FHCRC Protocol Cover Page

**FHCRC Protocol 1809:**

# A Phase II Trial Combining Radiolabeled BC8 (Anti-CD45) Antibody with Fludarabine and Low Dose TBI Followed by Related or Unrelated PBSC Infusion and Post-Transplant Immunosuppression for Patients with Advanced AML or High Risk Myelodysplastic Syndrome

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FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
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## 2. Introduction

*A. Protocol Summary*

This trial combines targeted hematopoietic radiation delivered by $^{131}$I-BC8 antibody with the non-myeloablative transplantation approach in an effort to improve disease control, without substantially increasing the toxicity of the transplant regimen, for patients with advanced acute myeloid leukemia (AML) beyond first remission, with primary refractory disease, or evolved from myelodysplastic or myeloproliferative syndromes; and patients with high risk myelodysplastic syndrome (MDS). Specifically, this study will assess the feasibility and safety for patients with advanced AML or high risk MDS treated with $^{131}$I-BC8 antibody at a starting dose of 22 Gy delivered to the normal organ receiving the highest dose combined with fludarabine and 2 Gy total body irradiation (TBI), plus cyclosporine (CSP)/mycophenolate mofetil **(**MMF), followed by matched related or unrelated allogeneic hematopoietic stem cell transplantation (HSCT). Determination of the maximum tolerated dose (MTD) will be the major study endpoint. The study will also estimate the rates of transplant-related mortality (TRM), disease response and disease-free survival, and determine rates of donor chimerism and graft versus host disease (GVHD).

*B. Overview*

Allogeneic HSCT using standard myeloablative preparative regimens for patients with high-risk AML has been associated with an approximate 50% 5-year mortality from non-relapse causes and more than 75% of these events occur within the first 200 days after transplantation. Moreover, the cumulative incidence of leukemic relapse remains high with standard unrelated HSCT and the probability of leukemia free survival (LFS) has been found to be <30% in patients transplanted in second remission or beyond[1]. We have used $^{131}$I-labeled-anti-CD45 antibody to deliver targeted hematopoietic irradiation to marrow, spleen and lymph nodes in an effort to improve leukemia cell kill and decrease relapse for advanced AML patients with encouraging results. Despite being able to deliver an additional average of 25 Gy to bone marrow and 50 Gy to spleen using targeted radiation therapy, high rates of TRM (~50%) and associated poor outcome has limited our success of conventional HSCT in combination with $^{131}$I-labeled-BC8 antibody (murine anti-human-CD45).

In an effort to reduce the TRM associated with allogeneic HSCT and generate a graft vs. leukemia (GVL) effect for advanced AML/MDS patients, reduced intensity non-myeloablative allogeneic conditioning regimens have been developed that have been designed to be immunosuppressive to permit donor engraftment and to limit systemic toxicity. These non-myeloablative approaches, however, have been most successful in patients with chronic leukemias or with acute leukemias in remission. The majority of patients with AML/MDS undergoing non-myeloablative HSCT while in active relapse have subsequently relapsed with their disease relatively rapidly after transplantation. The overall objective of this protocol is to reduce leukemic disease burden without adding excessive toxicity using targeted hematopoietic irradiation delivered by $^{131}$I-anti-CD45 antibody combined with the non-myeloablative transplantation regimen. It is unknown, however, if targeted hematopoietic irradiation delivered by $^{131}$I-anti-CD45 mAb can be added safely to the reduced intensity conditioning transplant regimen without a marked increase in TRM. Therefore, we will estimate the MTD and the rate of TRM among patients receiving a conditioning approach using fludarabine and 2 Gy TBI coupled with 22 Gy (starting dose) of radiation delivered by $^{131}$I-anti-CD45 mAb to the normal organ receiving the highest dose. We believe that the day 200 TRM rate using a non-myeloablative regimen will be less than the 50% seen with conventional HSCT. This reduction in

TRM should translate into an increased overall survival rate for patients receiving a "mini"-transplant, even if no improvement in the rate of relapse is detected. For example, for every 100 patients treated with standard allogeneic HSCT, a ~50% rate of relapse and a ~40% rate of day 200 TRM results in 30 patients surviving leukemia free. Conversely, a 20% improvement in the day 200 TRM rate for this same population of 100 patients transplanted using $^{131}$I-anti-CD45 antibody combined with the non-myeloablative transplantation regimen would translate into 40 patients remaining alive without disease.

## 3. Background

Despite many improvements in bone marrow transplantation over more than two decades, the disease-free survival for patients transplanted for advanced AML remains relatively poor. Relapse continues to be the major cause of failure for AML patients transplanted at advanced disease stage; relapse rates of up to 60% have been seen for patients transplanted while in relapse[2-4]. Rates of relapse for a given stage of myeloid malignancy are often lower for recipients of unrelated marrow as compared to related marrow, presumably because of a greater degree of graft-versus-leukemia (GVL) effect. Relapse rates in excess of 40% were still seen, however, in patients transplanted with refractory or relapsed acute leukemia in a recent series of 161 patients receiving allogeneic HSCT[1]. Moreover, 81% of the events seen in this study occurred within the first 100 days and 19% thereafter. Disease status at time of transplant was the most important prognostic factor with the worst leukemia-free survival (LFS) seen in the patients with the most advanced leukemia. The outcome was most encouraging in patients grafted during first complete remission (CR); the cumulative incidence of relapse in the first CR patients of this study was 19% at 4 years. Patients receiving allografts in relapse had a very poor outcome with only 7% LFS at 5 years due to both high relapse rates and high mortality from non-relapse causes. Efforts to decrease the incidence of disease relapse have primarily included intensifying the transplant preparative regimen. Several studies support a dose-response effect for myeloid leukemias to radiation and previous work by Clift *et al*. determined that increasing the TBI dose from 12 to 15.75 Gy in transplant preparative regimens could significantly decrease post-transplant relapse of acute and chronic myeloid malignancies [12% versus 35% for AML, 0% versus 25% for chronic myeloid leukemia, (CML)][5,6]. In each study, however, the higher TBI dose was associated with a higher rate of TRM, such that there were no significant differences in disease-free survival in either study. These and other studies confirm that conventional transplant preparative regimens are at the limit of normal organ tolerance. Therefore, more effective and yet more tolerable conditioning regimens are needed for patients with advanced AML considered for allogeneic transplantation.

The anti-leukemic effects of radioimmunoconjugates that selectively deliver irradiation to targeted leukemic blasts, with less toxicity inflicted on normal tissues, have been investigated as therapy for myeloid malignancies in the hope that cure rates could be significantly improved. The CD45 (T200) antigen is an attractive target for immunotherapy since the vast majority of hematologic malignancies express CD45, including 85-90% of acute myeloid leukemias, and this antigen is not found on tissues of non-hematopoietic origin[7-9]. An antibody reactive with the CD45 antigen targets the primary sites of leukemic cell involvement in AML (the marrow and spleen), as well as lymphoid tissue. By selecting an antibody reactive with normal myeloid precursors and all mature leukocytes, most malignant blasts, including blasts scattered in normal myeloid and lymphoid tissue, should receive relatively high doses of radiation given that the surrounding normal hematopoietic cells will be targeted as well. For this reason, $^{131}$I-anti-CD45 mAb (BC8) may offer clinical benefit both to patients with active disease and to those in remission. Encouraging phase I results have been demonstrated using $^{131}$I-BC8 antibody combined with

a transplant regimen employing 120 mg/kg cyclophosphamide (CY) and 12 Gy TBI for therapy of acute leukemias[10]. Biodistribution of trace-labeled antibody was initially determined in 41 patients with advanced AML or acute lymphocytic leukemia (ALL), and 3 patients with advanced myelodysplastic syndrome (MDS). The dose of $^{131}$I delivered to each patient was determined from dosimetry studies, and was escalated in cohorts of 3-7 patients to deliver estimated radiation doses of 3.5 Gy to 12.25 Gy to the normal organ receiving the highest dose. Most (84%) patients had favorable biodistribution of monoclonal antibody (mAb), with improved therapeutic ratios of radiation delivered to marrow and spleen as compared to liver (2.3 and 4.8 times higher than liver, respectively). Thirty-four patients were treated with the amount of $^{131}$I estimated to deliver from Dose level 1 (3.5 Gy) to level 6 (12.25 Gy) to the normal organ receiving the highest dose. The average estimated radiation absorbed doses delivered to marrow and spleen, average $^{131}$I doses, and Grade III/IV toxicities (defined using Bearman criteria) at each dose level are summarized in Table 1.

**Table 1:** FHCRC #557 (Phase I Study) -- $^{131}$I Activity Administered, Total Radiation Absorbed Doses, and Grade III/IV Regimen-Related Toxicities

| Gy to Liver (Dose Level) (number) | | mCi $^{131}$I Mean ± SD (range) | Gy to Marrow Mean ± SD (range) | Gy to Spleen Mean ± SD (range) | Grade III/IV Toxicities |
|---|---|---|---|---|---|
| 3.5 | (Level 1) (n=7) | 148±42 (76-206) | 7.3±1.7 (4-9) | 14.4±5.0 (7.5-22.1) | III GI – 1 pt. (1 N.E.) |
| 5.25 | (Level 2) (n=7) | 215±55 (142-309) | 12.6±2.5 (10.5-17.7) | 16.6±4.0 (9.1-21.7) | No (1 N.E.) |
| 7.0 | (Level 3) (n=6) | 250±83 (149-389) | 24.8±6.1 (13.2-30.6) | 36.5±13.8 (22.9-59.2) | IV Marrow – 1 pt. |
| 8.75 | (Level 4)[a] (n=3) | 304±92 (226-405) | 20.4±7.3 (14.7-28.6) | 52.4±10.0 (41.7-61.5) | No |
| 10.5 | (Level 5)[a] (n=6) | 255±73 (148-350) | 18.3±5.5 (11.6-24.0) | 61.7±21.1 (34.0-92.8) | Grade III Hepatic – 1 pt. |
| 12.25 | (Level 6) (n=2) | 549 (486-613) | 26.0 (24.1-28.0) | 55.2 (46.7-63.7) | Grade III Mucosal – 1 pt. Grade III+ Mucosal – 1 pt. |

[a]Three patients scheduled to be treated at these dose levels (two at Level 4, one at Level 5) had their radiation absorbed dose to liver limited so that dose to marrow would not exceed 28 Gy. These patients received 283, 275, and 459 mCi $^{131}$I delivering estimated radiation doses to liver of 4.8, 6.1, and 9.2 Gy respectively. These patients had no Grade III/IV toxicities and were not counted as evaluable patients for purposes of dose escalation.

Of 25 patients with advanced AML or MDS treated on study, three died of infection in the early post-transplant period, one died of infection 4 months post transplant, and one patient with poor engraftment after receiving a 4-HC-purged autologous marrow died 54 months post transplant with infection. Thirteen patients relapsed 2 to 77 months post transplant (Fig. 1). Seven patients survive disease free 15 to 89 months (median 65 months) post-transplant. Of the 9 patients with advanced acute lymphocytic leukemia (ALL), 2 died of infection, and 4 relapsed 0.5 to 11 months post-transplant. Three survive disease-free 23, 58, and 70 months post transplant. At the maximum tolerated dose (MTD) of 10.5 Gy, it was determined that $^{131}$I-anti-CD45 antibody in combination with CY and TBI could deliver up to an additional dose of 24 Gy to marrow and 50 Gy to spleen. This study

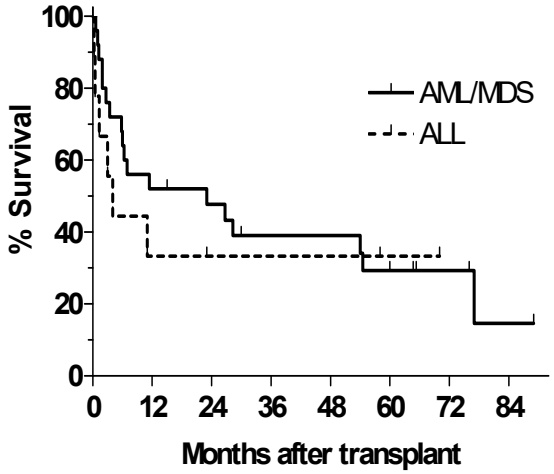



**Fig. 1**: Disease-free survival for patients receiving $^{131}$I-BC8 Antibody/Cy/12 Gy TBI on FHCRC Protocol #557.

demonstrated that ratios of at least 2 to 1 of radiation delivered to the target organs of marrow and spleen, as compared to the normal organ receiving the highest dose, can be reliably achieved in patients who are either in remission or acute leukemic relapse. An incremental dose of at least 10.5 Gy to the liver (with less to lung, kidney, and total body) delivered by $^{131}$I-labeled BC8 has been well-tolerated when administered in addition to cyclophosphamide 120 mg/kg and 1200 cGy TBI in patients under age 55.

