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Chimerism, Graft Survival, and Withdrawal of Immunosuppressive Drugs in HLA Matched and Mismatched Patients After Living Donor Kidney and Hematopoietic Cell Transplantation

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Thirty-eight HLA matched and mismatched patients given combined living donor kidney and enriched CD34$^+$ hematopoietic cell transplants were enrolled in tolerance protocols using posttransplant conditioning with total lymphoid irradiation and anti-thymocyte globulin. Persistent chimerism for at least 6 months was associated with successful complete withdrawal of immunosuppressive drugs in 16 of 22 matched patients without rejection episodes or kidney disease recurrence with up to 5 years follow up thereafter. One patient is in the midst of withdrawal and five are on maintenance drugs. Persistent mixed chimerism was achieved in some haplotype matched patients for at least 12 months by increasing the dose of T cells and CD34$^+$ cells infused as compared to matched recipients in a dose escalation study. Success of drug withdrawal in chimeric mismatched patients remains to be determined. None of the 38 patients had kidney graft loss or graft versus host disease with up to 14 years of observation. In conclusion, complete immunosuppressive drug withdrawal could be achieved thus far with the tolerance induction regimen in HLA matched patients with uniform long-term graft survival in all patients.

Abbreviations: ATG, anti-thymocyte globulin; CIN, chronic interstitial nephritis; CMV, cytomegalovirus; DCs, dendritic cells; DM, diabetes mellitus; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; GVHD, graft-versus-host disease; IS, immunosuppressive; MLR, mixed leukocyte reaction; MMF, mycophenolate mofetil; PKD, polycystic kidney disease; SLE, systemic lupus erythematosus; STR, short tandem repeats; TLI, total lymphoid irradiation

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Introduction

The goals of clinical transplantation tolerance are to prevent rejection of allogeneic cells, tissues, and organ grafts without the requirement for the lifelong use of immunosuppressive (IS) drugs. Although tolerance has been achieved in many preclinical models with or without the establishment of chimerism, recent reports of successful tolerance induction protocols in humans with living donors have been based on transient or persistent chimerism that develops after the infusion of donor cells from the blood or bone marrow that include hematopoietic progenitor cells (1–10).

In an early study of patients with multiple myeloma given fully HLA matched combined kidney and bone marrow transplants, complete IS drug withdrawal was achieved without rejection, nephropathy, or kidney disease recurrence thereafter in four of seven recipients (1). In a subsequent study by the same investigators, 4 of 10 HLA haplotype matched patients without malignancy were withdrawn from IS drugs for up to 9.2 years without subsequent graft dysfunction after establishment of transient mixed chimerism (2,3). Conditioning of these patients included pretransplant cyclophosphamide, thymic radiation, and anti-CD2 monoclonal antibodies (mAb) (1–3).

Our investigator group reported on 4 HLA mismatched kidney and enriched CD34$^+$ hematopoietic cell transplant patients conditioned with posttransplant total lymphoid

irradiation (TLI) and anti-thymocyte globulin (ATG) (4). Transient mixed chimerism for 2 to 3 months and IS drug withdrawal were achieved in two patients, but the short follow up after drug discontinuation did not allow for clear evaluation of the induction of tolerance (4). Subsequently, we reported on 16 HLA matched patients given the posttransplant TLI and ATG conditioning regimen and an infusion of enriched $CD34^+$ cells with a defined dose of T cells (5–7). Twelve of the latter patients with mixed chimerism for at least 6 months achieved tolerance and complete withdrawal of IS drugs without subsequent rejection episodes (5–7).

Investigators at Northwestern University reported on a study of 15 HLA mismatched kidney transplant patients given a pretransplant conditioning regimen of total body irradiation (TBI), fludarabine, and cyclophosphamide, followed by an infusion of donor hematopoietic cells (9). Recipients were treated with posttransplant cyclophosphamide to prevent graft versus host disease (GVHD) (8,9). Donor cells contained a mixture of enriched facilitator cells, hematopoietic progenitor cells, and T cells (8,9). Six out of 15 patients who developed complete chimerism were completely withdrawn from IS drugs without subsequent rejection (9).

In another tolerance study at Northwestern University, HLA matched kidney transplantation was performed without radiation or cyclophosphamide conditioning, but with repeated donor hematopoietic cell infusions over several months (10). Some of these patients developed transient chimerism, and five of ten were withdrawn from IS drugs at about 24 months posttransplant. Three of these five developed subsequent evidence of rejection or disease recurrence in the graft, and were returned to maintenance IS drug therapy (10).

In the current report, we provide updated data an initial cohort of 6 HLA mismatched patients, a second cohort of 22 HLA matched patients, and a new cohort of 10 HLA haplotype matched patients using the TLI and ATG conditioning regimen with a donor hematopoietic cell infusion to achieve mixed chimerism, tolerance and IS drug withdrawal.

Materials and Methods

Patients

Thirty-eight patients with end stage renal failure who were candidates for kidney transplantation, and who had living donors matched or mismatched for HLA A, B, C, DR, DQ, and DP antigens by high resolution DNA typing were enrolled.

Conditioning of recipients and collection of donor hematopoietic cells

TLI was administered to recipients as 10 doses of 80 or 120 cGy each to the supradiaphragmatic lymph nodes, thymus, subdiaphragmatic lymph nodes, and spleen during the first 10 days posttransplant as described previously (4,6). Rabbit ATG (Thymoglobulin, Genzyme) was given intravenously (1.5 mg/kg for each of 5 daily doses) starting with an intraoperative infusion. The protocol was approved by the Institutional Review Board of Stanford University, and all recipients and donors provided written informed consent.

