes should be clearly identified as such. Samples containing high levels of activity (>50 µCi) should be transported in shielded containers. All samples should be processed by personnel trained in the use of radioisotopes and sample volumes submitted to clinical laboratories will be as small as possible.

D. Potential alternative therapies will be discussed with all patients including observation alone, conventional chemotherapy and radiation therapy, and marrow transplantation with conventional conditioning regimens for which the patients are eligible.

E. Neither gender nor ethnicity are criteria for enrollment on this study. Based on previous enrollment experience at FHCRC/UW the expected gender and ethnicity distribution is shown in **table 7** below.

15.0 TARGETED/PLANNED ENROLLMENT

Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	1	2	3
Not Hispanic or Latino	17	24	41
Ethnic Category: Total of All Subjects *	18	26	44
Racial Categories			
American Indian/Alaska Native	0	1	1
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	1	1	2
White	15	22	37
Racial Categories: Total of All Subjects *	18	26	44

16.0 ADVERSE EVENT REPORTING

16.1 Adverse Event Definitions

- **Adverse Event**
  An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

- **Serious Adverse Event**
  A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:
  o Death.
  o Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
  o In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.

- o   Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- o   Congenital anomaly/birth defect.
- o   An important medical event that requires intervention to prevent one of the above outcomes.
- **Unexpected Adverse Event**
  An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

16.2   Monitoring and Recording AEs

Adverse events will be assessed by the investigator or qualified designee anddesigneeand recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure
- The action taken due to the adverse event
- The outcome of the adverse event.

16.3 Grading of the Severity of an Adverse Event

AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/About.html). If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event. However, the Bearman Scale of Regimen-Related Toxicity will be used for decisions regarding dose escalation/ de-escalation and invocation of stopping rules.

16.4 Attribution of Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal

of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).

- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated. For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition described in 21 CRF 312.32 as an AE for which there is a reasonable possibility that the drug caused the adverse event where "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reactions that are both serious and unrelated will be reported to the FDA as an IND safety report, in accordance with regulations under 21 CFR 312.32.

16.5 Adverse Event Reporting Period

AEs will be monitored and recorded in study-specific case report forms (CRFs). From the time of first exposure to an investigational agent (i.e., the start of the ^{111}In-DOTA-BC8 infusion) through day +30 post-transplant or through discharge prior to that date from the SCCA system to care of the patient's primary physician, non-hematologic adverse events of ≥ grade 3, possibly related events of grade 2 that have not previously been observed with components of the study regimen, and all serious adverse events will be captured in protocol-specific case report forms. Grade ≤4 hematologic toxicity is expected and will only be recorded as time to engraftment. Beyond day 30 after transplant/discharge from the transplant service until day 100, only SAEs and grade 4 and 5 toxicities will be collected. Beyond day 100, disease progression, development of myelodysplasia or secondary malignancies, and survival only will be collected. AEs with an onset date prior to the first exposure to an investigational product will not be recorded, except in the case of clinically significant worsening of the AE during the specified monitoring time frame. A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

The following events are *not* identified as AEs in this study:
- Disease progression or relapse. However, clinical events associated with progression/relapse may be reportable as AEs.

- Hospitalization for the purpose of facilitating conditioning and/or stem cell infusion is not considered an AE. Any AE requiring prolongation of this hospitalization will be recorded and subject to applicable SAE reporting.
- Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.
- Abnormal laboratory values will be identified and recorded as AEs only if clinical intervention is required as a result.

16.6 Adverse Event Reporting Requirements

16.6.1 Research Site Reporting Requirements

Classification of an event as serious or non-serious (see Section 16.1) determines the reporting procedures to be followed by the site for reporting the event to the IND Sponsor. The investigator must report events to the Fred Hutch IRB in accordance with the policies of the IRB.

TABLE 7: PI to IND Sponsor Reporting Requirements for Adverse Events

Classification		Reporting Time	Reporting Action	Contact Information
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team awareness	Email notification to IND Sponsor's Medical Monitor & ISIOC Administrator	Medical Monitor email: tillb@fredhutch.org ISIOC email: ISIOC@fredhutch.org
	All SAEs	Within 2 business days of research team awareness	Submit completed Institution-Sponsored IND SAE Reporting Form signed by PI or designated sub-Investigator	ISIOC Fax: 206-667-6068 ISIOC email: ISIOC@fredhutch.org
Non-serious Adverse Event		Per CRF completion guidelines	Record information on appropriate CRFs	N/A

*Research team is defined as the individuals listed on the delegation of authority log. Physicians listed on the study's delegation of authority log as transplant service attending physicians delegated authority to administer informed consent will not be considered part of the research team unless additional responsibilities related to the conduct of the study have been delegated to them by the Principal Investigator.

