ORIGINAL ARTICLE

Impact of belatacept conversion on kidney transplant function, histology, and gene expression - a single-center study

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ABSTRACT

Prior studies on belatacept conversion from calcineurin inhibitor (CNI) have been limited by an absence of postconversion surveillance biopsies that could underestimate subclinical rejection, or a case-controlled design. A total of 53 adult patients with allograft dysfunction underwent belatacept conversion (median: 6 months) post-transplant. At a median follow-up = 2.5 years, patient survival was 94% with a death-censored graft survival of 85%. Seven (13%) patients had acute rejection (including 3 subclinical) at median 6 months postconversion. Overall, eGFR improved (P = <0.001) from baseline = 31±15 to 40.2 ± 17.6 ml/min/1.73m^2 by 6 months postconversion, but then stayed stable. This improvement was also observed ($P < 0.001$) in comparison with a propensity matched control cohort on CNI, where eGFR stayed stable (mean ~ 32ml/min/1.72m^2) over 2-year follow-up. Patients converted < 6 months post-transplant were more likely to have a long-term improvement in kidney function. Paired gene expression analysis of 30 (of 53) consecutive pre- and postconversion surveillance biopsies did not reveal changes in inflammation/acute injury; although atrophy–fibrosis score worsened (mean = 0.28 to 0.44; $P = 0.005$). Thus, improvement in renal function with belatacept conversion occurred early and then sustained in comparison with controls where renal function remained unchanged overtime. We were unable to show molecular signals that could be related to CNI administration and regressed after withdrawal.

Transplant International 2020; 33: 1458–1471

Key words
belatacept, calcineurin inhibitors, immunosuppression, immunosuppression clinical, kidney clinical, molecular diagnostics, novel immunosuppressants

Received: 4 May 2020; Revision requested: 28 May 2020; Accepted: 7 August 2020; Published online: 7 September 2020

Introduction

The excellent long-term outcomes reported from the initial trial of Rostaing and Grinyo on low-immunologic risk kidney transplant patients with stable graft function spurred increasing interest in the conversion of calcineurin inhibitors (CNI) to belatacept [1,2]. In this trial, 7.1% (6/84) patients had acute rejection within six months of conversion to belatacept. In 2015, we reported our initial experience on the safety of belatacept conversion from tacrolimus in a small group of highly sensitized recipients with allograft dysfunction [3]. Since then, several other

doi:10.1111/tri.13718

conversion studies have examined this question [4-13]. Most of these studies were performed on low-immunologic risk Caucasian de novo kidney transplant patients. Renal function (as measured by estimated glomerular filtration rate; eGFR) improved variably by a mean of 6 to up to 43mL/min/1.73m2 by one-year postconversion. Postconversion acute rejection rates ranged from 4 to 20% with a one-year graft survival between 83 and 100%. There was a significant amount of intra- and inter-study variability in the choice of induction agents and maintenance immunosuppression used in these studies limiting the generalizability and interpretation of their findings. In addition, most studies did not have a case-controlled design that makes attribution of improvement in kidney function primarily to belatacept difficult. Finally, none of the previous studies incorporated postconversion surveillance biopsies despite a well-identified heightened acute rejection risk in the first few months postconversion [1]

In this single-center study, we report our long-term experience on 53 kidney transplant patients who underwent belatacept conversion from tacrolimus. To investigate the evolution of renal function after conversion, we matched our population to kidney transplant recipients from the Paris Transplant Group. Based upon prior studies which reported a significant early acute rejection risk, a majority of our patients received both pre- and postconversion surveillance biopsies. A subset of these paired biopsies were subjected to transcriptomic analysis using the Molecular Microscope Diagnostic System (MMDx; Alberta Transplant Applied Genomics Center, ATAGC, Edmonton, Canada).

Materials and methods

Our Institutional Review Board (IRB) approved this study. We retrospectively evaluated all "kidney-alone" adult transplant patients who were converted from tacrolimus to belatacept between November 2012 and December 2017 for acute/subacute allograft dysfunction and a biopsy-proven diagnosis of interstitial fibrosis/tubular atrophy (IFTA) without evidence of any rejection with a most recent follow-up of December 2018. A total of 711 adult kidney transplants were performed during this period, and 53 (7.5%) of those met criteria for inclusion in this study.

Immunosuppression and conversion to belatacept

All patients received induction immunosuppression with rabbit antithymocyte globulin (rATG; Thymoglobulin, Genzyme, Cambridge, MA) 6 mg/kg followed by triple-drug immunosuppression including tacrolimus, mycophenolate mofetil (MMF; 1–2 g/day), and prednisone (tapered to 5mg/day by 1-3 months post-transplant). All patients were confirmed to be Epstein–Barr virus (EBV)-seropositive and signed an informed consent prior to belatacept conversion. Belatacept was administered at 5 mg/kg on days 1, 15, 29, 42, and 57 and then monthly as described previously [1,3]. A weight change of $\geq$ 5 kg necessitated a change in the dose of belatacept over long-term follow-up. Given the presumed high risk of early rejection, MMF was increased usually by 500 mg/day (maximum dose 2.5 g/day) as tolerated on day 1 of the protocol. Tacrolimus weaning protocol was also modified as follows: 100% on day 1, 50% on day 15, 25% on day 29, and then off on day 42. Based upon our initial experience with acute rejection among patients undergoing belatacept conversion, tacrolimus taper was extended further among patients with leukopenia until they could tolerate an MMF dose of at least 1 g/day. Adverse events including serious infections or other major medical complications were recorded up till the most recent follow-up.

Biopsy processing

Biopsies were processed as described previously [3]. A portion (3-4mm) of a 16-gauge biopsy core was collected for gene expression analysis. The renal tissue was immediately stabilized in RNAlater® (Life Technologies, Carlsbad, CA, USA) and was refrigerated until shipping. Samples were shipped to ATAGC at room temperature for processing.

