Combined HLA-Matched Bone Marrow and Kidney Transplantation for Multiple Myeloma or AL Amyloidosis with End Stage Renal Disease

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Combined HLA-Matched Bone Marrow and Kidney Transplantation for Multiple Myeloma or AL Amyloidosis with End Stage Renal Disease

Short Title: Combined BMT and Renal Transplant for Hematologic Disorders with ESRD

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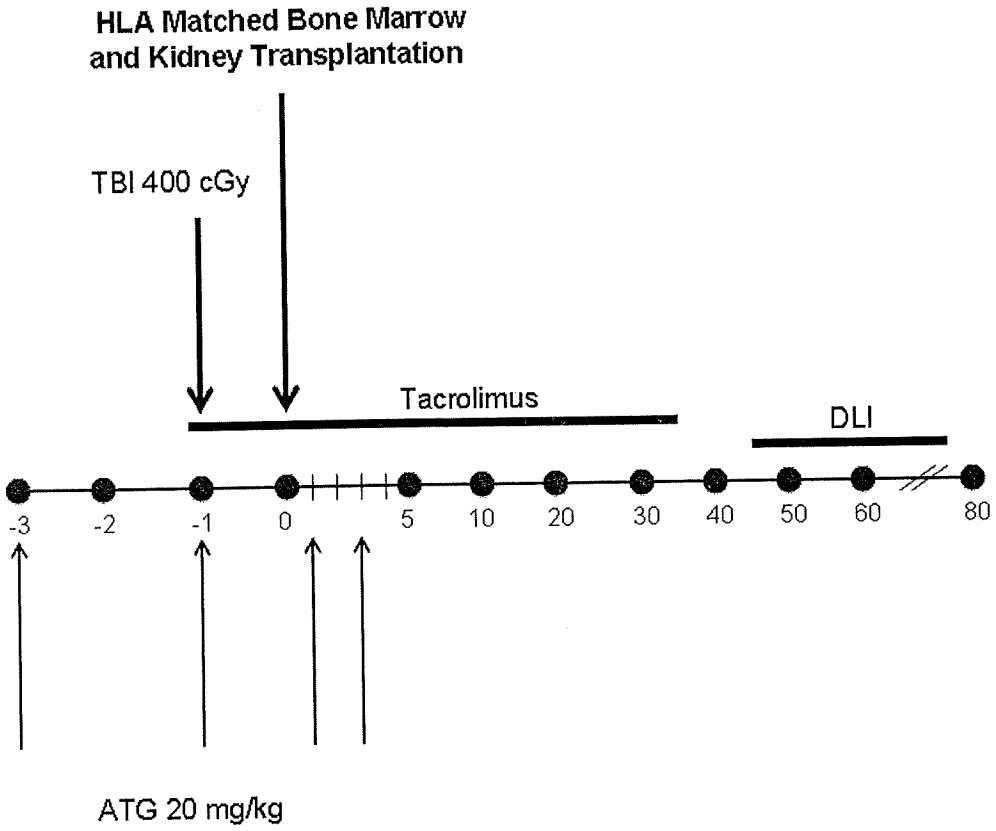
Basic Schema



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1. BACKGROUND AND SIGNIFICANCE

Beginning with the observations of Owen more than 50 years ago [1], it has been known that hematopoietic chimerism, when established in the fetus or neonate, leads to transplantation tolerance [2-4]. Subsequently, it was shown that a similar tolerant state could be achieved in adult rodents receiving allogeneic bone marrow transplantation (BMT) following lethal irradiation [5]. It was later shown that mixed allogeneic chimeras produced by reconstituting lethally irradiated mice with a mixture of T cell-depleted allogeneic and host-type marrow were specifically tolerant of donor tissue grafts [6, 7], with full immunocompetence that was superior to that of full allogeneic chimeras [7-11]. Murine mixed chimeras created with high doses of total lymphoid irradiation also demonstrated donor-specific tolerance [12-15]. Unfortunately, the potential toxicity of these regimens made them unsuitable for the clinical application of allograft tolerance induction. Furthermore, graft-vs-host disease (GVHD) presents a formidable obstacle to BMT when even partial human leukocyte antigen (HLA) barriers are transgressed [16], and amelioration of GVHD by donor marrow T cell depletion increases the risk of failure of marrow engraftment [17], which is high even with the use of unmanipulated marrow [18]. However, recent advances have brought the mixed chimerism approach to tolerance induction to the point that clinical application for selected patients is now possible. These began with the demonstration that fully major histocompatibility complex (MHC)-mismatched marrow engraftment and specific tolerance can be induced in mice receiving allogeneic BMT after non-myeloablative host conditioning with depleting anti-T cell monoclonal antibodies (mAbs), low dose (3 Gy) total body irradiation (TBI), and local thymic irradiation (TI) (7 Gy) [19]. We have further reduced the amount of conditioning used to achieve mixed chimerism by using high stem cell doses in place of TBI, and by replacing both recipient T cell depletion and thymic irradiation with a short course of treatment with co-stimulatory blockade [20-22]. The relatively low toxicity of the conditioning regimens that can be used to achieve mixed chimerism suggests an approach to using BMT for the induction of donor-specific transplantation tolerance and for the treatment of non-malignant diseases such as hemoglobinopathies. In these murine studies, GVHD has been avoided in part because the same reagents used to deplete recipient T cells or tolerize them to donor antigens are still present in the recipient when donor marrow is given, so that the much smaller number of T cells in these inocula are also depleted or inactivated.

Chimerism has generally been more difficult to induce in large animals than in rodents, at least in part due to differences in the types and amounts of irradiation and reagents used in the different species. Recent results in a porcine model have applied approaches developed in mice [19, 20] to achieve mixed chimerism with success [23] (and Fuchimoto et al, J.Clin.Invest.105:1779, 2000). In a non-human primate model, cynomolgus monkeys were conditioned with anti-thymocyte globulin (ATG) fractionated total body irradiation (TBI) (3 Gy), local thymic irradiation (TI)(7 Gy) and splenectomy before transplantation of MHC-mismatched bone marrow and kidney. Cyclosporine was given for four weeks after BMT [24, 25] with no further immunosuppression. Transient multi-lineage chimerism (falling to undetectable levels by flow cytometric analysis, typically by 6-8 weeks post-transplant) has been achieved in association with long-term stable graft function for over 15 years[26].

Mixed chimerism can also be used as an approach to exploiting the graft-vs-leukemia (GVL) effect of GVH-reactive donor T cells while avoiding GVHD. When non-tolerant donor lymphocytes are given to mixed chimeras several months following BMT, mixed chimeras convert to full donor chimeras, but GVHD does not occur [27]. The induction of such lymphohematopoietic GVH reactions without GVHD could provide an approach to separating GVL from GVHD in patients with hematologic malignancies. To make it more cytoreductive for such malignancies, we modified the non-myeloablative conditioning regimen described above by replacing TBI with cyclophosphamide. Treatment of mice with anti-CD4 and -CD8 mAbs on Day –5, 200 mg/kg cyclophosphamide on Day –1, and 7 Gy TI on Day 0 was only slightly myelosuppressive, and allowed fully MHC-mismatched allogeneic bone marrow to engraft and establish long-term mixed chimerism [28]. The administration of non-tolerant donor lymphocytes at 5 weeks post-transplant eliminated host hematopoietic cells, leading to full or nearly-full donor chimerism, without causing GVHD. These results suggested that induction of mixed chimerism with non-myeloablative conditioning followed at an appropriate time by DLI might allow lymphohematopoietic GVH reactions, and hence GVL effects, to eliminate hematologic malignancies without causing GVHD. Based on this information and on a primate mixed chimerism model developed by our group [24, 29,30], we initiated a clinical trial in the MGH BMT Unit of mixed chimerism induction followed by delayed donor leukocyte infusion (DLI) for the treatment of advanced hematologic malignancies. The encouraging results obtained in these studies are described in Section 1.1.

Multiple myeloma afflicts 2 to 4 people per 100,000 in various population groups, with increasing incidence with advancing age. Although a variety of effective therapies have been developed in recent years, none of these, including high dose chemoradiotherapy and autologous hematopoietic cell transplantation, have been shown to be curative[31]. While allogeneic BMT can be curative, probably due to an allogeneic graft-vs-leukemia (GVL) effect [32,33], this therapy is not widely applied because the age and general status of the myeloma population make these patients particularly susceptible to the morbidity and mortality associated with standard allogeneic BMT [34], and the chance of shortening survival with allogeneic BMT in patients with this relatively indolent disease is significant. Therefore, this patient population could benefit from allogeneic BMT with a less toxic conditioning approach followed by the delayed use of DLI to achieve GVL effects. A major complication of multiple myeloma, renal dysfunction induced by urinary light chain excretion, afflicts about 50% of patients with the disease. Myeloma patients with renal failure are not generally considered to be candidates for renal allotransplantation because of the poor prognosis associated with their underlying malignancy. Thus, combined renal transplantation with bone marrow transplantation from the same donor, using non-myeloablative, relatively non-toxic conditioning, has the potential to cure the myeloma or other underlying malignancy while allowing acceptance of a donor renal allograft without chronic immunosuppression.

Systemic AL amyloidosis is also a plasma cell neoplasm with an incidence of 0.5-1 per 100,000. Amyloidosis is characterized by the formation of extracellular deposition of fibrillary complexes of immunoglobulin light chains. Renal involvement is common with

nephrotic syndrome being the most common manifestation. Renal failure is uncommon but when it occurs presents a challenge similar to that of multiple myeloma, namely the reluctance to offer a renal transplant in a patient with an underlying neoplasm . Combined bone marrow and renal transplantation offer a potentially curative therapy for the amyloidosis and tolerance for the renal allograft. Similar to multiple myeloma patients must have treatment responsive disease and have received previously a bortezomib containing regimen and/or autologous stem cell transplantation which are standards of care for this disease.Proof of principle that this can be achieved has been obtained in a pilot clinical trial involving 10 patients as described in Section 1.1.

1.1. Pre-clinical and Clinical Data

The initial mixed chimerism protocol evaluated in participants with hematologic malignancies at MGH was based on the murine model described above [28], in which DLI at 5 weeks and later could convert mixed chimeras to full chimeras without causing GVHD. Although the approach developed in the animal studies is best suited to the treatment of relatively indolent hematologic malignancies, the novelty of the approach mandated initiation of studies in a group of participants with advanced and aggressive, otherwise refractory, hematologic malignancies. We therefore initiated a pilot trial using a non-myeloablative conditioning regimen (**Figure 1**), that was based on the murine regimen [28] and on the primate mixed chimerism model described above [28], for participants with refractory hematologic malignancies, who usually had a life expectancy of less than 6 months. They received related HLA-identical or up to two of six HLA (A, B, DR) antigen-mismatched donor bone marrow transplants.

