FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Title: A Study Evaluating Escalating Doses of ^{90}Y-DOTA-BC8 (anti-CD45) Antibody followed by Allogeneic Stem Cell Transplantation for High-Risk Acute Myeloid Leukemia (AML) Acute Lymphoblastic Leukemia (ALL), or Myelodysplastic Syndrome (MDS).

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Study Regimen: Radiolabeled (^{111}In/^{90}Y) DOTA-BC8 followed by Fludarabine, TBI, and Allogeneic Stem Cell Transplantation

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Investigator Statement:

I have carefully read Protocol 2468 entitled "A Study Evaluating Escalating Doses of ^{90}Y-DOTA-BC8 (anti-CD45) Antibody followed by Allogeneic Stem Cell Transplantation for High-Risk Acute Myeloid Leukemia (AML) Acute Lymphoblastic Leukemia (ALL), or Myelodysplastic Syndrome (MDS)" ("Protocol") version date 11/06/2015.

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

Allogeneic hematopoietic cell transplantation (HCT) is a widely used form of therapy for patients with hematological malignancies. In 2007, between 15,000 and 20,000 patients were treated with this procedure worldwide according to estimates from the International Bone Marrow Transplant Registry (IBMTR). Although HCT often offers the best, and sometimes the only chance for cure, the procedure too often fails to eradicate these patients' malignancies or is associated with fatal toxicities. It is therefore important to improve disease control for diseases such as advanced acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and high-risk myelodysplastic syndrome (MDS) patients without substantially increasing the toxicity of the conditioning regimen for patients undergoing HCT.

One recent advance, radioimmunotherapy (RIT), offers the potential to substantially improve HCT preparative regimens. With the use of radiolabeled monoclonal antibodies (Ab) it is possible to deliver high doses of radiation relatively specifically to bone marrow (BM), spleen and other sites of hematological malignancies while sparing other organs.[3-34] Our group has made considerable progress using iodine-131 (^{131}I) radiolabeled Ab to achieve the goal of developing preparative regimens with greater anti-tumor effects and overall less toxicity. We have used ^{131}I-labeled anti-CD45 Ab (BC8) to deliver at least 2-3-fold more radiation to BM, spleen and sites of leukemia than to any critical normal organ.

One significant barrier to expanding these results to other clinical and research centers is that many Nuclear Medicine departments have difficulty handling large quantities of ^{131}I. This is largely because the high-abundance (100%), medium-energy gamma component of ^{131}I and 8-day physical half-life require patients to be treated in radiation isolation, and poses a radiation exposure risk for staff and family. To address this challenge, we propose replacing ^{131}I with an alternative radionuclide to make our approach more feasible for Phase II studies at additional centers. Yttrium-90 (^{90}Y) has been selected as the therapeutic radioisotope for the current clinical trial because, in part, it is a pure ß-emitter that is commercially available in high specific activity and purity. Moreover, the beta particles from ^{90}Y have a high-energy (Emax = 2.28 MeV) with a greater tissue-penetrating range (up to 11 mm) that is thought to be most favorable for deposition of radiation in large tumor masses.[35]

In this study we hope to establish the safety, feasibility and optimized dose of ^{90}Y-labeled anti-CD45 Ab (^{90}Y-DOTA-BC8) in patients undergoing RIT treatment in conjunction with a standard reduced-intensity transplant regimen before HCT for refractory or relapsed AML or high-risk MDS. This clinical research protocol and the described study will be conducted in compliance with the IRB approved protocol, associated federal regulations and all applicable IRB requirements.

2.0 BACKGROUND

Allogeneic HCT is a widely used tool for the treatment of patients with hematological malignancies, however, relatively high-rates of relapse have been observed in patients with advanced leukemia and high-risk MDS. Efforts to decrease post-HCT relapse rates have focused largely on intensification of cytoreductive therapy, either by increasing the total body irradiation (TBI) dose or adding additional or alternative chemotherapy. Controlled randomized studies have shown that relapse rates can be

reduced by increasing the TBI dose.[36-37] However, in these studies the non-relapse mortality (NRM) was increased with the higher TBI dose, leading to no improvement in overall survival (OS).

2.1 Targeted hematopoietic irradiation delivered by radiolabeled Ab

Although studies have demonstrated benefit for improved tumor control with escalated doses of therapy, the toxicities that occurred as a result of efforts to increase the dose of therapy can be attributed to the non-specific targeting of the therapeutic agents.[36-37] In contrast, the use of Ab-targeted approaches to deliver radiation specifically to sites of disease while sparing normal organs offers hope for improved tumor control with minimally increased rates of toxicity. Hematologic malignancies in particular provide an optimal setting in which to investigate RIT, given their relative radiosensitivity and the comparative ease with which circulating Ab can gain access to cells in the BM, lymph nodes, and spleen.[38-39]

The efficacy of RIT depends on many variables, including properties of the targeted antigen (cell surface density, accessibility, shedding, heterogeneity of expression), the tumor (size, permeability, vascularity, and blood flow), and the Ab (binding site specificity, immunoreactivity, *in vivo* stability, avidity and rate of endocytosis). Although leukemia cells express a variety of lineage-specific antigens that might be targeted, CD33, CD45, and CD66 have emerged as the most successful antigens for RIT of leukemia.[1,27,30,34,40-48] Initial RIT studies by our group for the treatment of AML employed an anti-CD33 Ab conjugated to ^{131}I.[42] While initial localization of ^{131}I-anti-CD33 Ab to the BM was convincingly documented, the retention of ^{131}I in this tissue was short because of internalization of the Ab-antigen complex and rapid degradation of the Ab followed by excretion of small ^{131}I moieties from the tumor cells, contributing to a suboptimal therapeutic result.[11,42] Other investigators have pursued RIT for AML by targeting CD66 which is present on maturing hematopoietic cells, but not on leukemic blasts. CD66 targeting has been used to deliver homogenous radiation to normal cells throughout marrow, relying on bystander irradiation of leukemic blasts to improve the outcome of conditioning prior to HCT.[43,49]

Investigators have focused on the CD45 antigen as an attractive alternative target for RIT of leukemia and MDS. CD45 is a tyrosine phosphatase expressed as isoforms varying in size from 180 to 220 kD. This antigen is expressed on more than 70% of nucleated cells in normal BM, with an average copy number of approximately 200,000 molecules per cell.[11] The abundant expression of CD45 on virtually all leukocytes, including myeloid and lymphoid precursors in BM and mature lymphocytes in lymph nodes as well as more than 90% of AML and ALL samples, provides a high number of Ab binding sites in these tissues for patients in remission or relapse.[50-54] Available data suggest that CD45 is not shed into the bloodstream and is not rapidly internalized.[11,52] Though some have expressed concerns about targeting an antigen expressed as broadly as CD45, important advantages also must be recognized. Lineage-specific radiolabeled Abs such as those targeting CD45 may be superior to leukemia-specific radiolabeled Abs for patients in remission or relapsed patients with subclinical involvement of extramedullary tissues, since in these settings isolated malignant cells are surrounded by normal hematopoietic cells. Because the radiation from a radionuclide attached to an Ab bound to the surface of a cell can be emitted in any direction within a geographic area defined by the path length of the radionuclide, the isolated malignant cell may receive a significantly greater absorbed dose if the surrounding normal cells are targeted as well.

2.2 Clinical studies of RIT with ^{131}I-anti-CD45 Ab (BC8)

We have conducted a series of Phase I and II clinical trials employing ^{131}I-BC8 in conjunction with high-dose chemotherapy and HCT for AML, ALL, and MDS. Results of these trials will be summarized briefly.

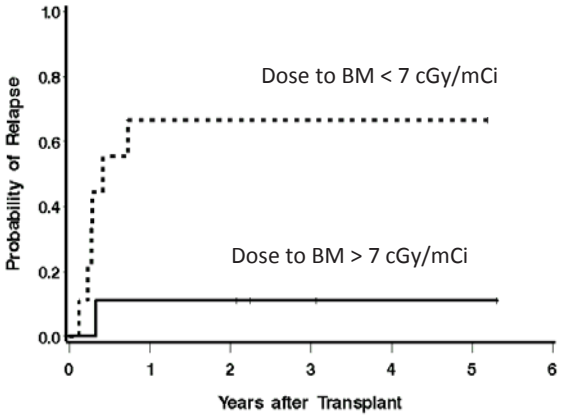

FIGURE 1: Probability of relapse for advanced AML patients who received ^{131}I-BC8 Ab combined with CY/TBI. Thick solid line = dose to BM > 7 cGy/mCi; thick dashed line = dose to BM < 7 cGy/mCi.[2]

Our initial study of ^{131}I-BC8 Ab in high-risk patients with advanced AML, MDS, or ALL was performed to determine the biodistribution of the targeted radiotherapy, as well as estimate the MTD of radiation delivered by ^{131}I-BC8 Ab when combined with 120 mg/kg CY and 12 Gy TBI.[30,34] When combined with CY and TBI, the estimated MTD of radiation delivered by ^{131}I-BC8 Ab to the liver, the normal organ receiving the highest dose was 10.5 Gy. Based on the average estimates of radiation absorbed doses, treatment at the MTD was projected to deliver supplemental radiation doses of 24 Gy to BM and 50 Gy to spleen. Based on these promising results, the same regimen was given in a Phase II study to 18 patients followed by CY/TBI and allogeneic HCT from matched related (n=9) or unrelated (n=9) donors. Seven of 18 treated patients (39%) are alive and disease-free after periods up to 5.5 years. Only 1 of 9 patients receiving a BM dose greater than 7 cGy/mCi relapsed, in contrast to 6 of 9 who relapsed after receiving doses less than 7 cGy/mCi to BM (**Fig. 1**).[2,55]

Since significant supplemental doses of hematopoietic radiation were tolerated when combined with the conventional transplant preparative regimen, we also performed a separate Phase II study of ^{131}I-BC8 Ab combined with standard busulfan (BU) and CY as a preparative regimen for patients with AML in first remission.[1] Forty-six patients were treated with 102-298 mCi ^{131}I delivering 5.3-19 (mean 11.3) Gy to BM, 17-72 (mean 29.7) Gy to spleen, and 3.5-5.25 Gy to the liver. The estimated 3-year disease-free survival (DFS) and NRM rates were 61% and 21%, respectively (**Fig. 2**). These results were compared to those from 509 similar patients in the IBMTR transplanted using BU/CY alone. After adjusting for differences in age and cytogenetics, the hazard of mortality among ^{131}I-BC8 treated patients was 0.65 times that of Registry patients (95% CI 0.39-1.08; p=.09). The encouraging results achieved with ^{131}I-BC8 Ab and BU/CY in younger patients led us to ask how this approach might be applied to older AML patients, since the outcome for AML patients over 55 is poor using standard chemotherapy, with a 5-year survival rate of <10%.[56-58]

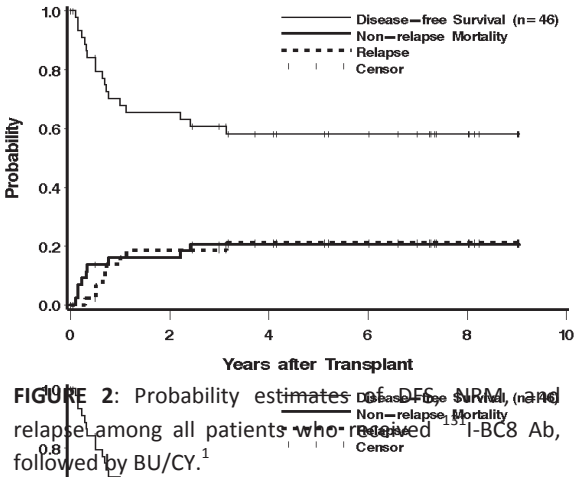

FIGURE 2: Probability estimates of DFS, NRM, and relapse among all patients who received ^{131}I-BC8 Ab, followed by BU/CY.[1]