Biopsy assessment

Preconversion biopsies were performed for one of the following indications: (i) rise in serum creatinine $\geq$ 20% above baseline; (ii) creatinine nadir $\geq$ 2.0 mg/dl post-transplant; or (iii) delayed graft function> 21 days post-transplant. Patients with a history of recent acute rejection underwent a repeat biopsy to confirm resolution of rejection prior to conversion. Postconversion indication biopsies were performed for acute allograft dysfunction defined as an unexplained rise in creatinine $\geq$ 20% above baseline. All patients underwent preconversion biopsies. A majority of patients underwent postconversion surveillance biopsies (n = 40; 75%). Of the remaining 13, two (4%) patients could not be biopsied due to death, four (8%) were deemed at a high risk of bleeding due to anticoagulation and prior episodes of bleeding, three (6%) had acute rejection prior to a surveillance

biopsy being scheduled, and four (7%) declined a surveillance biopsy due to stable graft function. "For-cause" biopsies ($n = 3$) performed postconversion were not included, and these patients were excluded from the paired analysis. After the MMDx platform became available at our center, thirty consecutive patients (57%) also underwent transcriptome analysis of paired pre- and postconversion biopsies.

Biopsies were graded based upon the Banff 2013 criteria [14]. A microvascular injury score (MVI) was calculated by adding the glomerulitis and peritubular capillaritis scores (g + ptc). All chronic semi-quantitative Banff scores were rated 0–3 [15]. A total chronicity score was calculated as the sum of four basic Banff qualifiers: chronic glomerular damage (cg), interstitial fibrosis (ci), tubular atrophy (ct), and vascular intimal thickening (cv), thus allowing for a total score ranging from zero to a maximum score of 12 as reported previously [16].

Microarray assessment

The details of microarray assessment have been reported previously [17]. Detailed protocols for microarray processing are available in the Affymetrix Technical Manual (www.affymetrix.com). The output was a.CEL file of measurements of expression of all probes sets.

Nearest neighbors analysis

As described previously, we used a K-Nearest Neighbors algorithm to determine which biopsies in the large multicenter fully phenotyped, reference set at Edmonton ($n = 530$) most closely resembled study samples in terms of their multivariate molecular distribution [18,19]. Molecular similarity is defined in terms of the three-dimensional Euclidean distance between samples in principal component analysis (PCA) space. The PCA used four molecular classifier scores (TCMR, ABMR, all rejection, tubular atrophy/fibrosis), and the summarized transcript scores for parenchymal transcripts (KT1) [20,21] and acute kidney injury (AKI transcripts or IRRATs) [22]. Additional classifiers were based on rejection-related histology lesions as described recently [17]

Antibody testing

All patients underwent repeat flow crossmatch and anti-HLA antibody testing at the time of initial biopsy, then at three months intervals for the first year post-transplant, and then at least yearly thereafter. The details of pretransplant and post-transplant antibody screening have been described by us previously [3]

Definitions and statistical analysis

For analysis purposes, eGFR was arbitrarily set at 10 ml/min for patients on dialysis. Estimated GFR was calculated based upon the CKD-Epi equation. Delayed graft function was defined as the need for dialysis within the 1st week of dialysis. Proteinuria was defined as $\geq$ 0.3g/g measured on a random spot urine protein/creatinine ratio. For comparison of eGFR before and after the switch to belatacept, a paired t-test was used. In addition, a random intercept–random slope (allowing for the slope to vary before 3 months and after) model was fitted for the longitudinal measurements of eGFR for individual patients. Missing values on follow-up were imputed using last observation carried forward (LOCF) analysis.

In order to assess the isolated effect of belatacept conversion on renal function, we matched our cohort to a cohort of patients derived from the Paris Transplant Group (INSERM; Institut national de la santé et de la recherche médicale) registry. Propensity score matching was done in a 1:3 ratio with the following parameters: recipient sex, recipient age, time from transplant to inclusion, interstitial fibrosis and tubular atrophy (ci + ct), microvascular inflammation (ptc + g), eGFR at inclusion, donor age, donor type, donor HTN, donor creatinine, HLA mismatch, DGF, DSA at transplant, and re-transplantation. Different ratios matching were tested (1:3, 1:4, and 1:5), and we decided to proceed with 1:3 matching based on the absolute standardized differences of the listed parameters (see Appendix S1).

Finally, we looked at the predictors that may predict a "renal response" to belatacept conversion. To assess this, we arbitrarily defined an eGFR improvement of> 5mL/min/1.73m2 at year postconversion to divide the patients in to two groups ("responders" and "nonresponders"). Internal validity for this dichotomy was provided by the fact that there was a significant difference in death-censored graft survival between responders and nonresponders (data shown in Results). External validity came from published studies where the average improvement with belatacept conversion has been> 5mL/min/1.73m^2 [4-13]. The following baseline variables (at time of conversion) were used to derive a stepwise logistic regression model: donor terminal creatinine, donor age, donor hypertension, delayed graft function, HLA mismatch, GFR at conversion, biopsy

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findings at conversion, molecular findings at conversion, proteinuria at conversion (yes/no), and time post-transplant to conversion (<6 or> 6 months postconversion).

Results

Demographic characteristics

Table 1 lists the characteristics of the 53 patients who were switched to belatacept. A majority of patients were African American (40/53; 75.5%). Many patients (17/53; 32%) were sensitized with a cPRA> 20% (median cPRA = 83%; range = 29-100%), and 17% (9/53) had donor-specific antibody at the time of transplant. Eight (15%) patients had a recent acute rejection prior to conversion (ABMR, $n = 6$; TCMR, $n = 2$) at a median of 4.1 months (range = 2.4-5.6 months) prior to conversion. All patients underwent a follow-up biopsy to confirm resolution of rejection prior to conversion. The median time on belatacept was 30 months (range 3-62 months) for the overall group. Of the patients with functioning allografts, the median time on belatacept was 24 months postconversion (range = 14-55 months).

Renal function trends

Overall average renal function improved from an eGFR of 30.8 ± 15.4 ml/min/1.73m^2 to 37.2 ± 16.8 ml/min/1.73m^2 at 3 months ($P < 0.001$) postconversion (Fig. 1). While there was a statistically significant improvement in eGFR compared with baseline at all time points postconversion, there was no statistical difference in eGFR trends beyond 3 months compared with 6 months (40.2 ± 17.6 ml/min/1.73m^2; $P = 0.5$), 12 months (42.8 ± 16.6 ml/min/1.73m^2; $P = 0.13$), and at most recent follow-up (42.4 ± 21.1 ml/min/1.73m^2; $P = 0.08$) at a median follow-up of 30 months postconversion. A mixed linear regression model suggested that the slope between the time of belatacept conversion was up trending at 0.82 ($P < 0.0001$) while the slope beyond 3 month was 0.41 ($P < 0.0001$). However, the difference between these slopes beyond 3 months was not statistically significant ($P = 0.62$).

