Immune Tolerance Network

Protocol ITN029ST

Immunosuppression Withdrawal for Pediatric Living-donor Liver Transplant Recipients

Short Title: *Immunosuppression Withdrawal for Pediatric Liver Recipients*

Version 5.0 (April 19, 2011)

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

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Protocol Approval

Protocol: ITN029ST	**Version 5.0 Date: April 19, 2011**
IND: Not Applicable	**Protocol Chair:** Sandy Feng, MD, PhD
Short Title: Immunosuppression Withdrawal for Pediatric Liver Recipients	

I have read protocol ITN029ST and I approve it. As the principal investigator, I agree to conduct this protocol using good clinical practices, as delineated in *ICH Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* (April 1996), and according to the criteria specified in the protocol.

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Principal Investigator (Print)

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Principal Investigator (Signature) **Date**

Synopsis

Title	Immunosuppression Withdrawal for Pediatric Living-donor Liver Transplant Recipients
Short Title	Immunosuppression Withdrawal for Pediatric Liver Recipients
Sponsor	National Institute of Allergy and Infectious Diseases
Conducted by	Immune Tolerance Network
Protocol Chair	[redacted]
Accrual Objective	20 participants
Study Design	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. Patients 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. Participants will undergo gradual withdrawal of immunosuppression and will be followed for a minimum of 8 years after completion of immunosuppression withdrawal. Immunologic and genetic profiles will be collected at multiple time points and compared between tolerant and nontolerant participants.
Pace of Enrollment and Study Duration	Enrollment is defined as starting on the day that immunosuppression withdrawal is initiated. Enrollment is planned for 2 years and will be limited to a maximum of two participants every 4 weeks. Maximum patient participation is up to 11 years; therefore, the total study duration is projected to be a maximum of 13 years.
Primary Endpoint	The proportion of participants who are successfully withdrawn from immunosuppression, which is defined as those who remain off immunosuppression for at least 1 year.
Inclusion Criteria	1. Living-donor liver transplantation from a parental donor. 2. Age less than 18 years at the time of transplantation. 3. At least 4 years since transplantation. 4. Availability and willingness of parental liver donor to participate in the trial. 5. Liver biopsy at screening demonstrating no evidence of acute or chronic rejection and a less than stage 2 fibrosis on the Ishak scale. 6. Negative urine pregnancy test at entry and agreement to use birth control during the study for women of childbearing potential. 7. Negative purified protein derivative (PPD) test results or history of appropriate treatment.
Exclusion Criteria	1. Indication for transplantation liver failure due to autoimmune disease, such as autoimmune hepatitis, primary sclerosing cholangitis, or primary biliary cirrhosis.

2. Hepatitis B infection, as defined by the presence of HB_SAg or active treatment for hepatitis B.
3. Hepatitis C infection, as defined by the presence of antibody against hepatitis C.
4. 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.
5. Transplantation of a second organ before, simultaneous with, or after liver transplantation; or liver retransplantation.
6. Aspartate or alanine aminotransferase (AST or ALT) of greater than 2 times the upper limit of normal.
7. Total bilirubin and direct bilirubin, and either alkaline phosphatase or gamma-glutamyl transferase (GGT) greater than 2 times the upper limit of normal.
8. Clinically significant change in hepatic function in the past 26 weeks.
9. GFR less than 40 mL/min/1.73 m^2.
10. Immunosuppression with
    a. 50% dose increase in a current agent within 26 weeks of screening, or
    b. more than one agent within 52 weeks of screening.
11. Any systemic illness requiring, or likely to require, immunosuppressive drug use.
12. Human immunodeficiency virus (HIV) infection.
13. Pregnancy or breast-feeding.
14. Unwillingness or inability to comply with study requirements and procedures.

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Glossary 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
PSS	Product Safety Scientist
RHO	Rho Federal Systems Division, Inc.
SAE	serious adverse event
SAP	statistical analysis plan
SMT	study management team
TMP	trimethoprim
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
WHO	World Health Organization

1. BACKGROUND

1.1 SUMMARY

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.

1.2 CLINICAL RATIONALE

1.2.1 Pediatric Liver Transplant Recipients

Historically, the pediatric patient has been instrumental in the initial establishment of, and subsequent innovation in, the field of liver transplantation. Both the first attempted liver transplant in 1963 and the first successful liver transplant in 1967 involved young children. Later, during the late eighties and early nineties, special challenges facing pediatric liver transplantation motivated the development and refinement of innovative surgical strategies (i.e., reduced-size, split, and, finally, living-donor liver transplantation techniques) that have permeated and changed not only pediatric but also adult liver transplantation.

