Supplementary Materials

Materials and Methods

Donor and recipient eligibility. Donors and recipients had to meet institutional criteria as suitable living transplant donors and recipients. Inclusion criteria for recipients included age 18 to 65 years, absence of donor-specific antibodies as assessed by flow PRA analysis, and receiving only a living donor kidney transplant. Women of childbearing age had to have a negative pregnancy test (urine testing acceptable) within 48 hours of receiving TBI and agree to use reliable contraception for one year after kidney/stem cell transplantation. Exclusion criteria included clinically active bacterial, fungal, viral or parasitic infection, pregnancy, previous radiation therapy at a dose which would preclude TBI, a positive flow cytometry crossmatch between donor and recipient, presence of donor specific antibodies, body mass index >35 or <18, and positive serologies for HBV, HCV, HIV.

Text: Detailed Subject Clinical Course Descriptions

Subject #1 was a 50-year-old male with renal failure secondary to membranous nephropathy who received a 5 of 6 HLA-matched living related donor (LRD) kidney and FCRx transplant from his sister in February 2009. Mobilized peripheral blood stem cell collection from the donor was complicated by high granulocyte contamination and resultant impaired product viability. The FCRx product was not administered. A subsequent iliac crest bone marrow harvest was performed intraoperatively during the donor nephrectomy and processed for FCRx. The post-transplant dose of cyclophosphamide was held due to safety concerns about the early nadir period in this patient population. Despite relatively low numbers of cell subsets in the product (**Table 1**), 30% donor chimerism was achieved at one month. Peripheral blood chimerism gradually decreased, however, and was lost after 5 months. Renal function was

excellent with a normal 6 month protocol biopsy and no evidence of GVHD. MMF was discontinued at 6 months, and tapering of tacrolimus initiated in light of donor-specific hyporesponsiveness in cell mediated cytolysis assays *in vitro*. At his one year standard of care visit, the subject presented with new onset proteinuria; a kidney transplant biopsy revealed recurrent membranous nephropathy. Tapering of tacrolimus was halted and the subject was treated with Rituximab (375 mg/m^2 weekly for one month). The proteinuria subsequently resolved. At the time of publication, he remained on tacrolimus monotherapy with normal renal function (**Fig. 1B**). A protocol biopsy at 24 months after transplant was free of acute or chronic rejection and confirmed disease quiescence.

Subject #2 was a 56-year-old male with end-stage renal disease (ESRD) due to hypertension who underwent a 3 of 6 HLA-matched living donor renal/stem cell transplant in April 2009. He tolerated the conditioning and transplant well and was discharged on postoperative day 2. He experienced immediate renal allograft function and the expected nadir for WBC and platelets of less than 2 weeks. He achieved 95% chimerism at month 1 and 100% at month 2. He had no evidence of GVHD or engraftment syndrome. A punch biopsy of a transient skin rash was indeterminate for infectious etiology or GVHD; the rash spontaneously resolved without treatment. At three months after transplant, the subject developed a febrile illness of uncertain etiology manifested by shortness of breath and hypotension that progressed to intercurrent sepsis and hypotension, requiring inotropic support and ventilation. The illness was presumed to be of viral etiology although viral cultures were negative. Workup including bronchoscopy, CT scan of the chest, abdomen and pelvis, as well as bone marrow biopsy which was negative for bacterial or viral pathogens. Bone marrow chimerism testing showed 100% donor chimerism at onset of the illness. Infectious disease workup subsequently demonstrated

pseudomonas on bronchoscopy for which the patient was treated. After several days, the patient was weaned from inotropic support and extubated. Serum creatinine was noted to be increasing and a renal transplant biopsy showed extensive hemorrhagic necrosis. Donor-specific antibody determination was negative. Hemodialysis was initiated. A nuclear medicine scan of the transplant showed absence of blood flow through the renal artery, and he underwent renal transplant nephrectomy. He recovered and was discharged from the hospital. Following a period of outpatient recovery, he underwent a living related renal transplant off study protocol. He continues to do well, with stable renal function on standard immunosuppression. The decision was made at the time of pancytopenia to infuse autologous stem cells collected preemptively for hematologic rescue, which was followed by the expected loss of donor chimerism. A careful and thorough review by the investigators and Data Safety Monitoring Board (DSMB) for the study concluded that occult viral sepsis was the most likely explanation.