Over the past decade the reduced-intensity HCT approach has also been applied to patients with AML, ALL and MDS in order to gain the benefit of a graft-vs-malignancy effect without the toxicities associated with a standard HCT regimen.[59-72] Although these studies mostly have been limited to low-risk remission patients, relatively high relapse rates remain a therapeutic dilemma in high-risk patients, suggesting that a graft-vs-leukemia (GvL) effect alone may be inadequate for advanced patients with AML.[73-76] Given the minimal toxicity of this reduced-intensity approach, we hypothesized that the anti-leukemic effect may be improved by the addition of targeted hematopoietic irradiation delivered by ^{131}I-BC8 Ab to the low-intensity conditioning regimen, without undue toxicity. To this end we performed a dose-escalation study adding targeted radiotherapy to our FLU/TBI reduced-intensity approach in 58 high-risk patients over the age of 50 with advanced AML and high-risk MDS. In this study we estimated the MTD of radiation delivered *via* ^{131}I-BC8 Ab to be 24 Gy to liver when combined with the reduced-intensity regimen for older patients. While this study was not designed to examine potential efficacy, we were encouraged by the observation that the 3-year disease-free survival was 38% for patients with active leukemia, a rate better than seen in the historical experience (**Fig. 3**).[77]

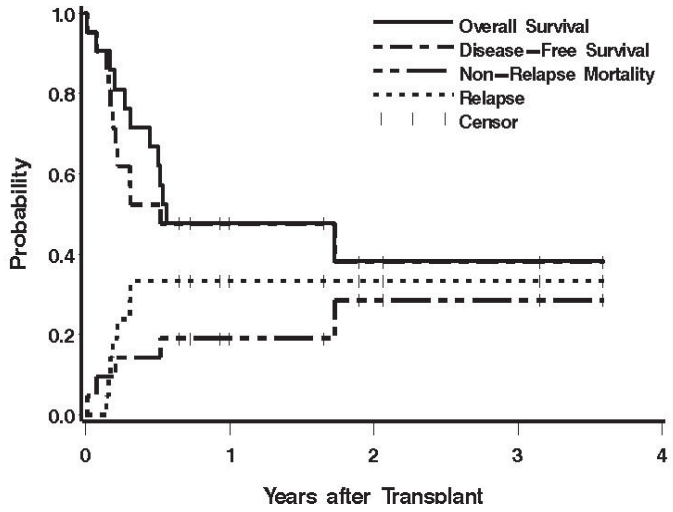

FIGURE 3: Estimates of the probability of OS, DFS, TRM, and relapse among patients treated at the MTD of 24 Gy of radiation delivered to the liver by the ^{131}I-BC8 Ab, followed by TBI/FLU.

2.3 Rationale for the use of Yttrium-90 labeled anti-CD45 antibody

We have used ^{131}I as the radiolabel in our prior clinical studies because it was readily available, there is extensive experience with its medical use, the technology for directly radiolabeling Abs with iodine is well established, and its gamma-ray component allows direct determination of labeled Ab biodistribution in the patient after a tracer infusion. However, the high-abundance gamma radiation component of ^{131}I requires that patients be treated in radiation isolation, and poses a potential radiation exposure risk for staff and family. This presents a major limitation to the exportability of this modality. To contemplate supplanting ^{131}I-anti-CD45 Ab with an alternative radionuclide for RIT of AML, ALL, and MDS requires careful consideration. Yttrium-90 has been selected as the therapeutic radioisotope for this proposed clinical trial because, in part, it is a pure ß-emitter that is commercially available in high specific activity and purity. Moreover, the beta particles from ^{90}Y have a high-energy with greater tissue penetrating range that is thought to be most favorable for deposition of radiation in large tumor masses.[35] These favorable characteristics must be balanced by the desirability of limiting localization of the radiation effect to antigen positive cells, and avoidance of unduly irradiating the BM stroma or normal bystander cells. We investigated whether ^{90}Y, which does not emit any photon radiation and does not require longer-term radiation isolation of the patient, might result in improved therapeutic ratios because of its higher energy beta particles (Emax = 2.28 MeV) and shorter decay half-life (2.7

days) compared to ^{131}I. These non-human primate studies suggested that the use of ^{90}Y as a radiolabel for anti-CD45 Ab results in an approximately similar ratio of radiation delivered to target as compared to non-target tissues as seen with ^{131}I.[78] However, the ability to treat patients with ^{90}Y without requiring radiation isolation, and the potential for improved homogeneity of radiation delivery within tissues given its longer beta-particle path-length, may provide therapeutic advantages.

The complete dosimetry of ^{90}Y-DOTA-BC8 Ab in humans is unknown, acceptable biodistributions of BC8 Ab labeled with a radiometal have yet to be demonstrated, and the toxicity profile of ^{90}Y-DOTA-BC8 needs to be defined. Moreover, we must establish that BC8 Ab labeled with ^{90}Y has the potential to adequately decrease relapse despite the absence of gamma emissions from this radionuclide. As a first step we will therefore investigate the use of ^{90}Y as the therapeutic radionuclide in this study

3.0 STUDY OBJECTIVES

3.1 Primary Objective

To estimate the MTD of radiation delivered *via* ^{90}Y-DOTA-BC8 when combined with FLU and 2 Gy TBI as a preparative regimen for patients aged ≥ 18 with advanced AML, ALL, and high-risk MDS.

3.2 Secondary Objectives

1. To determine disease response and duration of remission.
2. To determine the rates of engraftment and donor chimerism resulting from this combined preparative regimen, and to correlate level of donor chimerism with estimated radiation doses delivered to hematopoietic tissues *via* antibody.

4.0 STUDY DESIGN

4.1 Description of Study

This is an open label study conducted under an investigational new drug application (IND) submitted to the U.S. Food and Drug Administration (FDA). The study is a single-arm treatment protocol designed to meet the primary objective described above.

4.2 Endpoints

4.2.1 Primary Endpoint

The primary safety endpoint will be the proportion of patients who develop grades III/IV Bearman related toxicity within the first 100 days following HCT.

4.2.2 Secondary Endpoints

1. Rates of engraftment, chimerism, non-relapse mortality (NRM) and acute GvHD.
2. Achievement and duration of remission.
3. Overall and disease-free survival through day 100.
4. Estimation of absorbed radiation doses to normal organs, marrow and tumor.

4.3 Stopping Rules

Dose escalation/de-escalation will be implemented in this study to achieve the primary objective of estimating the MTD of ^{90}Y-DOTA-BC8 associated with a Grade III/IV (Bearman) RRT rate of 25%. As

described further under Statistical Considerations (section 17.0), additional stopping rules are in place related to graft rejection and rate of grade III-IV acute graft versus host disease (GvHD).

If sufficient evidence exists suggesting that the true rate of graft rejection exceeds 20%, the protocol will be suspended and reviewed by the investigators and other experts/committees as appropriate to determine whether the trial should be terminated, continued without change, or continued with revisions. Operationally, suspension would be triggered by graft rejection at any of the following observed proportions: 3/5, 4/10, 5/15, 6/20, 8/25, or 9/30. We have not seen graft rejection in any of our prior studies of radiolabeled BC8 Ab.

The protocol will also be suspended if sufficient evidence exists to suggest that the true rate of grades III-IV acute GvHD at day +100 is excessive (graded according to the established criteria described in **Appendix B**). Operationally, suspension would be triggered by any of the following observed proportions of GvHD: 4/5, 6/10, 8/15, 10/20, 12/25, or 14/30.

Proportions of graft rejection and grades III-IV acute GvHD will be evaluated after every 5th enrolled patient becomes evaluable. Each suspension rule will be evaluated among all patients enrolled, regardless of the dose of ^{90}Y-DOTA-BC8 administered.

5.0 SUBJECT SELECTION

5.1 Inclusions

1. Patients must have advanced AML, ALL or high-risk MDS meeting one of the following descriptions:
   - AML or ALL beyond first remission (i.e., having relapsed at least one time after achieving remission in response to a treatment regimen);
   - AML or ALL representing primary refractory disease (i.e., having failed to achieve remission at any time following one or more prior treatment regimens);
   - AML evolved from myelodysplastic or myeloproliferative syndromes; or
   - MDS expressed as refractory anemia with excess blasts (RAEB) or chronic myelomonocytic leukemia (CMML) by FAB criteria.
2. Patients not in remission must have CD45-expressing leukemic blasts. Patients in remission do not require phenotyping and may have leukemia previously documented to be CD45 negative (because in remission patients, virtually all antibody binding is to non-malignant cells which make up $\geq$ 95% of nucleated cells in the marrow).
3. Patients must be ≥ 18 years of age.
4. Patients should have a circulating blast count of less than 10,000/mm^3 (control with hydroxyurea or similar agent is allowed).
5. Patients must have an estimated creatinine clearance greater than 50/ml per minute by the following formula (serum creatinine value must be within 28 days prior to registration):

$$\text{CrCl} = \frac{(140\text{-age})\ (\text{Wt in Kg}) \times 0.85\ (\text{female}) \text{ OR } 1.0\ (\text{male})}{72 \times \text{serum Cr}}$$

6. Patients must have normal hepatic function (bilirubin, AST and ALT < 2 times the upper limit of normal).
7. ECOG ≤ 2 or Karnofsky ≥ 70.
8. Patients must have an expected survival of >60 days and must be free of active infection.
9. Patients must have an HLA-matched related donor or an HLA-matched unrelated donor who meets standard Seattle Cancer Care Alliance (SCCA) and/or National Marrow Donor Program (NMDP) or other donor center criteria for PBSC or bone marrow donation, as follows:
    - Related donor: related to the patient and genotypically or phenotypically identical for HLA-A, B, C, DRB1 and DQB1. Phenotypic identity must be confirmed by high-resolution typing.
    - Unrelated donor:
        - Matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing; OR
        - Mismatched for ***a single allele without antigen mismatching*** at HLA-A, B, or C as defined by high resolution typing but otherwise matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing.
        - Donors are excluded when preexisting immunoreactivity is identified that would jeopardize donor hematopoietic cell engraftment. This determination is based on the standard practice of the individual institution. The recommended procedure for patients with 10 of 10 HLA allele level (phenotypic) match is to obtain panel reactive antibody (PRA) screens to class I and class II antigens for all patients before HCT. If the PRA shows >10% activity, then flow cytometric or B and T cell cytotoxic cross matches should be obtained. **The donor should be excluded if any of the cytotoxic cross match assays are positive**. For those patients with an HLA Class I allele mismatch, flow cytometric or B and T cell cytotoxic cross matches should be obtained regardless of the PRA results. A positive anti-donor cytotoxic crossmatch is an absolute donor exclusion.
        - Patient and donor pairs homozygous at a mismatched allele in the graft rejection vector are considered a two-allele mismatch, i.e., the patient is A*0101 and the donor is A*0102, and **this type of mismatch is not allowed.**

5.2 Exclusions

1. Circulating human anti-mouse antibody (HAMA).
2. Prior radiation to maximally tolerated levels to any critical normal organ, or > 20 Gy prior radiation to large areas of the bone marrow (e.g., external radiation therapy to whole pelvis).
3. Patients may not have symptomatic coronary artery disease and may not be on cardiac medications for anti-arrhythmic or inotropic effects.
4. Patients with the following organ dysfunction:
    a. Left ventricular ejection fraction <35%
    b. Corrected DLCO <35% or receiving supplemental continuous oxygen

c. Liver abnormalities: fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction as evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis, or symptomatic biliary disease.

5. Patients who are known to be seropositive for HIV.
6. Perceived inability to tolerate diagnostic or therapeutic procedures.
7. Active CNS leukemia at time of treatment.
8. Women of childbearing potential who are pregnant (β-HCG+) or breast feeding
9. Fertile men and women unwilling to use contraceptives during and for 12 months post-transplant.
10. Inability to understand or give an informed consent.

6.0 DONOR SELECTION

Donors must meet HLA matching criteria as outlined in section 5.1.9 as well as standard SCCA and/or NMDP or other donor center criteria for PBSC or bone marrow donation.

For the very few occasions where we identify a donor HPC-A from a non-NMDP source, we have procedures in place through our unrelated donor office to collect the information necessary to comply with donor testing, screening, and declaration of donor eligibility according to 21 CFR 1271. We require that the donor testing be performed by a U.S. CLIA approved laboratory. In the very rare case where the donor testing is not able to be performed in a CLIA approved laboratory, or there is confirmatory testing that needs to be performed, or for any donor identified from Europe and at risk for CJD, we note this on the donor screening form and require that the unrelated donor Medical Director or the attending physician approves the use of the donor HPC-A product under Urgent Medical Need.

