DAIT/Rho STATISTICAL ANALYSIS PLAN
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ALLTOL
A Prospective Cohort Study of Operationally Tolerant Allograft Recipients

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SPONSOR: [redacted]

PREPARED BY: [redacted]

DAIT/Rho STATISTICAL ANALYSIS PLAN ACKNOWLEDGMENT AND SIGNATURE SHEET

ALLTOL
A Prospective Cohort Study of Operationally Tolerant Allograft Recipients

Approved: _____ Date: _____
[redacted]
Protocol Chair and Clinical Trial Physician

Approved: _____ Date: _____
[redacted]
NIAID Medical Monitor

Approved: _____ Date: _____
[redacted]
Rho Scientist

Approved: _____ Date: _____
[redacted]
Rho Statistician

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
BDR	Biomarker and Discovery Research
BiG	Bioinformatics Groups
CTCAE	Common Toxicity Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
eGFR	Estimated glomerular filtration rate
GGT	Gamma-glutamyl transferase
ITN	Immune Tolerance Network
KM	Kaplan Meier
MedDRA	Medical Dictionary for Regulatory Activities
NKF	National Kidney Foundation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation

1. PROTOCOL SYNOPSIS

Title	ALLTOL: A Prospective Cohort Study of Operationally Tolerant Allograft Recipients
IND Sponsor	NIAID
Conducted by	Immune Tolerance Network
Protocol Chair(s)	[redacted]
Accrual Objective	There is no upper limit on the number of study participants, but we estimate that approximately 70 participants may be enrolled from former ITN studies and from de novo referrals.
Study Treatment	N/A
Study Design	This trial is a multi-center, prospective, observational study in which operationally tolerant recipients of liver or kidney allografts will be followed longitudinally with annual collections of clinical data and biological samples. All participants will be followed for the duration of the trial regardless of changes in their tolerance status. Participants will be recruited along three main pathways: 1. Tolerant participants from current and past ITN trials; including those who have already completed trial participation and those who are anticipated to complete trial participation; 2. Tolerant participants referred by ITN affiliated investigators, academic and community transplant physicians and directly through outreach to transplant affinity groups such as the National Kidney Foundation (NKF); 3. Tolerant participants from the general transplant community who are reachable through general media channels such as clinicaltrials.gov, the ITN website, word-of-mouth referrals from existing participants and social media. Participants will be encouraged to complete their study visits on site. However, participants who are unable or unwilling to travel to a study site will have the option of completing their study visits remotely (Figure 1).
Study Duration	The trial will continue through January 31, 2021. On March 24, 2020 the study ended early due to the potential risk of the research participants and personnel as a result of the COVID-19 pandemic.
Primary Objective	The primary objective of this study is to identify and longitudinally follow individuals who have received a kidney or liver transplant and who have achieved a state of operational tolerance to their allograft. Operational tolerance is defined as meeting all of the criteria listed below: 1. Absence of biopsy-proven or clinical rejection, as determined by medical history 2. Maintenance off immunosuppressive medication, with the exception of short courses of steroids for non-allograft related conditions; e.g., asthma 3. Normal and stable allograft function (see Study Definitions). Allograft function tests used in primary endpoint calculations must be obtained in the absence of confounding factors. A Primary Endpoint Adjudication Committee (see Study Definitions) will review cases where confounding factors are suspected of having impact on the participant's test results and make recommendations toward resolution of the participant's status.

Primary Endpoint	The primary endpoint of this study is the time to loss of operational tolerance.
Secondary Endpoints	**Safety** The following endpoints will be assessed for all participants: 1. Time to development of de novo anti-HLA antibody or DSA 2. Time to the first episode of biopsy-proven or clinical acute rejection, steroid resistant rejection and chronic rejection 3. Time to graft loss, not including death with functioning graft **Mechanistic** Mechanistic endpoints will longitudinally examine the following parameters with regard to operational tolerance: 4. Time course of changes in previously identified tolerance signatures in individual participants. 5. Expression levels for a wide variety of genes measured by large-scale microarray or by PCR assessments. 6. Flow-cytometric measurements of cell populations distinguished by cell-surface phenotype. 7. miRNA levels in peripheral blood 8. Gut microbiome profile
Inclusion Criteria	Participants must meet *all* of the following criteria to be eligible for this study: 1. Recipient of single organ liver or kidney allograft from a living or deceased donor. 2. At screening, operationally tolerant defined as meeting both of the following criteria: a. Absence of any immunosuppressive therapy for ≥ 52 weeks prior to the screening visit. b. No evidence of allograft rejection in the 52 weeks prior to the screening visit, based on the medical history. 3. Normal allograft function (see Study Definitions) If the site investigator judges that a participant exhibits stable allograft function despite values outside these criteria, then the participant is eligible if the ITN clinical trial physician/protocol chair, NIAID medical monitor and a subject matter expert concur. 4. Receiving regular follow up for a kidney or liver transplant by a local physician. Participants must be willing to allow the study team to contact and share medical information with this local physician. *5.* Ability to sign informed consent.
Exclusion Criteria	Participants *who* meet *any* of the following criteria will *not* be eligible for this study: 1. Current malignancy requiring recent surgery, ongoing chemotherapy, or radiation. 2. Transplant of another organ. 3. Current drug or alcohol dependency. 4. Any medical condition that in the opinion of the principal investigator would interfere with safe completion of the trial. 5. Inability to comply with the study visit schedule and required assessments.
Stopping Rules	N/A

