DAIT/Rho STATISTICAL ANALYSIS PLAN 10FEB2020

ITN056ST OPTIMAL

VERSION: 1.0

DATE: 10FEB2020

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Document History

Version	Date	Change(s)	Author
0.1	07OCT2019	Initial Draft	[redacted]
0.2	30OCT2019	Updated per core call comments	[redacted]
0.3	20DEC2019	Updated per December SMT call comments	[redacted]
0.4	21JAN2020	Updated per January SMT call comments	[redacted]
1.0	10FEB2020	Updated per comments received from final SAP review	[redacted]

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LIST OF ABBREVIATIONS

BDR	ITN Biomarker and Discovery Research
BiG	ITN Bioinformatics Groups
CNI	Calcineurin Inhibitor
CTCAE	Common Toxicity Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
GFR	Glomerular Filtration Rate
IRB	Institutional Review Board
ITN	Immune Tolerance Network
ITT	Intent to Treat
MCS	Mental Component Score
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
PCS	Physical Component Score
PP	Per Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
ULN	Upper Limit of Normal

1. PROTOCOL SYNOPSIS

Title	Evaluation of Donor Specific Immune Senescence and Exhaustion as Biomarkers of Operational Tolerance Following Liver Transplantation in Adults
Short Title	Immune Senescence and Exhaustion Biomarkers of Operational Tolerance in Adult Liver Transplantation
IND Sponsor	NIAID
Conducted by	Immune Tolerance Network
Protocol Chair	James F. Markmann MD, PhD
Accrual Objective	60 liver transplant recipients and their living donors, if applicable
Study Treatment	Immunosuppression Withdrawal
Study Design	This trial is a multi-center, prospective, open label, non-controlled, nonrandomized, interventional cohort study in which 60 adult recipients of liver allografts will undergo gradual immunosuppression withdrawal. Liver recipients greater than 6 years post-transplant or greater than 3 years post-transplant and age greater than 50 years at time of screening will undergo liver biopsy and blood sampling before initiation of immunosuppression withdrawal. Participants may initiate withdrawal from calcineurin inhibitor (CNI) monotherapy or combination therapy with a CNI and prednisone or CNI and a mycophenolate compound. Eligible participants will undergo immunosuppressive withdrawal according to a pre-specified algorithm (see section 3.1.3) with the goal of achieving complete discontinuation of immunosuppressive medication between 24 and 45 weeks after initiation of withdrawal. Participants will undergo protocol biopsies at 1 and 3 years following drug discontinuation. Successfully weaned participants who remain rejection-free will undergo 3 years of follow-up after the last dose of immunosuppression. Participants who resume immunosuppression, due to biopsy-confirmed or presumed rejection, will undergo 3-3.5 years of follow-up. Study investigators and participants will remain blinded to the results of tolerance biomarkers for individual participants until the end of the study. The tolerance biomarker results will be evaluated as a group once the primary endpoint is reached. Participants will be enrolled by a consortium of US transplant sites. A parallel study with a harmonized clinical and mechanistic protocol is planned for a similar number of participants at EU sites.