While these contributions have been predominantly surgical and technical in nature, pediatric transplant recipients may now present a unique opportunity to explore tolerance. Specifically, there now exists a significant number of stable living-donor recipients who underwent transplantation as children, typically from parent donors. The current trial proposes to identify a cohort of stable, pediatric living-donor recipients 4 or more years after transplantation who are willing to undergo gradual immunosuppression withdrawal under close and prolonged supervision with an aim to identify and quantify those who are tolerant—defined as those able to maintain normal allograft function in the absence of immunosuppression. Previous reports regarding prospective withdrawal

trials would suggest that a significant proportion of such patients can be successfully and safely withdrawn. Releasing young patients from the lifelong burden of immunosuppression has obvious benefit and appeal, particularly considering the reality that complications and toxicities of immunosuppression (e.g., renal dysfunction, infection, malignancy, metabolic abnormalities, and growth retardation) represent the major threats to long-term quality of life and survival in this posttransplant population.[1]

1.2.2 Complications of Long-term Immunosuppression

The general risk of long-term renal dysfunction is illustrated by a review of 69,321 solid organ transplant recipients (median age 45 years) who were followed for a median of 36 months.[2] Chronic renal failure was reported in 16.5%; 29% of these recipients required dialysis. Among 2261 liver transplant recipients followed for 10 years, the cumulative incidence of chronic renal failure was 26%. Among pediatric non-renal-transplant recipients, the rate of chronic renal failure, defined as a serum creatinine of greater than 2.5 mg/dL or chronic dialysis, appears to be much lower. Of all heart and liver recipients undergoing transplantation between 1990 and 1999 who survived more than 1 year, 5.6% developed chronic renal failure; the actuarial risk at 5 years was 3%.[3] Among pediatric lung transplant recipients, a large, single center study of 125 one-year survivors reported that serum creatinine nearly doubled from pretransplant to 1-year post transplant and tripled by 7 years post transplant. Mean glomerular filtration rate (GFR), as estimated by the Schwartz formula, dropped from a baseline of 163 ± 5.9 mL/min to 69 ± 9.0 by 10 years after transplant. Five years after transplant, 38% of patients had GFR <60mL/min and seven patients developed end-stage kidney disease.[4]

More recently, detailed data has emerged from three single-center studies specifically regarding the renal function of pediatric liver transplant recipients 10 years after transplantation. All three reported that the calculated GFR by the Schwartz formula significantly overestimated actual GFR as measured by nuclear medicine renal isotopic clearance studies. Alonso reported on the 10-year calculated GFR for 32 pediatric liver transplant recipients from the University of Chicago.[5] Two patients had moderate renal insufficiency (calculated GFR < 60mL/min), whereas three had mild renal insufficiency (calculated GFR of 60–80mL/min).[5] The data from the two groups that measured GFR were quite different. The group from Lille, France reported on seven recipients.[6] Four of the seven had mild renal insufficiency (measured GFR 60–80mL/min) and two of the seven had moderate renal insufficiency (measured GFR of 20-60mL/min). Similarly, the group from Toronto reported on 26 of their 32 10-year survivors who underwent GFR measurements.[7] At 10 years post transplant, 20 of the 26 (77%) had abnormal renal function. Renal insufficiency was mild (measured GFR 75–100mL/min) for 4 (15%), moderate (measured GFR 50–75mL/min) for 15 (58%), and severe (measured GFR <50mL/min) for 1 (4%). Moreover, two additional patients did not undergo GFR measurement at their 10-year anniversary; one was hemodialysis dependent and the second had undergone combined liver and kidney transplantation 8 years after initial liver transplantation.

Therefore, while overall rates of renal failure are low, available data suggest that the prevalence of renal insufficiency is high. The concern is that children who have a longer potential life span after liver transplantation will "evolve from asymptomatic decreased glomerular filtration rate to severe renal impairment".[1] In children, progressive renal insufficiency leads to many well-characterized complications including poor growth, anemia, hypertension, secondary hyperparathyroidism/metabolic bone disease, and electrolyte abnormalities.[8] Although these morbidities become clinically apparent typically at GFR <30 mL/min, corresponding to chronic

kidney disease stages 4 or 5, children may be most sensitive to these metabolic derangements in chronic kidney disease stage 3. As pediatric transplant recipients mature into adulthood, concern focuses on the cardiovascular morbidity incurred by suboptimal renal function. It is clear that poor quality renal function is associated with cardiovascular morbidity independent of other known cardiovascular risk factors such as hypertension and dyslipidemia.[9, 10]

In addition to predisposing pediatric transplant recipients to metabolic abnormalities indirectly through renal dysfunction, immunosuppression predisposes directly to metabolic conditions such as hypertension, diabetes, and hyperlipidemia. In general, their impact on pediatric liver transplant recipients appears less than on their adult counterparts. However, at 10 years post transplant, Avitzur and associates reported that 8 of 32 patients (25%) required antihypertensive treatment, a rate consistent with several previous reports.[7, 11, 12] Moreover, a recent publication suggests that hypertension in pediatric liver transplant recipients may be substantially underdiagnosed. Del Compare and associates compared office blood pressure measurements (mean of three measurements 1 minute apart) with 24-hour ambulatory blood pressure monitoring (APBM) in stable pediatric liver transplant recipients 1.1–11.5 years (median 5.1 years) after liver transplantation who were not being treated with an antihypertensive medication.[13] Of 61 eligible patients, 32 were excluded: 18 (30%) because of chronic renal insufficiency (calculated GFR <80mL/min), 12 (20%) because of recent changes in immunosuppression, and 2 because of declined consent. Of the 29 stable patients with relatively normal renal function who were studied, 8 (28%) met criteria for hypertension by APBM; only 1 of the 8 was hypertensive based on office blood pressure measurements.[13] Overall, the incidence of insulin-dependent diabetes mellitus in 10-year liver transplant survivors has been reported at 6%.[7] Although 6 (26%) of 10-year pediatric liver transplant recipients had elevated fasting cholesterol and 10 (45%) had elevated triglycerides, respectively, none were prescribed lipid-lowering agents.[7] Again, although these metabolic abnormalities were most often mild and affected a modest proportion of patients, the potential for cumulative morbidity particularly over the anticipated long life of a pediatric transplant recipient span merits concern.

