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Avoidance of CNI and Steroids Using Belatacept – Results of the Clinical Trials in Organ Transplantation 16 Trial

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Abstract

Immunosuppression devoid of corticosteroids has been investigated to avoid long term comorbidities. Likewise, alternatives to calcineurin inhibitors have been investigated as a strategy to improve long-term kidney function following transplanion. Costimulatory blockade strategies that include corticosteroids have recently shown promise, despite their higher rates of early acute rejection. We designed a randomized clinical trial utilizing depletional induction therapy to mitigate early rejection risk while limiting calcineurin inhibitors and corticosteroids. This trial, CTOT-16, sought to evaluate novel belatacept-based strategies employing tacrolimus and corticosteroid avoidance. 69 kidney transplant recipients were randomized from 4 US transplant centers comparing a control group of with rabbit antithymocyte globulin (rATG) induction, rapid steroid taper, and maintenance mycophenolate and tacrolimus, to two arms using maintenance belatacept. There were no graft losses but there were 2 deaths in the control group. However, the trial was halted early due to rejection in the belatacept treatment groups. Serious adverse events were similar across groups. While rejection was not uniform in the belatacept maintenance therapy groups, the frequency of rejection limits the practical implementation of this strategy to avoid both calcineurin inhibitors and corticosteroids at this time.

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Supporting Information

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

Introduction

The excellent short-term outcomes of kidney transplant recipients in the current era are widely attributed to the introduction of new immunosuppressive agents such as calcineurin inhibitors (CNI) and safer, more efficacious immunosuppressive regimens. Current immunosuppressive management consists of T cell-depleting induction agents in approximately 70% of recipients, maintenance tacrolimus in combination with MMF in 93% of recipients, along with corticosteroids in 70% of recipients[1]. While the frequent use of tacrolimus and steroids speaks to their recognized efficacy in kidney transplantation, these agents are well-recognized contributing factors in the relatively stagnant and disappointing long-term outcomes in part due to cardiovascular disease and metabolic disorders, particularly diabetes[2]. Further, CNI may contribute to renal dysfunction and even kidney failure following both kidney and extrarenal organ transplantation[3, 4]. The adverse effects of CNI and corticosteroids have prompted investigators to explore alternative strategies to the long-term use of these agents in kidney transplantation, including: avoidance of one or both agents from the outset; seeking to reduce and minimize exposure over the long-term; and substituting other agents for CNI and/or corticosteroids.

While T-cell depletional induction has facilitated steroid avoidance, reducing cardiovascular and metabolic risks[5], these approaches have not fully succeeded due to rejection when CNI are avoided[6] or withdrawn[7, 8]. Further, a co-stimulatory receptor blockade strategy without depletional induction has demonstrated improved renal function as well as improved cardiovascular and metabolic risk profiles with belatacept treatment compared to cyclosporine[9–12] even out to seven years[10], but at the risk of early acute rejections.

Thus, we hypothesized that belatacept, when combined with effective induction therapy, would allow the long-term avoidance of both CNI and corticosteroids and tested this hypothesis in the CTOT-10 study[13]. In this study, the control arm was based on a large study demonstrating the feasibility of corticosteroid avoidance combined with alemtuzumab induction and tacrolimus and MMF maintenance therapy[5]. Due to an unacceptable rate of allograft thrombosis in the experimental/belatacept arms, enrollment was prematurely halted. Here, we present the safety and efficacy of the redesigned trial CTOT-16, where rATG replaced alemtuzumab induction. Although no allograft thrombosis or other thromboembolic events were noted in CTOT-16, the study was halted by the investigators at the recommendation of the Data Safety Monitoring Board due to an increased risk of acute cellular rejection in the experimental arms of the study.

Materials and Methods

Study Design and Interventions

Due to thrombotic events involving the renal allograft and increased rejection rates in the study arms, CTOT-10 was halted[13]. To address the dual concerns of thrombotic events and increased risks of ACR, the study was modified by substituting (rATG) for alemtuzumab and extending the duration of early “induction” with tacrolimus from three to five months. The Clinical Trials in Organ Transplant-16 (“Optimization of NULOJIX® (Belatacept) Usage As A Means of Avoiding Calcineurin Inhibitor (CNI) and Steroids in Renal Transplantation”;

NCT01856257; IND 111,783) was a one-year, open label, randomized prospective trial conducted at 4 transplant centers in the United States; one site did not enroll subjects prior to study closure (additional details in supplement).

The overall study schema is shown in Figure 1A and the immunosuppressive regimens are shown in Figure 1B. In group 1, recipients were induced with rATG and following a rapid methylprednisolone taper maintained on tacrolimus and mycophenolate mofetil. Group 2 received rATG induction, a rapid methylprednisolone taper and maintenance immunosuppression consisting of belatacept and mycophenolate mofetil. In an attempt to avoid depleting induction therapy and its potential adverse consequences, group 3 utilized the non-depleting agent basiliximab with a standard dosing regimen, a rapid methylprednisolone taper, a 5 month course of tacrolimus, and maintenance therapy consisting of belatacept and mycophenolate. Specific dosing and timing of therapy are shown in Table 1 and patients had close clinical monitoring during the withdrawal. Key inclusion and exclusion for study subject enrollment are shown in table 2.