Study Duration	Total study duration will be up to 391 weeks (7.5 years): • The enrollment phase will be up to 156 weeks (3 years). • The duration of the study for an individual participant may range from approximately 188 to 235 weeks, comprised of a screening phase of approximately 8 weeks, a withdrawal phase of approximately 24 to 45 weeks and a follow-up phase of approximately 156 to 182 weeks. The study primary endpoint, measured 52 weeks after the last participant's completion of immunosuppression withdrawal, could be achieved as early as 240 weeks (4.5 years), or as late as 265 weeks (5 years) after enrollment of the first participant.
Primary Objective	The primary objective is to determine whether a peripheral blood or graft lymphocyte phenotype of immune senescence or exhaustion is different between operationally tolerant and non-tolerant liver allograft recipients.
Primary Endpoint	The primary endpoint is the proportion of participants who achieve operational tolerance 52 weeks after completion of immunosuppression withdrawal defined by: a. No evidence of rejection since enrollment in the study. b. A liver biopsy at 52 weeks following discontinuation of all immunosuppression demonstrating absence of rejection per the Banff global assessment criteria1-3. The central pathology read will be used for this determination. c. A liver biopsy at 52 weeks following discontinuation of all immunosuppression demonstrating histological stability consistent with operational tolerance per Banff 2012 criteria, defined as the absence of the histological findings in Table 1. The central pathology read will be used for this determination. For the purposes of evaluating donor-specific exhaustion, operationally tolerant participants will be compared to those who fail immunosuppression withdrawal.
Secondary Endpoints	**Safety** 1. The proportion of participants who develop DSA or de novo anti-HLA antibodies after initiation of immunosuppression withdrawal. 2. The incidence, severity, and timing of acute rejection, steroid resistant rejection, and chronic rejection. 3. The incidence and progression of graft fibrosis in tolerant versus nontolerant patients. 4. The incidence of graft loss. 5. The incidence of all–cause mortality. 6. The incidence of study-related SAEs. **Effectiveness** 1. The proportion of operationally tolerant subjects who remain free of rejection at 3 years after completing immunosuppression withdrawal. 2. Changes in renal function (defined as estimated GFR calculated by CKD-EPI: http://www.qxmd.com/calculate-online/nephrology/ckd-

	epiegfr) in tolerant versus non-tolerant participants at 1, 2 and 3 years after completing immunosuppression withdrawal. 3. Changes in Quality of Life in tolerant versus non-tolerant participants and in all participants at baseline versus the end of study participation, as measured by the NIDDK Liver Transplantation Database Quality of Life Form (see Appendix 7). 4. Changes in SF-36 (see Appendix 6) in tolerant versus non-tolerant participants and in all participants at baseline versus the end of study participation. 5. Predictive value and the correlative value of the following parameters with regard to operational tolerance: a. Time post-transplant b. Recipient age **Mechanistic** 1. Mechanistic endpoints may assess both the predictive value and the correlative value of the following parameters with regard to operational tolerance: a. Phenotypic or molecular markers of immune senescence and/or exhaustion in T cells recovered from peripheral blood or liver tissue b. Donor-specific antibody c. Intra-allograft C4d d. Recipient anti-donor reactivity in vitro e. mRNA transcripts in blood and in liver allograft biopsies f. Thymic T cell output g. Peripheral blood and tissue miRNA expression h. Iron metabolism gene and serum proteins. i. Microchimerism by STR genotyping j. Gut microbiome profile
Inclusion Criteria	**Recipient** Recipient participants must meet all of the following criteria to be eligible for this study: 1. At the time of screening: • 18 to 50 years old and more than 6 years post-transplant OR • Greater than 50 years old and more than 3 years post-transplant 2. Recipient of either deceased or living donor liver transplant. 3. Recipient of single organ transplant only 4. Must have a screening liver biopsy that fulfills the criteria in Table 3 based on the central pathology reading 5. Liver function tests (Direct bilirubin, ALT) less than twice the upper limit of normal (ULN). ULN values for liver function tests will

	be defined by ranges from Harrison's Principles of Internal Medicine, 18th edition. 6. Receiving calcineurin inhibitor (CNI) based maintenance immunosuppression. Participants may also concurrently receive: • low dose mycophenolate mofetil (MMF ≤ 1500 mg daily) or mycophenolic acid (≤ 1080 mg daily) OR • prednisone ≤ 7.5 mg daily or equivalent corticosteroid. 7. Ability to sign informed consent **Living Donor** Living donor participants must meet all of the following criteria to be eligible for this study: 1. At the time of screening: ≥18 years old 2. Living donor of the liver allograft of an enrolled recipient participant 3. Ability to sign informed consent 4. Willingness to donate appropriate biologic samples
Exclusion Criteria	**Recipient** Recipient participants who meet any of the following criteria will not be eligible for this study: 1. History of HCV infection (defined as a positive HCV antibody test) 2. Positive antigen-antibody immunoassay for HIV-1/2 3. Serum positivity for HBV surface antigen or HBV-DNA 4. History of immune-mediated liver disease in which immunosuppression discontinuation is inadvisable (autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis) 5. Any medical condition associated with a likely need for systemic corticosteroid administration, e.g., reactive airways disease 6. Prospective baseline liver biopsy showing any of the following: a) acute rejection according to the Banff global assessment criteria1-3; b) early or late chronic rejection according to the Banff global assessment criteria1-3; c) inflammatory activity and/or fibrosis in excess of permissive criteria according to Banff 2012 criteria (see Table 3); d) any other histological findings that might