Infection has been reported as a major cause of late pediatric liver allograft loss and late pediatric liver recipient mortality.[14, 15] In particular, five different papers have reported on a total of 58 late deaths (>1 year after transplantation) that occurred in a total of 856 pediatric liver transplant recipients. Overwhelming infection was the most common cause, accounting for 35 (60%) of all deaths secondary to overwhelming infection.[14, 16-19] Although infectious complications do diminish over time after transplantation, presumably since immunosuppression decreases, they continue to cause morbidity between 5 and 10 years after transplantation. In a report of a single center's 32 pediatric liver transplant recipients who survived more than 10 years after liver transplantation, the majority developed infections requiring hospitalization or medical intervention between 5 and 10 years after transplantation.[7] Sixteen were diagnosed with viral infections (herpes zoster = 6; chickenpox = 4; CMV colitis/hepatitis = 2; EBV hepatitis = 1; parvovirus = 1; HCV = 1; and vulvar condyloma = 1), two with bacterial infections (mycoplasma = 1; periorbital cellulitis = 1), and one with a fungal infection (trichophyton). There were three additional infections in long-term recipients for whom no bacteriologic diagnosis could be made (pneumonia = 2; epididymitis = 1).

As for malignancy after transplantation, the most notable malignancies considered as directly related to immunosuppression are posttransplant lymphoproliferative disorder (PTLD) and skin cancer. In a single-center study, 19 of 335 pediatric transplant recipients (ages 0–17 years) developed PTLD, which was typically associated with Epstein-Barr virus (EBV).[20] Thirty-one developed EBV infection or reactivation without PTLD. Of those with PTLD, 32% died; of those with EBV without

PTLD, 6% died. Antibody-based treatment for rejection was common among those whose developed EBV-related disease. This report confirms the high mortality rate associated with EBV-related disease in patients receiving chronic immunosuppression after liver transplantation. With regard to skin cancer, the risks of squamous cell carcinoma and melanoma are increased by 65 and 3.4 times higher, respectively, in renal and cardiac transplant recipients than in the general population.[21] A retrospective single-center analysis of liver transplant recipients demonstrated a 4.5% incidence of malignancy with a 1.6% mortality rate.[22] Specific information on pediatric transplant recipients suggest that skin cancers typically do not occur during childhood.[23] Rather, in the largest series, skin cancers developed during the second decade after transplantation (range 5.5–292 months), at an average age of 26–28 years. As in adults, but even more so, there was reversal of the squamous cell to basal cell carcinoma ratio compared with the general population. Spread to lymph nodes was also more common in pediatric recipients than in adult recipients (9% vs. 6%). Finally, the third most frequent malignancy in pediatric kidney transplant recipients was anogenital cancer, accounting for 4% of tumors of pediatric transplant recipients. Similar to skin cancers, the tumors occurred on average 12 years (range 3.5–22 years) after transplantation, at a mean age of 27 years (range 20–39 years) childhood.[23]

1.2.3 Clinical Reports of Immunosuppression Withdrawal

1.2.3.1 Summary of Clinical Results

Reports from four transplant centers suggest that 19%–42% of liver transplant recipients are functionally tolerant.[24-29] These series included pediatric and adult recipients who underwent deceased- or living-donor liver transplantation for wide-ranging etiologies, including autoimmune disorders and viral hepatitis. Recipients were electively, incidentally (i.e., secondary to noncompliance), or obligatorily (i.e., secondary to development of a major contraindication to ongoing immunosuppression) weaned from immunosuppression. Episodes of subsequent rejection were easily reversed in nearly all cases; only one of the 54 reported cases of patients who experienced rejection required antilymphocyte therapy. In light of the major differences in the study populations, it is worthwhile to consider each of these experiences individually to clarify important differences between these historical cohorts and the one proposed in the current trial.

1.2.3.2 Deceased Donors

Three studies focus on recipients of deceased-donor liver transplants. The Kings College group reported on a cohort of 18 adult liver transplant recipients prospectively withdrawn from immunosuppression.[24] After 3 years, five recipients (28%) remained completely off immunosuppression. Of the remaining 13 recipients, 4 (22%) experienced allograft rejection, of which 1 was severe in grade; 8 developed a hepatitis-like picture, which for the majority of cases appeared to represent an exacerbation of findings identified in the prewithdrawal biopsies; and 1 recipient developed acute hepatic necrosis. Similar to the Pittsburgh approach, all treatment comprised corticosteroid pulses and /or resumption or escalation of baseline immunosuppression, including conversion to alternative agents without need for antilymphocyte therapy. The authors reported that transplantation for nonimmunologic liver disease, fewer HLA mismatches, and lower incidence of early rejection were associated with successful withdrawal.

