FINAL STATISTICAL ANALYSIS PLAN
Version 1.0

Immunosuppression Withdrawal for Pediatric Living-donor Liver Transplant Recipients

PROTOCOL NUMBER ITN029ST

SPONSOR

This clinical study is supported and conducted by the Immune Tolerance Network, which is sponsored by the National Institute of Allergy and Infectious Diseases.

Immune Tolerance Network

[redacted]

National Institute of Allergy and Infectious Diseases

[redacted]

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DOCUMENT VERSION CONTROL

Version Number	Date	Comments/Changes
0.1	06 October 2009	First Draft
1.0	23 April 2010	Final

APPROVALS

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LIST OF ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
AMA	antimitochondrial antibody
ANA	antinuclear antibody
ALKMA	anti-liver-kidney microsome antibody
ASMA	anti-smooth-muscle antibody
CFR	Code of Federal Regulations
CMV	cytomegalovirus
CRF	case report form
CsA	cyclosporine
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation

D5W	5% dextrose and water
DSMB	Data Safety and Monitoring Board
EBV	Epstein-Barr virus
EDTA	ethylenedinitrilo tetra-acetic acid
ELISPOT	enzyme-linked immunospot
GCP	good clinical practice
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
HCC	hepatocellular carcinoma
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IRB	institutional review board
IS	immunosuppression
INR	international normalized ratio
ICH	International Conference on Harmonization
IND	investigational new drug
IRB	Institutional review board
ITN	Immune Tolerance Network
ITT	intent-to-treat
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MMF	mycophenolate mofetil
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria for Adverse Events
NIAID	National Institute of Allergy and Infectious Diseases
NK cells	natural killer cells
PBC	primary biliary cirrhosis
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCP	*Pneumocystis carinii* pneumonia
PCR	polymerase chain reaction

PI	principal investigator
PP	per protocol
SAE	serious adverse event
SAEC	safety adverse event coordinator
SAP	statistical analysis plan
SMT	study management team
TMP	trimethoprim
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
WHO	World Health Organization

LIST OF IN-TEXT TABLES AND FIGURES

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1. INTRODUCTION

This statistical analysis plan only includes analyses related to the clinical endpoints. Mechanistic analyses will be performed at the ITN core facilities and a separate analysis plan will be created to detail the planned analyses. Relevant clinical data from the study will be submitted to the core facility to augment the mechanistic analyses.

2. CLINICAL BACKGROUND

Currently, transplantation of any solid organ incurs a lifelong burden of immunosuppression for the recipient. In spite of many advances, including the development of new agents, the basic premises of immunosuppression strategies remain unchanged and, as such, substantial metabolic, infectious, and neoplastic complications continue to threaten the recipient's life and well-being. Several reports, however, have shown that a significant proportion of liver recipients (19%–42%) can maintain normal allograft function without immunosuppression—the definition of "functional tolerance." Although drug weaning precipitates rejection in some recipients, most episodes are mild or moderate, are easily reversed, and do not result in long-term consequences.

These reports have motivated us to propose gradual and complete immunosuppression withdrawal in a highly selected subgroup of liver transplant recipients: those who underwent

living-donor liver transplantation as a child (<18 years of age) 4 or more years ago for diseases other than viral hepatitis and autoimmune liver disorders, who continue to have excellent graft function, and who are on a stable single-agent immunosuppression regimen. Recipients will be closely monitored during tapering to ensure expeditious recognition, diagnosis, and, if necessary, treatment of liver dysfunction.

The main clinical endpoints measure the outcome of immunosuppression withdrawal. They target the success rate of withdrawal; the duration for which recipients remain off of immunosuppression; and the overall incidence, severity, and timing of rejection. The current trial also encompasses a complementary scientific effort to identify, quantify, and characterize donor-specific immune responses, immunologic interactions, and genetic characteristics that may predict or correlate with functional tolerance.

3. PROTOCOL SUMMARY

3.1 Study Objectives

3.1.1 Primary Objective

The primary objective is to evaluate allograft tolerance in pediatric recipients of livers from parental living related donors.

3.1.2 Secondary Objectives

The secondary objectives of this study are

- To assess the safety of immunosuppression withdrawal in pediatric recipients of livers from parental living related donors;
- To assess the durability of allograft tolerance;
- To define profiles of immunologic and genetic features present before or during gradual withdrawal of immunosuppression that distinguishes tolerant and nontolerant allograft recipients.
- To define profiles of immunologic and genetic features associated with allograft rejection.

3.2 Study Endpoints

3.2.1 Primary Endpoints

The primary endpoint is the proportion of subjects who are successfully withdrawn from immunosuppression, which is defined as those who remain off immunosuppression for at least 1 year.

3.2.2 Secondary Endpoints

The following secondary endpoints are related to the safety of immunosuppression withdrawal.

- The proportion of subjects who have graft loss or who die after initiation of immunosuppression withdrawal.
- The time from the start of immunosuppression withdrawal to
  - the first episode of acute rejection requiring treatment,
  - the second episode of acute rejection not requiring treatment, or
  - the diagnosis of chronic rejection.
- The distribution of histologic severity among rejection episodes.
- The incidence of adverse events.
- Changes in renal function, blood pressure, cholesterol level, and glucose control.