Enrollment began on August 9, 2013, and primary outcomes were pre-specified to be analyzed at 12 months post-transplant. The trial protocol and any amendments were reviewed and approved by a NIAID-sponsored DSMB and the Institutional Review Board (IRB) of each site prior to study initiation or the implementation of subsequent protocol changes. The trial conduct was consistent with International Conference on Harmonization Good Clinical Practice (ICH GCP) and other applicable regulatory requirements. Due to a low rate of enrollment, randomization to group 3 was terminated in August 2014, with the aim of completing the study by maximizing enrollment in groups 1 and 2. The study was later halted on 04/15/2015 by the investigators at the recommendation of the Data Safety Monitoring Board due to an increased risk of acute cellular rejection in the experimental arms of the study. Subjects were informed of these results, and were converted to standard of care regimens with guidance by the investigators.

Investigational therapy and dosing

The investigational drug used in this trial was Nulojix® (belatacept) (NDC 0003–0371-13). Nulojix® is manufactured by Bristol-Myers Squibb (BMS, Princeton, NJ) and was supplied as 250 mg intravenous infusion-only vials (BMS Lot numbers: 2H62012, 3E76307, 4A83805, 4A82606, 4B79981, 4D82552 and 4J82939). The total infusion dose of Nulojix® was based on actual body weight at the time of transplantation, and not modified over the course of the study unless there was a change in body weight greater than 10%. Dosing details are in Table 1.

Outcome Measures

The hypothesis of this trial was that steroid-free belatacept-based regimens using induction with either rATG or basiliximab and tacrolimus would achieve superior renal function with comparable rates of rejection compared to a tacrolimus-based maintenance regimen. The primary outcome measure was the mean glomerular filtration rate (GFR) calculated for each treatment group using the Chronic Kidney Disease Epidemiology Collaboration Equation (CKD-EPI) at 52 weeks post-transplantation[14]. The study was designed to achieve 90%

power to detect a difference of at least 11.4 mL/min/1.73m^2 between group 2 (or 3) and group 1. Secondary outcome measures included histologic evidence of acute rejection with graft dysfunction in the first 24 weeks of treatment, cardiovascular and metabolic parameters such as new onset diabetes or impaired fasting glucose at 52 weeks, the incidence of treated diabetes, hemoglobin A1C measurements, and fasting lipid profiles (Supplementary Table 1). Biomarker assessments were performed at intervals and at the time of allograft biopsy (supplementary methods).

Safety Outcome Measures

An independent DSMB assessed cumulative safety data throughout the trial and provided NIAID with recommendations about study continuation. Safety events of interest included the incidence of death or graft loss, the incidence of thromboembolic events, the incidence of rejection, and the incidence of all adverse events and serious adverse events (SAEs). Moreover, we focused on any infections requiring hospitalization or systemic therapy, and specifically performed local surveillance for BK and CMV and EBV viremia.

Histopathological Assessment of Allograft Biopsies

All biopsies were analyzed at each transplant center utilizing Banff 2013 guidelines, the most current at study initiation[15]. Tissue blocks or stained slides were available to a central pathologist (ABF) in 71 of 76 biopsies obtained for allograft dysfunction. Correlation of local and central findings is shown in supplementary Table 2 demonstrating moderate agreement. Results herein are based on local pathology readings.

Statistical Analysis

All statistical analyses were performed using SAS software (SAS Institute, Cary, NC) and GraphPad Prism (GraphPad Software, San Diego, CA). See supplemental methods for full details.

Results

Sixty nine patients were enrolled in the trial using the study scheme shown (Figure 1). Of these, 29 were randomized to each of the first two groups while only 11 were enrolled in group 3 due to its early termination. The demographic characteristics of the three groups were similar (Table 3). Nearly half of enrolled subjects were African American, reflecting the participating study transplant centers' demographics. Despite the comparable mix of living and deceased donors, the rate of delayed graft function was higher in group 2 (31%) compared to groups 1 (21%) and group 3 (0%, p=0.04). There were 2 deaths in the control group 1: at day 285 due to toxoplasmosis and on day 161 due to hypertensive crisis and possible atypical hemolytic-uremic syndrome. There were no additional graft losses, no episodes of PTLD, and no episodes of allograft thrombosis or other thromboembolic events.

Analysis of renal function: primary endpoint

The primary endpoint of this study, eGFR at 52 weeks, was nearly identical in each of the three groups (Figure 2A), in spite of the increased incidence of rejection (see below) in the belatacept treated subjects. The estimated treatment group difference at W52 for group 2 vs.

group 1 was 3.5 mL/min/1.73m^2 with 95% CI of (−8.0, 15.0), p=0.54, and for group 3 vs. group 1 was 4.8 mL/min/1.73m^2 with 95% CI of (−10.5, 20.1), p=0.53. Although the change in slope of the eGFR was difficult to assess due to the occurrence and severity of rejection episodes in groups 2 and 3 (Figure 2B), on average there was a positive slope each month of 0.3, 1.3 and 0.8 in groups 1, 2 and 3 respectively (Figure 2A).