A small study involving nine prospectively weaned adult recipients has been reported recently from Spain.[27] Three recipients (33%) were successfully weaned; acute rejection was diagnosed in two; and

the remaining four developed nondiagnostic portal inflammation. Again, there was no need for antilymphocyte therapy.

The largest study comes from the University of Pittsburgh group who reported on 95 prospectively withdrawn recipients; at study entry, 31 were 20 years old or younger and the remaining were 21–68 years old.[25, 28] At last publication, 19% were completely off immunosuppression, 39% were still being tapered, 29% had experienced rejection, and 13% had withdrawn from the study. The pediatric cohort had better outcomes than the adult cohort did; it had a higher weaning success rate and a lower rejection rate. Of the 28 patients with rejection, 18 had biopsy-proven episodes, 7 had clinically suspected episodes, and 3 were withdrawn from the study because biopsy findings were suggestive of incipient chronic rejection although not diagnostic of chronic rejection. Treatment of recipients considered to have acute or chronic rejection comprised corticosteroid pulses and/or resumption or escalation of baseline immunosuppression, including conversion to alternative agents, typically from cyclosporine-based to tacrolimus-based regimens. Again, no recipient received antilymphocyte antibody therapy, and there were no graft losses related to allograft dysfunction.

Updated information regarding the Pittsburgh cohort was presented at the recent American Transplant Congress (Seattle, May 2005; Tables 1 and 2). Their withdrawal experience now encompasses 120 liver transplant recipients: 70 adults and 50 children. Overall, 33 (28%) were successfully weaned, 30 (25%) were still weaning, 47 (39%) had failed, and an additional 10 (8%) were on "hold": these 10 were no longer weaning but had not achieved any failure endpoints of acute or chronic rejection. Outcomes were distinctly better in the pediatric subgroup (n=50): 20 (40%) were successfully weaned, 23 (46%) were still weaning, and the remaining 7 (14%) had acute rejection and thus failed weaning (Table 2).

Table 1. Overall outcomes in the University of Pittsburgh study*

	Status/Outcome			
	Off drugs	**Withdrawing**	**Failure**	**Withdrawal on hold**
Patients, N (%)	33 (28)	30 (25)	47 (39)	10 (8)
Age at transplantation, yr	15.3	8.2	29.6	36.5
Transplantation to withdrawal start, yr	6.8	7.4	9.0	8.4
Withdrawal to outcome, yr	1.5	N/A	1.5	2.8
Current follow-up, yr	9.1 (2.5–12.6)	7.6 (2.0–11.0)	8.7 (2.7–9.3)	7.6 (4.0–6.8)

*Updated May 2005.

Table 2. Outcomes of children vs. adults in the University of Pittsburgh study*

Status/Outcome	Children N = 50	Adults N = 70
Off drugs	20 (40%)	13 (19%)
Withdrawing	23 (46%)	7 (10%)
Acute cellular rejection	7 (14%)	30 (43%)
Chronic rejection	0	1 (1%)
Duct injury	0	5 (7%)
Recurrent primary biliary cirrhosis	0	4 (6%)
On hold	0	10 (14%)

*Updated May 2005.

1.2.3.3 Living Donors

Perhaps the experience closest to the currently proposed trial was that from Kyoto University. This patient group, like the one we are proposing, was limited to pediatric recipients of living-donor liver transplants.[26, 29] An overall success rate for complete immunosuppression withdrawal of 42.6% (49 of 115 recipients) was observed. It should be noted, however, that the success rate was 69% (33 of 48) for those obligatorily or incidentally (nonelectively) weaned, and 24% (16 of 67) for those electively weaned. Overall, 20 patients (17%) experienced rejection: 12 (25%) in the nonelective and 8 (12%) in the elective weaning group. All rejection episodes were easily reversed except one occurring in a nonelectively weaned recipient who required OKT3 therapy. The authors could not identify any clinical parameters associated with successful weaning.

Similarly, the Kyoto group provided updated information regarding their withdrawal cohort at the American Transplant Congress (Table 3). As of June 2004, 55 patients were completely off of immunosuppression and 39 patients were continuing to wean. One patient developed chronic rejection which was successfully treated with return to a triple immunosuppression regimen. Unfortunately, the Kyoto group did not provide us with the number of patients for whom weaning was attempted to enable calculation of percentages.

Table 3. Outcomes in the Kyoto University study

	Transplantation 2001 N = 63	Update as of June 2004* N = Unknown†
Off drugs, *n*:	24 (38%)	55
Drug free time, mo Median (range)	23.5 (3–69)	Scheduled: 41 (10-81) EBV: 50 (9–124) Others: 57 (22–93)
Withdrawing, *n*:	23 (37%) §	39

	***Transplantation* 2001** **N = 63**	**Update as of June 2004*** **N = Unknown†**
Acute cellular rejection, *n*:	16 (25%) All episodes easily treated with tacrolimus ± corticosteroids	Unknown
Time of rejection, mo Median (range)	9.5 (1–63)	Unknown
Chronic rejection, *n*:	0%	1 Successfully treated with prednisone/tacrolimus/MMF

*Presented at the American Transplant Congress (Seattle, May 2005).
† Number of patients in whom withdrawal was unattempted is unknown.
§Six of these 23 withdrawal patients are taking less than once weekly tacrolimus.