Other secondary endpoints include

- Immunosuppression-free duration, defined as the time from discontinuation of immunosuppression to end of trial participation or to time of restarting immunosuppression;
- Results of mechanistic studies and clinical assessments at various time points that allow for the definition of profiles associated with tolerance;
- Results of mechanistic studies and clinical assessments that allow for definition of profiles associated with liver allograft rejection.

3.3 Stratification Variables

Selected analyses will present data grouped by clinical phenotypes of tolerant and not-tolerant. A subject is defined to be tolerant if s/he is successfully withdrawn from immunosuppression for at least one year. The definition of tolerant corresponds to the primary endpoint.

3.4 Overall Study Design and Plan

This is a prospective multicenter, open-label, single-arm trial in which 20 pediatric recipients of parental living-donor liver allografts will undergo gradual withdrawal of immunosuppression with the goal of complete withdrawal. Subjects on stable immunosuppression regimens with good organ function and no evidence of acute or chronic rejection or other forms of allograft dysfunction will be enrolled. Subjects will undergo gradual withdrawal of immunosuppression and will be followed for a minimum of 4 years after completion of immunosuppression withdrawal. Immunologic and genetic profiles will be collected at multiple time points and compared between tolerant and nontolerant subjects.

3.5 Study Population

Subjects will be selected from those pediatric recipients of parental living related donor hepatic allografts with adequate and stable graft function and no evidence of rejection or

significant allograft dysfunction. Subjects must meet all of the inclusion criteria and none of the exclusion criteria outlined in the following section.

3.5.1 Inclusion and Exclusion Criteria

Inclusion Criteria

- Living-donor liver transplantation from a parental donor.
- Age less than 18 years at the time of transplantation.
- At least 4 years since transplantation.
- Availability and willingness of parental liver donor to participate in the trial.
- Liver biopsy at screening demonstrating no evidence of acute or chronic rejection and a less than stage 2 fibrosis on the Ishak scale.
- Negative urine pregnancy test at entry and agreement to use a medically acceptable form of birth control during the study for women of childbearing potential.
- Negative purified protein derivative (PPD) test results or history of appropriate treatment.

Exclusion Criteria

- Indication for transplantation liver failure due to autoimmune disease, such as autoimmune hepatitis, primary sclerosing cholangitis, or primary biliary cirrhosis.
- Hepatitis B infection as defined by the presence of HBSAg or active treatment for hepatitis B.
- Hepatitis C infection as defined by the presence of antibody against hepatitis C.
- Serologic evidence of autoimmunity defined as abnormal antinuclear, anti-smooth-muscle, antimitochondrial, or anti-liver-kidney microsomal antibody titers greater than or equal to 1:160.
- Transplantation of a second organ before, simultaneously, or after liver transplantation; or liver retransplantation.
- Aspartate or alanine aminotransferase (AST or ALT) greater than 2 times the upper limit of normal.
- Total bilirubin and direct bilirubin, and either alkaline phosphatase or gamma-glutamyl transferase (GGT) greater than 2 times the upper limit of normal.
- Clinically significant change in hepatic function in the past 26 weeks.
- Glomerular filtration rate (GFR) less than 40 mL/min/1.73 m2.
- Immunosuppression with
    - 50% dose increase in a current agent within 26 weeks of screening, or
    - more than one agent within 52 weeks of screening.
- Any systemic illness requiring or likely to require immunosuppressive drug use.
- Human immunodeficiency virus (HIV) infection.
- Pregnancy or breastfeeding.
- Unwillingness or inability to comply with study requirements and procedures.

3.6 Treatment Regimens

The subjects in this study will be gradually withdrawn from immunosuppression with the goal of complete withdrawal. The algorithm for immunosuppression withdrawal is shown in Figure 1. For tacrolimus, high dose is defined as ≥0.08 mg/kg/day; low dose is <0.08 mg/kg/day. For cyclosporine, high dose is defined as ≥3 mg/kg/day; low dose is <3 mg/kg/day.

During the withdrawal process (see Figure 1), additional monitoring at the current immunosuppression dose level may be done before continuing on to the next scheduled dose reduction. In such instances, gradual withdrawal must resume, or a biopsy must be performed, within 4 weeks of the previously scheduled dose reduction.