The information in the Institution-Sponsored IND SAE Reporting Form must match or be reconciled with the information recorded in the adverse events section of the CRF and study database. For example, the same adverse event term should be used on both forms.

The investigator must report events to the Fred Hutch IRB in accordance with the policies of the IRB. The IND sponsor assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32.

16.6.2 Fred Hutch IND Sponsor Reporting Requirements

The sponsor assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32.

Each serious adverse event report received from the investigator will be evaluated by the Medical Monitor who will assess the seriousness of the event (see Section 16.1), the expectedness of the event (see Section 16.1), and the relationship to participation in the study (see Section 16.4). For regulatory reporting purposes, the IND Sponsor will determine expectedness relating to the investigational product using safety information specified in the Investigator Brochure. An event will be classified as related if either the investigator or the IND Sponsor determines that the event may be related to the study drug.

The IND Sponsor or its designee will provide all investigators with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. Investigators will be requested to provide written notification of safety report to the Fred Hutch IRB as soon as is practical, consistent with IRB requirements.

16.7   SAES Associated with ASCT

Certain events that are commonly observed as SAEs following ASCT are described here in order to facilitate assessments of attribution. SAEs that are identified as routinely experienced in the autologous transplant setting would typically be assessed as unrelated to elements of the radioimmunotherapy regimen used in this protocol. The following list represents some of the most frequent SAEs expected in this setting and is not intended to be comprehensive.

CTCAE Category	Toxicity
Blood and lymphatic system disorders	*As noted above, all patients undergoing HCT are expected to have ≤ Grade 4 pancytopenia as an intended therapeutic effect. These hematologic adverse events will be tracked and recorded only as time to recovery of blood counts/engraftment.* Febrile neutropenia
General disorders and administration site conditions	Fatigue Fever Rigors, chills
Gastrointestinal	Diarrhea Dysphagia Esophagitis Mucositis/Stomatitis Nausea Vomiting
Hemorrhage/Bleeding	Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia
Infections and infestations	*Infections may be associated with neutropenia following HCT*
Metabolism and nutrition disorders	Anorexia Dehydration Hypokalemia (e.g., potassium < 2.5 can result from wasting induced by HCT related medications)
Reproductive system and breast disorders	Reproductive system and breast disorders – Other: Sterility/infertility

17.0   DATA AND SAFETY MONITORING PLAN

This is a single institution trial where all patients are followed closely by the investigators. Additionally, the trial design provides rules for dose escalation depending upon the rate of development of Grade III/IV RRT (Bearman Scale). This design mandates ongoing review of the outcome of previous patients treated on study so that the appropriate Dose Level for the current patient can be assigned. The principal investigator, primary research nurse, and study data coordinator meet routinely (typically weekly, and not less than monthly) to review recently acquired data, stopping rules, and adverse events. The data recorded within the research charts and protocol database is compared with the actual data that is available from the medical record and/or clinical histories. Data detailed in the research case report forms includes the nature and severity of all significant toxicities, which are also reported as described above. All investigators on the protocol have received formal training in the ethical conduct of human research.

Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, FHCRC Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCRC Scientific Review Committee (SRC) and the FHCRC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

18.0   DATA MANAGEMENT/CONFIDENTIALITY

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique subject number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Additional clinical data may be made available from the Fred Hutch core database (Gateway), which is managed and verified independent of the research group.

The research team will maintain Case Report Forms (CRF) and associated research documentation for each patient treated under the protocol. This documentation includes both clinical data and study-specific documents for each patient. Additional study-specific documents and radiologic data are maintained by the UW Division of Nuclear Medicine. The Principal Investigator or a designee will verify completed CRFs against source documentation on an ongoing basis as they are completed for individual patients. CRFs should be complete and data entered into the study database within 120 days of transplant. Data required for analysis of patients treated on this protocol will be maintained in a password-protected study-specific database. Data from the CRFs are keyed directly into the database by authorized research staff and verified on an ongoing basis.

