STATISTICAL ANALYSIS PLAN

TREG ADOPTIVE THERAPY IN SUBCLINICAL INFLAMMATION IN KIDNEY TRANSPLANTATION

PROTOCOL NUMBER: CTOT-21

SHORT TITLE:	Tregs in Subclinical Inflammation
NCT #:	NCT02711826
CLIENT:	NIH/NIAD/DAIT
IND #:	16626
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VERSION HISTORY

SAP Version	Version Date	Change(s)	Rationale
1.0	17May2024	Initial Version	

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1. LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
AB	Antibody
AE	Adverse Event
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
BK	Human Polyomavirus 1
CDC	Centers for Disease Control and Prevention
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CRM	Common Response Module
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CTOT	Clinical Trials in Organ Transplantation
DAIT	Division of Allergy, Immunology, and Transplantation
DSA	Donor Specific Antibodies
DSMB	Data and Safety Monitoring Board
EBNA	Epstein-Barr Nuclear Antigen
EBV	Epstein-Barr Virus
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ELISPOT	Enzyme-linked Immunosorbent Spot
FDA	Food and Drug Administration
HB	Hepatitis B
HBcAB	Hepatitis B Core Antibody Test
HBSAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICU	Intensive Care Unit
IgM	Immunoglobulin M

IND	Investigational New Drug
IRB	Institutional Review Board
IS	Immunosuppression
ITT	Intent-to-Treat Sample
IVIg	Intravenous Immunoglobulin
kSORT	Kidney Solid Organ Response Test
LCA	Leukocyte Common Antigen
MedDRA	Medical Dictionary for Regulatory Activities
MFI	Mean Fluorescence Intensity
mITT1	Modified Intent-to-Treat Sample 1
mITT2	Modified Intent-to-Treat Sample 2
MMF/MPA	Mycophenolate Mofetil/Mycophenolic Acid
mRNA	Messenger Ribonucleic Acid
mTOR	Mammalian Target of Rapamycin
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institutes of Health
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative
REMS	Risk Evaluation and Mitigation Strategy
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
SOC	System Organ Class
TB	Tuberculosis
TdAP	Tetanus, Diphtheria, and Pertussis
VCA	Viral Capsid Antigen

2. PURPOSE OF THE ANALYSES

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and associated data displays to be included in the end of study analyses for Protocol CTOT-21 being conducted under IND 16626. This document provides details on the analysis population, derivation of variables, and statistical methods to be used in these analyses.

We will conduct one or possibly more analyses to evaluate whether inflammation is suppressed in the graft and markers of inflammation in graft and urine are reduced via an infusion of polyTregs in comparison to participants treated with CNI-based maintenance immunosuppression therapy.

3. PROTOCOL SUMMARY

Title	Treg Adoptive Therapy in Subclinical Inflammation in Kidney Transplantation
Short Title	Tregs Infusion for Inflammation Suppression
Clinical Phase	I/II
Number of Sites	7
IND Sponsor/Number	NIH/NIAID/DAIT/ #16626
Study Objectives	1. To assess the safety of polyTregs infusions and the safety of converting from CNI-based maintenance therapy to mTOR inhibitors after Treg therapy in adult kidney transplant recipients (see Study Definitions page). 2. To assess the efficacy of polyTregs infusions in reducing inflammation in adult kidney transplant recipients (see Study Definitions Page).
Study Design	This is a multi-center, open-label, randomized, controlled safety and efficacy trial with 2 cohorts, maintenance CNI-based immunosuppression therapy and polyTregs infusion, to achieve inflammation suppression in adult kidney transplant recipients. Eligible participants will either continue maintenance CNI-based immunosuppression therapy or will receive a single infusion of polyTregs ($550 \pm 450x10^6$). All participants will undergo biopsies to determine study eligibility and at 7 months after study group allocation. Participants who received a polyTregs infusion will undergo an additional biopsy 14 days after polyTregs infusion. The original study design included a third treatment arm of donor alloantigen reactive Tregs (darTregs). One participant was treated with darTregs prior to protocol version 9.0 in which this treatment arm was eliminated due to excessive manufacturing failures.
Primary Endpoint(s)	<u>Primary Safety Endpoint</u> Participants will be followed for safety for the full duration of study. The safety of polyTregs (Group 2) will be described in comparison with CNI-based maintenance IS therapy (Group 1) by: 1. The timing and incidence of Banff 2A or higher acute cell-mediated rejection and/or acute antibody mediated rejection, 2. The timing and incidence of study defined Grade 3 or higher infection <u>Primary Efficacy Endpoint</u> The change in inflammation as measured by the percentage area of cortex occupied by inflammatory cells using computer-assisted quantitative image analysis on the biopsy 7 months after group allocation, expressed as the percent change relative to the baseline biopsy. Primary Mechanistic Endpoint The immunologic profiles of kidney transplant recipients using graft common response module (CRM) gene expression of rejection and/or evidence of