Subject #3 was a 43-year-old male who developed ESRD due to polycystic kidney disease. He received a 1 of 6 HLA-matched living unrelated kidney/FCRx transplant in May 2009. The characteristic nadir, which is similar for all recipients, is shown in **Figure 2A**. He had 95% donor chimerism at one month after transplant. Chimerism has fluctuated between 63% and 100% with no evidence of GVHD or engraftment syndrome (**Fig. 2B**, **Table 2**). At 6 months, peripheral blood multilineage chimerism testing revealed the presence of 100% donor B cell, T cell, and myeloid cell production (**Fig. 2C**). T cell chimerism has consistently been 100% (**Table 3**). Flow cytometric crossmatch for anti-donor antibodies was negative at 1 month, 6 months, 1 year, and 2 years after transplant. At month 3, the recipient began to exhibit donor-specific hyporesponsiveness but regained immunocompetence to respond to HLA-disparate third-party alloantigen in *in vitro* mixed lymphocyte reaction (MLR) proliferative assays which

has persisted (**Fig. 2D**). Renal function has remained stable and within normal limits (**Fig. 1B**). Protocol biopsies 6 and 12 months after transplant were histologically normal, and MMF was discontinued at 6 months. Tacrolimus was reduced to subtherapeutic dosing at 9 months (trough 0-3 ng/ml) and discontinued at one year. A subsequent protocol graft biopsy 24 months after transplant (12 months of all immunosuppression) was histologically normal as assessed by H&E (**Fig. 3A**), masson trichrome (**Fig 3B**) and PAS (**Fig. 3C**). Donor chimerism has remained 100% more than one year after immunosuppression was withdrawn. Adverse events in this subject include an exanthema one month after transplant. GVHD was included in the differential diagnosis, but a biopsy revealed a sulfa-based drug rash. The rash resolved spontaneously and has not recurred. In addition, the subject developed a single dermatome varicella zoster reactivation at 9 months, which resolved and has not recurred. He is now 32 months after transplant with stable renal function.

Subject #4 was a 29-year-old male with ESRD due to Alports Syndrome who received a LRD kidney/FCRx transplant (3 of 6 HLA-match) June 2009. He received a deliberately reduced αβ-T cell dose (**Table 1)** because of unresolved concerns regarding the etiology of the rash in Subject #3, described above. His course after transplant was complicated by a wound infection successfully treated with intravenous antibiotics. Donor chimerism was 6% at one month, but chimerism was gradually lost by 3 months (**Table 2**). Donor-specific hyporesponsiveness persisted as assessed by MLR, and a protocol biopsy at 6 months was histologically normal. MMF was discontinued at 6 months and tapering of tacrolimus was initiated. However, a protocol biopsy at 12 months showed subclinical Banff 1A rejection despite a normal serum creatinine. Staining for C4d and donor-specific antibody assessment was negative. He was treated with a short course of intravenous corticosteroids and has been

maintained on therapeutic tacrolimus monotherapy (trough 5-8 ng/ml). Renal function has remained stable (**Fig. 1B)**, no donor-specific antibody has been detected, and follow-up allograft biopsies at 17 and 24 months after transplant were histologically normal.

The first four subjects represent a learning curve in this pilot study. Because of the loss of chimerism in Subjects #1 and #4, who both received lower numbers of αβ T cells, FC, and $CD34^+$ cells, all subsequent subjects in our study have received processed stem cell infusions modeled after the product administered in Subject #3 (**Table 1**), as well as both doses of cyclophosphamide. This has resulted in durable high levels of whole blood and CD3 cell chimerism in Subjects #5 through #8 as shown in **Tables 2 and 3**. No subject has exhibited engraftment syndrome, none has developed anti-donor antibody, and none has exhibited acute or chronic GVHD. All have exhibited stable renal function (**Fig. 1B**).