Cellular rejection was common in belatacept treated groups but de novo DSA not common

Biopsy proven rejection, defined as T-cell mediated rejection (TCMR) Grade IA or higher, was uncommon in group 1 (3%), compared to the belatacept treated recipients in groups 2 and 3 (34.5% and 36% respectively) (Table 4). Acute rejection was seen earliest in group 2, and throughout the course of the first 6 months (Figure 3). In contrast, most group 3 rejections were detected later in the post-transplant period, and were often coincident with the weaning of tacrolimus. Supplementary Figure 1 presents tacrolimus trough levels (ng/mL) plotted over time for groups 1 and 3; mean values were within the targeted range for both arms. However, in spite of these episodes, eGFR at early time points was not significantly affected between groups (Figure 2B).

Rejection episodes were treated according to each center's standard of care; rATG was used in all Banff grade IIA and III rejections as well as in 3 Banff IA and IB rejections. The protocol allowed investigators to discontinue belatacept based on biopsy results, which occurred in 3 subjects (one grade IIA and both III). At the time of the 4/15/2015 DSMB memo regarding acute rejection, 18 subjects in group 2 and 3 in group 3 were still receiving belatacept. However, 19/21 (90%) continued to receive belatacept using local hospital based supply as their standard of care.

Banff borderline rejection (BR) was detected in all groups but primarily in groups 1 and 2, and treated in 10 of 19 episodes with pulse corticosteroids and continuation of maintenance immunosuppressive therapy. One subject (group 1) had suspected AMR on biopsy without detectable de novo anti HLA donor specific antibody (dnDSA) at day 18 treated with plasmapharesis with eventual resolution. *De novo* DSA was detected in 4 subjects: 2 subjects in group 1 with class II DSA, (day 250 and day 365) and 2 subjects in group 2 developed both class I and II DSA (day 293 and day 357).

Biomakers of rejection were performed, analyzing urinary CXCL9 and CXCL10 in all subjects (see supplemental methods) as described in prior CTOT studies[7, 16]. Of 313 urine samples from 69 subjects available for analysis, the PPV was 50% (95% CI, 29.1% to 70.9%) and NPV 93.2% (95% CI, 81.3% to 98.6%), consistent with previous reports[7, 16]. Furthermore, 47 available allograft biopsies obtained at the time of allograft dysfunction were analyzed by tCRM gene expression score[17] (see supplementary methods). tCRM scores were significantly elevated in acute rejection (AR; 9.73±6.66) biopsies compared to no rejection (NR; 2.43±3.51; p=0.003), and with a trend toward increased elevation compared to borderline rejections (BR; 3.42±2.78; p=0.078) biopsies regardless of study group (Supplemental Figure 2). While the combined belatacept treatment groups had a higher percentage of subjects with positive tCRM results (65% vs. 25% in group 1; p=0.006), tCRM values and individual transcripts did not distinguish belatacept-associated

rejection from that associated with tacrolimus. Thus, biomarkers of immune injury operated similarly regardless of the use of belatacept therapy.

Analysis of cardiovascular and metabolic parameters and safety measures

While new onset diabetes mellitus after transplant (NODAT) was infrequent in all groups (n=2) by week 52, fasting hyperglycemia and treated diabetes was more common in group 1 (n=5) compared to groups 2 (n=2) and 3 (n=0). Similarly, hemoglobin A1C was higher at one year in group 1 (7.8±3.3) compared to group 2 (6.7±1.6; p=0.356) and group 3 (5.8±1.3; p=0.095). Systolic and diastolic blood pressures were similar across groups with most patients on anti-hypertensive treatments (group 1 62%, group 2 79% and group 3 73%) at one year. There were no significant differences in fasting total cholesterol or LDL levels between the groups at one year with 31–45% of individuals on lipid lowering agents.

Post-treatment adverse events occurred frequently across all treatment groups (Table 5) with 72% of subjects in group 1, 93% in group 2 (p=0.037 vs. group 1), and 82% in group 3 (p=ns), reflective of the complex nature of kidney transplantation. However, adverse events grade 3 or higher were more common in group 2 (76%) compared to groups 1 (59%, p=ns) and 3 (36%, p=0.029). Although not statistically significant, CMV viremia was more common in group 2 (21%) compared to group 1 (3%) and group 3 (9%) while BK viral infections were infrequent. Serious infections occurred in groups 1 and 2; however study therapy was not discontinued, and there was a single group 1 fatal infection (toxoplasmosis).

Discussion

Consistent with the results of CTOT-10[13], we found that CNI avoidance using belatacept resulted in higher rejection rates despite the use of depleting induction with rATG. The rates of rejection observed were high enough to halt study enrollment and would preclude adoption of this approach in clinical transplantation. Despite undesirable unacceptable rejection rates, we did not find a difference in renal function at one year, nor a preponderance of viral or other opportunistic infections, disproportionate to the belatacept treated groups. Moreover, hyperglycemia or overt diabetes though infrequent, were less common in the setting of belatacept therapy. Recent studies suggest that patient reported outcomes at one year[18] and improved quality of life are significantly better with belatacept treatment.[19] These results are encouraging, and again, suggest that specific patients at high risk for metabolic consequences following transplantation might benefit.