19.0   STATISTICAL CONSIDERATIONS

19.1 ^{90}Y dose escalation

One primary objective of this study is to estimate the MTD of ^{90}Y-BC8-DOTA that can be delivered prior to BEAM chemotherapy and ASCT in patients with relapsed/refractory lymphoma. The MTD is defined as the dose that is associated with a true DLT rate of 25%, where a DLT is defined as a therapy-related grade III or IV Bearman (transplant) toxicity within 30 days of transplant [80]. Dose escalation/de-escalation will be conducted by the "two-stage" approach introduced by Storer [81]. The starting dose level will be level 1 (10 Gy). In the first stage, single patients will be treated at escalating doses in 2-Gy increments (**Table 4**) until a DLT is observed**.** Once a DLT is observed, the second stage will begin at the next lower dose level and patients will be treated in cohorts of 4 according to the following rules. If no DLT is observed in a cohort of 4 the next cohort will be treated at a dose that is 2 Gy higher; if 1 of 4 experiences a DLT the next group will be treated at the same dose; if 2 DLT's are seen among 4 (or fewer) in a cohort, the next group of 4 will be treated at a dose that is 2 Gy lower. This algorithm will continue until 24 patients are treated in the second stage. Following the completed observation of the final patient, a two-parameter logistic model will be fit to the data, thereby generating a dose-toxicity curve based on the observed DLT rate at the various dose levels visited. Based on this fitted model, the MTD is estimated to be the dose that is associated with a DLT rate of 25%. It is possible that a patient will be entered on the protocol before all 4 patients in a cohort have been followed sufficiently long to evaluate toxicity. Such patients will be treated at the current dose level and will be used for purposes of fitting the dose-toxicity curve. These patients will not be used for purposes of dose-modification, however, nor will they be counted towards the total of 24 patients on the second stage for completion of the dose-adjustment phase of the trial.

19.2   Evaluation of efficacy

After completing sufficient enrollment to estimate the MTD of this approach, patients will be enrolled into a second cohort to evaluate its efficacy in terms of overall response rate, overall survival, and progression-free survival (PFS). The second primary endpoint of this study will be to estimate the rate of PFS at 1 year from ASCT when conditioned with ^{90}Y-BC8-DOTA + BEAM, which we will compare to a historical control. This study will not necessarily exclude patients that are traditionally felt to have poor outcomes from standard myeloablative conditioning (e.g., DLBCL failing to achieve remission after first salvage, HL with positive

functional imaging prior to ASCT) [62, 82]. Furthermore, this study will enroll a variety of histologies: B-NHL, T-NHL, and HL; indolent and aggressive. Thus, finding an accurate comparison to use as a historical control is challenging. Based on other reports, we will use the following benchmarks for 1-year PFS for each of these unfavorable-risk subgroups: for example, 10% for DLBCL that relapsed within 1 year of diagnosis (following a rituximab-based induction regimen); 30% for relapsed/refractory MYC+ DLBCL; 60% for rituximab-refractory FL, 40% for HL with a positive PET scan following salvage therapy, and 40% for relapsed/refractory T-NHL [62, 63, 83-85]. We'll assume that our proposed treatment will have roughly the same impact across these various histologies, and the ultimate benchmark that we'll use to assess potential efficacy will be a weighted average of these individual benchmarks, with the weights derived from the proportion of patients with each histology enrolled on the trial. For the current purposes, we'll assume that the overall benchmark to be used will be 30%. If the true 1-year PFS rate using the proposed approach is 54%, then 24 patients will provide 80% power to detect a statistically significant increased rate of PFS from the fixed rate of 30%, based on a one-sample chi-square test with one-sided significance level of 5%. We will include patients who were treated at the MTD in the dose-adjustment phase of the trial in this efficacy sample. Thus, the number of patients to be enrolled in this phase of the trial will be 24-n, where n is the number treated at the MTD in the Phase 1 portion of the trial.