More recently we have conducted a Phase II study of I-131-anti-CD45 antibody combined with CY/TBI for patients with advanced AML and MDS (FHCRC Protocol #1297). Twenty-six patients, 10 to 55 (median 39) years old, with AML (5 CR2, 18 relapse or refractory) or MDS (1 RAEB, 2 RAEBT) have received a test infusion of BC8 antibody trace-labeled with I-131. Favorable biodistribution, with estimated radiation absorbed dose to bone marrow and spleen > to liver (normal organ receiving the highest dose), was observed in 24 (92%) patients. Twenty patients received a therapy infusion of 0.5 mg/kg of antibody labeled with 127-389 (median 252) mCi of 131I, followed by 120 mg/kg CY over 2 days, 12 Gy TBI over 3 days and a matched related (n=9) or unrelated (n=11) HSCT. Since the toxicity with conventional transplant regimens is higher for patients with MURD, the initial target radiation absorbed dose to the liver from radiolabeled antibody was set lower (8 Gy) than that for patients with MRD (10 Gy). Three patients required a reduction from the planned liver dose to limit their marrow dose to the pre-established maximum of 33 Gy, with one patient receiving 8.4 instead of 10 Gy to liver, one receiving 6.9 instead of 8 Gy, and the third receiving 4 instead of 8 Gy. Mean estimated radiation absorbed doses from radiolabeled antibody were 21 + 8 Gy to marrow and 45 + 18 Gy to spleen, with a median of 2 (1-9.1) times greater radiation delivered to marrow than to liver. All patients engrafted (ANC > 500 cells/mm3) at a median of 18 (15-30) days post-HSCT. All patients experienced at least grade II (moderate) mucositis. Although 4 related and 3 URD recipients are alive and disease-free at 2.2 years (0.4 – 4.8 years) post-transplant, 6 patients have experienced TRM and 5 patients have experienced severe-to-fatal RRT. These results suggest that new therapeutic strategies are needed to reduce these rates of morbidity and TRM.

A follow-up phase II study is underway investigating the use of $^{131}$I-anti-CD45 mAb in a preparative regimen for patients with AML in first remission[11,12]. Fifty-six first remission patients have been studied with $^{131}$I-BC8 antibody. Fifty have had favorable biodistribution of a trace-labeled dose of antibody (89%). Forty-three patients (median age 44, range 16-55 years) have been treated with $^{131}$I-BC8 antibody combined with BU/CY, with the most recent 40 patients receiving estimated radiation doses of 5.25 Gy to the liver, the normal organ receiving the highest dose. The most recent 13 patients were treated on our current active protocol, Protocol #1470. The average estimated radiation absorbed dose delivered by I-131-BC8 antibody was 11 Gy to bone marrow and 29 Gy to spleen. Of the 43 patients treated to date, two are considered inevaluable, because they received PBSC off protocol. Neither of these patients experienced any Grade III/IV toxicities. One patient is alive and disease-free

24 months post-transplant, and the other inevaluable patient relapsed four months post-transplant. Five of the 41 evaluable patients (12%) have developed complications that were graded as Grade III/IV RRT, and three of these died, as did five additional patients from infection. Seven patients (17%) have relapsed 3 to 13 months post-transplant. Thus, 26 of 41 patients (63%) are surviving disease-free a median of 47 months post-transplant (Fig. 2). Thirty-six patients had cytogenetics at diagnosis and were categorized using current Southwest Oncology Group (SWOG) criteria for cytogenetic risk group as follows: favorable – 1; intermediate – 23; unfavorable – 11, unknown – 1. Two of the patients with intermediate-risk cytogenetics at diagnosis were considered in the unfavorable group because they developed AML following MDS or following treatment with alklyating agents. Fifteen of the 21 patients (71%) in the intermediate risk group are surviving disease-free, with two relapses (10%) and 4 non-relapse deaths (19%). Of the 13 patients in the unfavorable risk group, four have relapsed (31%) and three have died with RRT and/or infection (23%). These data appear promising since approximately 30% of patients with AML in first remission would be expected to develop leukemic relapse after being treated with standard BU/CY conditioning and stem cell transplantation[13].

As an alternative to conventional marrow transplant preparative regimens, several investigators have recently explored lower dose, non-myeloablative conditioning regimens. This approach is designed to achieve mixed donor chimerism with the goal of optimizing a GVL effect. The non-myeloablative transplant regimen employs pre-transplant immunosuppression with low dose TBI and fludarabine, followed by intensive post-transplant immunosuppression with cyclosporine and mycophenolate. The rate of day 200 TRM for patients treated with standard HLA-matched unrelated non-myeloablative transplantation has been found to be in the range of 10-15%, substantially less than that seen using conventional unrelated HSCT. Since 1997, more than 200 patients with a variety of hematologic malignancies, including 12 patients with AML, have been treated with a non-myeloablative regimen consisting of 2 Gy TBI + CSP/MMF, followed by PBSC infusions from HLA-phenotypically matched unrelated donors on a series of collaborative protocols at the FHCRC, Stanford, and the University of Leipzig[14-18]. Given early concerns of graft rejection seen in some patients using the mini-transplant approach, fludarabine has been included in the non-myeloablative regimen for patients regardless of their underlying diseases. Since the time of inclusion of fludarabine in protocols used at FHCRC and Stanford, there has been an approximate 20% graft rejection rate for patients receiving unrelated donor non-myeloablative allografts. Engraftment has been affected by the patient's diagnosis, degree of prior chemotherapy received, and source of stem cells, with the most impressive rates of engraftment seen in patients receiving stem cells from peripheral

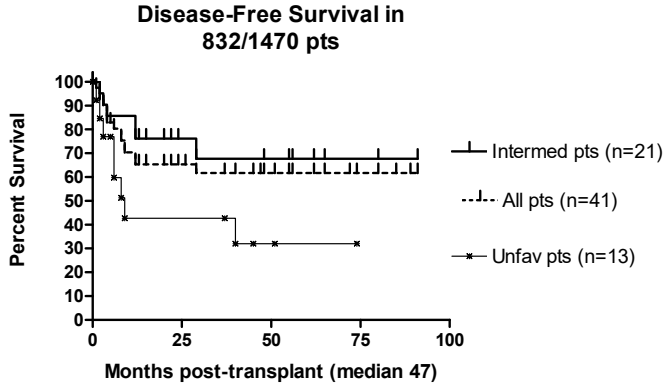



**Fig. 2**: Disease-free survival for patients with AML in first remission receiving a therapy dose of I-131-anti-CD45 antibody combined with BU/CY on FHCRC Protocols #832 and 1470. Thick solid line = intermediate risk subgroup defined using SWOG cytogenetic criteria and lack of antecedent MDS or treatment with alkylating agents; thick dashed line = all patients, and thin solid line = poor risk subgroup defined by SWOG criteria and presence of antecedent MDS or treatment with alkylating agents.

blood as opposed to stem cells harvested from bone marrow.

While this non-myeloablative transplantation approach has demonstrated impressive rates of engraftment with relatively low rates of non-hematologic toxicity, the GVL effect has been limited by the disease-stage at the time of transplantation and has been most effective in states of minimal residual disease. Three of 5 patients who received a non-myeloablative unrelated donor allograft while in first complete remission (CR) remain alive and disease-free 15 to 29 months after transplantation. Conversely, only 2 of 7 patients with more advanced AML remain disease-free at 15 months. The remaining advanced AML patients died following the unrelated donor non-myeloablative regimen, primarily due to progression or relapse of disease (1 of 3 in CR2, 3 of 3 beyond CR2, and 1 patient with primary refractory disease). For patients with advanced disease and large leukemic burden, the anti-leukemic efficacy of this mixed-chimerism approach may be improved by the addition of targeted hematopoietic irradiation delivered by $^{131}$I-anti-CD45 antibody to the relatively low-intensity therapy given in the non-myeloablative approach where the only anti-leukemic therapy is 2 Gy TBI.

In an on-going study (FHCRC protocol #1432), we have examined the combination of $^{131}$I-BC8 antibody with the non-myeloablative approach in patients over the age of 50 with advanced AML using HLA-matched related and unrelated donors. To date, 25 patients have been treated on protocol #1432 with adequate follow-up to assess toxicity and engraftment. All patients have achieved a complete remission and all patients have engrafted at a median of 15 days post-transplant. This experience suggests that this approach is feasible for patients with advanced AML who would be at high risk for TRM when using radiolabeled antibody combined with a conventional allogeneic transplant regimen. In this group, the slightly greater toxicity anticipated from the addition of $^{131}$I-BC8 antibody to 2 Gy TBI/FLU + CSP/MMF would be warranted if the targeted radiation delivered to marrow and spleen decreases the leukemic burden to the extent that the GVL effect resulting from engraftment of donor T cells may eradicate remaining cells.

The estimated MTD of radiation delivered to the normal organ receiving the highest dose by I-131-BC8 antibody on our Phase I study (Protocol #557) was 10.5 Gy, a dose level at which only 1 of 6 patients experienced Grade III (Bearman) regimen-related toxicity. However, Protocol #557 combined I-131-BC8 antibody with a full-dose, conventional transplant regimen of 120 mg/kg cyclophosphamide and 12 Gy TBI, whereas this protocol combines I-131-BC8 antibody with the much lower dose, less toxic "non-myeloablative" (or "mini") transplant regimen of fludarabine 90 mg/m$^2$ and 2 Gy TBI, followed by the immunosuppressive regimen of cyclosporine and mycophenolate mofetil. Patients receiving this non-myeloablative regimen alone have essentially no regimen-related toxicity other than cytopenias. In addition, the MTD has not been reached on our Phase I study (Protocol #1432) combining the reduced-intensity regimen with 131-BC8 antibody. This protocol will also combine I-131-BC8 antibody in a similar dose escalation format with 2 Gy TBI and FLU. We anticipate that the combined toxicity of 22 Gy (starting dose) delivered by I-131-BC8 antibody combined with fludarabine and 2 Gy TBI to patients under the age of 50 on this protocol will be even less that which is anticipated in the older patients treated with the same dose of I-131-BC8 antibody.. The primary goals of this study will be to determine the MTD, rates of TRM, feasibility, and safety for patients receiving $^{131}$I-BC8 antibody when combined with 2 Gy TBI + CSP/MMF.

## 4. Objectives

1. To evaluate the MTD and the TRM and toxicity of delivering $^{131}$I-BC8 (anti-CD45 antibody) at a starting dose of 22 Gy to the normal organ receiving the highest dose in combination with the

non-myeloablative regimen of fludarabine (FLU), 2 Gy TBI, cyclosporine (CSP), mycophenolate mofetil (MMF), and HLA-matched related or unrelated allogeneic hematopoietic stem cell transplant (HSCT) in patients 16 to 50 years old who have advanced AML or high risk MDS.

2. To estimate rates of 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.
3. To determine rates of disease relapse, graft vs. host disease, and 2-year disease-free survival in patients receiving $^{131}$I-BC8 antibody combined with FLU, 2 Gy TBI, CSP, MMF, and HLA-matched related or unrelated allogeneic HSCT.

