FHCC Protocol 2186 Cover Page

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Title: Hematopoietic Bone Marrow Transplantation for Patients with High-Risk Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), or Myelodysplastic Syndrome (MDS) using Related HLA-Mismatched Donors: A Trial Using Radiolabeled Anti-CD45 Antibody Combined with Immunosuppression Before and After Transplantation

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FRED HUTCHINSON CANCER RESEARCH CENTER

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Investigational Agent: Iodine (I-125, I-131) Conjugated Murine Monoclonal Antibody (BC8) to CD45

Study Regimen: Radiolabeled ^{131}I BC8 Antibody Combined with CY, Fludarabine, 2 Gy TBI, Tacrolimus, MMF and Haploidentical Allogenic Hematopoietic Marrow Transplant

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Investigator Statement:

I have carefully read Protocol 2186 entitled "Hematopoietic Bone Marrow Transplantation for Patients with High-Risk Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), or Myelodysplastic Syndrome (MDS) using Related HLA-Mismatched Donors: A Trial Using Radiolabeled Anti-CD45 Antibody Combined with Immunosuppression Before and After Transplantation" ("Protocol") version date 10/12/2016.

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

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1.0 INTRODUCTION AND BACKGROUND

1.1 Introduction

It is important to extend the option of hematopoietic cell transplantation (HCT) for potential therapy of hematologic malignancies to patients who do not have a readily available HLA-matched donor. This problem is especially acute for patients in ethnic minority groups. Almost all patients would have a related donor identical for one HLA haplotype (haploidentical) and mismatched at HLA-A, B or DR of the unshared haplotype. Moreover, it is important to improve disease control for advanced acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and high-risk MDS patients without substantially increasing the toxicity of the transplant regimen. In this protocol, we will combine targeted hematopoietic radiation delivered by ^{131}I-BC8 (anti-CD45) antibody with a combination of immunosuppressive agents including cyclophosphamide (CY) administered before and after HCT to facilitate engraftment and to delete highly alloreactive T-cell clones presumably involved in graft versus host disease (GvHD). Specifically, this study will assess the feasibility and safety for patients with advanced AML, ALL or high risk MDS of treatment with ^{131}I-BC8 antibody at a starting dose of 12 Gy delivered to the normal organ receiving the highest dose, combined with CY, fludarabine and 2 Gy total body irradiation (TBI), followed by transplantation with non-T-cell depleted bone marrow from haploidentical donors. On day +3, patients will be given a single dose of high-dose CY. On day +4, patients will begin prophylaxis for GvHD with tacrolimus and mycophenolate mofetil (MMF). Growth factor support with G-CSF will begin on day +4 and continue until recovery of neutrophils (>500 per µL). The primary endpoint is determination of the maximum tolerated dose (MTD) of targeted radiation. The study will also estimate rates of non-relapse mortality (NRM), disease response and disease-free survival, and determine rates of donor chimerism and acute graft-versus-host disease (GvHD) through day 100 post-transplantation.

1.2 Background

The combination of high-dose chemotherapy and allogeneic HCT is potentially curative therapy for a number of hematologic malignancies but is not an option for many patients who lack a readily available HLA-matched donor, either related or unrelated. The ability to use related, HLA-haploidentical donors for allogeneic HCT can expand this option to almost all patients, but stem cell transplants after myeloablative conditioning have been complicated by high rates of morbidity and mortality.[1-5] In the non-myeloablative setting, studies of HLA-haploidentical transplants for hematologic malignancies have been reported from two other transplant centers.[6, 7] In both studies, depletion of host and donor T-cells in vivo was accomplished using polyclonal or monoclonal antibodies specific for lymphocytes. Relatively high rates of relapse, severe GvHD, non-relapse mortality, and low rates of donor chimerism were observed by Spitzer et al.[6] Improved engraftment and reduced rates of acute and chronic GvHD were observed in the study by Rizzieri et al.,[7] but non-relapse mortality (NRM) was 31%, mostly due to infection including 14% of patients who developed CMV disease. Infection has continued to be a major cause of morbidity and mortality in studies of HLA-haploidentical transplants using myeloablative or non-myeloablative conditioning. In a recent study of nonmyeloablative HCT for hematologic malignancies using related, HLA-haploidentical donors carried out at the Hutchinson Center and Johns Hopkins Sidney Kimmel Cancer Center, mortality secondary to infection was <5%, suggesting that induction of tolerance by post-transplant CY may spare T-cell immunity to infection (submitted for publication). Developing a safe and effective approach to HCT from related, HLA-haploidentical donors

is an important goal since the graft-versus-leukemia (GVL) effect is expected to be augmented in this setting.[3, 4]

The central problem of HCT from related, HLA-haploidentical donors is controlling the potent alloreactivity of host T-cells that can lead to rejection of the graft or severe GvHD. Using the strategy of non-myeloablative HCT pioneered by Storb and colleagues at the FHCRC,[8] a protocol was developed with the goal of achieving complete donor T-cell engraftment, low rates of rejection and GvHD of acceptable severity by increasing the amount of immunosuppressive therapy before and after transplantation of HLA-haploidentical bone marrow. The key to the success of this protocol was the incorporation of the highly immunosuppressive alkylating agent, CY, before *and* after transplantation. The protocol was based on pre-clinical data from a mouse model of non-myeloablative HCT using haploidentical donors.[9] In this model, pre-transplant immunosuppression consisted of fludarabine and low-dose total body irradiation (TBI, 200 cGy) . High-dose CY was then given on day +3 post-BMT to delete highly alloreactive T-cells of the donor [for a review, see Mayumi, H., et al.[10]]. CY causes DNA damage and subsequent death of activated, proliferating T-cells.[10] Luznik, *et al.* showed that successful induction of stable, mixed chimerism could be achieved across total MHC disparity in congenic mice (6/6 H-2 mismatch, no minor histocompatibility differences) and in haploidentical strain combinations (3/6 H-2 mismatch, minor histocompatibilty differences) with a decreased incidence of GVHD compared to a set of control mice which were not given CY after HCT.[9] Another set of control mice not given a marrow graft recovered autologous hematopoiesis. Furthermore, it was found that addition of CY before HSCT was synergistic with fludarabine in preventing graft rejection and increasing the degree of donor chimerism, a finding also reported recently by Petrus *et al.*[11]

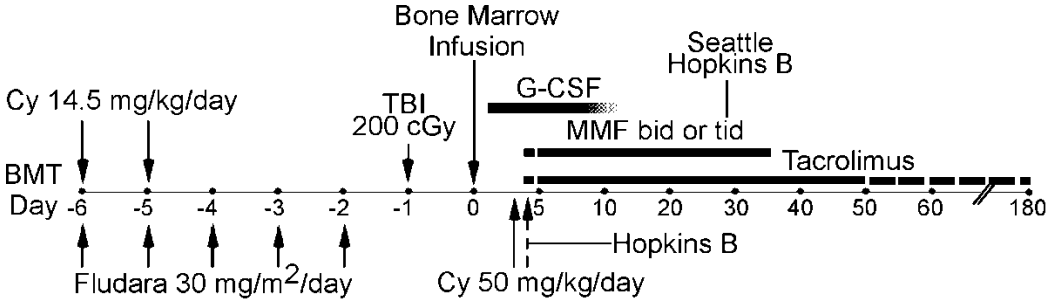
Using this approach, an initial clinical trial was initiated at Johns Hopkins in patients with high-risk hematologic malignancies. This dose-finding trial was designed to determine whether administration of CY as additional immunosuppression *before* HSCT might be required for engraftment.[12] In the first 13 patients it was found that CY at a dose of 14.5 mg/kg administered on days –6 and –5 improved the rate of donor engraftment. Eight of 10 patients receiving pre- and post-transplant CY achieved >90% donor T-cell engraftment by day +28. The two patients who rejected their grafts and recovered autologous hematopoiesis were patients with MDS who nonetheless remained in complete remission of their disease for at least one year post-transplant.

The Johns Hopkins trial has continued and a second clinical trial was opened at this Center in 2002 (FHCRC Protocol 1667). A joint report of these two studies has been submitted for publication. Patients with advanced hematologic malignancies (n=88) were studied in three cohorts as shown in the Table 1.1 and Schema below (Fig. 3.1). Cohorts differed by the total dose of post-transplantation CY (50 mg/kg versus 50 mg/kg x 2) and the dosing frequency of MMF (two versus three times daily).

Table 1.1: Cohorts of patients separated by dose of post-transplantation CY and the dosing frequency of MMF

Cohort	N	Doses of CY	MMF Dosing interval
Hopkins-A	20	1	Twice daily
FHCRC	28	1	Three times daily
Hopkins-B	40	2	Three times daily

Fig. 1.1: Schema



As shown in Table 1.2 below, the cohorts were not significantly different with respect to age, gender, ethnicity, sensitivity to last therapy given, or prior autologous transplantation.