19.3   Antibody dose adjustment

A secondary objective of this protocol is to identify the lowest antibody (BC8) dose (mg/kg) that is consistent with a favorable biodistribution rate ≥80% in lymphoma patients. A favorable biodistribution in a given patient will be defined by the target tissue receiving higher radiation dose per unit-administered activity (cGy/mCi) relative to all critical normal organs (lung, liver, kidney, etc.). To accomplish this goal, the dose to tumor sites (lymph nodes, marrow, spleen) and critical normal organs (lung, liver, kidney) will be calculated from the measurement data which includes the tumor biopsy data, conjugate views and SPECT/CT images for each patient and whole-body counts using methods previously published and described above [8, 57, 61, 86-89]. Towards this end, we will implement a "stopping rule" that will lead to escalation of the protein dose of BC8 used for the initial biodistribution study in each patient (see **Table 2**) if there is ever sufficient evidence to suggest that the true proportion of patients who have a favorable biodistribution following the infusion of ^{111}In-BC8-DOTA is less than 80%. Equivalently, if we have sufficient evidence to suggest that the proportion of patients who fail to achieve favorable biodistribution exceeds 20%, we will escalate the dose of BC8. Sufficient evidence will be taken to be any proportion of patients who fail for which the lower limit of the associated one-sided 80% confidence interval exceeds 20%. Operationally, this rule would activate if any of the following number-of-failures-among-patients-treated is seen: 2 of 4 or fewer, 3 of 7 or fewer, 4 of 11 or fewer, 5 of 15 or fewer, 6 of 20 or fewer, 7 of 24 or fewer, 8 of 27 or fewer, 8 of 28 or fewer, 9 of 32 or fewer, 10 of 37 or fewer, 11 of 41 or fewer, 12 of 46 or fewer, 13 of 50 or fewer, 14 of 52 or fewer. These rules have been listed out to the maximum possible number to be enrolled as the total sample size will depend on the number of patients treated on the dose-finding portion described above. If the true probability of "failure" is 10%, then the probability of escalation of BC8 is approximately .08; if the true probability of failure is 30% or 40%, then the probability of escalation is approximately .90 and .99, respectively (probabilities estimated from 5,000 simulations).

19.4   Estimation of dosimetry

We will also describe the estimated dose to tumor sites based on the tumor to normal organ ratios derived from dosimetry estimates coupled with the absorbed dose to normal organs based on the administered activity of ^{90}Y. This evaluation will be made among all patients and among those treated at the estimated MTD.

19.5   Stopping rules

Since the first phase of this protocol is a dose-finding study, there will be no early stopping rules for toxicity with the possible exception being that the lowest dose leads to unacceptable rates of DLT. Regarding efficacy, since many of these patients would be expected to have a relatively poor outcome from a standard autologous transplant, if >80% of patients experience progressive disease by day 30 following therapy the study may be suspended pending a careful review.

20.0   TERMINATION OF THE STUDY

Individual patients may choose to discontinue treatment at any time. Treatment may also be terminated at the discretion of the Principal Investigator if continuation of treatment is deemed to pose unacceptable toxicity to the patient. Specific reasons for withdrawal may include:

a. Patient request.
b. Unacceptable toxicity during dosimetry antibody infusion (see sections 14.0, 4.3, and 17.0 for complete toxicity descriptions and stopping rules).
c. Development of anti-mouse antibodies.

The study will be terminated upon complete accrual of patients, when toxicity criteria noted above are met, or at the discretion of the PI or sponsor.

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APPENDIX 1:   BEARMAN CRITERIA FOR REGIMEN-RELATED TOXICITY

Parameter	I (mild)	II (moderate)	III (severe)	IV (life threatening)
Allergic	Pruritus, rash	Generalized Urticaria	Anaphylaxis	Fatal
Renal Creatinine	1.5-2x increase	>2x increase No dialysis	Dialysis	Fatal
Pulmonary	Dyspnea	Interstitial pneumonia	Ventilatory support or $F10_2$ >50%	Fatal
Cardiac	Mild CHF No therapy needed	Moderate CHF Diuretics needed	Severe CHF Ejection Fraction <30%	Fatal
Hepatic *Bilirubin* *SGOT 2-* *Ascites*	2-6 mg/L 5 x increase Ascites <100 ml	60- 2mg/L >5 x increase Ascites >100 ml	>20 mg/dL Encephalopathy	Fatal
CNS	Transient somnolence	Somnolence >36 hr	Seizure or coma	Fatal
Stomatitis	Ulcerations	IV opiates	Intubation or aspiration pneumonia	Fatal
GI	Watery stools 0.5-2 L/d	Stools>2L/day Subileus	Hemorrhagic enterocolitis NG suction	Fatal
Bladder	Macroscopic Hematuria <7 days	Macroscopic hematuria >7 days	Sclerosing agents or surgery needed	Fatal

APPENDIX 2: POST TREATMENT EVALUATIONS

EVALUATION	TIME (MONTHS)[1]				
	1	**3**	**6**	**12**	**24** (continue annually until relapse/progression)
CBC	X	X	X	X	X
Chemistries[2]	X	X	X	X	X
Bone Marrow Biopsy[3]	X			X	X
Bone Marrow Aspirate[3]	X			X	X
SPEP[4]				X	X
CT/MRI[5]	X	X	X	X	X
PET-CT[6]	X	X	X	X	X
Pulmonary Function Testing				X	

[1]   Timepoints for these assessments are relative to the date of stem cell infusion.