	inflammation in biopsies at 2 weeks after infusion (Group 2) and 7 months after group allocation (Groups 1 and 2).
Secondary Endpoint(s)	<u>Secondary Endpoints for polyTregs – Clinical</u> 1. Proportion of participants exhibiting a relative decrease of 25% or more inflammation between the baseline kidney biopsy and the biopsy 2 weeks after polyTregs, measured as the percentage area of cortex occupied by inflammatory cells using computer-assisted quantitative image analysis. 2. Number of participants who exhibit a relative decrease of 50% or more inflammation on kidney biopsy at 2 weeks after polyTregs 3. Timing, incidence, and severity of polyTreg infusion reactions 4. Timing, incidence, and severity of culture-proven and clinically diagnosed infections after polyTreg infusion 5. Timing, incidence, and severity of acute rejection using Banff grading 6. Timing and incidence of BK viremia and cytomegalovirus (CMV) reactivation 7. Timing and incidence of >10% decrease in eGFR compared to baseline <u>Secondary Endpoints for mTOR Therapy – Clinical</u> 1. Incidence and timing of acute rejection, comparing participants who receive Tregs and convert to mTOR therapy to subjects who do not convert to mTOR therapy and participants in Group 1. <u>Secondary Endpoints – Mechanistic</u> 1. Persistence of infused Tregs in blood and biopsies using deuterium labeling and T-cell repertoire analysis (Group 2) 2. Cytokine and CRM mRNA and protein profiles in the urine as correlates of acute rejection and/or histologic evidence of inflammation and graft fibrosis (Groups 1 and 2) 3. Peripheral blood kSORT (kidney solid organ response test) mRNA expression of rejection/increased immune response (Groups 1 and 2)
Accrual Objective	14 participants total: 7 participants on CNI maintenance therapy 7 participants to receive polyTregs
Study Duration	6.5 Year Accrual, 12 Months Follow Up
Treatment Description	Subjects will be on maintenance immunosuppresiont herapy using CNI + MMF/MPA with or without steroids at the time of study entry. Eligible participants will be randomized to receive: 1. Standard CNI maintenance IS (no Tregs) 2. $550 \pm 450 \times 10^6$ polyTregs After receiving at least 300×10^6 polyTregs infusion, eligible participants will start mTOR inhibitor.

