

## **Renal Allograft Tolerance Through Mixed Chimerism**

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## Synopsis

Title	Renal Allograft Tolerance Through Mixed Chimerism
Short Title	Renal Allograft Tolerance
IND Sponsor	David H Sachs
IND #	BB-IND 10181
Trial Phase	Phase I/II
Conducted by	MGH, Transplant Surgery
Principal Investigators	A. Benedict Cosimi, MD David H Sachs, MD Tatsuo Kawai, MD, PhD
Accrual Objective	2 participants with end-stage renal disease (ESRD) and no evidence of prior sensitization, and their donors
Study Design	Pilot study
Pace of Enrollment and Study Duration	Enrollment will be limited to one patient every three months  Participants will be followed for a total of 5 years after combined bone marrow and kidney transplantation. Assessments will be performed for participant and graft survival, and long term adverse events.
Endpoint	<b>The primary endpoint</b> is Induction of transient mixed chimerism and renal allograft tolerance without “engraftment syndrome” or “acute kidney injury”.

## Recipient Inclusion Criteria

1. Male or female 18–60 years of age.
2. Candidate for a living-donor renal allograft with a one haplotype identical donor identified.
3. First or second transplant with either a living donor or cadaveric transplant as the first transplant.
4. Use of FDA-approved methods of contraception (those with less than a 3% failure rate) by all recipients from the time that study treatment begins until 104 weeks (24 months) after renal transplantation. (For further information on FDA- approved methods of contraception, see <http://www.fda.gov/ForConsumers/ByAudience/ForWomen/ucm118465.htm>)

5. Ability to understand and provide informed consent.
6. Serologic evidence of prior exposure to EBV.

### **Recipient Exclusion Criteria**

1. ABO blood group-incompatible renal allograft.
2. Evidence of anti-HLA antibody within 60 days prior to transplant as assessed by routine methodology (AHG and/or ELISA)
3. Leukopenia (WBC less than 2,000/mm<sup>3</sup>) or thrombocytopenia.
4. Seropositivity for HIV-1, hepatitis B core antigen, or hepatitis C virus (confirmed by hepatitis C virus RNA); or positivity for hepatitis B surface antigen.
5. Cardiac ejection fraction < 40% or clinical evidence of insufficiency.
6. Forced expiratory volume FEV<sub>1</sub> < 50% of predicted.
7. Lactation or pregnancy.
8. History of cancer other than basal cell carcinoma of the skin or carcinoma in situ of the cervix.
9. Underlying renal disease etiology with a high risk of disease recurrence in the transplanted kidney (such as focal segmental glomerulosclerosis, type I or II membranoproliferative glomerulonephritis).
10. Prior dose-limiting radiation therapy.
11. Known genetic disease or family history that may result in greater sensitivity to the effects of irradiation, or a physical deformity that would preclude adequate shielding or appropriate dosing during the irradiation component of the conditioning regimen.
12. Enrollment in other investigational drug studies within 30 days prior to enrollment.
13. Abnormal (>2 times lab normal) values for (a) liver function chemistries (ALT, AST, AP), (b) bilirubin, (c) coagulation studies (PT, PTT).
14. Allergy or sensitivity to any component of MEDI-507, tacrolimus, or rituximab.
15. Maintenance immunosuppression within 3 months prior to conditioning other than physiological doses of steroids, defined as ≤ 50 mg of hydrocortisone or dose equivalent.
16. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

### **Donor Inclusion Criteria**

1. Male or female 18–65 years of age.
2. For females of childbearing potential: a serum pregnancy test showing negative results.
3. Excellent health per conventional predonor history (medical and psychosocial evaluation).
4. Acceptable laboratory parameters (hematology in normal or near-normal range; liver function <2 times the upper limit of normal, and normal creatinine).
5. Negative for viral infection with HbsAg, HIV, HCV, or HTLV-1.
6. Cardiac/pulmonary function within normal limits (CXR, ECG).
7. Ability to understand and provide informed consent.
8. Meets standard institutional criteria for both bone marrow and kidney donation

### **Treatment Description**

Recipients will receive a conditioning regimen that starts with Rituximab on day –7 (and days –2, 5, 12), Whole Body Irradiation 150 cGy x2 on study days –5 and –4, followed by MEDI-507, a T-cell-depleting agent, will be administered on days –2, –1, 0, and 1. Thymic irradiation (700 cGy) will be given on study day –1, and combined renal and bone marrow transplant will be done on study day 0. Prednisone will be started at 2 mg/kg on day 4 and tapered off by day 20. Tacrolimus will be administered on study days –1 through 60, and then tapered if weaning criteria are met. Leukapheresis will be performed prior to initiating the conditioning regimen and these cells will be frozen

All patients who require a blood transfusion will receive only leukocyte-depleted and irradiated blood products for a period of at least 52 weeks following transplantation.

The proportion of patients not suffering “engraftment syndrome” and successfully weaned off immunosuppression will be assessed.

## Glossary of Abbreviations

AHR	acute humoral rejection
ALC	absolute lymphocyte count
ANC	absolute neutrophil count
APC	Antigen-presenting cell(s)
ATG	antithymocyte globulin
ATGAM	lymphocyte immune globulin
ATN	acute tubular necrosis
BMT	bone marrow transplantation
CBC	complete blood count
CFR	Code of Federal Regulations
CKBMT	Combined kidney and bone marrow transplant
CIS	carcinoma in situ
CML	cell-mediated lysis, cell-mediated lympholysis
CMV	cytomegalovirus
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic T lymphocyte
CVVH	continuous veno venous hemodialysis
ELISA	enzyme-linked immunosorbant assay
ELISPOT	enzyme-linked immunospot (assay)
ESRD	end-stage renal disease
GVHD	graft-versus-host disease
GCP	Good Clinical Practice
HLA	human leukocyte antigen
HTL	helper T lymphocyte
ICH	International Conference on Harmonisation
IND	investigational new drug
IRB	institutional review board
ITN	Immune Tolerance Network
LDA	limiting dilution analysis
MGH	Massachusetts General Hospital
MHC	major histocompatibility complex
MLR	mixed lymphocyte response, mixed lymphocyte reaction
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NK	natural killer (cell)
PBMC	peripheral blood mononuclear cell(s)
PCP	Pneumocystis carinii pneumonia
PRA	panel-reactive antibody; panel reactivity assay
PTLD	post-transplant lymphoproliferative disease
RTEC	renal tubular epithelial cell(s)
RT-PCR	real-time polymerase chain reaction
SAE	serious adverse event
SAEC	safety adverse event coordinator
SAP	statistical analysis plan
SI	Stimulation Index
TBI	total body irradiation

TI	thymic irradiation
TREC	T-cell receptor excision circle(s)
Treg	regulatory T cell

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## 1. BACKGROUND

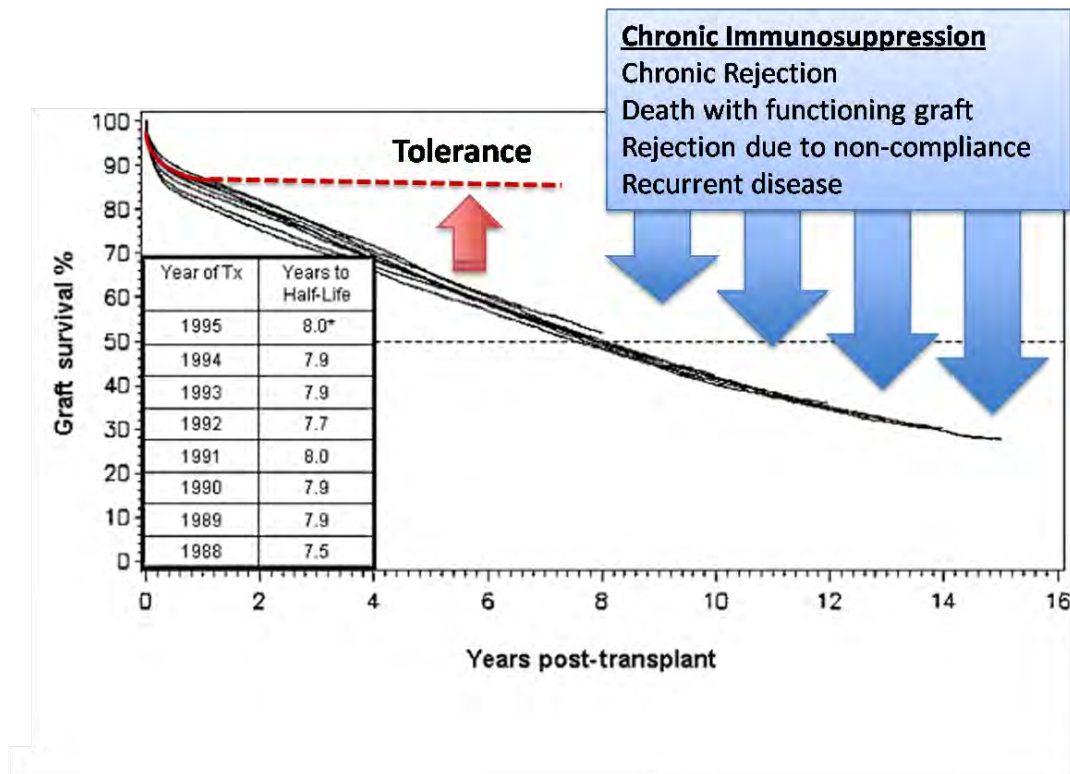
### 1.1 SUMMARY

#### Risk of long-term immunosuppression

Since the first successful human kidney transplantation by Murray et al. in 1956<sup>1</sup>, numerous immunosuppressive regimens have been developed for clinical application. As these more efficacious immunosuppressive drug combinations have successfully prevented or treated acute allograft rejection, short-term survival of organ transplants has significantly improved, resulting in solid organ transplantation becoming the therapy of choice for many end-stage organ diseases<sup>2,3</sup>.

However, chronic use of immunosuppressive drugs results in significantly increased risks of cardiovascular disease<sup>4-6</sup>, infection<sup>7-9</sup> and malignancies<sup>10-13</sup>. Immunosuppressive medications also cause various side effects, such as nephrotoxicity, de novo diabetes<sup>14-16</sup>, dyslipidemia<sup>17-20</sup> and neurotoxicity<sup>21,22</sup>. Cardiovascular disease has become the most common cause of mortality, accounting for 30-48% of the deaths after kidney transplantation<sup>10</sup>. Infection is also a common complication and remains the second most common cause of mortality after transplantation<sup>7-9</sup>. The risk of many common cancers has been observed to be higher in chronically immunosuppressed transplant patients. Such risk is particularly high in non-melanoma skin cancer and melanoma/leukemia, which are 30 and 3-fold higher than that in the general population, respectively<sup>11,23</sup>. Some studies report that the overall incidence of cancer after kidney transplantation is as high as 40% after 20 years of immunosuppressive therapy, in contrast to a 6% cumulative risk for cancer in an age- matched, non-transplanted control population<sup>13,24</sup>. Because of these morbidities, death with a functioning graft accounts for 16% of graft losses over the first 10 years after kidney transplantation<sup>25</sup>. Chronic nephrotoxicity from calcineurin inhibitor (CNI) treatment is also a serious side effect. Unfortunately, despite these toxicities that accompany the potent immunomodulatory effects of current therapeutic protocols, development of chronic rejection is not consistently prevented. Approximately 15-30% (dependent upon the assay utilized) of renal transplant recipients receiving maintenance immunosuppression eventually develop anti-HLA antibodies<sup>27</sup>. Chronic rejection, often in association with de novo alloantibody detection, accounts for about 20% of graft losses by 10 years after kidney transplantation<sup>25</sup>. In summary, the net effect of these limitations of currently available chronic immunosuppression protocols is an inexorable loss of previously functioning transplanted kidneys at an annual rate of about 5-7%<sup>28</sup> and minimal improvement of renal graft half life in the last 10 years<sup>2,3,29</sup> (Fig. 1)

**Figure 1. Long Term Results of Kidney Transplantation**



**Rationale of tolerance induction**

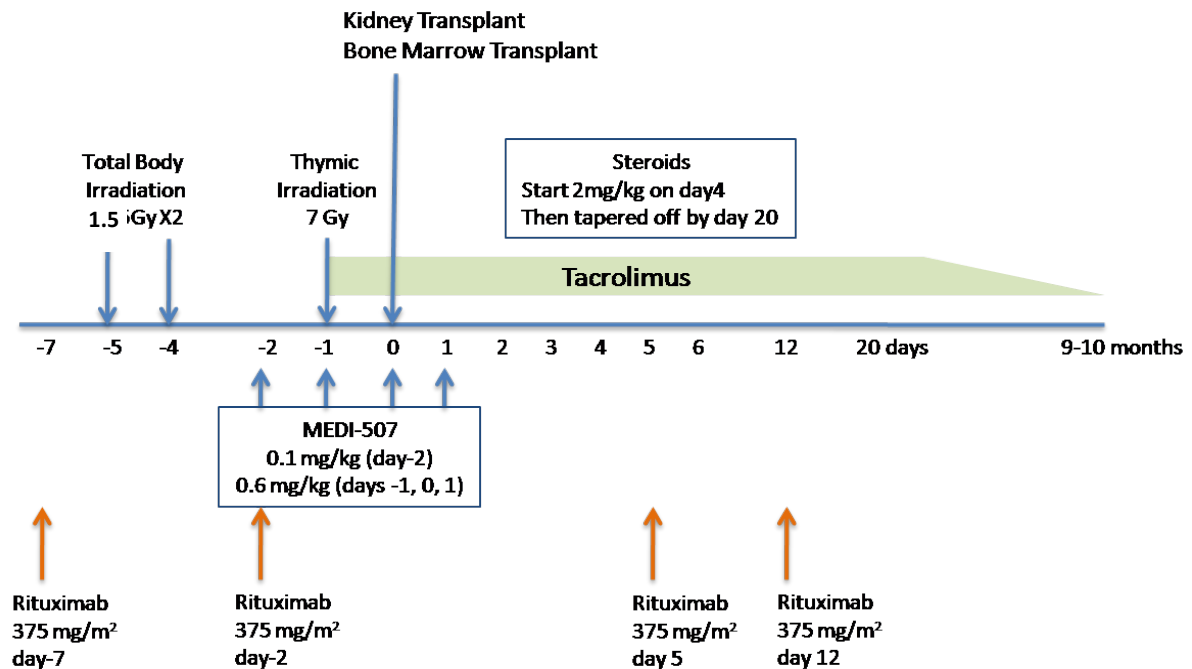
Induction of tolerance has been considered one strategy to improve the long-term results of organ transplantation. Operational tolerance (defined as the absence of a destructive immune response to a transplanted tissue without ongoing immunosuppression) is very rare in kidney transplant recipients. These observations have emphasized that additional conditioning or treatment is required for consistent induction of tolerance across major histocompatibility complex (MHC) barriers. In order to justify clinical application, development of such additional conditioning / treatment regimens must utilize approaches with short and long-term risks that do not outweigh the benefits over current standard of care. Presumably, if tolerance could be induced consistently, chronic rejection would be eliminated and the risks of cardiovascular disease, infection, malignancy and other drug-related complications should be considerably minimized. These advantages must be balanced against the potentially increased risks of infection and treatment-related morbidities in the early post-transplant period, which may be higher after administration of the conditioning regimens required for tolerance induction.

**Tolerance induction via the mixed chimerism approach**

Evidence that tolerance of kidneys can be induced via bone marrow transplantation in humans is illustrated by the fact that recipients of successful allogeneic bone marrow transplants who subsequently suffer end-stage renal disease have undergone kidney transplantation from the same donor without the need for immunosuppressive therapy. However, myeloablative conditioning for full allogeneic bone marrow transplantation, specifically for the induction of tolerance of the kidney allograft, is neither necessary nor desirable. Nonmyeloablative conditioning regimens have been shown to result

in mixed chimerism and donor-specific immunologic tolerance in a number of rodent model systems,<sup>32,33</sup> in miniature swine,<sup>34</sup> and in fully mismatched cynomolgus monkeys.<sup>35-37</sup> Our group has reported the successful induction of tolerance in patients with advanced multiple myeloma and renal failure through simultaneous bone marrow and renal transplantation from HLA matched donors (ITN study NKD01).<sup>38,39</sup> More recently, we have reported tolerance induction in patients with end-stage renal disease (ESRD) without myeloma who received haploidentical kidney and bone marrow transplants (ITN study NKD03)<sup>40</sup>. The originally studied protocol was revised (ITN036) to add rituximab and a short course of steroids and postoperative cyclosporine was replaced with tacrolimus to address the limitations of “engraftment syndrome” and development of donor specific antibody (DSA) observed in protocol (NKD03). Although recipients treated with ITN036 did not develop DSA or humoral rejection, “engraftment syndrome” continued to be observed. The goal of this study is to develop a nonmyeloablative regimen that can induce comparable levels of transient mixed chimerism without “engraftment syndrome”. The proposed conditioning regimen is depicted in Figure 2. Comparison with Figure 3 emphasizes that the only significant change from our most recently used conditioning is the substitution of TBI for cyclophosphamide on days -5 and -4. Our conditioning regimen in NHPs has always utilized TBI (100-150 cGy on days -5 and -4), with which the majority of recipients develop mixed chimerism and survive long-term without evidence of engraftment syndrome or allograft rejection. Nevertheless, cyclophosphamide had been selected for the initial clinical trials because, at that time, there was more extensive experience with this approach in human BM recipients. If the creatinine begins to rise or the level of donor chimerism falls by > 50% during the 2<sup>nd</sup> post-transplant week, Prograf will be discontinued and ATG will be administered (up to 5 doses over 10 days). Prograf administration will be resumed after ATG is discontinued. This addresses our observation of apparent synergistic CNI nephrotoxicity with that of engraftment syndrome (See below: Clinical studies).