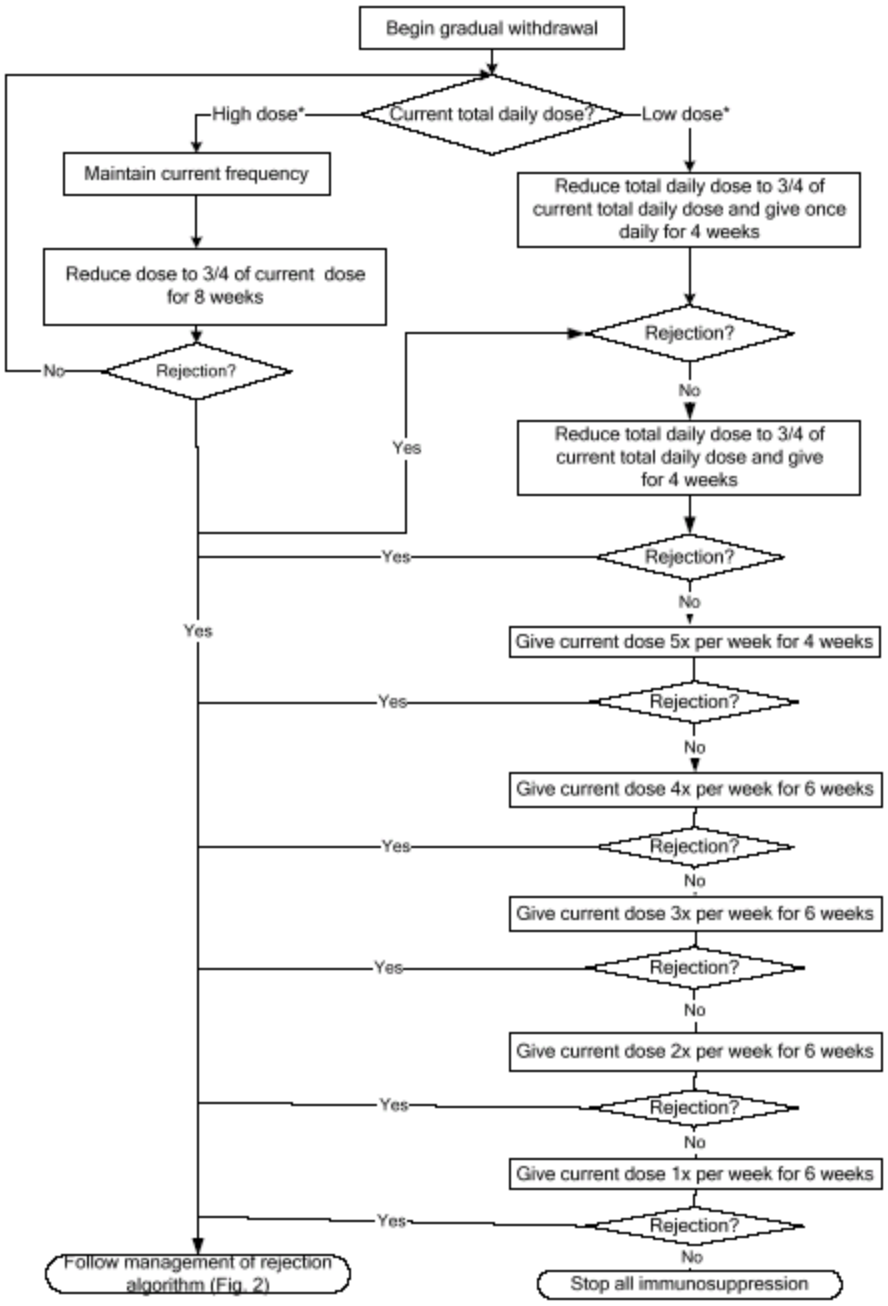

Figure 1. Immuosuppression withdrawal. (*See protocol section 5.1 for definition of *high dose* and *low dose*.)

3.7 Sample Size Determination

A total of 20 subjects will be enrolled in the study. There are no published data available with respect to the success of immunosuppression withdrawal exclusively in living-donor pediatric liver transplant subjects 4 years or more post transplant. The sample size of 20 is thus based on clinical experience and judgment in order to provide a broad, initial pilot estimate of the proportion of subjects meeting the primary endpoint in this subject population for which no prior data exist. For example, if 9 of 20 subjects are successfully withdrawn from immunosuppression, the point estimate of the success proportion is 45% (95% CI: 23.1%, 68.5%, based on the exact binomial method). If 4 of 20 are successfully tapered, the point estimate is 20% (95% CI: 5.7%, 43.7%). This broad, initial estimate can subsequently serve in the design of future immunosuppression withdrawal studies in this subject population.

4. 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 1 decimal place.
- In general, in frequency tables of variables with different categories (e.g., for discontinuation reason, race): if no subjects belong to a certain category, including Missing, across all treatment groups, then the printing of this category should be suppressed. Otherwise, if the number of subjects in a category for a treatment group is zero, then a zero should be displayed for the number, and the percentage should be left blank.
- Numeric variables will be summarized using n, mean, standard deviation (SD), median, min, max. The min/max will be reported at same level of significance as original data. The mean and median will be reported at 1 more significant digit than the precision of the data and SD will be reported at 2 more significant digits than the precision of the data. Descriptive statistics will be displayed in the order: n, mean, SD, median, min, max

- Following SAS default rules, the median will be reported as the average of the two middle numbers if the dataset contains even numbers.

- Test statistics including *t* and *z* test statistics will be reported to two decimal places.

- *P*-values will be reported to 3 decimal places if greater than 0.001. If less than 0.001 than report '<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.

- All statistical calculations will be performed using the SAS System version 9.1 (or higher).

- Tables and listings will be generated as RTF files with Courier New font, size 8. Figures will be generated as CGM files. Separate stacked files will be created for the tables, listings, and figures and converted to PDF prior to delivery.

- The program name, creation date, and data extraction date will be included in the footer of all displays (For example, Program: *Program Name* Creation Date: HH:MM/DDMMMYYYY Data Extraction Date: DDMMMYYYY)

- In general, columns with character values will have the header and column values left aligned. Numeric columns will be centered around their decimal place with headers also centered.

- In the first column, if text wraps onto another line indent 1 additional space. For subgroups, indent 2 spaces.

- Units of measurement - International units SI will be used for clinical laboratory data as a standard presentation. The metric system will be used whenever possible. Thus, weight will be in kilograms and height in centimeters. Temperature will be presented in Celsius degrees.

- For general footnotes, 'Note:' will come before any bracketed footnotes.