Donors received a 5 or 6 day course of granulocyte colony stimulating factor at a dose of 16 mcg/kg/day, and mononuclear cells were collected by 1 or 2 apheresis. $CD34^+$ cells were enriched with the use of an Isolex column (Baxter) and then a CliniMax column (Miltenyi) when the former was no longer available. Enriched cell products were cryopreserved until infusion into recipients. Column flow through cells were added back to $CD34^+$ cells to achieve a defined dose of $CD3^+$ T cells in the infusion as outlined in the Results section for different patient groups. The added back $CD3^+$ T cells were not purified in order to avoid the use of a second column, simplify the procedure, and take advantage of other cells that can facilitate $CD34^+$ cell engraftment.

Chimerism

Serial chimerism measurements were performed using DNA from blood mononuclear cells enriched for T cells, B cells, NK cells, and granulocytes on immunomagnetic beads (Miltenyi Biotec) coated with monoclonal antibodies to CD3, CD19, CD56, and CD15, respectively (6,11). The percentage of donor type cells was determined by analysis of polymorphisms in the lengths of short tandem repeats (STR) (11). The threshold for detection of chimerism by STR analysis is $\geq$1% of donor type cells.

Statistical analysis

Graft survival of patients enrolled in the tolerance protocols versus conventionally treated patients at the Stanford Medical Center was compared using Kaplan Meier analysis and the Greenwood formula. Graft survival of tolerance protocol patients was compared to that of conventionally treated patients in the national registry data base by nomogram analysis (12).

Results

Three cohorts of patients conditioned with posttransplantation TLI and ATG: Outcome of Cohort 1 (HLA mismatched patients)

The first cohort of 6 HLA mismatched patients were given transplants between 2000 and 2003, and four were the subject of a previous report (4). As shown in the patient characteristics of Cohort 1 in Table 1, the donors were related or unrelated and the number of HLA mismatches varied from three to six. The first four recipients received 10 doses of TLI of 80 cGy each and the last two received 10 doses of 100 cGy each in order to increase the levels and duration of chimerism. However, the increased dose of TLI did not result in improved chimerism (see below). Recipients were given intravenous infusions of 3.1–11.1×10^6 $CD34^+$ cells/kg without addition of column flow through cells such that the $CD3^+$ T cells dose was always less than 0.1×10^6 cells/kg in order to reduce the risk of GVHD. Posttransplant IS drugs were maintenance, prednisone, and cyclosporine without mycophenolate mofetil (MMF) (4).

None of the six patients developed chimerism that persisted beyond 3 months (Table 1). IS drug withdrawal criteria in this cohort was transient chimerism, with the

Table 1: HLA mismatched patient characteristics, donor cells, and outcome of Cohort 1

Patient #	Age/sex	ESRD cause	# HLA mismatches (haplo/unrelated)	$CD34^+$ cell dose ($\times 10^6\,kg^{-1}$)	$CD3^+$ cell dose ($\times 10^6\,kg^{-1}$)	SCr at last observation (mg/dL)	Duration of follow-up (mo)	Duration of chimerism (mo)	Duration off drugs (mo)
1	51/M	PKD	4 (Unrelated)	4.3	<0.1	1.2	160	2	3.5
2	28/M	Unknown	3 (Haplo)	3.1	<0.1	2.4	157	–	–
3	30/F	SLE	3 (Haplo)	3.1	<0.1	1.9	155	3	5.5
4	24/F	IgA	6 (Unrelated)	6.3	<0.1	1.3	151	–	–
5	48/M	PKD	3 (Haplo)	11.1	<0.1	1.3	129	–	–
6	32/M	IgA	3 (Haplo)	8.4	<0.1	1.4	128	–	–

PKD, polycystic kidney disease; SLE, systemic lupus erythematosus; IgA, IgA nephropathy.

absence of clinical rejection episodes, GVHD, and reactivity to donor cells in the mixed leukocyte reaction (MLR) (4). Patients 1 and 3, who met these criteria, were withdrawn from both IS drugs at the end of 12 months. These patients developed mild (Banff I) rejection episodes 3.5 and 5.5 months after drug discontinuation. The rejection episodes were treated, and the patients were placed on maintenance IS therapy with MMF and cyclosporine thereafter. IS drugs were not withdrawn from the other four patients without chimerism. Two of these (patients 2 and 5) had early rejection episodes that rapidly resolved after treatment. Patient 6 developed transient thrombotic microangiopathy. The current serum creatinine concentrations of the six patients are shown in Table 1 (range 1.2–2.4 mg/dL) at 128–160 months posttransplant. There were no graft losses.

Outcome of Cohort 2 (HLA matched patients)

The characteristics of 22 HLA matched patients (Cohort 2) given transplants from 2005 to 2013 are shown in Table 2.