2. INTRODUCTION

This statistical analysis plan (SAP) only includes analyses related to the primary and secondary clinical endpoints outlined in the protocol. Mechanistic analyses will be performed at the Immune Tolerance Network (ITN), and a separate analysis plan will be created to detail the planned analyses. Relevant clinical data from the study will be submitted to the ITN Biomarker and Discovery Research (BDR) and ITN Bioinformatics Groups (BiG) to augment the mechanistic analyses.

3. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following analyses and reporting conventions will be used:

- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form "n (%)." Percentages will be rounded to one decimal place.
- Numeric variables will be summarized using n, mean, standard deviation (SD), median, minimum (min), maximum (max). The min/max will be reported at the same level of significance as original data. The mean and median will be reported at one more significant digit than the precision of the data, and SD will be reported at two more significant digits than the precision of the data.
- The median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics including *t* and *z* test statistics will be reported to two decimal places.
- *P*-values will be reported to three decimal places if greater than or equal to 0.001. If less than 0.001, the value will be reported as "<0.001." A *p*-value can be reported as "1.000" only if it is exactly 1.000 without rounding. A *p*-value can be reported as "0.000" only if it is exactly 0.000 without rounding.
- Proportions will be expressed as percentages.
- Missing values will not be imputed unless explicitly stated.

If departures from these general conventions are present in the specific evaluations section of this SAP, then those conventions will take precedence over these general conventions.

4. ANALYSIS SAMPLES

The analysis sample will be comprised of all enrolled subjects that fulfill eligibility criteria and complete at least one mechanistic sample collection.

5. STUDY SUBJECTS

5.1. Disposition of Subjects

The disposition of all enrolled subjects will be summarized in tables and listed.

The numbers and percentages of subjects enrolled, screen failed, and accrued will be presented. The number and percent completing each yearly study visit as well as reasons for

early termination from the study will be presented. The breakdown of allograft type (liver vs. kidney) and previous ITN study participation vs. non-ITN study participation will also be shown.

5.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for the analysis sample defined in Section 4. Characteristics to be summarized include age (at study entry and time of transplant), race, ethnicity, sex, organ type, transplant indication, time from transplant, donor type, previous ITN study participation, date of operational tolerance and time off immunosuppression.

6. STUDY OPERATIONS

6.1. Protocol Deviations

Major protocol deviations (site-level and subject-specific) will be listed by site and by participant with information such as type of deviation, deviation sub-type, date of occurrence, details of the deviation, and the steps taken to address the deviation.

7. ENDPOINT EVALUATION

7.1. Overview of Efficacy Analysis Methods

7.1.1. Multicenter Studies

Study subjects will be recruited from 5 study sites. Due to the small number of subjects in the study, study data will be analyzed as a whole, and no formal accommodation for site-to-site variation will be made.

7.1.2. Assessment Time Windows

Visit 0 must occur within 60 days of enrollment. Each subsequent visit must occur yearly with a +/- 60-day window.

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

Furthermore, clinical data and mechanistic specimens that have already been collected from enrolled subjects from previous ITN study participation may be used to supplement the ALLTOL specimens and data, as needed.

7.2. Primary Endpoint

The primary endpoint of this study is the time to loss of operational tolerance.

Operational tolerance is defined as meeting all of the following criteria:

1. Absence of rejection (biopsy-proven or clinical rejection), as determined by medical history
2. Maintenance off immunosuppressive medication, with the exception of short courses of steroids for non-allograft related conditions; e.g. asthma

3. Normal and stable allograft function. Allograft function tests used in primary endpoint calculations must be obtained in the absence of confounding factors.