Table 1.2: Patient Characteristics

Treatment cohort	1 (Hopkins A)	2 (Seattle)	3 (Hopkins B)
Number of patients	20	28	40
Treatment location	Baltimore	Seattle	Baltimore
Post-transplantation Cy	50 mg/kg	50 mg/kg	100 mg/kg
MMF frequency	bid	tid	tid
Tacrolimus given on days:	4-50 (n=10) 4-180 (n=10)	4-90	5-180
Median Age (years)	49.5 (23-62)	40.5 (20-67)	47.5 (1-71y)
Sex			
Male	13/20 (65%)	16/28 (57%)	26/40 (65%)
Female	7/20 (35%)	12/28 (43%)	14/40 (35%)
Ethnicity			
White	16/20 (80%)	18/28 (64%)	32/40 (80%)
African-American	4/20 (20%)	5/28 (18%)	7/40 (17.5%)
Asian	0	1/28 (4%)	1/40 (2.5%)
Hispanic	0	2/28 (7%)	0
Native American	0	2/28 (7%)	0
Diagnosis			
AML	7 (35%)	13 (46%)	14 (35%)
ALL	2 (10%)	2 (7%)	2 (5%)
MDS	5 (25%)	0	1 (2.5%)
CML/CMML	3 (15%)	2 (7%)	4 (10%)
CLL	0	0	3 (7.5%)
HD	0	8 (29%)	5 (12.5%)
NHL	1 (5%)	2 (7%)	8 (20%)
MM/Plasmacytoma	2 (10%)	1 (4%)	2 (5%)
PNH	0	0	1 (2.5%)
Prior Treatment regimens (#)	2 (0-8)	5 (1-10)	3 (0-6)
Sensitive to prior treatment	14/15(93%)	33/37 (89%)	21/28 (75%)
Prior autologous transplant	4/20 (20%)	11/28 (39%)	10/40 (25%)

Abbreviations: Cy, cyclophosphamide; MMF, mycophenolate mofetil; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; PNH, paroxysmal nocturnal hemoglobinuria

The three cohorts also did not differ with respect to the age of the donor or the relationship of the donor to the patient. Diagnoses were similarly distributed over the three cohorts with the exception that Hodgkin's lymphoma was not present in the Hopkins A cohort. Patients in the Seattle cohort did receive a significantly greater number of prior regimens compared to either Hopkins cohort. The median number of prior regimens in this group was 5, compared to 2 in Hopkins A and 3 in Hopkins B.

As shown in Table 1.3 below for the entire group, half (44/88) of the donors were siblings of the patient, and a quarter each (22/88) of the donors were either parents or children of the patient. Donors differed

from their recipients by a median of 3 HLA antigens in both the host-versus-graft (HVG) and graft-versus-host (GVH) directions. About one-third of patients had at least four HLA antigens mismatched (71% in the FHCRC cohort).

Table 1.3: Donor and Graft Characteristics

Cohort	1 (Hopkins A)		2 (Seattle)		3 (Hopkins B)	
Median Age in years (range)	39 (20-63)		45 (21-66)		43.5 (22-69)	
Sex						
Male	11 (55%)		18 (64%)		20 (50%)	
Female	9 (45%)		10 (36%)		20 (50%)	
Relationship						
Parent	3 (15%)		10 (36%)		9 (23%)	
Sibling	11 (55%)		10 (36%)		23 (58%)	
Child	6 (30%)		8 (28%)		8 (20%)	
$CD3^+$ cells/kg x 10^7 Mean (SD)	3.26 (0.85)		4.49 (1.48)		4.07 (1.49)	
$CD34^+$ cells/kg x 10^6 Mean (SD)	4.21 (2.05)		5.64 (2.71)		4.21 (1.54)	
Infused MNC/kg x 10^8 Mean (SD)	1.18 (0.25)		1.68 (0.53)		1.48 (0.51)	
# HLA mismatches in GVH direction	Antigen	Allele	Antigen	Allele	Antigen	Allele*
Mean	*2.85*	*3.15*	*2.89*	*3.21*	*2.78*	*3.31*
0→	1	0	1	0	1	1
1→	2	3	2	3	1	0
2→	3	2	4	2	12	4
3→	7	4	13	9	18	15
4→	7	11	8	14	8	19
# HLA mismatches in HVG direction	Antigen	Allele	Antigen	Allele	Antigen	Allele*
Mean	*2.75*	*3.05*	*2.96*	*3.21*	*2.88*	*3.28*
0→	1	0	2	1	1	1
1→	4	4	1	2	1	0
2→	1	1	4	3	8	5
3→	7	5	10	6	22	14
4→	7	10	11	16	8	19

*Not done for one patient

Engraftment and chimerism

For the entire group of patients, the median times to hematopoietic recovery of neutrophils and platelets were 15 and 29 days, respectively as shown below in Figure 1.2.

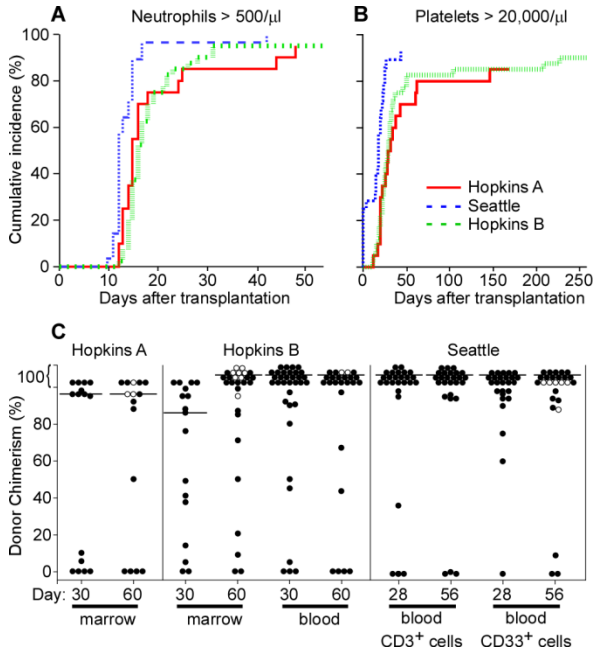


Fig. 1.2. Engraftment and chimerism. Cumulative incidence of (A) neutrophil and (B) platelet engraftment by cohort. (C) Percentage of donor chimerism at days 28-30 and 56-60 by cohort. Open circles represent the day 56-60 donor chimerism values for the 20 patients who are alive and event-free at the time of last follow-up. When two chimerism values are available for these patients, the open circle is shown for the marrow sample for the Hopkins cohorts and $CD33^+$ cells for the Seattle cohort.

By cohort the median times to neutrophil recovery were 15, 12, and 16 days and the median times to platelet recovery were 31, 20, and 30 in the Hopkins A, Seattle, and Hopkins B cohorts, respectively. In a multivariate analysis, a higher dose of $CD34^+$ cells in the graft was associated with faster recovery of both neutrophils and platelets, which may explain in part the faster engraftment times of the Seattle cohort. Graft failure occurred in a total of 15 out of 84 evaluable patients (18%): six out of nineteen (32%) in the Hopkins A cohort, three out of twenty-six (11%) in the Seattle cohort, and six out of thirty-nine (13%) in the Hopkins B cohort. All but two patients with graft failure experienced recovery of autologous hematopoiesis with a median time to neutrophil and platelet engraftment of 24 days (range 11-48 days) and 44 days (range 15-395 days), respectively. Adjusting for cohort, transplantation of female donor marrow into a male recipient increased the probability of graft failure (OR=6.23, 95% CI: 1.4-32.9; p=0.014), while increasing numbers of prior regimens decreased the risk of failure (OR=0.61, 95% CI: 0.36-0.90; p=0.007). Achievement of full donor chimerism was rapid after transplantation from an HLA-haploidentical donor (Fig. 1.2). Analysis of peripheral blood that was either: a) unfractionated (Hopkins cohorts) or b) separated by cell sorting into T-cell (CD3-positive) or granulocyte (CD33-positive) fractions (Seattle cohort) showed that with few exceptions, donor chimerism was virtually complete (>95%) by 2 months post-transplant.

Hospitalizations and transfusions

All patients received their treatment in the outpatient department and were discharged to their referring oncologist between 60 and 90 days after transplantation, unless complications requiring admission to the hospital supervened. The median number of hospitalizations prior to day 60 was 1 (range 0-4) for each patient cohort. The reason for admission was neutropenic fever in 56% of patients, non-neutropenic infection in 33%, and other causes, including acute GVHD, for the remaining 11%. A total of 36 patients (41%) did not require hospitalization within the first 60 days of transplantation. The median total number of hospital days prior to day 60 was 3, 6 and 2 for the Hopkins A, Seattle and Hopkins B, respectively. The median number of units of packed red blood cells transfused per patient was 8, 4, and 10 for the Hopkins A, Seattle, and Hopkins B cohorts. Three patients in the entire study did not require red blood cell transfusions.

Patients who are seropositive for cytomegalovirus (CMV) are known to be at high-risk for reactivating CMV after transplantation, regardless of the serologic status of the donor. In this study, CMV reactivation was observed in 20/60 (33%) high-risk patients (Table 1.4). The incidence of reactivation in high-risk patients differed across the cohorts but the median time to onset did not differ by cohort. Acute GVHD was diagnosed in 10/20 (50%) patients on or about the time of CMV reactivation. Despite the varying rates of CMV reactivation among the cohorts, with preemptive therapy the incidence of CMV disease was low. CMV pneumonia developed in only one patient in the Hopkins A cohort for an overall incidence of 5%. There was no CMV-associated mortality.

In the current era of anti-fungal prophylaxis, the incidence of invasive mold infections after transplantation, especially *Aspergillus*, has been an increasing problem. However, with the advent of anti-mold agents such as voriconazole, survival of patients with invasive mold infections has improved significantly. As shown in Table 1.4, proven or probable invasive mold infections post-transplant, all caused by *Aspergillus sp*, were observed in only 6 out of 88 patients, an incidence of 6.8%. Two patients in the Hopkins B cohort died from invasive *Aspergillus* infection, one while persistently neutropenic following graft failure.