7.0 INFORMED CONSENT OF SUBJECT AND DONOR

Subjects will be referred to University of Washington/Seattle Cancer Care Alliance for consideration of HCT and will be completely evaluated. The protocol should be discussed thoroughly with subject and family, and all known risks should be described. The 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.

Donors will be consented according to clinical consenting practices at SCCA (related donors) or NMDP or at other donor centers (unrelated donors).

8.0 SUBJECT REGISTRATION

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

9.0 PLAN OF TREATMENT

9.1 General 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 FLU, TBI, and allogeneic stem cell or bone marrow rescue. After informed consent, eligibility confirmation and registration, patients will receive an infusion of 0.5 mg/kg of ideal body weight of DOTA-BC8 trace labeled with ~5-10 mCi of ^{111}In to evaluate biodistribution and calculate the radiation absorbed doses to major organs and the whole body. The subsequent therapy infusion of ^{90}Y-DOTA-BC8 will deliver an amount of ^{90}Y calculated not to exceed the target dose to the critical normal organ receiving the highest radiation dose. The therapy dose will be administered on approximately day -12 of the preparative regimen, which will typically be approximately 1 to 2 weeks after the biodistribution dose. Biodistribution (dosimetric) infusions of ^{111}In-DOTA-BC8 will typically not require inpatient admission. Patients may be admitted overnight to the University of Washington Medical Center (UWMC) or to the UWMC Clinical Research Center (CRC) for therapeutic doses of ^{90}Y-DOTA-BC8 as needed depending on individual circumstances. Serum and urine samples will be collected from the first several patients enrolled to determine pharmacokinetics of radiolabeled DOTA-BC8.

FLU 30 mg/m^2 will be given on days -4, -3, and -2 prior to transplant. Patients then will receive 2 Gy TBI followed by infusion of unmanipulated PBSC or bone marrow on day 0. For GvHD prophylaxis, MMF will be delivered at 15 mg/kg every 12 hours starting on day 0 and continuing to day +27 (for patients with related donors), or every 8 hours starting on day 0 and continuing until day +40 and then tapered and stopped on day +96 (for patients with unrelated donors). CSP will be delivered at 3.75 mg/kg p.o. every 12 hours beginning on day -3 and continuing to day +56, then tapered to day +180 (for patients with related donors), or continuing to day +100 and then tapered to day +180 (for patients with unrelated donors).

Table 1 shows the general treatment schema for patients on this study, which combines ^{90}Y-DOTA-BC8 radioimmunotherapy with an established non-ablative regimen of FLU and 2 Gy TBI prior to PBSC or bone marrow infusion. **Tables 2 and 3** show specific treatment schema, including immunosuppressive regimens, for patients with matched related and unrelated donors.

TABLE 1: General treatment schema. The day of treatment is listed only to give a loose time-frame. An individual study calendar is created for each patient, with modifications permitted in consideration of clinical and logistical needs. It is anticipated that stem cells or bone marrow will be infused approximately 12 days after delivery of ^{90}Y-DOTA-BC8.

Day	Event
-21	^{111}In-DOTA-BC8 (~5-10 mCi) trace-labeled infusion Gamma camera imaging
-20	BM biopsy, gamma camera imaging
-19 thru -16	Gamma camera imaging
-12	^{90}Y-DOTA-BC8 therapeutic infusion
-4, -3, -2	FLU 30 mg/m^2/d
0	2 Gy TBI + PBSC or Bone Marrow infusion

TABLE 2: Specific treatment schema for patients with HLA-matched related donors. Treatment schema for PBSC transplants to establish mixed chimerism in patients with HLA-identical related donors.

Day	-21	-20	-12	-4	- 3	-2	0[b]	+ 27	+28	+ 56	+84	+180
^{111}In-DOTA-BC8[a]	●											
Bone Marrow Biopsy/Blood Sample		●										
^{90}Y-DOTA-BC8			●									
Fludarabine 30 mg/m^2/d				●	●	●						
TBI 200 cGy							●					
PBSC or Bone Marrow Transplant							●					
CSP					Start					Taper		Stop
MMF							Start[c]	Stop				
Chimerism Evaluations[d]									●	●[e]	●	
Bone Marrow Aspirate (and biopsy if needed)[f]									●		●	

[a] Followed by gamma camera imaging immediately post infusion, and at least two or three times total on days -20 through -16.

[b] Day 0 should be scheduled according to standard practice

[c] First dose should be given 4-6 hours after stem cell or bone marrow infusion

[d] Chimerism evaluations from blood at d 28, 56[e], and 84

[e] Only if <50% on d 28

[f] Samples for pathology, and cytogenetic analysis (if previous abnormality).

TABLE 3: Specific treatment schema for patients with HLA-matched unrelated donors: Treatment schema for PBSC or bone marrow transplants to establish mixed chimerism in patients with HLA-phenotypically matched unrelated donors.

Day	-21	-20	-12	-4	- 3	-2	0[b]	+ 28	+40	+ 56	+84	+96	+100	+180
^{111}In-DOTA-BC8[a]	●													
Bone Marrow Biopsy/Blood Sample		●												
^{90}Y-DOTA-BC8			●											
Fludarabine 30 mg/m^2/d				●	●	●								
TBI 200 cGy							●							
PBSC or Bone Marrow Transplant							●							
CSP					Start								Taper	Stop
MMF							Start[c]		Taper			Stop		
Chimerism Evaluations[d]								●		●[e]	●			
Bone Marrow Aspirate (and biopsy if needed)[f]								●			●			

[a] Followed by gamma camera imaging immediately post infusion, and at least two or three times total on days -20 through -16.

[b] Day 0 should be scheduled according to standard practice

[c] First dose should be given 4-6 hours after stem cell or bone marrow infusion

[d] Chimerism evaluations from blood at d 28, 56[e], and 84

[e] Only if <50% on d 28

[f] Samples for pathology, and cytogenetic analysis (if previous abnormality).

9.2 Investigational Drug Product

The investigational product for this study is DOTA-BC8 radiolabeled with either ^{111}In (biodistribution dose) or ^{90}Y (therapy dose). DOTA-BC8 is radiolabeled in the radiochemical facilities of the Division of Nuclear Medicine at the University of Washington. Following the labeling procedure the radiolabeled DOTA-BC8 is sterilized by filtration and tested for endotoxin content.

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 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 a bone marrow biopsy will be obtained over the next several days. One to two weeks after the biodistribution infusions, patients will receive a therapy infusion of ^{90}Y-DOTA-BC8.

9.3.1.1 ^{111}In-DOTA-BC8 dose and infusion rate

^{111}In-DOTA-BC8 will be administered at a total dose of 0.5 mg/kg ideal body weight for patients at or above ideal body weight, or actual body weight for patients below ideal body weight. The mixture of ^{111}In-DOTA-BC8 will be diluted to approximately 25 ml and infused at 7.5 mg/hour.

9.3.1.2 Blood sample collection

Blood samples (CMP and CBD) will be obtained pre-infusion, at the end of infusion and on the first day following infusion for CBD, BUN, creatinine, AST, ALT, and bilirubin.

9.3.1.3 Vital signs

Vital signs will be obtained prior to infusion of ^{111}In-DOTA-BC8 and monitored every 30 minutes (+/- 5 minutes) for the first 2 hours and then hourly (+/- 15 minutes) until the infusion is complete or more often if clinically indicated.

9.3.1.4 Premedication

^{111}In DOTA-BC8 will be administered through a central venous catheter. Premedications will include:

- acetaminophen 650 mg PO
- diphenhydramine 25-50 mg IV; may be given in divided doses if desired (e.g., 35 mg followed in one hour by 15 mg)
- ondansetron 8 mg IV
- ondansetron 8 mg IV to be repeated every 8 hours (+/- 15 minutes) until completion of infusion.
- hydrocortisone 100 mg IV
- hydrocortisone 100 mg IV to be repeated every 2 hours (+/- 15 minutes) until completion of 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
- D5 ½ NS to start prior to the antibody infusion and continue until completion of infusion

If grade II (NCI CTCAEv.4) allergic-type toxicity is encountered (see section 14.1.1), the infusion should be paused, the patient treated as indicated below, and the infusion not restarted until symptoms have subsided. If Grade III allergic toxicity is encountered and does not resolve using measures described below, the infusion must be terminated and not-restarted, and the patient will be off protocol. If other Grade II or III toxicities are encountered (see section 14.1.1), the infusion may be slowed or paused. If toxicity persists or progresses, the infusion will be terminated. If grade IV toxicity occurs, the patient will be off study.

Potential acute side effects and their planned management are as follows:

- fever: acetaminophen 650 mg (or 15 mg/kg) PO 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 PO or IV every 2-4 hours PRN
- low blood pressure: up to 500 ml normal saline bolus given IV over 30 min. may repeat x 1 PRN.
- nausea: diphenhydramine 25-50 mg PO or IV every 2-4 hours PRN; prochlorperazine 10 mg PO or IV every 6 hours PRN; granisetron 1 mg PO or IV every 12 hours PRN
- anxiety: lorazepam 0.5-2 mg (0.05 mg/kg) IV every 4 hours PRN

- cough, chest or throat tightness, wheezing: diphenhydramine and hydrocortisone may be repeated; albuterol nebulizer 2.5–5 mg up to every 1-2 hours PRN
- anaphylaxis: cessation of ^{111}In-DOTA-BC8 infusion and treat per institution standards

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.[26] 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 post ^{111}In-DOTA-BC8 infusion, followed by an additional two to three imaging time points over the course of 5 days. Each imaging session will consist of static anterior and posterior images of the chest, to include the thyroid and upper humeri, static anterior and posterior images of the abdomen, static anterior and posterior images of the pelvis, including the upper femurs, and anterior and posterior images of the whole body. During each imaging session a known source of ^{111}In will be imaged in a fixed geometry, in both static and whole body scanning modes, to account for radioactive decay and to determine gamma camera sensitivity.

Data obtained from gamma camera scans following the trace-labeled infusions of ^{111}In-DOTA-BC8 will be used to determine the ^{111}In biodistribution and to calculate 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 D**. 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 Marrow biopsies

A unilateral bone marrow biopsy (no aspirate) will be performed in the SCCA Procedure Suite on the day after ^{111}In-DOTA-BC8 infusion. If that sample is judged to be inadequate, a second sample may be obtained within 48 hours of the infusion. The biopsy section will be collected using NO FIXATIVE agents. A research technician from the Press Laboratory will prepare the research portion and send the remaining portion to SCCA Pathology for morphology.

Small sections from the biopsy will be weighed and counted using a gamma counter for ^{111}In content, with comparison to a quantitative standard of the infusion mix, allowing calculation of % injected dose/gram marrow of labeled ^{111}In-DOTA-BC8.

9.3.4 Requirements for Therapy

9.3.4.1 HAMA test

Blood samples for HAMA testing will be obtained the day prior to the planned therapeutic ^{90}Y-DOTA-BC8 infusion and must be negative in order for the patient to proceed on study.

9.3.4.2 Hepatic or Renal Toxicity

Blood samples described under section 9.3.1.2 above will be reviewed for evidence of hepatic or renal toxicity. If a patient experiences grade 3 toxicity, determination of whether the patient may proceed to the therapy dose will be made by the Principal Investigator (PI) (or designee) in consultation with the attending physician. Any patient who experiences grade 4 hepatic or renal toxicity will be withdrawn from the study.

9.3.4.3 Favorable biodistribution

Favorable biodistribution (*i.e.*, radiation dose to marrow greater than dose to liver) is *not* required for the patient to receive a therapy dose of ^{90}Y-DOTA-BC8. In previous patients receiving biodistribution doses on our other studies, the estimated doses to marrow and spleen were greater than doses to lung, kidney and total body even in those few patients whose marrow dose was slightly lower than liver dose. Therefore, for this group of patients who might not tolerate a conventional preparative regimen, we will proceed with a therapy infusion of ^{90}Y-DOTA-BC8 regardless of the ratio of radiation dose delivered to marrow compared to liver.