Comparison of renal function outcomes with controls

Table 2 shows the demographic characteristics of our cohort (IFTA cohort, $n = 53$) and the Paris cohort ($n = 159$). As compared to the control group, the belatacept group had improved eGFR at 24 months as compared to baseline. The belatacept group had a significant

Table 1. Demographic characteristics

n	53
Age (Mean ± SD), years	48 ± 10
Male gender	31 (58.5%)
African-American race	40 (75.5%)
Indication for Transplantation	
Re-transplant	12 (22.6%)
Hypertension	13 (24.5%)
Diabetes	8 (18.2%)
eGFR (Mean ± SD) at conversion	30.8 ± 15.4
<20	13 (24%)
20–40	26 (49%)
>40	14 (26%)
Proteinuria (Mean ± SD; g/g) at conversion	0.5 ± 0.6
cPRA at transplant (Mean ± SD, %)	26 ± 39
0–20%	36 (68%)
20–80%	6 (11%)
>80%	11 (21%)
Six-antigen HLA mismatch (Mean ± SD)	4.5 ± 1.4
Positive donor-specific antibody at transplant	9 (17%)
Positive donor-specific antibody at conversion	2 (3.7%)
Positive donor-specific antibody at most recent follow-up	4 (7.5%)
Deceased-donor kidney transplant	41 (77.4%)
Delayed graft function	32 (60.4%)
Median time to conversion; months (range)	6 (0.2-117)
<6 months post-transplant	26 (49%)
6–24 months post-transplant	18 (34%)
>24 months post-transplant	9 (17%)
Acute rejection < 6 months prior to belatacept conversion	8 (15%)
T-cell-mediated rejection	2 (3.7%)
Antibody-mediated rejection	6 (11.3%)
Tacrolimus trough at conversion (Mean ± SD; ng/ml)	5.8 ± 2.1
Mycophenolate dose at conversion (median, range; g/day)	2.0 (0.5-2.5)
Most recent mycophenolate dose (median, range; g/day)	1.5 (0.5-2.0)
Donor characteristics	
Kidney Donor Profile Index ((Mean ± SD; %)*	72 ± 24
Donor age (Mean ± SD), years	47 ± 15
African-American race	14 (28.3%)
Hypertension	24 (45.3%)
Terminal creatinine, mg/dl (Mean ± SD)	1.2 ± 0.6

CNI, calcineurin inhibitor; cPRA, calculated panel reactive antibody; eGFR, estimated glomerular filtration rate in ml/min/1.73m2; HLA, human leukocyte antigens; SD, standard deviation.

*Reported for deceased donors

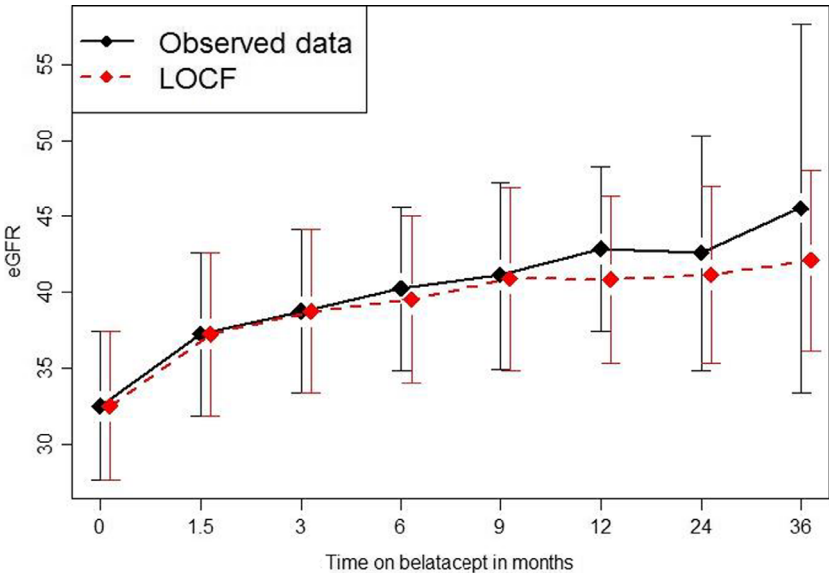

Figure 1 Trend in kidney function over 36 months postconversion. Kidney function across all time points postconversion. The slope between the time of belatacept conversion was up trending at 0.82 ($P < 0.0001$) while the slope beyond 3 month was 0.41 ($P < 0.0001$). However, the difference between these slopes beyond 3 months was not statistically significant ($P = 0.62$). *P*-values were derived using a mixed linear regression model. eGFR: estimated glomerular filtration rate (ml/min/1.73m^2); LOCF: last observation carried forward.

Table 2. Demographic comparison with propensity matched control cohort

Parameter	Belatacept cohort ($n = 53$)	Control cohort ($n = 159$)	*P*-value
Recipient age (mean ± SD)	48.12 ± 10.36	47.95 ± 13.59	0.942
Recipient gender (male, %)	31 (58%)	90 (57%)	0.936
Re-transplantation (*n*, %)	12 (23%)	35 (22%)	1
DSA at transplant (*n*, %)	9 (17%)	25 (16%)	1
DGF (*n*, %)	32 (60%)	97 (61%)	1
ABDR mismatch (mean ± SD)	4.53 ± 1.40)	4.40 ± 1.20	0.558
Donor creatinine ≥ 1.5 mg/dl	11 (21%)	35 (22%)	1
Donor HTN (*n*, %)	24 (45%)	63 (40%)	0.573
Deceased-donor transplant	41 (77%)	126 (79%)	0.923
eGFR at enrollment (mean ± SD)	30.87 ± 15.43	31.90 ± 12.02	0.658
Microvascular inflammation on biopsy at switch(mean ± SD)	0.64 ± 0.76	0.55 ± 0.91	0.490
IFTA (c + ct) at switch (mean ± SD)	2.00 ± 0.81	1.92 ± 1.00	0.582
Time from transplant to switch (or biopsy), year, mean ± SD	1.21 ± 1.90	1.07 ± 0.62	0.589
Donor age, mean ± SD	47.26 ± 15.11	48.48 ± 15.55	0.616

DGF, delayed graft function; DSA, donor-specific antibody; eGFR, estimated glomerular filtration measured in ml/min/1.72m2; HTN, hypertension; IFTA, interstitial fibrosis and tubular atrophy; SD, standard deviation.

progressive improvement in GFR (with trends as described above) in comparison with the matched Paris cohort where eGFR did not improve overtime but stayed stable (mean ~ 32mL/min/1.72m^2) up till a comparative time frame of 2 years (Fig. 2). There was no difference in the rate of 2-year graft loss between the two groups with 6/53 (11%) in the conversion group vs. 19/159 (12%) in the control group ($P = 1.0$).