Remarkably, despite the higher frequency of acute cellular rejection, a known risk factor for the development of DSA[20], dnDSA were infrequent, as has been reported in long term follow-up of belatacept-treated patients[10]. This is in spite of reduced tacrolimus, a known risk factor for dnDSA development[21]. Belatacept intrinsically fosters immunosuppression adherence, due to closely monitored, regular infusion visits. Other mechanistic work has attributed this effect to costimulatory blockade-induced effects on T follicular helper cell function and a reduction in clonal expansion of B cells[22] although this remains uncertain.[23]

Early acute rejection with this trial’s belatacept strategies was clinically unexpected, based on pre-clinical data that were quite encouraging. Further understanding the immunologic

mechanisms predisposing to rejection would provide insight into selecting therapies and assessing risk for transplant candidates. Molecular profiling using tCRM did not reveal any differences between groups with both Banff borderline grade IA rejections that were grade IA and higher behaving similarly in terms of magnitude of response. While speculated that belatacept-associated rejections involve novel upregulation of innate immune molecules, a recent small study noted no differences in implicated alloreactive pathways and innate immune activation among tacrolimus and belatacept treated recipients with allograft rejection[24]. In previous studies during conventional tacrolimus treatment[16] or withdrawal[7], the urinary chemokines CXCL9 and CXCL10 assisted in detecting inflammatory injury. However, detection of these chemokines do not uniquely identify rejection events, as these tests were unable to distinguish urogenital tract BK infection[25], and the detection during bacterial urinary tract infections has further limited clinical implementation. Further, the assay threshold is based on urine concentration that varies considerably based on recipient volume status and time of day[26]. Our results demonstrate concordance with acute rejection episodes, but infrequent sampling strategies continue to limit these tests as prediction markers. Enhanced sensitivity and rapid turnaround time may assist in developing this assay for clinical use in the future as it also correlates with subclinical rejection[25].

Two recent studies have examined steroid and CNI avoidance using belatacept and our results differ primarily due to a significantly higher frequency of rejection in the belatacept treated group. In an open label exploratory study of 89 patients, comparing steroid and CNI avoidance, with the CNI-fee arm utilizing a combination of belatacept and mTORi had comparable rates of acute rejection (4%) to conventional steroid avoidance treatment[27]. Rejection rates in all arms were quite low, attributed in part to induction with rATG as well as the use of mTORi in one arm. In the Belatacept-based Early Steroid Withdrawal Trial (BEST), 316 KTRs from 8 US Transplant centers were prospectively randomized to a standard of care regimen including rATG induction with maintenance CNI or two belatacept-based regimens that utilized either alemtuzumab or rATG to avoid both CNI and corticosteroids[28]. With a combined primary endpoint of patient death, allograft failure, or eGFR < 45 mL/min/1.73m^2 at one year, the study failed to show superiority in the belatacept arms and notably, biopsy proven acute rejections (cellular and antibody) were more significantly more common in both belatacept treatment groups (15.9 and 22%), regardless of induction therapy compared to the low rate in the control group (4.8%, p=0.024 and p<0001 respectively). Our findings are consistent with the results of the BEST Study albeit with higher baseline rates of rejection in all groups. This may be related to the increased frequency of factors commonly associated with increased immunologic risk such as African American race, deceased donor transplants and higher PRAs in CTOT-16. Both studies also showed that despite increased rates of cellular rejection renal function was preserved and development of DSA was infrequent in the groups treated with belatacept.

There are obvious limitations to this trial. While we intended a robust, randomized controlled clinical trial, the slow pace of deceased donor enrollment, coupled with belatacept-associated rejection events, led to study termination prior to target enrollment. In spite of these limitations, there are important lessons for future transplantation studies. The control group had a high frequency of adverse events, as expected in transplantation recipients with several years of accumulated morbidity on pre-transplant dialysis. This

demonstrates the complexity of trial design and the high risk nature of kidney transplantation that has been considered a barrier to new drug development[29]. However, the vast majority of our trial's adverse events were not sufficiently serious to lead to discontinuation from the study. Further, while the mean eGFR at week 52 in groups 2 and 3 was similar to the estimates used in our original power calculations, the group 1 estimated eGFR was about 10 mL/min/m^2 higher than originally anticipated. Therefore, had we observed these same differences between the two belatacept groups and the control group in the number of subjects we originally intended to investigate, the difference would have been non-significant. Another positive impact was that nearly half of recipients enrolled in the trial were African American, reflective of the transplant centers participating in this study. However, in many clinical trials, African Americans frequently account for less than 5% of enrolled subjects[30]. Study populations would benefit from genetic diversity to ensure that drug response (or failure) is representative of the disease population in the development of novel immunosuppressive therapies. Regardless, the broad clinical adoption of de novo belatacept as a means of avoiding CNI in kidney transplantation remains infrequent despite the perceived advantages in renal function and the low frequency of DSA.[31] Better defining *a priori* who will be unlikely to reject will assist in translating such approaches into clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure

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Abbreviations

(BEST)	Belatacept-based Early Steroid Withdrawal Trail
(BR)	Borderline rejection
(CNI)	Calcineurin inhibitors
(CKD-EPI)	Chronic Kidney Disease Epidemiology Collaboration Equation
(CTOT-16)	Clinical Trials in Organ Transplantation 16

(dnDSA)	*De novo* anti HLA donor specific antibody
(GFR)	Glomerular filtration rate
(IRB)	Institutional Review Board
(ICH GCP)	International Conference on Harmonization Good Clinical Practice
(NODAT)	New onset diabetes mellitus after transplant
(rATG)	Rabbit antithymocyte globulin
(SAEs)	Serious adverse events
(TCMR)	T-cell mediated rejection