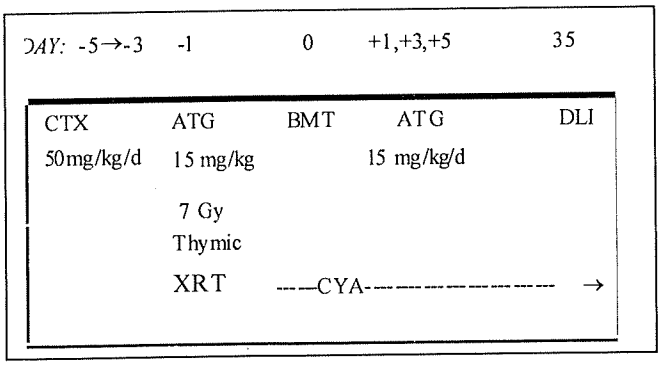

Figure 1. Pilot Trial Non-myeloablative Mixed Chimerism Regimen

The majority of participants on this protocol had refractory non-Hodgkin lymphoma, and a smaller number had refractory Hodgkin lymphoma, acute or chronic leukemia or multiple myeloma. Almost all participants had disease progression during chemotherapy prior to the transplant, and several had relapsed after an autologous stem cell transplant or progressed during or shortly after radiation therapy. Conditioning therapy consisted of cyclophosphamide (CTX) 50 mg/kg daily from Days -6 or -5 through -3; anti-thymocyte globulin, initially at a dose of 30 mg/kg, later reduced to 15 mg/kg given on Days -1, +1, +3 and +5 (now at 20 mg/kg/dose); 7 Gy thymic

irradiation (XRT) (for participants who had not received prior mediastinal radiation therapy) on Day –1; and cyclosporine (CYA) beginning on Day -1. DLI in participants without GVHD were initially scheduled for Days +35 and +56 post-BMT.

The results of this pilot trial were published previously[35,36]. Prominent outcomes included the uniform establishment of mixed chimerism after nonmyeloablative conditioning, even across HLA barriers (Figure 1), and potent and in some cases, sustained anti-tumor responses (Figure 2). Transplant related mortality was low (<10%). Conversion of mixed chimerism to full donor hematopoeisis occurred in the majority of patients who received DLI (Figure 3). Because of a high incidence of GVHD following DLI in the initial cohort of patients, the protocol was amended to use smaller numbers of T-cells and to delay or avoid the DLI if T-cell chimerism was increasing at the time of the intended DLI. These promising early results formed the basis of our subsequent combined bone marrow and kidney transplant trial for patients with multiple myeloma and kidney failure. The acceptable toxicity profile, the reliable induction of mixed chimerism, which could by itself induce specific tolerance for the kidney graft and also serve as a platform for delayed DLI, were the essential principles of the combined transplant trial.

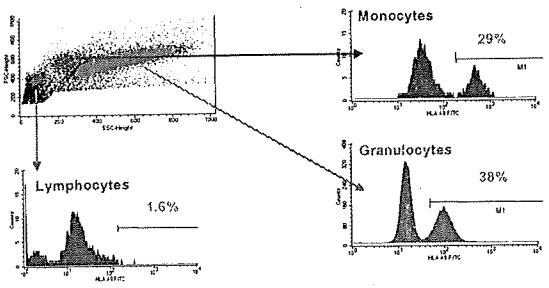

Figure 1. Participant that achieved remission and long-term survival for 3 years with long-term mixed or full chimerism and no ongoing GVHD

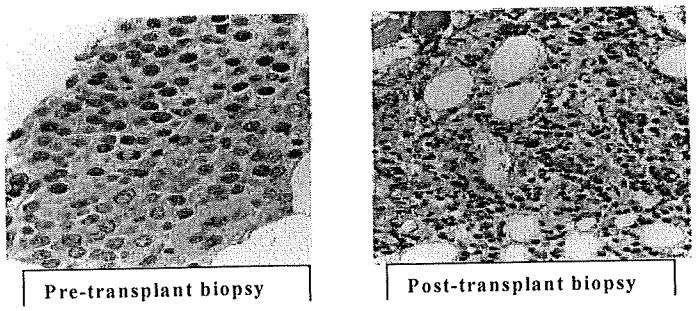

Figure 2. Pre- and Post-BMT bone marrow biopsies in a participant with refractory non-Hodgkin's lymphoma who achieved sustained complete remission on the mixed chimerism protocol. Pre-transplant biopsy demonstrates hypercellularity with diffuse involvement by predominantly small to medium-sized cleaved lymphoma cells (left) Post-transplant bone marrow biopsy (Day +106) shows trilineage hematopoiesis with no evidence of lymphoma (right).

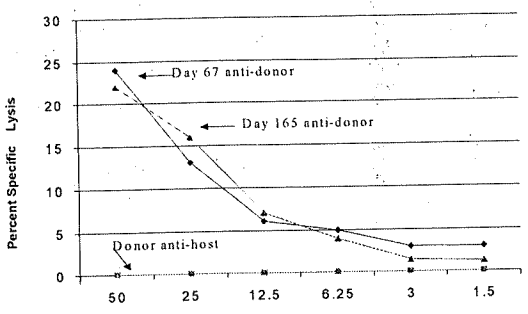

Figure 3. Conversion to full chimerism in recipients of prophylactic DLI on Day 35 +/- Day 56-61

1.2. Simultaneous Kidney and Bone Marrow Transplantation in a Participant with Renal Failure Secondary to Multiple Myeloma

Using a modified version of our original protocol, a simultaneous kidney and bone marrow transplant from the same HLA-identical living donor to a participant with multiple myeloma and consequent renal failure was performed in 1998[37]. The conditioning regimen shown in Figure 1 was modified in view of her lack of renal function, so that she received only two infusions of 60 mg/kg/day of cyclophosphamide on Days –5 and –4. Cyclosporine was begun on Day –1 (5 mg/kg

IV), given orally post-operatively, tapered to a dose of 200 mg/day by the time of discharge on Day 23, and discontinued by Day 73. Platelet and neutrophil engraftment were achieved by Day 12, and no major transplant-related complications developed. WBC chimerism was observed at high levels among T cells and non-T cells for the first 7 weeks post-transplant. However, T cell chimerism declined soon thereafter in association with an increase in host CD8 cell counts, and chimerism in all lineages became undetectable after Day 105 (Figure 4). Donor leukocyte infusions were given on Days 60 and 112, but these did not rescue donor hematopoeisis.

Renal function normalized shortly after transplantation, and no rejection episodes have occurred. She is currently more than 15 years post-transplant with normal renal function (creatinine 0.78 mg/dl in February,2014) with no immunosuppressive therapy since day 73 post-transplant. Approximately 3 years ago she was found to have slowly rising serum free kappa light chains. Two years ago she was found to have 10% plasma cells on bone marrow exam, consistent with early relapse of her myeloma. She has received multiple DLI with stabilization of her disease.

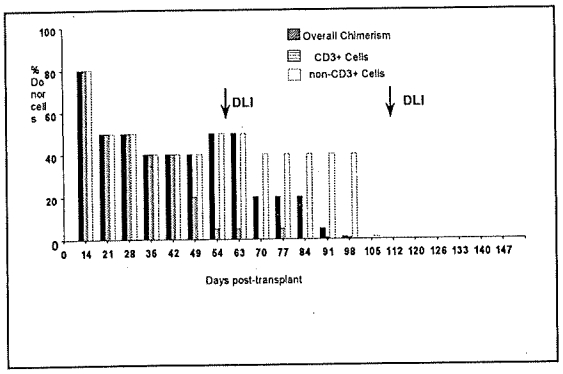

Figure 4. Chimerism at various times in a participant who received simultaneous kidney and bone marrow transplantation from her HLA identical sister.

Cytotoxic T-cell (CTL) assays performed on Days 67 and 165 showed a strong response to the donor (Figure 5), indicating that sensitization had occurred against donor minor histocompatibility antigens. In spite of this, the participant's kidney has been rejection-free off all immunosuppression since Day 73, suggesting that a state of split tolerance has been induced. This CTL response was no longer detectable by 22 months post-transplant, suggesting that a state of tolerance at the CTL level may have eventually developed.

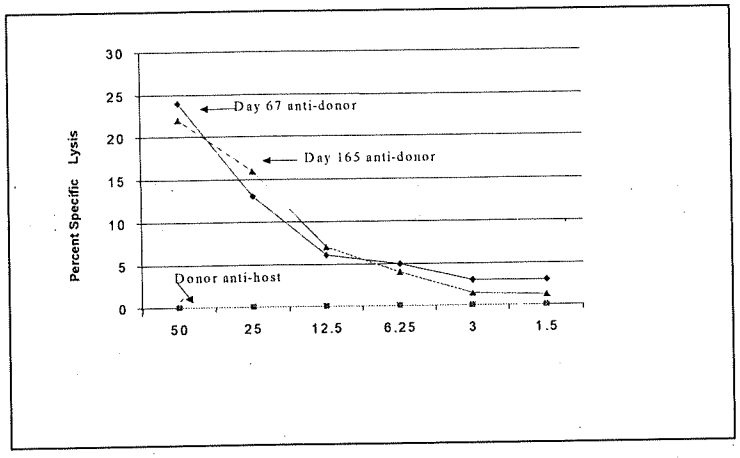

Figure 5. Sensitized anti-donor CTL response measured in bulk cell mediated lympholysis (CML) assay on Days 67 and 165 post-transplant. Participants periphral blood mononuclear cells (PBMC) drawn on Days 67 and 165 were stimulated in vitro with donor PBMC, and cytolytic activity on Cr51-labelled donor target was measured. A non-sensitized donor anti-pre-transplant host response is also shown.

Subsequently, 9 additional patients have undergone combined HLA-matched bone marrow and kidney transplantation for myeloma with renal failure[38,39]. The results of their transplant are shown in Table 1. Six of the patients are alive (two patients died from recurrent myeloma > 7 and > 12 years post-transplant, one patient died from recurrent myeloma and therapy related AML > 4 years post-transplant, and one patient died from metastatic melanoma 4 years post-transplant). Four patients are currently in complete remission (one after a second stem cell transplant); three have full donor chimerism and one patient has stable mixed chimerism.

TABLE 1

Combined HLA-Matched Kd/BMT for MM with ESRD

Pt#	Sustained Donor Chimerism	GVHD	Renal Graft Rejection	Myeloma Remission (years)	Current Status
001	No	No	No	15+	Alive, early relapse @ 15+ yrs
002	No	No	No	3	Died (relapse) @ 7+ yrs
003	Yes	Chronic	No	4	Died (relapse) @ 11+ yrs
004	No*	Chronic*	No	10+	Alive, 2nd CR*@ 11+ yrs
005	Yes	Acute+ chronic	No	None	Died (tAML) at 4+ years
006	No	No	Yes	7	Alive, ? early relapse @ 10+ yrs
007	Yes	Chronic	No	7+	Alive, CR @ 7+ yrs
008	Yes	Acute	No	4	Died (melanoma) @ 4 years
009^	Yes	No	No	3+	Alive, CR @ 3+ years
010	No*	No*	Yes	2	Alive, relapse @ 3+ years

*In CR following 2nd HSCT
^ TBI/ATG conditioning

Limitations of this approach have been the loss of donor chimerism in 5 of the 10 patients and the toxicities of the high dose cyclophosphamide including transient cardiotoxicity in two patients. Another patient developed cyclophosphamide induced cardiotoxicity on day -3 and her transplant was aborted. She later died from progressive myeloma.

