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Donor-specific Mesenchymal Stem Cell Infusion in Human and Non-human Primate Kidney Transplantation

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Abstract

We report the results of two independent, concurrently performed studies evaluating the safety and efficacy of donor-derived mesenchymal stromal cell (MSC) infusions in inducing immune-tolerance in nonhuman primate (NHP) and human kidney transplant recipients treated with depletion induction and belatacept/sirolimus maintenance. Fifteen NHPs received rhesus ATG induction and were divided in three groups: control (n=6), pre-transplant thymic irradiation (TI, n=4), and TI with monthly donor-MSC infusion (n=5). Sirolimus was discontinued at day-180, and belatacept at day-365 post-transplantation. In humans, six patients enrolled in ITN062ST underwent transplantation with alemtuzumab induction; four received 12 monthly donor-MSC infusions followed by immunosuppression withdrawal (ISW) if eligible. Donor-MSC infusion was acutely well-tolerated in humans and NHPs. Chimerism was not established, and tolerance was not induced in either study. Two of five NHPs that received MSCs rejected while on belatacept

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Trial Registration. [ClinicalTrials.gov](https://clinicaltrials.gov) - NCT03504241

monotherapy with detectable donor-specific antibody (DSA). Two patients did not initiate ISW due to de novo DSA and borderline rejection, and two patients failed ISW due to reversible rejection. In conclusion, donor-MSCs can be given to NHPs or humans repeatedly without acute consequences, but they neither lead to detectable chimerism nor induce tolerance. In a subset of recipients, infused MSCs can be sensitizing.

Keywords

kidney transplantation; costimulation blockade; T cell depletion; mesenchymal stromal cells

1. Introduction

Kidney transplantation is the standard of care treatment for patients with end-stage renal disease, and compared to dialysis, it is associated with decreased morbidity^{1,2}, improved survival³, and improved quality of life^{4,5} while being more cost effective⁶. However, kidney transplant recipients must remain on life-long immunosuppression to prevent allograft rejection (AR), often with deleterious long-term consequences. Calcineurin inhibitors (CNIs; e.g., tacrolimus) are the backbone of immunosuppressive regimens, with more than 90% of North American recipients on a CNI-containing regimen⁷ and thus being exposed to well-documented CNI-related morbidities including nephrotoxicity,⁸ cardiovascular events⁹, infections, and malignancies¹⁰.

Alternatives to CNIs have been investigated in kidney transplantation. Costimulation blockade (CoB) with belatacept, a modified CTLA4-Ig fusion protein, has emerged as a promising alternative to CNIs for selected patients¹¹. Belatacept blocks the B7-CD28 costimulation pathway, preventing T cell activation during the alloimmune response. Additionally, CoB therapy allows for activation-induced cell death of T cells, a key component in achieving peripheral tolerance *in vitro*¹². In particular, immunosuppression consisting of CoB combined with monoclonal or polyclonal depletion induction (e.g., thymoglobulin or alemtuzumab) and/or mechanistic target of rapamycin (mTOR) inhibitor (sirolimus or everolimus) treatment leads to excellent allograft function while displaying low rates of AR comparable to CNI-containing regimens^{13–15}. Despite the improved side-effect profile of CoB-based immunosuppression, patients continue to require life-long immunosuppression to prevent AR, although pilot clinical studies have shown that selected patients can be weaned to monotherapy CoB^{13,14}.

We conducted two independent, concurrent studies to evaluate the safety and efficacy of adding donor-derived mesenchymal stromal cell infusions (MSCs) to established depletion induction and CoB/mTOR inhibitor-based immunosuppressive maintenance regimens in order to facilitate complete immunosuppressive withdrawal. We chose MSC's as, unlike donor bone marrow, they are a non-hematopoietic source of donor cells that do not promote a risk of graft versus host disease. In the first study, we added monthly MSC infusions to a stringent nonhuman primate (NHP) model of kidney transplantation to assess this approach. The NHP study used polyclonal antibody depletion induction with the addition of thymic irradiation (TI) to potentiate the formation of MSC engraftment

and chimerism. In the second study, we added monthly MSC infusions to a published regimen of monoclonal T- and B-cell depletion with alemtuzumab and belatacept/ sirolimus maintenance immunosuppression^{13,14} (without thymic irradiation). This study, Tolerance by Engaging Antigen During Cellular Homeostasis (TEACH) (ITN062ST), was a single center, open label, dose-escalation clinical trial. The primary objective of both the NHP study and TEACH was to determine whether systemic donor-derived MSCs could promote operational tolerance in kidney transplant recipients who received lymphocyte depletion followed by maintenance immunosuppression with CoB and mTOR inhibition.

2. Methods

2.1 NHP study - Rhesus kidney transplantation, immunotherapy, and posttransplant monitoring

To assess the impact of TI and repeated MSC infusions on homeostatic repopulation and whether those interventions fostered tolerance following kidney transplantation, an NHP model of kidney allotransplantation was used (Table 1). Rhesus macaques (n=15) who had previously undergone left nephrectomy underwent kidney transplantation from maximally MHC-mismatched donor pairs using standard techniques followed by right nephrectomy as previously reported^{16,17}. They were randomized to receive one of three immunosuppressive regimens: control group (n=6; rhATG, belatacept, and sirolimus), TI group (control regimen plus 7Gy of thymic irradiation on POD# -3, n=4), and MSC+TI group (TI regimen plus monthly donor MSC infusions, n=5) (Figure 1a). Monthly infusions were chosen to coincide with monthly infusions of belatacept and the 12-month time course was chosen to cover the period of lymphocyte repopulation. Sirolimus was weaned at 6 months post-transplant and belatacept was weaned 1-year post-transplant. Animals were monitored daily for urine output and signs/symptoms of rejection. Bloodwork (CBC and chemistries) were obtained on post-operative days (POD) #1 and #3 and weekly/monthly thereafter. Blood samples were evaluated for lymphocyte phenotype and donor-specific antibodies (DSA) pre- and post-transplant (Supplementary document, Methods). The primary endpoint of the study was AR as determined by continued elevation of serum creatine after exclusion of technical complications. Kidney allografts were evaluated histologically for cell-mediated and antibody-mediated AR (Supplementary document, Methods).

2.2 NHP study - Rhesus MSC generation

Rhesus MSCs were generated from donor bone marrow and verified by surface staining for CD45, CD73, CD90, and CD105 (Figure S1a). Details of rhesus MSC expansion and preparation for infusion are included in the supplementary document.