- All listings will be sorted in order of treatment, subject, and time of assessment (e.g., visit, time, and/or event).

- Episodes of rejection will be determined by the diagnoses from the central pathologist. All references to rejection episodes in this Statistical Analysis Plan refer to central pathology reads.

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.

5. ANALYSIS POPULATIONS

Intent to treat (ITT) sample will be defined as all subjects who have signed informed consent and are enrolled. For the purpose of these analyses enrollment is defined as beginning the screening process for participation in the study.

Per protocol (PP) samples will be defined as all subjects in whom immunosuppression withdrawal is attempted.

- Per protocol 1 (PP1) sample will be defined as all subjects in whom immunosuppression withdrawal is attempted.
- Per protocol 2 (PP2) sample will be defined as all subjects without inclusion/exclusion criteria protocol deviations in whom immunosuppression withdrawal is attempted.

6. STUDY SUBJECTS

Subject Profiles will be created for a subset of the data collected. Profiles are similar to data listings however instead of listing clinical data by the type of data, profiles will list the data by subject in order to provide a comprehensive overview of the clinical status of each subject.

6.1 Disposition of Subjects

The disposition of enrolled subjects throughout the study will be listed and summarized in tables.
The numbers and percentages of subjects enrolled, completed, discontinued from immunosuppression withdrawal, terminated from protocol, in each analysis population, as well as reasons for terminating the protocol early will be presented. For subjects discontinuing immunosuppression withdrawal early, the reasons for discontinuing withdrawal early will also be presented. Analysis populations will be derived according to the definitions in Section 5.

Reasons for terminating the study early include:

- 'Voluntary Withdrawal'
- 'Death'
- 'Lost to Follow Up'
- 'Adverse Event'
- 'Protocol Deviation'

- 'Other'

Reasons for discontinuing study medication/treatment early include:

- 'Protocol Deviation'
- 'Voluntary Discontinuation from Weaning'
- 'Terminated from Study'
- 'Second episode of mild rejection not requiring treatment'
- 'Any episode of Rejection (mild, moderate, or severe) requiring treatment'
- 'Chronic rejection'
- 'Requirement for use of corticosteroid bolus therapy'
- 'Abnormal LFTs in the absence of biopsy-proven rejection that do not substantially improve within maximum interval'
- 'Other'

6.2 Protocol Deviations

Protocol deviations will be listed by site and subject with information such as deviation date and who identified it, deviation type and details, steps taken to resolve the deviation, and an indicator for whether the subject will continue in the trial. Protocol deviations will also be summarized in tabular format. The summary will be reported by clinical phenotype and will include the total number of protocol deviations, the number of subjects with at least one deviation, and indicators for whether the deviation was an inclusion/exclusion criteria deviation and whether the subject will continue in the trial.

7. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

7.1 Recipient and Donor Demographics

Summary descriptive statistics for demographic characteristics will be reported for the ITT sample, the PP1, and the PP2 sample by clinical phenotype.

Demographic data will include age at transplant, age at enrollment, sex, race (primary and secondary), ethnicity, weight at enrollment, and height; these data will be presented separately for recipients and donors in the following manner:

- Continuous data (e.g., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range as well as categorized into groups (e.g., Age of donors (years): 18-33, 34-49, 50-65)

- Categorical data (e.g., sex, race, and ethnicity) will be presented as frequencies and percentages.

Recipient demographic data will also be presented in a data listing by site and subject and will include birth date, age at enrollment, sex, primary and secondary race, ethnicity, height,

weight and BMI. Donor demographic data will be presented in a data listing by site and subject and will include birth date, age at enrollment, sex, primary and secondary race, and ethnicity.

8. MEASUREMENTS OF TREATMENT COMPLIANCE

Compliance to immunosuppression withdrawal schedules will be presented in a data listing by subject and dose interval and will include dose, frequency, start date, stop date, duration, expected duration per protocol, and tapering status for dose interval. Expected duration per protocol for each dose interval is described above in Figure 1 (Section 3.6).

9. EFFICACY EVALUATION

9.1 Overview of Efficacy Analysis Issues

9.1.1 Handling of Dropouts or Missing Data

All efforts will be made via the querying and monitoring phases to avoid missing data. In general, missing data will not be imputed.

Missing endpoint data for Kaplan-Meier analyses (time off immunosuppression) will be accounted for by censoring the data after the last time point where the subject's relevant endpoints are known. This approach allows all subjects to contribute data at all points where subject's treatment response are known, excluding them only when their data is unknown.

Endpoint data that is missing due to the subject being lost to follow-up will be imputed as failures in the primary endpoint analysis.

9.1.2 Multicenter Studies

Study subjects will be recruited from 3 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. If any one site is a large enroller, basic descriptive analyses of baseline demographics, physical examination findings, medical history, and key study endpoints will be repeated for each site individually in order to allow qualitative exploration of site-to-site variability.

9.1.3 Assessment Time Windows

All visits, except as noted below, should be completed within ±2 weeks of the scheduled time points in the Schedule of Events.

-Screening

- Interval between visits −2 and −1 will not exceed 9 weeks.
- Interval between visits −1 and 0 will not exceed 2 weeks.

-Initiation or interruption of gradual withdrawal of immunosuppression

- Immunosuppression withdrawal (visit 0) may begin on the same day as the liver biopsy

(visit −1) or up to 2 weeks afterwards.