Inclusion Criteria	Individuals who meet all of the following criteria are eligible for enrollment as study participants: 1. Participant must be able to understand and provide informed consent 2. Age ≥18 years of age at the time of study entry 3. Recipients of non-HLA identical living or deceased donor renal transplants 4. Protocol renal allograft biopsy at 5 months (± 8 weeks) after transplantation with Banff i1 and/or ti1 with concomitant t scores t0, t1, t2 or t3; Banff i2 and/or ti2 with concomitant t scores t0 or t1; and without v >0, [ptc + g] ≥2, C4d >1 (by IF), or C4d > 0 (by IHC) confirmed by central pathologist. Participants must not be treated for pathologic criteria (e.g., steroids). 5. eGFR ≥30 ml/min at the time of study entry 6. Maintenance immunosuppression consisting of tacrolimus, MMF/MPA ± prednisone (≥10 mg/day) 7. Current immunizations including TdAP, pneumococcal and seasonal influenza vaccines prior to study treatment, completed prior to randomization and no less than 14 days prior to planned manufacturing collection 8. Hepatitis B serologies must be: (1) Positive HB surface antibody, negative HB core antibody and negative HB surface antigen for recipients immune to Hepatitis B (2) Negative HB surface antibody, negative HB core antibody and negative HB surface for non-immune/HBV naïve recipients provided donor had negative HB core antibody and negative HB surface antigen at the time of donation 9. Negative TB test (PPD, interferon-gamma release assay, ELISPOT testing) within 1 year prior to enrollment. Participants with a history of TB (positive TB test without active infection) must have completed one of the latent TB infection treatment regimens endorsed by the CDC (Division of TB Elimination, 2016). Alternative regimens for latent TB infection eradication will be adjudicated by the site's infectious disease specialist. 10. Female participants with childbearing potential must have reviewed Mycophenolate REMS and have a negative pregnancy test upon study entry 11. Female participants of childbearing potential must agree to use FDA approved methods of birth control for the duration of the study; participants must consult with their physician and determine the most suitable method(s) that are greater than 80% effective (http://www.fda.gov/birthcontrol)
Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants: 1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol 2. History of malignancy; except adequately treated basal cell carcinoma

	3. History of graft loss from acute rejection within 1 year after any previous transplant 4. History of transplant renal artery stenosis 5. History of cellular rejection prior to enrollment that did not respond to steroids and/or subsequent creatinine after treatment for rejection greater than 15% above baseline 6. Known hypersensitivity to mTOR inhibitors or contraindication to everolimus (such as history of wound healing complications) 7. Any chronic illness requiring uninterrupted anti-coagulation after kidney transplantation 8. Post-transplant DSA >5000 MFI or post-transplant treatment with IVIg for DSA. Enrolled participants with post-transplant DSA >2000 MFI will not be eligible for mTOR conversion. 9. Positive HIV 1 or HIV 2 serology prior to transplantation 10. Known positive HBSAg, or HBcAb serology 11. Proteinuria with urine pr/cr > 1.0 g/g 12. Any condition requiring chronic use of corticosteroids >10mg/day at the time of study entry 13. Subjects requiring treatment for pathologic findings on study eligibility biopsy (see inclusion 4). 14. Active infection at the time of study entry 15. History of active TB or latent TB without adequate treatment (see inclusion 10). 16. Serum BK virus >1,000 copies/ml by PCR at the time of study entry 17. Hematocrit <27%; ANC < 1,000/μL; lymphocytes <500/μL; at the time of study entry 18. Participation in any other studies with investigational drugs or regimens in the preceding year 19. Any condition or prior treatment which, in the opinion of the investigator, precludes study participation 20. Unable to provide adequate biopsy specimen (paraffin embedded formalin fixed) from eligibility biopsy (3-7 months post-transplant) for quantitative analysis. 21. EBV naïve recipient of a kidney from an EBV positive donor, historically EBV naïve recipient with primary EBV infection at the time of screening (primary anti-VCA IgM, without antibody to EBNA), positive EBV PCR 22. Hepatitis C Virus AB positive participants with negative HCV PCR are eligible if they have spontaneously cleared infection or are in sustained virologic remission for at least 12 weeks after treatment. 23. Positive SARS-CoV2 testing by RT-PCR
Treg Infusion Inclusion Criteria	1. Individuals randomized to Group 2 who continue to meet all of the enrollment criteria are eligible for Treg infusion 2. Negative SARS-COV2 RTPCR testing within 1 week of Treg infusion