	make participation in the trial unsafe. Eligibility will be determined by the findings on the central biopsy reading. 7. Rejection within the 52 weeks prior to screening 8. Estimated GFR <40 ml/min as calculated by CKD-EPI method (to mitigate the risk of worsening renal failure should rejection occur and high level of CNI be required) 9. The need for chronic anti-coagulation that cannot be safely discontinued for a minimum of 1 week to safely perform a liver biopsy 10. Pregnant females and females of childbearing potential who are not using an effective method of birth control 11. Current drug or alcohol dependency 12. Inability to comply with the study visit schedule and required assessments, including frequent liver function monitoring and protocol biopsies 13. Inability to comply with study directed treatment 14. Any medical condition that in the opinion of the principal investigator would interfere with safe completion of the trial 15. Participation in another interventional clinical trial within the 4 weeks prior to screening **Living Donor** Living donor participants who meet any of the following criteria will not be eligible for this study: 1. Any medical condition, such as anemia, coagulopathy, etc., that in the opinion of the principal investigator would interfere with safe participation in the trial.

2. INTRODUCTION

This statistical analysis plan (SAP) only includes analyses related to the 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 data 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

Intent to treat (ITT) sample: All participants who provide informed consent for study participation and begin immunosuppression withdrawal.

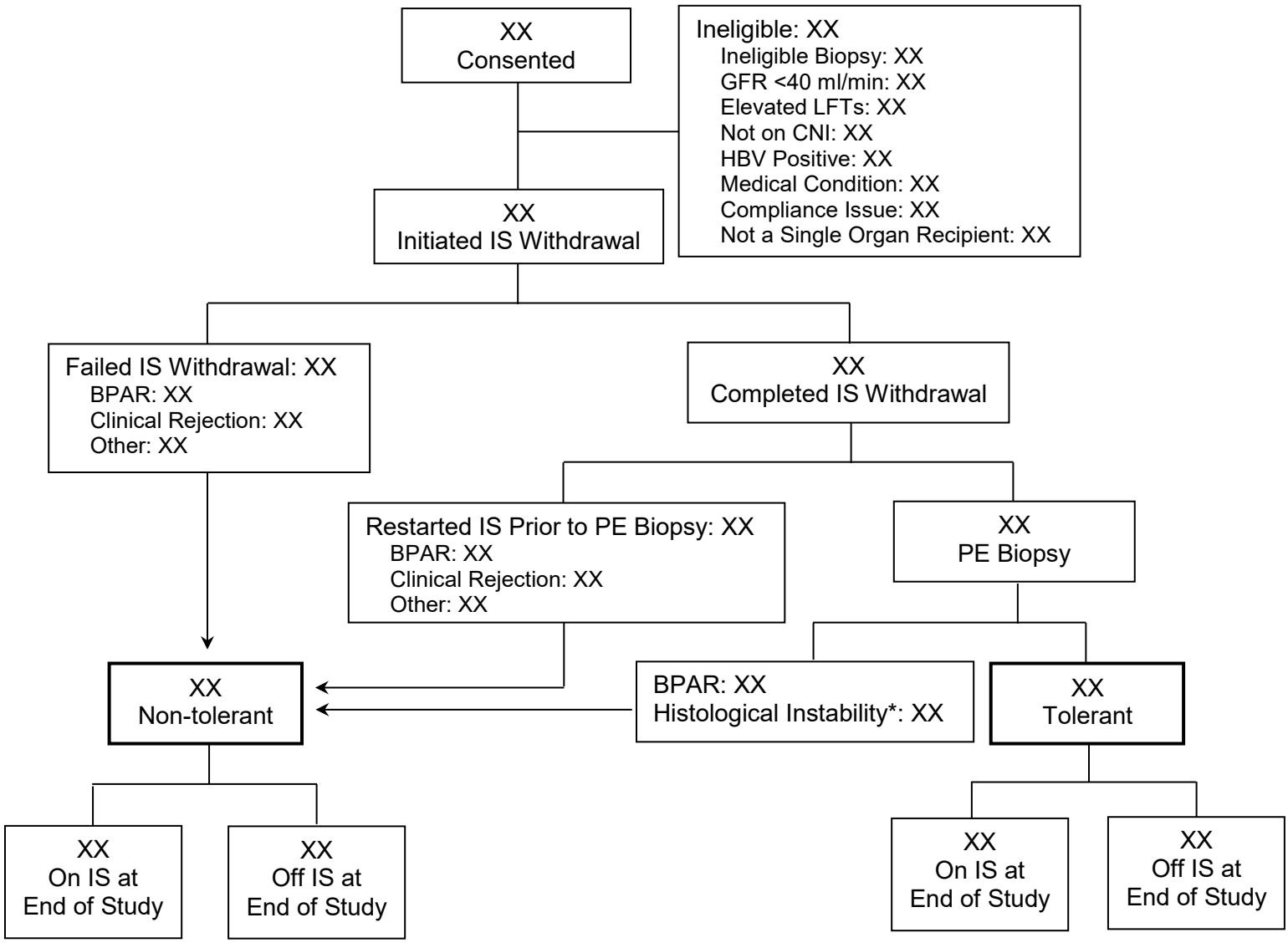
Per protocol (PP) sample: All participants who attempt immunosuppression withdrawal and do not have any unacceptable major protocol deviations. Subjects experiencing major protocol deviations may be excluded at the discretion of the study team during a blinded review of deviations.