**Figure 2. Conditioning regimen**



## 1.2 PRECLINICAL AND CLINICAL STUDIES

### 1.2.1 Preclinical Studies

We have previously demonstrated that the establishment of allogeneic mixed chimerism (i.e., survival of both host and donor lymphohematopoietic elements) with a nonmyeloablative conditioning regimen across MHC barriers can successfully achieve transplantation tolerance while otherwise maintaining full immunocompetence in mice,<sup>41,42</sup> in miniature swine,<sup>34</sup> and in fully mismatched cynomolgus monkeys.<sup>35-37</sup> Studies of the mechanism of tolerance induced by mixed chimerism have been carried out most extensively in mice, and have demonstrated that tolerance is induced centrally through a deletional mechanism, as established by analyses of T-cell receptor utilization.<sup>43</sup> Despite the more extensive mechanistic studies we have carried out in mice and miniature swine, we shall confine the preclinical data summarized here to our studies in cynomolgus monkeys, as these animals are most relevant to the present protocol.

The initially successful nonmyeloablative conditioning regimen developed for induction of mixed chimerism and renal allograft tolerance in MHC fully mismatched cynomolgus monkeys<sup>35</sup> consisted of total body irradiation (TBI), thymic irradiation (TI) and antithymocyte globulin. Splenectomy, bone marrow transplantation, and renal transplantation were performed on day 0 and cyclosporine was administered on days 0–28. Of 13 recipient monkeys undergoing this conditioning, 11 developed mixed chimerism and 10 survived long-term without allograft rejection.<sup>35,36,44</sup> In these long-term survivors, alloantibody and chronic vasculopathy were detected in only 1 monkey that had not developed clear macrochimerism. Furthermore, all long-term survivors showed donor-specific unresponsiveness in mixed lymphocyte response (MLR) assays<sup>35</sup> The longest surviving tolerant monkey was observed for more than 13 years post transplant, with a serum creatinine of 1.2 mg/dL and a BUN of 19 mg/dL; a renal biopsy at 10 years post transplant showed no diagnostic abnormalities, indicating that this protocol appears to not only avoid acute rejection but to prevent chronic rejection, which is the major cause for loss of long-term renal allografts. To our knowledge, of all published protocols leading to long-term survival of renal allografts in nonhuman primates, this mixed chimerism approach is the only one which avoids chronic rejection. Several modifications of our regimen utilizing either reduced doses of TBI, no TI, or no cyclosporine failed to induce chimerism, and all allografts were rejected; elimination of the splenectomy did not prevent the development of chimerism in all animals but resulted in graft loss.<sup>35</sup>

On the basis of further murine studies in our research center,<sup>43,45</sup> additional studies in 7 cynomolgus monkeys evaluated several modifications to the original protocol. The addition of costimulatory blockade with anti-CD40L antibody improved the reliability of engraftment and tolerance induction without the requirement for splenectomy<sup>37</sup>. Five monkeys received anti-CD40L added to the original regimen, including cyclosporine but without splenectomy, all developed chimerism, and none developed anti-donor antibodies. Of these five monkeys, one died due to a urethral stone on day 204, without evidence of allograft rejection, and one developed hemolytic uremic syndrome of unexplained origin; the other three monkeys remained stable without evidence of rejection for more than 8 years. These data suggested that improvement in T-cell depletion, with anti-CD40L as used in this study, could improve the reliability of engraftment and tolerance induction without the need for splenectomy.

Most importantly, our pre-clinical studies indicated that induction of transient mixed chimerism is sufficient to induce tolerance of MHC fully mismatched renal allograft. Because chimerism has always been transient, no GVHD has been observed in these monkeys. The recipients treated with TBI-based conditioning regimen lost their chimerism more slowly than humans treated with cyclophosphamide-based conditioning regimen, no “engraftment syndrome” or acute kidney injury have been observed in our monkeys.

### 1.2.2 Clinical Studies: Prior Studies

#### Introduction:

Since the seminal work reported by Billingham, Brent and Medawar on neonatal tolerance in 1956, numerous tolerance induction strategies have been identified in rodents. However, only a very limited number of these have been successfully translated to large animals and even fewer to primates. Among the few protocols that have been applied in humans, induction of donor chimerism, either transient or permanent, currently appears to be the most promising strategy to achieve renal allograft tolerance. Initial results of clinical trials for tolerance induction in three centers have so far been reported. Using TLI and DBMT, the Stanford group reported successful induction of stable chimerism and renal allograft tolerance but only in HLA identical kidney transplant recipients.<sup>46,47</sup> More recently, Leventhal et al. at Northwestern have reported use of an intensive conditioning regimen and donor hematopoietic stem cells for induction of tolerance in HLA-mismatched kidney transplant recipients<sup>48</sup>. Although the follow-up is still brief, persistent donor chimerism without GVHD has been reported, allowing weaning from all maintenance immunosuppression by 1 year in approximately half of their patients.

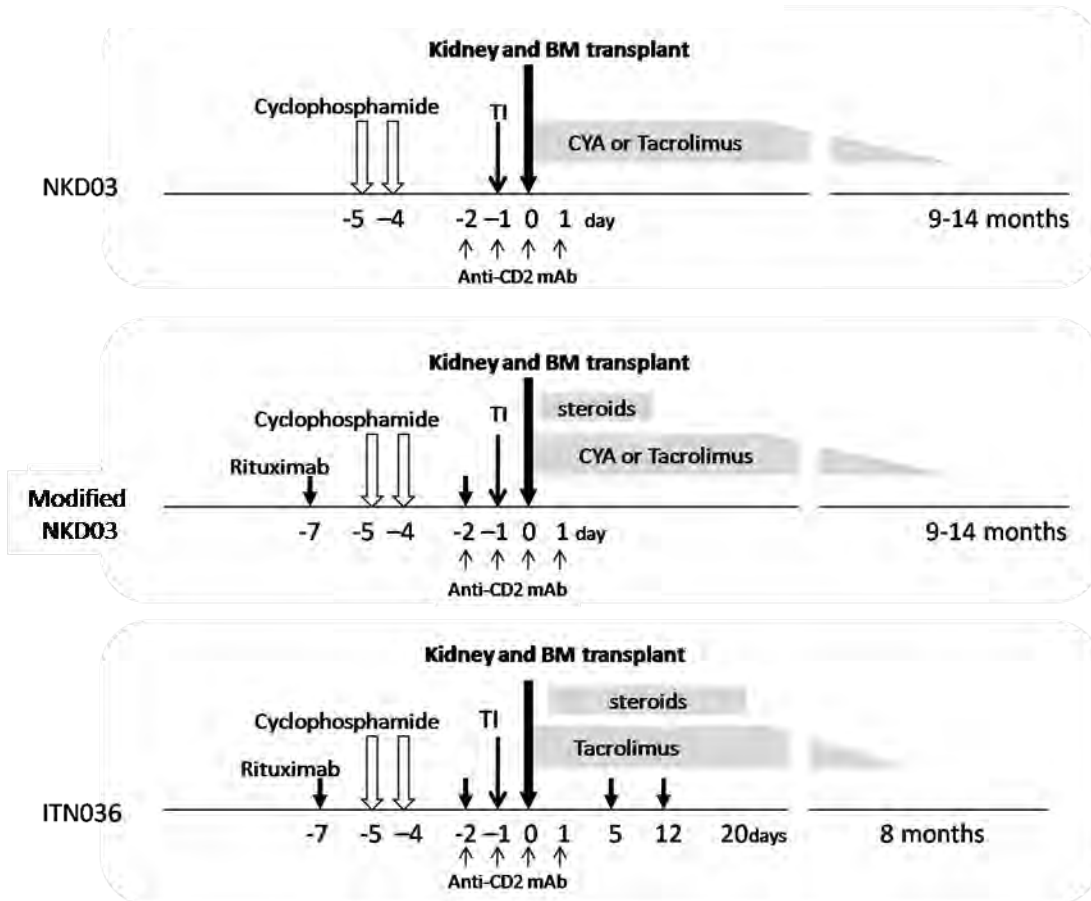
At Massachusetts General Hospital, based on decades-long basic studies in animal models<sup>32,35,37,49</sup>, we have applied combined kidney and donor bone marrow transplantation (CKBMT) for induction of allograft tolerance in both HLA matched and mismatched kidney transplant recipients<sup>38,39,50</sup>. The early results of the initial five mismatched patients who underwent CKBMT have been reported<sup>40</sup>. We subsequently extended this approach with a revised regimen to five additional patients. We have submitted for publication our observations in this later cohort as well as the longer-term follow-up (up to 10 years) of the previous recipients of HLA mismatched CKBMT (attached as Appendix 6 and briefly summarized here).

The three preparative conditioning regimens are depicted in Figure 3. The initial (NKD03) protocol was modified after treatment of the third subject who developed irreversible antibody mediated rejection, to include 1) administration of Rituximab, 375 mg/m<sup>2</sup>/dose on days -7 and -2; and 2) administration of prednisone, 2 mg/kg/d starting on the day of transplantation and then tapering over the next 10 post-transplant days (modified NKD03). Since subjects treated with this modified NKD03 still developed donor specific antibodies (DSA) after discontinuation of immunosuppression, the regimen was further modified (ITN036) to add two more doses of Rituximab (375 mg/m<sup>2</sup>/dose on post-transplant days 5 and 12, more prolonged administration of prednisone until day 20, and tacrolimus in place of CyA for the 5 most recently treated subjects. A total of 10 subjects, age 22-46, 6 males and 4 females, were enrolled into these studies (Table 2).



Donors were all HLA one haplotype mismatched parents or siblings. The first three subjects received the NKD03 regimen, the next two subjects received the revised NKD03 regimen. The last five subjects received the ITN036 conditioning. All patients developed transient multilineage mixed chimerism which became undetectable by 2-3 weeks post-CKBMT<sup>40,51</sup>. All subjects except for Subject #1 developed cytokine syndrome-like manifestations<sup>40,52</sup>. This syndrome has been temporally associated with the loss of peripheral chimerism as well as of the return of host-derived elements. The most concerning manifestation of this syndrome is acute kidney injury (AKI), which was observed after day 10 in all patients except for Subject #1. The biopsies taken during AKI showed severe endothelial injury with CD8<sup>+</sup> T cell infiltration, as reported in detail<sup>52</sup>. The peak of serum creatinine levels ranged from 3.5 to 15.4 during days 10-20. Among nine subjects who developed AKI, three recovered without additional treatment, two with Thymoglobulin and plasma exchange, one with Thymoglobulin alone and one with steroid pulse. Two kidney allografts failed to recover despite treatments due to associated humoral rejection and thrombotic micro-angiopathy.

**Figure. 3 Conditioning Regimens for Previous Clinical Trials**



Clinical courses of all subjects are depicted in Figure 4.

Subject #1 was a 22 y.o. female who has continued to be well with normal kidney allograft function without ongoing immunosuppressive therapy for more than 10 years. All four protocol

biopsies performed up to 8 years after CKBMT showed no evidence of rejection in light and electron microscopy. No DSA has been detected.

Subject #2 was a 22 y.o. man in whom all immunosuppressive medications were tapered off by day 422. He remained stable until year 7 following immunosuppression withdrawal. At that point he was first noted to have increased urinary protein. Allograft biopsy revealed no rejection but recurrence of his original disease, MPGN type 1. Mycophenolate mofetil (MMF) monotherapy for recurrent MPGN was initiated after the 8<sup>th</sup> year. He is currently stable without further increment of proteinuria.

Subject #3 was known to have positive PRA (50%) but ADA was not detectable by ELISA preoperatively. This subject developed severe irreversible acute humoral rejection on day 10. The protocol was subsequently modified with more strict inclusion criteria (PRA pretransplant detection against any specificity became a contraindication) plus the addition of two doses of pre-transplant rituximab and post-transplant prednisone up to day 10 (modified NKD03).

Subject #4 was a 25 y.o. man whose Immunosuppression was tapered and completely discontinued on day 244. This subject developed anti- HLA class II DSA, soon after complete discontinuation of immunosuppression. His five-year protocol biopsy revealed basement membrane duplication by electron microscopy with minimal interstitial fibrosis and no evidence of cellular or vascular rejection. Although his kidney allograft function has continued to be stable with serum creatinine of 1.6-1.9 mg/dl, he has recently developed mild proteinuria at 7 years post-transplant, and treatment with IVIG and MMF therapy have been recommended.

Subject #5 was a 46 y.o. male in whom immunosuppression was tapered and discontinued completely on day 272. He remained stable for 5 years with no evidence of rejection or C4d deposition in either light or electron microscopy. However, after suffering severe episodes of gout, his renal function became unstable with detection of DSA by ELISA at 6 years. Since DSA became positive with newly appeared C4d deposition in the biopsy, he received a short course of high dose IVIG (2g/kg) followed by rituximab. His *in vitro* CML and MLR assays have continued to show complete absence of anti-donor T cell responses. After these treatments, his renal function stabilized with no evidence of chronic humoral rejection and C4d staining returned to negative in the subsequent biopsy. He is currently doing well without maintenance immunosuppression.

Subject #6 developed AKI after day 10 but recovered without additional treatment. His immunosuppression was slowly tapered and completely discontinued at 8 months after CKBMT. He is currently at 3.5 years after transplantation and has been well, without immunosuppression and with a serum creatinine level of 1.5 mg/dl. Biopsy showed no evidence of rejection by either light or electron microscopy.

Subject #7 had her immunosuppression tapered and completely stopped at 8 months. She is currently at 3 years and 4 months after CKBMT with normal kidney function (serum creatinine 0.8 mg/dl) with no evidence of rejection or C4d staining in the 2 year protocol biopsy

Subject #8 developed AKI after day 10. The renal allograft biopsy on day 11 showed no rejection but endothelial injury. Another biopsy, performed on day 22 revealed intra-renal thrombotic microangiopathy, possibly due to tacrolimus toxicity. Tacrolimus was discontinued and three doses of anti-thymocyte globulin were administered with MMF. Despite these treatments, her kidney function did not recover and she was eventually returned to CAPD at 7 months after transplantation.

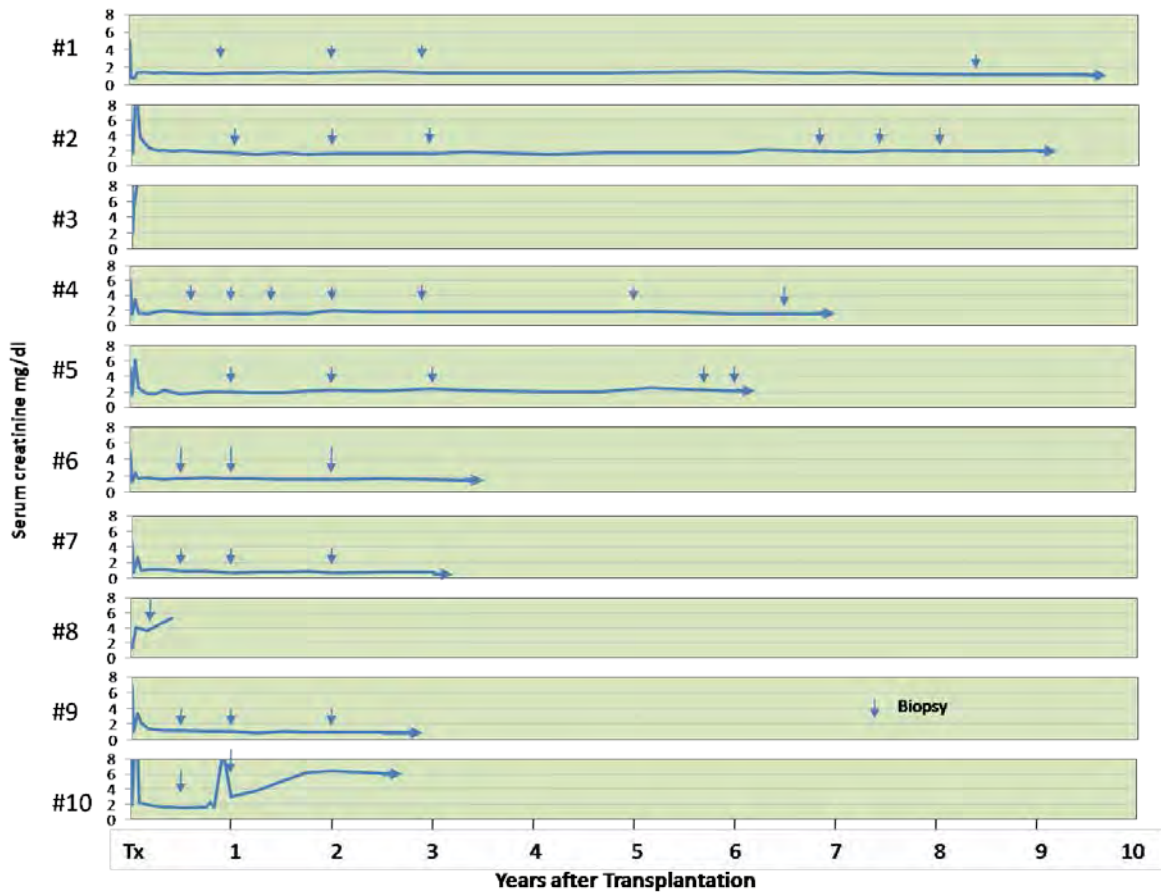
Subject #9 also developed transient AKI after day 10 and recovered without additional treatment. Her immunosuppression was discontinued at 8 months after transplantation. She is currently well at 3 years after CKBMT with normal kidney function (serum creatinine 1.0 mg/dl). The protocol biopsy at 2 years showed no evidence of rejection. No DSA has been detectable. Subject #10 developed AKI two weeks after CKBMT. His kidney function gradually returned to normal by 2 months after transplantation and a 6-month protocol biopsy did not show rejection. His immunosuppression was completely discontinued at 8 months. At 9 months, he developed acute pyelonephritis of the kidney allograft with moderately elevated serum creatinine (2.2 from 1.6 mg/dl). This was treated with antibiotics and the kidney function recovered to base line levels. However, 3 weeks after the resolution of his UTI, he developed severe acute cellular rejection (BANFF 2B) with no C4d staining. DSA has never been detectable by either ELISA or LUMINEX. He was treated with steroid pulses and anti-thymocyte globulin, following which his renal function improved but never fully recovered. Although he remains well, his compromised kidney function suggests that gradual progression to ESRD is likely.