1.2.3.4 Summary of Clinical Experience With Withdrawal

In summary, these four studies suggest that, 19%–43% of all liver transplant recipients and 40-43% of pediatric liver transplant candidates can be prospectively and successfully gradually withdrawn from immunosuppression and identified as functionally tolerant. Conversely, 14%–43% developed acute rejection. The remaining recipients failed withdrawal after developing nonspecific abnormalities that were not attributed to rejection, had withdrawal held without either succeeding or failing, or were still undergoing withdrawal.[25, 26, 28, 29] The four studies provide very consistent data regarding the severity and treatability of the rejection episodes: the majority of episodes were graded as mild to moderate and reversed without the use of antilymphocyte therapy. Finally, there were no instances of graft loss related to immunosuppression withdrawal.

1.2.4 Late Allograft Dysfunction in Uncontrolled and Controlled Settings

A fundamental premise of the current trial is that the acute rejection that occurs during controlled, closely supervised immunosuppression withdrawal as planned in the current trial is unlikely to have severe or lasting negative consequences. This is supported by reports of the several trials of gradual immunosuppression withdrawal presented above in which rejection was consistently observed to be mild to moderate and easily reversed.[24-29] In contrast to these experiences, late allograft dysfunction that occurs outside controlled immunosuppression withdrawal trials has been associated with variable results and more complications.[30-33]

1.2.4.1 Uncontrolled Settings

A major confounding factor in interpreting reports of allograft dysfunction after liver transplantation is the widely varying definition of "late," which has ranged from greater than 30 days to greater than 365 days after transplantation. Other factors include the variable etiologies (e.g., acute rejection vs. *de novo* autoimmune hepatitis) and additional factors (e.g., noncompliance or viral infection) that have frequently precipitated and/or complicated late allograft dysfunction.

With regard to "late" acute rejection, the literature on adult recipients is conflicting. Some reports suggest that late episodes of rejection are more refractory than early episodes to standard therapy[30-32] and that they predispose to the development of chronic rejection,[30, 31, 33] whereas other reports indicate

that their response to therapy is similar to that of early episodes[33, 34] and they do not predispose to chronic rejection.[32, 34]

Two reports focus on late rejection in pediatric recipients. The University of Pennsylvania group found that 32% (18 of 57) of recipients had rejection diagnosed more than 100 days after transplantation.[35] Seventeen patients (94%) responded well to therapy, with only one requiring OKT3. One recipient who had two episodes of early rejection and two episodes of late rejection did develop chronic rejection. Function was stabilized by conversion to tacrolimus-based immunosuppression.

The King's College group, however, reported a different experience. Twenty children were diagnosed with "late cellular" rejection greater than 6 months after transplantation.[36] At the time of diagnosis, 5 were considered to be on "adequate" immunosuppression while 15 were considered to be on "inadequate" immunosuppression. Ten of the 20 (50%) returned quickly to normal allograft function with treatment; only 1 of these required high-dose corticosteroids ("adequate" immunosuppression group) for reversal; 9 (all from the "inadequate" immunosuppression group) were treated simply with increased baseline immunosuppression or conversion from cyclosporine to tacrolimus.

The 10 other children, however, did not respond readily to treatment, including 3 who had recurrent or persistent CMV infection, of which 1 also had hepatic artery thrombosis; 1 who progressed to chronic rejection and underwent retransplantation; and 5 who progressed to *de novo* autoimmune hepatitis. These five were treated with immunosuppression designed for autoimmune hepatitis. All responded, although one has had several relapses of autoimmune hepatitis attributed to noncompliance. In retrospect, when late acute cellular rejection was diagnosed, three of these five already had histologic features suggestive of *de novo* autoimmune hepatitis (plasma cell infiltrates with perivenular dropout or bridging collapse) and two of five had detectable autoantibodies.

Therefore, the King's College experience suggests that uncomplicated late acute rejection responds readily to treatment. However, concurrent processes such as CMV infection or hepatic artery thrombosis make reversal of dysfunction more difficult. Moreover, serologic and/or histologic features of autoimmunity necessitate a different treatment approach (see section 1.2.3.1).

Posttransplant *de novo* autoimmune hepatitis was first described in 1995. Currently, the diagnosis can be made when a transplant recipient without previous diagnosis of autoimmune disease develops serologic evidence of autoimmunity (high immunoglobulin G titers with positive titers of antinuclear (ANA), smooth muscle (ASMA), mitochondrial (AMA) or liver-kidney microsomal (ALKMA) antibodies in conjunction with an allograft biopsy that shows histopathologic features of chronic hepatitis (portal and periportal hepatitis with lymphocytes and plasma cells, bridging collapse, and perivenular cell necrosis).[37] Although this does occur in adult recipients,[38, 39] it has been reported more often in pediatric recipients,[37, 40-42] with an estimated incidence of approximately 5% (2%–11%).