Patient 009 developed septic shock and multi-organ failure on day 0 following conditioning therapy cyclophosphamide/ATG and thymic irradiation. The acute deterioration in his condition (and positive blood cultures) were not apparent until

after his sibling donor's bone marrow had been harvested. Because of his clinical condition the donor nephrectomy and kidney transplant were not performed. After recovery, the patient was maintained on hemodialysis until one year later when the combined transplant procedure was reconsidered. Given his prior regimen related toxicities (including reversible cardiomyopathy), it was decided to substitute low dose total body irradiation (TBI) (200 cGy on day -1) for cyclophosphamide as conditioning therapy. Equine ATG (20 mg/kg on days 0 -1, +1, +3, +5) was given on the same schedule. His post-transplant course was uneventful. He developed early mixed chimerism followed by gradual and spontaneous conversion of the chimerism to full donor hematopoeisis in the T-cell and myeloid lineages by week 14 post-transplant (Figure 6). He is currently >5 years post-transplant, off all immunosuppression, with a creatinine of 1.47 mg/dl and minimal chronic GVHD (mild xerophthalmia).

Given the excellent tolerance of the conditioning regimen and achievement of spontaneous full donor chimerism, and a longstanding experience with low dose TBI (with fludarabine) at other centers as conditioning for stem cell transplantation for hematologic malignancies, it was decided to evaluate the efficacy of a revised low dose TBI regimen. Given the renal clearance of fludarabine, and the potential for serious neurologic toxicities of fludarabine in patients treated with renal failure, it was decided to substitute ATG for the fludarabine. As discussed above, ATG has also been incorporated into prior nonmyeloablative transplant regimens for hematologic malignancies at the MGH with good success in terms of inducing mixed chimerism as a platform for delayed DLI.

As our patient who had successful engraftment after 200 cGy of TBI with ATG had extensive prior therapy (icluding two prior autologous transplants), and sustained hematopoietic engraftment has been the biggest hurdle in our previous experience with high dose cyclophosphamide based conditioning, it was decided to use a TBI dose of 400 cGy and to shift the ATG schedule to include two doses of ATG prior to transplant (with two doses post-transplant). 300-400 cGy of TBI (with fludarabine) was used in a multi-center study at the Fred Hutchinson Cancer Research Center/Stanford/University of Leipzig for patients who had rejected a prior hematopoietic cell transplant with sustained donor chimerism achieved in 87% of patients[40].

One patient was enrolled on this protocol with systemic AL amyloidosis and therapy related MDS. She developed early, reversible, acute kidney injury due to engraftment syndrome and late (secondary) bone marrow graft rejection resulting in severe and prolonged pancytopenia. She required a second reduced intensity allogeneic stem cell transplant from the same bone marrow and kidney donor. She achieved engraftment with a high level of donor chimerism and has no evidence of kidney allograft rejection. Because of a higher rate of bone marrow graft rejection with some hematologic malignancies and the adverse consequences of the failure of meaningful autologous bone marrow recovery , the protocol was

amended to include only patients with multiple myeloma and systemic AL amyloidosis. Given our previous experience with the cyclophosphamide/ATG protocol where loss of chimerism was not associated with adverse hematologic consequences and in some cases was associated with a prolonged anti-tumor response, it is believed that patients with these disorders may benefit both from the standpoint of their neoplasm and kidney allograft tolerance.

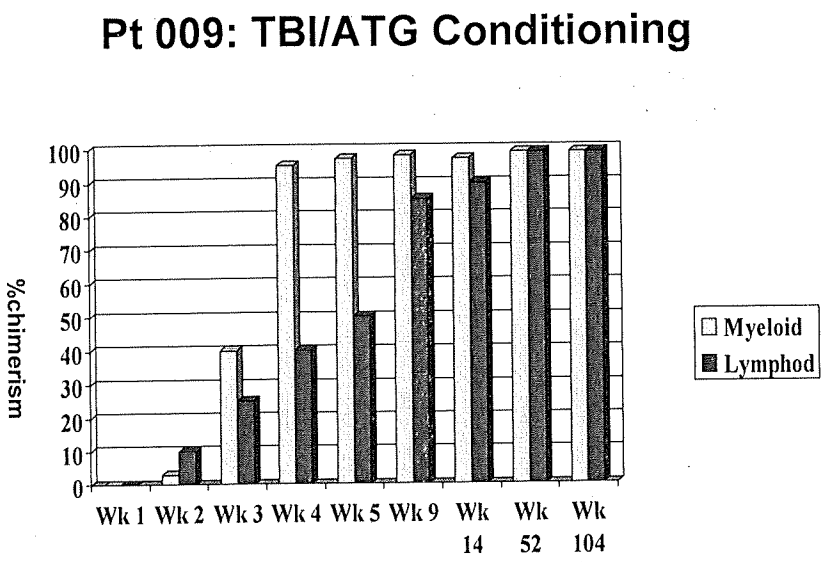

Figure 6: Myeloid and T-cell Chimerism following TBI/ATG and combined HLA-matched bone marrow and kidney transplantation

2. SPECIFIC AIMS

This pilot trial offers the unique opportunity for both the treatment of multiple myeloma or systemic AL amyloidosis for which hematopoietic stem cell transplantation would be appropriate and the reversal of end-stage renal failure, while avoiding the risks associated with long-term standard anti-rejection therapy used in renal transplantation. The primary objectives of this study are to assess renal allograft tolerance, assess anti-tumor response rate in multiple myeloma and amyloidosis, and assess complication rates for allogeneic BMT with donor renal allografting following a conditioning regimen in which TBI has been substituted for high dose cyclophosphamide.

3. SUBJECT SELECTION

3.1. Description of Study Population

Participants aged 18-65 with end-stage renal failure due to or in association with multiple myeloma or systemic AL amyloidosis for which hematopoietic stem cell transplantation would be appropriate will be considered for this clinical trial, pending the approval of the protocol by the hospital's Institutional Review Board. Potential recipients must have acceptable pulmonary, cardiac and liver function, be free of infection, and have an HLA-matched or one of six HLA antigen-mismatched related donor. Single antigen mismatched donors are included because experience in large BMT trials for hematologic malignancies has indicated that overall and disease-free survivals are similar in participants with HLA-identical and one antigen-mismatched related donors. Participants with end stage renal disease (ESRD) due to myeloma will be accepted with all stages of myeloma. Since ESRD usually reflects a high myeloma tumor burden, it is expected that all participants in this trial will have at least International Staging System (ISS) stage II, and most likely stage III, disease. Because there are no known curative therapies for multiple myeloma other than allogeneic BMT, and life expectancy is often short once ESRD develops as a consequence of the disease (with 50% mortality at 30 months and 100% mortality at 57 months in one series [41)], it is considered justifiable to use non-myeloablative allogeneic BMT in this group of participants, regardless of disease stage and prior therapies. Participants in whom the development of ESRD is not due to the underlying myeloma will be included if they have evidence of active myeloma, despite past treatment with standard therapies (patients must have been previously treated with a bortezomib containing regimen given reports of the reversibility of renal failure in some patients treated with these regimens). Similarly for patients with systemic AL amyloidosis renal transplantation is generally not offered because of the underlying amyloidosis and there are no potentially curative therapies other than allogeneic stem cell transplantation. Patients must have irreversible renal failure despite prior treatment with a bortezomib based regimen and/or autologous stem cell transplantation

No racial/ethnic or gender preferences will be considered in this trial, and every effort will be made to ensure that the participant population is representative of the entire area for all three institutions involved. Children under the age of 18 will be excluded because of concerns about the long-term risks of total body irradiation. Multiple myeloma and amyloidosis are diseases of adults, so this criterion will not result in significant exclusions of children. Since there is no gender predominance for multiple myeloma, it is expected that males and females will be enrolled equally into our trial.

3.2. Participant Inclusion Criteria

- Participants with end-stage renal failure due to or in association with multiple myeloma or systemic AL amyloidosis for whom hematopoietic cell transplantation is appropriate and a $\geq$ 50% five-year survival survival probability with transplantation is expected.

- Multiple myeloma (MM), ISS stage II or III in complete or very good partial remission

- AL amyloidosis without significant cardiac disease in partial or complete remission.

- Males or females 18 - 65 years of age.

- Participants must have an HLA-matched or one of six HLA A, B, or DR antigen-mismatched related donor, with high resolution molecular class I and II allele typing.

- Men and women of reproductive potential must agree to use a reliable method of birth control during the treatment, and women should do so for a period of 2 years following the transplant.

- Participants should be on dialysis or have a CrCl <20 ml/min.

- Patients should not have evidence of renal recovery of their renal failure over a 90 day period of therapy for their underlying malignancy or other blood disorder.

- Patients with a history of other malignancies excluding basal cell carcinoma of the skin and carcinoma in situ of the cervix with a disease-free survival interval of >2 years. Patients with the following malignancies must demonstrate a 5 year disease-free survival:
    - Breast cancer with positive nodes
    - Malignant melanoma (other than in situ)
    - Colorectal cancer (other than Dukes Stage A or B1)

- Patients with multiple myeloma must have received previous treatment with a bortezomib-based regimen.
- Patients with AL amyloidosis must have received treatment with a bortezomib-containing regimen and/or autologous stem cell transplantation

- Patients with a history of malignant melanoma must be reviewed by an independent oncologist prior to enrollment.

- Recipient ability to understand and provide informed consent.

3.3. Participant Exclusion Criteria

- Evidence of active infection as defined by: a) clinical syndrome consistent with viral or bacterial infection (e.g., influenza, URI, UTI) or b) fever with a

clinical site of infection identified, or c) microbiologically documented infection, including, but not limited to, bacteremia or septicemia.

- Participation in other investigational drug use at the time of enrollment.
- Contraindication to therapy with any one of the proposed agents (e.g., history of allergy to horse serum in ATG).
- Serologic positivity to HIV or HCV.
- Women of childbearing age in whom adequate contraception cannot be maintained.
- AST/ALT > 3 x normal or bilirubin > 1.5 x normal (unless due to Gilbert's syndrome).
- Pregnancy or uncontrolled serious medical illness not related to underlying myeloma.
- Cardiac ejection fraction < 40% by echocardiogram.
- FEV1 < 50% predicted or corrected DLCO < 50% predicted.
- ABO blood group incompatibility in the host-vs-graft direction.
- Diagnosis of myelodysplastic syndrome

3.4. Donor Inclusion Criteria

- HLA-matched or one of six HLA A, B, or DR antigen-mismatched related male or female donor 18-65 years of age.
- ECOG performance status 0 or 1.
- Excellent health per conventional pre-donor history (medical and psychosocial evaluation).
- Acceptable laboratory parameters (hematology in normal or near-normal range; liver function < 2 times the upper limit of normal and normal creatinine).
- Compatible ABO blood group.
- Negative donor lymphocyte crossmatch.

- No positive testing for viral infection (HbsAg, HIV, HCV, HTLV-1).
- Cardiac/Pulmonary evaluation within normal limits (CXR, EKG).
- Donor ability to understand and provide informed consent.

3.5. Participant Withdrawal Criteria

In addition to a participant being able to voluntarily withdraw from the study at any time, the Investigator may withdraw participants from the study under the conditions noted below.

3.5.1. When and How to Withdraw Participants from Trial

Participants will be immediately withdrawn from the protocol for serious and unexpected adverse experiences that appear to be related to the treatment regimen. Participants will also be withdrawn if they are unwilling or unable to comply with the protocol. Participants withdrawn from the study may not re-enroll under this protocol.

3.5.2. Type and Timing of Data Collected from Withdrawn Participants

Participants who withdraw from the study for any reason will be asked to continue follow-up evaluations on their original protocol schedule, except for unnecessary tests not related to their clinical care (e.g., monitoring of blood levels for medications no longer being taken). At a minimum, the participating center should track the participant and provide health status information, so that some safety information may be obtained through the original follow-up period.