**Table 1. Patient summaries for all CKBMT Trials**

	age	sex	Original disease	Graft Survival	Current Pathology	Current Creatinine (GFR)	Current medication	
NKD03	1	22	F	Alport's disease	>10 yrs	No Rejection	1.2 (81)	none
	2	22	M	MPGN type 1	>9 yrs	No Rejection MPGN recurrence	1.6-1.9 (39)	MMF (after 8 <sup>th</sup> year for MPGN)
	3	39	M	PCKD	10 days	AHR	NA	(re-Tx)
modified NKD03	4	25	M	Alport's disease	>7 yrs	CHR	1.6-1.9 (42)	MMF (after 7 <sup>th</sup> year for CHR)
	5	46	M	PCKD	>6 yrs	No rejection	2.0– 2.3 (51)	none
ITN036	6	35	M	Alport's disease	>3.5 yrs	No Rejection	1.5	none
	7	37	F	Reflux uropathy	>3.3 yrs	No Rejection	0.8	none
	8	36	F	FGS	0.5 yrs	TMA/AR	On CAPD	-
	9	22	F	MPGN type 1	>3 yrs	No Rejection	1.1	none
	10	26	M	Alport's disease	>2.8 yrs	Post -ACR	4-6	MMF/pred/ actinomycin

B cell depletion : Recovery of CD3<sup>-</sup>CD19<sup>+</sup> cells in two subjects (Subjects #1 and #2) treated with the original NKD03 regimen was observed by days 50 and 100, respectively. With two doses of pre-transplant rituximab (modified NKD03), depletion of CD3<sup>-</sup>CD19<sup>+</sup> cells was extended to day 150 (Subjects #4 and #5) but complete loss of CD3<sup>-</sup>CD19<sup>+</sup> cells from the peripheral blood was not observed. In contrast, peripheral blood CD3<sup>-</sup>CD19<sup>+</sup> cells were essentially undetectable for 6 months in all five subjects treated with 4 doses of peritransplant rituximab (ITN036). Of note although DSA has been detected at least transiently in 4 of 5 NKD03, no DSA has been detectable in all ITN036 subjects.

**Figure 4 Clinical Course**



Despite the encouraging results of successful long-term tolerance induction in our initial clinical trials, the major obstacle to more wide-spread application of this approach is the “engraftment syndrome”. AKI is a major manifestation of engraftment syndrome and has been observed at approximately day 10 in all subjects except for Subject #1. Subject #1 had been treated with immunosuppression for 10 years after her first transplantation (at age 12) before receiving CKBMT (at age 22), which may be the reason she was exempted from the engraftment syndrome. This syndrome has been described previously following both autologous and allogeneic bone marrow transplantation, which has been associated with the recovery of either host or donor type hematopoietic elements<sup>26</sup>. We note that the AKI of engraftment syndrome has never been observed in our MHC-mismatched monkey model, in which chimerism is also lost, but at a more gradual pace, usually over 2-3 months<sup>9,10,27</sup>. Our current interpretation of these findings is that the pre-transplant cyclophosphamide-based regimen chosen for the clinical trials<sup>14</sup>, in contrast to the non-myeloablative total body irradiation used in the monkey preparative regimen<sup>9,10,27,28</sup>, may be inadequately suppressive, allowing rapid homeostatic recovery of host memory T cells resulting in rapid rejection of the donor hematopoietic graft. Further supporting this hypothesis is the observation that AKI has also never been observed in our HLA-matched CKBMT recipients, in whom chimerism as in the NHP recipients typically disappears more slowly, over a period of 2-3 months<sup>12,13</sup>.

The proposed pilot protocol is planned to address the “engraftment syndrome” related AKI, which currently remains the only major obstacle to wider clinical application of this approach.

### 1.3 RISKS TO PARTICIPANTS

#### 1.3.1 MEDI-507

The following information for MEDI-507 was taken from the current investigator’s brochure.

The safety profile of MEDI-507 is based on over 600 patients with psoriasis, 70 patients with GVHD or renal transplantation, and 29 patients with lymphoproliferative disorders. The most serious adverse events in patients treated with MEDI-507 are:

- Infusion-related/cytokine-release syndrome
- Infections
- Secondary cancers

The following adverse events have been observed in patients treated with MEDI-507, and may be potentially related to MEDI-507 therapy:

Infusion-related/cytokine-release syndrome: Infusion-related reactions such as chills, pyrexia, and fatigue have been commonly seen in patients with cancer, GVHD and renal transplantation, and psoriasis. These events have been mild to moderate in severity, transient in nature, and have not recurred with rechallenge. Other associated events included nausea, vomiting, and hypotension.

Infections: T-cell antibodies may be immunosuppressive. Prolonged immunosuppression may increase the risk of infection, including opportunistic infections. Infections were common in both the cancer and GVHD studies. Infections occurred in 3 (16%) of 19 patients in MI-CP099, with 5 patients (26%) having positive CMV antigen. Three patients discontinued treatment after testing positive for CMV (protocol-specified reason for discontinuation of MEDI-507). In MI-CP107, there were two infection-associated SAEs (staphylococcal bacteremia and RSV infection) that occurred in 2 (20%) of 10 patients; both events were judged by the investigator as not being related to MEDI-507. In MI-CP042, infections occurred in 16 of 17 patients and were a primary cause of death in 6 patients. The most common infectious agents were staphylococci followed by CMV. Three patients developed infections with Aspergillus or other mycelial fungi. A single patient experienced cryptosporidiosis involving the lung and colon. In MI-CP046, infections were common in both MEDI-507 (19 [90%]) and placebo (12 [92%]) patients and the most common site of infection was blood. The most common isolates were the same among MEDI-507 and placebo patients, and included staphylococcus species, CMV and enterococcus. The incidence of fungal infections (primarily Candida) was higher in the pooled placebo group (7/13, 54%) than among MEDI-507 patients (4/21, 19%). In MI-CP049, infections were seen in 3 of 10 patients (30%).

Secondary cancers: Prolonged and sustained immunosuppression may result in an increased risk of developing certain types of cancer.

EBV-associated LPD, PTLD, and lymphoma have occurred in clinical trials of MEDI-507. In the two cancer trials of MEDI-507 (MI-CP099 and MI-CP107), EBV-LPD has been reported in five patients including two patients with LGL and one patient each with CTCL, peripheral T-cell lymphoma (PTCL), and ATL. Most of these patients (4 of 5) who developed EBV-LPD received  $\geq 3.4$  mg/kg/dose MEDI-507, and all cases were

judged as having either a possible relationship (2 of 5 patients) or probable relationship (3 of 5 patients) to MEDI-507. One patient with LGL developed a secondary leukemia (M4 type leukemia) 1 year after completing MEDI-507 therapy. The event was grade 5 (fatal) and judged as remotely related to MEDI-507 treatment.

In the GVHD studies of MEDI-507 (studies MI-CP042 and MI-CP049 in Table 3), EBV-LPD and PTLD have been reported in one patient each, and EBV-lymphoma has been reported in two patients. A case of AML relapse has also been reported.

In the study of renal transplant patients, one patient experienced a basal cell carcinoma of the skin on an area of actinic exposure reported during long-term follow-up. Two patients in the psoriasis studies had secondary malignancies (squamous cell carcinoma of the skin and MDS) following treatment with MEDI-507. Both patients had a prior history of these respective conditions, and were entered into a MEDI-507 psoriasis study in violation of protocol entry criteria that excluded patients with a history of cancer (although in the latter case, the condition was not disclosed at study enrollment).

Three cases of PTLD have been reported in investigator-initiated and compassionate use studies of MEDI-507.

### **1.3.2 Rituximab (Rituxan®)**

The following information for rituximab was taken from the Rituxan package insert.

Indications for use: Rituxan (Biogen Idec Inc. and Genentech USA, Inc., South San Francisco, CA) is a CD20-directed cytolytic antibody indicated for the treatment of the following:

- Non-Hodgkin's lymphoma (NHL).
- Rheumatoid arthritis (RA) in combination with methotrexate in adult patients with moderately- to severely-active RA who have inadequate response to one or more TNF antagonist therapies.

#### Common adverse events:

- NHL (□25%): infusion reactions, fever, lymphopenia, chills, infection and asthenia.
- RA (5%): hypertension, nausea, upper respiratory tract infection, arthralgia, pruritus, and pyrexia. Other important adverse reactions include infusion reactions, serious infections, and cardiovascular events.

#### Serious adverse events:

The following serious adverse events have been associated with use of Rituxan:

- Tumor lysis syndrome
- Severe mucocutaneous reactions
- PML
- Hepatitis B reactivation with fulminant hepatitis
- Cardiac arrhythmias and angina
- Bowel obstruction and perforation

For additional information, please refer to the package insert at the following web site:

<http://www.gene.com/gene/products/information/pdf/rituxan-prescribing.pdf>

Note: Most of the serious adverse events were related to tumor lysis in the treatment of cancer and are therefore not thought to be relevant to the current protocol.

### **1.3.3 Low Dose Total Body Irradiation**

Low dose TBI may cause bone marrow suppression, immune suppression, nausea, vomiting. There is a possibility that TBI with or without other anti-lymphocyte therapy may lead to a malignancy months or years following the transplant. Epidemiologic studies have established that low dose TBI (250-300 cGy) increases the life-time risk of malignancy by a factor of 1.2 over that observed in individuals without prior exposure to irradiation (33). In contrast, as described above, long-term immunosuppression is associated with a 2-30 fold increased risk of cancer (13, 24). Such data would appear to justify the use of low dose irradiation for tolerance induction.

Because of the risk of genetic damage, all women of childbearing years will have a pregnancy test before starting the treatment. Although reproductive organs will be shielded, TBI may also result in sterility, cause genetic damage to future offspring, or interfere in normal wound healing.

### **1.3.4 Thymic Irradiation**

Irradiation of the thymus gland may cause nausea, vomiting, mild skin irritation and inflammation of the esophagus (causing mild, temporary dysphagia). Occasionally, pericarditis or pneumonitis may occur. Hypothyroidism and secondary malignancies are additional potential risks.

There is a risk of multi-organ failure, including cardiac, renal, pulmonary, CNS, and hepatic failure. This risk is increased in patients who have already had significant chemotherapy or radiation therapy or both. These risks may be life threatening and lead to fatal complications.

There is a possibility that the chemotherapy with or without radiation therapy may lead to a 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.

### **1.3.5 Tacrolimus**

Side effects of tacrolimus include renal insufficiency, abnormal liver function studies, seizures, nausea, vomiting, confusion, hypomagnesemia, tremulousness, and increased risk of secondary malignancies. Co-administration with methylprednisolone will decrease hepatic metabolism and thus increase tacrolimus levels; convulsions have been reported with concomitant high doses of methylprednisolone. Additional information about tacrolimus can be found in the package insert at <http://www.astellas.us/docs/prograf.pdf>

### **1.3.6 Prednisone**

Side effects of corticosteroids include convulsions, headache, vertigo, mood swings, psychosis, congestive heart failure (CHF), hypertension, Cushing syndrome, menstrual irregularities, hyperglycemia, GI irritation, peptic ulcer, weight gain. Dermatologic effects may include thin skin, petechiae, ecchymosis, facial erythema, poor wound healing, hirsutism, and urticaria. Muscle weakness, loss of muscle mass, and osteoporosis may also occur. Ophthalmologic complications may include increased intraocular pressure, glaucoma, exophthalmos, and cataracts. Other complications may include immunosuppression and increased susceptibility to infection.

For further information about prednisone, see the package insert at <http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=16633>

### **1.3.7 GVHD**

Since induction of persistent chimerism is not expected with our nonmyeloablative regimen, the risk of GVHD is considered very low. No GVHD has been observed in 10 patients who received combined kidney and bone marrow transplantation from HLA-mismatched donors under a similar protocol (NKD03 and ITN 036; see section 1.2.2).

The risk of GVHD increases depending on the severity of the conditioning regimen. In allogeneic bone marrow transplantation with meloablative conditioning, acute GVHD and/or chronic GVHD are common complications (30-90% acute GVHD risk depending on the degree of HLA match and subject age group; 30%–50% chronic GVHD risk with conventional transplants). In our series of patients with neoplastic conditions receiving HLA-identical nonmyeloablative bone marrow transplants, the risk of GVHD in the absence of DLI has been approximately 30%, and with only a few exceptions, has been grade II GVHD confined to the skin. In recipients of a single DLI on day 35, the risk of acute GVHD has been approximately 40% (4 of 10 patients), and three of these have also been grade II GVHD of the skin, with or without the subsequent development of chronic GVHD. In 37 HLA-identical transplants using an ATG-containing regimen, we observed a 30% incidence of grade II or more acute GVHD overall. As noted in section 1.2.2, no GVHD was observed in ten non-HLA- identical bone marrow transplant recipients after treatment with our MEDI-507-containing conditioning regimen.

### **1.3.8 Renal Graft Failure**

The 2010 Annual Report of the U.S. Scientific Registry of Transplant Recipients and the Organ Procurement and Transplantation Network estimates 1, 5 and 10-year graft survival rates for renal allograft recipients of living donor allografts as 97%, 80% and 60%, respectively, based on over 64,000 transplants performed between 1998 and 2010. Thus, the short-term outcomes for living donor renal graft recipients using conventional treatment methods are excellent, but long-term results have not been satisfactory. Although 7/10 recipients who received previous regimens are doing well with the longest survival exceeding 10 years, the outcomes for the experimental intervention described in this protocol are unknown.

### **1.3.9 Infection**

While it is recognized that the transient pancytopenia and temporary T-cell depletion induced by conditioning for BMT under this protocol could theoretically lead to an increased risk of infection post transplant, our experience to date (with over 200 primate renal allografts performed with a similar type



of protocol, and with the 10 patients 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 antimicrobial treatments that are used.

#### **1.3.10 Bone Marrow Transplant-related Mortality**

There was no bone marrow transplant mortality in 10 patients treated with combined kidney and bone marrow transplantation. Additionally, no patient mortality has also been seen in 10 myeloma recipients who received CKBMT with similar conditioning regimen. {Spitzer, 1999 #42} {Spitzer, 2011 #3568}

#### **1.3.11 Renal Biopsies**

A wedge biopsy is performed on the day of transplant. Complications for the wedge biopsy include hemorrhage, renal infarction, infection, retention cysts, urine fistula, and hydronephrosis. All subsequent biopsies are performed percutaneously under ultrasound guidance with an 18-gauge needle. The biopsy will be performed only in patients 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 patients. The procedure is performed in the out-patient radiology suite, and the patient is monitored for stability in the transplant unit for approximately 8 hours prior to discharge on the evening of the biopsy. In the case of a rare but significant bleeding event resulting from the renal biopsy, transfusion with packed red blood cells may or may not become medically necessary.

#### **1.3.12 Leukapheresis**

Leukapheresis will be performed on the recipient prior to initiating the conditioning regimen and these cells will be frozen. In the unlikely event that the recipient develops GvHD, we will give the leukapheresis product to the participant (intravenous infusion), to treat the GvHD. If the participant does not develop GvHD, the leukapheresis product will be used for in vitro studies. Complications that may occur during leukapheresis include changes (increase or decrease) in blood pressure or pulse, bleeding, or hypocalcemia. Bleeding, fever, and infection from leukapheresis can also occasionally occur. On rare occasions, these complications may be life-threatening.

#### **1.3.13 Engraftment Syndrome**

Engraftment syndrome was observed in nine of the 10 patients in NKD03/ITN036. This syndrome, which is associated with the loss of chimerism and hematopoietic recovery of the recipient (typically during the second week after bone marrow administration), is characterized by fever, fluid retention, and renal dysfunction., all of which respond to steroids and are reversible. Acute kidney injury (AKI) is a major manifestation of engraftment syndrome and has been observed at approximately day 10 in all subjects except for Subject #1. This syndrome has been described previously following both autologous and allogeneic bone marrow transplantation, which has been associated with the recovery of either host or donor type hematopoietic elements 27. We note that the AKI of engraftment syndrome has never been observed in our MHC-mismatched monkey model, in which chimerism is also lost, but at a more gradual pace, usually over 2-3 months.9,10,28 Our current interpretation of these findings is that the pre-

transplant cyclophosphamide-based regimen chosen for the clinical trials,<sup>15</sup> in contrast to the non-myeloablative total body irradiation used in the monkey preparative regimen,<sup>9,10,28,29</sup> may allow rapid homeostatic recovery of host memory T cells, which may be associated with significant effector function, as suggested by the observation of CD8 T cell activation immediately following conditioning <sup>16</sup> and rapid rejection of the donor hematopoietic graft. Further supporting this hypothesis is the observation that AKI has also never been observed in our HLA-matched CKBMT recipients, in whom chimerism as in the NHP recipients typically disappears more slowly, over a periods of 2-3 months. In this pilot trial, we expect that AKI can be prevented by inducing more stable chimerism (but still expected to be transient chimerism) by substituting low dose TBI for cyclophosphamide.

### **1.3.14 ATG (Thymoglobulin or ATGAM)**

The most common adverse events associated with ATG are:

- Moderate fever and chills
- Leukopenia
- Although rare, the most common severe events associated with ATG are:
  - Allergic or anaphylactoid reactions
  - Serum sickness or serum sickness-like symptoms (fever, pruritus and rash associated with arthralgia or myalgia)

As with all immunosuppression, administration of ATG may be associated with an increased risk of infection and the development of malignancy (especially of the skin and lymphoid system). Thymoglobulin (1.5 mg/kg/day × 3–7 days) the course can be extended in the case of delayed graft function) is standard induction immunosuppression for both cadaveric and living-donor renal transplants at many centers.