Table 2: HLA matched patient characteristics, donor cells, and outcome of Cohort 2

Patient #	Age/sex	ESRD cause	Panel reactive antibody (%)	$CD34^+$ cell dose ($\times 10^6\,kg^{-1}$)	$CD3^+$ cell dose ($\times 10^6\,kg^{-1}$)	SCr at last observation (mg/dL)	Duration of follow-up (mo)	Duration of chimerism (mo)	Duration off drugs (mo)
1	48/M	Unknown	0	8.0	1	1.3	42	42[1]	36
2	39/F	FSGS	0	8.4	1	0.8	101	–	–
3	24/M	Dysplasia	0	12.5	1	1.6	95	4	–
4	52/M	Unknown	4	4.9	1	1.4	80	12	60
5	34/M	IgA	0	12.8	1	1.2	77	16	66
6	61/F	DM	0	12.2	1	1.0	76	2	–
7	23/F	SLE	0	16.7	10	1.2	72	32[1]	12[2]
8	33/M	Reflux	2	16.7	1	1.0	70	42[1]	63
9	29/F	Unknown	29	17.5	1	1.1	62	14	–
10	52/F	PKD	92	14.0	1	0.8	62	12	50
11	37/F	IgA	0	14.4	1	1.2	54	24[1]	47
12	36/F	PKD	0	10.0	1	1.5	51	36[1]	45
13	26/M	Unknown	0	6.6	1	1.3	43	12	29
14	22/F	Unknown	26	14.4	1	0.7	42	10	29
15	40/F	IgA	81	10.0	1	1.1	41	12	29
16	42/M	Type 1 DM	0	6.0	1	1.3	33	14	21
17	30/M	CIN	0	5.7	1	1.4	28	10	10
18	39/M	IgA	0	9.4	1	1.3	25	25	15
19	29/M	IgA	0	11.8	1	1.5	19	9	10
20	45/F	Unknown	0	14.0	1	0.9	14	14[1]	2
21	62/F	IgA	0	12.0	1	0.8	12	12[1]	2
22	54/F	Alport	54	4.3	1	1.0	7	7[1]	in taper

FSGS, focal segmental glomerulosclerosis; IgA, IgA nephropathy; DM, diabetes mellitus; SLE, systemic lupus erythematosus; PKD, polycystic kidney disease; CIN, chronic interstitial nephritis.

[1]Chimerism present on all assays for duration shown. No subsequent assays were performed. Chimerism lost in other patients at duration shown.

[2]Off MMF and Cyclosporin for 12 months and returned to MMF and prednisone after Lupus flare.

Chimerism considered absent when 2 consecutive whole blood samples showed less than 2% donor cells.

There were 12 females and 10 males between age 22 and 62 years. The outcome of 16 of these patients was reported previously (6). Donors were all HLA matched siblings, and all recipients were given 10 doses of 120 cGy each and 5 doses of ATG posttransplant. All recipients were given an infusion of column enriched $CD34^+$ cells ($4.3–17.5 \times 10^6$ cells/kg) and 21 were given a defined dose of 1×10^6 $CD3^+$ T cells as shown in Table 2. One patient with active lupus and immune hyperactivity was given 10×10^6 $CD3^+$ T cells/kg to further promote engraftment of $CD34^+$ cells. IS drug withdrawal criteria in Cohort 2 was changed to (1) persistent chimerism for at least 6 months, (2) lack of clinical rejection episodes, (3) lack of GVHD, and (4) lack of rejection on a protocol biopsy obtained within 2 weeks before the discontinuation of IS drugs.

The IS drug regimen in Cohort 2 was prednisone tapered to discontinuation after 10 days, standard dose cyclosporine starting at day 0 to be tapered such that discontinuation would occur after at least 6 months of chimerism, and standard dose of MMF for 30 days after the infusion of donor cells as reported previously (6). Seventeen of the 22 patients were successfully withdrawn from IS drugs at 6 to 14 months posttransplant without rejection episodes thereafter with an observation period of 2 to 66 months off drugs (median 29 months) (Table 2). One patient with lupus (patient 7) was returned to maintenance IS drugs after a systemic lupus flare. Patient 22 is in the midst of IS drug taper. Four patients did not meet drug withdrawal criteria due to clinical rejection episodes during early IS drug taper at 2–3 months (patients 3 and 6), microscopic rejection on a protocol biopsy (patient 9), or lack of chimerism associated with rapid return of focal segmental glomerulosclerosis (FSGS, patient 2).

Among the 16 patients who developed chimerism for at least 6 months and had IS drugs successfully discontinued without reinstitution, 7 had stable chimerism during and after IS drug withdrawal (Figure 1A), and 9 eventually lost chimerism (Figure 1B). Serial chimerism percentages are given for the lineage that had the highest peak percentage of donor type cells (either granulocytes or B cells), and arrows show the time of IS drug discontinuation. None of the six patients with peak chimerism levels below 65% within the first 60 days (Figure 1C) had a stable chimerism pattern and all lost chimerism during or after drug withdrawal. Thus, stable chimerism that persisted through the end of the cyclosporine taper, and at the last observation point (immunosuppression independent chimerism),was found only among 10 patients with high (>65%) early levels of chimerism (Figure 1A and C). Of the latter 10 patients, 8 had at least 20% chimerism in granulocytes or B cells at the time of cyclosporine discontinuation, and 7 of these maintained chimerism without subsequent loss. The range of serum creatinine concentrations in all the HLA matched patients at the last observation point was 0.7–1.6 mg/dL with up to 101 months of follow up (Table 2).