Table 1.4. CMV Reactivation and Invasive Mold Infection

	Hopkins A (n=20)	**Seattle (n=28)**	**Hopkins B (n=40)**
No. patients at high-risk for CMV reactivation	10 (50%)	24 (86%)	25 (63%)
No. high-risk patients with CMV reactivation	3 (30%)	15 (62.5%)	2 (8%)
No. high-risk patients with CMV disease	1 (10%)	0	0
Median days to onset of CMV reactivation (range)	47 (42-48)	30 (17-80)	47 (38-48)
No. patients with invasive mold infection	1 (10%)	3 (11%)	2 (5%)

Acute and chronic graft-versus-host disease

For the entire patient population, the cumulative incidences (CI) of grades II-IV and III-IV acute GVHD by day 200 were 35% and 10%, respectively (Fig. 1.3A).

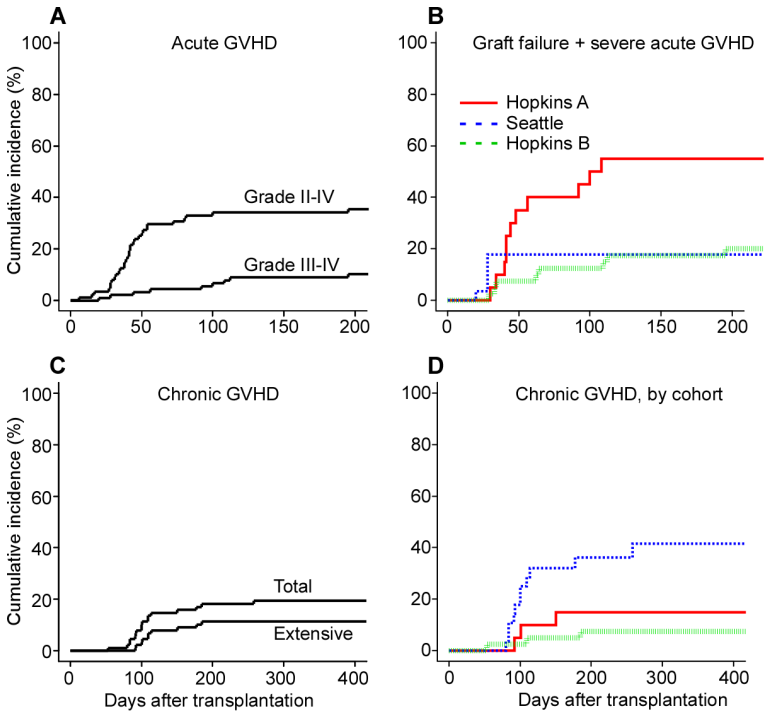


Fig. 1.3. Cumulative incidence of acute and chronic GVHD. (A) Cumulative incidence of acute GVHD grades II-IV and III-IV. (B) Combined cumulative incidence of severe (grades III-IV) acute GVHD and graft failure. (C) Cumulative incidence of total chronic and extensive chronic GVHD. (D) Cumulative incidence of total chronic GVHD by cohort.

There were no significant differences between cohorts in the incidence of acute grades II-IV and III-IV GVHD. Since graft failure and severe (grades III-IV) acute GVHD can both be manifestations of inadequate post-transplantation pharmacologic immunosuppression, the summed cumulative incidences of these events is shown in Figure 1.3B. The combined incidence of graft failure and severe acute GVHD in the Hopkins A cohort was 55% by 1 year, which motivated the addition of the second dose of post-transplantation Cy on day 4 and the change to thrice daily MMF in the Hopkins B cohort.

The cumulative incidences of total chronic and extensive chronic GVHD in the first year after transplantation for the entire population were 20% and 11%, respectively (Fig. 1.3C). For the Hopkins A, Seattle, and Hopkins B cohorts, the cumulative incidences of chronic GVHD at 1 year after transplantation were 25%, 43%, and 10%, respectively (Fig. 1.3D). The incidence of chronic GVHD in the Seattle cohort was significantly higher than in the Hopkins B cohort ($p = .004$) and marginally higher than in the Hopkins A cohort ($p = .08$). There was no significant difference between cohorts in the incidence of extensive chronic GVHD.

Non-relapse mortality (NRM) and relapse

Overall, the cumulative incidences of NRM at 180 days and 1 year after transplantation were 13% and 19%, respectively, and the cumulative incidences of relapse at 1 and 2 years after transplantation were 50% and 57%, respectively (Fig. 1.4A).

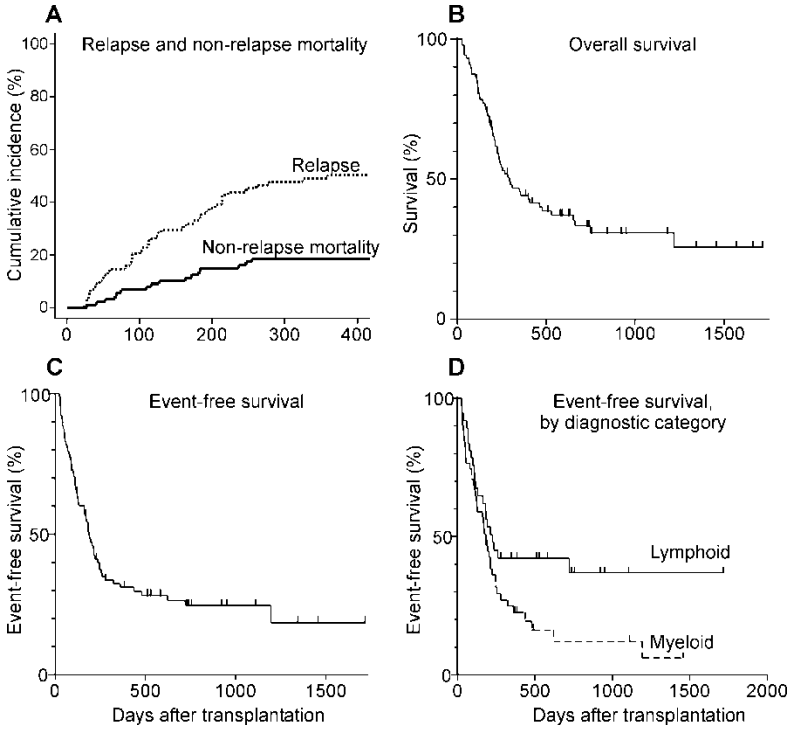


Fig. 1.4. Outcomes among nonmyeloablative haploidentical BMT recipients. (A) Cumulative incidence of non-relapse mortality and relapse. (B) Overall survival. (C) Event-free survival (D) Event-Free Survival by Disease Category (lymphoid versus myeloid).

There were no significant differences in the cumulative incidences of either NRM or relapse between cohorts. In a stratified multivariate analysis, patients with lymphoid malignancies had a significantly lower risk of relapse (HR 0.50, 95% CI .25-.99, $p < .046$) than patients with myeloid malignancies. In the same analysis, increasing age, graft $CD3^+$ cell dose, or number of prior regimens was not associated with a significantly increased incidence of relapse.

Overall and event-free survival (EFS)

With a median follow-up of 817 d (range: 112-1808 d) the actuarial overall survivals of the entire group at 1 and 2 years, respectively, were 50% and 37%, respectively (Fig, 1.4B) The actuarial event-free survivals (EFS) at 1 and 2 years was 33% and 24%, respectively (Fig. 1.4C). Overall and EFS survivals did not differ significantly between cohorts. However, compared to patients with myeloid malignancies, patients with lymphoid malignancies had a superior EFS at 1 year (42% versus 24%) and at 2 years after transplantation (36% versus 15%; $p = .04$, Fig. 1.4D).

A multivariate analysis of risk factors for event-free survival is presented in Table 1.5. Compared to patients with AML, patients with non-Hodgkin's lymphoma and Hodgkin's lymphoma had an improved event-free survival. Interestingly, an increasing number of HLA-antigen mismatches in the host-versus-graft direction was associated with a marginally improved EFS, whereas an increasing graft dose of $CD3^+$ cells was associated with a marginally worse EFS.

Table 1.5. Multivariate hazard ratios for event-free survival, stratified by cohort

		HR Confidence Limits		
Variable	**Hazard Ratio**	**Lower**	**Upper**	**p**
NHL	0.34	0.12	0.91	.03
HD	0.28	0.11	0.75	.01
ALL	0.46	0.13	1.57	.21
MDS	0.55	0.22	1.35	.19
CML	0.78	0.30	2.01	.60
Other dx	1.27	0.59	2.73	.54
# mismatches in HVG direction	0.77	0.59	1.01	.06
Graft $CD3^+$ cell dose	2.54	0.97	6.62	.06

Of 57 deaths, 41 occurred in patients with relapsed or progressive disease (Table 1.6). GVHD accounted for five of the sixteen deaths in the Hopkins A cohort but for only one out of 41 deaths in the other two treatment cohorts. Only 4 patients died of infection without GVHD.

Table 1.6 Causes of death among transplanted patients.