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

9.4.1 Selection of ^{90}Y-DOTA-BC8 dose for therapy

The total amount of ^{90}Y administered will be individualized based on the biodistribution of the trace-^{111}In labeled dose in each patient. The amount of ^{90}Y will be calculated to deliver the predetermined radiation dose (depending upon status of escalation schedule; see section 16.1) to the normal critical organ (almost always liver) predicted to receive the dose-limiting radiation dose. For each patient, the activity (mCi) of ^{90}Y needed will be confirmed by the PI (or designee) and Nuclear Medicine sub-investigator. In some patients with a high bone marrow to liver radiation dose ratio, there may be the possibility of adversely affecting engraftment by damage to the bone marrow supportive stroma resulting from delivering too much radiation to the marrow. These patients may have the ^{90}Y activity limited to that which would deliver the currently stipulated maximum dose to marrow (see section 16.2).

Table 4 shows the dose levels that will be used to estimate the MTD of ^{90}Y-DOTA-BC8 that can be delivered in combination with FLU plus 2 Gy TBI/CSP/MMF and allogeneic HCT.

TABLE 4: ^{90}Y-DOTA-BC8 Dose levels

Dose level	^{90}Y-DOTA-BC8 Estimated radiation dose to normal organ receiving highest dose (Gy)
1	6
2	8
3	10
4	12
5	14
6	16
7	18
8	20
9	22
10	24
11	26
12	28
13	30
14	32

9.4.2 Timing of ^{90}Y-DOTA-BC8 Therapeutic Dose

The ^{90}Y-DOTA-BC8 therapeutic dose will generally be infused approximately 1 to 2 weeks after the ^{111}In-DOTA-BC8 biodistribution dose, but can be delayed further if necessary because of clinical circumstances (as long as the patient has not developed HAMA). At least 6 days is typically required to allow calculation of radiation absorbed dose and ordering of the therapeutic dose of isotope. The day of administration of the therapy dose will generally be day –12.

9.4.3 ^{90}Y-DOTA-BC8 administration

^{90}Y-labeled DOTA-BC8 for therapy will be administered to each patient using an antibody dose and infusion procedures (including pre-infusion and PRN medications) identical to those used for dosimetry studies in the patient (see section 9.3.1) except that the hydration infusion, given at 200 cc/hr, should start prior to the ^{90}Y-labeled DOTA-BC8 infusion and continue for 2 hours after the radiolabeled antibody infusion is complete. Patients may receive the therapy infusion as an outpatient or may be admitted to the hospital overnight, depending on individual circumstances. ^{90}Y-DOTA-BC8 will be administered at a total dose of 0.5 mg/kg ideal body weight for patients at or above ideal body weight, or actual body weight for patients below ideal body weight. The mixture of ^{90}Y DOTA-BC8 will be diluted to at least 25 ml and infused at 7.5 mg/hour. Vital signs will be obtained prior to infusion and monitored every 30 minutes (+/- 5 minutes) for the first 2 hours and then hourly (+/- 15 minutes) until the infusion is complete or more often if clinically indicated. All appropriate shielding and radiation safety precautions will be followed during the administration.

If necessary, the patient, when able, will be instructed in drawing his or her blood samples from a central venous catheter and in taking his or her own vital signs using an automated blood pressure machine. Every patient will be instructed in the safe handling of radioactive body fluids. All blood,

urine, and tissue samples containing radioisotopes will be clearly identified as such. Samples containing high levels of activity (>50 µCi) will be transported in shielded containers. All samples will be processed by personnel trained in the use of radioisotopes.

9.5 Timing of PBSC or Bone Marrow Infusion

For patients with estimated marrow biologic half-times of less than 96 hours as estimated by the biodistribution dose, donor PBSC or bone marrow will normally be infused approximately 12 days after the therapy dose of ^{90}Y-DOTA-BC8. In the unlikely event that a patient has an estimated marrow biologic half-time longer than 96 hours, PBSC or bone marrow infusion will be delayed accordingly and the actual number of days prior to PBSC or bone marrow infusion that the therapy dose of antibody is administered will be adjusted. Treatments and schedule associated with PBSC or bone marrow infusion are further described below.

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

10.1 Fludarabine

Fludarabine will be administered at a dose of 30 mg/m^2/day on days –4, -3, and -2. Fludarabine will typically be given in the SCCA outpatient department.

10.2 Total Body Irradiation (TBI)

TBI will be administered at a dose of 2 Gy per standard practice on Day 0, followed by PBSC infusion. TBI will normally be administered between 11:00 a.m. and 2:00 p.m. to avoid proximity to administration of CSP/MMF.

10.3 Collection and Infusion of Donor PBSC

10.3.1 G-CSF Administration to Related Donors and PBSC Collection

Related donors will undergo G-CSF administration and PBSC collection as per SCCA standard clinical practice. The procedures described here may vary in accordance with allowable variances in clinical activities or as required by modifications to the SCCA Standard Practice Manual. Related donors typically will receive G-CSF 16 µg/kg/day for 5 consecutive days from day –4 to day 0. G-CSF will be administered by a subcutaneous daily injection beginning 4 days prior to day 0. The schedule of G-CSF administration and PBSC collections can only be ascertained once day 0 is identified. These doses will be administered daily in the SCCA Outpatient Department. The treatment regimen schedule and the schedule of G-CSF administration and PBSC collections must be confirmed with the personnel in the apheresis room. PBSC will be collected in the afternoon of day –1, stored in the refrigerator at 4°C overnight, and infused on day 0. If the collection on day –1 contains less than 5.0×10^6 CD34$^+$ cells per kg recipient weight, a second collection will be performed the following morning and transfused on day 0. Donors will preferably undergo vein-to-vein collections. If PBSC cannot be collected by a vein to vein technique, a percutaneous Mahurkar catheter will be inserted. General procedures will include the use of a standard apheresis machine (COBE Spectra, Lakewood Colo.), and processing up to 16 liters of whole blood during the collection.

10.3.2 G-CSF Administration to Unrelated Donors and PBSC Collection

Timing of PBSC collection for unrelated donors is prearranged through the donor center where the donor was procured and the schedule of G-CSF administration and stem cell collections can only be ascertained once day 0 is identified. PBSC will be collected according to the donor center standards and the physician responsible for PBSC collection will obtain informed consent from the donor. The target cell dose is 5.0 x 10^6 $CD34^+$ cells/kg of patient weight for unrelated donor stem-cell collection. If this collection goal is not met, the patient may be infused with all available cells as appropriate in accordance with standard practice. If the cell dose is below 2.5 x 10^6 CD34+ cells/kg, infusion will be at the discretion of the attending physician.

10.4 PBSC AND Bone Marrow Infusion

All patients will receive unmodified HSCT (PBSC or bone marrow) infusion on day 0 of the treatment regimen. Day 0 should be on a Tuesday to Thursday when possible.

10.5 Immunosuppression Dosing Schedules

Day –3: Begin CSP at 3.75 mg/kg PO Q12 hours (or 1.5 mg/kg IV Q12 hours if oral preparation is not tolerated). For patients with ***related*** donor, continue to day +56 and then taper to day +180. For patients with ***unrelated*** donor, continue to day +100 and then taper to day +180.

Day 0: Begin MMF at 15 mg/kg PO (or same dose IV if oral preparation is not tolerated). For patients with ***related*** donor, continue MMF Q12 hours to day +27. For patients with ***unrelated*** donor, continue MMF Q8 hours until day +40 and then taper to day +96.

CSP doses will be given Q12 hours, and MMF doses will be given Q12 hours (patient with ***related*** donor) or Q8 hours (patients with ***unrelated*** donor). Since significant nausea may accompany this immunosuppression, particularly in the days after TBI, regularly scheduled anti-emetic therapy is recommended for all patients for at least one week after transplant. Adjustments may be made to immunosuppression drugs, dosing, & schedule due to clinical reasons. See recommendations for CSP & MMF below.

10.6 Cyclosporine (CSP)

10.6.1 Dosing

For patients with matched related donors, CSP is given at 3.75 mg/kg PO Q12 hours from day –3 to day +56, and then is tapered to day +180 unless GVHD develops. For patients with matched unrelated donors, CSP is given from day –3 to day +100, and then is tapered to day +180. If there is nausea and vomiting at any time during CSP treatment, the drug should be given IV at 1.5 mg/kg Q12 hours. Both IV and oral doses of CSP are calculated using the adjusted weight.

10.6.2 Monitoring

Blood pressure, renal function (creatinine, BUN), electrolytes and magnesium will be followed at least once per week, or more often if clinically indicated, while receiving CSP.

10.6.3 Dose Adjustments

CSP whole blood "trough" levels (*i.e*., just prior to the next dose) will be evaluated on day 0 and adjusted if necessary to maintain blood levels that target 240-320 ng/ml. Dose reductions should only be made if CSP toxicity is present or at excessive levels in the absence of toxicity at the discretion of the Attending Physician. Further CSP determinations should be performed twice weekly until CSP is stopped unless excessive high levels are detected or toxicity is suspected, in which case more frequent monitoring will be performed as clinically indicated. In this group of patients, close monitoring of renal function is essential. Dose reductions for high levels without toxicity should be conservative (*e.g*., 25%), to avoid inadequate immunosuppression, particularly in the first month post-transplant.

10.6.4 Drug Interactions

Drugs that may affect CSP levels include: dilantin, phenobarbital (may lower CSP levels), steroids, fluconazole, voriconazole, posaconazole, ketoconazole, cimetidine (may increase CSP levels). A complete and current list of drugs that may affect CSP levels may be found in the BMT Standard Practice Manual.

10.7 Mycophenolate Mofetil (MMF)

10.7.1 Dosing

For patients with matched related donors, oral administration of MMF will be at 15 mg/kg Q12 hours (30 mg/kg/day) from the evening of day 0 (first dose to occur 4-6 hours after PBSC or bone marrow infusion) until day +27 post-transplant, at which point it will be stopped without tapering. For patients with matched unrelated donors, oral administration of MMF will be at 15 mg/kg Q8 hours (45 mg/kg/day), starting on day 0 and administered through day +40, at which point it will be tapered to day +96. Doses will be rounded to the nearest 250 mg. If the patient cannot tolerate oral MMF, the medication can be administered intravenously at the same dose. Both IV and oral doses are calculated using the adjusted weight.

10.7.2 Dose Adjustments

The principal adverse reactions associated with the administration of MMF include diarrhea, leukopenia, sepsis, and vomiting. If in the clinical judgment of the attending physician the observed toxicity is related to MMF administration, a dose adjustment may be made. Based on previous organ transplant studies, dose adjustments are likely to occur because of hematopoietic or gastrointestinal adverse effects (see section 14.0 "Toxicities and complications"). Based on previous experience in patients after HCT, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur such as peripheral blood chimerism studies, marrow aspiration, and review of marrow suppressive medications (e.g. bactrim). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for severe neutropenia (ANC $<100/mm^3$ for > 5 days) that persists after day 21 post-transplant. Dose reductions should be conservative (20%). After day 21, the use of G-CSF will be permitted for neutropenia. For severe toxicity related to MMF (grade IV neutropenia > 5 days refractory to G-CSF), MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides. In the event

of gastrointestinal toxicity that requires medication for control of persistent vomiting or diarrhea that is considered to be due to MMF, a 20% dose reduction will occur first and if there is no improvement, MMF will be reduced a further 20%. For severe G.I. toxicity related to MMF (severe refractory diarrhea, or overt gastrointestinal bleeding), the MMF may be temporarily stopped. Patients should be evaluated by a Gastroenterology consultant and discussed with the principal investigator before stopping MMF. Dose adjustments will be made based on occurrence of MMF toxicity.

10.8 Modifications of Immunosuppression

10.8.1 Modifications of immunosuppression for disease progression or relapse

Guidelines provided in this section are for patients who demonstrate either: i) progression of stable disease present at the time of transplant or ii) relapse of their underlying disease before discontinuation of immunosuppression has been completed. Persistence of stable, underlying disease does not in itself mandate accelerated taper of immunosuppression.

Patients with progressive disease defined as any evidence of relapse by morphologic or flow cytometric evaluation of a bone marrow aspirate will undergo rapid reduction of immunosuppression, after careful evaluation for GvHD, at the discretion of the Attending Physician, Principal Investigator, and/or study sub-investigator(s). In the event that patients with early disease progression or relapse do not have GvHD, immunosuppression should be discontinued.