The carcinogenic effects and the effect of ATG on fertility have not been established. ATG is contraindicated in patients with a history of allergy or anaphylaxis to rabbit proteins or to any product excipients, or who have active acute or chronic infections that contraindicate any additional immunosuppression.

For additional information on the risks associated with ATG, refer to the package insert for Thymoglobulin® (Genzyme) at the following website:

[http://www.thymoglobulin.com/home/thymo\\_pdf\\_pi.pdf](http://www.thymoglobulin.com/home/thymo_pdf_pi.pdf)

For additional information on the risks associated with ATGAM, refer to the package insert for Atgam® (Pfizer) at the following website:

[http://www.fda.gov/downloads/biologicsbloodvaccines/bloodbloodproducts/approvedproducts/license\\_dproductsblas/fractionatedplasmaproducts/ucm199603.pdf](http://www.fda.gov/downloads/biologicsbloodvaccines/bloodbloodproducts/approvedproducts/license_dproductsblas/fractionatedplasmaproducts/ucm199603.pdf)

## **1.4 RISKS TO DONORS**

### **1.4.1 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 last 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.

#### **1.4.2 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 0.033%. Although uninephrectomy of living donors has been rarely 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.

#### **1.4.3 Combined BMT and Kidney Donation**

The major risk to the donor for either nephrectomy or marrow procurement is that of general anesthesia. The simultaneous procurement of kidney and bone marrow requires approximately an additional hour of anesthesia as compared to a nephrectomy alone.

### **1.5 RATIONALE FOR SELECTION OF CONDITIONING REGIMEN**

All previously treated human subjects except for Subject #1 developed cytokine syndrome-like manifestations<sup>40,52</sup>. This syndrome has been temporally associated with the loss of peripheral chimerism as well as of the return of host-derived elements. The most concerning manifestation of this syndrome is acute kidney injury (AKI), which was observed after day 10 in all patients except for Subject #1. The biopsies taken during AKI showed severe endothelial injury with CD8<sup>+</sup> T cell infiltration (Fig. 4), as reported previously in detail<sup>52</sup>. The peak of serum creatinine levels ranged from 3.5 to 15.4 during days 10-20. Among nine subjects who developed AKI, three recovered without additional treatment, two with Thymoglobulin and plasma exchange, one with Thymoglobulin alone and one with steroid pulse. Two kidney allografts failed to recover despite treatments due to associated humoral rejection and thrombotic micro-angiopathy.

This syndrome has been described previously following both autologous and allogeneic bone marrow transplantation, which has been associated with the recovery of either host or donor type hematopoietic elements<sup>26</sup>. We note that the AKI of engraftment syndrome has never been observed in our MHC-mismatched monkey model, in which chimerism is also lost, but at a more gradual pace, usually over 2-3 months<sup>9,10,27</sup>. Our current interpretation of these findings is that the pre-transplant cyclophosphamide-based regimen chosen for the clinical trials<sup>14</sup>, in contrast to the non-myeloablative total body irradiation used in the monkey preparative regimen<sup>9,10,27,28</sup>, may be

inadequately suppressive, allowing rapid homeostatic recovery of host memory T cells resulting in rapid rejection of the donor hematopoietic graft. Further supporting this hypothesis is the observation that AKI has also never been observed in our HLA-matched CKBMT recipients, in whom chimerism as in the NHP recipients typically disappears more slowly, over a periods of 2-3 months<sup>12,13</sup>. We therefore propose to replace cyclophosphamide with low dose TBI in this clinical pilot study.

## **2. STUDY DESIGN**

### **2.1 DESCRIPTION**

This is a single center, pilot study of 2 adults with end stage renal disease and no evidence of prior sensitization, and their donors.

Recipients will receive a conditioning regimen comprising rituximab, low dose whole body irradiation, MEDI-507 and thymic irradiation prior to combined renal and bone marrow transplant. A short postoperative course of steroids will be administered. Tacrolimus will be given for a defined period and then tapered if specific criteria are met.

Prophylaxis will be provided for PCP, fungal/yeast infection, CMV, and perioperative infection.

The proportion of participants without engraftment syndrome and successfully withdrawn from immunosuppression will be assessed. Clinical outcomes will be compared with measurements of donor-specific tolerance and other immune parameters.

Participants will be followed for a total of 5 years post CKBMT. Assessments will be performed for participant and graft survival, and long-term adverse events. Participants who fail to complete immunosuppression withdrawal will be followed for 104 weeks (24 months) from the time they cease further reduction in immunosuppression.

### **2.2 CONSENT FOR PARTICIPATION**

For individuals identified as potential candidates for the trial, a consent addressing all aspects of the trial, including the planned withdrawal of immunosuppression will be obtained before any screening procedures are performed for evaluation of a participant's eligibility for the trial.

Informed consent will also be obtained from the donor and will be completed prior to performance of donor screening procedures..

### **2.3 STUDY ENDPOINTS**

#### **2.3.1 Primary Endpoint**

The primary endpoint of this study is successful induction of transient chimerism and renal allograft tolerance without "engraftment syndrome" or "acute kidney injury".

## **2.4 STOPPING RULES**

### **2.4.1 Ongoing Review**

The Principal Investigator will review safety data. Enrollment of participants in the trial and withdrawal of immunosuppression in current trial participants will be suspended at any time if any of these reviews concludes that there are significant safety concerns.

### **2.4.2 Review of Specific Adverse Events**

If the following events occur at any time during the study, they will be reviewed by the DMC. Any occurrence of death, graft loss, GVHD greater than grade II or prolonged neutropenia (> 14 Days) will result in suspension of enrollment pending DMC review.

- Death of a participant.
- Any malignancy (excluding basal cell carcinomas of the skin and CIS of the cervix) in two participants.
- Renal graft loss or irreversible organ failure in any participant, as evidenced by a return to dialysis after 1 week post-transplant or a sustained serum creatinine >3 times that established at post-transplant baseline. See Section 4.7.1 for definition of baseline creatinine.
- Humoral rejection in one participant.
- Acute cellular rejection greater than or equal to grade IIB in two participants.
- Acute GVHD greater than or equal to grade II in one participant.

### **2.4.3 Data Monitoring Committee**

The initial DMC meeting to review the study protocol will occur before the first patient receives CKBMT transplant. The review of participant data will occur 6 months after the first patient has received a transplant. Meetings will occur every 6 months thereafter. A DMC meeting will also occur if any of the specific adverse events in section 2.4.2 occur in enrolled participants.

## **2.5 ENROLLMENT, TIMING OF TRANSPLANT, AND LENGTH OF FOLLOW- UP**

For this study, enrollment is defined as the day on which the informed consent is signed.

Participants who complete immunosuppression withdrawal will be followed for a total of 5 years post CKBMT. additional Assessments will be performed for participant and graft survival and for long-term adverse events

Participants who fail to complete immunosuppression withdrawal will be followed for 104 weeks (24 months) from the time they cease further reduction in immunosuppression.

### **3. ELIGIBILITY**

#### **3.1 RECIPIENT INCLUSION CRITERIA**

Patients who meet all of the following criteria are eligible for enrollment as study participants:

1. Male or female 18–60 years of age.
2. Candidate for a living-donor renal allograft with a one haplotype identical donor identified.
3. First or second transplant with either a living donor or cadaveric transplant as the first transplant.
4. Use of FDA-approved methods of contraception (those with less than a 3% failure rate) by all recipients from the time that study treatment begins until 104 weeks (24 months) after renal transplantation. (For further information on FDA-approved methods of contraception, see <http://www.fda.gov/ForConsumers/ByAudience/ForWomen/ucm118465.htm>)
5. Ability to understand and provide informed consent.
6. Serologic evidence of prior exposure to EBV.

#### **3.2 RECIPIENT EXCLUSION CRITERIA**

Patients who meet any of these criteria are not eligible for enrollment as study participants:

1. ABO blood group-incompatible renal allograft.
2. Evidence of anti-HLA antibody within 60 days prior to transplant as assessed by routine methodology (AGH and/or ELISA)
3. Leukopenia (WBC less than 2,000/mm<sup>3</sup>) or thrombocytopenia (platelet count <100,000/mm<sup>3</sup>).
4. Seropositivity for HIV-1, hepatitis B core antigen, or hepatitis C virus (confirmed by hepatitis C virus RNA); or positivity for hepatitis B surface antigen.
5. Cardiac ejection fraction ≤ 40% or clinical evidence of cardiac insufficiency.
6. Forced expiratory volume (FEV<sub>1</sub>) < 50% of predicted.
7. Lactation or pregnancy.
8. History of cancer other than basal cell carcinoma of the skin or carcinoma in situ of the cervix.
9. Underlying renal disease etiology with a high risk of disease recurrence in the transplanted kidney (such as focal segmental glomerulosclerosis, type I or II membranoproliferative glomerulonephritis).

10. Prior dose-limiting radiation therapy.
11. Known genetic disease or family history that may result in greater sensitivity to the effects of irradiation, or a physical deformity that would preclude adequate shielding or appropriate dosing during the irradiation component of the conditioning regimen.
12. Enrollment in other investigational drug studies within 30 days prior to enrollment.
13. Abnormal ( $> 2 \times$  lab normal) values for (a) liver function chemistries (ALT, AST, AP), (b) bilirubin, (c) coagulation studies (PT, PTT).
14. Allergy or sensitivity to any component of MEDI-507, tacrolimus, or rituximab.
15. Maintenance immunosuppression within 3 months prior to conditioning other than physiological doses of steroids, defined as  $\leq 50$  mg of hydrocortisone or dose equivalent.
16. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

### **3.3 DONOR INCLUSION CRITERIA**

Donors who meet the following criteria will undergo nephrectomy and bone marrow aspiration under general anesthesia. Sufficient marrow will be obtained to provide at least  $2 \times 10^8$  nucleated cells per kilogram weight of the recipient.

1. Male or female 18–65 years of age.
2. For females of childbearing potential: a serum pregnancy test showing negative results.
3. Excellent health per conventional predonor history (medical and psychosocial evaluation).
4. Acceptable laboratory parameters (hematology in normal or near-normal range; liver function  $<2$  times the upper limit of normal, and normal creatinine).
5. Negative for viral infection with HbsAg, HIV, HCV, or HTLV-1.
6. Cardiac/pulmonary function within normal limits (CXR, ECG).
7. Ability to understand and provide informed consent.
8. Meets standard institutional criteria for both bone marrow and kidney donation

### **3.4 PREMATURE DISCONTINUATION OF STUDY THERAPY**

#### **3.4.1 Reasons for Premature Discontinuation of Study Therapy**

Study therapy is defined as initiation of any or all of the study medications or procedures described in section 5.

Study therapy will be discontinued for the following reasons:

- Hypersensitivity. If a participant develops a persistent hypersensitivity reaction to rituximab or MEDI-507 despite the measures described in sections 4.4.1, 4.4.1.2, 4.4.3.1 and 4.4.3.3
- Adverse experience. If a participant suffers from an adverse experience that, in the judgment of the principal investigator presents an unacceptable consequence or risk to the participant.
- Intercurrent illness or infection. If during the course of the study a participant develops an illness or infection that is not associated with the condition under study and that requires treatment not consistent with protocol requirements; or, if a participant develops an intercurrent illness that in the judgment of the principal investigator in any way justifies discontinuation.
- Protocol violation. If a participant cannot comply with the study protocol, and the protocol deviations are sufficient to jeopardize his or her well-being or the integrity of the study.

### **3.4.2 Follow -up for Participants Prematurely Discontinued from Study Therapy**

#### **3.4.2.1 Safety Follow-up Assessments**

All enrolled participants who discontinue study therapy, regardless of outcome, will complete a safety follow-up. The following assessments will be performed at each safety follow-up visit:

- Adverse events
- Concomitant medications
- Physical exam
- Hematology
- Serum and basic chemistries
- Thyroid function
- GVHD assessment
- Graft survival
- Chimerism if still detectable at the previous visit

#### **3.4.2.2 Safety Follow-up Visit Schedule**

Participants who discontinue study therapy will observe this visit schedule:

- A visit 2 weeks after discontinuation
- A visit every 4 weeks for 24 weeks
- A visit every 13 weeks for 78 weeks



Participants in safety follow-up will have the option of scheduling a telephone visit in lieu of a clinic visit every other visit when the visits are 13 weeks apart.

### **3.5 PREMATURE TERMINATION FROM THE STUDY**

#### **3.5.1 Reasons for Premature Termination from the Study**

Participants will be prematurely terminated from the study for the following reasons:

- Withdrawal of consent: If for any reason the participant withdraws consent during the study, the participant shall be terminated from the study.
- Lost to follow-up: Participants who fail to return for visits and do not respond to repeated contact by the site staff are considered “lost to follow-up.” Realistic efforts will be made to locate these participants and ask them to continue in the study or to attend the study termination visit so that we may collect closeout study data.
- Participants may only be replaced if they have been withdrawn from the study prior to receiving MEDI-507 and have had no study-related adverse events.

#### **3.5.2 Follow-up for Participants Prematurely Terminated from the Study**

Participants who wish to withdraw consent will be asked to complete the safety follow-up schedule specified in Section 3.4.2

Participants who are prematurely terminated from the study during or after irradiation will be advised of the risk of cytopenias and will be advised to arrange to be followed, particularly during the 4-week period following irradiation. Every attempt will be made to provide this follow-up for safety reasons, even if the participant has withdrawn consent.

## **4. STUDY MEDICATIONS AND PROCEDURES**

### **4.1 OVERVIEW OF CONDITIONING AND POST TRANSPLANT REGIMEN**

Participants will receive a conditioning regimen that starts with rituximab on days -7, -2, 5, and 12, TBI on days -5 and -4. MEDI-507 will be administered on days -2, -1, 0, and 1. Thymic irradiation will be given on day -1, and combined renal and bone marrow transplant will be done on day 0. Prednisone will be started at 2 mg/kg on day 4 and tapered off by day 20. Tacrolimus will be administered on days -1 through 60, and then tapered. This regimen is depicted in Figure 2.

The recipients will have a tunneled central line placed on Day -2 for study medication infusion and blood drawing. This will be removed post-transplant prior to discharge from the hospital.

Prophylaxis will be provided for PCP, CMV, and perioperative infection. All participants who require a blood transfusion will receive only leukocyte-depleted and irradiated blood products for a period of at least 52 weeks following transplant.

## **4.2 DONOR BONE MARROW HARVEST AND NEPHRECTOMY**

The donor will undergo nephrectomy and bone marrow aspiration under general anesthesia. Sufficient marrow will be obtained to provide at least  $2 \times 10^8$  nucleated cells per kilogram weight of the recipient.

## **4.3 LEUKAPHERESIS**

Leukapheresis will be performed x1 on the recipient prior to initiating the conditioning regimen.

## **4.4 CONDITIONING REGIMEN**

### **4.4.1 Rituximab (Days -7, -2, 5, and 12)**

Rituximab will be administered in a closely monitored setting by experienced staff knowledgeable about infusion reactions and their effective and timely management. There should be ready access to equipment and medications appropriate for management of infusion reactions. In particular, medications and fluids for the rapid management of such reactions, including intravenous fluids, epinephrine, antihistamines, inhaled bronchodilators, and corticosteroids, should be readily available.<sup>55</sup>

#### **4.4.1.1 Pretreatment**

Acetaminophen 650 mg PO  $\times$  1, along with diphenhydramine 50 mg PO, and hydrocortisone 100 mg IV  $\times$  1 will be given 30 minutes prior to each dose of rituximab on days -7, 5, and 12. These premedications will not be given before the dose of rituximab on day -2, as the subject will be premedicated before the first dose of MEDI-507 on day -2.

#### **4.4.1.2 Administration and Dosing**

Rituximab will be supplied by the local investigational pharmacy. Instructions for preparation and administration can be found in the package insert at:

<http://www.gene.com/gene/products/information/pdf/rituxan-prescribing.pdf>

Rituximab (375 mg/m<sup>2</sup>/dose) will be administered on days -7, -2, 5, and 12. Doses on days -7 and -2 will be administered several hours after hemodialysis if hemodialysis is required. Start, stop times and dosage for administration will be recorded.

The first rituximab solution for infusion will be administered intravenously at an initial rate of 50 mg/hour. Escalate the rate by 50 mg/hour every 30 minutes to a maximum of 400 mg/hour.

If hypersensitivity or an infusion-related event develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of the subject's symptoms.

The second dose of rituximab will be administered on day -2, after 2–3 hours of completing the first dose of MEDI-507 on day -2. If the first infusion was slowed or interrupted, the initial guidelines should

again be followed. Otherwise, the second infusion may be started at 100 mg/hour and titrated by 100 mg/hour every 30 minutes to a maximum of 400 mg/hour.

The third and fourth doses of rituximab will be administered in the same manner as the second dose.

#### **4.4.2 Low dose Total Body Irradiation (Days -5 and -4)**

##### **4.4.2.1 Pretreatment**

Participants will be treated with anti-emetics as per institutional standard.

##### **4.4.2.2 Administration**

Total Body Irradiation of 150 cGy will be administered on Day -5 and Day -4 for a total dose of 300cGy. Radiation sources and dose rates will be per institutional standards

#### **4.4.3 MEDI-507**

##### **4.4.3.1 Pretreatment**

Steroid premedication with intravenous methylprednisolone sodium succinate, 8 mg/kg up to a maximum dose of 500 mg, along with diphenhydramine 50 mg PO, and acetaminophen 650 mg PO will be administered prior to the first dose of MEDI-507 on day -2 for prophylaxis of possible first dose reactions.

Diphenhydramine and acetaminophen may be administered at the discretion of the investigator 2 hours prior to the second and subsequent infusions of MEDI-507 on days -1, 0, 1, but steroid premedication will not be given.