Cause of death	**Hopkins A (n=20)**	**Seattle (n=28)**	**Hopkins B (n=40)**
Relapse	10	12	19
Graft-versus-host disease	5		1
Infection		2	2
CNS hemorrhage			2
Unknown		1	1
Hepatorenal syndrome	1		
Secondary AML		1	

Targeted hematopoietic irradiation delivered by ^{131}I-anti-CD45 antibody

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 and lymphoid malignancies in the hope that cure rates could be significantly improved. The CD45 antigen is an attractive target for immunotherapy since the vast majority of hematologic malignancies express CD45, including 85-90% of acute myeloid and lymphoid leukemias. The CD45 antigen is not found on tissues of non-hematopoietic origin, and thus an antibody (Ab) reactive with the CD45 antigen targets the primary sites of leukemic cell involvement (the marrow and spleen), as well as lymphoid tissue. By selecting an Ab reactive with normal leukemic 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 Ab (BC8) may offer clinical benefit both to patients with active disease and to those in remission.

Prior studies have shown that escalating the dose of systemic therapy decreases the risk of relapse for patients with advanced AML, but also increases non-relapse mortality, resulting in no improvement in overall survival.[13] Early encouraging phase I results have demonstrated the feasibility of using ^{131}I-BC8 Ab combined with a transplant regimen employing 120 mg/kg CY and 12 Gy TBI for therapy of acute leukemias.[14] Biodistribution of trace-labeled Ab 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 Ab, with improved therapeutic ratios of radiation delivered to marrow and spleen as compared to liver (average 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 3.5 Gy to 12.25 Gy to the normal organ receiving the highest dose. At the time of initiation of this protocol, eight patients remained disease-free since treatment, surviving at 8.9 to 15 years (median 13.2 years) post-transplant; one additional surviving patient relapsed 6.4 years post transplant. Sixteen patients died following relapse at 3.8 to 141.9 months (median 16.6 months) post-transplant. Nine patients died from non-relapse related causes, from 8 days to 10.9 years (median 41 days) post transplant. At the maximum tolerated dose (MTD) of 10.5 Gy, it was determined that ^{131}I-BC8 Ab in combination with CY and TBI could deliver up to an additional dose of 24 Gy to marrow and 50 Gy to spleen. Therefore, this study 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.

Based on these results, we used the same preparative regimen in a Phase II study for patients 2-55 years old with AML beyond CR1, or with relapsed/primary refractory disease. Twenty-six patients, 10 to 55 (median 39) years old, with AML (5 CR2, 18 relapsed or refractory) or MDS (1 RAEB, 2 RAEBT) received a test infusion of BC8 Ab trace-labeled with ^{131}I. Favorable biodistribution, with estimated radiation absorbed dose to bone marrow and spleen $\geq$ to liver (normal organ receiving the highest dose), was observed in 24 (92%) patients. Eighteen patients received a therapy infusion of 0.5 mg/kg of Ab labeled with 127-389 (median 252) mCi of ^{131}I to deliver a planned dose of 8 Gy to the liver, in addition to 120 mg/kg CY over 2 days and 12 Gy TBI over 3 days, followed by matched related (MRD, n=9) or unrelated donor (URD, n=9) HCT. The initial target radiation absorbed dose delivered to liver from radiolabeled Ab was set lower (8 Gy) than that for HLA-MRD recipients (10 Gy) since regimen-related toxicity (RRT) with conventional transplant regimens was typically higher for patients receiving URD stem cells. Mean estimated radiation absorbed doses from radiolabeled Ab were 21 $\pm$ 8 Gy to marrow and 45 $\pm$ 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 $\geq$ 500 cells/mm^3) at a median of 18 (15-30) days post-HCT. All patients experienced at least grade II (moderate) mucositis. Survival and morbidity outcomes on this study are similar to that of historical controls treated with conventional CY/TBI regimens at the center during the same period. At the time of initiation of this protocol, seven of 18 patients (39%) were alive and disease-free at a median of 3.5 (2 to 5.5) years after transplant. Seven patients relapsed 44 - 266 days post-HCT. The estimated probability of relapse at 2 years is 38.9%. Nine patients received a dose to BM < 7 cGy/mCi and 9 other patients received > 7 cGy/mCi to BM. Only 1 relapse occurred in the higher dose group while 6 relapses were seen in the lower dose cohort. The hazard of relapse in the group that received < 7 cGy/mCi to BM is higher than that in the higher dose (> 7 cGy/mCi) group (HR=6.30, 95% CI 0.76-52.60, p=0.05 by log-rank test). These results suggest that a greater radiation absorbed delivered to BM may lead to lower post-transplant relapse rates

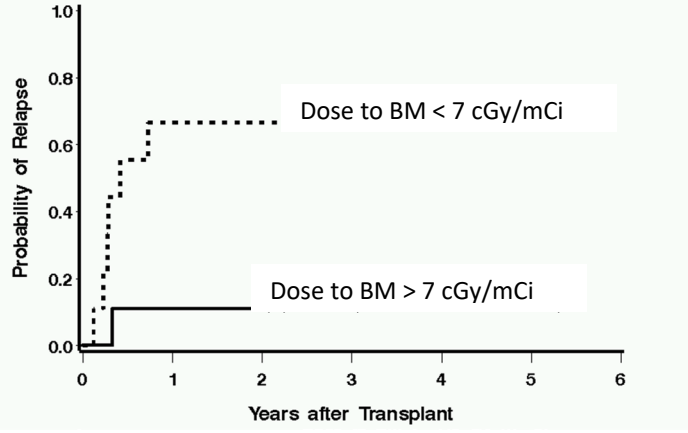


Fig. 1.5: Probability of relapse for patients with advanced AML who received a therapy dose of I-131-anti-CD45 Ab combined with CY/TBI on FHCRC Protocols #1297. Thick solid line = absorbed radiation dose to BM > 7 cGy/mCi; thick dashed line = absorbed radiation dose to BM < 7 cGy/mCi.

for patients with high-risk AML/MDS (Fig. 1.5).

Based on our demonstration that, in the majority of patients, greater estimated radiation doses could be delivered to marrow and spleen compared to liver, lung, and kidney, and that significant supplemental doses of hematopoietic radiation could be safely combined with a conventional transplant preparative regimen, a trial for patients with AML in first remission receiving HLA-matched related marrow was then initiated. Radiolabeled antibody was combined with BU/CY because a prospective randomized study in chronic phase CML had demonstrated lower toxicity with BU/CY[15] while retrospective comparisons of BU/CY and CY/TBI for transplant of AML in first remission have shown similar incidences of long-term disease-free survival.[16] In this Phase I/II study first remission AML patients received targeted irradiation delivered by ^{131}I-BC8 Ab combined with targeted BU (area-under-curve, 600-900 ng/ml) and CY (120 mg/kg). Fifty-two of 59 patients (88%) receiving a trace ^{131}I-labeled dose of 0.5 mg/kg BC8 murine Ab had higher estimated absorbed radiation in bone marrow and spleen than in any other organ. Forty-six patients were treated with 102-298 mCi ^{131}I delivering an estimated 5.3-19 (mean 11.3) Gy to marrow, 17-72 (mean 29.7) Gy to spleen, and 3.5 Gy (n=4) to 5.25 Gy (n=42) to the liver. The estimated 3-year non-relapse mortality and disease-free survival (DFS) were 21% and 61%, respectively (Fig. 1.6). These results were compared to those from 509 similar International Bone Marrow Transplant Registry patients transplanted using BU/CY alone. After adjusting for differences in age and cytogenetics-risk, the hazard of mortality among all antibody-treated patients was 0.65 times that of the Registry patients (95% CI 0.39-1.08; p=.09).

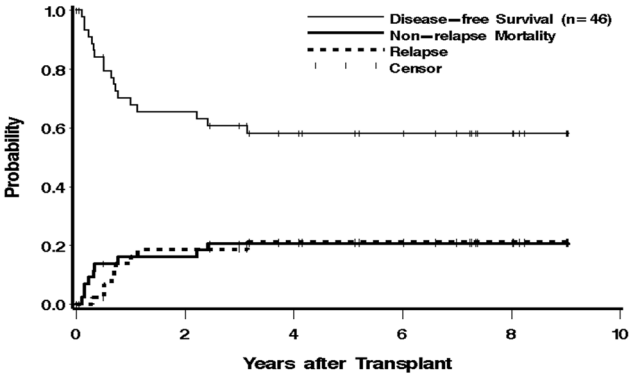


Fig. 1.6: Estimates of the probability of DFS, NRM, and relapse among all patients who received a therapeutic dose of ^{131}I-BC8 antibody, followed by BU/CY.

The encouraging results achieved with ^{131}I-BC8 Ab and BU/CY in younger patients led us to ask how this approach might be applied to older AML patients, since the outcome for AML patients over 55 is poor using standard chemotherapy, with a 5-year survival rate of <10%.[17-19] As noted above, recent success achieving stable donor chimerism following infusion of allogeneic peripheral blood stem cells (PBSC) after reduced intensity conditioning regimens affords an opportunity to safely induce a graft-vs-leukemia (GvL) effect with minimal acute morbidity. GvL effects, however, appear to be most potent in patients with low tumor burdens at the time of transplantation. Given the minimal RRT of this non-ablative approach, we hypothesized that the anti-leukemic effect in advanced disease patients may be improved by the addition of targeted hematopoietic irradiation delivered by ^{131}I-BC8 Ab to the low-intensity conditioning regimen. The MTD of radiation delivered via ^{131}I-BC8 Ab combined with the non-myeloablative regimen for older patients, however, was unknown. We have therefore conducted a Phase I clinical trial of targeted hematopoietic irradiation delivered by an ^{131}I-labeled anti-CD45 Ab (BC8) to determine the feasibility, safety and efficacy of this approach toward reducing the burden of disease before an established non-myeloablative regimen. This dose escalation study was designed to estimate the MTD of ^{131}I-BC8 Ab that can be combined with fludarabine (FLU) and low dose TBI followed by allogeneic transplantation. At the time of initiation of this protocol, 51 patients aged 50 to 74 (median 62) years old received a test infusion of BC8 Ab trace labeled with I-131. Six patients who received a test dose did not go on to be treated on this study. Two patients became positive for Human Anti-Mouse Ab (HAMA), two patients had reactions to the Ab and did not continue on study, one patient did not have

good uptake of the Ab with the dosimetry infusion, and one patient was hospitalized for fevers and pulmonary events approximately one week following the dosimetry infusion and did not recover sufficiently to proceed with the study.