- Additional monitoring at the current immunosuppression dose level may be indicated before continuing with the gradual withdrawal of immunosuppression. If so, the maximum interruption period may not exceed 4 weeks (see section 5.1).

- Liver biopsies

- A liver biopsy to determine eligibility at screening (visit −1) may be performed no later than 9 weeks after the initial screening visit (visit −2).
- If a for-cause biopsy is performed within 6 weeks of a scheduled study visit that includes a protocol biopsy, then all the tests (including ITN Core Laboratory blood draws, clinical assessments, etc.) for the scheduled visit can be conducted at the time as the for-cause biopsy.
- For subjects who have successfully withdrawn from immunosuppression, a biopsy will be performed within 4 to 8 weeks after the last dose of immunosuppressant was taken. In order for the biopsy to be performed within this time period, the window for the visit on which this biopsy occurs will be extended to ±6 weeks.

Unscheduled visits may also occur throughout the study.

Measurements from unscheduled visits, scheduled visits obtained outside the scheduled assessment time windows, and windowed measurements not closest to the scheduled target day will be included in listings only and not in by-visit tabular displays.

9.2 Efficacy Variables

9.2.1 Primary Efficacy Variable

The proportion of subjects who are successfully withdrawn from immunosuppression, defined as those who remain off immunosuppression for at least 1 year. Subjects with acute rejection occurring after 1 year following complete immunosuppression withdrawal will be considered successfully withdrawn for the purpose of the primary endpoint.

9.2.2 Secondary Efficacy Variables

1) Immunosuppression-free duration: a continuous variable measured in days and is defined as: date of end of trial participation or date of restarting immunosuppression – date of completing withdrawal. All subjects completing withdrawal at any time will be included.

2) Time to Rejection: time from initiation of immunosuppression withdrawal to first episode of acute rejection or first diagnosis of chronic rejection measured in days and defined as date of first episode of acute rejection or date of first diagnosis of chronic rejection – date of

initiation of immunosuppression withdrawal. All subjects initiating withdrawal (PP1 sample) will be included.

3) Graft Loss or Death:

Proportion of subjects who have graft loss or who die after initiation of immunosuppression withdrawal: a continuous variable taking on values between 0 and 1, the numerator is the total number of subjects who experience either graft loss or death after initiation of immunosuppression withdrawal and the denominator is the total number of subjects initiating immunosuppression withdrawal (PP1 sample). This analysis will also be done on the PP2 sample.

9.3 Analysis Methods

Analysis methods for clinical end points, described in detail below, are summarized in Table 9-1.

Table 9-1 Table of Efficacy Variables and Analysis Methods

Endpoint (section reference)	Analysis Technique (section reference)	Analysis Sample
Primary		
Proportion of subjects who are successfully withdrawn from immunosuppression ‡	Proportion with exact 95% confidence interval	PP1, PP2
Secondary		
1) Immunosuppression-free duration	Median Time and 95% CI	All subjects completing withdrawal
2) Time to Rejection	Median Time and 95% CI	PP1
3) Graft Loss or Death	Proportion with exact 95% confidence interval	PP1, PP2

‡ Defined as those who remain off immunosuppression for at least 1 year.

9.3.1 Primary Efficacy Analyses

The proportion of subjects in the PP1 and PP2 samples who are successfully withdrawn will be descriptively summarized with 95% confidence intervals using an exact binomial method.

9.3.2 Secondary Efficacy Analyses

1) Median immunosuppression-free duration will be estimated for all subjects completing withdrawal. Duration will be displayed graphically with a Kaplan-Meier curve and the corresponding median and two-sided 95% confidence interval.

2) Median time from initiation of immunosuppression withdrawal to first episode of acute rejection or to first diagnosis of chronic rejection will be estimated for the PP1 sample. Duration will be displayed graphically with a Kaplan-Meier curve and the corresponding median and two-sided 95% confidence interval.

3) The proportion of subjects experiencing graft loss or death after initiation of immunosuppression withdrawal will be descriptively summarized for the PP1 and PP2 samples as well as the ITT sample with 95% confidence intervals using an exact binomial method.

9.4 Examination of Subgroups

No subgroup analyses are planned.

10. SAFETY EVALUATION

10.1 Overview of Safety Analysis Methods

All safety analysis will be carried out using the PP1 sample and the PP2 sample defined in Section 5 unless otherwise noted. Missing safety information will not be imputed. These analyses will not be stratified by site. Safety will be analyzed through the reporting of adverse events, vital signs, physical examinations, and changes in routine laboratory values.

Listings will be prepared for all safety measurements. All listings will be sorted in order of subject ID and time of assessment (e.g., visit, time, and/or event).

10.2 Extent of Exposure

The displays that are used to describe the total burden of immunosuppression and treatment compliance (detailed in section 8 and 9.2.2) will also be used to assess treatment exposure.

10.3 Adverse Events

All adverse events will be coded using the MedDRA dictionary, including classification by system organ class (SOC) and preferred term. The severity of adverse events (AE) will be classified using the National Cancer Institute's Common Toxicity Criteria for Adverse Events toxicity scale.

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:

- Adverse events (AEs)
- Serious Adverse Events (SAEs)
- Deaths
- AEs reported as definitely, probably, or possibly related to immunosuppression withdrawal
- AEs reported by severity

The tabulations described above will not include classifications by SOC and preferred term.