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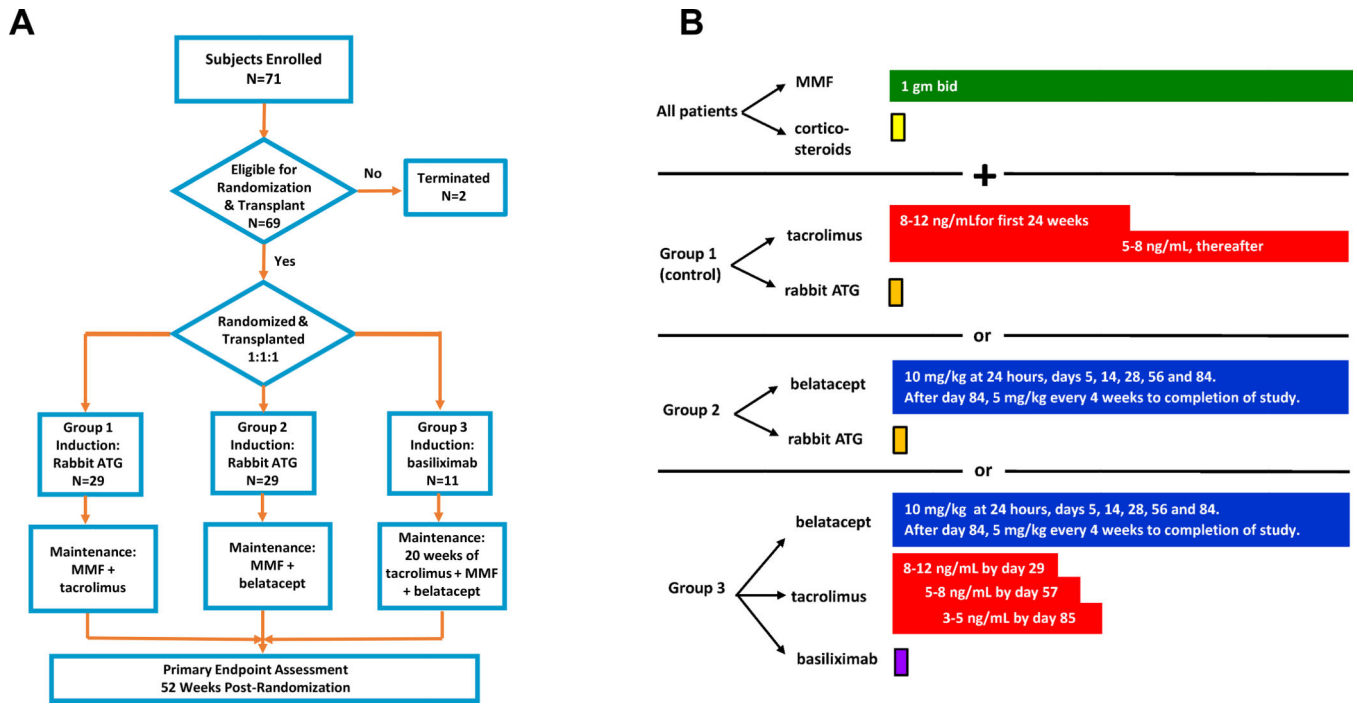

Figure 1:

Study design and therapy. Consort diagram of study design and enrollment (A). Assignments were made randomly to one of the groups (B), all of which received mycophenolate and rapid corticosteroid taper.