5. STUDY SUBJECTS

5.1. Disposition of Subjects

The disposition of all enrolled subjects will be summarized in a table and listed. The information will also be presented in a consort diagram as outlined below.

The numbers and percentages of subjects enrolled, screen failures, and those that initiated withdrawal, including details of withdrawal status, will be presented. The numbers and percentages of those who complete the study or are lost to follow-up with also be presented. Additionally, reasons for early termination from the study and the reasons for discontinuing withdrawal will be presented.



* Histology instability includes biopsy results that show no rejection per Banff, but show the presence of at least one of the histological findings in Table 1.

5.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for the ITT sample. Characteristics to be summarized include age at transplant, age at enrollment, race, ethnicity, sex, body weight at screening, height, donor type, graft type, reason for transplant, time from transplant to enrollment, and study entry medication type and dose.

6. STUDY OPERATIONS

6.1. Major 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 7 study sites. Due to the small number of subjects in the study and/or at a particular site, 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

Unscheduled visits may also occur throughout the study.

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

7.2. Primary Endpoint

The primary endpoint is the proportion of participants who achieve operational tolerance 52 weeks after completion of immunosuppression withdrawal defined by:

- No evidence of rejection since enrollment in the study.
- A liver biopsy at 52 weeks following discontinuation of all immunosuppression demonstrating absence of rejection per the Banff global assessment criteria. The central pathology read will be used for this determination.
- A liver biopsy at 52 weeks following discontinuation of all immunosuppression demonstrating histological stability consistent with operational tolerance per Banff 2012 criteria, defined as the absence of the histological findings in Table 1. The central pathology read will be used for this determination.

7.2.1. Computation of the Primary Endpoint

A participant will be considered operationally tolerant if they have successfully withdrawn from all immunosuppression and remain off all immunosuppression for 52 weeks, have no evidence of rejection since enrollment, and have a liver biopsy at 52 weeks read centrally that shows both

the absence of rejection and has histological stability consistent with Table 1. Otherwise, the participant will be considered non-tolerant.

Participants who are non-evaluable at 52 weeks following discontinuation of all immunosuppression time point due to inadequate follow-up, lack of biopsy, or premature study termination will be considered non-tolerant for the purpose of evaluating the primary endpoint.