## 5. Patient Selection

*A. Inclusions*

1. Patients with advanced AML defined as beyond first remission, primary refractory disease, or evolved from myelodysplastic or myeloproliferative syndromes; or patients with MDS expressed as refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEBT [*Note: classification removed under current WHO classification system*]), refractory cytopenia with multilineage dysplasia (RCMD), RCMD with ringed sideroblasts (RCMD-RS), or chronic myelomonocytic leukemia (CMML).
2. Patients not in remission must have CD45-expressing leukemic blasts or myelodysplastic cells. 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 $\geq$ 16 and $\leq$ 50 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):

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

6. Patients must have normal hepatic function (bilirubin, AST and ALT < 2 times the upper limit of normal).
7. Karnofsky score $\geq$ 70 or ECOG $\leq$ 2.
8. Patients must have an expected survival of >60 days and must be free of active infection.
9. Patients must have an HLA-identical sibling donor or an HLA-matched unrelated donor who meets standard Seattle Cancer Care Alliance (SCCA) and/or NMDP or other donor center criteria for PBSC donation. Related donors should be matched by molecular methods at the intermediate resolution level at HLA-A, B, C, and DRB1 according to FHCRC Standard Practice Guidelines and to the allele level at DQB1. Unrelated donors should be identified using matching criteria that follows the FHCRC Standard Practice Guidelines limiting the study to eligible donors that are allele matched for HLA-A, B, C, DRB1, and DQB1 (Grade 1; Appendix C), and accepting up to one allele mismatch as per Standard Practice Grade 2.1 for HLA-A, B, or C.

*B. Exclusions*

1. Circulating antibody against mouse immunoglobulin (HAMA).
2. Prior radiation to maximally tolerated levels to any normal organ.
3. Patients may not have symptomatic coronary artery disease and may not be on cardiac medications for anti-arrhythmic or inotropic effects.
4. Inability to understand or give an informed consent.
5. Patients who are seropositive for HIV.
6. Perceived inability to tolerate diagnostic or therapeutic procedures, particularly treatment in radiation isolation.
7. Patients who have previously undergone autologous or allogeneic HSCT.

## 6. Donor Selection

Donors must meet HLA matching criteria as outlined under section 5.A.9 as well as standard SCCA and/or NMDP or other donor center criteria for PBSC 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. Evaluation and Counseling of Patient and Donor

Patients will be referred here for consideration of a marrow transplant. The initial conference will be conducted by the outpatient attending physician. A second conference will be conducted by the Principal Investigator, or designated sub-investigator, to describe the details of radiolabeled antibody administration. The protocol will be discussed thoroughly with patient and family, and significant known risks to the patient described. The stem cell transplant procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the patient or, in the case of minors, to the patient's responsible family members. Informed consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Related donors will be counseled and consented in accordance with standard Seattle Cancer Care Alliance procedures and using the standard donor consent form. Unrelated donors will be counseled and consented according to the guidelines of the NMDP or other donor centers.

## 8. Protocol Registration

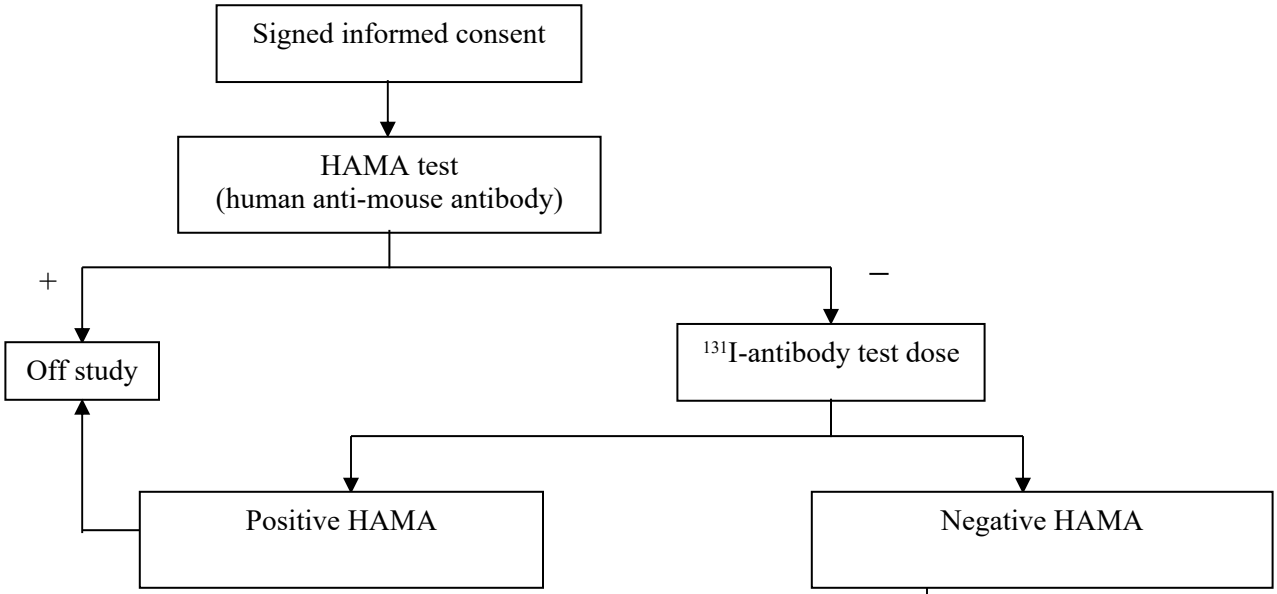
Patients will be assigned to a protocol by the Clinical Coordinator's office, which will register the patient with the Registration Office (206-667-4728) between 8:30 am and 4:00 PM, Monday through Friday. After hours, the Registration Office can be reached by paging 206-995-7437.

**9.**

## 9. Plan of Treatment

*A. Outline of Treatment Plan*

1. Basic Treatment Schema for Patients Meeting Inclusion Criteria:




| Transplant Day | Treatment |
|---|---|
| -10 to -14 | $^{131}$I-antibody therapeutic dose |
| -11 | Rest & radiation isolation |
| -10 | |
| -9 | |
| -8 | |
| -7 | |
| -6 | |
| -5 | |
| -4 | Fludarabine |
| -3 | Fludarabine; start cyclosporine |
| -2 | Fludarabine |
| -1 | |
| 0 | 2 Gy TBI, HSCT, start MMF |

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2. Treatment Schema and Post-transplant Immunosuppression for Patients with Matched, Related Donors

**Table 2**. Treatment schema for PBSC transplants to establish mixed chimerism in patients with HLA-identical sibling donors

| **Day** | -14 to -10 | -4 | - 3 | -2 | -1 | 0[a] | | + 27 | +28 | + 56 | +84 | +180 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$^{131}$I-BC8 Antibody Rx** | **X** | | | | | | | | | | | |
| **Donor Leukapheresis** | | | | | **X** | **X** | | | | | | |
| **TBI 200 cGy** | | | | | | **X** | | | | | | |
| **Fludarabine 30 mg/m$^2$/d** | | **X** | **X** | **X** | | | | | | | | |
| **PBSC Transplant** | | | | | | **X** | | | | | | |
| **CSP** | | | Start | | | | | | | Taper | | Stop |
| **MMF** | | | | | | Start[b] | | Stop[e] | | | | |
| **Chimerism Evaluations** | | | | | | | | | **X** | **X[c]** | **X[d]** | |

[a] Day 0 should be on a Tuesday to Thursday
[b] First dose should be given 4-6 hours after stem cell infusion
[c] Chimerism evaluation at d $^+$56 from blood only, and only if day $^+$ 28 chimerism is <50% donor
[d] Bone marrow aspirate on d $^+$84 for chimerism to also include samples for flow cytometry (and cytogenetic analysis if appropriate) to assess for minimal residual disease.
[e] MMF may be discontinued earlier than day +27 at the discretion of the study doctor in patients who are at least day +14 post transplant who exhibit no GVHD.

3. Treatment Schema and Post-transplant Immunosuppression for Patients with Matched, Unrelated Donors

**Table 3.** Treatment schema for PBSC transplants to establish mixed chimerism in patients with HLA-phenotypically matched unrelated donors.

| **Day** | -14 to -10 | -4 | - 3 | -2 | -1 | | 0[a] | +1 | + 40 | +96 | +100 | +180 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$^{131}$I-BC8 Antibody** | X | | | | | | | | | | | |
| **Donor Leukapheresis** | | | | X | X | | X | | | | | |
| **TBI 200 cGy** | | | | | | | X | | | | | |
| **Fludarabine 30 mg/m$^2$/d** | | X | X | X | | | | | | | | |
| **PBSC Transplant** | | | | | | | X | | | | | |
| **CSP** | | | Start | | | | | | | | Taper | Stop |
| **MMF** | | | | | | | Start[b] | | Taper[e] | Stop[e] | | |

| **Day** | +28 | +56 | +84 | | |
|---|---|---|---|---|---|
| **Chimerism studies[c]** | X | X[c] | X[d] | | |

Chimerism evaluations will be done on days: $^+$28, $^+$56 (only if donor is <50% on day $^+$ 28) and $^+$ 84 post-stem cell infusion
[a] Day 0 should be on a Tuesday to Thursday
[b] First dose should be given 4-6 hours after stem cell infusion
[c] Chimerism evaluation at d +56 from blood only, and only if day $^+$28 <50% donor
[d] Bone marrow aspirate for chimerism to include samples for flow cytometry (and cytogenetics if appropriate) to assess for minimal residual disease.
[e] MMF may be discontinued earlier, at the discretion of the study doctor, in patients who are at least day +14 post transplant who exhibit no GVHD.

*B. Radiolabeled Antibody Evaluation (Biodistribution Dose) and Treatment*

1. Biodistribution Study

The radiation absorbed doses for each organ delivered by $^{131}$I-labeled antibody will be estimated for each patient prior to administration of therapeutic amounts of $^{131}$I-labeled antibody. For this purpose 1 to 10 mg of antibody will be labeled with 4-10 mCi $^{131}$I. The $^{131}$I-labeled antibody will be mixed with unlabeled antibody to achieve 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. Estimates of radiation absorbed doses for each organ will be calculated after each infusion. These estimates will be used to determine the amount of $^{131}$I with which the therapeutic dose of isotope-antibody conjugate will be labeled.

a. Iodination and Characterization of Labeled Antibody

Antibodies will be labeled in the radiochemical facilities of the Division of Nuclear Medicine at the University of Washington, using established techniques (Chloramine-T) for the radioiodination of antibodies for human use. Following the labeling procedure, the antibody will be sterilized by filtration through a 0.1 μm filter and tested for endotoxin content. Since antibody labeled with therapeutic amounts of $^{131}$I must be infused within a few hours of labeling to avoid radiolysis, the results of sterility testing will not be available at the time of antibody infusion. $^{131}$I-labeled antibody immunoreactivity will be determined following each labeling procedure.

b. Non-radioactive Iodine Solution

The thyroid will be blocked with Lugol's Strong Iodine Solution, 5 drops three times daily PO (depending on patient weight), or saturated solution of potassium iodide (SSKI) 4 drops three times daily PO, starting two days prior to the morning of the biodistribution dose of labeled antibody and continuing for three weeks following the last infusion (unless limited by other medical conditions).

c. Vital Signs

Vital signs will be obtained prior to infusion and monitored every 30 minutes for the first 2 hours and then hourly until the infusion is complete or more often if clinically indicated.

d. Antibody Administration

Radiolabeled antibody will be administered through a central venous catheter. Premedications will include:

acetaminophen 650 mg PO,
diphenhydramine 25-50 mg IV,
ondansetron 8 mg IV,
hydrocortisone 100 mg IV,
D51/2NS to start with the antibody infusion and to continue until the antibody infusion is complete.
Hydrocortisone 100 mg IV will be repeated every 2 hours until the completion of the infusion.