Outcome of Cohort 3 (HLA haplotype matched patients)

The characteristics of patients in Cohort 3 are shown in Table 3, and all were given the same TLI (10 doses of 120 cGy each) and ATG (5 daily doses) regimen given to Cohort 2. However, the content of the donor cell infusion was changed to develop a dose escalation study of $CD3^+$ T cells to find a dose that would promote persistent mixed chimerism for at least 6 months. Increased numbers of infused donor T cells has been shown previously to facilitate the engraftment of hematopoietic progenitor cells, but the risks of GVHD have increased if complete instead of mixed chimerism occurred (13,14). The range of infused $CD34^+$ cells/kg was from 8 to 22×10^6 cells/kg in the current study (Table 3). The dose of $CD3^+$ T cells added to the $CD34^+$ cells started at 3×10^6 cells/kg in patient 1, and was increased to 10×10^6 cells/kg in the next four patients, 20×10^6 cells/kg in the next two and then to 50×10^6 cells/kg in the last three (Table 3).

Table 4 and Figure 2 show the early levels of chimerism achieved with the different doses of $CD3^+$ T cells and $CD34^+$ cells in the 10 patients. Chimerism with at least 30% donor type cells in at least 1 white blood cell lineage within the first 60 days was observed in patients 2 and 3 who were given the highest number of $CD34^+$ cells (15 and 22×10^6 cells/kg, respectively) along with 10×10^6 $CD3^+$ T cells/kg. Patients 1, 4, 5, 6, and 7 failed to achieve chimerism at this level when $3–20 \times 10^6$ $CD3^+$ T cells/kg were added to $8–12 \times 10^6$ $CD34^+$ cells/kg. However, when 50×10^6 $CD3^+$ T cells were added to $8–11 \times 10^6$ $CD34^+$ cells/kg in patients 8, 9, and 10 high levels of chimerism were achieved within the first 60 days with a peak of more than 70% donor type cells in all lineages in the case of patients 9 and 10 (Figure 2). Thus, the highest early levels of chimerism were achieved after the infusion of high doses of both T cells and $CD34^+$ cells. The doses of T cells were considerably higher (up to 50-fold) than that given to HLA matched patients.

Serial chimerism measurements for 12 months posttransplant in patients 2 and 3 are shown in Figure 3. Mixed chimerism persisted during this time interval, with the highest levels observed among granulocytes and the lowest levels among T cells. MMF was tapered starting at 6 months, and discontinued at 9 months in these two patients. Tacrolimus taper was started at 9 months, and was continuing at the last observation at 12 months. Discontinuation was planned between 12 and 15 months. Serum creatinine concentrations are shown in Figure 3, and remained stable without indication of clinical rejection episodes during the 12 month observation period.

Graft survival in HLA matched and mismatched transplant patients

Figure 4A shows kidney transplant recipient graft survival of HLA matched patients using Kaplan Meier analysis, and