##### **4.4.3.2 Administration and Dosing**

MEDI-507 is supplied as a clear colorless solution and does not contain a preservative. MEDI-507 bulk biological substance was produced by MedImmune, Inc and validated by TGA Sciences Inc. The bulk solution was sterilized by filtration and aseptically filled into prewashed, presterilized, depyrogenized vials. MEDI-507 is supplied at a concentration of 3.75 mg/mL in 4 mL vials that each contain 15 mg of MEDI-507. Sterile 0.9% normal saline for injection is used as diluent and supplied by MGH. The instructions for dose preparation and administration are given in Appendix 5.

MEDI-507 should be administered at approximately the same time each day, so that there are 24 hours between doses. MEDI-507 will be administered several hours after dialysis. The actual start and stop times of infusion will be recorded on the appropriate case report forms. If hypersensitivity or an infusion-related event develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of subject symptoms.

The first dose of MEDI-507 (day -2) will be 0.1 mg/kg, administered over 30 minutes using an infusion pump. The prepared 0.1-mg/kg dose will be supplied by the pharmacy to the investigator, who will administer the drug. The solution should be prepared to a final concentration of 0.1–0.3 mg/mL (30–100 mL).

The second and subsequent doses of MEDI-507 (days –1, 0, and 1) will be 0.6 mg/kg, administered over 1 hour using an infusion pump. The day 0 infusion can be completed before or after transplant as long as it is consistent with the timing of the last dose. The prepared 0.6-mg/kg dose will be supplied by the pharmacy to the investigator, who will administer the drug. For the second and subsequent doses, a concentration of 0.3–1.8 mg/mL (50- to 100-mL infusion volume) is permitted.

The infusion may be administered using a peripheral vein, or a central line, if the latter is in place. Since the compatibility with other intravenously administered medications is not known, the MEDI-507 solutions should not be infused through a common intravenous line used for other medications, unless the line is flushed prior to and after administration of MEDI-507. Precautions for “first-dose reactions” must be observed for the first and second doses of MEDI-507 (see section 4.4.3.3).

#### **4.4.3.3 Management of Reactions to Rituximab and MEDI-507**

“First-dose reactions” may occur upon the initial administration of rituximab or MEDI-507. These have the potential to be severe. Prophylaxis against first-dose reactions is described above in sections 5.4.1. and 5.4.3. In addition, the following procedures will be followed in assessing the occurrence of such reactions, and to manage adequately any acute toxic manifestations.

Qualified personnel will observe each participant for a period of 2 hours after the administration of each of the first 2 doses of MEDI-507. Vital signs will be collected at the end of each MEDI-507 infusion. A physician who is a member of the transplant team and who is familiar with this protocol must be available and in the facility throughout this time period. Additionally, a person who is expert in intubation must be on call, in the facility, and available for 2 hours after the first and second doses of rituximab and MEDI-507 in each subject. An emergency cart must be available. Participants must remain under observation for at least 2 hours after completion of the infusion of the first two doses of antibody.

Although acetaminophen and diphenhydramine are given prophylactically prior to administration of rituximab and MEDI-507, further doses of acetaminophen (650 mg PO or PR) and diphenhydramine (50 mg PO or IV) may be administered if fever or chills occur post study drug administration. Temperature and other physical symptoms of the subject must be documented prior to administration of either medication. Corticosteroids (methylprednisolone, 250 mg IV) may be administered if a severe reaction occurs. All symptoms of drug reactions and treatment for drug reactions must be recorded on the appropriate case report forms.

#### **4.4.4 Thymic Irradiation (Day –1)**

The 700 cGy of thymic irradiation are administered in a single dose on day –1. A field size of approximately 8 cm wide and 10 cm in longitudinal dimension will be used, with the midpoint of the upper edge of the field at the sternal notch. The dose will be calculated at a depth of approximately 6 cm, guided by results of a lateral chest roentgenogram for the approximate location of the thymus. A 10-MV machine at maximum dose rate should be used. No premedication for nausea should be required. The details regarding the field sizes, dose calculation, energy, beam spoiling, and dose rate will be recorded on the case report form.

#### **4.4.5 Tacrolimus Administration and Dosing (Day –1)**

Tacrolimus will be administered starting on day –1. The dose will be 0.05 mg/kg twice a day, and will be adjusted to provide a trough whole blood concentration of 8-12 ng/mL. Substitution of generic forms of tacrolimus is not permitted. Tacrolimus will be continued at this level, then tapered as described in section 5.4.8.

#### **4.4.6 Prednisone (Days 4–20, 22–42) and Methylprednisolone (Days 22–42)**

To further mitigate possible symptoms of engraftment syndrome, prednisone will be started at 2 mg/kg/day on day 4 and then tapered off by day 20.

Additional corticosteroids will be administered from days 22 to 42 if the participant meets any of the following criteria:

1. There has been no multilineage chimerism at any time post transplant.
2. There are clinical findings suggestive of rejection and there is biopsy confirmation of renal allograft rejection of Banff grade IA or greater.

#### **4.4.7 ATG Administration in the event of Engraftment Syndrome**

During the second post-operative week, if the creatinine begins to increase or the donor chimerism begins to precipitously fall ( $\geq 50\%$ ), Tacrolimus will be discontinued and ATG will be given. The recipients can receive up to 5 doses over a 10 day period during this time. Tacrolimus administration will be resumed after ATG is discontinued. Tacrolimus will subsequently be withdrawn as detailed in section 4.4.8.

##### **4.4.7.1 Preparation and Storage**

For information on the preparation, storage and handling of Thymoglobulin<sup>®</sup>, please refer to the package insert for Thymoglobulin<sup>®</sup> (antithymocyte globulin [rabbit]) at the following website: [http://www.thymoglobulin.com/home/thymo\\_pdf\\_pi.pdf](http://www.thymoglobulin.com/home/thymo_pdf_pi.pdf)

For information on the preparation, storage and handling of ATGAM, please refer to the package insert for Atgam<sup>®</sup> (lymphocyte immune globulin, anti-thymocyte globulin [equine]) at the following website: <http://www.fda.gov/downloads/biologicsbloodvaccines/bloodbloodproducts/approvedproducts/licensedproductsblas/fractionatedplasmaproducts/ucm199603.pdf>

##### **4.4.7.2 Pretreatment**

Before each dose of ATG, participants will be pre-medicated with:

- Methylprednisolone 100mg IV In addition participants will be pre-medicated with
- Acetaminophen 650 mg PO and
- Diphenhydramine 25 to 50 mg PO

##### **4.4.7.3 Administration and Dosage**

Participants may receive up to 5 intravenous doses of Thymoglobulin 1.5 mg/kg/dose or ATGAM 15mg/kg/dose during a 10 day time period. This should be given on a QOD schedule.

The first dose of ATG should be infused over a minimum of 6 hours into a high-flow vein. The vein may be peripheral or central per investigator discretion. Subsequent doses should be infused over at least 4

hours. ATG must be given under the supervision of appropriately trained medical personnel. At any sign of any infusion related side effects, the infusion rate should be slowed by 50%. In the rare instance that anaphylaxis is reported with ATG use, the infusion should be terminated immediately and medical personnel should be available to treat patients who experience anaphylaxis

#### **4.4.8 Immunosuppression Withdrawal**

##### **4.4.8.1 Eligibility Criteria for Immunosuppression Withdrawal**

Tacrolimus will be tapered beginning 60 days post transplant if all of the following conditions are met:

1. There has been stable renal function (Cr < 2.0 mg/dL) for > 30 days, unless a transient rise in creatinine has been demonstrated to be clearly related to a known cause of renal dysfunction such as tacrolimus toxicity, a urologic complication, other drug toxicity, etc.
2. There has been no evidence of Banff grade IA or greater rejection on a renal biopsy obtained within the past 4 weeks.
3. There has been detectable multilineage white blood cell chimerism at any level. For this purpose, chimerism will be assessed by flow cytometry.
4. There has been no evidence of GVHD.
5. There is no detectable antidonor HLA antibody.

Participants who do not meet these criteria the first time they are assessed will be reevaluated for withdrawal eligibility every 4 weeks.

Participants who do not meet withdrawal criteria by 78 weeks post transplant, however, will be considered ineligible for and to have failed immunosuppression withdrawal, and will begin safety follow-up as described in Section 3.4.2.

##### **4.4.8.2 Pace and Time of Completion of Immunosuppression Withdrawal**

Participants will withdraw from tacrolimus over no less than 12 weeks.

All withdrawal attempts will be completed no later than 104 weeks post transplant. Participants who do not complete withdrawal by this time will be considered to have failed immunosuppression withdrawal, and will begin safety follow-up as described in Section 3.4.2..

##### **4.4.8.3 Follow-up During and After Immunosuppression Withdrawal**

Participants will be followed intensively for evidence of allograft rejection during and after the completion of withdrawal. A chemistry panel including serum creatinine will be measured:

- Weekly during withdrawal and for 3 months after tacrolimus is discontinued
- Every 2 weeks for 26 weeks
- Monthly for 52 weeks

#### **4.4.8.4 Immunosuppression Withdrawal after Episodes of Rejection**

Participants who experience rejection prior to completion of immunosuppression withdrawal will be permitted to resume immunosuppression withdrawal only if they fulfill the following criteria:

1. Stability on maintenance calcineurin inhibitor monotherapy for at least 60 days.
2. A renal biopsy that demonstrates the absence of rejection, defined as less than Banff grade IA.

#### **4.4.8.5 Follow-up and Treatment for Participants who Fail Immunosuppression Withdrawal**

Participants who have failed immunosuppression withdrawal will begin safety follow-up as detailed in 3.4.2.2.

Participants in safety follow-up will have the option of scheduling a telephone visit in lieu of a clinic visit every other visit when the visits are 13 weeks apart.

Participants who fail immunosuppression withdrawal will be treated according to institutional standard at the discretion of the investigator.

Participants who experience rejection following completion of immunosuppression withdrawal will be permitted to restart immunosuppressive therapy and attempt withdrawal at a slower pace. If such participants experience a second episode of rejection at any time during the second attempt, they will not be allowed to undergo further immunosuppression withdrawal. They will be considered to have failed immunosuppression withdrawal and will begin safety follow-up as described in Appendix 7.

### **4.5 TRANSPLANT PROCEDURES**

#### **4.5.1 Bone Marrow Transplant (Day 0)**

##### **4.5.1.1 Preparation and Characterization of Bone Marrow Product**

Donor bone marrow ( $>1.5 \times 10^8$  nucleated cells /kg of recipient body weight) is prepared for infusion and characterized as outlined below. In the situation of minor ABO incompatibility, plasma will first be removed following centrifugation of the product. The bone marrow sample will be characterized as to the total volume, total nucleated cell content, total CD3+ T-cell content, and total CD34+ cell content.

##### **4.5.1.2 Infusion Procedures**

Allogeneic bone marrow will be rapidly infused intravenously without a filter as soon as possible after harvest. A total of 15,000 units of heparin is mixed with the marrow, which is infused at a rate of 300-500 mL/hour. The infusion begins in the operating room as soon as the vascular anastomosis of the renal allograft has been completed.

Protamine, 25 mg, is administered IV after completion of the first half of the bone marrow infusion. If a partial thromboplastin time measured after completion of the marrow infusion is greater than 60 seconds, the Protamine treatment will be repeated.

#### **4.5.2 Renal Transplant (Day 0)**

Prophylactic antimicrobial therapy will be administered prior to surgery according to institutional practice.

##### **4.5.2.1 Surgical Procedure**

The renal transplant is performed according to standard surgical techniques. A ureteral stent should be placed if surgically feasible.

##### **4.5.2.2 Intra-operative Renal Biopsy**

A wedge biopsy will be obtained on the day of transplant and sent for routine pathology diagnostics (please refer to section 5.4.2).

#### **4.6 PROPHYLAXIS AGAINST AND TREATMENT OF INFECTION**

##### **4.6.1 Pneumocystis and Urinary Tract Infections**

Trimethoprim and Sulfamethoxazole (TMP/SMX), one single- or double-strength tablet daily will be administered to all participants starting at the initiation of the conditioning regimen (day -7) and continuing until the day of transplantation. It will be restarted the day neutrophil counts exceed 500/ $\mu$ L and continued until 24 weeks post transplant.

In the event of allergy or intolerance to the components of TMP/SMX, atovaquone, 1.5 g PO each day will be used in its place for prophylaxis of *Pneumocystis carinii*.

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 pneumococcus and *H. influenza*.

Levofloxacin, 250–500 mg daily (as a suitable quinolone substitute), will be started at the onset of neutropenia (ANC <0.5) and continued until resolution of the neutropenia (ANC >0.5).

##### **4.6.2 Fungal and Yeast Infections**

Post-transplant prophylaxis for fungal infections will be administered at the discretion of the investigator.

##### **4.6.3 CMV Infection**

###### **4.6.3.1 Prophylaxis When at Least One of the Donor–Recipient Pair is Seropositive**



Cytomegalovirus prophylaxis is central to the successful outcome of BMT and organ transplantation. All BMT participants should receive seronegative or CMV “safe” blood (leukofiltered). The greatest risk for CMV infection in solid organ transplantation is for the donor seropositive (D+) into recipient seronegative (R-) combination. In hematopoietic stem-cell transplantation, CMV pneumonitis is most common in the D- into R+ combination. Given that CMV infection may occur in any combination in which at least one of the donor–recipient pair is seropositive, a uniform approach for such cases is most reasonable in terms of preventing invasive infection. To this end, each recipient under this protocol will:

1. Have the serologic status obtained for donor and recipient.
2. Be monitored weekly by quantitative CMV DNA PCR assay while neutropenic and following engraftment.
3. Receive Ganciclovir 5 mg/kg × 1 dose prior to renal transplantation (day –1).
4. Subsequently receive CMV prophylaxis per institutional standards after the ANC exceeds 1000.

#### 4.6.3.2 Prophylaxis in Individuals when Donors-Recipients are both Seronegative

Provided CMV-negative pedigreed blood products are utilized, there is a very low risk of CMV disease when both donor and recipient are seronegative. Therefore, in such cases, CMV prophylaxis will not be given. To prevent the 40% incidence of herpes simplex disease that occurs in transplant patients not receiving ganciclovir, oral acyclovir 400 mg TID or other approved antiviral therapy will be prescribed for 4–6 months.

#### 4.6.3.3 Treatment of CMV Infection

Initial treatment of CMV disease (defined by CMV PCR) is for a minimum of 2–3 weeks with intravenous ganciclovir at a dose of 5 mg/kg twice daily (with dosage adjustment for renal dysfunction), oral valganciclovir 900 mg PO BID, or other approved antiviral medication per institutional standards. CMV IgG (150 mg/kg q 4 weeks) will be added for seronegative participants at the discretion of the investigator. The endpoint of intravenous therapy is the documented clearance of virus from the blood as demonstrated by CMV antigenemia assay or quantitative PCR assay.

The risk of subsequent relapse is ~15%–20%, so treatment will be continued for at least 3 months. With 3 months of oral therapy following clearance of viremia, the rate of relapse is decreased substantially.

In participants with relapsing infection, initial treatment is repeated. CMV hyperimmune globulin (at a dose of 150 mg/kg IV once and 100 mg/kg IV q month × 3–6) is usually administered in conjunction with the intravenous ganciclovir, oral valganciclovir or other approved therapy for seronegative recipients at the discretion of the investigator. Treatment will be maintained in the setting of active GVHD.

Table 2. 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 a loading dose of 5 mg/kg. All patients (prophylaxis or therapeutic) receive leukocyte or CMV-negative blood; CMV-hyperimmune globulin (Massachusetts Red Cross) 150 mg/kg IV for the first dose and 100mg/kg × 4 at the discretion of the physician.

#### **4.6.4 Herpes Infection**

CMV prophylaxis as described in section 4.6.3 will also serve as prophylaxis against herpes infection.

### **4.7 ASSESSMENT OF ALLOGRAFT DYSFUNCTION AND TREATMENT OF RENAL REJECTION**

#### **4.7.1 Definition of Allograft Dysfunction and Indication for Biopsy**

Renal allograft dysfunction is defined as an increase in creatinine of 25% or greater compared to the baseline creatinine post transplant. Baseline is defined as an average of the 3 lowest creatinines during weeks 2-4 post transplant, excluding days on dialysis.

If allograft dysfunction is unexplained and persists for greater than 72 hours, a renal biopsy must be performed to rule out acute rejection. If the biopsy does not demonstrate rejection, further evaluation for other causes of renal dysfunction will be performed.

#### **4.7.2 Diagnosis, Grading and Monitoring of Rejection**

Renal allograft biopsies will be used to diagnose and grade rejection. Biopsies will be assigned a Banff grade according to the most current Banff Classification of Renal Allograft Pathology.

##### **4.7.2.1 Humoral (Antibody-mediated) Rejection**

Humoral rejection is defined according to the Banff Classification of Renal Allograft Pathology (see Appendix 2).

##### **4.7.2.2 Acute Cellular Rejection**

A Banff score of grade IA or above will constitute acute cellular rejection (see Appendix 2).

##### **4.7.2.3 Chronic Rejection**

Chronic rejection (both humoral and cellular) is defined according to the Banff Classification of Renal Allograft Pathology (see Appendix 2).

#### **4.7.3 Treatment of Rejection**

If participants show evidence of graft rejection, standard rejection therapy will be initiated according to institutional protocol. Specific therapy for rejection may include any or all of the following: Solumedrol, Thymoglobulin, Campath, or other antilymphocyte antibody therapies and local irradiation of the kidney allograft. Plasmapheresis, IVIg, Rituximab and Velcade may be utilized if humoral rejection is diagnosed. A renal biopsy will be performed within 14 days of completion of treatment for rejection to document response to therapy. After biopsy confirmation of resolution of rejection, the participant will be weaned to maintenance therapy as defined by institutional practices. Maintenance therapy may include any or all of the following medications: tacrolimus, cyclosporine, sirolimus, steroids, azathioprine, and/or MMF. 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.