DLI will not be given for progressive or relapsed disease on this protocol, and patients with relapse or progression would be eligible for other ongoing DLI protocols or treatment plans.

10.8.2 Modifications of immunosuppression for low donor chimerism

Immunosuppression should be discontinued as per protocol unless the patient develops GVHD or has falling donor chimerism without evidence of persistence, progression, or relapse of disease. In the latter situation, MMF should be continued at full dose for a month and then tapered over a month. CSP should be continued until MMF is discontinued and then tapered over 3 months. Guidelines for management of GvHD are provided under section 11.3.

10.9 Post-Transplant Growth Factors and Neutropenia

It is recommended that patients not receive growth factors during treatment with MMF before day 21 post-transplant because of the possible risk of potentiating MMF toxicity by forcing more progenitor cells into a dividing and proliferating state. G-CSF may be administered to patients who have severe neutropenia (ANC $<100/mm^3$ for >5 days) persisting after day 21 or if patients are neutropenic (ANC $<500/mm^3$) with active infection. If ANC drops to $<500/mm^3$ then prophylactic broad spectrum antibiotics may be given.

10.10 Intrathecal Therapy and Treatment of CNS Disease

Active CNS disease at time of treatment is an exclusion criterion for this protocol; however, patients with history of CNS disease will not be excluded. CNS assessment and prophylactic treatment will be managed in accordance with the SCCA Standard Practice Manual (with treatment modifications

determined by the Attending Physician if clinically indicated). All patients will have a diagnostic lumbar puncture performed during the initial pre-transplant workup in the outpatient department. Patients with past history or significant risk of CNS disease may receive prophylactic intrathecal therapy with methotrexate (dose per the Standard Practice Manual). Patients with evidence of CNS leukemic involvement will have instillation of methotrexate, and will also be considered by the Attending Physician to receive up to 18 Gy (10 fractions of 1.8 Gy) cranial or cranial-spinal irradiation as consolidation if indicated, beginning approximately day 32 post-transplant or as soon as engrafted, whichever comes later.

10.11 Infection Prophylaxis

Patients will receive prophylaxis for PCP, HSV and Candida as per standard practice manual. If amphotericin is required, patients should receive liposomal or other lipid-based amphotericin rather than regular amphotericin in order to reduce risk of nephrotoxicity that may compromise the administration of CSP.

11.0 ENGRAFTMENT AND GRAFT REJECTION

11.1 Donor Chimerism

Chimerism testing methods will be in accordance with Standard Practice Guidelines, with timing consistent with other reduced-intensity transplant protocols (*e.g.,* FHCRC 1938). Chimerism evaluations will be performed on peripheral blood on approximately days +28, +56 (if <50% at day +28), and +84. Mixed or full donor chimerism will be evidence of donor engraftment. Definitions of mixed and full chimerism, as well as increasing and low donor chimerism, are described in Section 13.0.

Patients who obtain mixed or full donor chimerism after reduced-intensity HCT, but subsequent chimerism studies cannot detect greater than 5% donor T-cells ($CD3^+$) as a proportion of the total - cell population, will be defined as having developed graft rejection.

Graft rejection can occur because of immunologic rejection of donor cells, or because of inadequate stromal support of the growth of donor cells. The risk of immunologic graft rejection is considered to be low for this protocol based on previous FHCRC mixed chimerism experience in which patients who had previously received intensive chemotherapy had <10% risk of graft rejection. In this protocol, additional immunosuppression may be provided by lymphohematopoietic irradiation from ^{90}Y-DOTA-BC8. However, in the event of marrow stromal damage from excessive radiation exposure, patients might have inadequate hematopoiesis yet not meet the previously defined criteria for graft rejection. Every effort will be made to determine graft status for patients with slow recovery of neutrophils. For patients who are critically ill and not expected to survive to day +28, an effort will be made to obtain a marrow sample prior to death in order to ascertain graft status. Similarly, chimerism studies will be performed prior to day +28 if necessary.

Patients with slow engraftment of neutrophils should be considered for treatment with hematopoietic growth factor as specified in section 13.0. Those patients with grade IV thrombocytopenia and neutropenia after day +21 that lasts >2 weeks and is refractory to growth factor support, in the absence of contributing cause such as medication (*e.g.,* MMF), sepsis, or CMV infection, will be considered to have never engrafted.

11.2 Evaluation of Chimerism

At days +28, +56 (if needed), and +84, patients will be evaluated for lymphoid or myeloid chimerism in the peripheral blood. Patients who have mixed or full donor chimerism at day +56 or later, and who have evidence of persistent, progressive or relapsed AML will be considered on a separate protocol for DLI, with the goal of maximizing the potential GvL effect. Patients who have stable mixed or full donor chimerism on day +56, whose disease is stable or responding, and who remain on full dose immunosuppression will continue to be observed, and will not be considered for DLI at that time. Patients with evidence of Grade II or greater GvHD also will not be considered for DLI.

11.3 Recommended Treatment of GvHD

After reduced-intensity HCT from 10/10 antigen matched unrelated donors, the incidence of grades II, III and IV acute GvHD in patients with sustained engraftment has been 46%, 4% and 0%, respectively. Acute GvHD has been readily controlled in most patients with high dose corticosteroids, but PUVA (psoralen activated ultraviolet light) has been required on occasion. Chronic extensive GvHD has occurred in 34% of patients. Acute GvHD and chronic GvHD will be graded according to established criteria (Appendices B and C).

11.3.1 Patients developing ≥ Grade 2 acute GvHD:

a. CSP 7.5 mg/kg/day PO in two divided doses. If there is concern of GI absorption, continue CSP prophylaxis using IV route (1.5 mg/kg Q12 hours).

b. Prednisone (up to 2 mg/kg/day) may be added if severe GvHD and no GvHD response by 72 hours to one week after the addition of CSP.

c. Patients with life-threatening grade III or IV acute GvHD and/or steroid refractory acute GvHD are eligible for institutional trials of GvHD therapy.

11.3.2 Patients with clinical extensive chronic GvHD:

LTFU consult should be obtained for patients with chronic GvHD and determination of the appropriate treatment for chronic GvHD will be based on the LTFU consult team recommendations.

12.0 EVALUATIONS

12.1 Patient Pre-Transplant Baseline Evaluation

Patient work-up will be in accordance with Standard Practice Policy Manual Evaluation Guidelines for Marrow Transplant Patients, with the following additional requirements:

a. 10 cc blood in one red top tube (10 cc) labeled “Protocol 2468” to the Lab (D3-395, Thomas Building) for analysis of HAMA.

b. CT scan of chest and abdomen for calculation of organ volumes (lungs, liver, spleen & kidneys). Other imaging studies as clinically indicated.

c. Unilateral bone marrow biopsy and aspirate for pathology and cytogenetics* within 30 days prior to consent signing/enrollment.

d. Data for relevant prognostic indices (*e.g.*, Relapsed AML Index, IPSS) will be recorded for all patients.

* Cytogenetics to include FISH panel for AML and MDS patients; NPM1, CEBPA, FLT3 mutations for AML patients only.

Certain baseline data collected as part of standard clinical work-up, including serum creatinine, estimated creatinine clearance, bilirubin, AST, ALT, leukocyte count, blast count, or cytopenias chest x-rays, and pulmonary function tests, will also be recorded as part of the research record.

12.2 Patient Evaluation with DOTA-BC8 Infusions

- Vital signs: prior to starting infusion, every 30 minutes (+/- 5 minutes) during first 2 hours of infusion, hourly (+/- 15 minutes) for remainder of infusion, and as clinically indicated.
- Blood samples:
  a. Before and after infusion, 3 cc lavender top tube for CBC with differential, and 6 cc green top tube for renal and liver function testing.
  b. 6 cc green top tube for renal and liver function testing one day after infusion.
  c. 10 cc blood in one red top tube (10 cc) to the Lab (room D3-395, Thomas Building) for HAMA one day prior to ^{90}Y-DOTA-BC8 Therapy infusion.
  d. Samples drawn for pharmacokinetics as described under section 12.3.

12.3 Pharmacokinetics (Pk)

12.3.1 Timing

The first several patients enrolled on the study will undergo collection of blood and urine samples for Pk studies. Initial Pk sampling will be based on the assumption that the study drug will distribute according to a two-compartment model. Although sampling may change with data accrual, the initial samples will be collected before and after ^{111}In/^{90}Y-DOTA-BC8 administration as follows:

- Blood: 2-5 cc in purple top tubes taken prior to treatment, and then after treatment at 15 minutes (+/- 5 minutes) and 1-2 hours (+/- 10 minutes) post infusion, and at 3-5 additional timepoints within the first 72 hours post infusion.
- Urine: samples will be collected during the first 2-3 days following infusion, either as aliquots from individual voids or based on 24-hour urine collection.

12.3.2 Handling of specimens

- Blood samples to be held at collection location for pickup by the lab technician (refrigeration is not required).
- Urine collections will be measured and recorded, an aliquot of up to approximately 2 cc removed and stored at room temperature (unless being held over the weekend, in which case refrigeration is preferable), and the remainder double-flushed down the toilet.
- It is important to provide the time of sample collections on the labels on tubes

12.4 Patient Evaluations During Conditioning and for First 100 Days Post-Transplant

See Standard Practice Policy Manual for "Evaluation Guidelines for Transplant Patients". Data from these standard laboratory studies, other clinically indicated studies, and clinical assessments by the primary care team will be reviewed regularly to assess for regimen-related toxicity, occurrence of Serious Adverse Events, and other potential post-transplant complications such as GvHD and infections.

The following additional studies and tests will be performed (**Table 5**):

a. Heparinized blood 10 cc to for quantitation of T-cell, NK-cell and granulocyte chimerism on approximately days +28, +56 (if <50% on day +28), and +84.

b. Bone marrow aspirate (and biopsy if unable to obtain adequate aspirate sample) to pathology on approximately days +28 and +84. Aspirate will be sent at each time point to cytogenetics, if patient's disease has a known cytogenetic abnormality and will also be sent for other informative disease markers.

c. Chronic GvHD screening between days +80 and +100 post-transplant.

d. 10 cc blood in one red top tube (10 cc) to the lab (D3-395, Thomas Building) for HAMA on approximately day +84.

12.5 Patient Evaluations Following 100 Days Post-Transplant

Following day +100 or discharge from the SCCA, patients will return to their primary care physicians under the guidance of the FHCRC/SCCA Long-Term Follow-Up After Hematopoietic Stem Cell or Bone Marrow Transplant General Guidelines for Referring Physicians. Patient follow-up will be performed primarily by the FHCRC Long-Term Follow-Up Department under the FHCRC/SCCA Master Protocol for Collection of Clinical Data and Storage of Leftover Specimens from Patients Treated According to FHCRC Protocols (Protocol #0999.209). The following data collected through this mechanism will be recorded for purposes of this study at 6, 9, 12, 18 and 24 months post-transplant (**Table 5**).

1. Survival.
2. Disease status.
3. Selected blood counts (WBC, hemoglobin, hematocrit, platelets).
4. Renal (creatinine, BUN) and hepatic (Bilirubin, AST, ALT) laboratory values. Estimated creatinine clearance will be calculated and recorded as part of the research record.
5. Presence/treatment of chronic GVHD.
6. Pulmonary function tests (12 months post-transplant)
7. Chest x-rays (12 months post-transplant)
8. Secondary malignancies.

Any of these data that are unavailable through standard long-term follow-up will be requested directly from the patient or local physician. After the two-year follow-up time point, we will continue to monitor any changes to survival, disease status, and secondary malignancies reported under the standard long-

term follow-up protocol, and will record this data for long-term analysis and reporting. 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.

TABLE 5: General Schedule of Patient Evaluations

	Baseline	Days			Months					Annual following 2 years
		+28	+56	+84	6	9	12	18	24	
HAMA (Press Lab)	●			●						
CT (chest, abdomen)	●									
Bone Marrow Biopsy/Aspirate to:										
Pathology	●[a]	●		●						
Cytogenetics	●[a]	●[b]		●[b]						
Blood to Hematopathology/CIL (Chimerism)	●[c]	●	●[d]	●						
GVHD screening				●[e]						
Data tracking for survival, disease status, blood counts, renal and hepatic lab values, presence/treatment of chronic GVHD, serious adverse events, secondary malignancies.					●	●	●	●	●	
Data tracking for survival, disease status, and secondary malignancies										●

[a] Bone marrow biopsy/aspirate within 30 days prior to enrollment.