If a participant intends to withdraw and forego further follow-up examinations, they should be asked to complete at least a single, comprehensive examination for study exit, equivalent to the final one-year evaluation, or as much of that exam as they are willing to complete. Documentation of a participant's decision and reason to not continue or complete requested testing should be placed in their study records.

3.5.3. Replacement of Withdrawn Participants

A participant may only be replaced if, in the absence of an adverse event, they withdraw prior to receiving the transplants.

3.5.4. Follow-up for Participants Withdrawn from Treatment

Participants will be followed on an intention-to-treat basis, data from all enrolled participants will be collected, irrespective of outcome, for the scheduled minimum follow-up period of 3 years.

4. SUBJECT ENROLLMENT

4.1. Methods of Enrollment

Patients and donors will be evaluated by both the Bone Marrow Transplant and Renal Transplant programs to determine eligibility. Patients and their donors will be assessed for eligilbity and wrtiten informed consent obtained by separate and independent teams within each program. Patients will be enrolled on this study if this is the preferred treatment plan, the inclusion criteria are met, and signed informed consent is obtained.

4.2. Procedures for Obtaining Consent

Eligible recipients who would like to participate in this study will provide informed consent at a pre-transplant visit with their primary bone marrow transplant physician. The consent will be documented by the patient's signature on the appropriate Partners Institutional Review Board approved consent form. The physician obtaining consent will also sign the form and a copy of the consent will be given to the patient. All subjects must be given a consent form describing this study and provided sufficient information to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedures. The consent form must be signed and dated by the participant, or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file. When existing patients are identified as eligible for this study, they will be given a consent form to take home and read. They will be given the contact information for the BMT research nurse and coordinator and may call with questions at any time. A follow up appointment will be made and the patient will meet with the research nurse and physician to speak about potentially participating in the study. All questions will be answered by the study team during this appointment.

Potential donors will be evaluated for their eligibility and counseled about participation in this study by separate members of the kidney and bone marrow transplant teams on a separate day than the evaluation of the reciepient. They will be given the opportunity to refuse to donate a kidney and bone marrow with assurance that the recipient will not know the reason that a kidney and bone marrow will not be donated. All potential donors will be informed about alternative treatments available to the recipient, will be given ample time to consider participation, and will be offered at least two different study staff members with whom they may ask questions. All eligibility criteria must be met before a donor can participate in this study. Informed consent will be documented by the patient's signature on the appropriate Partners Institutional Review Board approved consent form, as described above..

5. STUDY PROCEDURES

This trial is designed as an open-label, feasibility study of combined HLA-matched bone marrow and kidney transplantation for multiple myeloma or other hematologic disorder for which hematopoietic stem cell transplantation would be ordinarily indicated in association with end-stage renal failure.

5.1. Participation Duration and Follow-up

The active portion of this trial will begin 3 day prior to the transplant. Study-related data will be collected for a minimum of 3 years post-transplant. All participants will be followed indefinitely for survival.

The participant accrual rate is anticipated to be 3 recipient-donor pairs/year with a total enrollment goal of 12 eligible recipient-donor pairs. Therefore, the total study duration will be 7 years.

5.2. Pre-treatment Assessments: Receipient

Screening must be done within 42 days of enrollment. Enrollment evaluations are considered a single visit. A schedule for assessments is included in the Study Calendar in Section 5.5.

- Physical examination (including review of systems, vital signs, height/weight, performance status, and medical history)
- EKG, chest x-ray
- Cardiac function tests (Echocardiogram)
- Pulmonary function test (Spirometry and DLCO)
- Disease assessment and staging (skeletal survey, additional x-rays, CT scans, and MRIs as clinically indicated)
- Hematology including CBC w/ differential, platelets, PT-INR, PTT, blood type (ABO, Rh)
- Chemistries, including electrolytes, BUN, total bilirubin, SrCr, AST/ALT, LDH, Calcium, phosphorus, uric acid, total protein, albumin, AP, calcium, phosphorus, β2 microglobulin (multiple myeloma)
- Virology (serologic and nucleic acid assays for HIV, HCV, HBV, HbsAg, and serology for CMV, EBV, HSV, VZV)
- RPR
- PPD
- Serum pregnancy test
- HLA typing (A, B, DR with high resolution class I and II typing)

- Bone marrow aspirate and biopsy (for patients with hematologic malignancy and history of bone marrow involvement)
- Quantitative serum immunoglobulin analysis
- Serum and urine protein electrophoresis, immunoelectrophoresis, and M protein analysis (multiple myeloma)
- Radiographic survey of the bones (multiple myeloma)
- Evaluation by an independent oncologist of patients with a history of malignant melanoma

5.3. Pre-treatment Assessments : Donor

- Physical examination (including review of systems, vital signs, height/weight, performance status, and medical history)
- EKG, chest x-ray
- Hematology including CBC w/ differential, platelets, PT-INR, PTT, blood type (ABO, Rh)
- Chemistries, including electrolytes, BUN, total bilirubin, SrCr, AST/ALT, LDH, Calcium, phosphorus, uric acid, total protein, albumin, AP, calcium, phosphorus
- Virology (serologic and nucleic acid assays for HIV, HCV, HBV, HbsAg, and serology for CMV, EBV, HSV, VZV)
- RPR
- Serum pregnancy test
- HLA typing (A, B, DR with high resolution class I and II typing)
- Hemoglobin A1c
- Glucose tolerance test for family history of diabetes mellitus
- Anti-islet cell antibody testing for family history of type 1 diabetes mellitus

5.4.

5.4.1. Day -3

- Anti-thymocyte globulin (ATG, Atgam) 20 mg/kg IV over 4-6 hrs. A longer infusion time is acceptable in the event of an infusion-related reaction. ATG is prepared in 250-500 ml of ½ normal saline. The dose of ATG will be based on ideal or actual body weight, whichever is less.
- All participants will receive dexamethasone 10 mg IV 30 minutes before each ATG dose and 12 hours after the start of each ATG dose on Days-3, -1, +1 and +3. Each ATG infusion will also be preceded by Benadryl IV and Tylenol 650 mg.

- Begin antiviral prophylaxis with acyclovir (or a suitable substitute such as famciclovir or valacyclovir)

5.4.2. Day -1

- Anti-emetics: All patients should receive ondansetron (or equivalent 5HT3 receptor antagonist) prior to total body irradiation (TBI) and for at least 24 hours after TBI.
- ATG 20 mg/kg IV over 4-6 hrs.
- TBI 400 cGy will be given in two fractions of 200 cGy each on day -1. Radiation sources and dose rates will be according to institutional standards.
- Tacrolimus will be administered starting on Day –1. The dose will be 0.04 mg/kg orally twice daily until Day 0 when it will be reduced to 0.02 mg/kg twice daily because of the addition of fluconazole. The dose will be adjusted to provide a trough whole blood concentration of 5-10 ng/mL. If patients are unable to tolerate the oral formulation, IV tacrolimus can be used at a dose of 0.02 mg/kg daily (with dose adjustments to maintain a trough level of 5-10 ng/ml) over 20 hours.

5.4.3. Day 0 (Day of Transplant)

- The renal transplant is performed according to standard surgical techniques, preferably using an iliac fossa, extraperitoneal approach.

  Donor bone marrow (intended minimum of 2×10^8 nucleated cells/kg of recipient body weight) is prepared for infusion according to institutional standard procedures. A total of 15,000 units of heparin will be mixed with the marrow in order to prevent clotting of the product. In order to minimize the heparin at the time of infusion (given the risk of intra- and post-operative bleeding), the marrow will be plasma depleted and partially resuspended in saline. The plasma will removed by transferring the marrow product into standard blood transfer pack containers, centrifuging, and expressing the supernatant plasma with a plasma extractor into another transfer pack container. Intravenous 0.9% sodium chloride in a volume equivalent to approximately one half of
  the volume of the expressed plasma is then added to the cellular therapy product. This will also lower the volume of the infusion.
- The marrow will be infused at a rate of approximately 300-500 cc/hr. The infusion will begin in the operating room as soon as the vascular anastamosis of the renal allograft has been completed. A partial thromboplastin time will be measured half way through the infusion (or if bleeding seems excessive) and following completion of the infusion.

Protamine 25 mg may be given intravenously for a PTT of > 60 seconds or for an elevated PTT if that is believed to be the cause of bleeding.

- Post-operative management will follow institutional guidelines.
- Tacrolimus will be administered orally at 0.02 mg/kg twice daily or via IV at 0.02 mg/kg daily over 20 hrs.

5.4.4. Days 1 to 7

- ATG 20 mg/kg IV over 4-6 hrs (Days 1, 3).
- Tacrolimus administered orally at 0.02 mg/kg twice daily or via IV at 0.02 mg/kg daily over 20 hrs (Days 1, 2, 3, 4, 5, 6, 7).

5.4.5. Days 7 to 100

- Chimerism analyses Days 7, 14, 21, 28, 35, 49, 70, 100. Chimerism analyses will be discontinued if for 3 consecutive weeks there is no evidence of donor chimerism.
- Begin tapering of tacrolimus on Day 35 if T cell chimerism on Day 28 is >20% (median of chimerism range). The taper shall be performed progressively over the following 9 days with a goal of discontinuation by Day 44 in participants with evidence of progression of their underlying disease (malignancy) between the pre-transplant and Day 35 time points. In those without evidence of disease progression in this period, the tacrolimus taper will be carried out more slowly with the goal of discontinuation by Day 60. If evidence of GVHD or graft rejection is observed during the taper, the taper shall be suspended and anti-GVHD or anti-rejection therapy will be initiated (Day 35-60).
- Discontinue tacrolimus if no evidence of GVHD or rejection occurs during taper (Day 44 to 60).
- DLI (10^7 CD3 cells/kg) is administered 0 to 14 days following completion of the tacrolimus taper (i.e. between Days 45 and 74) in patients with an underlying malignancy if no GVHD or evidence for renal graft rejection is seen with the tacrolimus taper. The remainder of the donor PBMC will be cryopreserved in liquid nitrogen. DLI will not be administered if there is evidence of renal allograft rejection or active acute GVHD at the time of planned DLI, or biopsy-confirmed overall clinical ≥ grade II GVHD (see Appendix 3 for GVHD grading criteria) at any time. The decision regarding the timing of prophylactic DLI administration (on or following Day +44) will be made according to the guidelines in Table 1. The rationale for this decision algorithm is that increasing T cell chimerism is considered to be evidence of an ongoing graft-vs-host alloresponse, and may mediate graft-vs-tumor effects without the need for a DLI. Since DLI is associated with a significant risk of GVHD, it would be desirable to avoid this additional risk in participants with evidence of an ongoing graft-

vs-host reaction. The rationale for avoiding DLI if donor T cell chimerism is less than 20% is that our experience thus far has indicated that chimerism disappears completely following DLI in participants with less than or equal to 20% donor T cell chimerism [median of chimerism range given] near the time of DLI. Although it is not known whether the loss of chimerism in such participants would occur regardless of whether or not DLI is given, there is concern that this could precipitate graft rejection. However, if a participant shows evidence of disease progression, administration of DLI will be considered. If there is evidence of persistent malignancy following the tacrolimus taper, DLI may also be given despite T cell chimerism ≤20%. In participants with no evidence of malignancy following the tacrolimus taper, administration of DLI will be discussed with the Principal Investigator.