Treg Infusion Exclusion Criteria	1. Received any vaccination within 14 days prior to blood collection for Treg manufacture 2. Unacceptable Treg product 3. Positive pregnancy test for women of childbearing potential
mTOR Conversion Inclusion Criteria	1. Received at least 300 x 10^6 polyTreg infusion 2. Resolution of inflammation on the 2-week post-infusion biopsy as compared to the baseline biopsy, confirmed by central pathologist
mTOR Conversion Exclusion Criteria	1. Post-transplant DSA >2000 MFI 2. Any condition or clinical variable, which in the opinion of the site investigator, precludes conversion to mTOR
Study Stopping Rules	Any of the following after subjects have initiated their study regimen: 1. Any CTCAE grade 4 or higher infusion reaction 2. Any diagnosis of malignancy and post-transplant lymphoproliferative disease (except non-melanoma skin cancer) 3. Any graft loss 4. Acute Rejection (Banff grade 2A or higher) or acute antibody mediated rejection within 6 weeks after Treg infusion in 3 of first 5 participants 5. Any death 6. Inability to manufacture and supply polyTregs in 3 of 5 consecutive subjects assigned to Group 2 7. If there is a total of 8 failed lots of polyTregs at any time during the study

4. ANALYSIS SAMPLES

Modified Intent-to-Treat Sample 1 (mITT1) – The mITT1 sample consists of all subjects allocated to the CNI-based treatment regimen or received polyTregs while on study.

Modified intent-to-Treat Sample 2 (mITT2) – The mITT2 sample consists of all subjects who received polyTregs (Group 2).

5. STUDY SUBJECTS

5.1. Disposition of Participants

The disposition of all enrolled (i.e., consented) participants will be summarized in tables and listed, as necessary.

The numbers and percentages of various participant statuses will be presented. Reasons for screen failure will be presented. For participants discontinuing study treatment early, the reasons for discontinuing study treatment early will also be presented.

5.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for all participants in the analysis sample. Characteristics to be summarized include age, sex, race, and ethnicity. Additional characteristics may be included.

5.3. Prior and Concomitant Medications

If needed for the CSR, a prior and concomitant medication listing will be prepared.

5.4. Medical History

If needed for the CSR, a medical history listing will be prepared.

6. STUDY OPERATIONS

6.1. Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will complete a Protocol Deviation form. Protocol deviation reports will be compiled and reviewed by NIAID/DAIT and the DSMB. Sites will be responsible for reporting deviations to local IRBs, as per local requirements.

6.2. Randomization

Participants will be assigned to the treatment groups in an unblinded fashion using a variation of the Pocock and Simon adaptive randomization strategy. A web-based randomization system will be used in order to minimize the possibility of bias entering into those assignments and to maintain approximate equality in the group sizes. The initial treatment allocation will weight each treatment equally. In subsequent allocations, the less-populated arm will be weighted more in order to restore the balance between the groups. If a recipient is assigned to polyTreg and the recipient does not receive the intended polyTreg infusion, that recipient will be re-assigned to Group 1. The allocation schedule algorithm/scheme will not incorporate stratification variables.

6.3. Measurement of Treatment Compliance

Study subjects will have monitoring of trough levels at 1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months.

7. GENERAL ANALYSIS AND REPORTING CONSIDERATIONS

The following is a list of general analysis and reportion conventions to be applied for this study.

1. Categorical variables will be summarized using counts (n) and percents (%) and will be presented in the form n (%).
2. Continuous variables will be summarized using appropriate statistics (e.g., mean, median, standard deviation, min, max, interquartile range) using an appropriate level of precision.
3. Following SAS default rules, the median will be reported as the average of the two middle numbers if the dataset contains even numbers.
4. P-values will be reported to 3 decimal places if greater than 0.001. If less than 0.001 then report '<0.001'. Report p-values and significant levels as 0.05 rather than .05.
5. No preliminary rounding should be performed; rounding should only occur after analysis. To round, consider digit to right of last significant digit: if <5 then round down, if ≥5 then round up.
6. All listings will be sorted in order of subject, and time of assessment (e.g., visit, time, and/or event).
7. All analysis will be performed using the SAS System version 9.4 or higher.

7.1.1. Multicenter Studies

Study participants will be recruited from 7 study sites.

Following database lock, if potentially impactful site-to-site variation is noted, basic descriptive analyses of baseline demographics, medical history, or other key points will be repeated for each site individually in order to allow qualitative exploration of site-to-site variability.

7.1.2. Assessment Time Windows

Allowable visit windows are detailed in Section 10.6 of the protocol.