Acute rejection and graft/patient survival

Rejection and survival characteristics are listed in Table 3. Seven patients (13.2%) had an episode of acute rejection after belatacept conversion.

Of these seven, three (42%) were diagnosed with TCMR (two with TCMR1B and one with IIA) on 6-month postconversion surveillance biopsies. The first patient was successfully treated, and a follow-up biopsy six months later showed no residual inflammation. The second patient was treated with pulse steroids. She subsequently developed profuse diarrhea in the setting of inadvertent increased dose mycophenolate and Salmonella infection. Her mycophenolate was converted to azathioprine. This was followed by ABMR and de novo DSA formation at 1.5 years after conversion necessitating an allograft nephrectomy. A third patient with TCMR IIA on surveillance biopsy was treated only with pulse steroids due to disseminated shingles. His follow-

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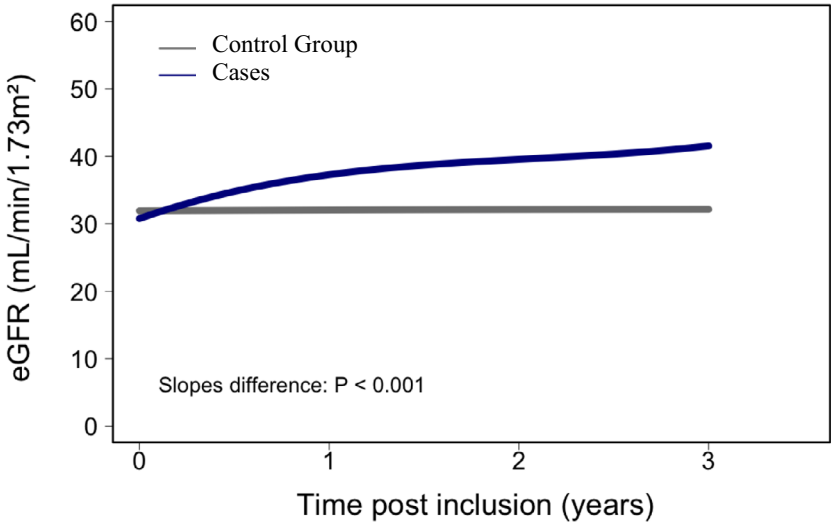

Figure 2 A Comparison of trends in renal function between IFTA (belatacept) group and control group. The slopes were significantly different with superior renal function noted in the belatacept group as compared to the control group.

Table 3. Patient outcomes postbelatacept conversion

xn	53
Total patients with acute rejection after belatacept conversion	7 (13.2%)
Clinical rejection*	4 (7.5%)
Subclinical rejection†	3 (4.3%)
T-cell-mediated rejection	5 (9.4%)
1A	1 (1.9%)
1B	3 (5.6%)
IIA	1 (1.9%)
Antibody-mediated rejection	2 (3.7%)
Time to rejection postconversion, median (range) in months	6 (1-20)
Median follow-up on belatacept; months (range)	30 (3-62)
De novo donor-specific antibody at most recent follow-up	3 (5.6%)
1-year death-censored graft survival	50 (94.3%)
1-year patient survival	50 (94.3%)
Overall death-censored graft survival	45 (84.9%)
Overall graft survival	42 (79.2%)
Overall patient survival	50 (94.3%)

*Diagnosis established on for-cause biopsy due to acute allograft dysfunction.

†Diagnosis established on surveillance biopsy postconversion per protocol for 2 patients and on surveillance due to a rise in DSA in 1 patient.

up biopsy showed persistent rejection. Subsequently, he had an aortic dissection and died to postoperative surgical complications.

One patient with progressive TMA had Banff Grade 1A rejection diagnosed one month after conversion. She progressed to graft loss six months postconversion despite treatment with eculizumab for presumed atypical hemolytic uremic syndrome. Her allograft nephrectomy specimen showed vascular changes consistent with chronic TMA without any ABMR. Another patient with donor-derived IFTA (KDPI = 92%) and presumed CNI-induced neurotoxicity had an abruption discontinuation of tacrolimus with conversion without a weaning protocol. He was diagnosed with TCMR 1B on a for-cause biopsy 1.5 months after conversion. He was treated with steroids only due to co-existent CMV disease. He lost his allograft due to an inadequate response to rejection therapy. One highly sensitized patient (cPRA = 88%) with a prior history of ABMR had a rise in immunodominant DSA (iDSA DQ6 6700->~22000MFI) at 16 months postconversion in the setting of reduction in mycophenolate dose for severe influenza. Her surveillance biopsy demonstrated subclinical ABMR that was treated with bortezomib (Takeda, MA) based therapy followed by a partial decline in DSA (iDSA DQ6 22000->14000MFI). She was the only patient with a previous history of prebelatacept conversion acute rejection (1/8; 12%; Table 1) to be diagnosed with rejection postconversion also. Two additional patients were noted to have DSA. One patient with nonadherence (as described below) lost her transplant 21 months postconversion. One additional patient with DSA at conversion continued to have unchanged DSA (Class I DSA ~ 1500 MFI) over one year of follow-up postconversion with no rejection on a follow-up surveillance biopsy.