7.2.1. Computation of the Primary Endpoint

Normal and stable allograft function is defined as liver function tests (ALT, GGT) that have not increase more than 50% above the participant's baseline values for liver transplant recipients OR serum creatinine that has not increase more than 25% above the participant's baseline value for kidney transplant recipients.

For participants with study data that suggests the loss of operational tolerance according to the criteria in Section 7.2, the Primary Endpoint Adjudication Committee will review each case to determine if there were any potential confounding factors that may have impacted the participant's results. This committee will be comprised of the Study Protocol Chair, NIH Medical Monitor, a subject matter expert, and statistician. The following information will be presented to the committee in a blinded fashion (void of all ID and site information):

a. Demographic and baseline characteristics (age at study entry, race, sex, ethnicity, organ type, transplant indication, age at transplant, transplant date, time from transplant, donor type, previous ITN study (Y/N), date off IS, time off IS).
b. ALT and GGT lab values presented over time graphically for liver recipients OR creatinine values presented over time graphically for kidney recipients. A listing of each relevant lab value will also be included.
c. All IS medication usage
d. Any rejection event information
e. Biliary complications or new diabetes diagnosis.

Additional information can be requested by the committee.

The committee will make a determination of each participant's tolerance status for participants with study data that suggests the loss of operational tolerance according to the criteria defined in section 7.2. The determination of the cases reviewed above will be recorded and signed off by the protocol chair and medical monitor.

7.2.2. Primary Analysis of the Primary Endpoint

The primary endpoint will be analyzed using Kaplan-Meier survival estimates and associated two-sided 95% confidence interval, using a delayed-entry model controlling for left-truncation of survival times through adjusting for the time from achieving operational tolerance to the time of study enrollment.

A participant will be considered to have lost operational tolerance if they experience rejection, have loss of stable allograft function (in the absence of confounding factors), or restart immunosuppression. The date of loss of operational tolerance (end date of operational tolerance) will be the first date on which the subject meets any of the criteria for loss of operational tolerance. Specifically,

- If tolerance is lost due to rejection, the date will equal the date of the biopsy.
- If tolerance is lost due to restarting immunosuppression, the date will equal the date of the first immunosuppression medication dosage.
- If tolerance is lost due to abnormal allograft function, the date will equal the first recorded abnormal lab date.

The time to loss of operational tolerance for all participants will be evaluated relative to time zero. Time zero will be considered 52 weeks from the participant's last documented dose of immunosuppression and in the absence of rejection or evidence for loss of normal and stable graft function since that last dose. If the exact date of the last dose is not known, the date will be imputed to the 15th of the month when the day is missing, and July 1st when both day and month are missing. The year must be known.

$$time\ to\ loss\ of\ operational\ tolerance = end\ date\ of\ operational\ tolerance - start\ date\ of\ operational\ tolerance$$

Subjects lost to death, voluntary withdrawal or lost to follow-up will be censored at the time of occurrence of these events. Subjects who were followed until study closure (24MAR2020) without losing tolerance will be censored at the time of their most recent data point.

7.2.3. Sensitivity Analyses of the Primary Endpoint

7.3. Secondary Endpoints

All secondary endpoints will be analyzed as described in Table 7-2.

Table7-2 Table of Secondary Endpoints and Analysis Methods

Secondary Endpoint	Method	Population
Time to development of de novo anti-HLA antibody or DSA.	The time to the first de novo anti-HLA antibody or DSA be expressed in days from time zero. Kaplan-Meier survival estimate and associated two-sided 95% confidence interval, using a delayed-entry model controlling for left-truncation of survival times through adjusting for the time from achieving operational tolerance to the time of study enrollment.	Analysis sample defined in Section 4
Time to the first episode of biopsy-proven or clinical acute rejection, steroid resistant rejection and chronic rejection.	The time to the first episode of biopsy-proven or clinical acute rejection, steroid resistant rejection or chronic rejection be expressed in days from time zero. Kaplan-Meier survival estimate and associated two-sided 95% confidence interval, using a delayed-entry model controlling for left-truncation of survival times through adjusting for the time from achieving operational tolerance to the time of study enrollment.	Analysis sample defined in Section 4
Time to graft loss, not including death with functioning graft.	The time graft loss, not including death with functioning graft, will be expressed in days from time zero. Kaplan-Meier survival estimate and associated two-sided 95% confidence interval, using a delayed-entry model controlling for left-truncation of survival	Analysis sample defined in Section 4

	times through adjusting for the time from achieving operational tolerance to the time of study enrollment.