De novo autoimmune hepatitis in pediatric liver recipients typically occurs several years after transplantation (often 2–4 years but as late as 13 years) when immunosuppression doses are commonly low. The overall incidence and time of development of *de novo* AIH is quite similar in the living-donor and deceased-donor liver transplant groups. The etiology is not fully clarified and may in fact be heterogeneous. Some have speculated that it can result from an immune response to a foreign antigen rather than a self-antigen. *De novo* hepatitis has been reported as an immune response directed against glutathione S-transferase T1 expressed in a donor liver by a transplant recipient

bearing the glutathione S-transferase T1-null genotype.[43] Viral and other infections have also been suggested as precipitating events as they may expose self-antigens and thereby disrupt peripheral tolerance. Moreover, some have speculated that calcineurin inhibitors might influence the development of AIH by activating autoreactive T-cell clones.[37, 39, 44] The literature recommends treatment with corticosteroids and azathioprine, at doses used to treat autoimmune hepatitis, rather than the immunosuppression protocols typically used to treat acute cellular rejection. The short-term response rates to such treatment have been reported to be high with biochemical and/or histologic improvement.[36, 37, 41] However, the long-term, natural history of *de novo* autoimmune hepatitis may be less favorable. Histologic progression to cirrhosis or the necessity for retransplantation has been reported.[42, 45] The expanded range of immunosuppression today may, however, decrease the likelihood of progressive *de novo* hepatitis. Kerkar and colleagues from Mt. Sinai have just reported their experience using rapamycin as rescue therapy for pediatric recipients with posttransplant (*de* novo and recurrent) autoimmune hepatitis that was unresponsive to corticosteroids and azathioprine.[40] Five nonresponders to standard therapy (two *de novo* and three recurrent) all responded to rapamycin, with normalization of liver function.

1.2.4.2 Controlled Settings

Several reports indicate that acute rejection that occurs during controlled immunosuppression withdrawal is most frequently mild to moderate and easily reversed.[24-29] Nearly all reported episodes have been successfully treated, typically with return to baseline immunosuppressants, with or without bolus corticosteroid therapy. To date, there has been no reported graft loss or patient death as a direct result of elective immunosuppression withdrawal. Presumably, the close surveillance regimen dictated by withdrawal protocols has ensured the expeditious detection, diagnosis, and treatment of allograft dysfunction that has resulted in resolution. The literature supports the intuitive concept that delay in diagnosis and treatment plays a substantial role in the more varied outcomes of allograft dysfunction that occur outside of close surveillance.[46]

As for chronic rejection, one study reported that 3 of 95 prospectively weaned patients were terminated from the withdrawal protocol because of "suspicion of incipient chronic rejection" without, however, the diagnosis of chronic rejection on liver biopsy.[25] All of these patients were adults. If there is histologic or serologic evidence of incipient chronic rejection or *de novo* hepatitis, the currently available armamentarium of immunosuppressants, many of which have been reported to be efficacious rescue therapies, provides some semblance of a safety net.[40, 47-49]

1.2.4.3 The Current Trial

There are several features of our trial design that should minimize the likelihood of an unfavorable outcome (Table 4). First of all, unlike previous controlled withdrawal studies, we are excluding all recipients who underwent transplantation for liver diseases that might recur.

Second, we require thorough biochemical, serologic, and histologic assessments aimed to detect recipients with subclinical allograft dysfunction to exclude them from participation. Only the King's College group performed prewithdrawal biopsies; those with mild, nonspecific abnormalities were allowed to withdraw. A significant number of their patients who could not be successfully withdrawn developed exacerbations of the abnormalities evident on their initial biopsies rather than acute rejection.

Third, our protocol incorporates serologic screening for markers of autoimmunity before enrollment and periodically during the withdrawal phase with an aim to identify those who may be evolving

toward *de novo* autoimmune hepatitis. Although *de novo* autoimmune hepatitis clearly occurs spontaneously, outside of controlled immunosuppression withdrawal, we are certainly concerned that this condition would be exacerbated by gradual withdrawal of immunosuppression.

Fourth, our study enrolls only pediatric recipients of parental living-donor grafts. Although there is doubt that HLA matching and donor-recipient relatedness result in a significant immunologic benefit, there is nevertheless a distinct thread of literature that suggests limited immunologic advantage to the parental living-donor setting. Some centers have reported that, while the incidence of acute rejection is comparable between living- and deceased-donor transplantation, that the acute rejection episodes that occur after living-donor transplantation are less severe and that living-donor liver transplantation is associated with substantially lower risk of chronic rejection than deceased-donor transplantation.[42, 50, 51]

Fifth, only parental living-donor recipients who are 4 or more years post transplant (compared with 2 years in the Kyoto report) are eligible for our withdrawal trial. Our patients will likely start on lower relative immunosuppression doses since they are further out from transplantation and have undergone more "natural weaning" by continued growth.

A sixth and important consideration is our requirement for evidence of good medical compliance for continued trial participation. A safety feature of supervised immunosuppression withdrawal is the close monitoring of liver function tests which is entirely dependent upon compliance.

Finally, we have set low thresholds to mandate liver biopsy to ensure swift detection of rejection, if and when it occurs. We believe that prompt treatment provides the best opportunity for a good response. Our protocol also specifies ongoing assessment of the severity and treatability of acute rejection episodes with concomitant stopping rules if unfavorable events occur with undue frequency.