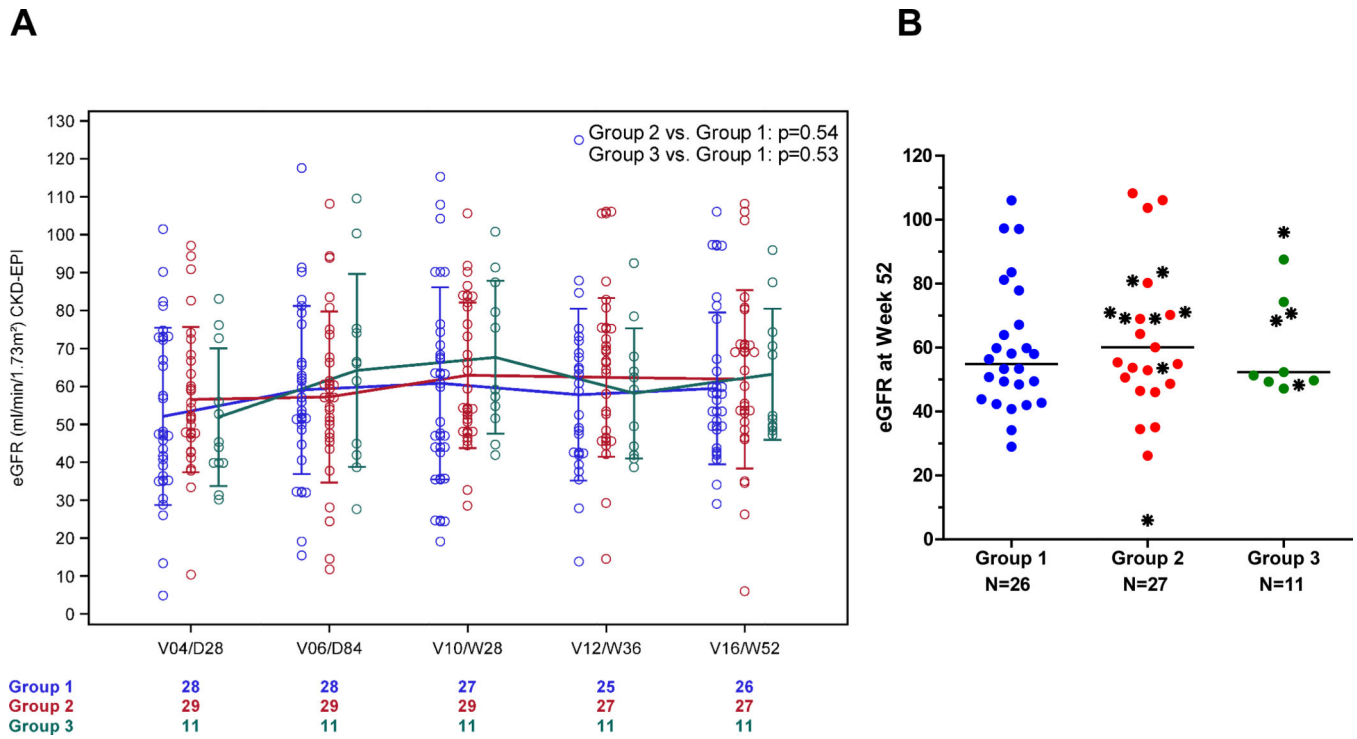

Figure 2.

Renal function during the first year post-transplant. A. Mean values (with standard deviation bars) of estimated glomerular filtration rate (eGFR) using the CKD-EPI equation are plotted over time for groups 1 (blue), 2 (red), and 3 (green). Individual eGFR values are plotted as circles within each group. A repeated measures linear mixed model yielded an estimated mean of 58.4 mL/min/1.73m^2 for group 1, 61.9 mL/min/1.73m^2 for group 2, and 63.2 mL/min/1.73m^2 for group 3 at week 52. The model resulted in an estimated treatment group difference at 52 weeks for group 2 vs. group 1 of 3.5 mL/min/1.73m^2 with 95% CI of (−8.0, 15.0), p=0.54, and for group 3 vs. group 1 of 4.8 mL/min/1.73m^2 with 95% CI of (−10.5, 20.1), p=0.53. B. eGFR at 52 weeks in each treatment group. The asterisks (*) indicate which subjects had rejection during the first year of enrollment.