Unscheduled visits may also occur throughout the study if creatinine increases or other concerns arise between regularly scheduled visits.

Generally, all data will be included in analyses, regardless of time of assessment.

7.1.3. Timing of Analyses

All analyses will be conducted following database lock.

7.1.4. Multiple Comparisons/Multiplicity

The analysis is primarily descriptive and will not be adjusted for multiple comparisons.

8. ENDPOINT EVALUATION

8.1. Primary Endpoint

The primary efficacy endpoint will be the change in inflammation as measured by the percentage area of cortex occupied by inflammatory cells using computer-assisted quantitative image analysis on the biopsy 7 months after study group allocation, expressed as the percent change relative to the baseline biopsy. The subject in the darTregs group will be excluded from efficacy analyses.

The safety of polyTregs (Group 2) and darTregs (Group 3) will be described in comparison with CNI-based maintenance IS therapy (Group 1) by:

1. The timing and incidence of Banff 2A or higher acute cell-mediated rejection and/or acute antibody mediated rejection.
2. The timing and incidence of study defined Grade 3 or higher infection.

8.1.1. Computation of the Primary Endpoint

- Primary Efficacy Endpoint
    - Percent change in inflammation at 7 months after group allocation relative to baseline biopsy.
- Primary Safety Endpoint
    - The timing and incidence of Banff IIA or higher acute cell-mediated rejection and/or acute antibody mediated rejection.
        - **Severe Acute Cell-Mediated Rejection**- Acute cell-mediated rejection was defined using the Banff 2007 criteria. Participants with a Banff grade of greater than or equal to IIA were determined to have met the endpoint. Severity is graded as IA, IB, IIA, IIB, or III, with IA being the mildest form of cellular rejection and III being the most severe form of cellular rejection.
        - **Acute Antibody Mediated Rejection**- Antibody mediated rejection was defined as diffusely positive staining for C4d, presence of circulating anti-donor antibodies, and morphologic evidence of acute tissue injury.
    - The timing and incidence of study defined Grade 3 or higher infection.
        - Severe infection was defined in the study as a Grade 3 or higher infection that was culture-proven and clinically diagnosed. Severity is graded as 1 through 5, with 1 being least severe to 5 being most severe. Grade 3 is any infection associated with hemodynamic compromise requiring pressors; any infection necessitating ICU level of care; any infection necessitating operative intervention; any infection involving the central nervous system; any infection with a positive fungal blood culture; any proven or probable aspergillus infection; any tissue invasive fungal infection; any

pneumocystis jiroveci infection. Grade 4 is any life-threatening infection. Grade 5 is any infection resulting in death.

8.1.2. Primary Analysis of the Primary Endpoint

The ANOVA method or Kruskal-Wallis test (or t-test for comparison of Groups 1 and 2) will be used to compare treatment groups on the mean or median percent change in inflammation at 7 months post-group allocation, relative to baseline biopsy. This method will be run on the mITT1 population, subset to participants that have available LCA data from the 7 month visit post-group allocation.

The Fisher's Exact or Cochran-Mantel-Haenszel tests will be used to compare the treatment groups on the incidence of severe acute cell-mediated rejection and/or acute antibody mediated rejection. This method will be run on the mITT1 population, subset to participants that have available Banff data.

The ANOVA method or Kruskal-Wallis test (or t-test for comparison of Groups 1 and 2) will be used to compare treatment groups on the mean or median time of event of severe acute cell-mediated rejection and/or acute antibody mediated rejection. This method will be run on the mITT1 population, subset to participants that have available Banff data.

The Fisher's Exact or Cochran-Mantel-Haenszel tests method will be used to compare the treatment groups on the incidence of severe infection. This method will be run on the mITT1 population.

The ANOVA method or Kruskal-Wallis test (or t-test for comparison of Groups 1 and 2) will be used to compare treatment groups on the mean or median time of event of severe infection. This method will be run on the mITT1 population.

8.1.3. Sensitivity Analyses of the Primary Analysis

No sensitivity analysis is planned.