Overall death-censored graft survival was 85% at a median follow-up of 30 months, range (3–62) postconversion (Fig. 3) with a 1-year graft survival of 94.3%. There were eight allograft failures at a median of

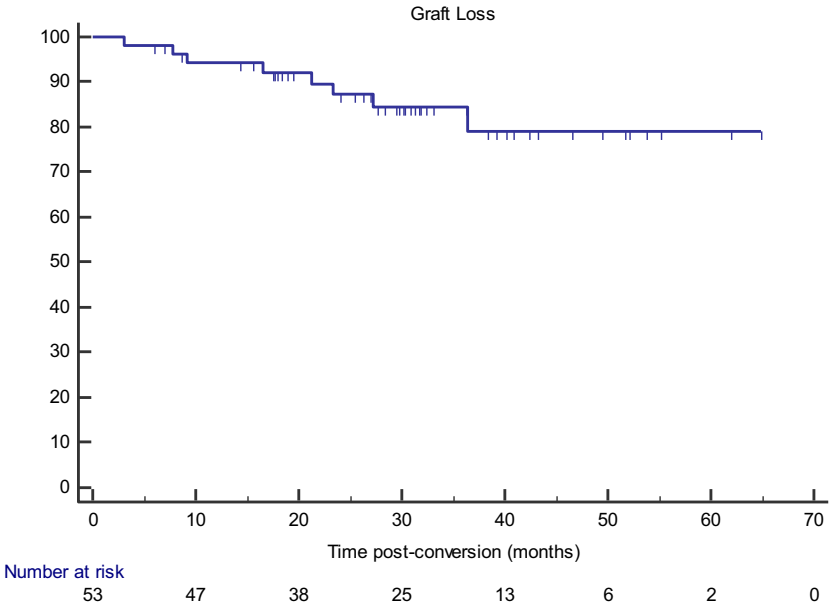

Figure 3 Kaplan–Meier graft survival curve for the overall population. Overall graft survival was 88.7% at a mean follow-up of 4.1 years postconversion.

9.1 months (range = 4.0-41.7 months) postconversion. Of the eight grafts (11.4%) that failed, two were lost to focal segmental glomerulosclerosis (FSGS), one due to recurrent pyelonephritis, one due to rejection in the setting of medication nonadherence, and one due to chronic allograft dysfunction. The remaining three allografts were lost as described above. Thus, three transplant losses (3/8; 37%) were related to rejection. Overall patient survival was 94%. Three patients died with functioning grafts; one patient died from intracranial hemorrhage in the setting of supra-therapeutic anticoagulation, one due to a presumed pulmonary embolism, and a third due to postaortic dissection associated surgical complications.

Histologic and gene expression analysis of pre- and postconversion biopsies

Overall, mean preconversion chronicity scores were 3.4 ± 2.0 for the entire cohort. Forty-three patients (81%) underwent at least one postconversion biopsy. Of these 43, three were done for cause (two for rise in creatinine, one for rising DSA) but a majority (n = 40; 75%) were done for surveillance at an average of 8.1 months (SD = 3.5 months) after conversion. Of these, two were diagnosed with subclinical acute TCMR (as described above). No acute rejection was noted on the remainder of the biopsies (38/40; 95%). Although sum of all chronic Banff classifiers (CI + CT+CV + CG) prior to conversion for these 40 patients remained statistically unchanged (3.8 ± 2.2 vs. 3.8 ± 2.1; P = 0.80; Fig. 4a), after conversion there was a slight trend toward worsening of the IFTA (ci + ct) scores (2.6 ± 1.4 vs. 3.1 ± 1.3; P = 0.10; Fig. 4b). Microvascular inflammation pre- vs. postconversion (0.68 ± 1.02 vs. 1.0 ± 0.98; P = 0.23) and total inflammation (total i-score) were not statistically different (0.7 ± 0.9 vs. 1.0 ± 0.8; P = 0.2).

After the molecular microscope platform was introduced at our center, both the pre- and postconversion surveillance biopsies for 30 consecutive patients were subjected to intra-graft mRNA-based gene expression analysis. An unsupervised analysis of the top 50 genes which were differentially expressed between the paired biopsies is reported in Table S1. After adjustment, there were no significant differences pre- vs. postconversion in terms of individual gene expression and additional candidate genes that may be associated with "CNI toxicity." We then analyzed a variety of gene signatures associated with rejection, acute kidney injury, and atrophy–fibrosis (Table 4). While there was a slight trend toward improvement in the injury-repair response-associated transcripts (IRRATs; P = 0.15), a worsening of the atrophy–fibrosis score was noted postconversion (P = 0.005; similar to the worsening of the IFTA score noted on histology).

Predictors of improvement in renal function

Due to an inadequate "event rate" of graft losses or substantial changes as well as results from our comparison with the Paris control group where eGFR stayed stable

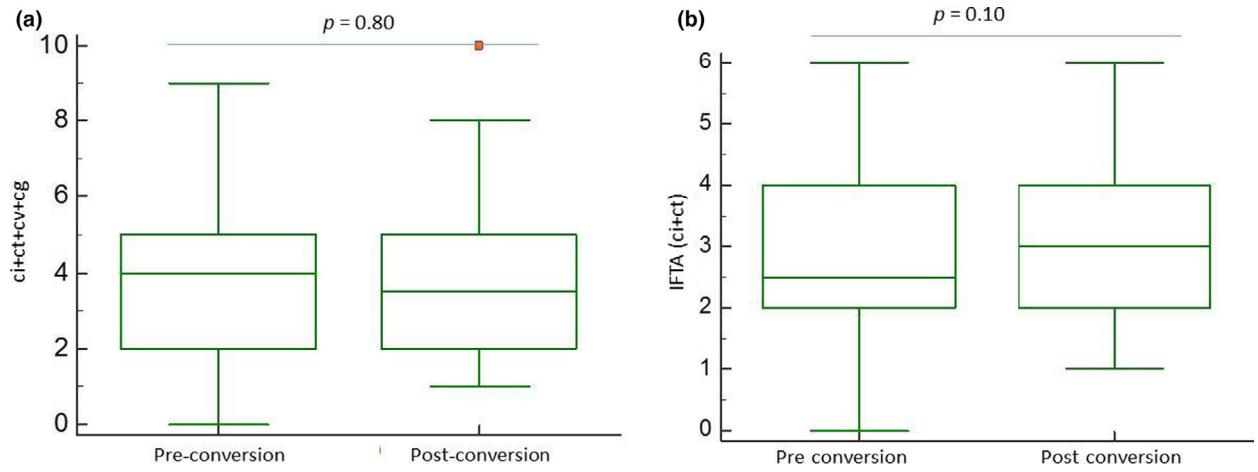

Figure 4 Pre- vs. postconversion biopsy chronicity scores. The sum of chronic Banff classifiers (CI + CT+CV + CG) is represented in (a). The sum (IFTA) of chronic interstitial fibrosis (ci) and tubular atrophy (ct) is represented in (b).