[b] Sample to cytogenetics if patient's disease has known cytogenetic abnormality.

[c] Blood may be drawn prior to baseline; e.g., at time of HLA typing.

[d] Chimerism at day +56 only if <50% at day +28.

[e] GVHD screening between day +80 and day +100.

13.0 DEFINITIONS

Complete remission: complete resolution of all signs of myelodysplasia or leukemia for at least four weeks with all of the following:

1. Normal bone marrow with blasts <5% with normal cellularity, normal megakarypoiesis, more than 15% erythropoiesis and more than 25% granulocytopoiesis.
2. Normalization of blood counts (no blasts, platelets > 100000/mm^3 , granulocytes >1500/mm^3)
3. No extramedullary disease.

Partial remission:

1. Improvement of hematological parameters in the peripheral blood
2. 50% decline in marrow blasts from pre-transplant level with >10% erythropoiesis and 25% granulocytopoiesis.

Non responder: All patients not qualifying for complete or partial remission.

Relapse:

1. After CR: >5% blasts in the bone marrow and/or peripheral blood. Confirmation of relapse by bone marrow analysis with more than 10% blasts.
2. After PR: increase of blasts cells in the marrow to >50% of those during PR.
3. Extramedullary disease confirmed cytologically or histologically.

Persistent Disease:

1. >5% blasts in the bone marrow by histology
2. presence of a clonal population of myeloid cells by flow
3. persistence/recurrence of a previously documented cytogenetic abnormality

Full Chimerism: >95% donor CD3+ T cells.

Mixed Chimerism: the detection of peripheral blood donor T cells (CD3+) and granulocytes (CD33+) as a proportion of the total peripheral blood T cell and granulocyte population, respectively.

Increasing Donor Chimerism: an absolute increase of 20% of CD3+ T cells over the chimerism evaluation of the previous month.

Decreasing Donor Chimerism: decreasing donor chimerism is defined as an absolute decrease of at least 20% of CD3+ T cell chimerism over the previous month.

Low Donor Chimerism: low donor chimerism is defined as <40% CD3+ T cells after HSCT. Low donor chimerism should always be confirmed with repeat peripheral blood T cell and granulocyte chimerism analysis.

Graft Failure: Primary graft failure is defined as failure to achieve neutrophil engraftment (ANC >500/μL) in patients surviving at least 28 days; secondary graft failure as neutrophil recovery followed by a decline in ANC to <500/μL unresponsive to growth factor stimulation.

14.0 TOXICITIES AND COMPLICATIONS

14.1 ^{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.

14.1.1 Acute toxicity associated with DOTA-BC8 infusion

The primary acute toxicities associated with administration of the BC8 Ab are allergic reactions. Allergic reactions to administration of foreign protein may result in fever, chills/rigors, hypotension, pruritis, urticaria, allergic rhinitis, conjunctivitis, cough, chest or throat tightness, wheezing/bronchospasm, dyspnea, anaphylaxis, Arthus reaction, vasculitis, and serum sickness. Nausea, vomiting, diarrhea, myalgia, arthralgia, headache, fatigue, and hand/foot skin reaction have also been observed with administration of BC8 antibody and are therefore similarly expected with DOTA-BC8. In addition, rapid

infusion of DOTA-BC8 may produce pulmonary, renal or hepatic toxicity as a result of lysis or agglutination of circulating cells. **Table 6** describes the grading of significant acute toxicities due to DOTA-BC8 infusion.

TABLE 6: Grading of Acute Toxicities Due to DOTA-BC8 Infusion (from NCI CTCAE version 4.0).

Parameters	1 (Mild)	2 (Moderate)	3 (Severe)	4 (Life-Threatening)
Immune system disorders: Allergic reaction	Transient flushing or rash; drug fever <38°C (<100.4°F); intervention not indicated	Intervention indicated; responds promptly to treatment	Prolonged recurrence of symptoms; hospitalization indicated	Life-threatening consequences; urgent intervention indicated
Investigations:				
1. Bilirubin	>ULN – 1.5 × ULN	>1.5 × ULN – 3.0 × ULN	>3.0 × ULN – 10.0 × ULN	>10.0 × ULN
2. AST	>ULN – 2.5 × ULN	>2.5 × ULN – 5.0 × ULN	>5.0 × ULN – 20.0 × ULN	>20.0 × ULN
3. Creatinine	>ULN – 1.5 × ULN	>1.5 × ULN – 3.0 × ULN	>3.0 × ULN – 6.0 × ULN	>6.0 × ULN

Premedication for and management of potential acute side effects are described under section 9.3.1.5. Patients experiencing acute allergic type toxicity during DOTA-BC8 infusion will have the infusion slowed or terminated. If grade 2 allergic toxicity is encountered, the infusion should be paused, the patient treated as indicated under section 9.3.1.5, and the infusion not restarted until symptoms have subsided. If grade 3 allergic toxicity is encountered and does not resolve using the measures described, the infusion must be terminated and not-restarted, and the patient will be off protocol. Other grade 2 or 3 acute toxicity may be treated and the infusion restarted. If toxicity persists or progresses, the infusion will be terminated. Patients with grade 4 acute toxicity will have the infusion stopped and further participation in the study will be terminated.

Hepatic and/or renal toxicity will not be evident during the DOTA-BC8 infusion but instead 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 first dose (associated with the biodistribution dose of radiolabeled DOTA-BC8), determination of whether or not the patient can proceed to the therapy dose will be made by the PI or his designee 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 first dose will not be eligible to receive the necessary second dose associated with the therapy dose of radiolabeled DOTA-BC8, and will be treated on an alternate protocol.

14.1.2 Toxicity associated with radiolabeled DOTA-BC8

The risk associated with the administration of ~5-10 mCi of ^{111}In is estimated to be low. 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 radioisotope ^{111}In is a relatively low-dose imaging agent that is widely used in clinical imaging applications.^{111}In is used as a tracer to follow biodistribution and to estimate the radiation absorbed

dose for ^{90}Y based on the assumption that both isotopes behave similarly *in vivo* when stably complexed by DOTA chelate. ^{90}Y-DOTA-BC8 that does not bind to cellular recognition sites is expected to be cleared rapidly by the kidneys, resulting in increased renal exposure to radiation. All patients will receive intravenous hydration before, during, and after the infusion in order to support adequate renal function and efficient clearance of circulating ^{90}Y-DOTA-BC8.

The major toxicity associated with the use of ^{90}Y-labeled antibodies is myelosuppression. This effect is partly due to dissociation of ^{90}Y from the DOTA molecule and subsequent accumulation of free ^{90}Y metal ion in bone that irradiates the bone marrow. The degree of myelosuppression is a function of residence time in the blood, chelate stability, and the amount of ^{90}Y administered. In addition, DOTA ligand complexes of M^{+3} metals, including yttrium, are known to be kinetically inert; therefore, release of free metal is expected to be minimal. Patients will undergo further intended myelosuppression from treatment with FLU and TBI prior to HCT.

In previous studies using high doses of targeted radiation delivered via ^{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 post-transplant in 7 of the first 74 patients treated (9%). These events include dyspnea and hypoxia at NCI CTC/CTCAE Grade 3 and above, bronchiolitis obliterans organizing pneumonia (BOOP), and pneumonitis/pulmonary infiltrates. In the same patient population, 16 of 34 (47%) patients surviving more than 100 days post-transplant had later reports of pulmonary events during follow-up. Most of these were mild (e.g., cough), but 4 patients showed radiographic changes suggestive of pneumonitis and/or fibrosis.

Additional radiation-related toxicities may include damage to various organs such as the skin, lungs, liver or kidney. Radiation exposure is also associated with a risk of secondary malignancy. There is a rare risk of veno-occlusive disease (VOD), which causes abnormal liver function, body swelling from water retention and pain in the right side of the abdomen. Patients will be informed about these potentially fatal risks.

14.2 Fludarabine

14.2.1 Description

Fludarabine monophosphate is a purine antimetabolite that, after administration, undergoes rapid conversion in plasma to the nucleoside 2-fluoro ara-A (F-araA). F-araA subsequently enters cells where it is phosphorylated to F-araATP and the monophosphate F-araAMP. Once activated, F-araATP inhibits DNA polymerase and ribonucleotide reductase. The monophosphate F-araAMP, once incorporated into DNA, is an effective DNA chain terminator. Following IV administration, the drug is metabolized to 2-F-araA and widely distributed in tissues. 2-F-araA is excreted primarily in urine and has a terminal elimination half-life of 7-12 hr.

14.2.2 Storage and administration

Fludarabine monophosphate is commercially available as a 50 mg/vial which is reconstituted with 2 ml of sterile water, resulting in a 25mg/ml solution. The desired dose is further diluted to concentrations of

0.04-1 mg/ml in normal saline or 5% dextrose (50-100ml) for injection and administered by IV infusion. Fludarabine will be administered by IV infusion over 30 minutes in a dose of 30 mg/m^2/day on days -4 to -2.

14.2.3 Side effects and toxicity

Clinical toxicities of fludarabine monophosphate include: myelosuppression, primarily lymphopenia and granulocytopenia, alopecia, rash, dermatitis, nausea, vomiting, anorexia, stomatitis, diarrhea, somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness, hepatocellular toxicity with elevation in serum transaminases, and interstitial pneumonitis. These effects are reversible when the drug is discontinued. Immunosuppression observed with the use of fludarabine increases the risk of infection which can be life-threatening.

14.3 Cyclosporine (CSP)

See SCCA standard practice manual for information about administration, toxicity and complications.

14.4 Mycophenolic Acid Mofetil (MMF)

14.4.1 Description

MMF is the morpholinylethylester prodrug of the active immunosuppressant mycophenolic acid (MPA). This active metabolite is a noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase, particularly the type II isoform that is more prominent in activated lymphocytes. As a result of the inhibition of de novo purine synthesis, proliferation of T- and B-lymphocytes is blocked and antibody production is inhibited. There are no pharmacokinetic interactions with ganciclovir, cotrimoxazole, oral contraceptives or cyclosporine.

14.4.2 Storage and administration

MMF is commercially available in an oral and an intravenous formulation. The oral formulation is supplied in 250 mg hard gelatin capsules and can be stored at room temperature. MMF for i.v. administration is supplied as a lyophilized powder in a glass vial containing the equivalent of 500 mg.

14.4.3 Side effects and toxicity

Side effect profiles include diarrhea, leukopenia, sepsis, allergic reactions, and vomiting. MMF use during pregnancy carries a significant risk of miscarriage or birth defects. An increase in certain types of infection mainly from the herpes virus family (CMV, HSV & VZV) and candida has been reported. There have been reports of progressive multifocal leukoencephalopathy (PML), sometimes fatal, and of Pure Red Cell Aplasia (PRCA) in patients receiving MMF as part of an immunosuppressive regimen. MMF has not been studied extensively in patients after HCT. Most common side effects known from studies in patients with solid organ transplants are hematologic (decline in WBC and hematocrit) and gastrointestinal (nausea, vomiting, diarrhea, G.I. bleeding). Experience has shown that nausea can be successfully treated with anti-emetics, and that side effects often respond to a decrease in dose. Several etiologic factors may cause alterations in G.I. and hematologic parameters in the setting of HCT. MMF dose adjustments will therefore be made when clinically indicated if the responsible physician ruled out other possible causes. Dose adjustments should be discussed with the principal investigator. With the

exception of hypophosphatemia there seems to be no difference in side effects between i.v. and oral MMF administration and efficacy is the same with both administration routes.

15.0 ADVERSE EVENT REPORTING

15.1 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:
  - Death.
  - 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).
  - 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.
  - Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
  - Congenital anomaly/birth defect.
  - 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.

15.2 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.

15.3 Grading of the Severity of an Adverse EventAEs 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.

15.4 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.

15.5 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 +100 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 +100, disease progression, development of 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.

15.6 Adverse Event Reporting Requirements

15.6.1 Research Site Reporting Requirements

Classification of an event as serious or non-serious (see Section 15.1) 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-	ISIOC Fax: 206-667-6068 ISIOC email:

			Investigator	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.