Subject #5 was a 40-year-old male with ESRD secondary to chronic glomerulonephritis. He underwent a combined renal/FCRx transplant from a 1 of 6 HLA-matched living unrelated donor in February 2010. Chimerism was 100% at 1 month, 92% at 3 months, 94% at 5 months, 100% at 6 months, and has persisted at 100% through 15 months (**Table 2**). He began to exhibit a donor-specific tolerant profile at month 3 with responses to PHA and third-party allo-antigen but not to donor in MLR. T cell chimerism was 100% at 6 months and has persisted (**Table 3**). He was maintained on tacrolimus monotherapy (trough levels 0-3 ng/ml) from month 9 to 12, and all immunosuppression was discontinued 12 months after transplant after a normal protocol biopsy. Renal function has remained stable and there is no evidence of GVHD. His last serum creatinine was 1.3 mg/dl (**Fig. 1B**).

Subject #6 is a 39-year-old female who developed ESRD secondary to ureteral reflux. She underwent a first renal transplant 34 years ago. Function of her living donor transplant was

lost after 34 years due to chronic allograft nephropathy. She underwent a second renal and FCRx transplant from a 2 of 6 HLA-matched living unrelated donor in March 2010. She exhibited 100% donor chimerism at 1 month, which has persisted (**Table 2**). T cell chimerism has consistently been 100% donor (**Table 3**). Her 12 month protocol biopsy showed no evidence of rejection and all immunosuppression was withdrawn. She is currently off all immunosuppression without evidence of GVHD. Renal function is stable, with the most recent serum creatinine 0.8 mg/dl (**Fig. 1B**).

Subject #7 was a 35-year-old male with ESRD due to hypertension who received a 3 of 6 HLA-matched LRD kidney/FCRx transplant in April 2010. He has consistently exhibited 100% donor whole blood (**Table 2**) and T cell (**Table 3**) chimerism through 12 months follow-up. Renal allograft function has been normal (**Fig. 1B**). A protocol biopsy at 6 months was normal and MMF was discontinued. The subject declined his 12 month protocol biopsy. His immunosuppression was withdrawn at 12 months and he has maintained stable renal function without evidence of GVHD.

Subject #8 was a 46-year-old female with ESRD due to polycystic kidney disease who received a 1 of 6 HLA-matched living unrelated kidney/FCRx transplant in July 2010. Her course was uncomplicated, with a characteristic nadir for white blood cells and platelets. Whole blood and T cell chimerism have consistently ranged from 95 to 100% throughout follow-up (**Tables 2 and 3**). The protocol biopsies at 6 months and 12 months were histologically normal and she was withdrawn from all immunosuppressive therapy at one year after transplant. Renal function has remained within normal limits (**Fig. 1B**).

Supplemental Figures

Fig. S1. Reconstitution of immune cell subsets in recipients of combined stem cell and kidney transplants. A) αβ-TCR^{+} cells, B) CD4^{+} T cells, C) CD8^{+} T cells, D) ratio of CD4/CD8 T cells, E) CD19^{+} B cells, F) CD14^{+} monocytes, G) CD56^{+} NK cell subpopulations; Y axis in panels A-C, E-G denotes absolute cell number in 10^{3}/ml; X axis denotes months after transplant. Panel D illustrates the ratio of CD4^{+}/CD8^{+} cells and panel H the ratio of CD4^{+}/CD25^{+}/CD127^{lo}/FoxP3^{+} phenotypic regulatory T cells to CD4^{+}/CD25^{-}/CD62L^{-} T_{eff} cells.