Biopsy results may be as early as 44 weeks following the discontinuation of all immunosuppression. This is because a for-cause liver biopsy visit that occurs within 8 weeks prior to a scheduled protocol visit takes the place of a scheduled protocol visit, thus a for-cause biopsy within 8 weeks of Visit 204 can be evaluated for the primary endpoint. Any biopsies done earlier than 44 weeks prior to discontinuation of all immunosuppression will be reviewed for inclusion by the SMT on a case by case basis. Any primary endpoint biopsy performed later than 52 weeks (+4 weeks) will be utilized.

All immunosuppression dosing information is collected as a daily record. Therefore, any immunosuppression information will not have an associated window.

7.2.2. Primary Analysis of the Primary Endpoint

The proportion of participants who are identified as operationally tolerant with a corresponding two-sided 95% exact binomial confidence interval. This will be analyzed using the ITT and PP samples.

The results will be presented in a table as shown below.

	Total (N = XX) n (%)
Proportion Tolerant [1]	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)

[1] Subjects who remain off immunosuppression for at least 52 weeks without evidence of rejection and demonstrate histological stability, as determined by a biopsy performed 52 weeks after the completion of immunosuppression withdrawal, are considered tolerant.

7.3. Secondary Endpoints

All secondary endpoints will be analyzed as described in Table 7-2. Mock displays for these results are outlined in the Appendix.

Table7-2 Table of Endpoints and Analysis Methods

Endpoint	Method	Population
Primary		
The proportion of participants who achieve operational tolerance 52 weeks after completion of immunosuppression withdrawal.	Proportion of participants who achieve operational tolerance 52 weeks after completion of immunosuppression withdrawal with a corresponding two-sided 95% exact binomial confidence interval.	ITT, PP
Secondary		
Safety		
The proportion of participants who develop DSA or de novo anti-HLA antibodies after initiation of immunosuppression withdrawal.	Proportion of participants who develop DSA or de novo anti-HLA antibodies after initiation of immunosuppression withdrawal with a corresponding two-sided 95% exact binomial confidence interval.	ITT, PP
The incidence, severity, and timing of acute rejection, steroid resistant rejection, and chronic rejection.	**Acute Rejection:** Incidence will be measured as the proportion of participants who have acute rejection (per Banff criteria) with a corresponding two-sided 95% exact binomial confidence interval. Severity will be measured according to the Banff grade. Acute rejection can be expressed as mild, moderate or severe. This will be summarized descriptively with counts and percentages. **Steroid Resistant Rejection:** Incidence will be measured as the proportion of participants who have steroid resistant rejection (rejection requiring antibody treatment) with a corresponding two-sided 95% exact binomial confidence interval. All steroid resistant rejections are considered severe in nature and nothing further will be summarized. **Chronic Rejection:** Incidence will be measured as the proportion of participants who have chronic rejection (per Banff criteria) with a corresponding two-sided 95% exact binomial confidence interval.	ITT, PP

	Severity will be measured according to the Banff grade. Chronic rejection can be expressed as early or late. This will be summarized descriptively with counts and percentages. For all rejection types, timing will be expressed as the time from the initiation of immunosuppression withdrawal to the time of the first biopsy showing acute rejection and will be summarized descriptively. All data presented will use the central read.	
The incidence and progression of graft fibrosis in tolerant versus non-tolerant patients.	Fibrosis will be looked at in two ways—by ISHAK and LAFSc. ISHAK is a measure taken directly from the central pathology read. LAFSc is calculated as the sum of three measures that summarize fibrosis in the portal tract (portal_fibrosis per central read), sinusoids (disse_fibrosis per central read), and centrolobular vein (central_fibrosis per central read). For both ISHAK and LAFSc, a subject will be considered as having graft fibrosis if the score is greater than or equal to 2. Incidence of graft fibrosis will be summarized descriptively with a proportion and corresponding two-side 95% exact binomial confidence interval using Ishak Score and LAFSc, separately, for tolerant and non-tolerant subjects. Change in ISHAK score from baseline biopsy to the last available biopsy will be calculated. Descriptively statistics will be calculated separately for tolerant and non-tolerant subjects. This same process will be repeated for LAFSc. All scores utilized will be from the central read.	ITT, PP
The incidence of graft loss.	Proportion of participants who have lost their liver graft with a corresponding two-sided 95% exact binomial confidence interval.	ITT, PP
The incidence of all–cause mortality.	Proportion of participants who have died with a corresponding two-sided 95% exact binomial confidence interval.	ITT, PP