15.6.2 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 15.1), the expectedness of the event (see Section 15.1), and the relationship to participation in the study (see Section 15.4). 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.

15.7 SAES Associated with Hematopoietic Cell Transplantation (HCT)

Certain events that are commonly observed as SAEs following HCT are described in **Appendix E** in order to facilitate assessments of attribution. SAEs that are identified as routinely experienced in the allogeneic transplant setting would typically be assessed as unrelated to elements of the investigational regimen used in this protocol. Symptoms associated with GvHD are described under Appendices B and C.

16.0 DOSE ESCALATION

16.1 Dose to Normal Organ

Dose escalation will be conducted by the "two-stage" approach introduced by Storer.[79] The MTD will be defined as the dose that is associated with a true DLT rate of 25%. A DLT will be defined as a Grade III/IV

regimen-related toxicity (Bearman scale) occurring within 30 days post-transplant. Patients at either stage are considered evaluable after survival of 30 days post-transplant, or earlier if Grade III/IV regimen-related toxicity (RRT) occurs before that time.

In this study an initial conservative ^{90}Y starting dose has been selected to deliver an estimated 6 Gy to the normal organ that receives the highest dose. This starting dose is based on data that utilized ^{90}Y-ibritumomab tiuxetan (Zevalin®) anti-CD20 RIT as part of a high-dose therapy regimen in the setting of autologous HCT for non-Hodgkin lymphoma (NHL). These groups have shown that employing standard (0.4 mCi/kg) doses of ^{90}Y-ibritumomab tiuxetan based approach as part of a HCT regimen is feasible, efficacious and safe, without a negative impact on engraftment.[80-81] Additional investigators have explored the use of escalating doses of ^{90}Y-ibritumomab tiuxetan in combination with high-dose chemotherapy and autologous HCT for NHL.[82-86] These studies have safely delivered ≥0.5 mCi/kg with doses estimated to deliver >15 Gy to critical organs (liver in most cases) when combined with high-dose chemotherapy and autologous HCT. In addition, the estimated MTD of radiation delivered to the normal organ receiving the highest dose of ^{131}I-BC8 Ab on our Phase I study (Protocol #1432) was 24 Gy delivered to the liver. Given the differences in radionuclides utilized between Protocol #1432 and this study, combined with prior experiences using ^{90}Y-ibritumomab tiuxetan, we anticipate that a dose of 6 Gy delivered by ^{90}Y-DOTA-BC8 will represent a conservative starting point resulting in less toxicity than has been observed using 24 Gy delivered by ^{131}I-BC8 Ab or using higher doses of ^{90}Y as reported by other groups.

Single patients will be entered on the first stage, and escalation by 2 Gy increments (in the radiation dose delivered to the normal organ receiving the highest dose; see Table 4) will occur until a patient experiences a Grade III/IV regimen-related toxicity (Bearman scale) or DLT, at which point the second stage will begin at the next lower dose level. If the first patient (*i.e.*, at the starting dose level) has DLT, de-escalation will occur by 2 Gy increments until the first dose at which a patient does not have DLT, at which point the second stage will begin at that dose level.

In the second stage, patients will be entered in cohorts of 4 evaluable patients. If the current cohort is treated at dose level k, the next cohort will be treated according to the following: level k-1 if 2 DLT's are encountered among the 4 or fewer patients in the cohort; level k if 1 DLT is observed within the current cohort; level k+1 if 0 DLT's are seen in the current cohort. These rules are followed until a minimum of 20 evaluable patients have been treated at the second stage and have been evaluated for DLTs through day 100 following transplant. It is possible that a patient will be entered on the protocol before a patient currently enrolled on Stage 1 or all 4 patients in a cohort in Stage 2 have been followed sufficiently long to evaluate toxicity (30 days post-transplant). Such patients will be treated at the current dose level and will be used for purposes of fitting the dose-response curve upon completion of the second stage. However, these patients will not be used for purposes of dose modification unless required in the interests of patient safety based on the clinical judgment of the principal investigator, nor will they be counted towards the required number of patients for completion of 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-response curve based on the observed toxicity 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 toxicity rate of 25%.

16.2 Dose to Bone Marrow and Graft Failure

Initially no patient will receive a dose of ^{90}Y estimated to deliver more than 43 Gy to the marrow (5 Gy less than the 48 Gy from ^{131}I-BC8 Ab tolerated on Protocol #1432). Patients for whom the amount of ^{90}Y required to deliver the stipulated dose to liver would result in the delivery of more than 43 Gy to marrow will have the ^{90}Y dose limited to the amount which would deliver 43 Gy to marrow. If 0 of 3, or no more than 1 of 6 patients treated at this estimated dose to marrow experiences graft failure, we will then escalate in increments of 5 Gy to marrow in those patients as allowed by the marrow:liver radiation absorbed dose ratio (i.e., without exceeding intended estimated radiation absorbed dose to liver). The MTD of radiation dose to marrow will be estimated to be the dose below any dose where $\geq$ 2 patients of up to 6 treated experience graft failure.

16.3 Dose Limits to Other Normal Organs

Although the RIT approach is intended to deliver myeloablative doses of absorbed radiation to sites of disease, it will be important to ensure patient safety and minimize the risk of radiation toxicity in normal organs. In this study the total radiation dose will be limited to ensure that critical organs receive radiation absorbed doses below known tolerance limits. The total dose includes contributions from both external and internal radiation sources. The internal radiation component is the absorbed dose to normal organs from ^{90}Y-DOTA-BC8. The external radiation dose in this study is 200 cGy of TBI. For patients who have previously been treated with external beam radiotherapy, the prior radiation dose delivered will also be factored into determination of the maximum radiation dose permitted for that individual. Although there are several published standards for the tolerance limits for radiation absorbed doses for critical normal organs, the most widely quoted are those established by Emami *et al*. for high dose rate, external gamma radiation therapy delivered by a linear accelerator.[87] While the Emami *et al.* values may not directly equate to tolerance limits for radiation absorbed doses to critical normal organs delivered by very low dose rate internal radiation therapy using beta-particle emitting radionuclides, we will adopt them as a conservative approach to patient safety. **Table 8** shows the functional dose limits that we will follow.

TABLE 8: Functional Dose Limits to Normal Organs

Organ[a]	Maximum whole organ TD 5/5 limit (cGy)[87]	Radiation to be delivered via TBI (cGy)	Maximum radiation dose permissible from Y-90 (cGy)
Kidneys	2300	200	2100
Bladder wall	6500	200	6300
Brain	4500	200	4300[b]
Heart	4000	200	3800
Stomach	5000	200	4800
Small intestine	4000	200	3800
Upper large intestine	4500	200	4300

Organ[a]	Maximum whole organ TD 5/5 limit (cGy)[87]	Radiation to be delivered via TBI (cGy)	Maximum radiation dose permissible from Y-90 (cGy)
Lower large intestine	6000	200	5800
Liver	3000	200	2800[c]

[a] Lungs are not accessible and are not measured using standard dosimetric methods due to rib attenuation.

[b] Maximum Y-90 radiation dose to brain in patients with prior evidence of CNS leukemic involvement may be further decreased based on anticipated external cranial or cranial-spinal irradiation as described under section 10.10.

[c] Actual dose to liver is expected to be lower, reflecting dose escalation schema described under section 16.1.

16.4 Additional Clinical Dose Limits and Modifications

In some patients, the amount of ^{90}Y required to deliver the target dose to normal organ, as determined by biodistribution studies, may be deemed clinically inappropriate by the investigator (e.g., if the amount of ^{90}Y activity (mCi) is significantly higher than has been previously delivered in a clinical or research setting). In this event, the patient may be treated at a lower dose based on use of a prior dose level or use of an alternate dose determination mechanism, such as mCi/kg or mCi/m^2, at the discretion of the investigator in conjunction with the attending physician and a Nuclear Medicine representative.

17.0 STATISTICAL CONSIDERATIONS

The primary objective of this study is to estimate the MTD of ^{90}Y-DOTA-BC8 when combined with a reduced intensity conditioning regimen. The MTD will be defined as the dose of ^{90}Y-DOTA-BC8 used in combination with the reduced-intensity HCT conditioning regimen that is associated with a Grade III/IV RRT or true DLT rate of 25%. In this protocol for patients with advanced AML, ALL, or high-risk MDS, the dose adjustment schema is designed to target a 25% rate of Grade III/IV RRT since these patients are at such high-risk of relapse post-transplant and could benefit from the delivery of maximum radiation doses to leukemic cells in BM and spleen. Details of the dose-modification algorithm are contained in section 16.1.

A secondary objective of this study is to determine if engraftment can be achieved safely among patients with high-risk hematologic malignancies who undergo HCT using DOTA-BC8 RIT. Although we have not seen rejection in any of our prior radiolabeled BC8 Ab studies, in this protocol if sufficient evidence exists suggesting that the true rate of graft rejection exceeds 20%, the protocol will be suspended and reviewed by the investigators and other experts/committees as appropriate to determine whether the trial should be terminated, continued without change, or continued with revisions. Sufficient evidence will be taken to be a lower limit of the appropriate 80% one-sided confidence interval associated with the estimated proportion of rejections in excess of 0.20. These proportions and associated confidence intervals will be calculated after every 5th patient enrolled is evaluable. Operationally, graft rejection at any of the following observed proportions would trigger a suspension: 3/5, 4/10, 5/15, 6/20, 8/25, 9/30, 10/35, or 11/40.

In addition to graft rejection, the study will be suspended if there is sufficient evidence to suggest that the true rate of grades III-IV acute GvHD at day +100 is excessive (graded according to the established criteria at the FHCRC). Sufficient evidence for grades III-IV GvHD will be taken to be an observed proportion of GvHD whose lower limit to the associated one-sided 80% confidence interval exceeds 35%. As with rejection, these proportions will be evaluated after every 5th enrolled patient becomes evaluable. Operationally, any of the following observed proportions of GvHD would lead to study suspension: 4/5, 6/10, 8/15, 10/20, 12/25, 14/30, 16/35, or 18/40.

Each suspension rule will be evaluated among all patients enrolled, regardless of the dose of ^{90}Y-DOTA-BC8 administered.

Table 9 summarizes the operating characteristics of each of these rules, each rule considered independent of the other.

TABLE 9: Characteristics of Suspension Rules

Number of patients	True rate of graft rejection	Probability of suspension[1]	True rate of GVHD	Probability of suspension[2]
20	.15	.12	.25	.04
35	.15	.13	.25	.05
20	.35	.80	.50	.66
35	.35	.90	.50	.83

[1] Represents estimate of the probability of study suspension by associated number of patients due to excess graft rejection.

[2] Represents estimate of the probability of study suspension by associated number of patients due to excess GvHD.

Each estimated from 5,000 Monte Carlo simulations.

Because the anti-leukemic effect of ^{90}Y-DOTA-BC8 combined with this reduced-intensity regimen is unknown, but likely to increase at higher dose levels, we will follow disease response and duration of remission, but rates of relapse post-transplant, or failure to enter remission, will not halt the study.

18.0 TARGETED/PLANNED ENROLLMENT

Ethnic Category	**Sex/Gender**		
	Females	Males	Total
Hispanic or Latino	1	1	2
Not Hispanic or Latino	13	15	28
Ethnic Category Total of All Subjects*	14	16	30
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	11	12	23
Total of All Subjects	14	16	30

19.0 TERMINATION OF 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 PI and IND sponsor may terminate the study at any time. The IRB and FDA also have the authority to terminate the study should it be deemed necessary. Stopping rules associated with lack of efficacy and excessive toxicity are detailed in the statistical section.

20.0 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 communicate routinely (typically weekly) 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.

21.0 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.

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APPENDIX A: BEARMAN CRITERIA FOR REGIMEN-RELATED TOXICITY

The criteria for toxicity were developed to convey the following considerations about the toxicity of the preparative regimen. It is the responsibility of the principal investigator to try and distinguish toxicities due to the preparative regimen from these due to other features of transplantation.

Grade 1 Development of transient chemical abnormalities which are not of major clinical consequence and which reverse without requiring major medical interventions. In general, the intent of this toxicity scale is to observe transient target organ toxicity which is reversible.