The mixture of $^{131}$I-labeled BC8 antibody (4-10 mCi) will be diluted to approximately 25 ml and infused intravenously at 7.5 mg/hour.

If grade II allergic-type toxicity is encountered (see section 13.A.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, 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 11.A), 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 (or 1 mg/kg) PO or IV every 2-4 hours PRN.
Nausea: lorazepam 0.5-2 mg (0.05 mg/kg) IV every 4 hours PRN
diphenhydramine 25-50 mg (1 mg/kg) IV every 4 hours PRN
ondansetron 8mg IV every 8 hours for first 24 hours from start of infusion, then every 8 hours PRN for nausea.
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 antibody infusion and treat per institution standards.

e. Antibody and Blood Samples

Aliquots of the infusion mix will be taken prior to infusion, to be used as quantitative standards for assessment of counts in blood and marrow. A blood sample (5-7 ml) will be obtained at the time of marrow biopsy. This sample will be obtained at the SCCA. These samples will be used for analysis of antibody clearance.

f. Gamma Scanning

Serial gamma camera images will be obtained in conjunction with dedicated computers at 0 hours (i.e. end of infusion) on day 0, and on at least two of the following days: day 1, 2, and 3 post infusion of antibody, to be stored on removable soft disks and magnetic tape. Images will include anterior and posterior head/neck, anterior and posterior chest with upper humeri, abdomen, and pelvis with upper femurs. At each imaging time a source of $^{131}$I will be counted and imaged on the gamma camera in a fixed geometry to account for radioactive decay and changes in camera sensitivity during the study so that body tissue activity curves and areas of interest, etc. can be temporally compared more accurately.

Images will be inspected for general body distribution of activity, especially liver, kidney, bone marrow and spleen, and relative counts at various times over the organs for any organ with activity above background will be recorded and compared to the fixed $^{131}$I source. The methods used to calculate estimated radiation absorbed doses to each tissue or organ per mCi I-131 administered are described in Appendix D. Reporting of dosimetry will include the MIRD/OLINDA-EXM calculations for all 25 organs included in the program. These

calculations will include the use of quantitative data from serial gamma camera images performed after the biodistribution dose of trace I-131-BC8 antibody for all organs with activity above background. Specifically, we will include estimated dosimetry for thyroid and bladder, although neither will be considered a dose-limiting organ.

g. Marrow Biopsies

A unilateral bone marrow biopsy (no aspirate) will be performed at least one time on the day after antibody infusion (*i.e.* ~ 24 hours). If that sample is judged to be inadequate, a second sample may be obtained within 48 hours of the infusion. This will be performed in the SCCA Procedure Suite. Small sections will be weighed and counted via gamma counter for $^{131}$I content, with comparison to a quantitative standard of the infusion mix, allowing calculation of % injected dose/gram marrow of labeled BC8 antibody. The biopsy section will be collected using NO FIXATIVE agents. A research technician from the Laboratory (D3-377) will prepare the research portion for the Lab and send the remaining portion to SCCA Pathology for morphology. A portion of the biopsy may be analyzed using flow cytometry (for detection of bound antibody).

h. Laboratory Samples

A blood sample for HAMA will be obtained the day prior to planned therapy infusion. Blood samples (CMP and CBC/CBD) will be obtained pre-infusion, at the end of infusion and on day 1 for CBC, BUN, creatinine, AST, ALT, and bilirubin.

2. Requirements for Therapy

All patients with a negative HAMA test will be eligible for $^{131}$I-labeled antibody infusion regardless of the estimates of radiation absorbed doses from the dosimetric analysis.

*C. Selection and Timing of $^{131}$I-Labeled Antibody Dose for Therapy*

$^{131}$I-labeled antibody for therapy will be administered to each patient in a protein dose and infusion schedule identical to that used for dosimetry studies in the patient (see section 9.B.1). The therapeutic dose will be generally infused 6 to 14 days after the trace-labeled biodistribution dose, but can be delayed further if necessary because of clinical circumstances (as long as the patient has not developed HAMA). In previous patients, serum antibody concentrations after the dosimetry infusion have declined to much less than 10% of their initial level by this time. At least 6 days is 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 (see 9.C.2 below)

1. Selection of Isotope Dose

The total amount of $^{131}$I administered will be individualized based on the biodistribution of the trace-labeled dose in each patient. The dose of $^{131}$I will be calculated to deliver 22 Gy (at the starting dose) to the normal critical organ (almost always liver) predicted to receive the highest estimated dose of radiation, unless that would result in an unacceptable marrow dose (initially >43 Gy; see sections 13.C and 19.B for further discussion). For each patient, the dose level at which the patient will be treated will be discussed by the P.I. (or his designee) and Drs. Rajendran, Eary, or Shields (or designee).

2. Timing of Peripheral Blood Stem Cell (PBSC) Infusion

For patients with estimated marrow biologic half-times of less than 96 hours as estimated by the biodistribution dose, donor PBSC will normally be infused 12 days after the therapy dose of $^{131}$I-BC8 antibody. In the Phase I dose-escalation trial, the longest measured marrow biologic half-time was 86 hours. In the unlikely event that a patient has an estimated marrow biologic half-time longer than 96 hours, PBSC infusion will be delayed until the estimated radiation dose rate in marrow is < 2 mR/hour, and the actual number of days prior to PBSC infusion that the therapy dose of antibody is administered will be adjusted accordingly.

*D. 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.

*E. Total Body Irradiation*

TBI will be administered at a dose of 2 Gy (6-7 cGy/min delivered from a linear accelerator) on Day 0, followed by PBSC infusion. TBI will be administered between 11:00 a.m. and 2:00 p.m. to avoid proximity to CSP/MMF administration.

*F. Immunosuppression*

1. Cyclosporine (CSP)
   a. For patients with matched sibling donors, CSP is given at 3.75 mg/kg PO Q12 hours from day –3 to day +56, then is tapered to be discontinued on day +180 unless GVHD develops. For patients with matched unrelated donors, CSP is given from day –3 to day +100. The CSP starting dose is 3.75 mg/kg PO Q12 hours and continues to day +100. CSP is then tapered to end at 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. Alternative formulations of CSP may be substituted if nausea and vomiting are persistent according to the Standard Practice Manual and at the discretion of the Attending Physician.

   b. Guidelines for CSP 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 discretion of the Attending Physician in the absence of toxicity. 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.

   c. Blood pressure, renal function (creatinine, BUN), electrolytes and magnesium will be followed at least three times per week while receiving CSP.

d. Drugs that may affect CSP levels include: dilantin, phenobarbital (may lower CSP levels), steroids, fluconazole, ketoconazole, cimetidine (may increase CSP levels).

2. Mycophenolate Mofetil (MMF)

a. Oral MMF will be started 4-6 hours after PBSC infusion on day 0. The drug should be given IV at the same dose level as oral MMF if there is nausea and vomiting at any time preventing oral administration. Doses will be rounded to the nearest 250 mg. Both IV and oral doses are calculated using the adjusted weight. For patients with matched sibling donors, oral administration of MMF will be given daily at 15 mg/kg Q12 hours (30 mg/kg/day) until day +27 post-transplant, at which point it will be stopped without tapering. For patients with matched, unrelated donors, MMF will be given daily at 15 mg/kg Q8 hrs (45 mg/kg/day) until day +40 post-transplant and then tapered off by day + 96. In the case of impending graft rejection with markedly low (<40%) donor T cell chimerism after HSCT, MMF should be continued at full dose or, if MMF taper has been initiated, reinstitution of full dose MMF should occur. Also in this case, if MMF has been discontinued, MMF should be reinitiated at full dose. Note that for patients exhibiting no signs of GVHD at day +14 post transplant, MMF may be discontinued sooner than described above at the discretion of the study doctor.

b. Guidelines for MMF dose adjustment: 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 non-myeloablative transplant studies, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. The etiology of the neutropenia should be thoroughly investigated with the use of peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications, such as Sulfa based drugs (*e.g.* Bactrim). Conservative dose adjustments of ~20% will only be made for grade IV neutropenia persisting after day +21 post-transplant once all other potential causes of marrow toxicity have been ruled out. After day 28, the use of G-CSF will be permitted for neutropenia. 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 be made or the drug may be given IV. For severe toxicity related to MMF (grade IV neutropenia > 5 days refractory to G-CSF, severe refractory diarrhea, or overt gastrointestinal bleeding), the MMF may be temporarily stopped. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF). MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

*G. Collection and Infusion of Donor PBSC*

1. G-CSF Administration to Related Donors and PBSC Collection

Related donors 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 before 10:00 a.m. each day in the 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 4ºC overnight, and infused on day 0. If the collection on day –1 contains less than 5.0 × 106 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 l of whole blood during the collection.

**Treatment Schema for Donor**

| **Day** | **-4** | **-3** | **-2** | **-1** | **0** |
|---|---|---|---|---|---|
| G-CSF 16 μg/kg/SQ | X | X | X | X | X |
| PBSC collection | | | | X | X |

2. G-CSF Administration to Unrelated Donors and PBSC Collection

Timing of PBSC collection for unrelated donors is prearranged through the NMDP or other donor center guidelines 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 NMDP or other 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 will be infused with all available cells at the discretion of the Attending Physician.

3. PBSC Infusion

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

*H. Myelosuppression and Post-Transplant Growth Factors.*

Grade IV myelosuppression will be defined as a decrease in ANC to < 500/$mm^3$ and/or platelet count to ≤ 20,000/$mm^3$. If myelosuppression occurs, a bone marrow aspirate and biopsy should be performed to exclude disease progression. Samples should be sent for chimerism analysis by FISH or VNTR. Patients with myelosuppression will be managed as follows:

a. G-CSF (5μg/kg/day S.C.) will be started in patients who are > 28 days after HSCT with a hypoplastic marrow and an ANC of < 500/mm3.
b. Thrombocytopenic patients will receive platelet transfusion as per standard care.
c. Prophylactic broad spectrum antibiotics *e.g.* ciprofloxacin, while ANC < 500/$mm^3$.
d. In cases of suspected MMF toxicity, refer to section 9.F.2 for management recommendations.
e. In cases of suspected ganciclovir toxicity, consider changing to foscarnet.

It is recommended that patients not receive growth factors during treatment with MMF 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

<500/mm$^3$). If ANC drops to < 500/mm$^3$ then prophylactic broad spectrum antibiotics should be given, *e.g.* PO ciprofloxacin.

*I. Intrathecal Therapy and Treatment of CNS Disease*

All patients will have a diagnostic lumbar puncture performed during the initial pre-transplant workup in the outpatient department. Patients with past history of CNS disease may be considered for instillation of methotrexate as per the Standard Practice Manual. Citrovorum factor will be administered to those patients who have high serum methotrexate according to the guidelines found in the Standard Practice Manual. Patients with CNS disease will not be excluded. Those patients with evidence of CNS leukemic involvement will have instillation of methotrexate (dose per Standard Practice Manual). This regimen may be modified by the Attending Physician as clinically indicated. Patients with evidence of CNS leukemic involvement 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.

*J. Infection Prophylaxis*

Patients will receive prophylaxis for PCP, HSV, and candida as per FHCRC Standard Practice Manual. If amphotericin is required, patients should be considered to 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.

*K. Modifications of Immunosuppression for Low Donor T Cell Chimerism and Disease Progression*

Patients with progressive disease defined as any evidence of relapse (see Section 12 for definition) by morphologic or flow cytometric evaluation of a bone marrow aspirate will undergo rapid reduction of immunosuppression at the discretion of the Attending Physician, study P.I., and/or study sub-investigator(s). Persistence of disease, in itself does not mandate accelerated taper of immunosuppression. 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. This section provides guidelines for management of patients with low donor chimerism and disease progression.