For participants with findings “suspicious” for acute cellular rejection (less than grade IA rejection), additional maintenance immunosuppressive therapy will not be instituted even if they are treated for rejection based on clinical need. However, these participants will be rebiopsied within 3–4 weeks, closely monitored for further histological or clinical evidence of rejection, and considered for subsequent tacrolimus taper and discontinuation.

For participants who receive antilymphocyte antibody therapy, antiviral and antipneumocystosis prophylaxis will be administered for an additional 10 weeks at the discretion of the investigator.

#### **4.8 PROHIBITED MEDICATIONS**

No concomitant immunosuppressants aside from rituximab and MEDI-507 (or others specified in this protocol) may be administered during the conditioning regimen. If such therapy is required, the conditioning regimen must be stopped, and the participant will be considered to have prematurely discontinued study therapy (section 4.4). The administration of any live or investigational vaccine is prohibited for all participants on the study from 30 days prior to transplantation (day -30) until 60 days following transplantation (day 60).

#### **4.9 DRUG ACCOUNTABILITY**

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator is required to maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed. Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log containing the patient identification number and the date and quantity of drug dispensed will be maintained for each participant.

### **5. STUDY PROCEDURES**

#### **5.1 GENERAL ASSESSMENTS**

Informed consent: Written informed consent will be obtained before any study-related assessments or procedures are performed.

- Physical examination

- At Screening
  - At each inpatient or outpatient visit
- Medical history: Medical history will include psychosocial evaluation and previous transplant history
  - At Screening
- Cardiac function—echocardiogram.
  - At Screening
- Pulmonary function—chest x-ray and PFTs (DLCO, spirometry & Lung volumes)
  - At Screening
- GVHD: GVHD will be staged and graded as in Appendix 3.
  - Daily while hospitalized
  - At each outpatient visit until chimerism undetectable
- Leukapheresis
  - Prior to Day -7
- Protocol Renal biopsy
  - Day 10
  - Month 6
  - Month 9
  - Year 1
  - Year 2
  - Year 3

## 5.2 LABORATORY ASSESSMENTS

- Hematology—CBC with differential and platelets
  - At Screening
  - Daily while hospitalized
  - At each outpatient visit
- Serum chemistry—ALT, AST, BUN, AP, bilirubin, albumin, total protein
  - At Screening
  - Weekly while hospitalized
  - At each outpatient visit
- Basic chemistry—electrolytes, glucose, BUN, and creatinine
  - Daily while hospitalized
  - At each outpatient visit
  - See section 4.9.3 for monitoring during and after immunosuppression withdrawal
- Coagulation studies—PT/INR and PTT
  - At screening
  - Week 1
  - Day 10 (with biopsy)
  - Month 9 (with biopsy)
  - Every 6 months thereafter
- Thyroid function—T3, FT4, and TSH
  - At Screening
  - Every 6 months thereafter
- Serum pregnancy test

- At Screening
  - HLA typing – by intermediate resolution for HLA-A, B, C, DRB1, DQA1 and DQB1
    - At Screening
  - ABO typing
    - At Screening
  - T and B Cell crossmatch by Flow Cytometry and Cytotoxicity
    - At Screening
  - HLA alloantibodies per routine methodology (AHG and/or ELISA)
    - At Screening
    - Prior to Initiation of Day -7 Treatment
    - Weekly - Weeks 1-6
    - Every other month for 3 years
    - Year 4
    - Year 5
  - Viral and syphilis serology—CMV IgG, HIV I/II, HbsAg, HbsAb, HCV, EBV, and syphilis
    - At Screening
  - Tacrolimus levels
    - Daily Post-Transplant while hospitalized
    - At each outpatient visit
    - May discontinue when level is undetectable after immunosuppression withdrawal
  - BK virus PCR (blood)
    - Week 1
    - Every other month thereafter
  - Chimersim
    - 2x/ Week starting on Day 7 until chimerism is undetectable
  - Research Testing
- This is a 2 patient pilot trial, no funding is provided for in vitro research studies. A minimal volume of blood ( $\leq 40$  cc / week) will be drawn and stored for potential future research studies. If the leukapheresis that is collected pre-transplant is not used to treat GVHD, this will be used for potential future research studies as well.

## **5.3 RENAL BIOPSY**

### **5.3.1. RENAL CORE BIOPSIES**

Participants will undergo surveillance renal core biopsies Day 10-12, 6 months, 1 Year, 2 Years and 3 Years post transplant. These biopsies will be used to monitor function and to screen for subclinical rejection.

### **5.3.2 INTRA-OPERATIVE WEDGE BIOPSY**

A 3 × 5-mm wedge biopsy will be obtained on the day of transplant to assess for any evidence of donor disease.

### **5.3.3 For-cause Biopsies**

For-cause biopsies will be obtained to confirm suspected rejection following unexplained allograft dysfunction.

## **6. ADVERSE EVENTS**

### **6.1 OVERVIEW**

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events, Version 4.0 (May 28, 2009).

### **6.2 DEFINITIONS**

#### **6.2.1 Adverse Event**

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial.

An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first.

#### **6.2.2 Serious Adverse Event**

An SAE or reaction is defined as any adverse event that suggests a significant hazard, contraindication, side effect, or precaution. This includes but is not limited to any of the following events:

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered treatment related or not.
- A life-threatening event: A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability.
- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the above definitions.

### **6.2.3 Unexpected Adverse Event**

An adverse event is considered “unexpected” when its nature or severity is not consistent with applicable product information described in the safety information provided in the investigator’s brochure for MEDI-507 or package inserts for study medications in section 4. The package inserts that apply are those for Rituxan (rituximab), Thymoglobulin, ATGAM, prednisone, Prograf (tacrolimus).

## **6.3 COLLECTING ADVERSE EVENTS**

Adverse events will be collected from the time the participant is enrolled until 30 days after the participant completes the study. Prior to the beginning of study treatment, only adverse events related to screening assessments will be reported.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant’s safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate case report form(s).

The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

## **6.4 GRADING, ATTRIBUTION AND REPORTING OF ADVERSE EVENTS**

### **6.4.1 Grading Criteria**

The study site will grade the severity of adverse events experienced by ITN study participants according to the criteria set forth in the National Cancer Institute’s Common Toxicity Criteria for Adverse Events Version 4.0 (published May 28, 2009). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site:

<http://ctep.cancer.gov/reporting/ctc.html>.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

#### 6.4.2 Attribution Definitions

Adverse events will be categorized for their relation to each of the following:

- MEDI-507
- Total Body Irradiation
- Rituximab
- Tacrolimus (or other CNI)
- Other study medications and procedures

The relation, or attribution, of an adverse event to MEDI-507 and other study medications and procedures will be determined by the site investigator. The investigator will also record the determination of attribution on the appropriate CRF and/or SAE reporting form. The relation of an adverse event to MEDI-507 or other study medications and procedures will be determined using the descriptors and definitions provided in Table 6.

Table 3. Attribution of adverse events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related.
Related Categories		
2	Unlikely	The adverse event is doubtfully related.
3	Possible	The adverse event may be related.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

#### 6.4.3. Adverse Event Reporting Requirements

Adverse events that are  $\leq$  Grade 2 will not be reported.

## 7. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN



## **7.1 ANALYSIS SAMPLES**

Given a sample size of 2 participants, the analyses will be descriptive. However, to the extent possible, estimates obtained in the study will be interpreted in the context of the data from historical cohorts.

## **8. QUALITY CONTROL AND QUALITY ASSURANCE**

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented.

## **9. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE**

### **9.1 STATEMENT OF COMPLIANCE**

This trial will be conducted in compliance with the protocol, with current good clinical practices (GCP), the principles of the Declaration of Helsinki, and with all applicable regulatory requirements.

Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics review committee or institutional review board. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

### **9.2 INFORMED CONSENT**

The informed consent form is a means of providing information about the trial to a prospective subject and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before entering the study, taking study drug, or undergoing any study-specific procedures. Consent materials for participants who do not speak or read English must be translated into the participants' appropriate language.

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The attending physician, in the presence of a witness, will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he/she may withdraw from the study at any time, for any reason.

### **9.3 PRIVACY AND CONFIDENTIALITY**

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number, and these numbers rather than names will be used to collect, store, and report participant information.

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## **Appendix 1. Diagnosis and Classification of Selected Opportunistic Infections**

### **Cytomegalovirus Infections**

1. Seroconversion: Change from CMV seronegative status to seropositive after transplantation as determined by an accepted serologic test, using the manufacturers criteria for seroconversion. The type of test used should be specified.
2. Shedding (urine, throat): A positive culture, whether conventional or by rapid antigen test ("shell vial"), without evidence of clinical disease.
3. Viremia or antigenemia: A positive culture from blood or buffy coat, whether by conventional culture, or rapid antigen test ("shell vial"), or immunochemical testing.
4. Disseminated/syndrome: A positive culture from blood or buffy coat, or another site, as above, in association with a compatible clinical illness consisting of fever and leukopenia, with or without liver function test abnormalities.
5. End organ disease (specify site): Clinical signs plus a positive culture of a normally sterile site, preferably the organ involved, or positive histology with appropriate clinical signs (gut, lung, retina, liver, etc.). Must be considered presumptive if positive clinical signs or laboratory test results are found, but no definitive evidence of viral invasion is present (positive culture, presence of antigen, positive histology).

### **Herpes Zoster Infections**

1. Localized: Dermatomal distribution of a single or 2 adjacent dermatomes with no crossing the midline.
2. Skin dissemination: Lesions in more than 2 dermatomes, crossing the midline, or multiple isolated lesions on the skin, with no evidence of internal organ involvement (e.g. brain, lungs, liver, gut, peritoneum).
3. Disseminated: Internal organ involvement by histology or compatible clinical syndrome with characteristic skin involvement.

### **Candida Infections**

1. Mucosal: Mucosal involvement (oropharynx, GU tract) without systemic signs or symptoms attributable to Candida.
2. Candidemia: Positive culture of blood or intravenous access device tip, with or without fever.
3. Invasive: Positive culture of a normally sterile site, or positive histologic evidence of tissue invasion (skin [hematogenous], gut, liver, spleen, retina, lung, kidney), including positive blood culture in absence of an infected intravenous device.

## Appendix 2. Current Banff Classification of Renal Allograft Pathology (Banff 2007)<sup>1</sup>

### 1. Normal

### 2. Antibody-mediated changes (may coincide with categories 3, 4 and 5 and 6)

Due to documentation of circulating antidonor antibody, C4d,<sup>1</sup> and allograft pathology

C4d deposition without morphologic evidence of active rejection

C4d+, presence of circulating antidonor antibodies, no signs of acute or chronic TCMR or ABMR (i.e. g0, cg0, ptc0, no ptc lamination (<5 layers by electron microscopy), no ATN-like minimal inflammation). Cases with simultaneous borderline changes are considered as indeterminate

#### Acute antibody-mediated rejection<sup>2</sup>

C4d+, presence of circulating antidonor antibodies, morphologic evidence of acute tissue injury, such as (Type/Grade)

Type I. ATN-like minimal inflammation

Type II. Capillary and/or glomerular inflammation (ptc/g >0) and/or thromboses

Type III. Arterial—v3

#### Chronic active antibody-mediated rejection<sup>2</sup>

C4d+, presence of circulating antidonor antibodies, morphologic evidence of chronic tissue injury, such as glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries

### 3. Borderline changes: 'Suspicious' for acute T-cell mediated rejection (may coincide with categories 2 and 5, and 6)

This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2 or t3) with minor interstitial infiltration (i0 or i1) or interstitial infiltration (i2, i3) with mild (t1) tubulitis

### 4. T-cell mediated rejection (TCMR, may coincide with categories 2 and 5 and 6)

Acute T-cell mediated rejection (Type/Grade):

IA. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)

IB. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)

IIA. Cases with mild to moderate intimal arteritis (v1)

IIB. Cases with severe intimal arteritis comprising >25% of the luminal area (v2)

III. Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)

Chronic active T-cell mediated rejection

'chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)

5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology (may include nonspecific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features)

Grade

I. Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)

II. Moderate interstitial fibrosis and tubular atrophy (26–50% of cortical area)

III. Severe interstitial fibrosis and tubular atrophy/ loss (>50% of cortical area)

6. Other: Changes not considered to be due to rejection- acute and/or chronic (For diagnoses see table 14 in (49); may include isolated g, cg, or cv lesions and coincide with categories 2, 3, 4, and 5)

<sup>1</sup>Please refer to Banff 2007 classification paper <http://onlinelibrary.wiley.com/doi/10.1111/j.1600-6143.2009.02987.x/abstract>

<sup>2</sup>Suspicious for antibody-mediated rejection if C4d (in the presence of antibody) or alloantibody (C4d+) not demonstrated in the presence of morphologic evidence of tissue injury.

B. Sis<sup>1,2,\*</sup>, M. Mengel<sup>1,2</sup>, M. Haas<sup>3</sup>, R. B. Colvin<sup>4</sup>, et al., Banff '09 Meeting Report: Antibody Mediated Graft Deterioration and Implementation of Banff Working Group. Am J Transplant, 2009;10:464-471 <http://onlinelibrary.wiley.com/doi/10.1111/j.1600-6143.2009.02987.x/abstract>

### Appendix 3. Staging and Grading of Graft-Versus-Host Disease

#### Organ Staging of Graft-Versus-Host Disease

Stage	Skin	Liver	Gut
1	Maculopapular rash, <25% of body surface <sup>a</sup>	Bilirubin 2–3 mg/dL <sup>b</sup>	500–999 mL diarrhea/day <sup>c</sup> , or persistent nausea with histologic evidence of GVHD in stomach or duodenum
2	Maculopapular rash, 25%–50% of body surface <sup>a</sup>	Bilirubin 3.1–6 mg/dL <sup>b</sup>	1000-1499 mL diarrhea/day <sup>c</sup>
3	Maculopapular rash, > 50% of body surface <sup>a</sup>	Bilirubin 6.1–15 mg/dL <sup>b</sup>	1500 or more mL diarrhea/day <sup>c</sup>
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl <sup>b</sup>	Severe abdominal pain with or without ileus

<sup>a</sup> Use “Rule of Nines” to determine extent of rash.

<sup>b</sup> Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.

<sup>c</sup> Downgrade one stage if an additional cause of elevated bilirubin has been documented.

#### Overall Clinical Grading of Severity of Acute GVHD <sup>a</sup>

Grade	Degree of Organ Involvement
I	Stage 1-2 rash and no liver or gut involvement
II	Stage 3 rash, or stage 1 liver involvement, or stage 1 gut involvement
III	None to stage 3 skin rash with stage 2–3 liver, or stage 2–4 gut involvement
IV	Stage 4 skin rash, or stage 4 liver involvement

<sup>a</sup> Adapted from Thomas ED, Storb R, Clift RA, et al. Bone-marrow transplantation (second of two parts). N Engl J Med. April 24, 1975;292(17):895-902.



## Appendix 4. Donor Evaluations

### **Organ Transplantation Protocol for Donor Screening**

The initial examination of potential living donors will include:

- Evaluation by appropriate staff psychiatrist.
- HLA typing (by Intermediate resolution) HLA-A, B, C, DRB1, DQA1 and DQB1.. B- and T-cell crossmatch tests will also be performed.
- Determination of ABO blood group.

If the donor is found to be mentally suitable and compatible for blood and tissue types, the following will be performed:

- Health history questionnaire
- Physical examination by physician
- Blood tests:
  - Comprehensive metabolic panel
  - Hepatic panel
  - Lipid panel
  - CBC, Diff, Sed Rate,
  - Retic
  - PT, PTT
  - EBV antibodies
  - CMV IgG
  - RPR
  - CMV antibodies
  - Ab to Hep B core Ag, total Hepatitis B surface antigen Hepatitis B surface antibody  
Hepatitis C antibody
  - HIV I/II screen
  - HTLV1 antibody
  - Blood to QC for monoclonals for chimerism testing
- Urine tests:
  - Urinalysis
  - Urine culture
  - 24-hour urine collection for total protein and creatinine clearance
- Chest x-ray (PA and LAT)
- EKG

### Collecting Donor Adverse Events

Adverse events related to study procedures will be collected from the time the donor is enrolled until 10 days after all study procedures have been completed. Adverse events will be followed until they have resolved or until 30 days after the donor terminates from the study, whichever comes first.

### Reporting Donor Adverse Events

All donor adverse events except those  $\leq$  grade 2 will be reported .

## Appendix 5: MEDI-507 Dose Preparation Instructions

### 1. GENERAL INSTRUCTIONS

- ❖ MEDI-507 is provided as a preservative-free, sterile solution at a concentration of 3.75 mg/mL. Each single-use vial contains approximately 4 mL of solution (15mg/vial).
- ❖ The solution appears clear with occasional protein strands and particles.
- ❖ MEDI-507 must be stored between 2° and 8°C (36–46°F).
- ❖ The solution must not be frozen.

### 2. DOSE PREPARATION/ADMINISTRATION:

- ❖ Select the appropriate number of vials of MEDI-507 required to prepare the patient's dose.
- ❖ MEDI-507 vials should come to room temperature prior to dose preparation.
- ❖ Inspect each vial selected for dose preparation.
- ❖ To avoid foaming, the vial should not be shaken. A vial should only be used once to prepare a single dose.
- ❖ The solution in each vial should be carefully inspected for turbidity. If the solution is not clear, quarantine the vial(s) at refrigerated 2° and 8°C (36–46° F) temperature for institution drug accountability.
- ❖ MEDI-507 must be prepared using aseptic technique.
- ❖ MEDI-507 must be diluted for intravenous administration in a polyvinyl chloride (PVC) infusion bag containing 0.9% Sodium Chloride for injection.
- ❖ Once a patient's dose is prepared, it must be administered within 24 hours.
- ❖ If it is not administered within this interval, a new dose must be prepared.
- ❖ The pharmacist or site personnel should slowly draw the calculated dose volume plus 0.5 mL from the vial(s) using an appropriately sized syringe (additional 0.5 mL for priming filter and needle). Attach a 25 mm 0.22 micron Millex GV filter to the syringe, attach a new needle and slowly prime the syringe to the calculated dose volume. The dose volume of MEDI-507 is then added to a polyvinyl chloride (PVC) infusion bag containing 0.9% Sodium Chloride, USP.