The incidence of study-related SAEs.	Proportion of study-related SAEs as assessed by the NIAID Medical Monitor with a corresponding two-sided 95% exact binomial confidence interval.	ITT, PP
Efficacy		
The proportion of operationally tolerant subjects who remain free of rejection at 3 years after completing immunosuppression withdrawal.	Proportion of tolerant participants who remain off all IS and free of rejection 3 years after completing immunosuppression withdrawal with a corresponding two-sided 95% exact binomial confidence interval.	Tolerant Subjects
Changes in renal function (defined as estimated GFR calculated by CKD-EPI) in tolerant versus non-tolerant participants at 1, 2 and 3 years after completing immunosuppression withdrawal for tolerant subjects and failure of withdrawal for non-tolerant subjects.	Baseline eGFR will be calculated from data collected at Visit 0. If Visit 0 data is missing, Visit -1 data will be used. Data collected closest to 1-year post completion/failure, within +/- 2 months, will be used to calculate eGFR at 1. The same process will be followed for 2 and 3-years after completion/failure. 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 Change in eGFR will be calculated as: 1-year post completion/failure eGFR – baseline eGFR Thus positive value will indicate increase kidney function. Changes will be summarized descriptively by tolerance status.	ITT, PP
Changes in Quality of Life in tolerant versus non-tolerant participants and in all participants at baseline versus the end of study participation, as measured by the NIDDK Liver Transplantation Database Quality of Life Form.	The questionnaire filled out at Visit 0 will serve as the baseline questionnaire. The questionnaire filled out closest to the end of study participation will serve as the end of study questionnaire. For subjects	ITT, PP

	with complete follow-up, this will be the questionnaire completed at Visit 208 or Visit 306. The questionnaire will be summarized into five domains as outlined by Bell et al. The domains include measures of disease, psychological status, personal function, social and role function, and general health perception. The change in each domain will be calculated as: Domain Score at End of Study – Domain score at Baseline. The baseline, end of study, and change from baseline domain scores will be summarized descriptively by tolerance status. If the data permits, a spaghetti plot visualizing each component score over time will be produced. Each graph will have two panels—one depicting the tolerant subjects, the other depicting the non-tolerant subjects.	
Changes in SF-36 (see Appendix 6) in tolerant versus non-tolerant participants and in all participants at baseline versus the end of study participation.	The questionnaire filled out at Visit 0 will serve as the baseline questionnaire. The questionnaire filled out closest to the end of study participation will serve as the end of study questionnaire. For subjects with complete follow-up, this will be the questionnaire completed at Visit 208 or Visit 306. The SF-36 questionnaire will be scored in accordance with the scoring algorithm (Version 2 scoring algorithm used via quality metric) and summarized with the Physical Component Score (PCS) and Mental Component Score (MCS). The change in the component scores will be calculated as: Component Score at End of Study – Component Score at Baseline. The baseline, end of study, and change from baseline domain scores will be summarized descriptively by tolerance status. If the data permits, a spaghetti plot visualizing each component score over time will be produced. Each graph will have two panels—one depicting the tolerant subjects, the other depicting the non-tolerant subjects.	ITT, PP

Predictive value and the correlative value of the following parameters with regard to operational tolerance: *a. Time post-transplant* *b. Recipient age*	A logistic regression model will be utilized to estimate the probability of operational tolerance. Time post-transplant and recipient age will be included in the model as continuous covariates. Odds ratios and 95% confidence intervals will be presented.	ITT, PP

8. SAFETY EVALUATION

8.1. Overview of Safety Analysis Methods

All safety analyses will be carried out on participants in the ITT sample. Screen failure data will be presented separately as specified below. Missing safety information will not be imputed. These analyses will not be stratified by site.

8.2. 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 18.0). The severity of AEs will be classified using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. 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.

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:

- All AEs
- AEs indicated as serious
- AEs with an outcome of death
- AEs that were reported as being related to immunosuppression withdrawal, blood draw, and/or biopsy
- AEs reported by severity
- AEs reported in >10% of subjects

In addition, AEs classified by MedDRA SOC and preferred term will be summarized overall for each of the following:

- All AEs

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 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. Percentages will be based on the number of subjects in the safety population.