2.3 TEACH Trial – Patients, kidney transplants and protocol immunosuppression

The Immune Tolerance Network trial ITN062ST TEACH was a phase-I, open label, dose-escalation trial designed to assess the safety and efficacy of donor-derived mesenchymal stromal cells after transplant to facilitate immunosuppression withdrawal in adult recipients of primary, human leukocyte antigen (HLA)-non-identical, live donor kidney transplants. One recipient was haplo-identical and the others were less well matched (Table 2). The Division of Allergy, Immunology and Transplantation (DAIT) of the National Institute

of Allergy and Infectious Disease (NIAID), National Institutes of Health (NIH) was the sponsor of this trial which was conducted under US FDA IND 17846. Safety oversight was provided by an independent NIAID-sponsored DSMB and by a NIAID Medical Monitor (NDB). All subjects (donors and recipients) were enrolled under a Duke University Institutional Review Board-approved protocol following informed consent, and the study was registered with [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03504241) (NCT03504241). Only Epstein–Barr virus (EBV) seropositive and DSA-negative kidney transplant candidates were eligible.

As shown in Figure 1b, patients received induction with 30mg alemtuzumab IV, one hour following pretreatment with acetaminophen (650 mg po), diphenhydramine (50 mg IV) and methylprednisolone (500mg IV). The first dose of 10mg/kg belatacept (IV) was given within 24 hours after transplantation. Subsequent doses were given on days 5 and 14, then every 2 weeks for a total of 5 doses, and then every 4 weeks through 24 weeks post-transplant. After this, the dose was changed to 5mg/kg every 4 weeks. Belatacept was provided for the study by Bristol-Myers Squibb Co. Sirolimus was started on day 1 and adjusted to maintain trough levels of 8–10ng/ml.

The study was designed to have three dosing cohorts of two recipients each, receiving 12 infusions of donor-derived MSCs every four weeks starting at week six posttransplantation. Monthly infusions were chosen to coincide with monthly infusions of belatacept and the 12-month time course was chosen to cover the period of lymphocyte repopulation. The planned doses in the three cohorts were 10^4 , 10^5 and 10^6 cells/kg/dose, with dose escalation being contingent on safety and tolerability of the dose given in the previous cohort. Study enrollment was terminated before recruitment commenced in the third cohort, see below. Belatacept was administered prior to each MSC infusion. The MSC product was administered by intravenous (IV) infusion over 5–30 minutes 30 minutes after premedication with acetaminophen, diphenhydramine, and hydrocortisone. Subjects were monitored for infusion reactions for 4 hours post-infusion.

2.4 TEACH Trial – Generation of MSCs

Human MSCs (hBM-MSCs) were generated at the Robertson GMP Cell Manufacturing Laboratory, Duke University, from donor bone marrow cells harvested on the day of kidney donation. The MSCs were expanded to passage 2 (P2), harvested, and cryopreserved for the required dosing at 2 million MSCs/kg per dose. The phenotype of MSCs was verified as CD90⁺CD73⁺CD105⁺CD166⁺CD29⁺CD44⁺CD274⁺HLA-I⁺ with combined total of contaminating cells including endothelial or hematopoietic in origin < 10% of any cell preparation. Full details of MSC manufacturing are included in the supplementary document.

2.5 TEACH Trial – Immunosuppression withdrawal (ISW) and monitoring

Subjects with stable graft function, no DSA or rejection, and who had received at least six doses of MSCs, were eligible to initiate sirolimus withdrawal between 52–104 weeks post-transplant. Those who successfully withdrew from sirolimus and remained stable on belatacept monotherapy for at least 24 weeks were eligible to discontinue belatacept. Subjects who developed one episode of rejection or DSA during or following sirolimus

withdrawal resumed full-dose sirolimus, but could re-attempt sirolimus withdrawal and be weaned to belatacept monotherapy (but not belatacept withdrawal) after resolution of rejection or DSA.

Subjects were followed for three years of follow-up after successful ISW, or for two years after failing ISW. (See Supplementary document, methods, for details of monitoring).

Protocol-directed biopsies were performed prior to initiating sirolimus withdrawal, and again prior to belatacept withdrawal. For-cause biopsies were obtained as needed. Biopsies were evaluated and scored according to the Banff 2017 classification¹⁸. Subjects with biopsy proven acute rejection received treatment as per the site standard of care.

The primary endpoint was the proportion of subjects who achieved operational tolerance 52 weeks after completion of ISW, as defined by no episodes of acute rejection after initiation of ISW, stable renal function (estimated GFR > 60 ml/min/1.73m² and urine protein: creatinine <0.5), and no DSA after completion of ISW. The 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate the eGFR⁽¹⁹⁾. Secondary clinical endpoints included the (1) proportion of subjects who remained stable on belatacept monotherapy for >48 weeks without rejection or DSA (2) incidence, severity, and timing of rejection (3) incidence of DSA (4) incidence of AEs attributable to MSCs (5) incidence of select AEs including ≥grade 3 infection, malignancy or wound complications (6) incidence of graft loss, and (5) death. A key mechanistic endpoint was the prognostic value and correlation over time of T cell proliferation (Ki67) with successful ISW. Data analyses are descriptive.

3. Results

3.1 NHP Study Results

3.1.1 Addition of TI and repeated donor MSCs fails to induce tolerance in a NHP model of kidney transplantation: Two NHPs were censored due to technical failure (H23K in TI group; H34Y in MSC+TI group) (Table 1). Two NHPs were censored due to acute gastrointestinal dilation²⁰ (i.e. bloat) leading to NHP demise (DFF8 in control group; H59T in the MSC+TI group) and one NHP was censored due to bleeding in the setting of thrombocytopenia (H23K in the TI group). There was no evidence of rejection at time of sacrifice of the censored animals. Two NHPs were censored following episode of graft dysfunction/rejection in the setting of viral infections (DG83 in the control group – SV40+ infection; H55F in the control group – LCV infection).

There was overall no statistically significant survival difference between all three groups and no survival advantage with addition of TI or MSC+TI (Figure 2). NHPs in the control group and TI groups were successfully weaned to belatacept monotherapy but rejected their allografts once fully off immunosuppression. In contrast to the two other groups, two NHPs in the MSC+TI group rejected their allograft while on belatacept monotherapy (H70D, H75K) associated with the development of DSA. These findings suggested that addition of TI or MSCs did not facilitate tolerance induction in our NHP model and further indicated a potential sensitizing effect of MSCs.

3.1.2 Thymic irradiation leads to thymic involution but does not lead to

donor chimerism: NHPs in the TI and MSC+TI group received 7Gy of radiation to the thymus on POD#-3. The thymus was targeted through CT guidance (Figure S2a). Compared to gender- and aged-matched NHPs, NHPs who received TI had evidence of thymic involution on histology (Figure S2b). At necropsy, there was no evidence of donor DNA chimerism at the thymus in either the TI or MSC+TI groups (data not shown). NHPs in the TI group experienced in the post-operative phase a significant increase in the percentage of CD4⁺ recent thymic emigrants (RTE) T cells compared to control NHPs (Figure S2c). This difference was not observed a month post-operatively (data not shown).