### 3. ANCILLARY ITEMS REQUIRED:

- ❖ 25 mm 0.22 micron Millex GV filter.
- ❖ Polyvinyl chloride (PVC) infusion bag containing 0.9% Sodium Chloride, USP

## Appendix 6: Long-term results of HLA mismatched combined kidney and bone marrow transplantation without maintenance immunosuppression

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### Contribution:

Tatsuo Kawai, A. Benedict Cosimi, David H. Sachs, Megan Sykes, Thomas Spitzer and Robert B. Colvin worked for study design, clinical management, data analyses and manuscript writing. Nina Tolckoff-Rubin, Waichi Wong, Winfred W. Williams and Rex-Neal Smith participated in clinical management. Susan L. Saidman, Fred Pfeffer, Ben Sprangers, Brittany Shonts, Sam LoCascio, Heather Morris performed in vitro assays and data analyses.

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### Summary:

**Background:** We report the long-term results (up to 10 years) of HLA mismatched kidney transplantation without maintenance immunosuppression (IS) following combined kidney and bone marrow transplantation (CKBMT).

**METHODS:** Five previously reported subjects underwent CKBMT after a nonmyeloablative conditioning regimen (NKD03) including cyclophosphamide, thymic irradiation, anti-CD2 mAb with or without pre-transplant rituximab. Five subsequent subjects received a revised regimen (ITN036), in which four doses of peri-transplant rituximab were added.

**RESULTS:** All 10 subjects developed transient chimerism becoming undetectable by day 21 without GVHD. Associated with the loss of chimerism, 9/10 developed transient acute kidney injury (AKI) beginning around day 10. Two kidneys failed: one at week 2 secondary to humoral rejection and one at 6 months secondary to thrombotic microangiopathy. IS was discontinued 8-14 months post-transplant in the remaining 8 subjects. One developed acute rejection 2 months later and has remained on chronic IS. Five of the remaining 7 are off IS with no evidence of rejection for periods of 3-10 years. In two subjects, treatment with mycophenolate mofetil alone was initiated 7-8 years after IS withdrawal due to recurrent MPGN in one and probable chronic humoral rejection in the other. Despite in vitro T cell assays showing loss of anti-donor T cell responses by 6–15 months after transplantation, donor specific antibody (DSA) became detectable in 4/5 NKD03 subjects. In contrast, DSA has not been detectable in the ITN-036 subjects who received more intensified anti-B cell treatment.

**CONCLUSIONS:** Long-term stable tolerance appears inducible despite induction of only transient chimerism via CKBMT. Our observations emphasize the necessity for adequate B cell depletion during the initial 6 months until T cell tolerance is established. Observations in both the successful and unsuccessful cases suggest additional modifications of this approach that will lead to the development of this approach.

### Introduction:

Short-term results following organ transplantation have been significantly improved by the use of increasingly efficacious immunosuppressive agents. However, their chronic use results in significant morbidity, especially from an increased incidence of cardiovascular disease,<sup>1</sup> infection,<sup>2</sup> malignancies,<sup>3</sup> de novo diabetes<sup>4</sup> and other metabolic derangements. Unfortunately, the potent immunomodulatory effects of current therapeutic protocols do not prevent the development of chronic rejection, despite their administration being pushed to toxic levels. Therefore, induction of tolerance, defined as the absence of destructive immune responses to a transplanted tissue without ongoing immunosuppressive therapy, remains the ultimate goal of organ transplantation.

Since the seminal work reported by Billingham, Brent and Medawar on neonatal tolerance in 1956, numerous tolerance induction strategies have been identified in rodents. However, only a very limited number of these have been successfully translated to large animals and even fewer to primates. Among the few protocols that have been applied in humans, induction of donor chimerism, either transient or permanent, currently appears to be the most promising strategy to achieve renal allograft tolerance. Initial results of clinical trials for tolerance induction in three centers have so far been reported. Using TLI and DBMT, the Stanford group reported successful induction of stable chimerism and renal allograft tolerance in HLA identical kidney transplant recipients.<sup>5,6</sup> More recently, Leventhal et al. at Northwestern have reported use of an intensive conditioning regimen and donor hematopoietic stem cells for induction of tolerance in HLA-mismatched kidney transplant recipients.<sup>7</sup> Although the follow-up is still brief, persistent donor chimerism without GVHD has been reported, allowing weaning from all maintenance immunosuppression by 1 year in half of the patients.

At Massachusetts General Hospital, based on decades-long basic studies in animal models,<sup>8-11</sup> we have applied combined kidney and donor bone marrow transplantation (CKBMT) for induction of allograft tolerance in both HLA matched<sup>12-14</sup> and mismatched kidney transplant recipients.<sup>15</sup> The early results of the initial five mismatched patients who underwent CKBMT have been reported.<sup>15</sup> We subsequently extended this approach with a revised regimen to five additional patients. We report here our observations in this later cohort as well as the longer-term follow-up (up to 10 years) of the previous recipients of HLA mismatched CKBMT.

## METHODS

**Study Subjects:** 10 subjects with end stage renal disease, aged 22-46, have been enrolled into the studies. All had one HLA haplotype-mismatched parent or sibling donors (Table 1).

**Conditioning Regimen:** The original preparative conditioning regimen (Fig. 1, NKD03) consisted of 60 mg/kg/d of cyclophosphamide administered i.v. on days -5 and -4 with respect to transplantation; 0.6 mg/kg/dose of humanized anti-CD2 mAb (MEDI 507, MedImmune Inc., Gaithersburg, MD) on days -2, -1, 0 and +1; 5 mg/kg cyclosporine A (CyA, Novartis Pharmaceuticals Inc., East Hanover, NJ) i.v. on day -1; and thymic irradiation (700 cGy) on day -1. Hemodialysis was performed before and 14 hours after each dose of cyclophosphamide. On Day 0, kidney transplantation was followed by i.v. infusion of unprocessed donor bone marrow (DBM). Oral CyA (Neoral, Novartis Pharmaceuticals, Inc.) was administered postoperatively at 8 to 12 mg/kg/day with target trough blood levels of 250-350 ng/ml, then tapered and discontinued over several months. The protocol was modified after treatment of the third subject (see Results), to include 1) administration of Rituximab, 375 mg/m<sup>2</sup>/dose on days -7 and -2; and 2) administration of prednisone, 2 mg/kg/d starting on the day of transplantation and then tapering over the next 10 post-transplant days (modified NKD03). Since subjects treated with this modified NKD03 still developed donor specific antibodies (DSA) after discontinuation of immunosuppression, the regimen was further modified (Fig.1) to add two more doses of Rituximab (375 mg/m<sup>2</sup>/dose) on days 5 and 12, more prolonged administration of prednisone until day 20, and tacrolimus in place of CyA for the 5 most recently treated subjects (ITN036). Tacrolimus was slowly tapered over several months and completely discontinued at 8 months after confirming no rejection by a 6 month protocol biopsy. All treatment regimens were approved by the Massachusetts General Hospital Institutional Review Board (IRB) and developed in collaboration with the Immune Tolerance Network (ITN).

**Biopsies:** Kidney allograft biopsies were taken per protocol at day 0, 6, 12, 24 and 36 months. Later protocol biopsies were done from 5-8 years (Fig. 2). Indication biopsies were taken for episodes of graft dysfunction. All biopsies were processed for routine light microscopy, immunofluorescence (including C4d stains) and electron microscopy by techniques previously reported<sup>15,16</sup>.

**In Vitro Immunologic Assays:** Standard MLR and CML assays were performed using the methods detailed previously.<sup>13</sup> LDA to quantify cytotoxic T-lymphocyte precursor frequencies and IL-2-producing Th frequencies were performed as described.<sup>17</sup>

**Detection of donor specific antibodies:** Serially collected pre and post transplant sera samples were tested for the presence of HLA antibodies using ELISA kit (LAT Class I & II, One Lambda, Canoga Park, CA).

## RESULTS:

### **Study subjects:**

A total of 10 subjects, age 22-46, 6 males and 4 females, were enrolled into these studies. Their original kidney diseases include Alport's syndrome (n=4), polycystic kidney disease (n=2), membranoproliferative glomerulonephritis (MPGN) type 1 (n=2), reflux uropathy (n=1) and focal glomerulosclerosis (n=1) (Table 1). The first three subjects (#1 - #3) received the NKD03 regimen; the next two subjects (#4 and #5) received the modified NKD03 regimen. The last five subjects (#6 - #10) received the ITN036 protocol (Fig. 1).

### **Induction of transient chimerism:**

As in the previously reported patients, all additional subjects developed transient multilineage mixed chimerism which became undetectable by 2-3 weeks post-CKBMT.<sup>15,18</sup>

### **Engraftment syndrome and acute kidney injury (AKI):**

We previously described the cytokine syndrome-like manifestations observed after CKBMT as "engraftment syndrome".<sup>15,16</sup> In fact, the symptoms have been temporally associated with the loss of peripheral chimerism as well as of the return of host-derived hematopoietic elements. The most troublesome manifestation of this syndrome is acute kidney injury (AKI), which was observed after day 10 in all patients except for Subject #1. The biopsies taken during AKI showed severe endothelial injury with CD8<sup>+</sup> T cell infiltration, as reported previously in detail.<sup>16</sup> The peak serum creatinine (Cr.) level ranged from 3.5 to 15.4 mg/dl during days 10-20. Among the nine subjects who developed AKI, three recovered without additional treatment, two with Thymoglobulin and plasma exchange, one with Thymoglobulin alone and one with pulse steroid therapy. Two kidney allografts failed to recover due to associated humoral rejection (Subject #3) and thrombotic micro-angiopathy (Subject #8).

### **Long-term clinical course of subjects in NKD03 (Subjects #1-5)**

Initial results, after follow-up periods of up to 4 years, in the first five patients, were previously reported.<sup>15</sup> As in that report, except for Subject #3, who retrospectively probably had DSA prior to transplant and suffered early graft failure due to acute antibody-mediated rejection, all renal allografts are functional with the longest survival now exceeding 10 years (Subject #1). Four protocol biopsies performed in Subject #1 up to 7.5 years after CKBMT showed no evidence of rejection (Figs. 3A-C). Subject #2 remained stable until year 7 following immunosuppression withdrawal (Fig. 2). At that point, urinary protein first became detectable. Allograft biopsy revealed no rejection but recurrence of his original disease, MPGN type I (C3 glomerulopathy) (Figs. 3D-F). Mycophenolate mofetil (MMF) monotherapy for recurrent MPGN was initiated after the 8<sup>th</sup> year. He is currently stable without worsening proteinuria. Subject #4 received the modified NKD03 regimen (Fig. 1). As previously reported, protocol biopsies up to day 731 stained positive for C4d,<sup>15</sup> but did not have features of active rejection. However, his five-year protocol biopsy revealed glomerular basement membrane duplication by light and electron microscopy with capillaritis and continued C4d deposition with minimal interstitial fibrosis (Fig. 3I). Although his kidney allograft function has remained stable with serum Cr of 1.6-1.9 mg/dl (Fig. 2), proteinuria became detectable after 7 years post-transplant, and a brief course of IVIG and ongoing MMF therapy has been recently initiated. Subject #5 remained stable for 5 years with no evidence of rejection or C4d deposition. Subsequently, after suffering multiple severe episodes of gout, his renal function deteriorated and C4d deposition became detectable for the first time in the biopsy at 6 years. He was treated with high dose IVIG (2g/kg) and 2 doses of rituximab. After these treatments, his renal function stabilized. A protocol biopsy at 6.3 months showed minimal transplant glomerulopathy (cg1) (Figs. 3J, K) with negative C4d staining again became negative (Fig. 3L). He is currently doing well without maintenance immunosuppression.

### **Clinical course of subjects in ITN036 (Subjects #6-10)**

Subject # 6 developed AKI after day 10 but recovered without additional treatment. His immunosuppression was slowly tapered and completely discontinued at 8 months after CKBMT. He is currently doing well more than 3.5 years after transplantation, without ongoing immunosuppression, and with a serum Cr. level of 1.5 mg/dl. A recent biopsy showed no evidence of active rejection (Figs. 3M and N). There was de novo C4d staining involving peritubular capillaries (Fig. 3O), although he continued to have no evidence of DSA by ELISA assay. Immunosuppressive therapy for Subject #7 was discontinued at 8 months. She is currently over 3 years after CKBMT with normal kidney function (serum Cr. 0.8 mg/dl) (Fig. 2) with no evidence of rejection or C4d staining in the 2 year protocol biopsy (Figs. 3P-R). Subject # 8 failed to recover from AKI, and the biopsy performed on day 22 revealed arterial intimal matrix expansion with infiltrating mononuclear cells and arterial fibrinoid necrosis without C4d deposition in peritubular capillaries. No DSA was detectable. Differential diagnosis included acute cellular rejection type III versus intra-renal thrombotic microangiopathy, possibly due to tacrolimus toxicity. Tacrolimus was discontinued and three

doses of anti-thymocyte globulin were administered with MMF. Despite these treatments, her kidney function gradually failed and she was returned to CAPD at 7 months after transplantation. Subject #9 developed transient AKI after day 10 and recovered without additional treatment. Her immunosuppression was slowly tapered and was discontinued at 8 months after transplantation. She is currently well at 3 years after CKBMT with normal kidney function (serum Cr. 1.0 mg/dl). The protocol biopsy at 2 years showed no evidence of rejection (Figs.3S-U). In Subject #10, kidney function gradually returned to normal by 2 months after AKI and a 6-month protocol biopsy did not show rejection. His immunosuppression was discontinued at 8 months. One month later, he developed acute pyelonephritis in the kidney allograft with moderately elevated serum Cr. (2.2 from 1.6 mg/dl). This was treated with antibiotics and the kidney function recovered to base line. However, 3 weeks after the resolution of his infection, he developed severe acute cellular rejection (Banff 2B)(Fig. 3V) with no C4d deposition (Fig. 3X). DSA were negative. He was treated with steroid pulses and anti-thymocyte globulin, following which his renal function improved but never fully recovered. A renal biopsy 6 months later showed interstitial and intimal fibrosis without active inflammation (Fig. 3W). Although he remains well almost 3 years post-transplant, his compromised kidney function suggests that gradual progression to ESRD is likely (Fig. 2).

### **In vitro immunological assays**

#### Anti-donor T cell responses:

MLR, CML, CTLp and HTLp assays in NKD03 subjects were previously reported.<sup>15,19</sup> All NKD03 subjects developed donor specific nonresponsiveness (DSN) between 3 to 18 months. Serial in vitro immunological assays to test anti-donor T cell responses were also performed in the four ITN036 subjects who discontinued their immunosuppression. In these subjects, DSN or donor-specific hyporesponsiveness (DSH) were observed in MLR by 3 to 18 months (Table 2). DSN or DSH were also observed by 3 to 18 months but some responses against the donor returned positive in Subjects #7 and #9. LDA-HTLp also showed DSH by 3 to 18 months, but anti-donor HTLp response was detectable at 18 months in Subject 9.

#### Anti-donor B cell responses (Fig. 4B):

DSA has never been detected in Subject #1. DSA was transiently detected only once in Subject #2 with no evidence of rejection. Anti-HLA class II DSA has been persistently positive in Subjects #4 and #5. DSA in Subject #4 has been associated with C4d deposition and glomerulopathy, whereas DSA in subject #5 has been weak with only transient C4d deposition and no rejection (Fig. 3).

In contrast to NKD03 subjects, no DSA has been detectable in any of the ITN036 subjects by solid phase assays (ELISA).