If donor T cell chimerism shows a progressive increase between Days 28 and 42, administration of the DLI will be suspended until a constant or decreasing level of T cell chimerism has been maintained for at least 2 consecutive determinations. If T cell chimerism is stable or decreasing after Day 28 (and at least 20% donor [median of chimerism range given] by Day 42) and there is evidence of disease progression, the DLI will be administered between Day 45 and 58. It is believed that an observation period of one to two weeks following discontinuation of tacrolimus will permit avoidance of DLI in participants who may develop GVHD when tacrolimus prophylaxis is removed, but that a prolonged observation period may be detrimental in participants with progressive disease. The prophylactic DLI will usually be suspended indefinitely if GVHD develops or if T cells demonstrate complete donor chimerism, but this decision will be individualized according to the status of the underlying disease. Such decisions will be made by the Principal Investigator upon discussion of the individual participant. If participants are without evidence of disease progression by Day 35, the tacrolimus taper will be conducted more slowly, with the goal of discontinuation by Day 60 and DLI administration within two weeks following discontinuation if the above conditions are met.

TABLE 2

Day 28 T cell chimerism	>20%	>20%	≤20%	≤20%
Disease progression Day 35	No	Yes	No	Yes
Tacrolimus taper[1]	Days 35-60	Days 35-44	Days 35-60	Discuss with Principal Investigator

DLI[2]	Days 61-74	Days 45-58	Discuss with Principal Investigator	Discuss with Principal Investigator

[1] Tacrolimus taper will be stopped and appropriate therapy initiated if GVHD or rejection develops.
[2] Prophylactic DLI will not be given if GVHD develops during or after tacrolimus taper, if biopsy-proven ≥ Grade II GVHD is present before the time of planned DLI, or if T cell chimerism is completely donor or is increasing at the last determination before planned DLI. DLI may also be held if T cell chimerism declines to ≤ 20% at the last determination before the planned DLI, depending on the status of the myeloma (to be discussed with the Principal Investigator). In the case of increasing T cell chimerism, DLI may be given after mixed T cell chimerism stabilizes on 2 consecutive determinations.

5.4.6. 6 Months to 3 Years Post-transplant

- Recipient PBMC collection and storage (at Month 6, 1 year, 2 years, 3 years)

5.5. Post-transplant Monitoring

A listing of post-transplant follow-up evaluations is provided in the Study Calendar in Section 5.5.

5.5.1. Chimerism Studies

- Chimerism will be monitored using microsatellite analysis of MACS-separated CD3+ (T cells) which will be performed when T cells are present at >30 cells/mm^3 (Days 7, 14, 21, 28, 35, 42, 49, 70, 100, month 6, year 1, year 1.5, year 2). If the participant becomes negative for donor cells or achieves full donor chimerism within the first 100 days following transplant, further testing will be performed weekly for 3 weeks and then stopped unless clinically indicated.

5.5.2. Disease Evaluation

- Bone marrow biopsy and aspirate (pre-transplant, Day 100, 6 months, year 1, year 2).
- Quantification of serum and urine M protein and serum free light chain analyses (Days 35, 70, 100, month 6, year 1, year 1.5, year 2, year 3).
- Quantitative immunoglobulin analysis (Days 7, 14, 21, 28, 42, 56, 70, 84, 100, month 6, year 1, year 1.5, year 2, year 3).
-

5.5.3. Laboratory Evaluation

- Hematology: CBC w/diff, platelets (daily while hospitalized, then weekly up to 100 days, then at month 6, and years 1, 1.5, 2, 3).

- Chemistries: BUN, serum creatinine, Na, Cl, K, CO2, Glu, Ca, Phos, AST, ALT, T.bili/D.bili, Alk Phos, LDH (daily while hospitalized, then weekly up to 100 days, then at month 6, and years 1, 1.5, 2, 3).
- Tacrolimus levels will be measured at least once weekly while hospitalized, then as clinically indicated.
- Cytomegalovirus (CMV) viral load by PCR weekly through day 100
- Epstein Barr virus (EBV) viral load every other week through day 100, then as clinically indicated

5.5.4. Renal Biopsies

Renal biopsies will be performed as clinically indicated for suspected rejection.

5.6. Study Calendar

	STUDY VISIT																									
		Day																					Month				
Assessment	**Screen**	-3	-1	0	1	2	3	5	7	14	21	28	35	42	49	56	63	70	77	84	91	100	6	12	18	24	36
Physical Exam, ROS, EKG, Chest X-ray	X																									
Medical History	X																									
Echo or MUGA	X		X																							
PFTs	X																									
Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events[1]	X								X	X	X	X				X						X	X	X	X	X	X
Hematology Labs[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation Labs	X																										
Chem Panel[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CMV viral load[12]									X	X	X	X	X	X	X	X	X	X	X	X	X	X					
EBV viral load									X			X		X		X		X		X		X					
Virology[3]	X																										
Thyroid Stimulating Hormone	X																						X	X	X	X	X
Pregnancy test	X																										
Urinalysis	X																										
24-hr Urine	X																										
Chimerism Analysis[4]	X								X	X	X	X	X	X	X			X				X	X	X	X	X	

HLA/ABO Typing, Lymphocyte Crossmatch	X																													
Anti-microbial Prophylaxis[5]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							
Antiemetics			X																											
ATG		X	X		X		X																							
TBI			X																											
Tacrolimus Medication[6]			X	X	X	X	X	X	X	X	X	X	X	X	X	X														
Tacrolimus Levels				X[7]	X	X	X	X	X	X	X	X	X	X	X															
Combined Kidney and BM Transplantation				X																										
		Day																								Month				
Assessment	**Screen**	-3	-1	0	1	2	3	5	7	14	21	28	35	42	49	56	63	70	77	84	91	100	6	12	18	24	36			
Post-op Transfusion				X[8]																										
DLI[9]														X																
Disease Evaluation (X-ray, CT, skeletal survey)	X																					X		X	X	X	X			
BM Biopsy	X																					X	X	X	X	X	X			
Urinary Light Chain Analysis	X												X					X				X	X	X	X	X	X			
Serum and Urine Electrophoresis and Immunoelectroph-oresis*	X																													
Serum and Urine M Protein Analysis*	X												X					X				X	X	X	X	X	X			
Quantitative Serum Immunoglobulins*	X				X				X	X	X	X		X		X		X		X		X	X	X	X	X	X			

[1] Assessed throughout the study.
[2] Performed daily while hospitalized. CBC with diff, Plt, BUN, Cr, Na, Cl, K, CO2, Glu, Ca, Phos, AST, ALT, T.bili/D.bili, Alk Phos, LDH.
[3] HIV, HCV, HbsAg, RPR.
[4] If the participant becomes negative for donor cells or achieves full donor chimerism within the first 100 days following transplant, further testing will be performed weekly for 3 weeks and then stopped unless clinically indicated.
[5] See Appendix 2 for specifics.
[6] Oral Tacrolimus will begin tapering on Day 35 if T-cell chimerism on Day 28 is >20%. Discontinue by Day 60 if no evidence of GVHD or rejection occurs during taper (see Section 5.3.5 and Table 2 of protocol).
[7] Tacrolimus levels at least 3x/week while inpatient, then weekly as outpatient, and as clinically indicated.
[8] As needed.

[9] DLI may be administered 0-14 days following completion of Tacrolimus taper (ie. Between Days 45 and 74) if no GVHD or rejection is seen with Tacrolimus taper.
[10] Visits are +/- 3 days for Days 7, 14, 21, 28 and then +/- 7 days for Days 56 and 100.
[11] Visits are +/- 1 month.
[12] By PCR.

5.7. Medications Permitted and not Permitted Before or During Trial

Bactrim and ganciclovir antimicrobial prophylaxis will be suspended between day of transplant and the time of neutrophil engraftment (>500/μl).

5.8. Procedures for Monitoring Participant Compliance

Weekly clinic evaluations will determine whether or not the participants are following instructions regarding notification of physicians of symptoms of infection, rejection, or GVHD. If participants are on tacrolimus, blood levels will provide an assessment of drug compliance. If participants do not appear for clinic appointments, the study nurse and/or physician will contact them.

5.8. Donor Follow-up

Based on Organ Procurement and Transplant Network (OPTN) guidelines, donors will be evaluated at discharge or 6 weeks after donation, whichever comes first, and at 6 months, 1 year and 2 years after donation.

6. STATISTICAL CONSIDERATIONS

This section reflects the change in eligibility criteria to restrict patient population to those with myeloma or amyloidosis only. This change was made following a discussion with the DSMB, after the first patient on the trial with MDS experienced late bone marrow graft rejection. The objective of this pilot study is to evaluate the feasibility and efficacy of the combined HLA-matched bone marrow and kidney transplantation using low dose TBI based conditioning for participants with multiple myeloma or amyloidosis, with end-stage renal failure. Given the novelty of this treatment strategy, this pilot study will accrue 12 eligible patients with multiple myeloma or amyloidosis. In addition to monitoring aGVHD and CTCAE toxicity rate, this revised section also incorporates interim plans to monitor the rate of renal graft rejection and the rate of achieving sustained chimerism.

6.1. Identification of Direct CRF Input Data and Other Source Data

Data will be obtained from a variety of sources including laboratory notebooks, automated instrument output files, and clinical participant charts. No case report forms will serve as source documentation. Data from these source materials will be input into a standardized set of case report forms that will be used in this single center study.

6.2. Primary Endpoint

The primary endpoint is the renal allograft rejection rate at 6 months post-transplant. The study will be considered promising if this rate is less than 10%, and not promising if the rate is $\geq$ 40%. Based on this information, the treatment strategy will be considered acceptable if $\leq$2 patients experience graft rejection by 6 months post-transplant. Using this design, the probability of accepting the treatment is 0.89 if the true rejection rate is 10%, and the probability is 0.083 if the true rejection rate is 40%. Table 1 displays the operating characteristics of this design.

Table 1: Operating characteristics for the primary endpoint

	True but unknown renal graft rejection rate			
	10%	**20%**	**30%**	**40%**
Probability of observing $\leq$2 renal graft rejections in 12 patients	**0.89**	**0.56**	**0.25**	**0.083**

6.3. Safety Monitoring

The following events will be monitored continuously to ensure patient safety: NCI Common Terminology Crtieria for Adverse Events (CTCAE) grade 4 or higher toxicity that are deemed to be (possibly, probably, or definitely) related to treatment; grade III-IV acute GVHD (aGVHD); rate of renal graft rejection, and the proportion of patients who achieve sustained chimerism. Accrual will not stop during the following interim analysis. Rather, these rules will serve as guidelines, and when unexpectedly high event rate is observed, the DSMB will be notified to decide whether the study should be terminated early.

6.3.1. Monitoring Grade 4 or Higher Toxicity

The expected rate of grade 4 or higher treatment related toxicity on this study is 20%. Among the first 6 eligible patients, if 3 or more patients are observed with grade 4 or higher toxicity, the DSMB may consider terminating the study early. Using this rule, the probability that the study may be terminated early is 0.099 if the true grade 4 or higher toxicity is 20% and 0.82 if the true toxicity rate is 60%. Table 2 gives the operating characteristics of this monitoring rule.

Table 2: Operating characteristics for monitoring treatment related toxicity

	True but unknown grade 4 or higher toxicity rate				
	20%	30%	40%	50%	60%
Probability of observing 3 or more grade 3 or higher toxicity in the first 6 patients	**0.099**	**0.26**	**0.46**	**0.66**	**0.82**

6.3.2. Monitoring aGVHD

The expected rate of grade III-IV aGVHD by day 180 is 10%. Among the first 6 eligible patients, if 2 or more patients develop grade III-IV aGVHD, the DSMB may consider to terminate the study early. Using this rule, the probability that the study may be terminated early is 0.11 if the true grade III-IV aGVHD is 10% and 0.89 if the rate is 50%. Table 3 gives the operating characteristics of this monitoring rule.