3.1.3 Development of donor-specific antibodies (DSA) following repeat MSC

infusions: NHPs in the MSC + TI group received monthly donor MSC infusions starting on POD#30 (Figure 1a). These infusions were well tolerated with no adverse reactions noted at the time of infusion. No NHPs experienced sudden worsening in renal function following MSC infusions. However, two NHPs in the MSC+TI group developed DSA to donor MSCs (Figure 3a, 3b) and donor lymphocytes (Figure 3c, 3d) temporally associated with their AR while on belatacept monotherapy (H70D, H75K). While 2 NHPs in the control group and 1 in the TI group developed DSA while under belatacept monotherapy (Figure S3a, S3b) both maintained normal allograft function for several weeks post-full immunosuppression wean. Unstimulated donor MSCs expressed MHC class I molecules and expressed both MHC class II molecules as well as CD40, a co-stimulatory molecule, under interferon gamma (IFN- γ) and tumor necrosis factor alpha (TNF- α) stimulation (Figure S1b). These findings show that, in our study, donor MSCs expressed both MHC and costimulatory molecules under inflammatory conditions and potentially had a sensitization effect leading to AR on belatacept monotherapy.

3.1.4 Addition of TI and MSC did not significantly alter homeostatic

repopulation: Animals in both TI groups trended toward lower T cell counts at the time of transplant. rhATG induction produced significant CD4⁺ and CD8⁺ T cell depletion with gradual repopulation in all treatment groups (Figure 4a). To assess the repopulating T cell phenotype post-depletional induction, PBMCs were segregated into naïve (T_{Naïve}, CD28⁺CD95⁻) and effector memory (T_{EM}, CD28⁻CD95⁺) subsets based on surface CD28 and CD95 expression (Figure S4). As shown in Figure S5, no substantial patterns distinguished the groups besides baseline lymphopenia.

The repopulation of CD20⁺ cells in animals receiving was significantly suppressed in all groups (Figure 4b). Additional analysis of CD20⁺ cells based on the surface CD27 and IgD expression (Figure S6) showed that the base immunosuppression regimen significantly inhibited repopulation of unswitched (CD27⁺IgD⁺), switched (CD27⁺IgD⁻), and exhausted (CD27⁻IgD⁻) memory cells after transplantation (Figure S7). Additional pretransplant TI significantly delayed switched memory cell repopulation. Posttransplant MSC infusion did not prevent the repopulation of naïve and memory B cells.

3.2 TEACH Trial Results

3.2.1 Infusion of donor MSCs were acutely well-tolerated: The ITN TEACH trial enrolled its first subject on July 30, 2018. The last subject discontinued ISW and moved to safety follow-up on September 6, 2023. Altogether, eight subjects were screened and six underwent transplantation (Figure 5); their characteristics are summarized in Table 2. All patients tolerated alemtuzumab induction and belatacept therapy. One patient discontinued sirolimus therapy given concern for focal segmental glomerulosclerosis and switched to MMF. One patient was initially placed on MMF post-transplant given a complex wound closure and concern for delayed wound healing on sirolimus; MMF was converted to sirolimus after the wound healed. Four subjects received MSCs. Two did not initiate MSC infusions: one due to a technical graft loss and one due to *de novo* post-transplant DSA prior to MSC infusion. One subject had grade 1 and 2 MSC-related adverse events: fever and headache. There were no deaths. Supplemental Table 1 summarizes the other adverse events in this trial.

3.2.2 Immunosuppression withdrawal: Table 3 and Figure S8 summarize the outcomes of patients who received donor MSCs. In cohort 1, subject 1 was ineligible to initiate ISW at 52 weeks due to *de novo* DSA. Although DSA declined over time, it did not resolve completely during the study follow up period and ISW was not attempted. Subject 2 had borderline TCMR on the pre-sirolimus withdrawal surveillance biopsy, which was treated with steroids. Sirolimus withdrawal was successfully attempted after 52 weeks of clinical stability and this subject has now been on belatacept alone for >2.5 years.

In cohort 2, both subjects initiated sirolimus withdrawal. Subject 6 developed graft dysfunction during withdrawal and was treated for presumed AR with an increase in the sirolimus dose. Subject 3 developed grade IIB acute TCMR 16 weeks after completing sirolimus withdrawal that was treated with steroids and resumption of sirolimus. Sirolimus withdrawal was not re-attempted in either subject. In all, there were three episodes of acute rejection in three of four subjects who received MSC: two biopsy-proven and one presumed. There were no cases of biopsy proven AMR or chronic rejection. *De novo* DSA were detected in three subjects, one of whom (subject 1) had received MSCs (Table 4).

Of the four subjects who received MSCs, one subject in cohort 1 developed BK viremia, peak viral load 15800 copies/ml at 4 months post-transplant. Viral load decreased to <500 copies/ml by 6 months post-transplant, and did not increase. CMV or EBV reactivation was not observed post-transplantation. All subjects are alive and well with stable graft function (Figure S8).

3.2.3 Homeostatic T cell repopulation post-depletional induction: As previously reported^{13,14,21}, alemtuzumab induction prior kidney transplants produced profound T and B cell depletion followed by a slow repopulation of CD20⁺, CD4⁺ and CD8⁺ cells (Figure 6a). Granulocytes and monocytes were not significantly depleted. The repopulating naïve and memory cells posttransplantation were assessed based on the surface CD45RA and CCR7 expression (Figure S9a). As shown in Figure 6b, the repopulation of CD4⁺ T_C, T_{Naïve}, and T_{EM} cells was gradual and consistent with that reported in

patients not receiving MSCs^{13,14,21}. The recovery of CD8⁺ T_{EM} cells was significantly delayed. Homeostatic proliferation of CD4⁺ and CD8⁺ cells, measured by intracellular Ki67 expression, a nuclear protein expressed in proliferating cells²², persisted through for at least 9 months post-depletional induction (Figure S9b, Figure 6c). The immune phenotype of Ki67⁺ cells was predominantly T_{EM} cells pretransplantation, and the frequency of this subset decreased over time (Figure 6c). The Ki67⁺ T_{Naïve} cells also repopulated gradually.