B cell depletion (Fig. 4C): Recovery of CD3<sup>+</sup>CD19<sup>+</sup> cells in two subjects (Subjects #1 and #2) treated with the original NKD03 regimen was observed by days 50 and 100, respectively. With two doses of pre-transplant rituximab (modified NKD03), depletion of CD3<sup>+</sup>CD19<sup>+</sup> cells was extended to day 150 (Subjects #4 and #5) but complete loss of CD3<sup>+</sup>CD19<sup>+</sup> cells from the peripheral blood was not observed. In contrast, peripheral blood CD3<sup>+</sup>CD19<sup>+</sup> cells were <5/mm<sup>3</sup> for 6 months in all five ITN036 subjects treated with 4 doses of peritransplant rituximab.<sup>18</sup>

## **DISCUSSION**

We report here that induction of long-term (up to 10 years) stable renal allograft function without maintenance immunosuppression can be reproducibly achieved after induction of transient lymphohematopoietic chimerism in recipients of HLA-mismatched CKBMT. Since chimerism is transient, there has been no GVHD observed in our preclinical<sup>9,10</sup> and clinical studies<sup>15</sup>, which is a major advantage of this approach for tolerance induction. We hypothesize that the mechanism of tolerance induction after transient chimerism in these recipients differs from that observed in murine models, in which chimerism persists indefinitely.<sup>8,20</sup> Our NHP studies have consistently demonstrated the necessity for DBM engraftment resulting in measurable donor chimerism, especially in lymphoid lineages, for induction of tolerance.<sup>9,10,21</sup> We have therefore concluded that the mechanism of tolerance induction by mixed chimerism in primates is likely to involve both central and peripheral pathways. We postulate that initially, donor cells traveling to the thymus lead both to deletion of newly developing T cells and generation of regulatory cells which are required to down-regulate any anti-donor responses that were not eliminated at the time when chimerism was established. Consistently, we have demonstrated marked enrichment for Tregs in the first 6-12 months following conditioning in the patients in both of these series (please cite Andreola AJT here) and additional studies are suggestive of an early wave of Treg emigration from the thymus in these patients (B. Sprangers et al, manuscript in preparation). However, we have only occasionally, and only in the first year post-transplant, been able to demonstrate augmentation of anti-donor reactivity by depleting Tregs for *in vitro* assays,<sup>19</sup> We therefore postulate that donor-specific Tregs emigrating from the thymus may concentrate in the renal allograft itself, and we are currently addressing this possibility in ongoing studies. However, given the complete and long-lasting donor-specific unresponsiveness in Treg-depleted T cells of our patients and the apparent absence of donor-specific T cell precursors or of suppressive activity suggested by late LDAs,<sup>19</sup> we have suggested that donor-specific T cells are ultimately deleted by continual encounter with donor antigens in a non-inflammatory context in the graft.<sup>19</sup>

In our monkeys, costimulatory blockade, with agents directed against CD40/CD154<sup>10</sup> or B7/CD28 (manuscript in preparation) were helpful in achieving consistent tolerance. In our clinical protocol, we have used anti-CD2 monoclonal antibody which provides both T-cell depletion and costimulatory blockade via the CD2/LFA-3 interaction<sup>15</sup>. Our studies have also emphasized that the kidney allograft itself appears to play a critical role in the induction and maintenance of tolerance. This point is illustrated directly by our in vitro studies, in which patients receiving combined kidney and BMT develop donor-specific unresponsiveness,<sup>15,19</sup> whereas those receiving similar BMT regimens in a cancer treatment protocol, without a kidney graft, demonstrated preserved anti-donor responses following loss of peripheral blood chimerism.<sup>17</sup> Consistently the same conditioning/transient chimerism regimen that achieves renal allograft tolerance has failed to induce tolerance of isolated heart allografts in monkeys.<sup>22</sup> Interestingly, heart allograft tolerance can be achieved by co-transplanting the kidney from the same donor, and heart allografts that had survived long-term are promptly rejected if the kidney allograft is removed.<sup>23</sup> Presumably, the donor-specific regulatory cells generated during the chimeric state require continuing interaction with as yet undefined specific cells or antigens in the kidney allograft for maintenance of the tolerance. One potentially important renal cell is the renal tubular epithelial cell, that has been reported to participate in the induction of allospecific tolerance in rodents and humans.<sup>24-26</sup>

Despite the encouraging results of successful long-term tolerance induction in our initial clinical trials, several obstacles to more widespread application of this approach have been identified. The most troubling of these is the “engraftment syndrome”. AKI is a major manifestation of engraftment syndrome and has been observed at approximately day 10 in all subjects except for Subject #1. This syndrome has been described previously following both autologous and allogeneic bone marrow transplantation, which has been associated with the recovery of either host or donor type hematopoietic elements<sup>27</sup>. We note that the AKI of engraftment syndrome has never been observed in our MHC-mismatched monkey model, in which chimerism is also lost, but at a more gradual pace, usually over 2-3 months.<sup>9,10,28</sup> Our current interpretation of these findings is that the pre-transplant cyclophosphamide-based regimen chosen for the clinical trials,<sup>15</sup> in contrast to the non-myeloablative total body irradiation used in the monkey preparative regimen,<sup>9,10,28,29</sup> may allow rapid homeostatic recovery of host memory T cells, which may be associated with significant effector function, as suggested by the observation of CD8 T cell activation immediately following conditioning<sup>16</sup> and rapid rejection of the donor hematopoietic graft. Further supporting this hypothesis is the observation that AKI has also never been observed in our HLA-matched CKBMT recipients, in whom chimerism as in the NHP recipients typically disappears more slowly, over a periods of 2-3 months.<sup>12,14</sup> However, marrow rejection may not be the driving force for this syndrome, since CD8 activation is observed as early as Day 0,<sup>16</sup> before sensitization could occur. Unfortunately, our efforts to test this hypothesis, by using a cyclophosphamide-based regimen in monkeys, have been precluded by unusually severe toxicities of this agent in this species.<sup>30</sup>

The second obstacle observed in the first study (NKD03) subjects was the frequent development of either transient or persistent DSA, despite specific loss of anti-donor T cell responses. In NKD03, DSAs were detectable in most subjects except for Subject #1. Despite occasional (Subject #2) or persistent (Subjects #4 and #5) detection of DSA, only Subject #4 developed chronic glomerulopathy with C4d deposition. Studies are underway to evaluate the possible usefulness of the Luminex approach to identifying DSA complement fixation (C1q) as a means of clarifying the clinical relevance of these variably detected antibody levels in allograft recipients not on chronic immunosuppression. In contrast to NKD03 subjects, with the more intensified anti-B cell treatment afforded by adding rituximab to the conditioning regimen for ITN036 subjects, there has been no evidence of DSA. Since anti-HLA antibodies are thought to represent T cell-dependent responses, these observations suggest that B cells in NKD03 subjects might have been sensitized before T cell tolerance was established by mixed chimerism.<sup>15</sup> Since anti-donor HTLP responses can be observed up to 18 months after CKBMT (Fig. 4A), it may be critical to prevent B cell activation during the early post-transplant period in order to establish robust tolerance. Although rituximab does not affect serum immunoglobulin levels, antigen-specific antibody titers, or antibody secreting plasma cell numbers, it does effectively deplete peripheral mature B cells, likely including germinal center B cells<sup>31</sup>. The germinal center is the nidus of immunological activation, where close collaboration between antigen driven T and B cells occurs<sup>32</sup>. Thus B cell depletion by rituximab can prevent humoral immune responses and antibody class switching<sup>31</sup> and its use in ITN036 appears to have largely removed this obstacle to tolerance induction via this approach.

The third obstacle to more widespread application of our tolerance technology is its requirement for living donor transplantation, as this strategy involves treatment of the recipient beginning 6 days prior to transplantation. To address this obstacle, we have recently developed a novel regimen for “delayed tolerance” induction, in which the recipient initially undergoes organ transplantation with conventional immunosuppression, followed by conditioning and DBM transplantation at a later date. In our NHP studies, we have shown that such “delayed tolerance” can be induced by modifying the post-transplant treatment to avoid generation of donor-specific memory T cell responses prior to induction of mixed chimerism<sup>29,33</sup>. In addition to making it possible to utilize deceased donors for renal transplants, successful application of this new procedure could permit



treatment of patients with functioning transplants who want to discontinue immunosuppression for a variety of reasons and could also help to extend tolerance to other non-renal organs.

In conclusion, our clinical studies have shown that immune-suppression free stable renal allograft function can be reproducibly achieved for periods now extending to 10 years by induction of transient chimerism through DBMT. Further modifications of the protocol are planned to address the “engraftment syndrome” related AKI, which is the current major barrier to continued development of this approach.

### Figure Legends

#### Fig. 1: Anti-donor responses- T cell responses

Anti-donor or anti-third party LDA-HTLp in ITN-036 subjects are shown. LDA-HTLp also showed DSH by 3 to 18 months, but anti-donor HTLp response was detectable at 18 months in Subject 9.

#### 1A: Detection of donor specific antibody (DSA)

DSAs are detected by ELISA.

NKD03: Subject #1: DSA has never been detectable. Subject #2: DSA was only transiently detected once around 7 years.

Subject 3: Although pre-transplant DSA was negative by ELISA, retrospective Lumindex showed positive anti-class I DSA (B44) before transplantation. The subject developed acute humoral rejection on day 10 with DSA (B44 and DR4). Subjects 4 and 5: DSA became positive soon after stopping his immunosuppression with or without C4d deposition and chronic rejection (See Fig. 3G-L).

ITN036: In contrast to NKD03 subjects, No DSA has been detectable in any ITN036 subject.

#### Figure 2: CD3<sup>+</sup>CD19<sup>+</sup> cell depletion after CKBMT

In two subjects treated with the NKD03 regimen, the recovery of CD3<sup>+</sup>CD19<sup>+</sup> cells (B cells) were found by day 50 (Subject #1) and day 120 (Subject #2) (black lines). With addition of two doses of pre-transplant rituximab (modified NKD03), B cells were never depleted completely and recovered by days 150 and 180 (blue lines). With 4 doses of peri-transplant rituximab (ITN036), B cells were significantly depleted (less than 1-2/mm<sup>2</sup>) for 180 days (red lines).

**Fig. 3:** Late renal allograft biopsies (all protocol biopsies unless noted).

**A-C (Subject #1 at 7.5 years):** The protocol biopsy shows no evidence of rejection by light microscopy (LM) (A). The glomeruli are normal by electron microscopy (EM) (B) and no C4d staining is detectable by immunofluorescence (IF) (C).

**D, E, F (Subject #2 at 8 years):** An indication biopsy for proteinuria shows prominent lobular mesangial expansion with widespread glomerular basement membrane (GBM) duplication (D, arrow). Granular dense deposits in the duplicated GBM are seen by EM (E). Granular and segmental staining for C3 along the GBM and in the mesangium is evident by IF, but no immunoglobulin was detected (F), indicative of recurrent C3 glomerulopathy (originally classified as MPGN, type I).

**G, H, I (Subject #4 at 5 years):** Widespread GBM duplication (arrow) and glomerulitis is seen by LM (G). EM shows prominent duplication of the GBM without deposits and reactive endothelial cells (H). C4d deposition is detected in peritubular capillaries (I). These findings are indicative of chronic, active antibody mediated rejection, which at this time was subclinical.

**J, K, L (Subject #5 at 6 years):** The biopsy shows minimal glomerulitis by LM (J), slight segmental GBM duplication by LM (K) and EM (K) and no C4d deposition by IF (L).

**M, N, O (Subject #6 at 2 years):** LM shows normal glomeruli with rare foci of interstitial mononuclear inflammation affecting <5% of the cortex. EM shows minimal focal GBM duplication and normal endothelium (N). C4d was present in the majority of the peritubular capillaries (O).

**P, Q, R (Subject #7 at 2 years):** LM is within normal limits (P). EM reveals a normal GBM and endothelium; foot process effacement is present in a minority of the capillaries (20%) (Q). There is no C4d deposition (R).

**S, T, U (Subject #9 at 2 years):** The kidney biopsy is within normal limits and shows no evidence of rejection by LM (S) with normal glomeruli by EM (T). There is no evidence of recurrent MPGN. No C4d is detected (U).

**V, W, X: (Subject #10):** Indication biopsy at 9.5 months shows acute cellular rejection with endarteritis (V). A protocol biopsy six months later shows complete resolution of the inflammatory process and residual interstitial and intimal fibrosis (W). No C4d deposition is detectable at 9.5 months (X) or at other times.

Light microscopy stains: H&E, A, M, V; PAS, D, G, J, P, S. Immunofluorescence stains: C4d, C, F, I, L, O, R, U, X.

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Fig. 1

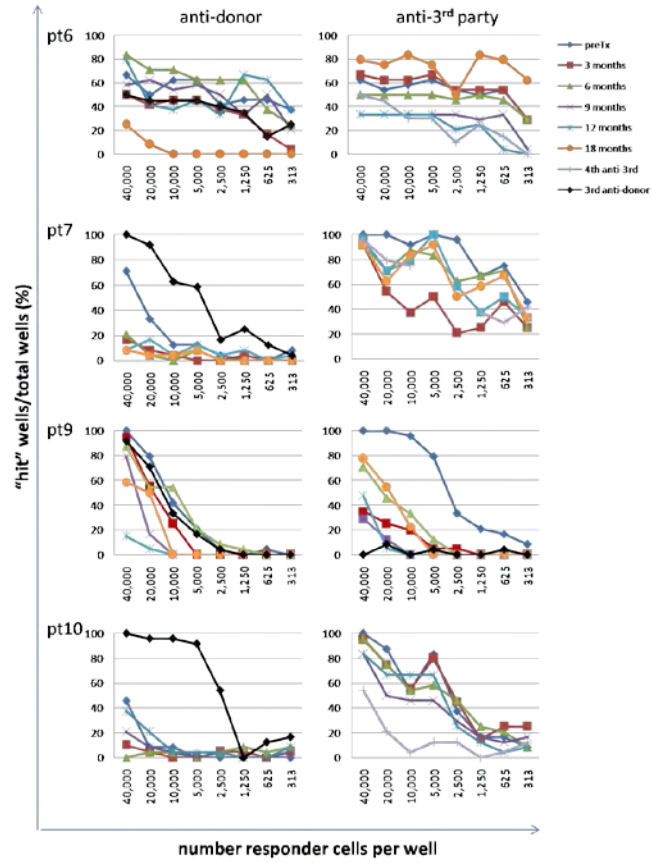


Fig. 1A

		pre	1 y	2 yrs	3 yrs	4 yrs	5 yrs	6 yrs	7 yrs	8 yrs	9 yrs	10 yrs
NKD03	#1	-	-	-	-	-	-	-	-	-	-	-
	#2	-	-	-	-	-	-	-	-	DQ9	-	-
	#3	-	DR4									
	#4	-	-	DR17	DR17				DR17	DR17		
	#5	-	-	-	DR53	DR53	DR53	DR53				
ITN036	#6	-	-	-	-	-						
	#7	-	-	-	-	-						
	#8	-	-									
	#9	-	-	-	-							
	#10	-	-	-	-							

Fig. 2

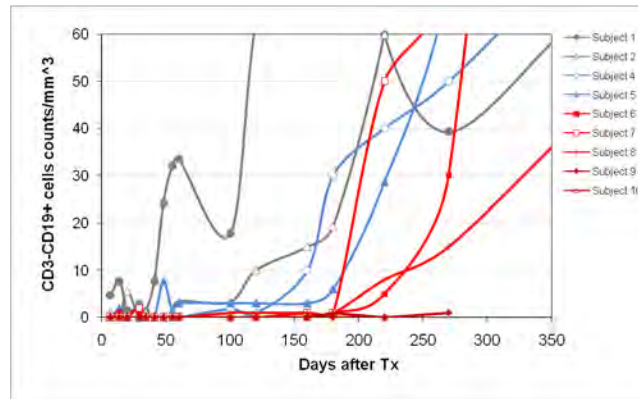
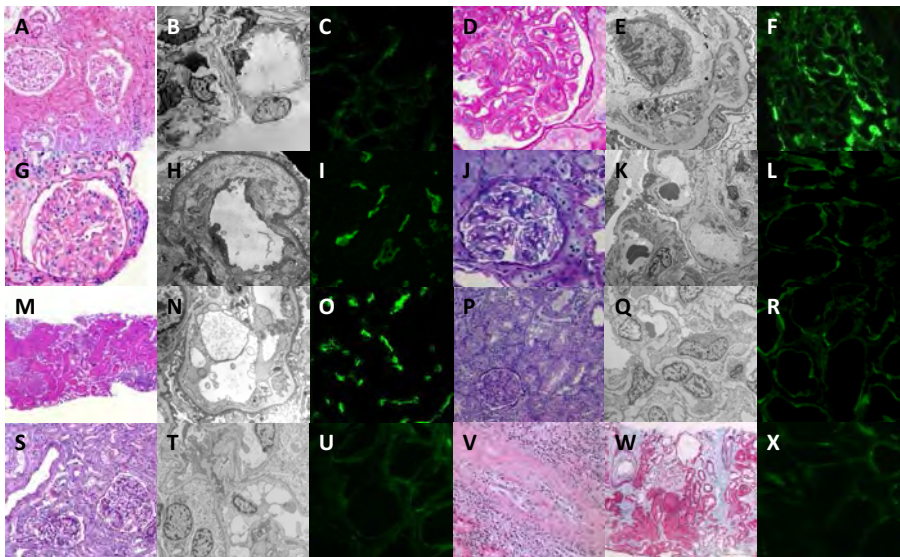


Fig. 3



**Appendix 7: Schedule of Events for Safety Follow-up**

Time										M6		Y1				Y2
Week	0	2	6	8	10	13	14	18	22	26	39	52	65	78	91	104
Follow-up for participants prematurely discontinued from study therapy (section 3.4.4.2)																
Visit		101	102		103		104	105	106	107	108	109	110	111	112	113
Assessments																
Adverse events		X	X		X		X	X	X	X	X	X	X	X	X	X
Concomitant medications		X	X		X		X	X	X	X	X	X	X	X	X	X
Physical examination		X	X		X		X	X	X	X	X	X	X	X	X	X
Hematology		X	X		X		X	X	X	X	X	X	X	X	X	X
Serum and basic		X	X		X		X	X	X	X	X	X	X	X	X	X
Thyroid function		X	X		X		X	X	X	X	X	X	X	X	X	X
GVHD assessment <sup>1</sup>		X	X		X		X	X	X	X	X	X	X	X	X	X
Graft survival		X	X		X		X	X	X	X	X	X	X	X	X	X
Chimerism <sup>2</sup>		X	X		X		X	X	X	X	X	X	X	X	X	X
Follow-up for participants prematurely terminated from study (section 3.5.2)																
Visit	201			202												
Assessments																
Adverse events	X			X												
Concomitant medications	X			X												
Physical examination	X			X												
Hematology	X			X												
Serum and basic	X			X												
Thyroid function	X			X												
GVHD assessment <sup>1</sup>	X			X												
Graft survival	X			X												
Chimerism <sup>2</sup>	X			X												
Follow-up treatment for participants who fail immunosuppression withdrawal (section 4.4.8.5)																
Visit	301				302					303	304	305	306	307	308	309
Assessments																
Adverse events	X				X					X	X	X	X	X	X	X
Concomitant medications	X				X					X	X	X	X	X	X	X
Physical examination	X				X					X	X	X	X	X	X	X
Hematology	X				X					X	X	X	X	X	X	X
Serum and basic	X				X					X	X	X	X	X	X	X
Thyroid function	X				X					X	X	X	X	X	X	X
GVHD assessment <sup>1</sup>	X				X					X	X	X	X	X	X	X
Graft survival	X				X					X	X	X	X	X	X	X
Chimerism <sup>2</sup>	X				X					X	X	X	X	X	X	X

1 Can discontinue GVHD assessment once chimerism is not detectable.

2 Perform test until chimerism is no longer detectable. Chimerism testing will be done by Flow Cytometry. In the event that HLA antigens (needed for Flow cytometry analysis) cannot be detected by available monoclonal antibodies, STR/Microsatellite methodology will be used.