Forty five patients over 50 years of age with advanced AML or high-risk MDS (> 5% blasts), with no other treatment options at our Center, received a therapy infusion of 0.5 mg/kg of Ab labeled with 246 to 932 mCi ^{131}I delivering an estimated 5.2 to 45.9 (mean 27.5) Gy to bone marrow, 39.6 to 155 (mean 86.1) Gy to spleen, and 12-24 Gy to the liver (dose-limiting organ). In 44 patients, this was followed by FLU (30 mg/m^2 daily for 3 days), 2 Gy TBI and matched related (14) or unrelated (30) PBSC infusion combined with cyclosporine and mycophenolate mofetil. The first patient entered on this protocol had a matched related donor and was treated with I-131 Ab combined with 2 Gy TBI and matched related PBSC. At that time, FLU was to be added for patients with unrelated donors only. The protocol was then modified to add FLU to the treatment regimen for all patients on this protocol. At the time of initiation of this protocol, of the 45 patients treated, 11 patients were alive and disease free with the longest being 33 months post-transplant. Two additional patients were alive following relapse. Eighteen patients have died following relapse, and 14 patients have died of other causes: 7 from infections, 5 from pulmonary causes, and 1 each from refractory GVHD and cerebral bleed. The pulmonary events include 2 instances of bronchiolitis obliterans organizing pneumonia (BOOP), 1 alveolar hemorrhage, 1 aspiration pneumonitis leading to respiratory failure, and 1 resistant pneumonia that occurred approximately 2 years post treatment. These events represent the majority of the Grade III/IV (Bearman) toxicities that have impacted dose escalation/de-escalation on this study.

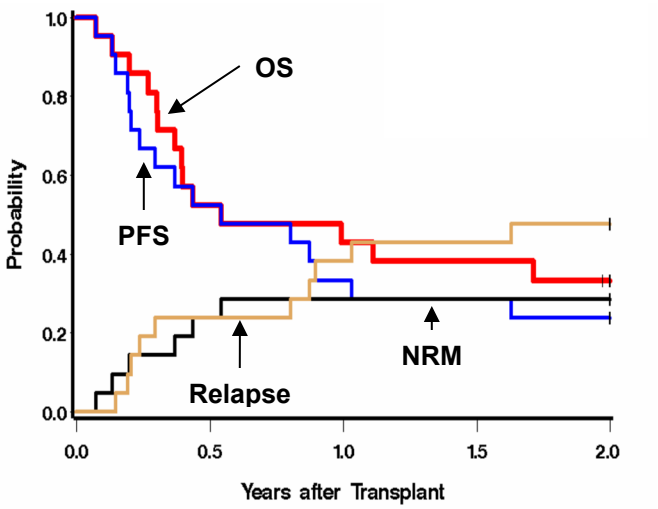


Fig. 1.7: Estimates of the probability of PFS, NRM, and relapse among all patients who received a therapeutic dose of ^{131}I-BC8 Ab, followed by FLU/TBI.

While it remains to be determined whether this approach will reduce post-transplant relapse rates for older patients with high-risk AML/MDS, the results are sufficiently encouraging such that we now propose to combine targeted hematopoietic radiation delivered by ^{131}I-BC8 Ab with the transplantation approach using haploidentical donors in an effort to improve disease control for advanced acute leukemia and high-risk MDS patients. Specifically, this study will assess the feasibility and safety for patients with advanced AML/ALL/MDS treated with a starting dose of 12 Gy ^{131}I-BC8 Ab delivered to the normal organ receiving the highest dose, combined with FLU and 2 Gy TBI, plus CSP, followed by haploidentical allogeneic HCT and immunosuppression using tacrolimus and MMF. Nonrelapse-related mortality (NRM) will be the major study endpoint to determine the maximum tolerated dose of radiation when used as part of this regimen. The study will also estimate the rates of disease response and disease-free survival, and determine rates of donor chimerism and GvHD.

2.0 OBJECTIVES

1. To estimate the maximum tolerated dose of radiation delivered via ^{131}I-BC8 antibody when combined with pre- and post-transplant CY, FLU, 2 Gy TBI, tacrolimus, MMF, and a

haploidentical allogeneic hematopoietic marrow transplant in patients who have advanced AML. ALL, or high risk MDS.

2. To estimate rates of immune reconstitution, engraftment, and donor chimerism resulting from this combined preparative regimen.
3. To determine rates of disease relapse, acute GvHD, and day 100 disease-free survival in patients receiving ^{131}I-BC8 Ab combined with CY, FLU, 2 Gy TBI, tacrolimus, MMF, and HLA-haploidentical allogeneic HCT.

3.0 SUBJECT SELECTION

A. Inclusions

1. Patients with advanced AML or ALL 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 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. 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 $\geq$ 18 years of age.
4. Patients should have a circulating blast count of less than 10,000/mm^3 (control with hydroxyurea or similar agent is allowed).
5. Patients must have a creatinine clearance greater than 50/ml per minute by the following formula (test must be performed within 28 days prior to registration):

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

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 a related donor who is identical for one HLA haplotype and mismatched at the HLA-A, -B or DRB1 loci of the unshared haplotype with the exception of single HLA-A, -B or DRB1 mismatches.

B. Exclusions

1. Circulating antibody against mouse immunoglobulin (HAMA).
2. Prior radiation to maximally tolerated levels to any critical normal organ.
3. Cross-match positive with donor
4. Patients may not have symptomatic coronary artery disease and may not be on cardiac medications for anti-arrhythmic or inotropic effects.
5. Patients with the following organ dysfunction:
    a. Left ventricular ejection fraction <35%
    b. Corrected DLCO <35% and/or receiving supplemental continuous oxygen

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

6. Patients who are known seropositive for HIV.
7. Perceived inability to tolerate diagnostic or therapeutic procedures, particularly treatment in radiation isolation.
8. CNS involvement with disease refractory to intrathecal chemotherapy and/or standard cranial-spinal radiotherapy.
9. Women of childbearing potential who are pregnant (β-HCG$^+$) or breast feeding
10. Fertile men and women unwilling to use contraceptives during and for 12 months post-transplant.
11. Inability to understand or give an informed consent.

4.0 DONOR SELECTION

Donors must meet HLA matching criteria as outlined under section 5.A.9 as well as standard Seattle Cancer Care Alliance (SCCA) criteria for Allogeneic bone marrow donation. Preference should be given to donors who are mismatched at the HLA-A, -B and –DRB1 loci.

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.

5.0 INFORMED CONSENT OF SUBJECTS AND DONOR

Subjects will be referred to University of Washington/Seattle Cancer Care Alliance for consideration of HCT and will be completely evaluated. The protocol should be discussed thoroughly with subject and family, and all known risks will be described. The procedure and alternative forms of therapy will be presented objectively and the risks and hazards of the procedure explained. Consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. A summary of the conference should be dictated for the medical record detailing what was covered.

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

6.0 SUBJECT REGISTRATION

Patients will be assigned to a protocol by the Clinical Coordinator who 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.

7.0 PLAN OF TREATMENT

Treatment will be initiated in the outpatient department for the dosimetric dose of ^{131}I-BC8 antibody and subsequent gamma scans. All patients will be admitted to lead-lined radiation isolation rooms at the University of Washington Medical Center (UWMC) for the therapeutic dose of ^{131}BC8 antibody and will remain in isolation for approximately 5-10 days, as described under section 7.C.1.d. Patients will then continue transplant regimen on an outpatient basis unless clinically indicated.

A. Outline of treatment plan (see Table 7.1)

The day of treatment is listed only to give a loose time-frame. An individual study calendar is created for each patient, with modifications permitted in consideration of clinical and logistical needs. It is anticipated that stem cells will be infused approximately 14 days after delivery of the ^{131}I-BC8 therapy dose.

~Day -23
Dosimetric Dose ^{131}I-BC8 Antibody Rx

~Day -22 to -20
Gamma scans

~Day -14
Therapeutic Dose ^{131}I-BC8 Antibody Rx

Days -6, -5
FLU 30 mg/M^2 iv qd
CY 14.5 mg/kg iv qd
Mesna (dosed at 100% CY dose)

Days –4 to –2
FLU 30 mg/M^2 iv qd

Day –1
200 cGy TBI at 6-7 cGy/min

Day 0
Infuse processed marrow allograft

Day +3
CY 50 mg/kg
(Must be administered 60-72 hr post-BMT)
Mesna (dosed at 100% CY dose)

Day +4

Begin Tacrolimus 1 mg IV and MMF 15 mg/kg po tid

Begin G-CSF (5 μg/kg/d) IV or SC, continue until ANC>500/mm^2 x 3d

Day +28

Assess chimerism in peripheral blood

Day +35**

Discontinue MMF

Day +56

Assess chimerism in peripheral blood

If day +28 <50 % donor

Day +84

Assess disease status; assess chimerism in peripheral blood

Immune reconstitution studies

If no GVHD, start Tacrolimus taper

Day +180

Discontinue Tacrolimus (under care of primary hematologist/oncologist)

****MMF may be discontinued earlier than day+35 at the discretion of the study doctor in patients who have no GVHD**

Table 7.1: Outline of treatment plan

	Days before transplant								
	~23	~14	*Radiation Isolation*	−6	−5	−4	−3	−2	−1
Antibody test dose	●								
Antibody therapy dose		●							
Fludarabine				●	●	●	●	●	
Cyclophosphamide and Mesna				●	●				
Total body irradiation									●

Bone Marrow Transplant: Day 0

	Days after transplant				
	3	4	35	84	180
**Cyclophosphamide and Mesna	●				
G-CSF		*Start**			
MMF[#]		*Start*	*Stop*[#]		
Tacrolimus		*Start*	→	*Taper*	*Stop*

*G-CSF will continue until absolute neutrophil count (ANC) is greater than 500/mm^2 for three consecutive days.