4. Discussion

In the two independently conducted studies reported herein, we tested the hypothesis that exposure to a chronic, peripherally distributed form of donor antigen (MSCs) would foster a reduced need for immunosuppression following kidney transplantation. They were based on previous studies in humans^{13–15} and NHPs^{16–17} that depletion followed by belatacept and non-CNI-based maintenance therapy can allow selected individuals to wean to monotherapy belatacept. As one mechanism postulated to reduce AR in recipients on CoB is antigen directed activation induced cell death, we reasoned that additional antigen might accelerate this process. While MSC infusion was overall well-tolerated without obvious side effects, they failed to induce operational tolerance in NHPs or humans. Moreover, while these are small experiences, there was a concerning suggestion that the MSCs might contribute to sensitization rather than tolerance. Together, these studies suggest that addition of systemic donor MSC infusions to a CoB-based regimen is feasible but does not facilitate operational tolerance and may have the potential to sensitize the recipients. We have chosen to present the two studies together to strengthen the negative findings, which would be less compelling if shown in a single model system.

In the first study, we investigated TI and MSCs as means to promote tolerance in a CoB-based regimen in a stringent NHP kidney transplant model. TI is ubiquitously used in chimerism-based tolerance induction protocols²³, as it is thought to prevent intra-thymic rejection of donor progenitor cells and foster re-population of the thymus with donor cells²⁴. We noted thymic involution following TI in our NHP model. However, we failed to detect any donor chimerism in the thymus or any evidence that TI added to a CoB-based regimen could lead to operational tolerance. A possible explanation for the failure to generate donor chimerism in the thymus was the insufficient load of donor antigen and cells. As such, we investigated whether addition of donor MSCs, used in our NHP study as a delivery vehicle of donor antigen, could lead to operational tolerance and donor chimerism. Additionally, MSCs are known to have immunosuppressive properties as they inhibit T cell responses²⁵ and induce apoptosis of activated T cells²⁶ *in vitro*. Furthermore, infusions of MSCs have been associated with significantly prolonged allograft survival in a preclinical models²⁷ and have been anecdotally noted to lead to tolerance in humans²⁸. Despite this, we failed to note donor chimerism at the thymus in the MSC+TI group and failed to induce operational tolerance in our preclinical model. Congruently, no patients in TEACH, a parallel clinical trial, were able to successfully wean off immunosuppression, though consistent with our prior experience, no patient required CNIs and one weaned to belatacept monotherapy. Clearly, these studies do not support TI and MSCs as an approach toward operational tolerance following depletional induction and CoB-based immunosuppression.

Importantly, MSC infusions were overall acutely well-tolerated, both in our preclinical model as well as in the TEACH trial, as previously described by others²⁶. Indeed, there were no serious adverse events noted at the time or immediately following infusions. However, we noted incidences of AR and development of DSA under belatacept monotherapy in the MSC+TI group, something not seen in the other treatment groups. Concurrently, 3 out of 4 patients in the TEACH trial that received MSCs developed AR and one recipient developed DSA. This contrasts with a prior clinical trial using similar immunosuppression that found no evidence of rejection in the first-year post-transplant^{13,14}. These data suggest that infusion of donor MSCs could have had a sensitizing effect. Indeed, we noted that both unstimulated NHP and human MSCs expressed MHC class I molecules and under inflammatory conditions could also express MHC class II molecules and costimulatory proteins, essential components to generate an allo-response. Third-party MSCs have been suggested to have immunosuppressive properties²⁹, and unlike donor bone marrow do not carry with them the risk of graft versus host disease. It is possible that intentionally mismatched MSCs could have a salutary effect without the risk of sensitization.

There are several limitations to this study. First and foremost, our conclusions are limited by the low numbers both in our NHP study and the TEACH trial. Different cohorts received different MSC doses in the TEACH trial which added to the heterogeneity of the study. We also did not collect mechanistic data on Treg function, which could be influenced by MSCs. Regardless, the consistent suggestion in both the NHP and human trials that sensitization occurs after donor-derived MSC infusions warrants caution, particularly given that AR was seen in both studies. Of note, all donor-recipient pairs were significantly mismatched (intentionally so in the NHP model), and this may have influenced the outcomes, skewing toward alloimmunity.

In summary, we have performed two separate trials adding donor-derived MSC infusions to depletion induction, followed by belatacept and rapamycin maintenance immunosuppression. Though practically feasible, MSC infusions did not induce tolerance and in a subset of patients, they were sensitizing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

Allan D Kirk reports financial support was provided by National Institutes of Health. Allan D Kirk reports financial support was provided by Immune Tolerance Network. If there are other authors, they declare that they have no

known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All NHP study data have been stored in the secured server of Department of Surgery/IRB/ Duke University. Data from the ITN TEACH Trial will be made openly available in Trial Share at <https://www.itntrialshare.org> once the study is completed.

Abbreviations:

AR	Allograft rejection
CoB	Costimulation blockade
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMV	Cytomegalovirus
DSA	Donor specific antibody
EBV	Epstein–Barr virus
eGFR	Estimated glomerular filtration rate
HLA	Human leukocyte antigen
IFN-γ	Interferon gamma
ISW	Immunosuppression withdrawal
MSC	Mesenchymal stromal cells
mTOR	Mammalian target of rapamycin
NHP	Nonhuman primate
PCR	Polymerase chain reaction
POD	Post-operative day
RhATG	Rhesus anti-thymocyte globulin
TI	Thymic irradiation
TNF-α	Tumor necrosis factor alpha