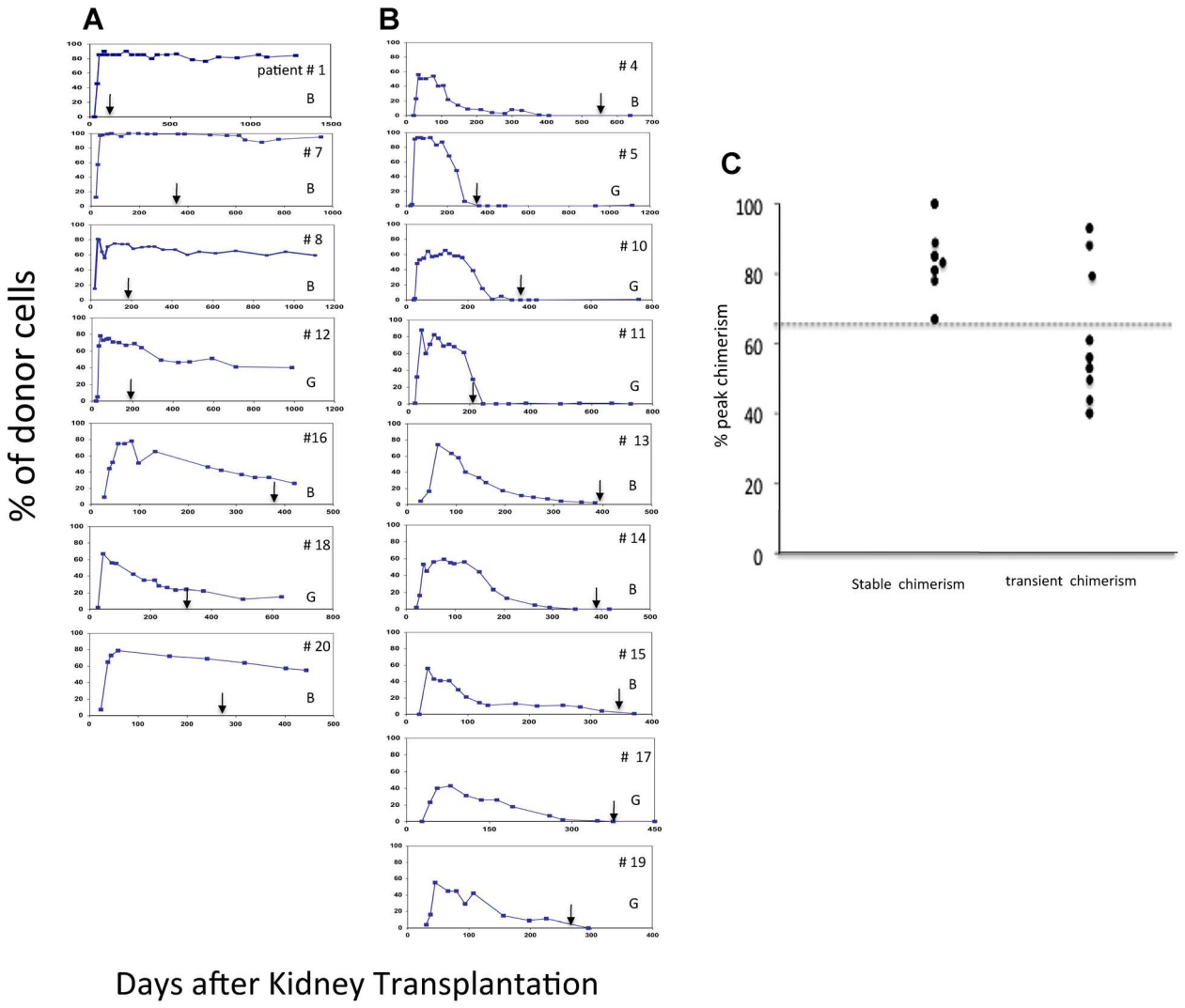

Figure 1: Tolerant HLA matched patients had either stable mixed chimerism or ''metastable'' transient chimerism. (A) Shows the serial percentages of donor type cells among the granulocytes (G) or B cells (B) depending on which lineage had the highest peak level in tolerant HLA matched patients with stable mixed chimerism. Arrows show the time point of IS drug discontinuation. (B) Shows the serial percentages in tolerant matched patients who lost chimerism during or after IS drug withdrawal (transient chimerism). Chimerism among NK cells and T cells was always lower than that of granulocytes or B cells (data not shown). (C) Shows the peak percentages of chimerism among granulocytes or B cells in the first 60 days posttransplant among the patients with stable mixed chimerism or ''metastable'' transient chimerism. Horizontal line at 65% shows that peak chimerism of less than 65% was observed only in patients with transient chimerism.

compares the first 20 HLA matched patients enrolled in the tolerance induction protocol with control patients given conventional IS therapy at the Stanford Medical Center during the same period of time. The patients enrolled in the tolerance induction protocol had 100% graft survival over an observation period of over 8 years from 2005 to 2013. Figure 4A also shows data from the HLA matched controls with an observation period of over 12 years from 2001 to 2013. The actuarial graft survival of the control patients was about 86% after 8 years, and the graft survival difference between tolerance protocol and control patients was not statistically significantly different (Greenwood formula) due to the small number of protocol patients.

Figure 4B compares the graft survival of the combined group of the first 20 HLA matched and the first 10 HLA mismatched patients (total 30) enrolled in the tolerance protocols with that of conventionally treated living donor kidney transplant patients with similar pretransplant characteristics and similar initial IS drug therapy whose data is recorded in the US national registry maintained by

Table 3: HLA haplotype matched patient characteristics, donor cells, and outcome of Cohort 3

Patient #	Age/sex	ESRD ause	# HLA mismatches	$CD34^+$ cell dose ($\times 10^6\,kg^{-1}$)	$CD3^+$ ell dose $\times 10^6\,kg^{-1}$)	SCr at last observation (mg/dL)	Duration of follow-up (mo)	Duration of chimerism (mo)
1	46/M	IgA	3	12	3	1.7	39	2
2	24/F	SLE	1	15	10	0.9	22	>12
3	35/F	Unknown	3	22	10	1.1	18	>12
4	33/M	Unknown	3	9	10	1.3	18	–
5	26/F	SLE	3	8	10	1.1	9	2
6	21/M	Unknown	3	8	20	1.2	8	2
7	47/F	GN	3	11	20	1.1	6	2
8	26/F	GN	3	8	50	1.5	3	>3
9	55/M	Obstruction	3	10	50	1.9	3	>3
10	34/M	IgA	3	11	50	1.6	2	>2

IgA, IgA nephropathy; SLE, systemic lupus erythematosus; GN, unspecified glomerulonephritis.

the Organ Procurement Transplant Network/Scientific Registry of Transplant Recipients (OPTN/SRTR). Eighteen co-variate parameters of the protocol patients including donor and recipient age, gender and BMI, number of HLA mismatches, race of donor and recipient, and anti-rejection drugs were matched with those of registry patients using a nomogram that accurately predicts 5 year graft survival based on the co-variates (12). Figure 4B shows the observed 5 year graft survival of protocol patients (100%) versus the predicted graft survival of all patients in the registry with matched characteristics. The registry patients were predicted to have about 83% 5 year graft survival, and the latter survival was significantly lower than the observed survival as judged by the nomogram analysis ($p < 0.04$).

Safety outcomes in HLA matched and mismatched transplant patients

During the period from 2001 to 2013, there was one death among the patients given transplants in the tolerance protocols. The first HLA matched patient enrolled in Cohort 2 with a history of coronary artery disease died suddenly while bicycle riding. No autopsy was obtained. This patient had excellent graft function at the last observation point and had been off IS drugs for about 3 years at the time of death. With regard to malignancies, one patient in Cohort 1 developed thyroid cancer 7 years after transplantation that was successfully treated with radioactive iodine, and is in complete remission. Another patient in Cohort 2 was diagnosed 2 years after transplantation with early stage breast cancer. She was treated with surgery, and is in complete remission. One patient (#2) with early relapse of FSGS in the transplanted kidney had no evidence of disease activity on maintenance IS drugs at 101 months posttransplant, and one patient (#7) with relapse of systemic lupus including lupus nephritis was on maintenance IS drugs with minimal disease activity at 72 months.