1. Engraftment and Graft Rejection

Mixed or full donor chimerism will be evidence of donor engraftment. VNTR analysis or FISH studies will be used to quantitate chimerism. The same assay should be used in a given patient for repeated studies of chimerism. When possible, FISH analysis will be used for sex-mismatched donor-recipient combinations rather than VNTR studies. Definitions of mixed and full chimerism, as well as increasing and low donor chimerism, are described in Section 12.

Patients who obtain mixed or full donor chimerism after non-myeloablative HSCT, but subsequent chimerism studies cannot detect greater than 5% donor T cells (CD3+) as a proportion of the total T 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 131I-BC8 antibody. 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. All patients will have marrow aspirates and/or biopsies performed at approximately day 28 and 84 for assessment of in vitro marrow stromal growth. 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 both graft and marrow stromal status. Similarly, VNTR 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 9.H. 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.

2. Evaluation of Chimerism

At days +28, +56, and +84, patients will be evaluated for lymphoid and myeloid chimerism. In addition, bone marrow will be sent for histological examination, flow cytometry, and cytogenetic studies (if the patient had a known cytogenetic abnormality prior to transplant) at days +28 and +84 to evaluate for relapse/persistence of AML. 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.

3. Modifications of Immunosuppression

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 AML. 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.

*L. Recommended Treatment of GVHD:*

After non-myeloablative HSCT 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 (Appendix B).

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 use IV route (1.5 mg/kg Q12 hours).
   b. Prednisone (2 mg/kg/day) to 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.

2. Patients with Clinical Extensive Chronic GVHD:
   a. 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.

*M. ABO Incompatibility*

All patients with ABO incompatibility should be evaluated and treated as according to the standard practice manual. Acute hemolysis has been seen rarely in patients who had a minor ABO mismatch with their donor, and these patients should be monitored and treated aggressively if there is any evidence of hemolysis.

**10. Patient Evaluations**

*A. Patient Pretransplant Baseline Evaluation*

See Standard Practice Policy Manual for standard "Evaluation Guidelines for Marrow Transplant Patients." In addition, obtain the following:

1. CT scan of chest and abdomen for calculation of organ volumes. Other imaging studies as clinically indicated.
2. 10 cc blood in red top tube to D3-377 Thomas building for analysis of HAMA (Human Anti-Mouse Antibody).
3. Unilateral bone marrow biopsy and aspirate for pathology, flow cytometry, and cytogenetics within 30 days prior to consent signing/enrollment.
4. To subsequently determine the host or donor origin of relapse, heparinized peripheral blood from the patient and donor will be drawn and sent to:
   a. Cytogenetics Lab *if* patient/donor are sex mismatched.
   b. Clinical Immunogenetics Lab (E-514) to do chimerism analysis by DNA. Pretransplant samples to be sent to allow storage of DNA for evaluation of post-transplant chimerism.

*B. Post Transplant Patient Evaluation*

See Standard Practice Policy Manual for standard "Evaluation Guidelines for Marrow 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:

1. Heparinized blood 10cc to clinical chimerism lab for quantifying of T cell and granulocyte chimerism on days +28, +56, and +84.
2. Bone marrow aspirate to clinical chimerism lab for chimerism studies on days +28 and +84.

3. Bone marrow aspirate (and biopsy if unable to obtain adequate aspirate sample) to pathology and cytogenetics (if previous cytogenetic abnormality was present) on days +28 and +84.
4. Thyroid function (TSH) at approximately day 84 workup.
5. Chronic GVHD screening between days 80 and 100 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 Transplant General Guidelines for Referring Physicians*, with additional recommendations for thyroid testing (and initiation of treatment if clinically indicated) at 6, 9, 12, 18 and 24 months after transplant, and then annually if still normal. 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:

1. Survival.
2. Disease status.
3. Hypothyroidism.
4. Selected blood counts (WBC, hemoglobin, hematocrit, platelets).
5. Renal (creatinine, BUN) and hepatic (Bilirubin, AST, ALT) laboratory values.
6. Presence/treatment of chronic GVHD.
7. Serious adverse events.
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 and disease status reported under the standard long-term follow-up protocol, and will record this data for long-term analysis and reporting.

## 11. Drugs and Toxicities

*A. $^{131}$I-BC8 antibody:*

$^{131}$I-BC8 antibody will be given at a dose of 0.5 mg/kg ideal body weight, or actual body weight for patients below ideal body weight. The antibody itself can cause side effects including fever, chills, nausea, vomiting, diarrhea, hypotension, dyspnea, joint pain, rash, and anaphylaxis. See section 9.B.1.d for premedications and infusion guidelines, and 13.A.1 for details regarding grading of and response to antibody-related toxicities. The biodistribution dose is labeled with 4-10 mCi $^{131}$I, which by itself does not cause symptoms. This dose is usually administered as an outpatient in the UWMC General Clinical Research Center or current treating location. Patients who are not stable at the end of infusion are admitted for overnight observation. The therapy dose is labeled with larger amounts of $^{131}$I, as determined by the cGy/mCi $^{131}$I estimates calculated from the biodistribution antibody infusion for the normal organ receiving the highest dose and the current limit of radiation dose delivered to marrow (section 13.B). The therapy dose of $^{131}$I can cause nausea and vomiting, in addition to myelosuppression and other organ toxicities seen with high dose radiation.

Engraftment syndrome (ES) is an early complication of stem cell transplants that occurs around neutrophil engraftment. One patient experienced a life threatening side effect of ES about 2 weeks after stem cell transplant under this protocol. Usual symptoms of ES include fever around the time of engraftment, shortness of breath, weight gain, sudden rise in white blood count, and skin rash.

*B. Fludarabine:*

Fludarabine will be administered in the outpatient department. The dose of fludarabine used in this protocol is non-myeloablative, but does cause significant immunosuppression. Fludarabine can lower the white blood cell count, in particular the CD4+ T-cells. The immunosuppression observed with the use of fludarabine increases the risk of infection, which can be life threatening. Possible other side effects include nausea and vomiting.

*C. Total Body Irradiation:*

TBI will be given in one 2.0 Gy fraction from a linear accelerator at a rate of 6-7 cGy/min. Dosimetry calculations are performed by the radiation therapist. At the dosage used, side effects are not expected. Nevertheless, there may be fever, alopecia, parotitis, diarrhea, reversible skin pigmentation, mucositis and late effects including cataract formation, growth retardation, pulmonary damage, carcinogenesis, and sterilization.

*D. CSP:*

See standard practice manual and section 9.F.1 for information about administration, toxicity and complications. Side effects are generally reversible and may include renal insufficiency and failure, hypomagnesemia, paresthesias, tremor, seizures, visual disturbances, paresis, disorientation, depression, confusion, somnolence, coma, nausea, hypertension, hemolytic-uremic syndrome, hyperglycemia, gynecomastia, and hypertrichosis.

*E. Mycophenolate Mofetil (MMF):*

See section 9.F.2 for information about MMF administration and dosage adjustments. Previous clinical studies in patients after allografting suggest that the principal adverse reactions associated with the administration of MMF include neutropenia, nausea, vomiting, diarrhea, and on one occasion bloody diarrhea. There may also be a higher incidence of certain viral infections (CMV, VZV, HSV). MMF use during pregnancy carries a significant risk of miscarriage or birth defects. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in gastrointestinal and hematologic parameters. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the principal investigator and documented in the medical records and the clinical reporting form (CRF).

## 12. Definitions

*A. Complete Remission:* complete resolution of all signs of 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 >100,000/$mm^3$, granulocytes >1,500/$mm^3$).

3. No extramedullary disease.

*B. 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.

*C. Non Responder:* all patients not qualifying for complete or partial remission.

*D. 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.

*E. 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,

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

*G. 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.

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

*I. 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.

*J. 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.

## 13. Radiolabeled Antibody - Toxicities and Complications

Two separate sorts of toxicities are anticipated, those due to antibody infusions (see 13.A below) and those due to the radiation delivery and combined preparative regimen. In the prior Phase I dose-escalation study of $^{131}$I-labeled BC8 antibody for patients with advanced AML, ALL, and MDS a dose of 10.5 Gy delivered to the liver by $^{131}$I-labeled BC8 has been well-tolerated when administered in addition to cyclophosphamide 120 mg/kg and 12 Gy TBI. In this study, however, we will decrease the TBI to 2 Gy and use fludarabine, which may be less toxic than cyclophosphamide. Therefore, by using 22 Gy (starting dose) of $^{131}$I-BC8 antibody with the non-myeloablative allogeneic transplant regimen, we do not anticipate the same degree of regimen-related toxicities (RRT) seen in full transplants. Conversely, we do expect more RRT compared with other non-myeloablative regimens that do not employ radiolabeled antibody therapy.

*A. Toxicity Attributable to Monoclonal Antibody Infusions*

Allergic reactions to administration of foreign mouse protein may include fever, urticaria, bronchospasm, anaphylaxis, Arthus reaction, vasculitis and serum sickness. In addition, rapid infusion of antibody may produce pulmonary, renal or hepatic toxicity as a result of lysis or agglutination of circulating cells. Grading of acute toxicities due to antibody infusion is shown in Table 3.

1. Modifications for Acute Toxicity

Patients experiencing acute toxicity (*i.e*. allergic type reactions) during antibody infusion will have the infusion slowed or terminated as described in section 9.B.1.d. If Grade III allergic-type toxicity is encountered, the infusion must be terminated and not-restarted, and the patient will be off protocol. Other moderate (grade II - III) acute toxicity may be treated and the infusion restarted. Patients with grade IV 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 antibody infusion but instead will be determined by laboratory samples obtained at the end of infusion and the following day. If a patient experiences Grade III hepatic or renal toxicity after the biodistribution dose, determination of whether or not the patient can proceed to the therapy dose will be made by the P.I. or 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 IV hepatic or renal toxicity after the biodistribution dose will not receive the therapy dose and may be treated on an alternate protocol.

**Table 3:** Grading of Acute Toxicities Due to Antibody Infusion (from NCI CTCAE version 3.0).

| Parameters | I (Mild) | II (Moderate) | III (Severe) | IV (Life-Threatening) |
|---|---|---|---|---|
| Allergic reaction/ hypersensitivity (including drug fever) | Transient flushing or rash; drug fever <38°C (<100.4°F) | Rash; flushing; urticaria; dyspnea; drug fever ≥38°C (≥100.4°F) | Symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension | Anaphylaxis |
| Hepatic:<br>1. Bilirubin<br>2. AST | >ULN – 1.5 × ULN<br>>ULN – 2.5 × ULN | >1.5 × ULN – 3.0 × ULN<br>>2.5 × ULN – 5.0 × ULN | >3.0 × ULN – 10.0 × ULN<br>>5.0 × ULN – 20.0 × ULN e | >10.0 × ULN<br>>20.0 × ULN |
| Renal:<br>Creatinine | >ULN – 1.5 × ULN | >1.5 × ULN – 3.0 × ULN | >3.0 × ULN – 6.0 × ULN | >6.0 × ULN |

2. Long-Term Toxicities

Hypothyroidism is the most common long-term effect of radiation exposure resulting from the $^{131}$I therapy dose and has been seen in approximately 70% of patients receiving this treatment. Some incidence of avascular necrosis (AVN) has been seen in patients following treatment with $^{131}$I-BC8; however, it is not clear whether this represents any increase from AVN rates typically seen following transplant and use of prednisone for treatment of GVHD.

*B. Specifics of Radiolabeled Antibody Administration: Therapeutic Dose*

The therapeutic dose of $^{131}$I-BC8 antibody will be administered according to the same schedule as the trace-labeled (biodistribution) dose. The patient will continue taking non-radioactive iodine drops following the biodistribution dose until a minimum of three weeks after the therapeutic infusion (unless limited by other medical conditions). 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. The patient will be admitted to a special lead-lined isolation room at UWMC on the morning of the therapeutic infusion (generally day –12) and will remain there until total body activity is less than 30 mCi $^{131}$I, as estimated by a measurement of < 7 mR/hour one meter from the patient or as directed by the UWMC Radiation Safety Officer based on the public dose calculation from Nuclear Regulatory Commission Regulatory Guide 8.39. The patient can then be discharged, and can remain an outpatient to complete treatment unless admission is medically indicated.