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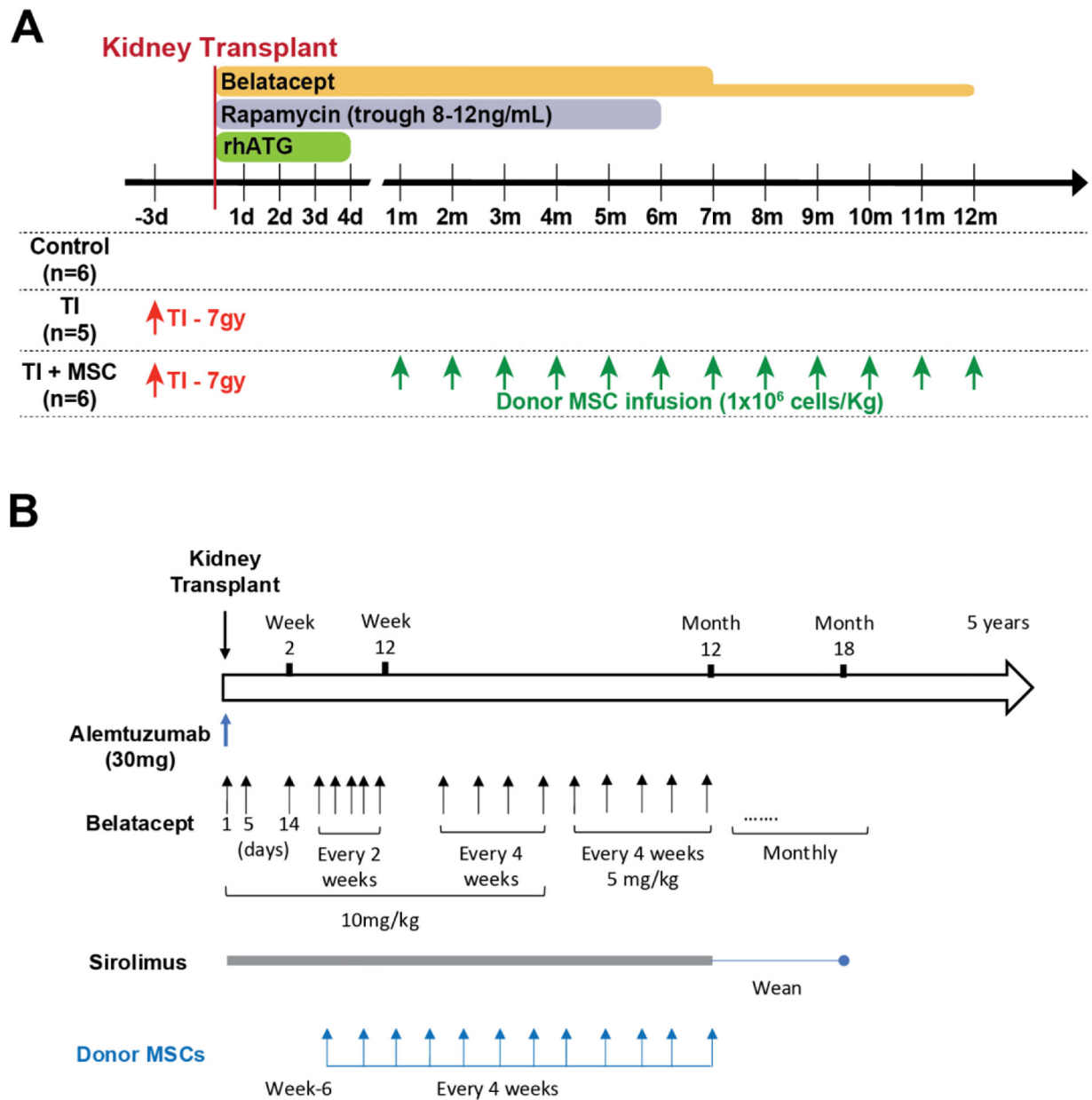


Figure 1. NHP and TEACH trial study designs.

(A) Schematic of the design used in the NHP study to assess the effect of TI and MSCs on alloimmunity following kidney allotransplantation. Primates were divided in 3 groups. Recipients underwent kidney allotransplantation on POD#0. The study ended following episode of AR. (B) Schematic of the design used in the TEACH Trial. There were two cohorts with recipients receiving donor MSCs infusions comprised of either 10^4 or 10^5 cells/kg/dose.

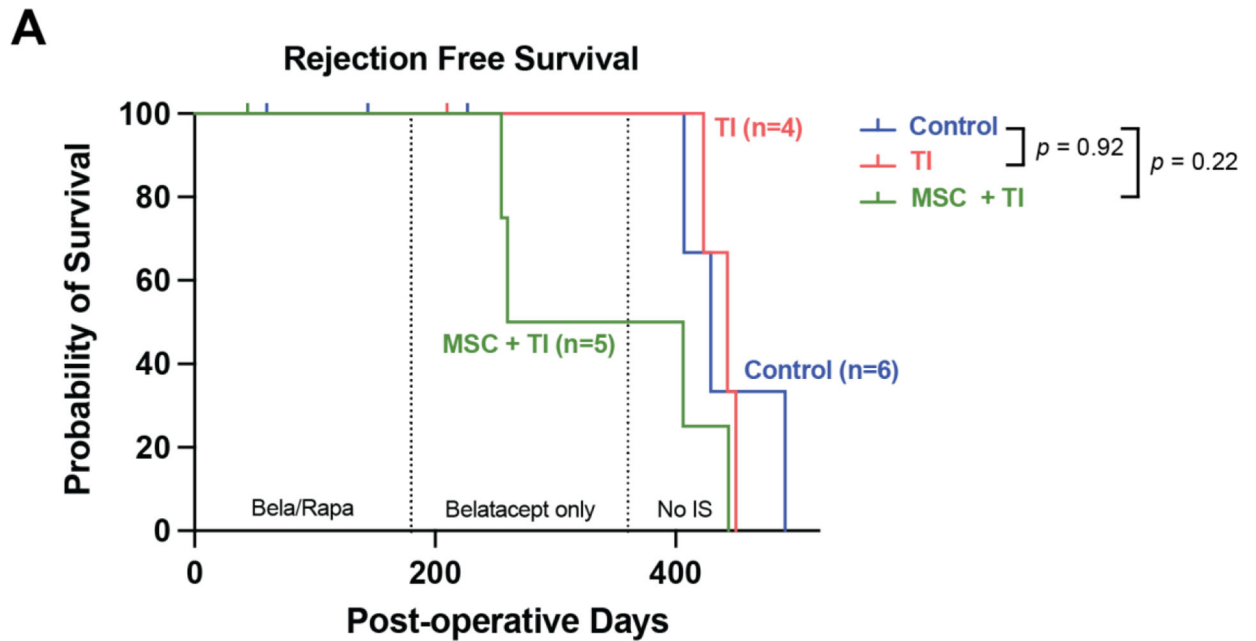


Figure 2. Allograft survival following kidney allotransplantation.

(A) Overall rejection-free survival. There was no AR under immunosuppression in both the control and TI group. Two primates in the MSC + TI group rejected their allograft under Belatacept monotherapy (H70D, H75K). All primates experienced AR once immunosuppression was fully weaned.