Table 3: Operating characteristics for monitoring aGVHD

	True but unknown grade III-IV aGVHD rate				
	10%	20%	30%	40%	50%
Probability of observing 2 or more grade III-IV aGVHD in the first 6 patients	**0.11**	**0.34**	**0.58**	**0.77**	**0.89**

6.3.3. Monitoring renal graft rejection rate

Among the first 6 eligible patients, if 2 or more patients experience renal graft rejection, the DSMB may consider to terminate the study early. Using this rule, the probability that the study may be terminated early is 0.11 if the true rate is 10% and 0.89 if the rate is 50%. Table 4 gives the operating characteristics of this monitoring rule.

Table 4: Operating characteristics for monitoring renal graft rejection rate

	True but unknown renal graft rejection rate				
	10%	20%	30%	40%	50%
Probability of observing 2 or more patients with renal graft rejection in the first 6 patients	**0.11**	**0.34**	**0.58**	**0.77**	**0.89**

6.3.4. Monitoring the rate of achieving sustained chimerism

The proportion of patients who achieve sustained chimerism, defined as ≥ 20% donor in both the CD33+ and CD3+ T-cell populations for a minimum of 6 months, is expected to be 90%. Among the first 6 eligible patients, if 4 or less patients achieve sustained chimerism, the DSMB may consider to terminate the study early. Using this rule, the probability that the study may be terminated early is 0.11 if the true rate of achieving sustained chimerism is 90% and 0.89 if the rate is 50%. Table 5 gives the operating characteristics of this monitoring rule.

Table 5: Operating characteristics for monitoring rate of achieving sustained chimerism

	True but unknown rate of achieving sustained chimerism				
	50%	60%	70%	80%	90%
Probability of observing 4 or less patients achieving sustained chimerism in the first 6 patients	**0.89**	**0.77**	**0.58**	**0.34**	**0.11**

1.1. Secondary Endpoints and Analysis Plan

Secondary endpoints include long term renal graft acceptance rate, cumulative incidence of grade II-IV aGVHD, progression-free survival (PFS) and overall survival (OS). PFS is defined as time from transplant to disease progression or death, whichever occurred first, and patients who are alive without disease progression will be censored at last day known alive. OS is defined as time from transplant to death, or last day known alive. Given the small sample size, analysis will be mainly descriptive. For easier interpretation, proportions of patients with renal graft acceptance (1- proportion of graft rejection) at 6 months post-transplant will be reported, along with 90% confidence interval (CI). Long term data regarding proportions of patients with sustained renal graft acceptance will also be calculated and reported.

OS and PFS will be estimated using the Kaplan-Meier method.

1.2. Accrual

Accrual rate is expected to be 3 eligible patients per year, therefore it will take approximately 4 years to complete accrual of 12 eligible patients, after the amendment activates.

1.3. Description of "Stopping Rules" for Individuals

Participants will be removed from the study if they experience serious and unexpected adverse events related to the treatment regimen prior to receiving the transplant, or if they are unable or unwilling to comply with the protocol.

2. RISKS AND DISCOMFORTS

2.1. Risks

Participation in this trial is associated with certain risks for both the recipient and donor.

2.1.1. Risks to the Recipient

- **Total body irradiation.** TBI may cause nausea, vomiting, mouth sores and diarrhea. It may cause lowering of blood counts with a resultant risk of infection or bleeding. Other side effects include dry mouth, parotitis, late cataract formation, and occasional pneumonitis (unlikely at this low dose). TBI may result in late (secondary) cancers.
- **Tacrolimus. Side effects** of tacrolimus include renal insufficiency, abnormal liver function studies, hypertension, seizures, nausea, vomiting, excessive hair growth, confusion, hypomagnesemia, tremulousness, and increased risk of secondary malignancies.
- **Anti-thymocyte globulin.** Side effects of ATG include immune suppression, nausea, vomiting, fever, chills, myalgia, and serum sickness (characterized by

fever, rash, arthralgias and joint swelling, and abnormalities of kidney function).

- **Multi-organ failure.** There is a risk of multi-organ failure including cardiac, renal, pulmonary, CNS, and hepatic failure. This risk is increased in participants who have already had significant chemotherapy or radiation therapy or both. These risks may be life threatening and lead to fatal complications.
- **Relapse.** Relapse of the underlying malignancy may occur particularly in patients who are not in CR at the time of their transplant. There is also a possibility that the radiation therapy may lead to a secondary malignancy months or years following the transplant. Because of the risk of genetic damage, all women of childbearing years will have a pregnancy test before starting the treatment.
- **Secondary Malignancy.** There is a possibility that immunosuppressive therapy for the transplant and the exposure to radiation therapy may lead to a secondary malignancy months or years following the transplant.This risk is difficult to quantify given that secondary,malignancy risk is dependent on patient age, underlying disease, dose of TBI (which in this protocol is substantially less than the myeloablative TBI doses ($\geq$ 12Gy) that have been used for most hematopoietic cell transplants, and the presence of GVHD particularly chronic GVHD. Nonetheless, an approximately 3% risk of secondary myeloid malignancy (AML or MDS) has been reported after allogeneic BMT and an approximately two to fourfold increased risk of late solid tumors compared to the general population has been observed in this patient population. Skin cancer is prevelant particularly in patients with chronic GVHD and if long term imunosuppression is required.. Post-transplant lymphoproliferative disorder (PTLD) is a risk of the systemic immunosuppression required for kidney and bone marrow transplantation but should be a lower risk than after non-HLA matched donor kidney and bone marrow transplantation. With tolerance induction and discontinuation of immunosuppression, this risk should be even lower.
- **Graft-vs-host disease.** Acute and/or chronic GVHD are common complications of allogeneic transplantation (30-90% acute GVHD risk depending on degree of HLA match and participant age group; 40-60% chronic GVHD risk with conventional transplants). In our series of 10 patients receiving HLA-identical combined bone marrow and kidney transplants, 2 developed acute and 3 chronic GVHD.
- **Renal graft rejection:** This occurred in 2 of ten patients in our previous trial, but was transient, and was effectively managed with systemic immunosuppressive therapy. Both patients achieved normal or near normal long term renal function, one who requires no immunosuppressive therapy and one who is on low dose immunosuppressive therapy for GVHD

- **Bone marrow graft rejection:** While half of the patients in our previous trial lost evidence of donor chimerism over time, in no instance did it cause significant impairment of autologous marrow recovery and in 3 of the 5 cases was associated with prolonged anti-tumor responses. The first patient on this trial with MDS had late bone marrow graft rejection and failed to recover meaningful autologous marrow reconstitution, necessitating a second reduced intensity allogeneic stem cell transplant from the original bone marrow and kidney donor. For this reason the protocol has been amended to include only patients with multiple myeloma and systemic AL amyloidosis
-
- **Risks of surgery.** While it is recognized that the transient pancytopenia and temporary T cell depletion induced by conditioning for BMT under our protocol could theoretically lead to an increased risk of infection post-transplant, our experience with over 100 primate renal allografts performed with a similar type of protocol and with the 2 participants receiving a simultaneous kidney and marrow transplant at MGH has been that infection is not a major problem. This probably reflects the high level of attentiveness to potential infectious complications among the surgical and BMT staff and the prophylactic anti-microbial treatments that are used.
- **Transplant-related mortality.** In our previous trial of non-myeloablative BMT for hematologic malignancies upon which this protocol is based, the incidence of transplant-related, non-disease-related mortality was < 10%. There has been no transplant related mortality among the 11 patients who have received a combined HLA-matched bone marrow and kidney transplant.
- **Renal biopsy.** This procedure is performed percutaneously under ultrasound guidance with an 18-gauge needle. It will only be performed in participants with platelet counts >50,000. The most frequent complication of temporary, self -limited bleeding occurs in approximately 2% of allograft recipients at the MGH. There has been no significant allograft dysfunction or loss of an allograft due to a biopsy in over 500 consecutive participants. The procedure is performed in the outpatient Radiology suite, and the subject is monitored for stability in the Transplant Unit for approximately 8 hours prior to discharge on the evening of the biopsy.

2.1.2. Risks to the Donor

- **Bone marrow donation.** Risks of bone marrow donation are related to either the administration of anesthesia or the operative procedure itself. Risks of anesthesia include but are not limited to postoperative nausea, vomiting, fatigue and rare life-threatening allergic reactions or hemodynamic instability. Most donors experience postoperative discomfort at the sites of bone marrow harvest. On average the soreness and bruising lasts for approximately 2-3 weeks post-harvest. A small percentage of donors (less than 10%) experience prolonged (weeks or months) pain or sciatic type pain, likely as the result of inflammation of

the nerve roots from local bleeding or edema. Rare complications of bone marrow harvesting include local infection (including reports of osteomyelitis), pelvic fracture, phlegmon formation, pancreatitis, ileus, and fat embolism.

Fatal complications following bone marrow harvesting are exceptionally rare and have been related to cardiopulmonary complications secondary to anesthesia or other catastrophic events such as pulmonary embolism.

- **Kidney donation.** Risks of kidney donation include the same anesthesia related complications described above. The major postoperative morbidity associated with donor surgery includes pneumonia, wound infection, hematoma, phlebitis, pulmonary embolism, and urinary tract infection. The surgical complication rate for kidney donation is approximately 2%. The perioperative mortality for a living kidney donor is estimated to be 0.03%. Although uninephrectomy of living donors has been associated with proteinuria and hypertension 6-10 years following nephrectomy, careful study of long-term outcome in kidney donors (up to 23 years) and army personnel who lost kidneys due to trauma during World War II (45 years follow up) did not substantiate an increased risk of hypertension or renal failure with uninephrectomy.

2.2. Procedures to Minimize Risks to the Recipient

2.2.1. Evaluations

- Extensive pre-transplant medical evaluations to ensure adequate underlying organ function will be performed.
- Specific interventions to provide palliation include anti-emetic therapy to prevent and ameliorate radiation side effects including nausea and vomiting, prophylactic anti-infectives to prevent opportunistic infections, and other symptomatic measures as needed.
- A physical therapy consult will be obtained on admission to assess level of activity. Participants will be followed by the physical therapy team following the transplant to assist in preserving muscle tone, strength, and coordination.
- Outpatients will have a formal evaluation by the Bone Marrow Transplant social worker prior to admission. When appropriate, a psychiatric evaluation will be undertaken at that time. Following transplant, the participants will be followed closely by the social worker and the Psychiatric team as needed.

2.3. Treatment of Rejection Episodes

If participants show evidence of graft rejection, as evidenced by increasing serum creatinine levels, a diagnostic renal biopsy will be obtained, and standard rejection therapy will be initiated. This consists of pulse Solumedrol 500 mg IV over 20 minutes. If rejection persists after 2 Solumedrol pulses, OKT3 treatment 5 mg IV daily or Thymoglobulin 1 mg/kg IV daily will typically be given for 5-10 days, depending on the time of return of creatinine levels to baseline. If the rejection

episode occurs after discontinuation of Tacrolimus, Tacrolimus therapy will be re-initiated during the period of anti-lymphocyte antibody treatment. Prednisone and mycophenolate will be added to the maintenance therapy, and maintenance Neoral or Gengraf, Prednisone, and mycophenolate dose reductions will be conducted according to the usual clinical criteria over time (see Appendix 1: Acute Renal Rejection Treatment Protocol). It is recognized that graft failure and return to dialysis may occur despite these measures, and such an outcome will be included in the consent forms and discussed with the participant.