Table 5 summarizes additional safety features of the 38 patients during the first year after transplantation. Eight patients developed infections. None of the viral infections required hospital readmission, and all were transient with resolution either after anti-viral therapy or with reduction of immunosuppression. Five patients had hospital readmissions for neutropenic fever, ureteral stricture, acute rejection, or pyelonephritis (Table 5). Nadir white blood cell counts of less than 1×10^3 cells/mm^3 were observed in

Table 4: Donor cell content and chimerism in HLA mismatched recipients (Cohort 3)

Patient number	$CD3^+$ cell number/kg ($\times 10^6$)	$CD34^+$ cell number/kg ($\times 10^6$)	Chimerism (>30% donor type cells)
1	3	12	–
2	10	15	+
3	10	22	+
4	10	8	–
5	10	8	–
6	20	8	–
7	20	11	–
8	50	8	+
9	50	10	+
10	50	11	+

Median $CD34^+ = 11 \times 10^6\,kg^{-1}$.
>14 $CD34^+ = 6/33$.
<8 $CD34^+ = 6/33$.

Table 5: Patient safety outcomes in the first year, all cohorts

Infection	8
Herpes zoster	4
BK viremia	2
CMV disease	1
Acute pyelonephritis	1
Acute cellular rejection episode	5
Hospital readmission	5
Neutropenic fever	1
Ureteral stricture	1
Acute rejection	2
Acute pyelonephritis	1
Nadir WBC $< 1 \times 10^3\,mm^{-3}$	3
GVHD	0

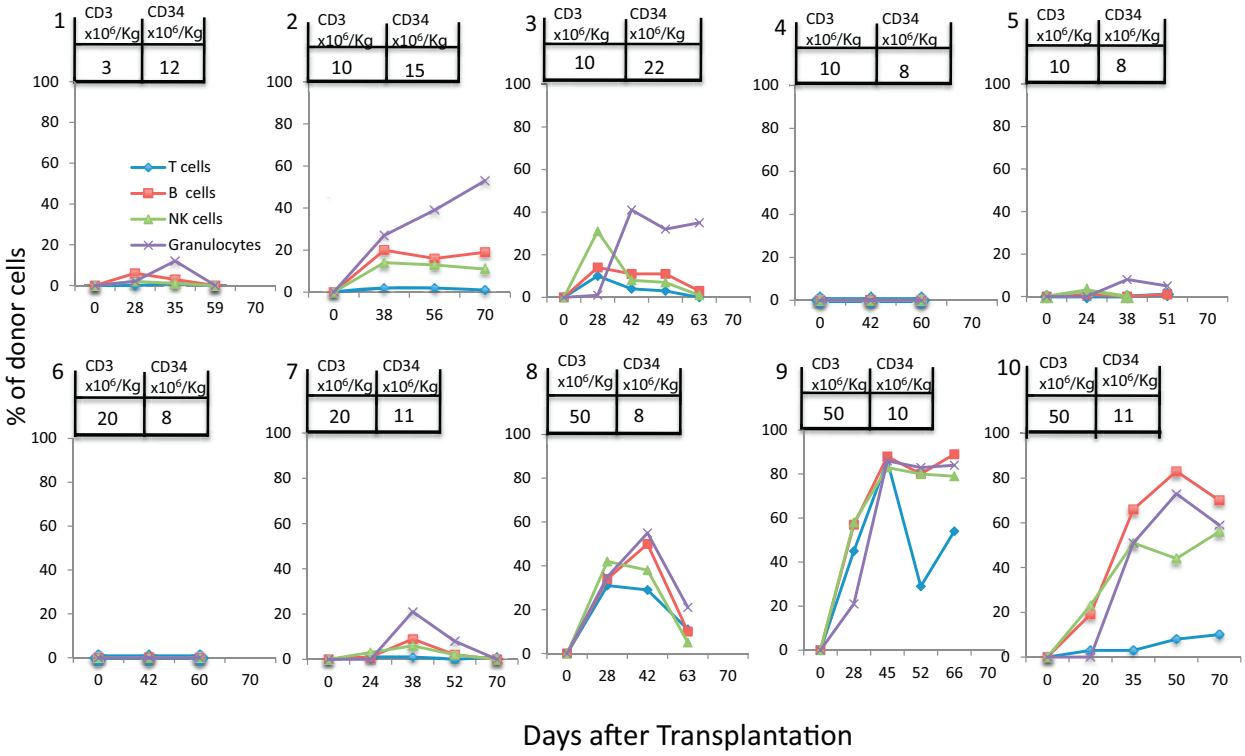

Figure 2: Chimerism levels within the first 70 days in haplotype matched patients increased in patients infused with high levels of $CD3^+$ T cells and/or high levels of $CD34^+$ cells. Serial percentages of donor type cells among granulocytes, T, B, and NK cells are shown in the 10 patients enrolled in the dose escalation study of donor T cells. The numbers of T cells and $CD34^+$ cells/kg infused are shown in the boxes. Highest chimerism levels were achieved after the infusion of 50×10^6 T cells and at least 10×10^6 $CD34^+$ cells/kg in patients 9 and 10.