A data listing will be provided for all AEs. A separate data listing will be presented for screen failures.

8.3. Deaths and Serious Adverse Events

Serious adverse events (SAEs) will be listed and summarized in the same manner described in Section 8.2. A separate display listing and summarizing death, including time of death relative to ISW and cause of death, will also be created.

8.4. Clinical Laboratory Evaluation

Clinical laboratory measurements include serum chemistry and hematology. Results will be converted to standardized units where possible.

Laboratory data will be plotted to show patterns over time for alkaline phosphatase, AST, ALT, GGT, total bilirubin, direct bilirubin, creatinine, WBC, and glucose. For these tests, 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. All other tests will not be plotted.

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

8.5.1. Vital Signs

Descriptive statistics of vital signs results will be summarized overall. Data listings sorted by subject, vital sign parameter, and time of assessment will be provided for vital signs measurements.

8.5.2. Physical Examinations

An indication of whether the examination was performed and the date of the examination were collected. Because details of events that are abnormal and meet AE reportable criteria will be entered on the medical history or AE form as appropriate, this data will be summarized with medical history and AEs, respectively.

8.5.3. Other Safety Measures

8.5.3.1. IS Minimization

The baseline IS dose (dose immediately prior to the initiation of withdrawal) will be compared to IS dose at the end of study participation for each subject that initiated IS withdrawal. Each comparison will be classified as 'more', 'less', or the 'same'. The count and percentage of each category will be presented.

For subjects that do not switch medication types (i.e. start on tacrolimus monotherapy and finish on tacrolimus monotherapy) this will be a straight numeric comparison of total daily dose.

For subjects on multiple drugs or subjects that switch medication types (i.e. start on tacrolimus and switch to cyclosporine), the SMT will complete a blinded review of the data and make a determination of 'more', 'less' or 'same'.

8.5.3.2. Rejection Treatment

Immunosuppression medication changes will be summarized for each rejection episode. Specifically, the count and percentage of each episode receiving a dose increase, conversion to a different immunosuppression medication, an addition of a second immunosuppression drug, administration of corticosteroids and administration of an antibody treatment.

8.5.3.3. Resolution of Rejection

Baseline ALT and GGT values are calculated as the average of the two following time points: the value taken at the screening visit and the value taken 7 (+/- 2 days) before the initiation of immunosuppression withdrawal. If only a single value is collected prior to initiation of withdrawal, this single value will be used.

For subjects with a baseline value less than the upper limit of the normal range (ULN) an episode of allograft dysfunction begins when either the ALT or GGT value is greater than or equal to 2 times the ULN. The ULN for ALT is 41 U/L and for GGT is 58 U/L.

For subjects with a baseline value greater than or equal to the ULN, an episode of allograft dysfunction beings when either the ALT or GGT value is greater than or equal to 2 times the baseline value.

An episode of allograft dysfunction involving elevated ALT (with or without elevated GGT), will be considered as resolved when ALT is ≤ 1.5 times baseline levels. This includes cases where the GGT elevates initially but does not resolve before ALT also elevates. If allograft dysfunction involved elevated GGT alone, it will be considered resolved when GGT is less than or equal to 1.5 times baseline.

The resolution of rejection will be expressed as the time from the beginning of allograft dysfunction to the time to the resolution of rejection, per the definitions defined above. Time will be expressed as days and summarized descriptively.

8.5.3.4. Stopping Rules

Events contributing to the stopping rules will be summarized in a listing with relevant details.

9. OTHER ANALYSES

10. INTERIM ANALYSES AND DATA MONITORING

No formal interim efficacy analysis was planned.

The progress of the study will be monitored by the Data and Safety Monitoring Board (DSMB). The Transplant DSMB will be chartered to review safety data and to make recommendations regarding continuation, termination, or modification of the study. The DSMB will formally review the safety data at least yearly.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the National Institute of Allergy and Infectious Diseases (NIAID) medical monitor or protocol chair to warrant review, or when an event occurs that could contribute to a predefined stopping rule specified in the protocol.