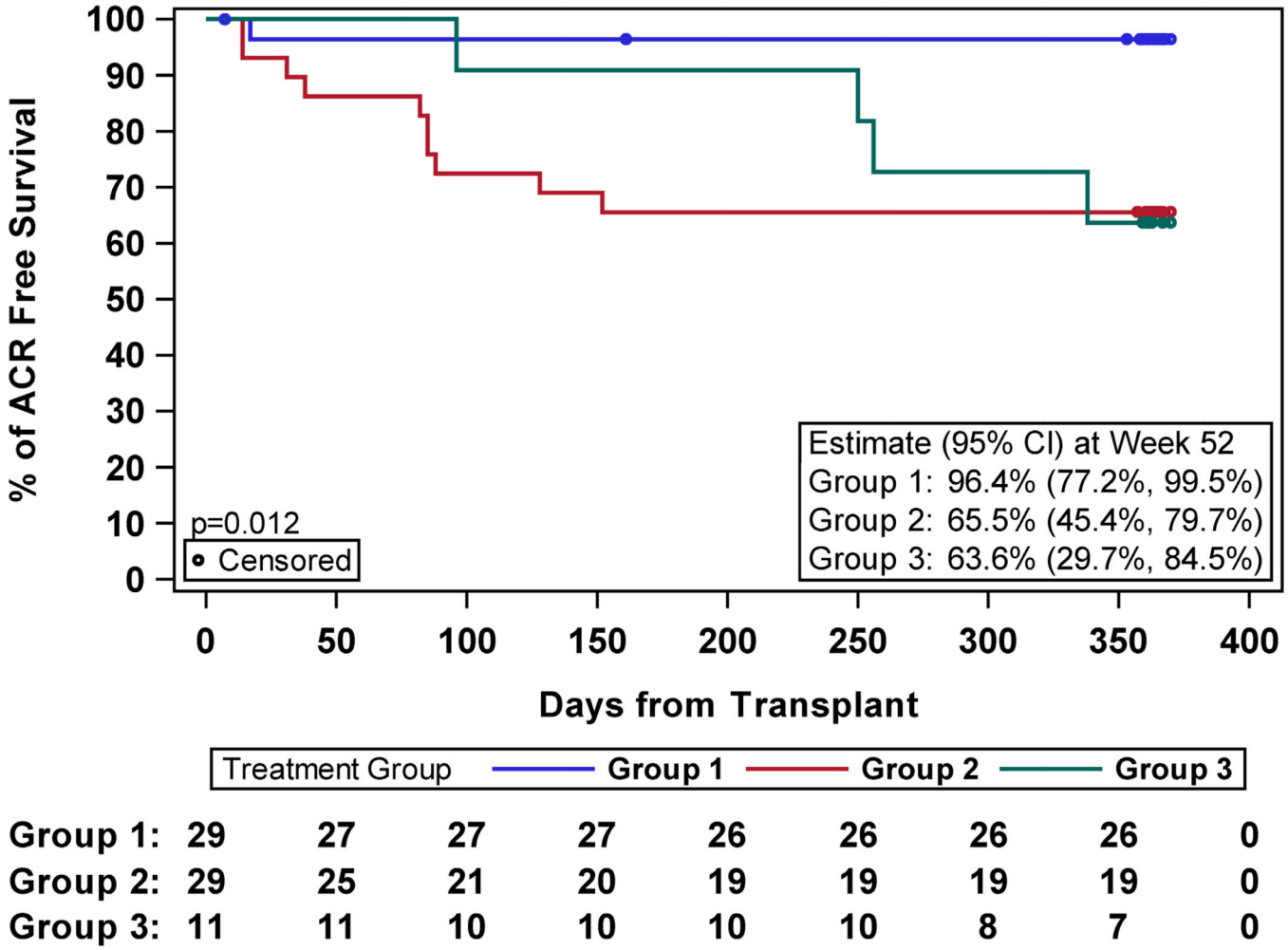

Figure 3.

Freedom from biopsy-proven acute cellular rejection (≥ Banff grade IA rejection based on local read) by treatment group. Number of subjects at risk is presented at selected days post-transplant. Subjects are censored at day of last follow-up or death. Follow-up truncated at day 370 for illustration purposes. CI=confidence interval.

Table 1.

CTOT 16 therapy and timing of administration

Immunosuppression Regimen	Dose and Timing	Treatment Group
Thymoglobulin® Rabbit antithymocyte Globulin	Target dosage is 6mg/kg total over 3 to 4 days. The recommended route of administration is intravenous via a high flow vein. Thymoglobulin should be infused over a minimum of 6 hours for the first infusion and over at least 4 hours for subsequent infusion. The infusion will be monitored per center practice. Premedication with corticosteroids, acetaminophen and/or anti-histamine should occur 1 hour prior to the infusion per center standard operating procedure.	Groups 1 and 2
Simulect® (basiliximab)	The first 20 mg dose administered within 2 hours prior to transplantation surgery. The second 20 mg dose given 4 days after transplantation.	Group 3
Nulojix® (belatacept)	NULOJIX® (belatacept) administered at 10mg/kg beginning 24 hours (+/− 12 hours) from the time of reperfusion, and then at days 5, 14, 28, 56 and 84. There must be at least 4 hours in between the completion of the Thymoglobulin infusion and start of the belatacept infusion on Day 1. After 84 days, subjects will receive belatacept at the maintenance dose of 5 mg/kg every 4 weeks until completion of the trial	Group 2 and 3
Prograf® (tacrolimus) or generic	Tacrolimus administered at a dose of 0.1mg/kg PO BID beginning on the day of surgery or post-operative day 1 depending upon when during the day the surgery is completed, then adjusted to target trough levels of 8–12 ng/ml during the first 24 weeks post-transplant, then adjusted to target trough levels of 5–8 ng/ml thereafter.	Group 1
Prograf® (tacrolimus) or generic	Tacrolimus administered at a dose of 0.1mg/kg PO BID, beginning on the day of surgery or post-operative day 1 depending upon when during the day the surgery is completed. The dosage will be adjusted to achieve the following therapeutic trough levels: 8–12 ng/ml by day 29, 5–8 ng/ml by day 57, 3–5 ng/ml by day 85 and then stopped	Group 3
CellCept® (mycophenolate mofetil), Myfortic®, or generic	CellCept® (Mycophenolate Mofetil- MMF) administered at a target dose of 1000 mg PO BID beginning on the day of surgery or post-operative day 1 (max. MMF dosing 2G per day). MMF adjusted based on clinical complications. After 1 year, dosing may be modified at the discretion of the site investigator. Myfortic® (mycophenolate sodium) may be used as a replacement for MMF, dosed at 720 mg PO BID.	All treatment groups
Medrol® (methylprednisolone)	Methylprednisolone administered at a target dose of 500 mg beginning on the day of transplant, and tapered to 250 mg day 1 post-transplant, 125 mg day 2 post-transplant, 60 mg day 3 post-transplant, 30 mg day 4 post-transplant and day 5 post-transplant 0 mg if therapeutic tacrolimus level achieved.	All treatment groups

Table 2.