3 of 38 patients, and GVHD was observed in none. Five patients developed cellular rejection episodes during the first year that resolved with anti-rejection therapy. One of these patients (haplotype matched patient 4) developed a vascular rejection with endothelial cell injury with similar features to those identified as an ''engraftment syndrome'' (13)).

Discussion

The goal of the study was to determine whether tolerance could be induced in 38 fully HLA matched or HLA mismatched kidney and hematopoietic cell transplant patients such that IS drugs could be withdrawn safely without kidney graft loss. Three cohorts of patients given posttransplant conditioning with TLI and ATG were enrolled over a period of 14 years (22 fully HLA matched patients and 16 HLA mismatched), and graft survival was 100% during the 14 years. Conventionally treated living donor HLA matched patients at Stanford observed for 8 years showed actuarial graft survival of about 86%. Comparison of the combined matched and mismatched protocol patients to conventionally treated living donor kidney transplant recipients with similar transplant characteristics in the OPTN/SRTR registry using nomogram analysis showed a significant improvement in 5 year graft survival.

The improved graft survival of the tolerance protocol patients could be due to the tolerance regimen regardless of whether IS drugs were discontinued, and the results suggest that there were persistent immune changes that attenuated the risk of acute and chronic rejection during the 14 year observation period. An alternative explanation is that the improvement was due to a ''center effect'' related to socioeconomic and other differences in the patient groups, and/or to the ''study effect'' in which protocol patients were studied more closely than conventional patients, and the close follow up improved graft survival. Multi-center randomized controlled trials in which tolerance protocol and conventionally treated patients are compared could be used to distinguish the different explanations.

Successful IS drug withdrawal was achieved in 16 of the 22 HLA matched patients without subsequent rejection episodes with follow up off IS drugs for up to 66 months (median 29 months). One of the matched patients is undergoing drug taper. HLA matched patients who were successfully withdrawn from IS drugs showed two patterns of chimerism; one in which chimerism was stable

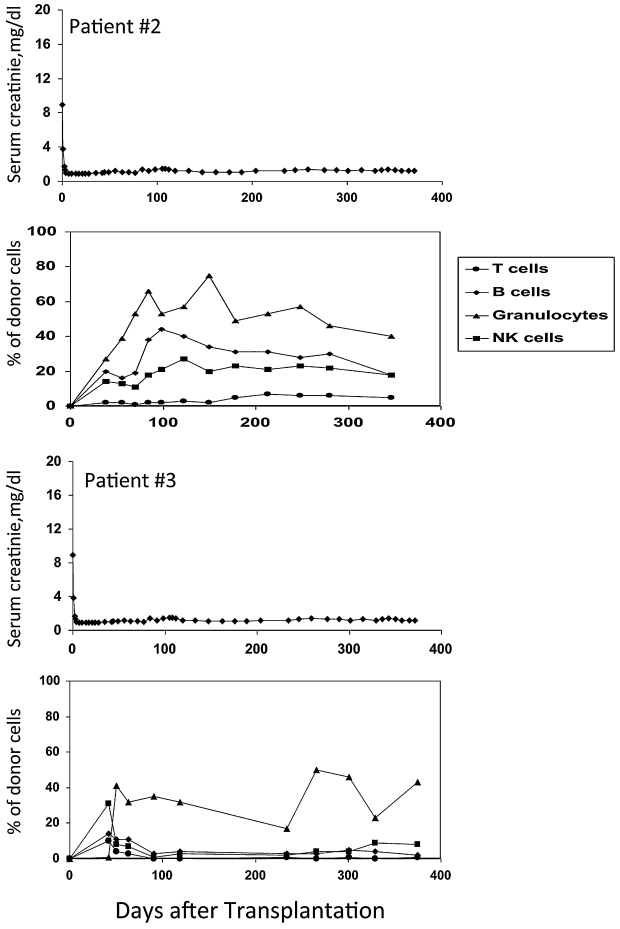

Figure 3: Serial serum creatinine concentrations and chimerism levels in haplotype matched patients 2 and 3 during 12 months after kidney transplantation. There were no clinical rejection episodes and chimerism persisted.