In addition, a summary table of adverse events classified by MedDRA SOC and preferred term will be provided for each of the following:

- AEs
- AEs by maximum severity
- AEs by relationship to immunosuppression withdrawal.

The summary tables will present the total number of events as well as the number and percentage of subjects experiencing the events by clinical phenotype. When reporting the number of AEs, if the same AE occurs for a subject on multiple occasions the event will be counted once for each occurrence. When reporting the number of subjects experiencing the events, a subject will only be counted once if they ever experience an event within the particular system organ class or preferred term. Percentages will be based on the number of subjects categorized in each clinical phenotype.

Separate data listings will be provided for all AE and AEs leading to treatment discontinuation/study termination ordered by subject id.

10.4 Deaths, Serious Adverse Events, and Other Significant Adverse Events

10.4.1 Deaths and Serious Adverse Events

SAEs will be listed and summarized in the same manner described in section 10.3. Separate displays listing and summarizing death, including time to death and cause of death will also be created.

10.4.2 Histologic Severity of Rejection Episodes

The Banff global assessment grade indicating histologic severity of rejection episodes will be summarized for the PP1, PP2 and ITT samples by clinical phenotype. Counts and percentages will be based on the number of rejection episodes.

10.4.3 Graft Loss

Data listings will be provided for graft loss, including the date of graft loss, study day, and primary cause of graft loss.

10.4.4 Allograft Dysfunction Episodes

Data listings will be provided for allograft dysfunction episodes, including the date, final clinical diagnosis, whether follow-up biopsies were performed, date of biopsies, and whether treatment was provided for rejection.

10.4.5 Change in Renal Function, Blood Pressure, Cholesterol, and Glucose Control

Renal function is measured by creatinine and GFR. The GFR for each subject will be estimated by the Schwartz Formula:

$$\text{GFR (mL/min/1.73 m}^2\text{)} = \frac{k(height)}{creatinine};$$

where $k = 0.33$ in premature infants, $k = 0.45$ in full term infants to 1 year old, $k = 0.55$ in children 1 year old up to 13 years old, $k = 0.65$ in adolescent males greater than 13 years old (k remains 0.55 for adolescent females greater than 13 years old); height is measured in cm; serum creatinine is measured in mg/dL.

Renal function, blood pressure, cholesterol, and glucose results and their respective changes from baseline will be summarized by visit for subjects in the PP1, PP2 and the ITT samples by clinical phenotype.

Spaghetti plots for post-screening visits will be created for renal function measurements, blood pressure, cholesterol, and glucose.

10.4.6 Infections

Infection site, start date, stop date, culture site, result, and organism will be listed for all infections for all subjects.

10.5 Clinical Laboratory Evaluation

Clinical laboratory measurements include serum chemistry, urinalysis, and hematology. Results will be standardized to the international system of units (SI), where possible. All clinical laboratory evaluations were analyzed at study sites or at local laboratories where each subject resides. Laboratory results and the change from baseline will be summarized by visit for subjects in the PP1 sample. Results for chemistry and hematology labs will be listed in separate listings.

All laboratory tests will be listed, but if there are duplicate laboratory tests for one study period then the earliest observation will be used in the table summaries and graphic displays. They will be sorted by subject ID, laboratory parameter and time of assessment. Laboratory normal ranges will be included and out-of-range flags (H or L) will be used to denote values that are above (H) or below (L) the relevant reference range. Reference ranges will be obtained from The Harriet Lane Handbook[1].

Spaghetti plots for baseline and post-screening visits will be created for liver function tests including: AST, ALT, alkaline phosphatase, GGT, total bilirubin, and direct bilirubin.

Autoantibodies and quantitative immunoglobulins results and the change from baseline will be summarized using descriptive statistics by visit for subjects in the PP1 and PP2 samples as well as listed in separate listings.

Subject serology will be summarized by number and percentages for subjects in the PP1 and PP2 samples. CMV and EBV Reactivation will be summarized by descriptive statistics by visit for subjects in the PP1 and PP2 samples. Serology and CMV and EBV Reactivation variables will also be presented in separate listings.

10.6 Vital Signs, Physical Findings, and Other Observations Related to Safety

10.6.1 Vital Signs

Descriptive statistics of vital signs results and change from baseline of vital signs will be summarized for the PP1 and PP2 samples. Data listings will be provided for vital signs measurements. They will be sorted by subject, vital sign parameter and time of assessment. Data reported in English units will be converted to corresponding metric units (e.g., cm, kg, degrees C).

10.6.2 Physical Examinations

A listing of the physical examination data will be created. Physical exam status at baseline and change in physical examination will be summarized at each post-baseline visit by body system. The categories of change include no change, improved, and worsened.

11. EXPLORATORY ANALYSES

All analyses performed on the PP2 sample will also be performed on the PP2 sample excluding subjects with selected liver pathologies as exploratory analyses.

12. OTHER ANALYSES

12.1 Use of Medications

Medications reported on the CRF will be categorized into prior, concomitant or after for analysis. Prior, concomitant or after medications will be identified by comparing the medication start and stop dates with the date of initiation of immunosuppression withdrawal. Prior medications will have both the medication start and stop date prior to the first dose of study medication date. After medications will have both the medication start and stop date after the last dose of study medication date. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study medication by at least one day.