Table 4. Comparison of immunosuppression withdrawal trial designs

	Adult or pediatric	**Deceased or living donor**	**Inclusion of HCV/HBV/ AIH/PBC/ PSC?**	**Screening for de novo AIH?**	**Time after tx**	**Prewith-drawal biopsy?**	**Postwith-drawal biopsies?**
King's College	Adult	Deceased	Yes	No	> 5yr	Yes	Yes
University of Pittsburgh	Adult and Pediatric	Deceased	Yes	No	≥ 5yr for elective weaning	No	No
Kyoto University	Pediatric	Living	No	No	> 2 yr for elective weaning	No	No
ITN trial	**Pediatric**	**Living**	**No**	**Yes**	**≥ 4 yr**	**Yes**	**Yes**

Overall, we anticipate that our rate of successful withdrawal will be at least 20% but possibly higher, based on published literature of previous elective withdrawal trials, and in particular, the experience with pediatric liver transplant recipients.[24-29, 37, 40] Features of the transplant recipients in the current trial, as outlined above, may however, favor a higher success rate and a higher safety rate.

1.3 SCIENTIFIC RATIONALE

1.3.1 Rationale for Immune Studies

While the clinical reports regarding immunosuppression withdrawal do provide a context of clinical expectations for our proposed study, they, unfortunately, have yielded little information regarding the mechanism(s) of functional tolerance or biomarkers that may predict, characterize, or identify functional tolerance. Three of the four experiences involved recipients of livers from deceased donors and, as such, were severely limited in the performance of tolerance studies by the lack of extant donor tissue. The Kyoto experience, which comprised solely pediatric recipients of living-donor liver transplants, did begin to explore potential mechanisms of functional tolerance with very limited findings. They suggested that downregulation of interferon-gamma secretion may be a mechanism responsible for the observed donor-specific hyporeactivity in mixed lymphocyte reactions.[52] Clearly, much more can and must be done to elucidate mechanisms and biomarkers of operational tolerance, as we are currently proposing.

If biomarkers of clinical tolerance can be identified, our serial evaluations of peripheral blood and liver tissue before, during, and after withdrawal should enable us to delineate the time course of their presence. It is hoped that an emerging signature of tolerance will yield clues about the operational mechanism(s) of tolerance. We plan to enroll patients who will undergo drug withdrawal without any peritransplant "tolerizing" immunosuppression protocol; therefore, our study does not induce tolerance because it is simply designed to uncover preexisting, but unidentified, tolerance.

An integral part of the success of this study is the identification of immune cell or molecular markers that can segregate patients who have successfully achieved tolerance following withdrawal of immunosuppression vs. those patients who failed. Through the discovery of such markers it is hoped that predictive markers can be extrapolated, such that parameters measured before withdrawing immunosuppression would help guide whether or not drug withdrawal should be attempted. Thus, the use of surrogate markers could create a powerful means for stratification of patients into those "potentially tolerizable" or those who will likely never achieve tolerance.

Using many of the latest cellular immunological techniques combined with molecular profiling, the measurements of peripheral blood and liver biopsy material will be done in patients before, during, and after immunosuppression withdrawal. Importantly, assays will also be performed at time of rejection so that comparisons can be made between withdrawal successes and failures.

1.3.2 Unique Immunobiology of the Liver

Of all solid organs that are transplanted, the liver has been regarded historically and consistently as being immunologically privileged—the most resistant to immunologic attack and damage. Presentation of antigens via the portal venous system has long been recognized as more likely to result in a tolerizing response than presentation via the systemic venous system. In several allogeneic models ranging from rodent to canine to swine, minimal or even no exposure to immunosuppression has resulted in successful and durable graft function after transplantation.[53-56]

In the human arena, the liver is unique among transplanted solid organs in several ways. First, while episodes of acute rejection commonly connote deleterious outcomes for nearly all other solid organs that we transplant, acute rejection after liver transplantation has had no such long-term connotations.[57, 58] Second, it is well known that some episodes of acute rejection may resolve spontaneously without treatment.[59-61] Third, although chronic rejection in varied manifestations represents a major threat to the longevity of other transplanted solid organs, its incidence and therefore, its importance, is substantially less in the post-liver-transplant setting, particularly in the age of modern immunosuppression.[62] Furthermore, it has been suggested that the advent of tacrolimus immunosuppression has nearly eliminated the threat of chronic rejection for pediatric liver transplant recipients,[63, 64] and that living-donor liver transplants may be particularly immune to chronic rejection.[42] Finally, while the importance of humoral mechanisms of acute and chronic allograft has recently emerged for other solid organs, there is little literature to substantiate their relevance and importance in the setting of liver transplantation.

At present, the biologic basis for these observed differences between the liver and other solid organs remains imprecisely defined. While the liver's unique regenerative capacity enabling repair of injury may be important to buffer the impact of immunologic attack, most believe that the primary reason for the liver's privileged position of relative resistance against immunologic attack is predominantly immunologic in nature and results from the unique anatomy and function of the liver.[65, 66] The liver has two blood supplies—the portal venous and the hepatic arterial systems—which mixes in the hepatic sinusoids, specialized blood channels in the liver which are lined by fenestrated endothelial cells and lack a discrete basement membrane. This unique architectural arrangement and the sluggishness of sinusoidal flow facilitates the entry of circulating antigens and immune cells into these blood spaces where they can interact with endothelial cells, hepatocytes, Kupffer cells, and other resident cells.