[2]   Chemistries = creatinine, LDH, bilirubin.

[3]   Only when clinically indicated, BM biopsy/aspirate PCR (only patients that had prior positive PCR studies), flow cytometry, cytogenetics as performed at baseline per standard practice.

[4]   Only patients with a prior documented monoclonal protein are required to undergo follow up SPEP testing

[5]   CT/MRI = chest, abdomen and pelvis.

[6]   Post-treatment PET-CT scan at 1 month is strongly recommended (but is not required by the study) if baseline scan was positive. Subsequent PET-CT scans are to be considered (but are not required by the study) until complete treatment response has been documented by a negative PET-CT.

Following relapse or disease progression, patients will be followed annually for survival and development of secondary malignancies. Following other significant diagnoses and/or therapies that would confound assessment of the relationship between the study treatment and adverse events (e.g., further therapy intended to maintain disease remission), patients will be followed annually for disease status, survival, and development of secondary malignancies.

APPENDIX 3: METHODS USED TO CALCULATE RADIATION ABSORBED DOSES TO PATIENTS

a. Scientific Basis for Internal Dosimetry Calculations

Radiation absorbed doses will be calculated for each patient's normal organs and tissues, the whole body, and for imageable tumors using methods recommended by the Medical Internal Radiation Dose (MIRD) Committee of The Society of Nuclear Medicine (Loevinger et al., 1991). The MIRD methods account for both the penetrating gamma and the non-penetrating beta radiation (electrons) emitted by radioactivity distributed throughout the body. In the MIRD schema, dosimetry calculations are based on a series of direct measurements of the organ biodistribution of radiolabeled antibody in individual patients. These include gamma-camera images and quantitative activity measurements of the radionuclide used (^{111}In as a tracer for yttrium-90-labeled antibody) in the major imageable source organs, tumor tissue, red marrow, and the total body at various time-points post-infusion. When available, the patient-specific organ masses are used for internal dose calculations rather than generic model values. The mathematical foundations for application of these methods to critical organs in high-dose radioimmunotherapy are well-established (Fisher 1994, 2000; Fisher et al. 2009).

b. Rationale for the Use of Indium-111 to Predict Yttrium-90 Biodistribution for Deciding the Administered activity of Therapy

Because ^{90}Y is a pure beta-emitter, it cannot be imaged accurately or conveniently in the patient. We assume that the biodistribution of the trace-labeled ^{111}In antibody faithfully represents the biodistribution of ^{90}Y antibody in the cancer patient.   Preclinical studies by others showed that the biodistribution of ^{111}In antibody usually correlates with ^{90}Y antibody biodistribution (Fisher et al. 2009). Therefore, it is common practice to use ^{111}In antibody measurement data to predict the biodistribution of ^{90}Y antibody. The correlation may be only partly correct, however, because partial dissociation of ^{90}Y and ^{111}In from the immunoconjugate may occur *in vivo;* some of the free ^{90}Y deposits on bone surfaces, and some of the ^{111}In preferentially goes to the testes due to natural uptake of indium by germ cells. During radiolabeling, quality control usually allows less than 2% unbound ^{111}In in the radioimmunoconjugate. We will not measure the unbound fraction in serum after injection. For dosimetry calculations, however, we will assume that the biodistributions of ^{111}In and ^{90}Y labeled antibody are equivalent, recognizing that without further correction this assumption may lead to underestimates of ^{90}Y dose to bone surfaces and red marrow, and to overestimates of ^{90}Y dose to the testes.

c. Direct Measurements in Patients

(i) Conjugate-view quantitative planar imaging with anterior and posterior measurements is the most widely used method for assessing source-organ activity in patients. The conjugate view method does not require knowing the depth of the source region and does not depend on assumptions inherent in single-view phantom simulations, but does incorporate correction for background, scatter, and photon attenuation.