Table 4. A comparison of molecular changes between preconversion and postconversion surveillance biopsies (n = 30 patients)

	Presumed range of "normal"*	Preconversion biopsy (mean)	Postconversion biopsy (mean)	*P*-value
Aggregate scores				
Total rejection score	0.0–0.30	0.083	0.13	0.16
TCMR molecular score	0.0–0.10	0.01	0.04	0.28
ABMR molecular score	0.0–0.20	0.08	0.08	0.82
Global disturbance score (inflammation)	−3.8 to 0.03	−1.08	−0.74	0.53
Acute kidney injury score	−0.60 to 0.39	0.26	0.14	0.16
Atrophy–fibrosis score	0.0–0.7	0.28	0.44	0.005
Glomerulitis score	0.0–0.22	0.136	0.169	0.31
Peritubular capillaritis score	0.0–0.27	0.129	0.170	0.34
Interstitial inflammation score	0.0–0.1	0.027	0.037	0.19
Tubulitis score	0.0–0.16	0.040	0.055	0.298
Transplant glomerulopathy score	0.0–0.24	0.094	0.095	0.98
Rejection-Related Transcripts				
Interferon-gamma- and rejection- induced transcripts		0.420	0.493	0.40
Endothelium-associated transcripts		0.019	0.041	0.49
Quantitative cytotoxic T-cell-associated transcripts		0.572	0.697	0.26
Donor-specific antibody selective transcripts		0.142	0.200	0.26
Kidney Injury Transcripts				
Injury-repair response-associated transcripts		0.200	0.056	0.15
Kidney parenchymal transcripts		−0.261	−0.159	0.24
Kidney solute carrier transcripts		−0.535	−0.318	0.33

ABMR, antibody-mediated rejection; TCMR, T-cell-mediated rejection.

*2.5th–90th percentiles of the reference set

over 2 years of follow-up on CNI therapy, we performed an exploratory analysis using an arbitrary definition of improvement in eGFR> 5mL/min/1.73m^2 by 6 months postconversion to classify patients as "responders" or "nonresponders." The change in eGFR from preconversion to 6 months was significantly different ($P < 0.001$) between the two groups with a median 0mL/min/1.73m^2 (range: −16 to + 3mL/min/1.73m^2) among "nonresponders" and a median + 13 (range=+5 to + 48 ml/min/1.73m^2) among "responders." There was a significant difference in death-censored graft survival between responders and nonresponders (mean 57 months vs. 31 months postconversion; $P = 0.02$) with only 2 (of 32; 6%) graft loss among responders

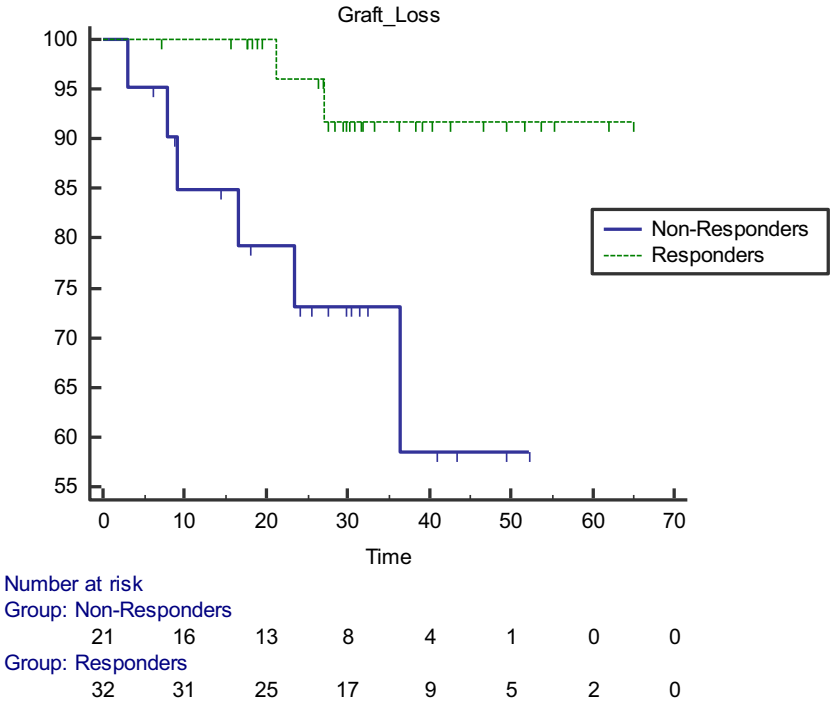

Figure 5 Graft loss trends between "responders" vs. "nonresponders." There was a significant difference in death-censored graft survival between responders (94%) and nonresponders (72%; $P = 0.02$).

and 6 graft losses (of 21; 28%) among nonresponders (Fig. 5). An analysis of biologically plausible and statistically different variables that associated with response (vs. nonresponse) is described in Table 5. Only time to conversion of < 6 months post-transplant was independently associated (Odds Ratio: 4.2; $P = 0.02$) with "response" at 6 months postconversion.

Adverse events

Adverse events are listed in Table 6. In total, 64 adverse events occurred during 88.5 patient years of belatacept exposure. Eight (15%) patients required hospitalization postconversion, with urinary tract infections being the most common indication. Six (11.3%) patients developed cytomegalovirus (CMV) associated disease. Three (out of 6; 50%) were CMV serodiscordant which put them at risk of primary infection. BK polyomavirus viremia was noted in one patient (1.9%), 14 days after conversion. This responded to reduction in the dose of mycophenolate.