*C. Dose to Bone Marrow*

Initially, no patient in this study will receive a dose of $^{131}$I estimated to deliver more than 43 Gy to the marrow (+ 2 Gy TBI = 45 Gy total, *i.e.* 5 Gy more than the 28 Gy from $^{131}$I-BC8 antibody + 12 Gy TBI previously shown to be tolerated). Patients for whom the amount of $^{131}$I required to deliver the stipulated dose to liver would result in the delivery of more than the acceptable level of radiation to marrow will have the $^{131}$I dose limited to the amount which would deliver the maximum acceptable level (see section 19.B).

*D. Patients who become ineligible for therapeutic antibody infusion after biodistribution dose*

Patients who become ineligible for infusion of the therapeutic $^{131}$I-BC8 antibody will be considered for an alternate protocol. The patient's case will be presented to the Patient Care Conference to obtain a consensus opinion for the most appropriate protocol and treatment plan in this circumstance. Potential causes for ineligibility include development of a positive HAMA test or Grade IV toxicities after the biodistribution dose (section 13.A.1).

## 14. Special Considerations

A. All patients will require placement of a large bore right atrial catheter.
B. All patients receiving therapeutic amounts of $^{131}$I-labeled antibodies will be treated in single rooms at the University Hospital with appropriate shielding to minimize exposure of health care personnel and to prevent contamination of the room with radioisotope. Patients will be instructed in self-care procedures, *e.g.* vital sign monitoring, blood drawing from central venous catheter as much as possible.
C. All blood, urine, and tissue samples containing radioisotopes will be clearly identified as such. Samples containing high levels of activity (>50 uCi) will be transported in shielded containers. All samples will be processed by personnel trained in the use of radioisotopes.

## 15. Targeted/Planned Enrollment Table

**Table 4:** Targeted/Planned enrollment
PROTOCOL #1809

| TARGETED/PLANNED ENROLLMENT: Number of Subjects (*must provide actual numbers. I.e. no range)* | | | |
|---|---|---|---|
| **Ethnic Category** | *Sex/Gender* | | |
| | Females | Males | Total |
| Hispanic or Latino | 1 | 1 | 2 |
| Not Hispanic or Latino | 6 | 12 | 18 |
| Ethnic Category Total of All Subjects* | 7 | 13 | 20 |
| **Racial Categories** | | | |
| American Indian/Alaska Native | | 1 | 1 |
| Asian | 1 | 1 | 2 |
| Native Hawaiian or Other Pacific Islander | | 1 | 1 |
| Black or African American | | 1 | 1 |
| White | 5 | 8 | 13 |
| Racial Categories: Total of All Subjects * | 7 | 13 | 20 |

## 16. Data Safety and Monitoring Plan

*A. Plans for assuring data accuracy and protocol compliance*

Clinical research personnel (monitors) who are contracted or assigned by the FHCRC Clinical Trials Support Office (CTSO) will conduct site visits to the investigational facilities. The purposes of these site visits are to verify the accuracy of the study data, assess compliance with the protocol and with GCP regulations, and assure the timely and complete reporting of safety (adverse event) data. Each visit will include review of relevant documents and data, including those at each institution at which a patient was treated while on study, and those kept in the UWMC Nuclear Medicine patient file, as stipulated in the Monitoring Plan and Monitor Orientation documents which are part of BB-IND 3771 files. The frequency of the site visits will be at least twice per year, in accordance with FHCRC policies. Monitoring reports will be submitted to the Principal Investigator, the sponsor of BB-IND 3771, and the Protocol Data Monitoring Committee after each visit. The Principal Investigator agrees to provide the monitors access to the clinical supplies, the dispensing and storage areas and records, the patients' medical records and research files, the case report forms and data listings, and the IND and study files.

*B. Monitoring the progress of trial and safety of participants*

This is a single institution trial where all patients are followed closely by the investigators. The trial design provides rules for dose de-escalation depending upon the rate of development of Grade III/IV RRT (Bearman Scale) in previous patients at a given Dose Level (Section 19). 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 investigators meet at least monthly to review the course of each patient on study to identify the incidence of RRT or other adverse events requiring expedited reporting (see below). Rules for dose de-escalation and termination of the study are detailed under Section 19.

In this protocol for patients with advanced AML, the dose adjustment schema is designed to accept a 25% rate of Grade III/IV RRT given that 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 bone

marrow and spleen. Patients on this study represent a high risk population for whom HSCT offers the only chance of potential cure, and whose survival following conventional HSCT would be at most 20% for those patients young enough to tolerate a conventional, full-dose preparative regimen. Because of this, as well as the use of the dose adjustment schema noted above, strict stopping rules for rates of death, severe GVHD, or lack of efficacy will not be used. In addition to the regularly scheduled review of patient outcome by the investigators, results will be summarized in the Annual Renewal Report to the IRB, and the investigators will comment regarding the implications of these results for ongoing risk-benefit ratios for patients. In any year in which more than 15 patients are treated on study, an interim report will be submitted to the IRB addressing these issues after the first 15 patients for that year are treated and evaluable through day 90 post-transplant.

*C. Plans for assessing compliance with requirements regarding the reporting of adverse events*

Detailed guidelines are provided in Appendix E for the definition of Adverse Events and delineation of AE's requiring expedited reporting to the IRB and FDA or reporting in Annual Reports. Dr. Orozco and the research nurse and/or the data coordinator will meet routinely to review the interval course for every patient enrolled on this protocol who is being treated on an SCCA HSCT team, whether pre or post-transplant. The review of each patient's course will be documented on a patient specific "AE review sheet", which will be filed in the patient research record. In Dr. Orozco's absence (*e.g.* for travel or illness), review will be supervised by a designated physician sub-investigator.

## 17. Termination of Study Participation by Individual Patients

A. Patient request.
B. Unacceptable toxicity during dosimetry antibody infusions.
C. Development of anti-mouse antibodies.

## 18. Records

Clinical Statistics maintains a patient database at FHCRC to allow storage and retrieval of patient data collected from a wide variety of sources. The investigator will ensure that data collected conform to all established guidelines for coding, collection, key entry and verification. Each patient is assigned a unique patient number (UPN) to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room and are maintained by the FHCRC data collection staff. Access is restricted to personnel authorized by the Division of Clinical Research. Information forwarded to the NCI and the FDA about patients on this protocol refers to patients by their UPN only.

The study nurse and/or data coordinator maintains a Case Report Form (CRF) and associated research documentation for each patient treated under the auspices of BB-IND 3771, including all patients on this study. This documentation includes both clinical data and IND-specific documents for each patient (including, but not limited to Absorbed Dose Estimate report from Dr. Fisher, the UW notice of Patient inclusion, the Dosimetry and Therapy Radiopharmaceutical Dose Sheets, Dosimetry and Therapy Radiolabeled Antibody Infusion Records, and HAMA reports). Additional IND-specific documents are kept in the UWMC Nuclear Medicine patient study files. All data required for analysis of patients treated on this protocol will be maintained in a password-protected IND-specific Access

Database application. This single Access application will be used to retrieve the data for analysis on all patients treated under the auspices of IND 3771, and will include two sources of data. The first source of data is the Fred Hutchinson core database (Gateway). The second type of data will be antibody-specific and other CRF data. These data will be keyed directly into the database by the research nurse or other authorized research staff and will be extracted from the CRF forms or source documents maintained as part of each patient's research documentation. Dr. Orozco or a designee will review data entry on an ongoing basis, as it is completed for individual patients, by cross-checking the entered data against the source documents or the CRFs, and will initial and date the data printouts as documentation that the accuracy of entered data has been verified.

## 19. Dose Escalation/De-Escalation, Statistical Considerations, and Termination of Study

*A. Dose Escalation/De-Escalation*

Dose escalation/de-escalation will be conducted by the "two-stage" approach introduced by Storer[19]. The starting dose level will be a total isotope dose estimated to deliver Dose Level 6, 20 Gy to the normal organ receiving the highest dose. 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 – Table 5) will occur until a patient experiences a Grade III/IV regimen-related toxicity (Bearman) or dose-limiting toxicity (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 (Table 5) until the first 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 (i.e. patients not dying within the first 30 days of infection, unless they clearly have Grade III/IV RRT). 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 or more DLT's are encountered 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 total of 20 evaluable patients have been treated at the second stage. Following the completed observation of the $20^{th}$ 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%. If the estimate of the slope parameter for the fitted curve is not positive and finite, the geometric mean of the dose level used for the last cohort and the dose level that would have been assigned to the next cohort is taken as the MTD. 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. 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. These patients will not be used for purposes of dose modification, however, nor will they be counted towards the total of 20 patients for completion of the second stage.

**Table 5: Dose Levels for Escalation/De-Escalation**

| Dose Level | Gray delivered by $^{131}$I-BC8 antibody to normal organ receiving the highest dose (liver) |
|---|---|
| 1 | 10 Gy |
| 2 | 12 Gy |
| 3 | 14 Gy |
| 4 | 16 Gy |
| 5 | 18 Gy |
| 6 | 20 Gy |
| 7 | 22 Gy (starting dose level) |
| 8 | 24 Gy |
| 9 | 26 Gy |
| 10 | 28 Gy |

*B. Dose to Bone Marrow and Graft Failure*

Initially no patient will receive a dose of $^{131}$I estimated to deliver more than 43 Gy to the marrow (+ 2 Gy TBI = 45 Gy total, i.e. 5 Gy more than the 28 Gy from $^{131}$I-BC8 antibody + 12 Gy TBI tolerated on Protocol #557). Patients for whom the amount of $^{131}$I required to deliver the stipulated dose to liver would result in the delivery of more than 43 Gy to marrow will have the $^{131}$I 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 (see below), 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.

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 have not rejected the donor graft. In this protocol, additional immunosuppression may be provided by lymphohematopoietic irradiation from $^{131}$I-BC8 antibody. However, in the event of marrow stromal damage from excessive radiation exposure, patients might have inadequate hematopoiesis yet not meet the criteria for graft failure. Every effort will be made to determine graft status for patients with slow recovery of neutrophils. All patients will have marrow aspirates and/or biopsies performed at approximately day 28 and 84 for assessment of *in vitro* marrow stromal growth. 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 both graft and marrow stromal status. Similarly, VNTR 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. Those patients who fail to achieve an ANC $\geq$ 500/mm$^3$ for two consecutive days by day 40, in the absence of contributing cause such as medication, sepsis, or CMV infection, will be considered to have graft failure.

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*C. Statistical Considerations*

This is a Phase II dose-escalation study whose primary objective is the determination of the maximum tolerated dose of radiation delivered to normal organs by $^{131}$I-BC8 antibody when combined with the non-myeloablative regimen of 2 Gy TBI + CSP/MMF. For this group of patients at extremely high risk of relapse after marrow transplantation, it is reasonable to accept a rate of grade III/IV regimen-related toxicity as high as 25% if anti-leukemic efficacy is improved by the therapy. Thus in this study we will accept Grade III/IV RRT in an average of 1 of 4 patients, and the dose escalation/de-escalation schema outlined above is based upon this. Monte Carlo analysis of a thousand simulated trials for each of 3 different scenarios of "True" Grade III/IV toxicity rates demonstrate the range of potential estimates of MTD which would arise from this dose-escalation design.

We anticipate treating 25 to 30 patients, with the final number treated dependent upon the extent of dose escalation and the number of patients treated at each dose level to achieve 20 patients evaluable for RRT in the second phase of the trial.

Because the anti-leukemic effect of $^{131}$I-BC8 antibody combined with this non-myeloablative 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.