The number and percentage of subjects receiving prior, concomitant and after medications will be presented overall and by medication class. When reporting the number of subjects receiving the medication, a subject will only be counted once if they ever received the medication within the medication class. Percentages will be based on the number of subjects in the PP1 sample. Separate data listings will be provided for prior, concomitant and after medications.

12.2 Immunosuppression Medication History

Number and percentage for types of medication given during induction, given post-discharge, and used within the 12 months prior to enrollment will be summarized for all subjects by visit in the ITT sample. Separate data listings will be provided for induction, post-discharge, and detailed 12 month prior to enrollment to current immunosuppresion.

12.3 Medical History

The number and percentage of subjects with clinically significant diseases including allergies and current or past medical procedures other than the disease under study, by body system will be listed and summarized for the ITT sample.

12.3.1 Liver Transplant Specific Medical History

The liver transplant specific medical history includes indication for liver transplant, whether subject has experienced infections associated with liver transplant and the infection type, and whether subject has experienced malignancy since the liver transplant. A data listing will be created for liver transplant specific medical history data for the ITT sample.

12.3.2 Rejection History

Rejection episode date, histological grading and treatment for rejection will be listed for all rejection history for the ITT sample.

12.3.3 Prescreening Biopsies

Reason for biopsy, BANFF global assessment grade, other findings and Ishak fibrosis score will be listed for all pre-screening biopsies for the ITT sample.

12.3.4 Blood Typing

The number and percents for blood type and Rh type for all subjects and their donors will be summarized. Blood typing data for all subjects in the ITT sample and their donors will also be listed.

13. INTERIM ANALYSES AND DATA MONITORING

Per protocol version 4.0, there are not any planned interim analyses for this study.

The protocol chair, the ITN clinical trial physician, the NIAID medical monitor, and the NIAID Transplant Data and Safety Monitoring Board (DSMB) will periodically review safety data. Enrollment of subjects in the trial and withdrawal of immunosuppression in current trial subjects will be suspended at any time if any of these reviews concludes that there are significant safety concerns. Stopping rules are described in Section 3.5 of the protocol.

The progress of the study will be monitored by the NIAID Data and Safety Monitoring Board (NIAID DSMB). The NIAID 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. The discontinuation of study treatment will also be periodically reported to the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the NIAID medical monitor or protocol co-chairs to warrant review, or when an event occurs that could contribute to a pre-defined stopping rule specified in the Protocol.

Findings will be reported to IRBs and health authorities.

14. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

The principal features of the plan for statistical analysis of the data are outlined in this SAP. Any changes in these principal features will require a SAP amendment, which will be subject to review by the independent DSMB, the study sponsor(s), and the regulatory agencies. These changes will be described in the final report as appropriate.

15. REFERENCES

1. The Harriet Lane Handbook, Fifteenth edition, Editors George K. Siberry MD, MPH; Robert Iannone, MD; pg 119-121. (Publisher - Mosby INC).

16. APPENDICES

16.1 Schedule of Events

Appendix 1. Schedule of Events: Gradual to Complete Withdrawal Plus 3 Months of High-intensity Follow-up

	Withdrawal Plus 3-Month High-intensity Follow-up														
Monthly visits	**–2**	**–1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
				13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
General Assessments															
Informed consent	X														
Demographic history	X														
Medical history	X														
Liver transplant: specific medical history	X														
Physical examination	X	X				X			X			X			X

	Withdrawal Plus 3-Month High-intensity Follow-up														
Monthly visits	**–2**	**–1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
				13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Vital signs	X	X				X			X			X			X
Inclusion/exclusion criteria		X													
PPD skin test	X														
Telephone consultation			X	X	X		X	X		X	X		X	X	
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Site and Local Laboratory Assessments															
Hematology	X[1]					X			X			X			X
Comprehensive chemistry	X[1]														X
Basic chemistry						X			X			X			
Liver panel		X	Every 2 weeks starting at visit 0[2]												
Autoantibodies	X					X			X			X			X
Quantitative immunoglobulins	X					X			X			X			X
Viral serology[3]	X														
Urine hCG	X														
Glomerular filtration rate (creatinine and height)	X														
Hemoglobin A_{1C}	X														
Tacrolimus or cyclosporine serum levels		X													
Central Laboratory Assessments															

[1] Can be performed within 9 weeks before the liver biopsy visit.

[2] LFTs must be performed upon diagnosis of allograft dysfunction or rejection and at the resolution of rejection (see sections 5.5.2 and 5.5.3.1). Additional LFTs may be performed during rejection at the investigator's discretion.

[3] See section 6.3 for a description of the viral tests to be performed.

	Withdrawal Plus 3-Month High-intensity Follow-up														
Monthly visits	**–2**	**–1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
				13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Whole blood–quantitative PCR for CMV reactivation	X					X			X			X			X
Whole blood–quantitative PCR for EBV reactivation	X					X			X			X			X
Liver Biopsies															
Liver biopsies[4]		X													X[5,6]
Tolerance Assessments[7]															
Whole blood–flow cytometry panel staining	X[8]					X			X			X			X
Frozen PBMC–T-cell assays [9]	X[8]					X			X			X			X
Liver biopsy–histology		X													X[5,6]
Whole-blood–gene expression profiling	X[8]					X			X			X			X
Liver biopsy RNA–gene expression profiling		X													X[5,6]
Serum–secreted cytokines	X[8]					X			X			X			X
Serum–HLA alloantibodies	X[8]								X						X
Whole blood DNA–HLA genotypes[9, 10]	X[8]														

[4] Additional biopsies will be done to rule out rejection if necessary.