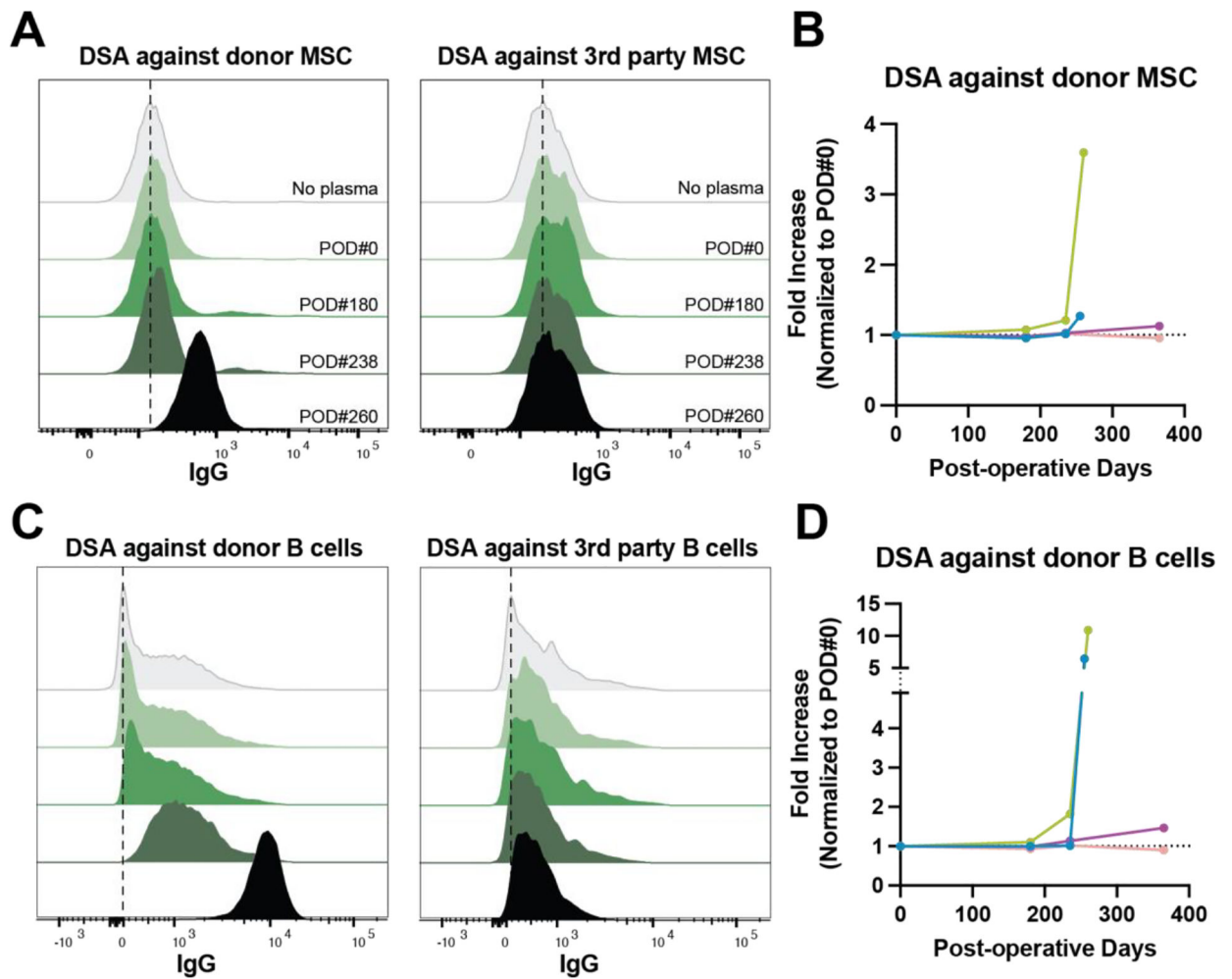


Figure 3. Development of DSA under Belatacept monotherapy in the MSC + TI group.

(A) Evaluation of DSA against donor MSC and 3rd party MSC at various timepoints post-transplantation for H75K. Sirolimus was discontinued on POD#180 and primates remained on Belatacept monotherapy until POD#365. (B) Two out of four primates (H75K and H70D) developed increased DSA titers against donor MSCs while under Belatacept monotherapy. (C) Evaluation of DSA against donor B cells and 3rd party B cells at various timepoints post-transplantation for H75K. (D) Two out of four primates (H75K and H70D) developed increased DSA titers against donor B cells while under Belatacept monotherapy.