2.4. Treatment of Disease Recurrence

This therapy will be individualized according to the treatment that participants have already received, their responses, and their disease stages. Given that end-stage renal disease is usually an indication of advanced multiple myeloma, most participants on this trial will have already undergone other anti-myeloma therapies. These often include treatment with novel agents such as bortezomib and lenalidomide, corticosteroids, and high-dose chemotherapy with autologous stem cell transplantation. Other malignancies will have also likely been treated prior to transplantation. In the event that relapses occur in participants on this study, we will consider treatment with donor leukocyte infusions and withdrawal of immunosuppressive therapy where appropriate, salvage chemotherapy, or other experimental therapies. Consideration of all these therapies will be individualized according to the participants' prior therapies, disease responsiveness, chimerism status, presence of GVHD, etc.

3. POTENTIAL BENEFITS

3.1. Benefits

Combined renal transplantation with bone marrow transplantation from the same donor, using non-myeloablative, relatively non-toxic conditioning, has the potential to cure the underlying malignancy or other blood dyscrasia while allowing acceptance of a donor renal allograft without chronic immunosuppression. This therapy has been undertaken in an attempt to offer potential cure to a group of patients with no other curative therapies available to them, to lessen hematopoietic cell transplant-related toxicities, induce mixed hematopoietic chimerism and resistance to subsequent DLI-induced GVHD, to take advantage of the potentially powerful anti-tumor activity of adoptive immunotherapy (via DLI), and to offer renal transplantation and hence freedom from dialysis to this group of patients who is otherwise ineligible for renal transplantation.

4. MONITORING AND QUALITY ASSURANCE

4.1. Independent Monitoring of Source Data

All participant-related data are recorded on standard inpatient flow sheets and entered into unique bone marrow transplant and organ transplant computerized databases at the clinical sites.

4.2. Data Safety Monitoring Board Charter

Membership
The DSMB will consist of three physicians, two of whom are independent of the MGH (Dr David Avigan from BIDMC and Dr Joe Antin from the DFCI) and one of whom is an oncologist in the Myeloma Center at the MGH (Dr. Noopur Raje).
Responsibilities
The responsibility of the DSMB is to review the clinical outcomes of the patients enrolled on the protocol, with an emphasis on adverse events and excessive toxicities, both expected and unexpected. Disease-free and overall survival will be reviewed and causes of mortality (nonrelapse and relapse related) will be fully evaluated. The DSMB will be cognizant of the stopping rules for the protocol as defined in protocol section 6.3 and inform the principal investigator and the PHRC of any concerns about continuation of the study.
Timelines
The DSMB will meet after every three subjects are enrolled or at least yearly should less than three subjects be enrolled during that time frame.
All DSMB reports will be submitted to the PHRC in real-time (defined as submission of reports within two weeks of the meetings).
It is expected that the principal investigator will respond in writing to the DSMB report and make any recommended protocol revisions within two weeks of the reports.

4.3. Specification of Safety Parameters

The following measures are recorded as clinical safety measures for this study. The Study Calendar in Section 5.5 indicates frequency of each measure.

a) Adverse event and toxicity assessments (recorded at any time necessary)
b) Labs: hematology and chemistry
c) Renal function tests
d) Immunosuppressive drug (tacrolimus) levels
e) Assessment of Graft-vs-host Disease
f) Opportunistic infections

4.4. Adverse Event Reporting

For **interventional** studies, the principal investigator must complete appropriate Adverse Event Form and forward to the IRB of record for the protocol within the required time frame for reporting, but in no case beyond these time frames. Investigators are encouraged to report events as soon as possible within the required time frame. Regular reporting is within 5-7 days of the event and rapid reporting is

within 24 hours of the event. Refer to the table below for reporting requirements for **interventional** studies.

Interventional Studies			
SEVERITY OF EVENT	NATURE OF EVENT	RELATIONSHIP OF EVENT	REPORTING TO PHRC
Serious	Expected* Unexpected	Definitely Probably Possibly Unlikely Unrelated	Report immediately (within 24 hours of event) by telephone, fax, or email followed by a full written report using the PHRC Adverse Event Form within 5 working days/7 calendar days
Mild Moderate	Unexpected	Definitely Probably Possibly	Report in writing using the PHRC Adverse Event Form within 5 working days/7 calendar days
Mild Moderate	Unexpected	Unlikely Unrelated	Summarize events in progress report at continuing review unless the study is being monitored by the sponsor and the FDA under an IND or IDE, in which case FDA regulations do not require reporting to the IRB
Mild Moderate	Expected	Definitely Probably Possibly Unlikely	Summarize events in progress report at continuing review
(1) Report all serious adverse events that occur after active study participation for a minimum of 30 days post study discontinuation or as specified in the protocol, whichever time period is greater if the event is thought to be possibly, probably, or definitely related to the study drug, biologic, device or other study-related intervention or diagnostic procedure. (2) *Reporting of expected serious adverse events to the IRB is not required prior to continuing review for NIH-sponsored cooperative Group trials, such as NCI sponsored oncology trials or certain AIDS trials. Refer to the sponsor's protocol for specific reporting requirements.			

Serious adverse events (21 CFR 312.32) are events that result in any of the following outcomes: death; a life threatening experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability/incapacity; or a congenital anomaly/birth defect. In addition, events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may

jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Mild adverse events: Awareness of signs or symptoms, but easily tolerated; are of minor irritant type; causing no loss of time from normal activities; symptoms would not require medication or a medical evaluation; signs and symptoms are transient.

Moderate adverse events: Discomfort severe enough to cause interference with usual activities; persistent or requiring treatment.

Unexpected adverse events (21 CFR 312.32) are defined as any event, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigative plan or elsewhere in the current application, as amended. "Unexpected", as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents.

Expected adverse events are defined as any event, the specificity or severity of which is consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is consistent with the risk information described in the general investigative plan or elsewhere in the current application, as amended.

4.5. Adverse Event Severity Grading

Severity grades are assigned by the study site to indicate the severity of adverse experiences. The CTCAE document can be viewed at the NCI website (http://ctep.cancer.gov/reporting/CTCAE.html). The purpose of using the NCI-CTCAE system is to provide a standard language to describe adverse events, to facilitate tabulation and analysis of the data, and to facilitate the assessment of the clinical significance of treatment-related toxicities. The NCI-CTCAE provides a term and a grade that closely describes the adverse event. Copies of the grading scales and event descriptions are provided to each participating site. The NCI-CTCAE grade for each adverse event should be associated with a severity category: Grade 1 (Mild), Grade 2 (Moderate), Grade 3 (Severe), and Grade 4 (Life-threatening).

If the adverse event is not included in the NCI-CTCAE, the following general definitions should be used in determining severity:

Grade 1 Mild	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, e.g., aspirin for simple headache, acetaminophen for post-surgical pain). Mild adverse effects are an expected consequence of the bone marrow transplant protocol used here, and standard supportive therapies (per institutional guidelines) are permitted.
Grade 2 Moderate	Mild to moderate limitation in activity, some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 3 Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible.
Grade 4 Life-threatening	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required, hospitalization or hospice care probable.
Grade 5 Death	Death.

4.6. Relation to Therapy

- **Associated:** There is a reasonable possibility that the event may have been caused by the test product and/or procedure. This definition applies to those adverse events that are considered definitely, probably and possibly or unlikely related to the test article.
- **Definitely Related:** An adverse event that follows a temporal sequence from administration of the test product and/or procedure; follows a known response pattern to the test article and/or procedure; and, when appropriate to the protocol, is confirmed by improvement after stopping the test product (positive dechallenge: and by reappearance of the reaction after repeat exposure (positive rechallenge); and cannot be reasonably explained by known characteristics of the participant's clinical state or by other therapies.
- **Probably Related:** An adverse event that follows a reasonable temporal sequence from administration of the test product and/or procedure; follows a known response pattern to the test product and/or procedure, is confirmed by improvement after dechallenge; and cannot be reasonably explained by the known characteristics of the participant's clinical state or other therapies.

- **Possibly Related:** An adverse event that follows a reasonable temporal; sequence from administration of the test product and/or procedure and follows a known response pattern to the test product and/or procedure, but could have been produced by the participant's clinical state or by other therapies.
- **Unlikely Related:** An adverse event that may still be related to the test product and/or procedure but is more likely explained by the participant's disease state or other therapies.
- **Not Associated:** An adverse event for which sufficient information exists to indicate that the etiology is not related to the test product and/or therapy.
- **Unrelated:** An adverse event that does not follow a reasonable temporal sequence after administration of the test product and/or procedure; and most likely is explained by the participant's clinical disease state or by other therapies. In addition, a negative dechallenge and/or rechallenge to the test article and/or procedure would support an unrelated relationship.

4.7. Data Handling and Recordkeeping

Standard procedures will be used to ensure that data are as complete and accurate as possible. All participant-related data are recorded on standard inpatient flow sheets and entered into unique bone marrow transplant and organ transplant computerized databases at the clinical sites. In analyses, a full accounting will be made for all data items. For any outcome measure, cases with incomplete values will be omitted

4.8. Procedures for Reporting any Deviations from Original Statistical Plan

The principle features of the design of this study and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principle features would require a protocol amendment, which would be subject to review by the PI.

4.9. Selection of Participants to be Included in Analyses

Participants will be followed on an intention to treat basis. Data from all enrolled participants will be collected, regardless of level of compliance with, or the outcome of, planned therapy.

5. REGULATORY AND ETHICAL OBLIGATIONS

5.1. Declaration of Helskinki

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the participant.

5.2. Informed Consent

Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their families. Consent forms describing in detail the treatment procedure and risks are given to the participant and the donor and written informed consent is required prior to starting treatment. Informed consent forms will be IRB approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants can feel free to discuss the study with their surrogates or think about it prior to agreeing to participate. The participants can withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. No modification or waiver of elements of the consent or requirements for documentation of consent has been authorized by the hospital Institutional Review Boards. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. There is no financial incentive to participate in this study. There is no concern that individuals will feel coerced by economic pressure. Children under the age of 18 will not be considered as recipients or donors in this study. In order to assure that participants understand the information provided in the informed consent document, they will be asked to state in their own words their understanding of the information provided about the study and as reviewed in the informed consent document.

Participant confidentiality is strictly held in trust by the participating Investigators, their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

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Appendix 1

Acute Renal Rejection Treatment Protocols

I. MGH Acute Cellular Rejection Protocol

A. Steroid Pulse Treatment

1. Daily Prednisone dosage unchanged
2. Solumedrol, 500 mg IV in 50 cc D5W, given as a 20 minute infusion;

Monoclonal Ab Treatment

If rejection (biopsy-confirmed) persists after 2 Solumedrol pulses, OKT3 or other mAb Rx may be added (only for participants not sensitized after previous therapy). OKT3 may be used initially if rejection grade is IIb or more.