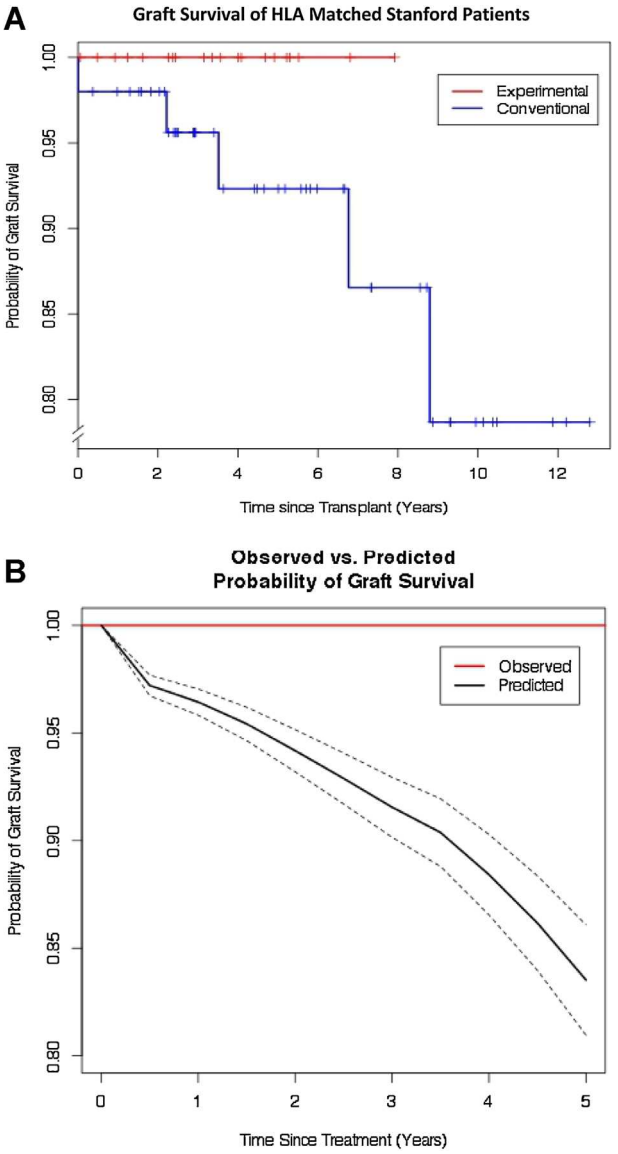

Figure 4: Graft survival among patients enrolled in the tolerance protocol was compared to that of conventionally treated patients at Stanford or in the OPTN/SRTR registry data base. (A) Compares Kaplan–Meier curves of death censored graft survival among the first 20 enrolled fully HLA matched patients in the tolerance protocol to that of 49 HLA matched patients given conventional therapy at Stanford during the past 12 years ($p = 0.14$; likelihood ratio test). (B) Compares the 5 year death censored graft survival of the first 20 enrolled fully HLA matched patients plus the first 10 enrolled HLA mismatched patients in the tolerance protocol (observed) to the predicted survival of patients with similar transplant characteristics in the entire national registry data base using nomogram analysis ($p = 0.04$). The p value was obtained by calculating the probability of no graft failures in the sample of enrolled patients given the graft survival function among registry patients. Dashed lines show 95% confidence interval for registry patients.

during and after cyclosporine withdrawal, and another in which chimerism that persisted for at least 6 months was lost during or after cyclosporine withdrawal (''metastable'' chimerism). The mechanisms of lack of rejection of kidney grafts after discontinuation of IS drugs in patients with loss of chimerism is not clear. One possibility is that the chimerism persists in tissues other than the blood, for example in the thymus, such that negative selection of host T cells can still take place. Preclinical studies of tolerance after mixed chimerism indicated that donor dendritic cells (DCs) in the thymus mediate negative selection (16). Another is that chimerism below the level of detection of STR analysis is sufficient to maintain tolerance, and a third is that immune regulation by suppressive immune cells such as Tregs in the host tissues or in the donor organ transplant maintain tolerance. Preclinical models have provided considerable evidence for the latter mechanism (17–19). The tolerant HLA matched patients showed specific unresponsiveness to donor alloantigens in the MLR (6).

A dose escalation study of donor T cells was undertaken to determine whether a minimum effective dose of T cells and $CD34^+$ cells could be identified that resulted in persistent chimerism for at least 6 months in the 10 HLA haplotype matched patients in Cohort 3. A second goal of the dose escalation was to find a dose that would result in peak chimerism of at least 65% in the first 60 days based on the data from matched patients that showed a correlation between high levels of early chimerism and stable mixed chimerism after IS drug discontinuation.

Results from Cohort 3 indicated that persistent chimerism for at least 12 months could be achieved with infusion of 10×10^6 T cells/kg when high doses (15 and 22×10^6 cells/kg) of $CD34^+$ cells were given. However, the latter patients did not achieve at least 65% chimerism at 60 days. The ability of these patients to undergo successful drug withdrawal will be the subject of a subsequent report with larger numbers of patients. When the dose of T cells was escalated to 50×10^6 cells/kg and the dose of $CD34^+$ cells was 10×10^6 cells/kg, then early high levels of chimerism were achieved. Donor cells with the latter composition will be administered to an enlarged cohort of patients to determine the reproducibility of the achievement of early high chimerism, and whether these patients will show stable mixed chimerism during and after IS drug withdrawal as was the case with the HLA matched patients.

The three cohorts did not show the high incidence of some adverse events reported in other tolerance induction studies. In particular, the high incidence of severe neutropenia (<500 white blood cells/mm^3) and/or thrombocytopenia (<20 000 platelets/mm^3) associated with conditioning with TBI and cyclophosphamide (9,20,21), was observed in less than 5% of patients in the current study. In addition, the high incidence of ''engraftment syndrome'' observed in patients with loss of chimerism within 2 weeks (15) was observed in only one of 38 patients in the current study.

In conclusion, the tolerance protocol described herein was safe and about 75% of HLA matched patients were successfully withdrawn from IS drugs. Increasing the dose of donor T cells was necessary to achieve mixed chimerism for at least 1 year in HLA mismatched patients. It is too early to determine whether stable mixed chimerism and/or successful IS drug withdrawal can be achieved in HLA mismatched patients. The data on graft survival and IS withdrawal suggests that the tolerance protocol may be a better option than conventional IS therapy for matched patients.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Authors S.S., R.L., and E.G.E. are co-founders of SERC Therapeutics, a cell therapy company with a focus on organ transplantation which provided no funding for the study.

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