Two patients developed cryptococcal meningitis, 1.9 and 3.5 years, respectively, after conversion. One patient was diagnosed with pulmonary cryptococcosis 1.4 years postconversion. Belatacept dose was reduced by 50% (2.5mg/kg) and spaced out to 6 weeks for two doses and then re-instituted every 4 weeks at 5mg/kg after recovery from the episode. Mycophenolate dose was reduced by 50% also. All three patients maintained stable allograft function in the long term despite

Table 5. Comparison of important variables between responders and nonresponders

Variable	Responders ($n = 32$)	Nonresponders ($n = 21$)	*P*-value
Donor terminal creatinine (mean ± SD), mg/dl	1.3 ± 0.6	1.0 ± 0.4	0.07
Delayed graft function	22 (68.8%)	10 (47.6%)	0.1
Conversion < 6 months post-transplant	20 (62.5%)	6 (28.6%)	0.02
eGFR at conversion	30 ± 17	31 ± 13	0.37
Proteinuria (≥0.3g/g) at conversion	13 (41%)	12 (57%)	0.27
Interstitial fibrosis/tubular atrophy (ci + ct)	2 (0-6)	2 (0-6)	0.8
Chronic vasculopathy (cv)	0.8 ± 1.1	0.4 ± 0.7	0.22
Median (range) atrophy–fibrosis score*	0.16 (0.03-0.63	0.29 (0.15-0.83)	0.15

eGFRm estimated glomerular filtration rate (1.73 ml/min/m2).

*Molecular atrophy–fibrosis score available only on 18 responders and 12 nonresponders.

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Table 6. Major adverse events

Adverse events		Notes
n	53	
Hospitalization	8 (15%)	100% due to infections
Bacterial Infections		
Surgical wound infection	4 (7.0%)	
Urinary tract infection	13 (24.5%)	54% (7/13) females
Bacteremia	3 (5.7%)	100% UTI associated
Clostridium difficile colitis	1 (1.9%)	
Viral		
Significant CMV Viremia	6 (11.3%)	Median 383 days postconversion (range: 27-1289)
BK polyomavirus viremia	1 (1.9%)	14 days postconversion
Fungal		
Cryptococcosis	3 (5.7%)	1.4, 1.9, and 3.5 years postconversion
Diarrhea	18 (34%)	Average MMF dose 2 g/day in patients with diarrhea
Colonic biopsy-proven MMF toxicity	5/18 (27.7%)	3 improved with dose reduction; 2 improved with conversion to Azathioprine
Unknown cause	8/18 (44.4%)	Resolved without any changes
CMV colitis	4/18 (22.2%)	Resolved with therapy
Clostridium difficile colitis	1/18 (5.5 %)	Resolved with therapy
Leukopenia, Grade 3 or higher	7 (13.2%)	

CMV, cytomegalovirus; MMF, mycophenolate mofetil.

liposomal Amphotericin B therapy and did not develop de novo DSA on follow-up. No cases of lymphomas, skin cancers, or any other malignancies were observed.

Diarrhea (18/53, 34%) was the most common adverse event noted in the study. These patients were on a higher average dose of mycophenolate (1.97 ± 0.34 g/day vs. 1.68 ± 0.44 g/day) compared with the rest of the group at the time of diarrhea onset. Of the 18 patients who suffered from diarrhea, five (27.7%) were found to have colonic biopsy-proven mycophenolate-associated toxicity. Three responded to dose reduction. Two patients had to be converted to azathioprine 2 mg/kg/day. One patient continued to do well, but the second patient developed antibody-mediated rejection and lost her allograft (details described above). We were unable to ascertain the cause of diarrhea in 8 (of 18; 44.4%) patients, but their symptoms resolved with symptomatic anti-diarrheal therapy only.

Discussion

In this study, we report our extended experience on belatacept conversion for 53 patients with allograft dysfunction that encompasses more than 90 patient years of belatacept exposure. All patients received similar induction and maintenance immunosuppression. Thirty-eight (out of 44; 86%) patients in our study had one or more risk factors that might be considered high immunologic risk (African-American race, high cPRA, re-graft, or prior history of acute rejection). We report that even in this relatively high-risk group, renal function improved significantly by an average eGFR of 9.9ml/min/1.73m2. These results confirm and expand on previously published data [3-9]. These studies had variable limitations related to lack of African-American representation, absence of re-grafts, sensitized patients, and the use of variable induction or maintenance immunosuppressive protocols. In addition, postconversion surveillance biopsies were not performed which does not allow for an estimation of subclinical rejection rates. Finally, a major limitation of almost all previous studies has been an absence of a control cohort of patients maintained on CNI therapy.

Belatacept-based immunosuppression studies have reported acute rejection rates ranging from 5% to up to 55% [23]. Given our largely high immunologic risk population, we performed postconversion surveillance biopsies on most of our patients. Reassuringly, surveillance biopsies did not show any worsening of either total inflammation or microvascular inflammation. Despite a high degree of sensitization, only one patient developed de novo DSA on follow-up. A few noteworthy points can be gleaned from our experience. First, we chose a slightly prolonged tacrolimus taper (over 42 days) to minimize the risk of early acute rejection reported in previous studies. Second, we increased the dose of MMF by at least 500 mg/day for all patients at the time of conversion. Based upon the observation that

several patients developed diarrhea on higher doses of MMF in the long term (Table 6), we have now tailored our approach to reduce MMF dose to ≤ 2 g/day once the postconversion surveillance biopsy shows no histologic evidence of rejection. A total of seven patients (13%; 3 subclinical) were diagnosed with acute rejection. Consistent with previous experience, a majority of patients were diagnosed with acute rejection within the first 6 months of conversion [24]. One patient who developed acute rejection had his tacrolimus stopped without a weaning period after conversion to belatacept. A second patient developed TCMR in the setting of a rapid reduction of MMF to 500 mg/day due to worsening leukopenia. Based upon these two experiences, we now use an induction belatacept dose of 10mg/kg in patients where CNI needs to be immediately discontinued [5,6]. Among patients with leukopenia who can be maintained on tacrolimus, we extend the CNI taper by 1-3 months until they can tolerate a MMF dose of ≥ 1 g/day.