1. One red top tube (10cc) to tissue typing lab pre-OKT3 Rx
2. Solumedrol: 500 mg IV, 1-4 hours before initial OKT3 dose
3. Solucortef: 100 mg IV, 30 minutes after initial OKT3 dose
4. OKT3: 5 mg IV, daily x 10-14 days
5. Prednisone dose unchanged
6. Neoral or Gengraf is reduced by at least 50%
7. After at least 3 days of OKT3 discontinue Neoral or Gengraf and start FK506 (24 hours after Neoral or Gengraf has been discontinued) at 0.05 mg/kg bid PO, aiming for levels of 10-15 ng/ml to be reached before OKT3 completion.
8. If on MMF, reduce dose by 50% until 3 days prior to discontinuing OKT3, then return to pre-rejection maintenance dose. MMF dosage may be further altered if leukopenia or thrombocytopenia develops.
9. If on Imuran, reduce Imuran to 50 mg/day until 3 days prior to discontinuing OKT3; then return to pre-rejection maintenance level.

B. Thymoglobulin Treatment (for Participants Sensitized to OKT3)

1. Thymoglobulin is usually begun at a dose of 1 mg/kg (in 200 cc of ½NS).
2. Subsequent daily dosage is usually 0.75-1.0 mg/kg mg/kg (in 200 cc of ½NS) and may be modified dependent upon lymphocyte monitoring. Thymoglobulin will be administered for 5-10 days.
3. Prednisone dosage unchanged.
4. Neoral or Gengraf is reduced by at least 50%.
5. After at least 3 days of Thymoglobulin discontinue Neoral or Gengraf and start FK506 (24 hours after Neoral has been discontinued) at 0.05 mg/kg bid PO, aiming for levels of 10-15 ng/ml to be reached before ATGAM completion.
6. If on MMF, reduce dose by 50% until 3 days prior to discontinuing Thymoglobulin, then return to pre-rejection maintenance dose. MMF

dosage may be further altered if leukopenia or thrombocytopenia develops.

7. If on Imuran, reduce Imuran to 50 mg/day until 3 days prior to discontinuing ATGAM; then return to pre-rejection maintenance level.

Appendix 2

Institutional Guidelines for Antimicrobial Prophylaxis

1. Antimicrobial Prophylaxis for Surgery

Cefazolin, 1 g IV will be administered in the pre-operative period just prior to the transplant procedure. In penicillin/cephalosporin allergic participants in whom a cephalosporin is contraindicated, vancomycin, 1 g IV and aztreonam 500 mg IV will be administered in place of cefazolin. Only a single dose of prophylactic antibiotic is required.

2. Antimicrobial Prophylaxis for Pneumocytosis and UTI

Trimethoprim/sulfamethoxazole (TMP/SMX) 1 single strength tablet daily starting at the initiation of the conditioning regimen (Day -3) and continuing for the first 6 months post-transplant will be administered to all participants. This prophylaxis is interrupted beginning on the day of transplantation until the day when neutrophil counts exceed 500/μl. In the event of allergy or intolerance to the components of TMP/SMX, atovaquone, 1.5 g PO each day or an alternative prophylaxis regimen will be used. Because of the potential for bone marrow suppression with TMP/SMX, participants must be monitored carefully during the first month post-transplant, and the alternative regimen used in the case of possible TMP/SMX-related bone marrow suppression. After the first month following normalization of hematologic status, participants should be switched back to TMP/SMX if there is no contraindication. This prophylaxis may be interrupted briefly in the peri-operative period if participants are unable to take oral medications. Consideration will be given to extending the period of prophylaxis for an additional 6 months if it is felt to be clinically indicated, since TMP/SMX provides prophylaxis against the majority of isolates of the pneumococcus and H. influenzaesplenectomy prophylaxis, below.

Levofloxacin 250-500 mg daily (or a suitable quinolone substitute) will be started at the onset of neutropenia (ANC < 0.5) and continued until resolution of the neutropenia

3. Fungal/yeast Prophylaxis

Clotrimazole troches 10 mg three times a day will be administered starting on Day -3 and continuing for the first 3 months post-transplant. Alternatively, Nystatin swish and swallow, 5cc PO BID can be used. Fluconazole 200 mg daily will be started on transplant Day 11 and continued until at least resolution of neutropenia. Clotrimazole or Nystatin therapy may be held during fluconazole administration. This prophylaxis may be interrupted briefly in the peri-operative period if participants are unable to take these medications.

4. CMV Infection

Anti-viral prophylaxis: Cytomegalovirus prophylaxis is central to the successful outcome of bone marrow transplantation (BMT) and organ transplantation. All participants receive an anti-viral prophylaxis specifically adjusted to the expected risk of viral activation. Acyclovir (400 mg PO TID or 5 mg/kg/ q8h IV) or a suitable substitute such as oral famciclovir or valacyclovir will be used routinely in participants not at risk for primary CMV infection (donor and recipient sero-negative). All BMT participants should receive sero-negative or CMV "Safe" blood (leukocyte reduced). Weekly CMV DNA (by PCR) assays are obtained for participants at risk of infection.

Regimens:
Treatment of Symptomatic CMV Disease: The standard of care for treating symptomatic CMV disease is: 1) a minimum of 2-3 weeks of intravenous ganciclovir at a dose of 5 mg/kg twice daily (with dosage adjustment for renal dysfunction); 2) oral valganciclovir 900 mg PO BID; or, 3) other approved antiviral medication per institutional standards. The end point of intravenous therapy is the documented clearance of virus from the blood as demonstrated by CMV quantitative PCR assay. In sero-positive individuals, the risk of subsequent relapse is ~15-20%, which can be essentially eliminated by following intravenous therapy with oral valganciclovir, 450-900 mg daily (adjusted for renal function) for 3 months. With 3 months of oral therapy following clearance of viremia, the rate of relapse is ~20%. In participants with relapsing infection, CMV hyperimmune globulin (at a dose of 150 mg/kg IV once and 100 mg/kg IV every month x 3-6) can be administered in conjunction with the intravenous ganciclovir therapy in sero-negative recipients. Treatment is maintained in the setting of active GVHD.

Dosing Nomogram for Treatment of CMV Infection

Serum Creatinine (mg/dl)	Intravenous Dose (mg/kg)	Frequency
<2.0	5	Q12h
2-3	5	Daily
3-5	1.25	Daily
>5.0	*1.25	QOD
Hemodialysis	5	Post- Dialysis
Peritoneal Dialysis	*2.5	Daily
*After loading dose of 5 mg/kg. All participants (prophylaxis or therapeutic) receive: leukocyte or CMV-negative blood: CMV-hyperimmune globulin (Massachusetts Red Cross) 150 mg/kg IV for first dose and 100 mg/kg x 4 at the discretion of the physician.		

Prevention of CMV Disease in High Risk Patients: In the BMT participant, the greatest risk for pulmonary disease, in particular, is in the mismatched combination, Donor sero-negative and Recipient sero-positive (D-/R+). Such CMV disease will increase the chance of GVHD and other opportunistic infections and therefore merits prophylaxis. The incidence of CMV infection after allogeneic BMT is 20-55% and causes parenchymal disease in 10-35%.

a. Solid Organ Participants at Risk for Primary Disease (or BMT Participants R+):

Prophylaxis for all such individuals is advocated, since the risk of clinical disease is > 50%. The regimen advocated is intravenous ganciclovir at a dose of 5 mg/kg on Day –4 followed by oral valganciclovir at a dose of 450-900 mg daily once neutropenia has resolved (ANC > 0.5). An alternate approved antiviral therapy may be used per institutional standards. **Dosage adjustment in the face of renal dysfunction is required. Neutropenia is usually treated with GCSF, not by dose reduction of ganciclovir due to risk of resistant viral strains.**

b. Participants (R+) Being Treated with Antilymphocyte Antibodies: (Antithymocyte Globulin or OKT3):

The risk of CMV disease with induction antilymphocyte antibody therapy rises to 65%. The administration of intravenous ganciclovir at a dose of 5 mg/kg/day (50% of therapeutic dose with dosage adjustment for renal dysfunction) for the duration of the antilymphocyte antibody therapy, followed by oral valganciclovir 450-900 mg daily (adjusted for renal function) for 3 months essentially eliminates symptomatic CMV disease. An alternate approved antiviral therapy may be used per institutional standards.

c. Prophylaxis in Sero-negative Individuals Receiving Allografts from Sero-negative Recipients (D-R-):

Provided CMV pedigreed blood products are utilized, there is essentially no risk of CMV disease other than via sexual contacts. In order to prevent the 40% incidence of herpes simplex disease that occurs in transplant participants not receiving ganciclovir, these individuals are prescribed oral acyclovir 400 mg *TID* for 4-6 months, or other approved antiviral therapy.

5. Anti-viral Prophylaxis During Anti-lymphocyte Therapy for Treatment of Rejection

If anti-lymphocyte therapy (ATG or OKT-3) for treatment of rejection is required, either intravenous ganciclovir, 5 mg/kg I.V. once daily or oral valganciclovir (450-900 mg po once daily) corrected for renal function (see package insert) should be administered during the period of administration of the anti-lymphocyte agent, plus an additional 2 days, followed by valganciclovir 450-900 mg daily (adjusted for renal function) for an additional 8 weeks. For donor CMV(-), recipient CMV(-) pairs, oral acyclovir or oral valacyclovir may be substituted for the oral valganciclovir. Activation of CMV infection will be treated according to institutional protocol.

Appendix 3

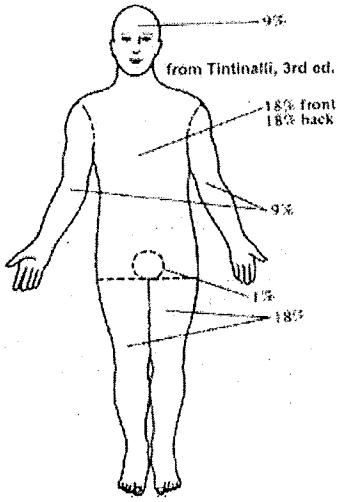
Graft-versus-Host Disease Staging and Grading Table

Clinical Stage of Acute Graft-versus-host Disease (GVHD) According to Organ System

Note: Staging is performed only if GVHD is the cause of at least one organ's dysfunction.

Stage	Skin	Liver	Gut
1	Maculopapular rash < 25% of body surface[1]	Bilirubin 2-3 mg/dL[2]	> 500 ml diarrhea per day[3] (> 5 ml/kg in children), or persistent nausea with histologic evidence of GVHD in stomach or duodenum
2	Maculopapular rash 25 - 50 % of body surface[1]	Bilirubin 3.1 - 6 mg/dL[2]	> 1000 ml diarrhea per day[3] (> 10 ml/kg in children)
3	Maculopapular rash > 50% of body surface[1]	Bilirubin 6.1 - 15 mg/dL[2]	> 1500 ml diarrhea per day[3] (> 15 ml/kg in children)
4	Generalized erythroderma with bullous formation	Bilirubin > 15 mg/dL[2]	Severe abdominal pain with or without ileus.

1. Use "Rule of Nines" (below) or burn chart to determine extent of rash.
2. Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
3. Downgrade one stage if an additional cause of diarrhea has been documented



Appendix 4

ECOG Performance Status*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

*Eastern Cooperative Oncology Group (ECOG) status table as published in American Journal Clinical Oncology:

Oken M. M., Creech R. H., Tormey D. C., Horton J., Davis T.E., McFadden E. T., Garbone P. P. *Toxicity and Response Criteria of the Eastern Cooperative Oncology Group*. AM J Clin Oncl 5:649-655, 1982.