As discussed in section 19.B, we do not anticipate difficulties with graft rejection on this study. However, if graft rejection in excess of 20% was experienced, we would need to re-evaluate this approach. If there ever exists sufficient evidence suggesting that the true rate of graft rejection exceeds 20%, the protocol will be suspended and alternative approaches explored. 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 .20. These proportions and associated confidence intervals will be calculated after every 5$^{th}$ patient enrolled is evaluable. Operationally, any of the following occurrences would meet the limits of this stopping rule: 3 or more of the first 5, 4 or more of the first 10, 5 or more of the first 15, 6 or more of the first 20, 8 or more of the first 25, 9 or more of the first 30, or 10 or more of the first 35 patients enrolled experience graft rejection.

The following table summarizes the operating characteristics of this stopping rule.

| Number of Patients | True Rate of Graft Rejection | Probability of Stopping[1] |
|---|---|---|
| 10 | .10 | .02 |
| 20 | .10 | .03 |
| 10 | .30 | .37 |
| 20 | .30 | .64 |
| 10 | .40 | .64 |
| 20 | .40 | .90 |

[1]Represents estimate of the probability of stopping by associated number of patients due to excess graft rejection. Estimated from 5,000 Monte Carlo simulations.

We anticipate that the proposed regimen will result in better disease control. We will also thus follow disease response and duration of remission and Kaplan-Meier estimates of disease-free survival and overall survival will be determined. However, because the anti-leukemic effect of $^{131}$I-BC8 antibody combined with this non-myeloablative regimen is unknown, the expected rate of relapse post-

transplant or failure to enter remission after treatment is unpredictable. If the rate of relapse or refractory disease exceeds 50% within the first 200 days post-transplant, similar to rates seen using standard myeloablative unrelated donor SCT, we would need to re-evaluate this approach.

Lastly, we will calculate descriptive statistics on the number and percent of toxicities and compare the results obtained with historical patients treated with $^{131}$I-BC8 antibody + 12 Gy TBI. The cumulative incidence of GVHD and the proportion of patients achieving donor chimerism, and the respective 95% confidence intervals, will be reported.

## 20. Other Criteria if Applicable:

See appendix A for the Bearman criteria for grading regimen-related toxicity and Appendix B for grading of acute GVHD.

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## APPENDIX A

## CRITERIA FOR 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 $pO_2$ 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
## GRADING OF GVHD[a]

Severity of Individual Organ Involvement

| | | |
|---|---|---|
| Skin | +1 | a maculopapular eruption involving less than 25% of the body surface |
| | +2 | a maculopapular eruption involving 25-50% of the body surface |
| | +3 | generalized erythroderma |
| | +4 | generalized erythroderma with bullous formation and often with desquamation |
| Liver | +1 | bilirubin (2.0-3.0 mg/100 ml) |
| | +2 | bilirubin (3-5.9 mg/100 ml) |
| | +3 | bilirubin (6-14.9 mg/100 ml) |
| | +4 | bilirubin > 15 mg/100 ml |
| Gut | | Diarrhea is graded +1 to +4 in severity. Nausea and vomiting and/or anorexia caused by GVHD is assigned as + 1 severity. |
| | | The severity of gut involvement is assigned to the most severe involvement noted. Patients with visibly bloody diarrhea are at least a stage +2 gut and grade +3 overall. |
| Diarrhea cause. | +1 | < 1000 ml of liquid stool/day in the absence of infectious/medical |
| | +2 | >1,000 ml of stool/day |
| | +3 | >1,500 ml of stool/day |
| | +4 | 2,000 ml of stool/day |

Severity of GVHD

| | |
|---|---|
| Grade I | +1 to +2 skin rash<br>No gut or liver involvement |
| Grade II | +3 skin rash *or*<br>+1 gastrointestinal involvement and/or +1 liver involvement |
| Grade III | +2 to +4 gastrointestinal involvement and/or<br>+2 to +4 liver involvement with or without a rash |
| Grade IV | Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death |

[a] From Graft-vs-Host Disease, Sullivan, Keith M in Hematopoietic Cell Transplantation. Ed: E. Donnall Thomas, Karl G. Blume, Stephen J. Forman. Pages 518-519.*

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## APPENDIX C

**Class I HLA-A, -B, -C Antigens (or Equivalents) That Must Be Identified for Donor Matching**

| HLA-A | HLA-B | HLA-B | HLA-C |
|---|---|---|---|
| A1 | B7 | B45(12) | Cw1 |
| A2 | B703(7) | B46(15) | Cw2 |
| A3 | B8 | B47 | Cw3 |
| A11 | B13 | B48(40) | Cw4 |
| A23(9) | B14 | B49(21) | Cw5 |
| A24(9) | B62(15) | B50(21) | Cw6 |
| A25(10) | B63(15) | B51(5) | Cw7 |
| A26(10) | B70(15) | B52(5) | Cw8 |
| A29(19) | B75(15) | B53 | |
| A30(19) | B76(15) | B54(22) | |
| A31(19) | B77(15) | B55(22) | |
| A32(19) | B21 | B56(22) | |
| A33(19) | B18 | B57(17) | |
| A34(10) | B27 | B58(17) | |
| A36 | B35 | B59 | |
| A43 | B37 | B67 | |
| A66(10) | B38(16) | B73(7) | |
| A68(28) | B39(16) | B78(5) | |
| A69(28) | B60(40) | B81(7) | |
| A74(19) | B61(40) | | |
| A80 | B41 | | |
| | B42 | | |
| | B44(12) | | |

## APPENDIX D

## METHODS USED TO ESTIMATE RADIATION ABSORBED DOSES TO PATIENTS

Absorbed radiation doses are calculated for each patient's normal organs and tissues, the whole body, and for tumor masses. Doses are calculated using the formal methods that are recommended by the Medical Internal Radiation Dose (MIRD) Committee of The Society of Nuclear Medicine (a, b, c). These methods account for both the penetrating gamma and the non-penetrating beta radiation emitted by radioactivity distributed throughout the body. Dosimetry calculations are based on a set of direct measurements in individual patients. These include, based on gamma-camera measurements of iodine-131 activity in the major source organs, tumor tissue (the red marrow) and the total body at various time-points post-infusion of the radiolabeled antibody. Red marrow biopsies indicate the activity concentration in marrow at specific time points. Organs for which measurement data are obtained are called "source organs." Organs, tissues, and the whole body for which radiation absorbed doses are estimated, are called "target organs."

Direct Measurements in Patients

Conjugate-view quantitative planar imaging with anterior and posterior measurements is the most widely used method for assessing source-organ activity in patients (d, e). 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.

After a tracer level of iodine-131 (usually about 5 to 8 mCi) on the monoclonal antibody is administered, the patient is imaged using collimated anterior and posterior planar gamma-camera imaging. Images are acquired over the source organs using a large-field-of-view camera. Anterior plus posterior conjugate view are obtained using a collimated system with the photon energy window set over the relevant energy peak at about ± 15-20% (full width at half-maximum). Images will include head/neck, chest with upper humeri, abdomen, and pelvis with upper femurs.

Regions of interest are selected for the major source organs, such as the liver, spleen, red marrow space, and occasionally for the lungs and kidneys (when suitable images can be obtained), and for any other tissue with activity above background, including thyroid and bladder. Measurements are also made of representative background tissue (such as the thigh muscle), and a counting standard with and without the patient in the camera field-of-view. The outlines for the regions of interest are drawn by a technician from the acquired image, and counts are obtained from the selected regions. The counts are appropriately decay-corrected to a radionuclide standard. Patient thickness and the distance from the gamma camera to the source organ are determined. The geometric mean of the anterior and poster counts is obtained for each region of interest. Counts are then corrected for attenuation, geometry, and background.

Total body measurements will be obtained using an external, non-imaging gamma probe at a distance of 4 meters from the patient to quantify the absolute I-131 activity in the patient, over time, as a fraction of the total administered activity.

Sampling Times

Selecting an appropriate number of counting times requires a trade-off between the desire for sufficient data, while economizing the overall costs and minimizing patient inconvenience. Our objective is to select the fewest time points that will provide a reasonable description of the time-activity curve. The minimum number of data measurement points is typically four. These include one

measurement at or near the zero time point (time of radionuclide infusion), plus additional measurements at about 18, 44, and 66 hours post-infusion. These analyses provide an estimate of the fraction of the administered activity that resides in each source organ (and in the total body) at each measurement time post-infusion.

Time-Activity Curves

The sequential measurement data are plotted to determine the cumulated activity and residence time for each source organ. Plotted are the fractions of the total administered activity that are present at each measurement time point. Time-activity curves are constructed from the measurement data and are integrated out to infinite time to determine the residence times ($\tau$, hours) for each source organ, the red marrow, and for the whole body. The points are fitted to an exponential or sum of exponentials. An estimate of the long-term tail of the time activity curve may be made by fitting an exponential function to the last two points.

We plot the *effective* fractions present (as measured), rather than the values that were decay-corrected from a radionuclide standard, because internal doses are approximately proportional to the integral areas under the effective time-activity curves.

Residence Time Calculations: Integrating the Time-activity Curves

The residence time 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. Thus, the residence time is the area under the fractional time-activity curve. The residence times are the basic input value to the software packages MIRDOSE2 and MIRDOSE3 (f) (Oak Ridge Associated Universities, Oak Ridge, Tennessee) and OLINDA-EXM (Vanderbilt University, Nashville, Tennessee) that implement the MIRD dosimetry schema..

The cumulated activity, $\tilde{A}_h$, and residence time, $\tau_h$, are determined by integrating the area under the time-activity curves for the clinical measurement data for each source organ and the remainder tissues. The integrations are carried to infinity for accuracy and simplicity.

Residence times for red marrow are determined from a set of gamma camera measurements of iodine-131 activity in marrow spaces (usually the right and left acetabula), and from a bone marrow biopsy that is weighed and then counted in a standard well-type counter. The time-activity curve is developed from the marrow counts, and then the curves are adjusted to exactly cross through the data point for the marrow activity concentration. The long-term tail of the exponential may be estimated by curve-fitting.

According to the MIRD schema, the cumulated activity, $\tilde{A}_h$, is proportional to the sum of all nuclear transformations during the time of interest in a source organ, h. The cumulated activity is also called the time integral of $A_h(t)$, or

$$\tilde{A}_h = \int A_h(t)\, dt, \qquad (1)$$

where the units of cumulated activity are given in $\mu$Ci-hr or Bq-sec. If a single exponential is fit to the data points representing simple clearance, then the activity in the source organ at any point in time, t, may be estimated by the equation:

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$$A_h(t) = A_h(0) \exp(-\lambda t), \quad (2)$$

where $A_h(0)$ is the y-axis intercept of the exponential equation, and where $\lambda$ is the long-term *effective* (not corrected for radioactive decay) constant. The effective clearance constant $\lambda$ is:

$$\lambda. = (\ln 2)/(T_{1/2}\text{eff}), \quad (3)$$

and therefore the effective clearance halftime, $T_{1/2}$eff, is:

$$T_{1/2}\text{eff} = (\ln 2)/\lambda = 1.443 / \lambda. \quad (4)$$

The integral of the single exponential for the cumulated activity, or area under the curve is:

$$\int A_h(t)\, dt = A_h(0)\, (1/\ln 2)\, (T_{1/2}\text{eff}), \text{ and} \quad (5)$$

$$\int A_h(t)\, dt = A_h(0)\, (1.443)\, (T_{1/2}\text{eff}), \quad (6)$$

where $A_h(0)$ is the y-axis intercept. It follows that the residence time, $\tau_h$, is:

$$\tau_h = [A_h(0)/A_o]\, (1.443)\, (T_{1/2}\text{eff}). \quad (7)$$

If a multi-exponential function is needed to describe the pharmacologic uptake, retention, clearance, and physical decay processes, the time-activity curves are described using multiple exponential functions of the form:

$$y = A \exp(-\lambda_1 t) + B \exp(-\lambda_2 t) \ldots \quad (8)$$

where $\lambda_1$ and $\lambda_2$ are the effective retention constants. If a multi-exponential function is fit to the measurement data, then the integral of the function (or area under the curve) is simply

$$\int y\, dt = A/\lambda_1 + B/\lambda_2 \ldots . \quad (9)$$

Patient-specific Dosimetry

The residence times for each source organ are corrected for actual patient mass (if known from CT-imaging volumetrics) using a method described by Fisher *et al.* (g, h). Methods for this correction for patient-specific dosimetry are described in the following paragraphs.