(ii) Biodistribution Imaging

After a tracer quantity of ^{111}In (usually 111 to 222 MBq or 3 to 6 mCi) labeled to the monoclonal antibody is administered, the patient is imaged using collimated anterior and posterior planar gamma-camera imaging. Biodistribution imaging begins immediately after infusion of ^{111}In trace-labeled antibodies on day 0. A nuclear medicine camera with a medium energy collimator, with photopeak settings at 171 and 245 keV, and a symmetric 15% window around each photopeak, is used for imaging. Image regions of interest include head and neck, chest with upper humeri, abdomen, and pelvis with upper femurs. Regions of interest are always selected for the major source organs, such as the liver, spleen, heart volume, red marrow space, occasionally for the lungs and kidneys (when suitable images can be obtained), and for any other tissue with activity above background, including thyroid, testes, and bladder.

Normal organs and tumors are visualized when concentrations of ^{111}In antibody in the organ or tumor are greater than in the adjacent systemic activity. Since ^{111}In exhibits significant non-specific localization in the liver, spleen, cardiac blood pool, kidneys, urinary bladder and bone marrow, no additional imaging (e.g. ^{99m}Tc liver-spleen, lung or renal scans) will be required to delineate these organs. Background regions are drawn for each organ/region similar to the methods described above. In addition, attenuation correction factors are determined for the chest, abdomen, and pelvis using the images described previously.   We image tumor sites that have well-defined uptakes and retention of ^{111}In radiopharmaceuticals, allowing us to determine the percent of administered activity per gram of tumor tissue for all tumors selected for dose assessment. Imaging of selected tumors may be conducted for various time points, and time-activity curves can be constructed and integrated for tumor dose assessments.

Measurements are also made of representative background tissue (such as the thigh muscle), and of a counting standard with and without the patient in the camera field-of-view. The outlines for the regions of interest are drawn by a technologist from the acquired images, and counts are obtained from the selected regions. The counts are decay-corrected to an ^{111}In standard. Patient thickness and the distance from the gamma camera to the source organ are determined. The geometric mean of the anterior and posterior counts is obtained for each region of interest. Counts are then corrected for attenuation, geometry, and background. Total-body measurements are obtained using whole-body gamma camera sweep in anterior and posterior projections or by an external, non-imaging gamma probe at a distance of 4 meters from the patient, to quantify the total ^{111}In activity remaining in the patient, over time, as a fraction of the total administered activity.

Volumes of organs such as liver, spleen, kidneys and lungs are obtained from CT scan reports. A standard containing a known amount of ^{111}In (approximately 1-2 mCi, in a 250 cc tissue culture flask) is available for each patient study. The exact activity and time of measurement is recorded on the flask and in the patient's data record. A fluid filled sheet source large enough to cover the entire field of view of the camera and containing about 2 mCi ^{111}In is used for transmission and attenuation imaging prior to all patient studies.

(iii) Attenuation

Prior to antibody infusion, all patients will have measurement made of their chest, abdomen, and pelvis attenuation by transmission scans. A fluid filled sheet source, large enough to cover the entire useful field of view of the camera with gamma rays (16x25-inches and ½- inch thick for our camera), is loaded with

approximately 2 mCi of ^{111}In. Uniform distribution of the isotope throughout the sheet source is ensured. The source is placed on the lower detector, and the scanning table is brought into place over the source. The upper detector is lowered to one patient thickness from the table, and a five minute image is acquired. The patient lies on the table, is positioned so that his chest, abdomen, and pelvis are in the field of view, and a five-minute image is taken. The observed ratio of counts in the flood source activity counted with and without the patient overlying is the attenuation correction factor for the various areas (organs) of interest.