In our study, we report a rapid improvement in eGFR within the first 3 months of conversion followed by sustained stability thereafter (Fig. 1). These findings mirror those noted in previous studies although a previous randomized controlled trial on this topic did show statistical improvement in eGFR beyond 12 months postconversion [4,7,25]. While ours was a review of a prospectively designed protocol, the primary limitation remains the lack of a randomized control group. We accounted for this limitation with the development of a propensity matched cohort from a well-defined largely homogenous cohort of patients maintained on CNI therapy. We found that there was an improvement of renal function in our cohort when compared to the control cohort. It is possible that local immunosuppression protocols, racial and other demographic differences could account for these differences. Nevertheless, external validity of our analysis comes from the fact that the clinical variables chosen for the matching have been validated for prognostication in a large multi-continental multicenter study by the Paris group that included patients from our center [26]. In addition, our observations are similar to the data reported by Bertrand et al who reported an improvement in GFR in patients converted to belatacept for chronic vasculopathy (cv lesions) when compared to a control cohort where GFRs remained stable [7]

Another limitation of previous studies has been an absence of analysis on the factors that may predict a response to belatacept. We used a definition of a GFR improvement> 5mL/min/1.73m2 at 6 months postconversion to define patients into "responders" and "nonresponders." While this definition was arbitrary, we found that there was a significant difference in long-term graft survival between these two groups providing internal validity to our definition. Although our sample size is limited, our results suggest that early conversion (< 6 months post-transplant) was independently associated with an improvement in kidney function. This is similar to previous findings from Europe [11]. A numerically higher number of patients with a high donor terminal creatinine and delayed graft function had a response indicating that these patients likely had acute kidney injury (? donor-derived) rather than chronicity. In keeping with this hypothesis, responders had a lower molecular atrophy–fibrosis score than nonresponders. Future larger studies would be required to expand on our early exploratory findings that may allow for tailoring future conversion trials.

In our study, we also performed LOCF analyses which confirmed the suggestion from previous studies that significant eGFR improvements were limited to the first few months of conversion [4,7,25]. Thus, these trends provide some insight into the possible mechanisms behind the improvement. The primary mechanism for "acute CNI nephrotoxicity (CNIT)" is a reversible renal afferent arteriolar vasoconstriction with an attendant drop in glomerular filtration [27]. Therefore, it seems intuitive to expect an early improvement in GFR if CNIs are discontinued. Previous studies which involved merely CNI discontinuation without any substitution showed similar early improvements further solidifying this premise [28]. In a case-controlled study, Abdelwahab reported that there was no difference in the slope of inverse creatinine during the 12-month period after conversion in patients converted to belatacept early post-transplant vs. patients maintained on belatacept [10]. Initial studies by Nankivell suggested that almost all kidney transplants developed chronic CNIT over long-term follow-up [29,30]. A major limitation of these early studies was the lack of a control arm. Subsequent studies have questioned the specificity of many of the "pathognomonic" CNIT lesions, for example, arteriolar hyalinosis [30-34]. In our study, we compared intra-graft gene expression in a large cohort of biopsies prior to and then ~ 7 months postconversion. Somewhat surprisingly, neither an analysis of individual genes (Table S1), nor an analysis of gene sets related to parenchymal kidney injury (Table 4) showed any clear changes overtime. We did observe a possible numerical improvement in the injury-repair response-associated transcripts in the preconversion vs.

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the postconversion biopsies ($P = 0.15$). Our results are not consistent with previous studies that identified unique CNIT signatures which were enriched for genes associated with fibrosis and early tubulo-interstitial damage [35,36]. A few factors could explain these discrepant findings. The study by Maluf et al used a histologic diagnosis of CNIT to identify molecular signatures related to this process. This might lend itself to error in light of the nonspecific nature of histology attributable to CNIT in general. The study by Vitalone compared 12-month surveillance biopsies between patients on de novo CNI ($n = 17$) vs. belatacept ($n = 18$). Our study design was different in that we used individual patients as their own controls rather than a case-controlled design. It is also possible that a larger and more homogenous sample could have revealed more meaningful differences.

Unfortunately, we did not see an improvement in gene signatures related to fibrogenesis. Similarly IFTA scores trended toward worsening on follow-up biopsy. Many factors contribute to the progression of atrophy–fibrosis such as ischemia–reperfusion injury, aging, and somatic cell senescence due to donor age. Previous studies have suggested that parenchymal wounding, regardless of time after transplant, triggers a prolonged time-dependent response-to-wounding program in the affected nephrons [37]. Thus, evolution to atrophy–fibrosis in these settings may not represent ongoing injury but rather a response to early wounding. In the absence of serial surveillance biopsies from patients on CNI, we were unable to suggest whether atrophy–fibrosis on CNI and belatacept patients would have continued to progress or not. Atrophy–fibrosis in these patients is may not be due to CNI toxicity but rather to other injuries and thus will evolve on belatacept according to the natural history of the response to wounding. In summary, it could be hypothesized that clinical "CNIT" is more likely due to a complex interplay of donor factors, concentration–effect, pharmacogenetics, pharmacokinetics, simultaneous exposure to other nephrotoxins, and peri-operative and postoperative hemodynamic insults that could worsen in the setting of CNI-induced vasoconstriction [27]

Our report does have some limitations. This was a single-center single-arm study with no comparator arm. The consideration of switching to belatacept was based upon clinician judgment rather than standardized histologic criteria that could have led to some bias. Although our sample size was small, paired transcriptome analysis on a subset of kidney biopsies did not reveal any changes in transcripts related to fibrosis. Further studies with recruitment of additional patients are needed to confirm these findings.

Authorship

Gaurav Gupta and Dhiren Kumar: involved in research design, writing of paper, performance of research and data analysis. Marc Raynaud: involved in writing of paper, performance of research, analytic tools and data analysis. Jessica Chang: involved in performance of research, analytic tools and data analysis. Pamela Kimball: involved in performance of research and data analysis. Le Kang: involved in writing of paper and analytic tools. Marlon Levy, Amit Sharma, Chandra Bhati, H. Davis Massey and Chelsea Kidd: involved in writing of paper and performance of research. Layla Kamal: involved in research design and writing of paper. Idris Yakubu: involved in research design, writing of paper and data analysis. Anne L. King and Philip Halloran: involved in research design, writing of paper and performance of research.

Funding

The authors have declared no funding.

Conflict of interest

G Gupta has given lectures for Thermo Fisher; has received honoraria from Alexion, CareDx, Mallinckrodt, Relypsa; and has received research funding from Gilead. P F Halloran holds shares in Transcriptome Sciences Inc., a University of Alberta research company with an interest in molecular diagnostics; has given lectures for Thermo Fisher; and is a consultant for CSL Behring. The other authors have no disclosures to declare.

Acknowledgements

We thank our Research Nurse Coordinator Ms. Mary Baldecchi for help with processing samples for molecular analysis.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Histogram analyses for propensity matching.

Table S1. Intra-graft differential gene expression.

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