** Corticosteroids may not be used as an anti-emetic agent and should not be administered until 24 hours after the completion of post-transplantation cyclophosphamide, unless used for adrenal support or during a medical emergency (e.g. treatment of anaphylaxis).

[#] MMF may be discontinued earlier than day +35 at the discretion of the study doctor if patient has no GVHD

B. Indwelling central venous catheter

Placement of a double lumen central venous catheter will be required for administration of IV medications and transfusion of blood products.

C. Radiolabeled Antibody Evaluation (Biodistribution Dose)

1. Biodistribution study

The radiation absorbed doses for each organ delivered by ^{131}I-labeled Ab will be estimated for each patient prior to administration of therapeutic amounts of ^{131}I-labeled Ab. For this purpose 1 to 10 mg of Ab will be labeled with 4-10 mCi ^{131}I. The ^{131}I-labeled Ab will be mixed with unlabeled Ab 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 the infusion. These estimates will be used to determine the amount of ^{131}I with which the therapeutic dose of isotope-Ab conjugate will be labeled. The therapy dose is labeled with larger amounts of ^{131}I, as determined by the cGy/mCi ^{131}I estimates calculated from the biodistribution Ab infusion for the normal organ receiving the highest dose, the dose level at which the patient is to be treated, and the current limit of radiation dose delivered to marrow.

a) Iodination and Characterization of Labeled Antibody

Abs 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 several 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 Ab will be administered through a central venous catheter. Premedications will include:

i. acetaminophen 650 mg PO

ii. diphenhydramine 25-50 mg IV or ranitidine 50mg IV over 20-30 minutes for patients who do not tolerate diphenhydramine
iii. ondansetron 8 mg IV
iv. hydrocortisone 100 mg IV.
v. D51/2NS to start with the Ab infusion and to continue until the antibody infusion is complete.
vi. Hydrocortisone 100 mg IV will be repeated every 2 hours until the completion of the infusion.

The mixture of ^{131}I-labeled BC8 Ab (4-10 mCi) will be diluted to approximately 25 ml and infused intravenously at 7.5 mg/hour. Infusion of the biodistribution dose will occur at the UW Medical Center.

Specifics of Radiolabeled Antibody Administration - Therapeutic Dose: The therapeutic dose of ^{131}I-BC8 Ab will be 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 with appropriate shielding to minimize exposure of health care personnel and to prevent contamination of the room with radioisotope 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. 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. The patient can then be discharged, and can remain an outpatient to complete treatment unless admission is medically indicated.

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 (2-5ml) will be obtained at the time of marrow biopsy (see section 7.C.1.g below). This sample will be obtained at the SCCA. These samples will be used for analysis of Ab clearance.

f) Gamma scanning

Serial gamma camera images will be obtained 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. Whole body counts will be measured at each time point by a gamma probe.

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.

For all patients, 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 Ab. The biopsy section will be collected using NO FIXATIVE agents. A research technician from the Press Laboratory will prepare the research portion for the Press 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

Patients must be HAMA negative on a blood sample obtained the day prior to therapy. All patients will be eligible to receive a therapy dose of ^{131}I-BC8 Ab unless they are positive for HAMA prior to the planned dose, even if their estimated radiation dose to marrow is less than liver. In previous patients receiving biodistribution doses on our other studies, the estimated doses to marrow and spleen were greater than doses to lung, kidney and total body even in those few patients whose marrow dose was slightly lower than liver dose. Therefore, for this group of patients who would not tolerate a conventional preparative regimen, we will proceed with a therapy dose of ^{131}I-BC8 Ab regardless of the ratio of radiation delivered to marrow as compared to liver.

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

^{131}I-labeled Ab 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. This dose will be administered following the patient's inpatient admission to radiation isolation at the University of Washington Medical Center. 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 our previous patients, serum Ab 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 –14.

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 the predetermined radiation dose to the normal critical organ (almost always liver) predicted to receive the highest estimated dose of radiation. For each patient, the dose level at which the patient will be treated will be discussed by the P.I. (or his designee) and Dr. Rajendran (or his designee). In some patients with a high bone marrow: liver radiation dose ratio, there may be the possibility of adversely affecting engraftment by damage to the bone marrow supportive stroma resulting from delivering too much radiation to the marrow. These patients may have the ^{131}I dose limited to that which would deliver the currently stipulated maximum dose to marrow (see further discussion under section 12.2.B).

2. Timing of Marrow Infusion

   For patients with estimated marrow biologic half-times of less than 96 hours as estimated by the biodistribution dose, donor marrow will be infused ~14 days after the therapy dose of ^{131}I-BC8 Ab. In the unlikely event that a patient has an estimated marrow biologic half-time longer than 96 hours, marrow infusion will be delayed until the estimated radiation dose rate in marrow is < 2 mR/hour, and the actual number of days prior to marrow infusion that the therapy dose of Ab is administered will be adjusted accordingly.

E. Conditioning regimen

1. Fludarabine:
   a. Description: Fludarabine monophosphate is a purine antimetabolite that, after administration, undergoes rapid conversion in plasma to the nucleoside 2-fluoro ara-A (F-araA). F-araA subsequently enters cells where it is phosphorylated to F-araATP and the monophosphate F-araAMP. Once activated, F-araATP inhibits DNA polymerase and ribonucleotide reductase. The monophosphate F-araAMP, once incorporated into DNA, is an effective DNA chain terminator. Following IV administration, the drug is metabolized to 2-F-araA and widely distributed in tissues. 2-F-araA is excreted primarily in urine and has a terminal elimination half-life of 7-12 hr.
   b. Storage and Administration: Fludarabine monophosphate is commercially available as a 50 mg/vial which is reconstituted with 2 ml of sterile water, resulting in a 25mg/ml solution. The desired dose is further diluted to concentrations of 0.04-1 mg/ml in normal saline or 5% dextrose (50-100ml) for injection and administered by IV infusion. Fludarabine will be administered by IV infusion over 30 minutes in a dose of 30 mg/M^2/day on days -6 to -2.

2. Cyclophosphamide (14.5 mg/kg Adjusted BW*, unless IBW>ABW, then ABW per Standard Practice Guidelines)
   a. Description: CY is an alkylating agent which prevents cell division primarily by cross-linking DNA strands. CY is cell cycle non-specific. CY is not stem cell toxic.
   b. Storage and administration: CY for injection is commercially available in 2000mg vials which are reconstituted with 100 ml sterile water. The concentration of the reconstituted product is 20mg/ml. The calculated dose will be diluted further in 250-500

ml of Dextrose 5% in water. Each dose of CY will be administered as an IV infusion over 1 hr on day -6 and day -5. Cyclophosphamide pre- and post hydration with normal saline will be administered outpatient for 2 hours pre- and 8 hours post cyclophosphamide, or inpatient for 4 hours pre- and 8 hours post cyclophosphamide.
*Adjusted BW = [(ABW – IBW) 0.25] + IBW

3. Mesna:
    a. Description: Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxasophosphorines (CY and ifosphamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxasophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxasophosphorines. Administration and patient monitoring will be per standard practice.
    b. Storage and Administration: Mesna is commercially available in 200 mg, 400 mg and 1000 mg vials containing a 100 mg/ml solution. Each dose of mesna will be diluted further in 50 ml of normal saline to be infused over 15 min. Mesna dose will be based on the cyclophosphamide dose being given. The total daily dose of mesna will be equal to 100% of the total daily dose of CY.

4. Total body irradiation (200 cGy) will be given on day –1 at a rate of 6-7 cGy/min per Standard Practice Guidelines. Dosimetry calculations are performed by the radiation therapist. Refer to Standard Practice Manual for information about administration, toxicity, and complications.

5. As the testes may serve as a sanctuary for ALL, male patients with ALL will receive 1600 cGy of testicular radiation per Radiation Oncology. The testicular radiation can be given concurrently with conditioning.

F. ***Bone marrow infusion***

The donor marrow harvest will occur on Day 0 per standard procedures. The total nucleated cell (TNC) goal will be based on the recipient's ideal body weight per standard practice protocol. In the absence of ABO or other red cell incompatibility, the marrow product will be directly infused into the recipient without manipulation. If major or minor ABO incompatibility exists with significant donor or recipient antibody titers, the product will be manipulated according to standard practice procedures.