Inclusion and Exclusion Criteria for CTOT 16 Trial

Inclusion Criteria	Exclusion Criteria
• Male or Female, 18–65 years of age at the time of enrollment; • Ability to understand and provide written informed consent; • Candidate for primary renal allograft from either a living or deceased-donor; • No known contraindications to study therapy using NULOJIX® (belatacept); • Female participants of childbearing potential must have a negative pregnancy test upon entry; • Female and male participants with reproductive potential must agree to use FDA approved methods of birth control during participation in the study and for 4 months following completion of the study; • Negative crossmatch or a PRA of 0% on historic and admission sera as determined by each participating study center. • A documented negative TB test within the 12 months prior to transplant. If documentation is not present at the time of transplantation, and the subject does not have any risk factors for TB, a TB-specific interferon gamma release assay (IGRA) may be performed. • Transplanted kidney from an ABO compatible donor.	• Need for multi-organ transplant; • Recipient of previous organ transplant; • EBV sero-negative (or unknown) recipients; • Active infection including hepatitis B, hepatitis C, or HIV; • Individuals who have required treatment with prednisone or other immunosuppressive drugs within 1 year prior to transplant; • Individuals undergoing transplant using organs from KDPI > 85% donors; • HLA identical living donors; • Individuals at significant risk of early recurrence of the primary renal disease including FSGS and MPGN type 2 or any other disease that in the opinion of the investigator is at increased likelihood of recurrence and which may result in rapid decline in renal function; • Known history of thrombotic events or risk factors; including any of the following: 1. Factor V Leiden, elevated homocysteine, positive lupus anticoagulant, elevated anticardiolipin antibody, heparin induced thrombocytopenia 2. A family history of a heritable thrombotic condition, 3. Recurrent DVT or PE, 4. Unexplained stillborn infant or recurrent spontaneous abortion or other congenital or acquired thrombotic disorder. 5. At the discretion of the investigator, a history of thrombosis of a dialysis access graft, fistula, or indwelling catheter/device may not be considered an exclusion criterion. • Any condition that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements; • Use of investigational drugs within 4 weeks of enrollment; • Known hypersensitivity to mycophenolate mofetil (MMF)or any of the drug's components; • Administration of live attenuated vaccine(s) within 8 weeks of enrollment. • Blood type A2 and A2B donors into blood type B recipients.

Table 3.

Demographics of CTOT 16 population

Characteristics	Group 1 (N=29)	Group 2 (N=29)	Group 3 (N=11)
Deceased Donor – n (%)	14 (48.3)	15 (51.7)	6 (54.5)
Delayed Graft Function – n (%)	6 (20.7)	9 (31.0)	0
Median Age (range)	47 (34 – 62)	46 (20 – 61)	44 (23 – 59)
Male – n (%)	20 (69.0)	21 (72.4)	7 (63.6)
Race – n (%) White Black Asian Other	 9 (31.0) 16 (55.2) 1 (3.4) 3 (10.3)	 11 (37.9) 13 (44.8) 3 (10.3) 2 (6.9)	 4 (36.4) 5 (45.5) 0 2 (18.2)
Dialysis Dependent – n (%)	26 (89.7)	23 (79.3)	7 (63.6)
Median time on dialysis in months (range)	26 (1 – 180)	60 (2 – 189)	50 (15 – 120)
Recipient Current cPRA (%) Median (range) >0% – n (%)	n=23 0 (0 – 99) 9 (39.1)	n=22 0 (0 – 26) 5 (22.7)	n=6 11 (0 – 82) 3 (50.0)
HLA mismatches (ABDR), median (range)	4 (0 – 6)	5 (2 – 6)	5 (2 – 6)
CMV status – n (%) D+/R+ D+/R− D−/R+ D−/R−	 6 (20.7) 5 (17.2) 4 (13.8) 4 (13.8)	 10 (34.5) 1 (3.4) 5 (17.2) 2 (6.9)	 2 (18.2) 1 (9.1) 0 1 (9.1)
rATG Total Dose – n (%) 3–6 mg/kg 6 mg/kg	 5 (17.2) 24 (82.8)	 4 (13.8) 25 (86.2)	 n/a

Table 4.

Acute cellular rejection episodes in treatment groups

Group	Banff Rejection Grade # Subjects, # Episodes						Subjects with ACR n (%)	Days Post-Transplant to ACR (Mean ± SD)	Treated Rejection # Subjects, # Episodes
	Borderline	IA	IB	IIA	IIB	III			
Group 1 (N=29)	6, 9	1, 1	1, 1	0, 0	0, 0	0, 0	1 (3.4)	17	7, 11
Group 2 (N=29)	8, 9	4, 5	1, 2	4, 4	0, 0	2, 2	10 (34.5)	72 ± 47	14, 20
Group 3 (N=11)	1, 1	2, 2	2, 2	0, 0	0, 0	0, 0	4 (36.4)	235 ± 101	4, 5

Table 5.

Summary of common post-treatment adverse events grade 2 or higher

Adverse Event – n (%)	**Group 1 (N=29)**	**Group 2 (N=29)**	**Group 3 (N=11)**
Total # of AEs	**118**	**124**	**32**
# of Subjects with an AE	21 (72.4)	27 (93.1)	9 (81.8)
Anemia	7 (24.1)	4 (13.8)	3 (27.3)
Leukopenia or Decrease in WBC	8 (27.6)	6 (20.7)	2 (18.2)
Gastrointestinal Complaints	5 (17.2)	6 (20.7)	3 (27.3)
Infections			
Any AE for Infection	14 (48.3)	16 (55.2)	3 (27.3)
CMV Viremia	1 (3.5)	6 (20.7)	1 (9.1)
CMV Disease	1 (3.5)	0	0
BK Viremia	0	1 (3.4)	1 (9.1)
BK Nephropathy	0	3 (10.3)	0
UTI	6 (20.7)	9 (31.0)	1 (9.1)
Other noteworthy infections n, type	1 Toxoplasmosis 1 Clostridium difficile colitis 1 wound infection 1 bacterial peritonitis	1 Mucormycosis 1 Clostridium difficile colitis 1 bacteremia	0