8.1.4. Secondary Analyses of the Primary Endpoint

No secondary analysis of the primary endpoint is planned.

8.2. Secondary Endpoints

All secondary endpoint analyses will use the mITT1 population, unless otherwise specified.

8.2.1. Endpoints

Variable: Secondary incidence endpoints including:

Endpoint	**Time Frame**
polyTreg infusion reactions	405 days post-group allocation
Culture-proven and clinically diagnosed infections	405 days post-group allocation

Acute rejection using Banff grading	405 days post-group allocation
BK viremia after polyTregs infusion	405 days post-group allocation
CMV reactivation after polyTregs infusion	405 days post-group allocation
>10% decrease in eGFR compared to baseline	405 days post-group allocation
Acute rejection after converting to mTOR therapy after polyTregs infusion	69 days post-group allocation to 405 days post-group allocation
Proportion of participants exhibiting >=25% decrease of inflammation between baseline kidney biopsy and the week 2 kidney biopsy	Baseline biopsy to Week 2 kidney biopsy
Proportion of participants exhibiting >=50% decrease of inflammation between baseline kidney biopsy and the week 2 kidney biopsy	Baseline biopsy to Week 2 kidney biopsy
Proportion of participants exhibiting >=25% decrease of inflammation between baseline kidney biopsy and the month 6 kidney biopsy	Baseline biopsy to 6 month kidney biopsy (for polyTregs infusion group). Baseline biopsy to 7 months post-group allocation (for maintenance therapy group).

Analysis: For each of the above incidence endpoints, the proportion of participants with events will be reported by treatment group and compared using Fisher's Exact test or a Cochran-Mantel-Haenszel test.

Population-level summary:

- **polyTreg infusion reactions** - mITT 2
- **Acute rejection using Banff grading** – mITT1, subset to participants that have available Banff lab data
- **Acute rejection after converting to mTOR therapy after polyTregs infusion**- mITT2
- **Proportion of participants exhibiting >=25% decrease of inflammation between baseline kidney biopsy and the week 2 kidney biopsy** – mITT1, subset to participants with available LCA data from week 2 visit
- **Proportion of participants exhibiting >=50% decrease of inflammation between baseline kidney biopsy and the week 2 kidney biopsy** – mITT1, subset to participants with available LCA data from week 2 visit
- **Proportion of participants exhibiting >=25% decrease of inflammation between baseline kidney biopsy and the month 6 kidney biopsy** – mITT1, subset to participants with available LCA data from 6 month visit
- All other endpoints will use the mITT1 sample

Variable: Secondary timing endpoints including:

Endpoint	**Time Frame**
polyTreg infusion reactions	405 days post-group allocation
Culture-proven and clinically diagnosed infections	405 days post-group allocation
Acute rejection using Banff grading	405 days post-group allocation
BK viremia after polyTregs infusion	405 days post-group allocation
CMV reactivation after polyTregs infusion	405 days post-group allocation
>10% decrease in eGFR compared to baseline	405 days post-group allocation
Acute rejection after converting to mTOR therapy after polyTregs infusion	69 days post-group allocation to 405 days post-group allocation

Analysis: For each of the above timing endpoints, the mean or median time of event (study days since start of treatment) will be reported by treatment group and compared using ANOVA or the Kruskal-Wallis test (or t-test for comparison of Groups 1 and 2).

Population-level summary:

- **polyTreg infusion reactions** – mITT2
- **Acute rejection using Banff grading** – mITT1, subset to participants that have available Banff lab data
- **Acute rejection after converting to mTOR therapy after polyTregs infusion**- mITT2
- All other endpoints will use the mITT1 sample

Variable: Secondary severity endpoints including:

Endpoint	**Time Frame**
polyTreg infusion reactions	405 days post-group allocation
Culture-proven and clinically diagnosed infections	405 days post-group allocation
Acute rejection using Banff grading	405 days post-group allocation

Analysis: For each of the above severity endpoints, the count of participants will be reported by treatment group and compared using Fisher's Exact test or a Cochran-Mantel-Haenszel test.