Findings will be reported to Institutional Review Boards (IRBs).

11. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

12. REFERENCES

Belle SH, Porayko MK, Hoofnagle JH, Lake JR, Zetterman RK. Changes in quality of life after liver transplantation among adults. National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Liver Transplantation Database (LTD) Liver Transpl Surg. 1997; 3: 93–104.

13. APPENDIX

Table X. Safety Endpoints

	Total (N = XX)
Development of DSA or de novo anti-HLA antibodies, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)
Incidence of acute rejection, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)
Severity of acute rejection per Banff criteria, n (%)	
Mild	X (X.X%)
Moderate	X (X.X%)
Severe	X (X.X%)
Time to first acute rejection, median (IQR)	X.X (X-X)
Incidence of steroid resistant rejection, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)
Time to first steroid resistant rejection, median (IQR)	X.X (X-X)
Incidence of chronic rejection, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)
Severity of acute rejection per Banff criteria, n (%)	
Early	X (X.X%)
Late	X (X.X%)
Time to first chronic rejection, median (IQR)	X.X (X-X)
Graft Loss, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)
All–cause Mortality, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)
Study-related Serious Adverse Events, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)

Table X. Long-term Tolerance

	Total (N = XX)
Tolerant Subjects Free of Rejection 3 years after Completing Immunosuppression Withdrawal, n (%)	X (X.X%)
95% Exact Binomial Confidence Interval	(X.X, X.X)

Table X. Graft Fibrosis in Tolerant vs. Non-tolerant Subjects

	Tolerant (N = XX)	Non-tolerant (N = XX)	Total (N = XX)
Graft Fibrosis, n (%)			
ISHAK	X (X.X%)	X (X.X%)	X (X.X%)
LAFsc	X (X.X%)	X (X.X%)	X (X.X%)
95% Exact Binomial Confidence Interval			
ISHAK	(X.X, X.X)	(X.X, X.X)	(X.X, X.X)
LAFsc	(X.X, X.X)	(X.X, X.X)	(X.X, X.X)
Change from Eligibility Biopsy to End of Study Biopsy, median (IQR)			
ISHAK	X.X (X-X)	X.X (X-X)	X.X (X-X)
LAFsc	X.X (X-X)	X.X (X-X)	X.X (X-X)

Table X. GFR in Tolerant vs. Non-tolerant Subjects

	Tolerant (N = XX)	Non-tolerant (N = XX)	Total (N = XX)
Change in eGFR[1], median (IQR)			
Year 1	X.X (X-X)	X.X (X-X)	X.X (X-X)
Year 2	X.X (X-X)	X.X (X-X)	X.X (X-X)
Year 3	X.X (X-X)	X.X (X-X)	X.X (X-X)

[1] Estimated GFR (eGFR) is calculated by the CKD-EPI method.

Table X. Quality of Life Measures in Tolerant vs. Non-tolerant Subjects

	Tolerant (N = XX)	Non-tolerant (N = XX)	Total (N = XX)
Change in SF-36[1], median (IQR)			
Physical Component Score (PCS)	X.X (X-X)	X.X (X-X)	X.X (X-X)
Mental Component Score (MCS)	X.X (X-X)	X.X (X-X)	X.X (X-X)
Change in NIDDK[1], median (IQR)			
Measures of Disease	X.X (X-X)	X.X (X-X)	X.X (X-X)
Psychological Status	X.X (X-X)	X.X (X-X)	X.X (X-X)
Personal Function	X.X (X-X)	X.X (X-X)	X.X (X-X)
Social and Role Function	X.X (X-X)	X.X (X-X)	X.X (X-X)
General Health Perception	X.X (X-X)	X.X (X-X)	X.X (X-X)

[1] Change is calculated as the difference between the last available QOL assessment and the QOL assessment collected at baseline.

Table X. Logistic Regression Model Predicting Operational Tolerance

Variable	
Time post-transplant	
Odds ratio per increase of 1 year (95% CI)	X.X (X.XX, X.XX)
Recipient Age	
Odds ratio per increase of 1 year (95% CI)	X.X (X.XX, X.XX)