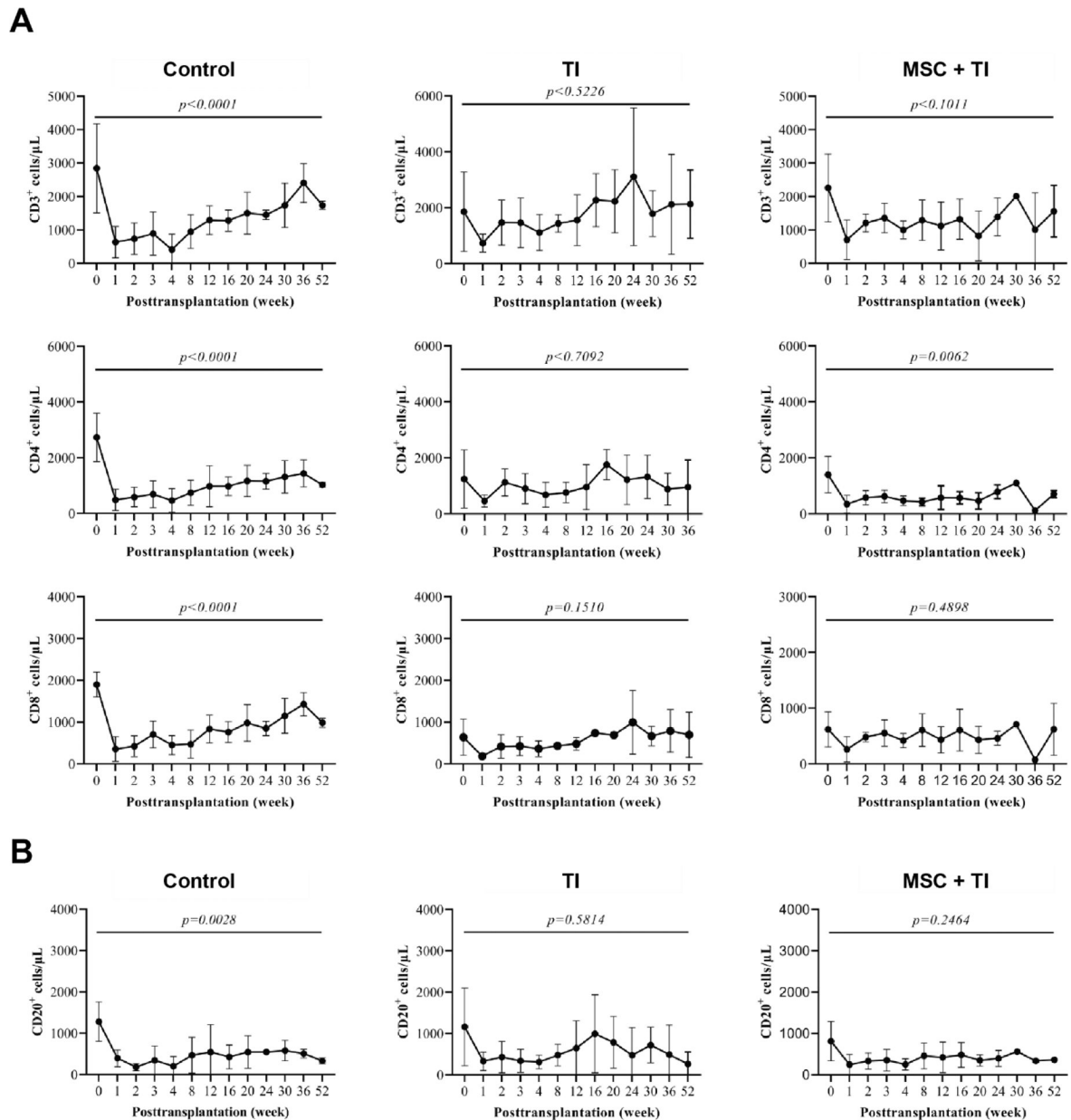
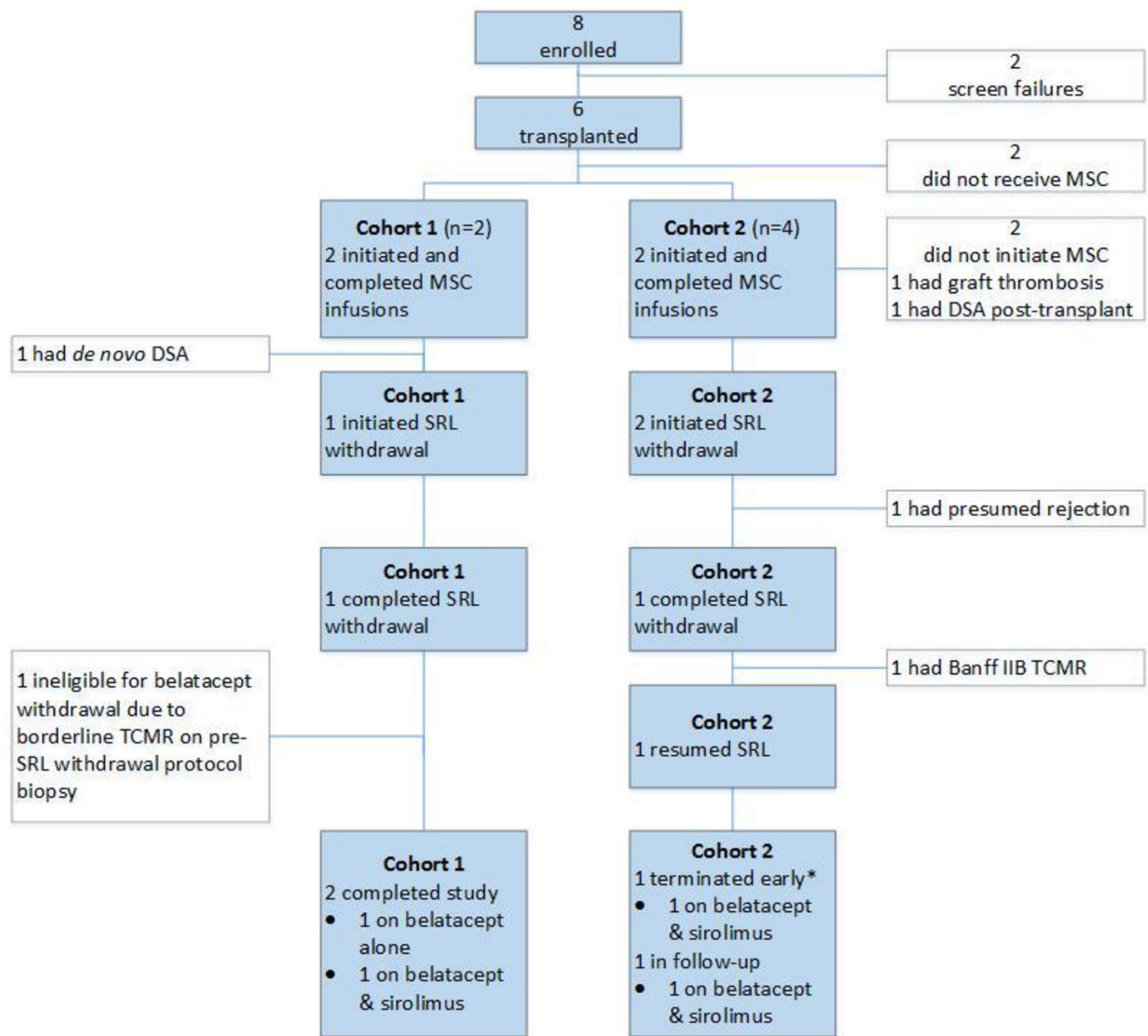


Figure 4. Depletion and repopulation of T and B cells in the NHP study.

(A) Absolute numbers of CD3⁺, CD4⁺, and CD8⁺ cells following transplantation in all groups. Primates in all groups experienced profound depletion of CD3⁺, CD4⁺, and CD8⁺ cells post-transplant followed by delayed repopulation. (B) Absolute numbers of CD20⁺ cells following transplantation in all groups. Primates in all groups experienced profound depletion of CD20⁺ cells post-transplant followed by delayed repopulation.



*As per patient preference at 420 days post-transplant

Figure 5.
Flow of subjects in the TEACH Trial.