Grade 2 Development of chemical or laboratory abnormalities which are persistent and which may represent target organ damage which may not be readily reversed. It is anticipated that at this dose of drug, the toxicity obtained would be manageable by clinical methods but may interfere with other therapies.

Grade 3 Development of major clinical, chemical or laboratory abnormalities which represent maximum toxicities without being fatal. This grade of toxicity is designed to be the dose limiting toxicity. Further, the development of this degree of toxicity cannot be considered to be a regular acceptable but rather an occasional toxicity (<20%), acceptable given the severity of the disease. Included in this would be the need for life support techniques such as renal dialysis and ventilation therapy.

Grade 4 Fatal.

A. Cardiac

Grade 1 EKG voltage decrease by 25% or less, resting tachycardia with weight gain but responsive to diuretic therapy.

Grade 2 EKG voltage decrease by 25-50%, congestive heart failure responsive to diuretic therapy, arrhythmias manageable with medical therapy.

Grade 3 Symptomatic CHF unresponsive to diuretic therapy, decrease in EKG voltage by more than 50%, life-threatening arrhythmia.

Grade 4 Fatal toxicity.

B. Bladder

Grade 1 Microscopic hematuria for more than 7 days.

Grade 2 Macroscopic hematuria.

Grade 3 Hemorrhagic cystitis requiring transfusion and placement of an indwelling catheter to remove clots, or cystoscopy with or without installation of sclerosing agents.

Grade 4 Fatal toxicity.

C. Renal

Grade 1 Increase in creatinine up to twice baseline value.

Grade 2 Increase in creatinine above twice baseline value but not requiring dialysis.

Grade 3 Requirement of dialysis.

Grade 4 Fatal toxicity.

D. Pulmonary

Grade 1 Decrease in pO2 or dyspnea without an infiltrate or, at most, a transient patchy infiltrate.

Grade 2 Transient interstitial pneumonia (either idiopathic or unbiopsied) that does not require ventilatory support).

Grade 3 Interstitial pneumonia requiring ventilatory support.

Grade 4 Fatal toxicity.

E. Hepatic

Grade 1 Transient elevations in liver function tests less than those listed as Grade 2 toxicity.

Grade 2 Hepatic dysfunction with bilirubin elevation greater than 5, SGOT increase greater than five-fold or ascites.

Grade 3 Hepatic failure including hepato-renal syndrome, hepatic encephalopathy, bilirubin elevation greater than 20, or Grade 3 VOD as defined by Shulman et al.

Grade 4 Fatal toxicity.

F. Central Nervous System

Grade 1 Transient somnolence.

Grade 2 Somnolence more than 36 hours or other signs of CNS toxicity.

Grade 3 Seizures or coma.

Grade 4 Fatal.

G. Stomatitis

Grade 1 Ulceration or pain but not prohibiting oral intake.

Grade 2 Painful ulceration prohibiting oral intake.

Grade 3 Severe ulceration requiring intubation.

Grade 4 Fatal.

H. Gastrointestinal

Grade 1 Watery stools, but less than 6 stools/day.

Grade 2 Watery stools more than 6-12 stools/day, or hemorrhagic enterocolitis not requiring transfusion, or transient ileus.

Grade 3 Hemorrhagic enterocolitis requiring transfusion, or ileus requiring nasogastric suction.

Grade 4 Fatal.

APPENDIX B: ACUTE GVHD ASSESSMENT

Staging by Individual Organ Involvement

SKIN: measured by rash first appearing generally between 10 and 70 days after transplant. (excludes rashes of known viral or other origin)

Stage	Description
1	Maculopapular rash <25% BSA
2	Maculopapular rash 25 – 50% BSA
3	Generalized erythroderma
4	Generalized erythroderma with bullous formation and desquamation

LIVER*: measured by total serum bilirubin

Stage	Description
1	2.0 – 2.9 mg/dL
2	3.0 – 5.9 mg/dL
3	6.0 – 14.9 mg/dL
4	≥ 15.0 mg/dL

GUT:** includes only diarrhea occurring after Day +21

Score	Adult	Pediatric***
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day	upper GI (anorexia, nausea, vomiting) with diarrhea of <555 mL/m^2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m^2/day diarrhea
3	≥ 1500 mL/day diarrhea	>833 mL/m^2/day diarrhea
4	severe abdominal cramping, bleeding or ileus caused by GVHD	

* In cases where another cause of hyperbilirubinemia antedated the onset of rash, the liver score should be decreased by one stage.

** In cases where peak GI symptoms are exacerbated by a cause other than GVHD, the gut score should be decreased by one stage.

*** Pediatric patients <17 years of age

Overall Grade

The determination of an overall GVHD grade should be based on the organ stage, response to treatment and whether GVHD was a major cause of death.

Overall Grade	Organ Stage	Qualifying Conditions	Additional Qualifying Conditions
I	Stage 1 -2 skin	No liver or gut	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD.
II	Stage 3 skin or Stage 1 liver or Stage 1 gut	N/A	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD, but glucocorticoid treatment after the onset of GVHD was generally sufficient to control the disease.
III	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**without** GVHD as a major contributing cause of death	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD and that additional treatment after the onset of GVHD did not readily control the disease.
IV	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**with** GVHD as a major contributing cause of death	GVHD was resistant to both the prophylactic immunosuppressive regimen and any additional treatment after the onset of the disease.

APPENDIX C: CHRONIC GVHD GRADING*

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores, 60%, > 15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below.

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nails	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase $\geq$ 3x upper limit of normal, AST or ALT $\geq$4x upper limit of normal or total serum bilirubin $\geq$ 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

Laboratory testing and diagnostic indicators of chronic GVHD*

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as FEV1 < 80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV1/FVC by > 12% within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/μl), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

* From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

APPENDIX D: 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 and the whole body using standard 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. The pharmacokinetics and biodistribution of the radiolabeled antibody will vary from one patient to the next. Therefore, in the MIRD schema, dosimetry calculations are based on a series of direct measurements of the organ biodistribution of radiolabeled antibody. These include gamma-camera images and quantitative activity measurements of the radionuclide used (^{111}In as a surrogate 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. Marrow tissue biopsies are weighed and counted to determine the activity concentration in marrow at specific time points. 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, and activity over time in the individual organs cannot be easily delineated. The photon-emitting indium-111 labels the antibody to show the biodistribution of ^{90}Y-antibody. 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 confirm 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 during therapy. In absolute terms, this correlation may not be completely correct, however, because partial dissociation of ^{90}Y and ^{111}In from the immunoconjugate may occur *in vivo;* some of the free ^{90}Y may deposit on bone surfaces, and some of the ^{111}In may preferentially go 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, in some cases, lead to minor underestimates of ^{90}Y dose to bone surfaces and red marrow, and to minor 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 DOTA-BC8 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 DOTA-BC8 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. Images include chest with upper humeri and thyroid, abdomen, and pelvis with upper femurs. Regions of interest are always selected for the major source organs that visualize above background, which may include, but are not limited to, the liver, spleen, heart volume, red marrow space, lungs, kidneys, thyroid, and testes.

Normal organs and tumors are visualized when concentrations of ^{111}In antibody in the organ or tumor are greater than those in the surrounding tissue. Background regions are drawn for each organ or region of interest similar to the methods described above. In addition, attenuation correction factors are determined for the chest and abdomen using the methods described below. We may 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 tissue for all cancerous areas selected for dose assessment. Imaging of selected tumors may be conducted for various time points, and time-activity curves may be constructed and integrated for tumor dose assessments.

Measurements are also made of representative background tissue and of an imaging standard 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 the ^{111}In imaging standard. The geometric mean of the anterior and posterior counts is obtained for each region of interest. Counts are then corrected for attenuation, decay, and background. Total-body measurements are obtained using whole-body gamma camera images in both anterior and posterior projections, to quantify the total ^{111}In activity remaining in the patient, over time, as a fraction of the total administered activity.

(iii) Attenuation

Prior to antibody infusion, all patients will have measurement made of their abdomen attenuation by transmission scans. A fluid filled sheet source, large enough to cover the entire useful field of view of the camera, is loaded with approximately 0.5 – 0.7 mCi of ^{111}In. Uniform distribution of the isotope throughout the sheet source is ensured. The source is placed on the lower detector, and with the patient on the scanning table and their abdomen in the field of view, the upper detector is lowered into place. A five minute transmission image is acquired. Without moving the detectors and with the scanning table alone between them, another five minute image (flood) is acquired. 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 organs of interest.

(iv) Sampling Times

Selecting an appropriate number of imaging/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 data-collection time points that will still provide a reasonable evaluation of the correct mathematical function that best describes the activity-time curve. A minimum of three to four data measurement points will typically be required for ^{111}In-DOTA-BC8 imaging. Imaging includes one set of measurements immediately post ^{111}In-DOTA-BC8 infusion, followed by an additional two to three imaging time points on Days 1 and 2 post infusion. For example, a patient could have images performed during the morning and afternoon of Day 1, and the afternoon of Day 2, post infusion. These analyses provide data to 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. Time-Activity Curves

The sequential measurement data are plotted to determine the cumulated activity and residence times for each source organ. We plot the fractions of the total administered activity observed at each measurement time point. We plot the *effective* data (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. We then select an appropriate mathematical function (equation) with parameters that best represent the data, and we determine the equation parameters by least-squares linear regression. We then integrate the best-fit equations to infinite time to determine the residence times (τ, hours) for each source organ, tumor, the red marrow, and the whole body. We usually fit the biodistribution data to an exponential or sum of exponentials. An estimate of the slope of the long-term tail of the time-activity curve may be made by fitting an exponential function to the last two points.

e. Residence Time Calculations: Integrating the Time-activity Curves

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 to infinity, as described in the previous paragraph. Residence time values are the basic input number required by 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 patient dose assessment on this protocol.

f. Patient-specific Dosimetry

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 obtained by CT-imaging (Rajendran et al., 2004). The residence times for each source organ will be corrected in OLINDA-EXM using actual organ mass and patient weight. Actual patient weights and organ sizes vary among patients, but we can correct for those differences using actual organ masses from CT volumetrics.

g. Estimate of Dose to Testes

We recognize that free ^{111}In may deposit in the testes due to natural biokinetic processes, but that ^{90}Y may not. Therefore, we will be careful not to attribute counts from ^{111}In in the testes to ^{90}Y during therapy.

h. Estimate of Dose to the Bone Marrow:

Red marrow dosimetry is always challenging in radioimmunotherapy because it is difficult to assess the highly variable concentration of radioactivity in marrow and 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 will employ quantitative imaging of ^{111}In in defined marrow spaces (acetabulum, sacrum, femoral head, or lumbar vertebrae) by direct imaging. We will also obtain and count a representative bone marrow biopsy specimen in a standard well-type counter. We will plot a time-activity curve from the marrow imaging, and then we will normalized the curves the known value from the biopsy specimen. We will obtain a residence time by integrating the mathematical function represented by the normalized exponential obtained by least-squares regression analysis.

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APPENDIX E

Potential Adverse Events Associated or Expected with Hematopoietic Cell Transplantation

1. Graft versus host disease: GVHD is a major toxicity associated with the infusion of allogeneic donor stem cells. GVHD may be acute or chronic and may affect multiple organ systems, including the skin, liver, and GI tract.

2. Opportunistic infections, including viral and fungal infections, can result in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

3. Gastrointestinal toxicity. Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

4. Cardiac toxicity. Cardiotoxicity (congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with the chemotherapy agents and TBI used in the regimen and these sequelae may prove lethal.

5. Pulmonary toxicity. Diffuse interstitial pneumonitis of unknown etiology and diffuse alveolar hemorrhage occurs with some regularity after BMT and interstitial fibrosis occurs much more rarely. Both are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

6. Hepatic toxicity. Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Calcineurin inhibitors may cause elevation of ALT/AST.

7. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from calcineurin inhibitors and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive multi-agent conditioning plus TBI.

8. Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

9. Central nervous system toxicity. Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

10. Marrow aplasia. Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days after the pretransplant conditioning regimen. Transfusion of platelets and red

blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

11. Miscellaneous. Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible after TBI. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone and increased susceptibility to infection. MMF can cause spontaneous abortions and birth defects. Hospitalization during conditioning and recovery period is expected to be 5-9 weeks in duration.