(iv) <u>Sampling Times</u>

Selecting an appropriate number of counting times requires trade-offs between having sufficient data and economizing the imaging costs and minimizing patient inconvenience. Our objective is to select the fewest time points that will provide a reasonable description of the activity-time curve. The total number of data measurement points is typically three or four, depending on shape of the uptake-retention function. Imaging includes one measurement at or as close to time zero (time of radiopharmaceutical infusion), plus additional measurements at least on two of the three days to 72 hours post-infusion, as practical. These analyses provide an estimate of the fraction of the administered activity that resides in each source organ and in the total body at each measurement time post-infusion.

d. <u>Time-Activity Curves</u>

The sequential measurement data are plotted to determine the cumulated activity and residence times for each source organ. Plotted are the fractions of the total administered activity observed at each measurement time point. Time-activity curves are constructed from the measurement data and are integrated to infinite time to determine the residence times (τ, hours) for each source organ, tumor, the red marrow, and the whole body. The points are fitted to an exponential or sum of exponentials. An estimate of the long-term tail of the time-activity curve may be made by fitting an exponential function to the last two points. We plot the *effective* fractions present (as measured), rather than the values that were decay-corrected from a radionuclide standard, because internal doses are calculated from the integral areas under the effective time-activity curves.

e. <u>Residence Time Calculations: Integrating the Time-activity Curves</u>

The residence time (Bq-sec/Bq or μCi-hr/μCi administered) for a source organ is the fraction of the administered activity in a source organ over time to complete decay, obtained by integrating the time-activity curve. Residence times are the basic input value to software packages (MIRDOSE2 and MIRDOSE3 computer programs, Oak Ridge Associated Universities, Oak Ridge, Tennessee) and OLINDA-EXM (Vanderbilt University, Nashville, Tennessee) that implement the MIRD dosimetry schema. We will use OLINDA-EXM for this project.

The cumulated activity, $\tilde{A}_h$, and residence time, τ_h, are determined by integrating the area under the activity-time curves for the clinical measurement data for each source organ and the remainder tissues. The integrations are carried to infinity for accuracy and simplicity. Residence times for red marrow are determined from a set of gamma camera measurements of ^{111}In activity in marrow spaces (usually the right and left acetabula)The long-term tail of the exponential may be estimated by curve-fitting.

f.   Patient-specific Dosimetry

Actual patient weights and organ sizes vary from those used in the standard MIRD dosimetric models. Since organ dose is approximately proportional to the inverse of target mass, a correction should be made for patient weight and organ mass when actual organ weights are known from CT-imaging. The correction involves recalculating the S values for each of the source-target combinations where patient-specific organ volumes are known. The recalculated S values account for both the gamma component specific absorbed fraction of energy and the mass over which the beta component is averaged.   For most radionuclides, the beta self-irradiation dose in a source organ is the greater contributor to total organ dose (usually more than 90 percent of the total).

The residence times for each source organ are corrected for actual patient mass (if known from CT-imaging volumetrics) using a method described by Rajendran et al. (2004). This method corrects the beta component but does not correct for the lesser-important gamma component. For individual organs and for the whole-body, one multiplies the calculated source-organ residence time, τ_h, by the ratio of the defined reference man or reference woman organ mass to the known organ mass:

$\tau_{new} \approx (\tau_h)(m_{MIRD}/m_{actual})$.

This correction may be appropriate when most of the organ dose is due to non-penetrating radiation. The new residence time for each source organ and for the remainder tissues may then be entered into MIRDOSE to estimate normal organ and whole-body doses (in rad/mCi or mGy/MBq administered) for the patient. A similar correction is made in OLINDA-EXM when patient organ mass is known.

g.   Estimate of Dose to Testes

In male patients with significant testicular uptake of ^{111}In, we estimate the ^{90}Y dose to testes with corrections for ^{111}In disassociation and thickness of tissue due to their superficial position in anterior scans. Regions may be drawn around the testicular uptake on serial anterior gamma camera images. Care is taken to separate the penile activity by instructing the patient to physically move the penis away from the testes during each scanning session. A suitable background is used to correct the counts, but no attenuation correction factor is used because the testes are positioned to the anterior view without any significant attenuating tissues.

h.   Estimate of Dose to the Bone Marrow:

Red marrow dosimetry is challenging because it is difficult to assess 1) the highly variable concentration of radioactivity in marrow, and 2) the mass of red marrow in the patient. Common approaches to marrow dosimetry often rely on unreliable estimates of radiolabeled antibody concentration in red marrow or in red marrow relative to the concentration in circulating blood or blood plasma.   Rather than measure blood plasma activity and make uncertain assumptions, we employ quantitative imaging of defined marrow spaces (acetabulum, sacrum, pelvis, femoral head, or lumbar vertebrae) by repetitive direct counting. Indium-111 activity can sometimes be quantified in lumbar vertebral bodies L2-L4 after injection. These measurements provide data for evaluating the red marrow time-activity curve.

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