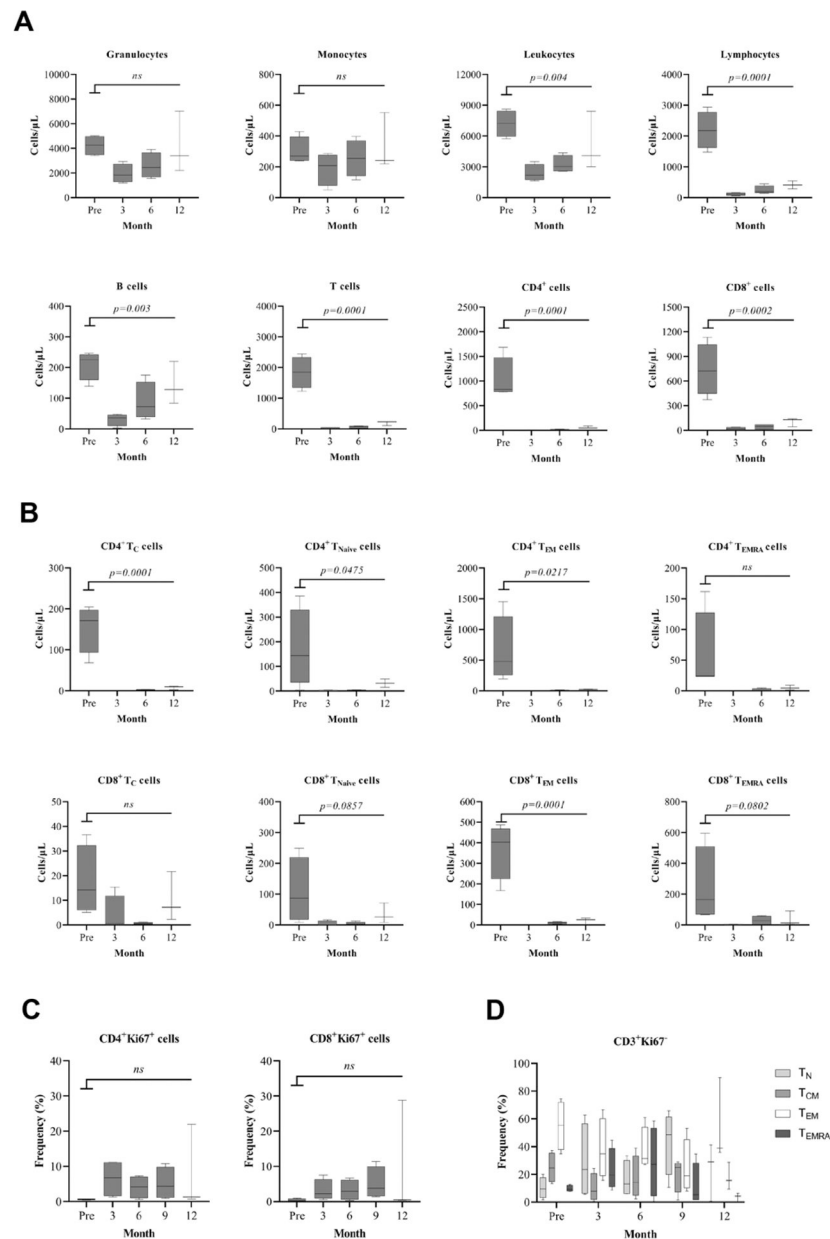


Figure 6. Depletion and repopulation of cells in the TEACH trial.

(A) Absolute counts of granulocytes, monocytes, leukocytes, lymphocytes, B cells, T cells, CD4⁺ cells, and CD8⁺ cells pre- and post-transplant. (B) Absolute counts of CD4⁺/CD8⁺ T central memory cells (T_C cells), CD4⁺/CD8⁺ T naïve cells (T_{Naive} cells), CD4⁺/CD8⁺ T effector memory cells (T_{EM} cells), and CD4⁺/CD8⁺ T EMRA cells (T_{EMRA} cells) pre- and post-transplant. (C) Frequency of CD4⁺Ki67⁺ cells and CD8⁺Ki67⁺ cells pre- and post-transplant. (D) T cell phenotype of CD3⁺Ki67⁺ cells pre- and post-transplant.

Table 1.

Summary of outcomes in the NHP study.

Immunotherapy	NHP ID	Rejection-free survival (days)	Immunosuppression withdrawal	Reason for censoring	Histology of allografts
Control	H731	491	Belatacept & Sirolimus	N/A	ACR borderline
	DG83	144	None	SV40+ infection	ACR Type IIA
	DFF8	60	None	Bloat	No evidence of ACR
	H727	429	Belatacept & Sirolimus	N/A	ACR Type III
	H55F	227	Sirolimus	LCV infection	ACR Type IIA
	H52C	407	Belatacept & Sirolimus	N/A	ACR Type III
TI	H45Y	450	Belatacept & Sirolimus	N/A	ACR Type III
	H759	423	Belatacept & Sirolimus	N/A	ACR Type III
	DFX7	210	Sirolimus	Thrombocytopenia/bleed	ACR Type III
	H23K	5	None	Primary graft dysfunction	No evidence of ACR, ATN
	H649	443	Belatacept & Sirolimus	N/A	ACR Type IIA
TI + MSC	H50T	444	Belatacept & Sirolimus	N/A	ACR Type IIB
	H13R	406	Belatacept & Sirolimus	N/A	No evidence of ACR
	H34Y	1	None	Technical failure	N/A
	H59T	44	None	Bloat	No evidence of ACR
	H70D	255	Sirolimus	N/A	ACR Type III
	H75K	260	Sirolimus	N/A	ACR Type III

Table 2.

Summary of patient characteristics in the TEACH Trial.

	Subject	Age	Sex	Race	Cause of ESRD	Donor age	Donor sex	Donor race	Donor mismatch
Cohort 1	1	32	Female	White	Diabetes mellitus	50	Female	White	A2, B1, C1 DR2, DQ2, DP1
	2	47	Male	White	Hypertension	26	Female	White	A2, B1, C1 DR2, DQ2, DP1
Cohort 2	3	35	Female	White	Polycystic kidney disease	44	Female	White	A01, B1, C1 DR1, DQ1, DP0
	4	58	Male	White	Polycystic kidney disease	59	Female	White	A0, B2, C1 DR1, DQ1, DP1
	5	61	Female	White	Polycystic kidney disease	60	Male	White	A1, B2, C1 DR2, DQ1, DP1
	6	57	Female	White	Diabetes mellitus and hypertension	58	Male	White	A2, B2, C2 DR1, DQ2, DP2

Table 3.

Outcomes of study subjects who received MSCs in the TEACH Trial.

	Subject	Number of MSC Doses	Initiated ISW	Completed ISW	Acute rejection during the study	De novo DSA		Immunosuppression at the last visit	eGFR at the last visit ² (ml/min/1.73 m ²)
						Class I	Class II		
Cohort 1 (10 ⁴ cells/kg per dose)	1	12	No	NA	None	HLAB8	HLADR7, HLADQA1	Belatacept Sirolimus	87.1
	2	12	Yes	SRL withdrawal only	Borderline TCMR on protocol biopsy	None	None	Belatacept Sirolimus ¹	88.3
Cohort 2 (10 ⁵ cells/kg per dose)	3	10	Yes	SRL withdrawal only	Banff IIB acute TCMR after SRL withdrawal	None	None	Belatacept Sirolimus	85.5
	6	6	Yes	No	Presumed acute rejection during SRL withdrawal	None	None	Belatacept Sirolimus	40.4

¹ SRL withdrawal was successfully completed after patient's last visit in the study.² eGFR was calculated using 2021 Chronic Kidney Disease Epidemiology Collaboration equation.

Table 4.
Study subjects who developed DSA during the TEACH Trial.

Cohort	Subject	Received MSC?	HLA specificities		Time of first detection of DSA		Peak MFI		Time of resolution		AMR during the study?
			Class I	Class II	HLA Class I	HLA Class II	HLA Class I	HLA Class II	HLA Class I	HLA Class II	
1	1	Yes	B8	DR7 DQA1	18 weeks	18 weeks	8456	7091	72 weeks	48 weeks	No
2	4	No	B13	-	44 weeks	-	759	-	60 weeks	-	No
	5	No	B51	-	3 weeks	-	1571	-	20 weeks	-	No