G. Post-transplant immunosuppression

1. Cyclophosphamide: A single dose of CY (50mg/kg Adjusted BW*, unless IBW>ABW, then ABW per Standard Practice Guidelines) will be given on day +3 after transplant (within 60-72 hr of marrow infusion). CY will be given as an IV infusion over 1-2 hr (depending on total dose) with Mesna and appropriate hydration as described above in Section 7.E.2. Monitoring of urine output and for hematuria will be performed similarly.
    a. Corticosteroids may not be used as an anti-emetic agent and should not be administered until 24 hours after the completion of post-transplantation cyclophosphamide, unless used for adrenal support or during a medical emergency (e.g. treatment of anaphylaxis).

2. Tacrolimus and MMF:
    a. Description: Tacrolimus, also known as FK-506, is a macrolide immunosuppressive agent. Tacrolimus inhibits lymphocytes by forming a complex with FKBP-12, calcium, and calmodulin, leading to the decrease in the phosphatase activity of calcineurin. Calcineurin mediates the first intracellular signal required for T-cell activation after antigen recognition by the T-cell receptor. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants and for prophylaxis of GvHD in the setting of HCT. It is also used for immunosuppression after kidney, cardiac, pancreas, pancreatic islet cell and small bowel transplantation. This drug is well-absorbed orally. It is metabolized in the liver by unknown mechanisms, but demethylation and hydroxylation have been proposed based on in vitro studies. The metabolized products are excreted in the urine.
    b. Storage and administration: Tacrolimus is commercially available in capsule form (0.5, 1.0 and 5.0 mg) and as a sterile solution in 1mL ampules (5mg/mL) for IV administration. Starting on day +4, tacrolimus will be given at a dose of 0.03 mg/kg/d (for patients <30 kg) and 1mg/d (for patients >30kg) IV over 1-2 hr and should be changed to a PO dosing schedule as tolerated once a therapeutic level (5-15 ng/ml) is achieved. Serum levels of tacrolimus should be measured on day +8 or as frequently as needed to establish a therapeutic level and then weekly thereafter with the dose adjusted accordingly to maintain a level of 5-15 ng/ml. Tacrolimus will be tapered after day +86 (adapted dose-reduction to be discontinued by day +180) if there is no evidence of GVHD.

3. Mycophenolic Acid Mofetil (MMF):
    a. Description: MMF is the morpholinylethylester prodrug of the active immunosuppressant mycophenolic acid (MPA). This active metabolite is a noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase, particularly the type II isoform that is more prominent in activated lymphocytes. As a result of the inhibition of de novo purine synthesis, proliferation of T- and B-lymphocytes is blocked and antibody production is inhibited. There are no pharmacokinetic interactions with ganciclovir, cotrimoxazole, oral contraceptives or cyclosporine.
    b. Storage and administration: MMF is commercially available in an oral and an intravenous formulation. The oral formulation is supplied in 250 mg hard gelatin capsules and can be stored at room temperature. MMF for iv administration is supplied as a lyophilized powder in a glass vial containing the equivalent of 500 mg. Starting on day +4, MMF will be given orally or intravenously at a dose of 15 mg/kg tid. If orally, doses will be rounded to the nearest 250 mg (capsules are 250 mg).

H. Modification of immunosuppression for early disease progression or relapse

Guidelines provided in this section are for patients who demonstrate either: i) progression of stable disease present at the time of transplant or ii) relapse of their underlying disease before discontinuation of immunosuppression has been completed. Patients fulfilling the criteria for relapse or progression should undergo a reduction in immunosuppression after careful evaluation for GvHD. In the event that patients with early disease progression or relapse do not have GvHD, immunosuppression should be discontinued. Persistence of stable, underlying disease itself does not mandate accelerated reduction of immunosuppression.

I. Growth Factor Support

Patients will receive G-CSF at 5 μg/kg/d [rounded to the nearest vial size (300 μg or 480 μg)] SC starting at day +4 and continuing until the ANC >500/mm^2 for 3 consecutive days.

J. Infection prophylaxis and therapy

Patients will receive prophylaxis and therapy for bacterial, fungal and viral infections according to Standard Practice Guidelines. Standard CMV monitoring and prophylaxis should commence at the time of transplant and should continue as appropriate.

K. 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 history of CNS disease or evidence of current CNS leukemic involvement will not be excluded, and may be considered for instillation of methotrexate as per the 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.

8.0 PATIENT AND DONOR EVALUATION

A. HLA-typing of patient and potential donors (pre-transplant evaluation and secondary endpoint)

As broad a range of potential donors as possible should be considered. Included would be parents, siblings, and eligible children. HLA-typing should be at least intermediate resolution for HLA-A, HLA-B and HLA-DRB1.

Blood samples should be sent to the Clinical Immunogenetics Lab (CIL) for HLA-typing (green top tube, 10 cc).

B. Donor

Donors will be evaluated according to Standard Practice Guidelines, including blood sample storage by CIL for subsequent determination of donor chimerism.

C. Patient - Pre-transplant Baseline Evaluation

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

a. 10 cc blood in red top tube labeled “Protocol 2186” to Press Lab (D3-395, Thomas Building) for analysis of HAMA (Human Anti-Mouse Antibody).
b. Thyroid function (TSH) test.
c. CT scan of chest and abdomen for calculation of organ volumes (lungs, liver, spleen, kidneys). Other imaging studies as clinically indicated.
d. Unilateral bone marrow biopsy and aspirate for pathology, flow cytometry, and cytogenetics within 30 days prior to consent signing/enrollment.
e. Heparinized blood sample (green top tube, 10 cc) as a pre-transplant reference for subsequent determination of donor chimerism sent to the CIL.

D. Patient Evaluation During Conditioning and for First 100 Days Post-Transplant

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 (within ±7 days of target days listed below):

1. Heparinized blood 10 cc to hematopathology for quantitation of T cell, NK cell and granulocyte chimerism on days +28, +56 (only if day +28 <50 % donor), and +84.
2. Bone marrow aspirate to chimerism lab for chimerism studies on approximately days +28 and +84.
3. Bone marrow aspirate (and biopsy if unable to obtain adequate aspirate sample) to pathology on approximately days +28 and +84. Aspirate to flow cytometry at each time point and to cytogenetics, if patient's disease has a known cytogenetic abnormality.
4. Thyroid function test (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.

9.0 TOXICITIES AND COMPLICATIONS

A. ^{131}I-BC8 Antibody

^{131}I-BC8 Ab 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 Ab itself can cause side effects including fever, chills, nausea,

vomiting, diarrhea, hypotension, dyspnea, joint pain, rash, and anaphylaxis. 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 (GCRC) or current treating location. Patients who are not stable at the end of infusion are admitted for overnight observation at University Hospital. The therapy dose of ^{131}I can cause nausea and vomiting, in addition to myelosuppression and other organ toxicities seen with high dose radiation.

Two separate sorts of toxicities are anticipated, those due to antibody infusions and those due to the radiation delivery and combined preparative regimen.

1. 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 Ab may produce pulmonary, renal or hepatic toxicity as a result of lysis or agglutination of circulating cells. Table 9.1 describes the grading of the most common acute toxicities due to Ab infusion.

Table 9.1: Grading of Acute Toxicities due to Antibody Infusion (from NCI CTCAE version 3.0):

Parameters	1 (Mild)	2 (Moderate)	3 (Severe)	4 (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, ± urticaria; i.v. med(s) indicated; allergy-related edema/angiodema; hypotension	Anaphylaxis
Hepatic:				
1. Bilirubin	>ULN – 1.5 × ULN	>1.5 × ULN – 3.0 × ULN	>3.0 × ULN – 10.0 × ULN	>10.0 × ULN
2. AST	>ULN – 2.5 × ULN	>2.5 × ULN – 5.0 × ULN	>5.0 × ULN – 20.0 × ULN	>20.0 × ULN
Renal:				
Creatinine	>ULN – 1.5 × ULN	>1.5 × ULN – 3.0 × ULN	>3.0 × ULN – 6.0 × ULN	>6.0 × ULN

Modifications for Acute Toxicity: Patients experiencing acute allergic type toxicity during Ab infusion will have the infusion slowed or terminated. If **grade 2 allergic toxicity** is encountered, the infusion should be paused, the patient treated as indicated below, and the infusion not restarted until symptoms have subsided. If **grade 3 allergic toxicity** is encountered and does not resolve using measures described above, the infusion must be terminated and not-restarted, and the patient will be off protocol. **Other grade 2 or 3 acute toxicity** may be treated and the infusion restarted. If toxicity persists or progresses, the infusion will be terminated. Patients with **grade 4 acute toxicity** will have the infusion stopped and further participation in the study will be terminated.

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

Pruritus: diphenhydramine 25-50 mg (or 1 mg/kg) PO or IV every 2-4 hours PRN or ranitidine 50mg IV over 20-30 minutes for patients who do not tolerate diphenhydramine

Nausea: lorazepam 0.5-2 mg (0.05 mg/kg) IV every 4 hours PRN

diphenhydramine 25-50mg (1 mg/kg) IV every 4 hours PRN or

ranitidine 50mg IV over 20-30 minutes for patients who do not tolerate diphenhydramine

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

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 3 hepatic or renal toxicity** after the biodistribution dose, determination of whether or not the patient can proceed to the therapy dose will be made by the P.I. or his designee in consultation with the attending physician, and will depend in part upon the rate of recovery from the laboratory abnormalities. A patient experiencing **grade 4 hepatic or renal toxicity** after the biodistribution dose will not receive the therapy dose and will be treated on an alternate protocol.