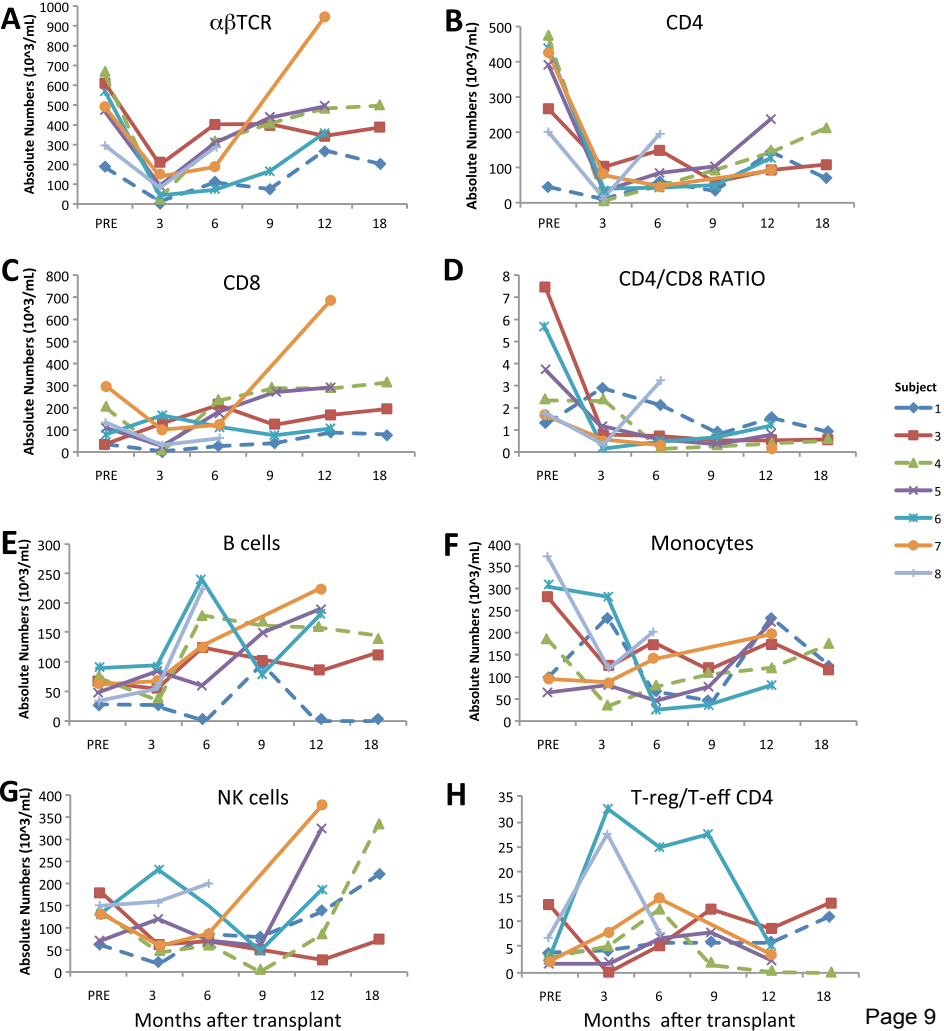
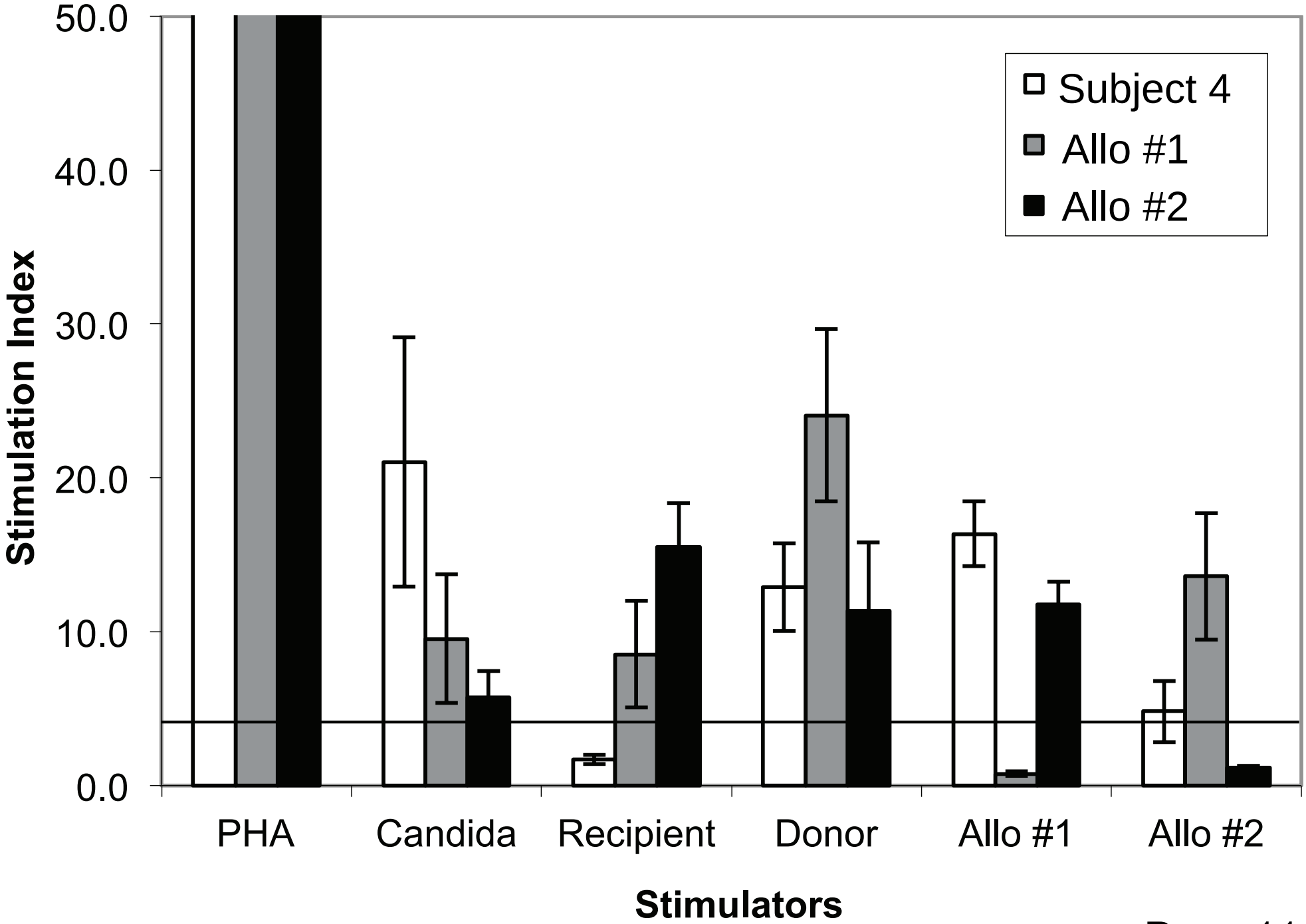