[5] Will be performed within 4 to 8 weeks after the last dose of immunosuppressant is taken.

[6] Will be performed only at visit 12 and not at visit 24.

[7] If a rejection episode occurs, samples for all mechanistic assessments that are scheduled for monthly visits 12 and 24 (except for whole blood–flow cytometry panel staining) will be collected at this time.

[8] Please try to collect blood at assigned visit -2; if this is not possible, collect at visit −1. If not able to collect at visit −1, please collect at visit 0.

[9] To be collected from living donor and nondonor parents after they have signed the informed consent; blood draw can be done at any visit during the recipient's trial participation.

[10] Collection from the subject may be deferred to any visit during the trial depending on subject's weight and blood volume status.

Appendix 2. Schedule of Events: Medium-intensity Follow-up

	Medium-intensity Follow-up [11]											
Monthly visit	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
	M13	**M14**	**M15**	**M16**	**M17**	**M18**	**M19**	**M20**	**M21**	**M22**	**M23**	**M24**
General Assessments												
Physical examination						X						X
Vital signs						X						X
Telephone consultation	X	X	X	X	X		X	X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Study Site and Local Laboratory Assessments												
Hematology						X						X
Comprehensive chemistry						X						X
Liver panel	X	X	X	X	X		X	X	X	X	X	
Autoantibodies						X						X
Quantitative IgG						X						X
Glomerular filtration rate (creatinine and height)	X[2]											X
Hemoglobin A_{1C}	X[2]											X
Central Laboratory Assessments												
Whole blood–quantitative PCR for CMV reactivation[12]	X[13]					X						X
Whole blood–quantitative PCR for EBV reactivation [2]	X[3]					X						X
Liver Biopsies												
Liver biopsies[14, 15]												X
Tolerance Assessments[16,17]												
Whole blood–flow cytometry panel staining						X						X

[11] Subjects enter medium-intensity follow-up after completing 3 months of high-intensity follow-up or after failing immunosuppression withdrawal (see section 5.3). Subjects who have successfully completed immunosuppression withdrawal will remain in medium-intensity follow up for 24 months. Subjects who fail immunosuppression withdrawal will remain in medium-intensity follow-up for 12 months and then be discharged from the study.

[12] Will be performed *only* at visit M1 and not at visit M13.

[13] Do not collect if last sample was collected less than 6 weeks before visit M1.

[14] Additional biopsies will be performed if necessary to rule out rejection.

[15] Will be performed *only* at the end of the second year (i.e., month 24) of medium-intensity follow-up for those who have successfully withdrawn. Will *not* be done for those who experienced rejection and have completed 1 year of medium-intensity follow-up.

[16] If a rejection episode occurs, samples for all mechanistic assessments that are scheduled for monthly visits M12 and M24 (except for whole blood–flow cytometry panel staining) will be collected at this time.

[17] Do not collect for subjects who have failed immunosuppression withdrawal.

	Medium-intensity Follow-up [11]											
Monthly visit	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
	M13	**M14**	**M15**	**M16**	**M17**	**M18**	**M19**	**M20**	**M21**	**M22**	**M23**	**M24**
Frozen PBMC–T-cell assays						X						X
Liver biopsy–histology [4]												X
Whole-blood–gene expression profiling						X						X
Liver biopsy RNA–gene expression profiling[4]												X
Serum–secreted cytokines						X						X
Serum–HLA alloantibodies						X						X

Appendix 3. Schedule of Events: Low-intensity Follow-up

	Low-intensity Follow-up[1]											
Monthly visit	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**	**L10**	**L11**	**L12**
	L13	**L14**	**L15**	**L16**	**L17**	**L18**	**L19**	**L20**	**L21**	**L22**	**L23**	**L24**
General Assessments												
Physical examination						X						X
Vital signs						X						X
Telephone consultation		X		X				X		X		
Adverse events		X		X		X		X		X		X
Concomitant medications		X		X		X		X		X		X
Study Site and Local Laboratory Assessments												
Hematology						X						X
Comprehensive chemistry						X						X
Liver panel		X		X				X		X		
Autoantibodies						X						X
Quantitative IgG						X						X
Glomerular filtration rate (creatinine and height)												X
Hemoglobin A_{1C}												X
Central Laboratory Assessments												
Whole blood–quantitative PCR for CMV reactivation						X						X
Whole blood–quantitative PCR for EBV reactivation						X						X
Liver Biopsies												
Liver biopsies[2, 3]												X
Tolerance Assessments[4]												
Whole blood–flow cytometry panel staining												X
Frozen PBMC–T-cell assays												X
Liver biopsy–histology [2]												X
Whole blood–gene expression profiling												X
Liver biopsy RNA–gene expression profiling [2]												X
Serum–secreted cytokines												X
Serum–HLA alloantibodies												X

[1] Subjects enter low-intensity follow-up after completing medium-intensity follow-up.
[2] Additional biopsies will be performed if necessary to rule out rejection.
[3] Will be performed at the end of the second year (i.e., monthly visit L24) of low-intensity follow-up.
[4] If a rejection episode occurs, samples for all mechanistic assessments that are scheduled for monthly visits L12 and L24 (except for whole blood–flow cytometry panel staining) will be collected at this time.