7.4. Examination of Subgroups

In addition, the following subset analyses will be performed on all study endpoints where the data permits:

a. Allograft type (liver vs. kidney)
b. Previous ITN study participation vs. non-ITN study referral

All subgroup analyses will be descriptive in nature.

7.5. Other Endpoints

The following endpoints will be examined in an exploratory nature. All analyses will be dependent upon the availability of the data.

Exploratory Endpoint	Method	Population
Time to all-cause mortality	The time to all-cause mortality will be expressed in days from time zero. Kaplan-Meier survival estimate and associated two-sided 95% confidence interval, using a delayed-entry model controlling for left-truncation of survival times through adjusting for the time from achieving operational tolerance to the time of study enrollment.	Analysis sample defined in Section 4
Changes in renal function (defined as estimated GFR (eGFR) calculated by CKD-EPI over time in renal transplant recipients	eGFR will be calculated using the CKD-EPI equation: $GFR = 141 * \min(Scr/\kappa,1)^{\alpha} * \max(Scr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018$ [if female] * 1.159 [if black] Scr: serum creatinine (mg/dL) κ: 0.7 for females and 0.9 for males α: -0.329 for females and -0.411 for males All available eGFR will be displayed graphically over time in two separate ways—as absolute values and as percent change from baseline.	Kidney transplant recipients of the analysis sample defined in Section 4
Changes in liver tests (ALT, GGT) over time in liver transplant recipients	All available ALT and GGT will be displayed graphically over time in two separate ways—as absolute values and as percent change from baseline.	Liver transplant recipients of the analysis sample defined in Section 4

Time to incidence of biliary complications in liver transplant recipients	The time to the earliest of biliary stricture requiring stenting, biliary obstruction, or cholangitis will be expressed in days from time zero. Kaplan-Meier survival estimate and associated two-sided 95% confidence interval, using a delayed-entry model controlling for left-truncation of survival times through adjusting for the time from achieving operational tolerance to the time of study enrollment.	Liver transplant recipients of the analysis sample defined in Section 4
Incidence of diabetes or use of concomitant medications for hypertension and/or hyperlipidemia	The proportion of participants that indicate that they have been newly diagnosed with diabetes (i.e. do not have diabetes at study entry) or have recorded using a medication for hypertension and/or hyperlipidemia with a corresponding two-sided 95% exact binomial confidence interval.	Analysis sample defined in Section 4

8. SAFETY EVALUATION

8.1. Overview of Safety Analysis Methods

All safety analyses will be carried out using all enrolled subjects unless otherwise noted. Missing safety information will not be imputed.

8.2. Adverse Events

Only serious adverse events (SAEs) are collected. All SAEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA] version 19.0). The severity of SAEs will be classified using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. Each SAE 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.

An overall summary table will be developed to report the number of events and the number and percentage of subjects having at least one event in the following categories:

- SAEs with an outcome of death
- SAEs related to study mandated blood draw

In addition, SAEs classified by MedDRA SOC and preferred term will be summarized for all SAEs.

Summary tables will present the total number of events as well as the number and percentage of subjects experiencing the events. If a subject experiences the same SAE on multiple occasions, the event will be counted once for each occurrence when reporting the number of SAEs. 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. Percentages will be based on the number of enrolled subjects.

8.3. Deaths

A listing summarizing death, including time of death and cause of death, will be created.

8.4. Clinical Laboratory Evaluation

Clinical laboratory measurements include serum chemistry, urinalysis, hematology, and a fasting lipid panel. Results will be converted to standardized units where possible.

Laboratory data for ALT and GGT will be plotted to show patterns over time for liver recipients. Creatinine and urine albumin-creatinine ratio will be plotted for kidney recipients. Glucose and fasting lipid data will be plotted for all subjects. Data will be plotted as a spaghetti plot where each subject's values will be plotted and connected by line segments, forming one line per subject. For all labs listed above, descriptive statistics of both absolute values and percent change from baseline will be presented in tabular format.

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

8.5.1. Vital Signs

Vital signs were not collected.

8.5.2. Physical Examinations

Physical examinations were not performed as part of this study.

9. INTERIM ANALYSES AND DATA MONITORING

No formal interim efficacy analysis was planned.

10. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

Changes in eGFR will only be calculated using the CKD-EPI method since height was not collected. Height is required to calculate eGFR using the Schwartz formula.

11. REFERENCES