Population-level summary:

- **polyTreg infusion reactions** – mITT2

- **Culture-proven and clinically diagnosed infections** – mITT1, subset to those with culture-proven and clinically diagnosed infections
- **Acute rejection using Banff grading** – mITT1, subset to participants that have available Banff lab data

8.3. Other Endpoints

Any mechanistic data received, aside from data that's been noted above in sections 8.1 and 8.2, will be summarized using appropriate statistics, provided that the data is sufficient and complete to do so.

9. SAFETY EVALUATION

9.1. Overview of Safety Analysis Methods

General safety analyses will be carried out using the mITT1 population defined in Section 4 (with the addition of the one subject who received darTregs), unless otherwise noted. Missing safety information will not be imputed. These analyses will not be stratified by site.

Safety will be analyzed in each treatment group through the reporting of AEs, vital signs, and changes in routine laboratory measures.

Listings will be prepared, if needed to fulfill regulatory reporting obligations, for all safety measurements. All listings will be sorted in order of subject identifier and time point of assessment (e.g., visit and/or time).

9.2. Extent of Exposure

If needed to fulfill regulatory reporting obligations, a listing summarizing polyTreg and darTreg infusion status will be created.

9.3. Adverse Events

All AEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA] version 18.1). The severity of AEs will be classified using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0. Each AE is entered on the electronic case report form (eCRF) once at the highest severity. As such, no additional data manipulation is needed to identify events.

A summary table will present the total number of events as well as the number and percentage of subjects experiencing the events organized by relatedness and severity for all AEs and SAEs separately. If a subject experiences the same AE on multiple occasions, the event will be counted once for each occurrence when reporting the number of AEs. When reporting the number of subjects experiencing the events, a subject will only be counted once if they experience an event within the particular SOC or preferred term. A data listing will be provided for all AEs. Separate listings will be provided for the CSR as needed.

9.4. Deaths, Serious Adverse Events, and Other Significant Adverse Events

Serious adverse events (SAEs) will be listed. If needed to fulfill regulatory reporting obligations, a separate display listing summarizing death, including time of death relative to treatment start date and cause of death, will also be created.

9.5. Clinical Laboratory Evaluation

If needed to fulfill regulatory reporting obligations, a separate listing summarizing clinical laboratory measurement data will also be created.

9.6. Vital Signs, Physical Findings, and Other Observations Related to Safety

9.6.1. Vital Signs

If needed to fulfill regulatory reportion obligations, a separate listing summarizing vital signs related to safety will be created.

9.6.2. Physical Examinations

If needed to fulfill regulatory reporting obligations, listings of physical examination data will be produced.

9.6.3. Other Safety Measures

No other safety measures have been identified.

10. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

The analysis populations for the endpoints 1) incidence of severe acute cell-mediated rejection and/or acute antibody mediated rejection, 2) incidence of acute rejection, 3) severity of acute rejection, and 4) timing of acute rejection were changed from the modified intent-to-treat population to the modified intent-to-treat population subset to participants that have available Banff data.

The analysis population for the severity of culture-proven and clinically diagnosed infections endpoint was changed from the modified intent-to-treat population with polyTregs infusion to the modified intent-to-treat population subset to those with culture-proven and clinically diagnosed infections.

The analysis populations for the endpoints 1) percent change in inflammation at 7 months after group allocation relative to baseline biopsy and 2) proportion of participants exhibiting >=25% relative decrease of inflammation between baseline kidney biopsy and the 6 month kidney biopsy were changed from the modified intent-to-treat population with polyTregs infusion to the modified intent-to-treat population subset to participants that have available LCA data from 6 month visit.

The analysis populations for the endpoints 1) proportion of participants exhibiting >=25% relative decrease of inflammation between baseline kidney biopsy and the week 2 kidney biopsy and 2) proportion of participants exhibiting >=50% relative decrease of inflammation between baseline kidney biopsy and the week 2 kidney biopsy were changed from the modified intent-to-treat population with polyTregs infusion to the modified intent-to-treat population subset to participants that have available LCA data from Week 2 visit.

11. REFERENCES

None

12. APPENDICES

None

13. ATTACHMENTS

None