A
αβTCR
Absolute Numbers (10^3/mL)
PRE 3 6 9 12 18
B
CD4
Absolute Numbers (10^3/mL)
PRE 3 6 9 12 18
C
CD8
Absolute Numbers (10^3/mL)
PRE 3 6 9 12 18
D
CD4/CD8 RATIO
PRE 3 6 9 12 18
Subject
1
3
4
5
6
7
8
E
B cells
Absolute Numbers (10^3/mL)
PRE 3 6 9 12 18
F
Monocytes
Absolute Numbers (10^3/mL)
PRE 3 6 9 12 18
G
NK cells
Absolute Numbers (10^3/mL)
PRE 3 6 9 12 18
Months after transplant
H
T-reg/T-eff CD4
PRE 3 6 9 12 18
Months after transplant

Fig. S2. Anti-donor reactivity at 24 months after transplant in transiently chimeric Subject #4. Lymphocytes from the recipient (clear) and two third-party donors were co-cultured with PHA, Candida, or irradiated stimulators from donor or third-party in a MLR assay. A stimulation index ≥ 3 (horizontal line) indicates positive reactivity.


50.0
40.0
30.0
20.0
10.0
0.0
Stimulation Index
Subject 4
Allo #1
Allo #2
PHA
Candida
Recipient
Donor
Allo #1
Allo #2
Stimulators