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. Fludarabine

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

C. Cyclophosphamide

Clinical toxicities of CY include alopecia, nausea and vomiting, headache and dizziness, hemorrhagic cystitis, cardiotoxicity, immunosuppression, myelosuppression, pulmonary fibrosis, increased hepatic enzymes and syndrome of inappropriate anti-diuretic hormone (SIADH).

D. Mesna

At the doses used for uroprotection, mesna is virtually non-toxic. However, adverse effects which may be attributable to mesna include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

E. Tacrolimus

Adverse reactions include tremor, headache, insomnia, nausea, diarrhea, hypertension, and renal dysfunction (hyperkalemia, increased BUN and creatinine).

Drugs that may increase blood levels of tacrolimus include: macrolide antibiotics, antifungals (fluconazole and itraconazole), calcium channel blockers, cimetidine, danazol, methylprednisone and metoclopramide. Drugs that may decrease blood levels of tacrolimus include: phenobarbital, phenytoin, carbamazepine, rifamycins and the anti-fungal agent caspofungin.

F. Mycophenolic Acid Mofetil (MMF)

Side effects and toxicity: Side effect profiles include diarrhea, leukopenia, sepsis, allergic reactions, and vomiting. MMF use during pregnancy carries a significant risk of miscarriage or birth defects. An increase in certain types of infection mainly from the herpes virus family (CMV, HSV & VZV) and candida has been reported. There have been reports of progressive multifocal leukoencephalopathy (PML), sometimes fatal, and of Pure Red Cell Aplasia (PRCA) in patients receiving MMF as part of an immunosuppressive regimen. MMF has not been studied extensively in patients after HCT. Most common side effects known from studies in patients with solid organ transplants are hematologic (decline in WBC and hematocrit) and gastrointestinal (nausea, vomiting, diarrhea, G.I. bleeding). Experience has shown that nausea can be successfully treated with anti-emetics, and that side effects often respond to a decrease in dose. Several etiologic factors may cause alterations in G.I. and hematologic parameters in the setting of HCT. MMF dose adjustments will therefore be made when clinically indicated if the responsible physician ruled out other possible causes. Dose adjustments should be discussed with the principal investigator. With the exception of hypophosphatemia there seems to be no difference in side effects between i.v. and oral MMF administration and efficacy is the same with both administration routes.

1. If an observed toxicity related to MMF administration occurs in the clinical judgement of the investigator, the MMF dose will be adjusted. Based on previous observations in patients after nonmyeloablative HCT, the side effect most likely to occur will be neutropenia due to myelosuppression. Severe gastrointestinal toxicity such as gastrointestinal hemorrhage has been very rare after nonmyeloablative HCT. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. co-trimoxazole or ganciclovir). Dose adjustments will not be made for neutropenia unless it is severe or persists after day +21. In the rare event of gastrointestinal toxicity that requires medical intervention including 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 at the same dose. For severe toxicity related to MMF (grade 4 neutropenia refractory to G-CSF, severe refractory diarrhea, or overt gastrointestinal bleeding), the MMF may be temporarily stopped. MMF should be restarted at 20% reduced dose when the underlying toxicity subsides. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and Case Report Forms (CRF). MMF will be discontinued on day +35. However, MMF may be discontinued earlier than day +35 at the discretion of the study doctor in patients with no GVHD.

G. Marrow infusion

As per Standard Practice Guidelines.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Event Definitions

- **Adverse Event**
  An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- **Serious Adverse Event**
  A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:
    1. Death.
    2. Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
    3. In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.
    4. Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
    5. Congenital anomaly/birth defect.
    6. An important medical event that requires intervention to prevent one of the above outcomes.
- **Unexpected Adverse Event**
  An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

10.2 Monitoring and Recording AEs

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

- Description of the adverse event using concise medical terminology

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

10.3 Grading of the Severity of an Adverse Event

AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event. However, the Bearman Scale of Regimen-Related Toxicity will be used for decisions regarding dose escalation/ de-escalation and invocation of stopping rules.

10.4 Attribution of Adverse Event

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

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated. For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition

described in 21 CRF 312.32 as an AE for which there is a reasonable possibility that the drug caused the adverse event where "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reactions that are both serious and unrelated will be reported to the FDA as an IND safety report, in accordance with regulations under 21 CFR 312.32.

10.5 Adverse Event Reporting Period

AEs will be monitored and recorded in study-specific case report forms (CRFs). From the time of first exposure to an investigational agent (i.e., the start of the biodistribution dose) through day +100 post-transplant (or discharge prior to that date from the SCCA system to care of the patient's primary physician), all adverse events and all serious adverse events will be captured in protocol-specific case report forms. Beyond day +100, serious adverse events, development of secondary malignancies, disease progression and survival will be collected through 24 months post-transplant. AEs with an onset date prior to the first exposure to an investigational product will not be recorded, except in the case of clinically significant worsening of the AE during the specified monitoring time frame. A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

All deaths except those from relapse of malignancy, but otherwise regardless of attribution, that occur within the first 100 days following transplant will undergo expedited reporting. If the patient has returned to the care of the referring physician prior to day 100 post-transplant, the death will be subject to expedited reporting based on the time we learn of the death. Deaths occurring after 100 days will not undergo expedited reporting although they will be included in Annual Reports and publications of the study.

The following events are *not* identified as AEs in this study:

- Disease progression or relapse. However, clinical events associated with progression/relapse may be reportable as AEs.
- Hospitalization for the purpose of facilitating conditioning and/or stem cell infusion is not considered an AE. Any AE requiring prolongation of this hospitalization will be recorded and subject to applicable SAE reporting.
- Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.

10.6 Adverse Event Reporting Requirements

10.6.1 Research Site Reporting Requirements

Classification of an event as serious or non-serious (see Section 10.1) determines the reporting procedures to be followed by the site for reporting the event to the IND Sponsor. The investigator must report events to the Fred Hutch IRB in accordance with the policies of the IRB.

TABLE 10.1: PI to IND Sponsor Reporting Requirements for Adverse Events

Classification	Reporting Time	Reporting Action	Contact Information

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

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

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

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

10.6.2 Fred Hutch IND Sponsor Reporting Requirements

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

Each serious adverse event report received from the investigator will be evaluated by the Medical Monitor who will assess the seriousness of the event (see Section 10.1), the expectedness of the event (see Section 10.1), and the relationship to participation in the study (see Section 10.4). For regulatory reporting purposes, the IND Sponsor will determine expectedness relating to the investigational product using safety information specified in the Investigator Brochure. An event will be classified as related if either the investigator or the IND Sponsor determines that the event may be related to the study drug.

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

10.7 SAES Associated with Hematopoietic Cell Transplantation (HCT)

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

11.0 DEFINITIONS

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

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

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:

1) 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.

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

12.0 STATISTICAL CONSIDERATIONS (submitted as a separate document)

13.0 DATA AND SAFETY MONITORING PLAN

This is a single institution trial where all patients are followed closely by the investigators. Additionally, the trial design provides rules for dose escalation depending upon the rate of development of Grade III/IV RRT (Bearman Scale). This design mandates ongoing review of the outcome of previous patients treated on study so that the appropriate Dose Level for the current patient can be assigned. The principal investigator, primary research nurse, and study data coordinator communicate routinely (typically weekly) to review recently acquired data, stopping rules, and adverse events. The data

recorded within the research charts and protocol database is compared with the actual data that is available from the medical record and/or clinical histories. Data detailed in the research case report forms includes the nature and severity of all significant toxicities, which are also reported as described above. All investigators on the protocol have received formal training in the ethical conduct of human research.

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

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

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

14.0 DATA MANAGEMENT/CONFIDENTIALITY

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

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

15.0 TARGETED/PLANNED ENROLLMENT

TARGETED/PLANNED ENROLLMENT: Number of Subjects (must provide actual numbers. i.e. no range)			
Ethnic Category	*Sex/Gender*		
	Females	Males	Total
Hispanic or Latino	2	2	4
Not Hispanic or Latino	19	27	46
Ethnic Category Total of All Subjects*	21	29	50
Racial Categories			
American Indian/Alaska Native	1	2	3
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	3	3	6
White	15	22	37
Racial Categories: Total of All Subjects *	21	29	50

16. Termination of Study Participation by Individual Patients

A. Patient request.
B. Unacceptable toxicity during dosimetry antibody infusion (see section 11.A.1 for complete toxicity descriptions and stopping rules).
C. Development of anti-mouse antibodies.

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APPENDIX A: BEARMAN CRITERIA FOR REGIMEN-RELATED TOXICITY

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

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

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

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

Grade 4 Fatal.

A. Cardiac

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

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

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

Grade 4 Fatal toxicity.

B. Bladder

Grade 1 Microscopic hematuria for more than 7 days.

Grade 2 Macroscopic hematuria.

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

Grade 4 Fatal toxicity.

C. Renal

Grade 1 Increase in creatinine up to twice baseline value.

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

Grade 3 Requirement of dialysis.

Grade 4 Fatal toxicity.

D. Pulmonary

Grade 1 Decrease in 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: 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 $\pm$ 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 (τ, 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 computer programs (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, τ_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 μCi-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:

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

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

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

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, τ_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 λ_1 and λ_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).

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, τ_h, by the ratio of the defined reference man or reference woman organ mass to the known organ mass:

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

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

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

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

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

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, 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 C: POTENTIAL ADVERSE EVENTS ASSOCIATED OR EXPECTED WITH HEMATOPOIETIC CELL TRANSPLANTATION

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

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

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

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

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

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

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

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

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

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

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

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