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

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A less-accurate, but more convenient "short-cut" method for correcting for known organ mass is given below. This short-cut method corrects the beta component but does not correct for the lesser-important gamma component. For individual organs and for the whole-body, the correction may be made by multiplying the calculated source-organ residence time, $\tau_h$, by the ratio of the defined reference man or reference woman organ mass to the known organ mass:

$$\tau_{new} \approx (\tau_h)(m_{MIRD}/m_{actual}). \quad (10)$$

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

Dose Calculations

In the MIRD schema, the absorbed dose, *D*, to target organs or tissues ($r_k$), and the whole body, is calculated from the sum of the products of the cumulated activity $\tilde{A}$ (μCi-hours) in each source organ ($r_h$) and the S value (the absorbed dose per unit cumulated activity in rads per μCi-hour, or gray per becquerel-second) for each source-target organ pair, according to the MIRD equation

$$D(r_k \leftarrow r_h) = \sum_h \tilde{A}\, S(r_k \leftarrow r_h), \quad (11)$$

where the cumulated activity $\tilde{A}$ in a source organ is also the product of the residence time $\tau$ and the administered activity $A_0$. Thus,

$$\tilde{A} = \tau A_0, \quad \text{and} \quad \tau = \tilde{A}/A_0 \quad (12)$$

The dose calculations are implemented by the MIRDOSE2 and MIRDOSE3 computer software for medical internal dose assessment (Oak Ridge Associated Universities, Oak Ridge, Tennessee) or OLINDA-EXM (Vanderbilt University, Nashville, Tennessee).

Relevant to the use of MIRDOSE2 and MIRDOSE3 software, The S values used for the calculations MIRDOSE2 computer software are the same as those that were previously implemented in ICRP Publication 30 (i). Revised S values for marrow and the skeleton are implemented in MIRDOSE3 (f). S values for solid tumors are estimated by extrapolation using normal organs of similar size and location in the body. In this leukemia trial, we assume that the cancer cells are in the red marrow, and therefore the dose to red marrow and the dose to leukemia cells are assumed to be the same. MIRDOSE2 S values for red marrow are used when appropriate, since a new evaluation of the S values at the University of Florida has shown that MIRDOSE3 S values may underestimate the mean dose per unit cumulated activity to red marrow.

Therapy Doses

The radiation absorbed doses that are estimated for the tracer infusion of iodine-131-labeled monoclonal antibody are extrapolated linearly to higher levels of activity that are later administered for the therapeutic infusion.

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## APPENDIX E

## SERIOUS ADVERSE EVENT (SAE) DEFINITION AND REPORTING GUIDELINES

HSCT involves the administration of high doses of chemotherapy and or radiation and is associated with a high rate of adverse events which are to be considered "Expected" in the setting of HSCT. For patients on this protocol, there is also an expected incidence of side effects associated with the administration of the biodistribution and therapy dose of I-131-BC8 antibody. While we will use the Bearman Scale of Regimen-Related Toxicity detailed in Appendix A of the protocol for decisions regarding 1) continuation on study depending on antibody infusion-related toxicities (Section 13.A.1. and 2.) dose adjustment or invocation of stopping rules, for the purposes of SAE reporting, and defining which adverse events require expedited SAE reporting or reporting in the Annual Report to the FDA for BB-IND 3771, we will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0, (**http://ctep.cancer.gov/forms/CTCAEv3.pdf**). The criteria for "Expected" toxicities are outlined separately for: 1) antibody infusion-related toxicities, and 2) transplant-related toxicities and adverse events.

*A. Grading of Acute Toxicities due to I-131-BC8 Antibody Infusion:*

In our experience in over 130 patients receiving a biodistribution dose of I-131-BC8 antibody, approximately 31% developed Grade II allergic side effects, and 6% developed Grade III allergic side effects when graded using our protocol-delineated 5 point scale (Section 13.A.1), where the absence of side effect is considered "0". All patients had resolution of these side effects, and only two of these patients did not complete their antibody infusion. No patient had worse side effects upon resumption of antibody infusion (in those for whom the infusion was held temporarily because of their side effects), and very few patients had side effects during their therapy infusion of I-131-BC8 antibody that were more severe than the patient had experienced during his/her biodistribution infusion.

Thus the following events are to be considered expected as a consequence of the infusion of I-131-BC8 antibody, and will only be undergo expedited reporting as SAEs if they are of $\geq$ Grade 4 severity by the NCI CTCAE scale, or if they result in hospitalization:

| | |
|---|---|
| Chills | Conjunctivitis |
| Fever | Urticaria |
| Hypotension | Myalgia |
| Nausea | Arthralgia |
| Vomiting | Arthritis |
| Diarrhea | Headache |
| Allergic | Fatigue |
| reaction/hypersensitivity | Hand/foot skin reaction |
| Dyspnea | |
| Allergic rhinitis | |

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The following events may be expected, but will undergo expedited SAE reporting if they are of ≥ Grade 3 severity:

SGOT (AST) elevation
SGPT (ALT) elevation

Many patients entered on this study will have limited marrow reserve because of either recent therapy or because of leukemic relapse. It is expected that patient may have transient leukopenia and/or thrombocytopenia following the biodistribution dose of I-131-BC8 antibody, and that the patient may require a platelet transfusion because of the transient effect on circulating platelet count. Leukopenia, lymphopenia, neutropenia and thrombocytopenia will not be reported as SAEs. Life-threatening infection or bleeding will undergo expedited SAE reporting.

Other adverse events that are unexpected will undergo expedited reporting if they are of Grade 3 severity with possible, probable or definite attribution, and if they are of ≥ Grade 4 severity regardless of attribution.

*B. Grading of Transplant-Related Toxicities and Adverse Events*

All patients undergoing HSCT are expected to have Grade 4 pancytopenia as an intended therapeutic effect, and thus this will not be reported as an SAE. Graft rejection or Graft Failure will undergo expedited reporting as an SAE. Certain Grade 4 toxicities under particular categories in the CTCAE are common in the HSCT setting and do not represent a life-threatening event as detailed. These toxicities are not likely to be particularly due to the administration of the investigational agent as part of the preparative regimen, and will not undergo expedited reporting as an SAE. These include:

Fatigue
Fever
Anorexia (as defined under "Gastrointestinal")
Vomiting
Hypokalemia (e.g., potassium < 2.5 can result from wasting induced by amphotericin)
Sterility/infertility

Any other Grade 4 toxicity occurring within 100 days of transplant will undergo expedited SAE reporting, regardless of attribution. This will include SAEs due to infection or complications associated with GHVD. The following events are to be considered expected in the setting of HSCT, and will not be reported in an expedited fashion unless they are of ≥ Grade 4 severity:

| **CTCAE Category** | *Toxicity* | |
|---|---|---|
| Cardiac (General) | Edema<br>Hypertension | Hypotension |
| Coagulation | DIC<br>Thrombotic microangiopathy | |
| Constitutional Symptoms | Graft versus host disease<br>Rigors, chills<br>Stem cell infusion complications | Veno-Occlusive Disease (VOD)<br>Weight gain associated with VOD |

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| **CTCAE Category** | *Toxicity* | |
|---|---|---|
| Dermatology/Skin | Hand-foot skin reaction<br>Pruritis/itching<br>Rash/desquamation | |
| Gastrointestinal | Anorexia<br>Colitis<br>Dehydration<br>Diarrhea<br>Dysphagia<br>Esophagitis | Gastritis<br>Ileus<br>Mucositis/Stomatitis<br>Nausea<br>Typhlitis<br>Vomiting |
| Hemorrhage/Bleeding | Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia<br>Hemorrhage, GI – Lower GI NOS<br>Hemorrhage, GI – Rectum<br>Hemorrhage, GI – Stomach | Hemorrhage, GU – Bladder<br>Hemorrhage, GU – Vagina<br>Hemorrhage, pulmonary/ upper respiratory – nose<br>Hemorrhage, pulmonary/ upper respiratory – Respiratory tract NOS |
| Hepatobiliary/Pancreas | Hepatic enlargement<br>Pancreatitis | |
| Infection | Catheter-related infection<br>Febrile neutropenia<br>Infection with grade 3 or 4 neutropenia | Infection with unknown ANC<br>Infection without neutropenia |
| Metabolic/Laboratory | Acidosis<br>Albumin, serum-low (hypoalbuminemia)<br>Alkaline phosphatase elevation<br>Alkalosis<br>ALT, SGPT<br>Amylase elevation<br>AST, SGOT<br>Bilirubin (hyperbilirubinemia)<br>Creatinine<br>GGT elevation<br>Hypercalcemia<br>Hypercholesterolemia | Hyperglycemia<br>Hyperkalemia<br>Hypermagnesemia<br>Hypernatremia<br>Hypertriglyceridemia<br>Hyperuricemia<br>Hypocalcemia<br>Hypoglycemia<br>Hypokalemia<br>Hypomagnesemia<br>Hyponatremia<br>Hypophosphatemia<br>Lipase elevation |
| Musculoskeletal/Soft Tissue | Muscle weakness | |
| Pain | Pain – abdomen NOS<br>Pain – bladder | |
| Pulmonary/Upper Respiratory | Cough<br>Hypoxia<br>Pleural effusion | Pneumonitis/pulmonary infiltrates<br>Voice changes/dysarthria |
| Renal/Genitourinary | Bladder spasms<br>Renal failure | Urinary electrolyte wasting<br>Urinary frequency/urgency<br>Urinary retention |

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| **CTCAE Category** | *Toxicity* |
|---|---|
| Sexual/Reproductive Function | Persistent amenorrhea |
| Syndromes | Tumor lysis syndrome |
| | |

Engraftment syndrome is an early complication of stem cell transplants that occurs around neutrophil engraftment. One patient experienced a life threatening side effect of ES about 2 weeks after stem cell transplant under this protocol. Usual symptoms of ES include occurrence or re-occurrence of fever around the time of engraftment, shortness of breath, weight gain, sudden rise in white blood count, and skin rash.

Grade 3 toxicities which are considered unexpected and possibly, probably or definitely related to the investigation drug will undergo expedited SAE reporting.

The primary transplant hospitalization will not be reported itself as an SAE, although ≥Grade 4 toxicities occurring during the hospitalization will be reported, as above. All hospitalizations other than the hospitalization for administration of the therapy dose of I-131-BC8 antibody and the primary transplant hospitalization will undergo expedited reporting as an SAE.

All deaths except for those from relapse of malignancy, but otherwise regardless of attribution, which occur within the first 100 days will be undergo expedited reporting as an SAE. If the patient has returned to the care of the referring physician prior to day 100 post-transplant, we will report the death as an SAE with expedited reporting based on the time we learn of the death. Deaths and events occurring after 100 days will not undergo expedited SAE reporting although they will be included in Annual Reports and publications of the study. Grade 3 toxicities that are considered "expected" will be reported in the Annual Report. Patients will be followed for long-term toxicities that may be related to the administration of I-131-BC8 antibody, such as hypothyroidism, and such toxicities will continue to be reported in the Annual Report.

All adverse events meeting criteria for expedited reporting will be reported within 7 days to the IRB and the FDA. Because IRB reports are routed through the Protocol Office, every effort will be made to notify the Protocol Office within 5 days in order to allow time for processing. When that is not possible, the Protocol Office will be notified that immediate processing is required. Notification will consist of a completed "Fred Hutchinson Cancer Research Center/Clinical Research Division/Institutional Review Office Serious Adverse Event Report" and a brief narrative letter to the FDA. Notification will be made by the principal investigator, a sub-investigator, or the Study Coordinator/Research Nurse.