This design is likely integral to the liver's unique immunobiology, which has been delineated only recently.[66] Compared with other organs and peripheral blood, the liver has a unique complement of lymphocytes. Natural killer (NK) and NK-like T cells strikingly account for approximately 60% of resident lymphocytes compared with approximately 15% in the peripheral blood compartment. The liver is also enriched for $CD8^+$ over $CD4^+$ T cells.[67] When challenged by pathogens, the liver has the demonstrable capability to generate a protective immune response. However, since portal venous flow constitutively exposes the liver to nonpathogenic foreign antigens (e.g., food derivatives, environmental toxins, and bacterial products), the liver must also possess potent mechanisms that suppress immune activation. One of the scientific goals of this proposal is to define the immunologic interactions that critically determine the nature and scope of the immune response.

1.3.3 Planned Immunologic and Genetic Assessments

A variety of immunologic and genetic assessments will be performed at several time points to study changes in immune responses during and after immunosuppression withdrawal. These will include measures of cellular reactivity, antibody response, and patterns of gene expression. Assessments will be performed on peripheral blood samples and on liver tissue. The results of immune and genetic assessments will be compared between tolerant and nontolerant individuals; that is, between subjects who are successfully withdrawn from immunosuppression and those who experience rejection or some other complication during tapering.

1.4 POTENTIAL BENEFITS AND RISKS TO HUMAN SUBJECTS

1.4.1 Potential Benefits

In the current trial, participants undergo gradual withdrawal of immunosuppression. Previous clinical experience, described in section 1.2, demonstrates that a proportion of these participants will likely be able to be withdrawn completely from immunosuppression. The long-term risks of immunosuppression are detailed in section 1.2.1. This approach provides study participants the possibility of graft survival in the absence of immunosuppression. The thorough clinical and laboratory monitoring of potential adverse events during the gradual withdrawal process allow the risks of withdrawal to be closely managed.

1.4.2 Risks

Standard care in liver transplantation currently includes lifelong administration of immunosuppression, typically a calcineurin inhibitor with or without corticosteroids or other immunosuppressants. In the current trial the gradual withdrawal of immunosuppression among eligible participants is likely to increase the risk of acute allograft rejection. In this case additional diagnostic procedures and the reinstitution of immunosuppression may be required. This could lead to an increased risk of opportunistic infection.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

To evaluate allograft tolerance in pediatric recipients of livers from parental living related donors.

2.2 SECONDARY OBJECTIVES

2.2.1 Safety of Immunosuppression Withdrawal

To assess the safety of immunosuppression withdrawal.

2.2.2 Duration

To assess the durability of allograft tolerance.

2.2.3 Tolerance Predictive Profiles

To define profiles of immunologic and genetic features present before or during gradual withdrawal of immunosuppression that distinguish tolerant and nontolerant allograft recipients.

2.2.4 Rejection Profiles

To define profiles of immunologic and genetic features associated with allograft rejection.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a prospective multicenter, open-label, single-arm trial in which 20 pediatric recipients of parental living-donor liver allografts will be gradually withdrawn from immunosuppression with the goal of complete withdrawal. Patients 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. Participants will undergo gradual withdrawal of immunosuppression and will be followed for a minimum of 8 years after completion of withdrawal. Immunologic and genetic profiles will be collected at multiple time points and compared between tolerant and nontolerant individuals.

3.2 ASSENT AND CONSENT FOR PARTICIPATION

Consent must be obtained from individuals eligible for screening assessments before any screening assessments are performed to determine their eligibility for trial participation. Participants who meet the eligibility criteria (defined in sections 4.2 and 4.3) and have continued willingness to participate will then be enrolled in the study.

Requirements for the assent and informed consent process will depend on the participant's age:

4–6 years A parent or legal guardian will sign the consent form for the participant.

7–12 years The participant will sign the assent form and a parent or legal guardian will sign the consent form.

13–17 years The participant and a parent or legal guardian will both sign the consent form.

≥18 years Only the participant will be required to sign the consent form.

Informed consent for blood drawing must also be obtained from the parent who was the liver donor and will be requested from the parent who is not the donor.

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

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

3.3.2 Secondary Endpoints

3.3.2.1 Safety of Immunosuppression Withdrawal

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

3.3.2.2 Duration

Immunosuppression-free duration, defined as the time from discontinuation of immunosuppression to end of trial participation or to time of restarting immunosuppression.

3.3.2.3 Tolerance Predictive Profiles

Results of mechanistic studies and clinical assessments at various time points that allow for the definition of profiles associated with tolerance.

3.3.2.4 Rejection Profiles

Results of mechanistic studies and clinical assessments that allow for definition of profiles associated with liver allograft rejection.

3.3.2.5 Assessment of Fibrosis Over Time for Participants Undergoing Extended Follow Up

The change in periportal and pervenular fibrosis on liver biopsy over time will be analyzed only for participants who remain off immunosuppression and enter Extended Participant Follow Up (see Appendix 4).

3.4 RATIONALE FOR SELECTION OF STUDY POPULATION

The study population is defined in section 4.1. The proposed study aims to prospectively identify a cohort of stable patients who underwent parental living-donor liver transplantation when they were less than 18 years of age. This choice stems from the following:

- The concerning incidence of renal dysfunction, hypertension, and metabolic abnormalities related to long-term use of immunosuppressive medications in long-term (5–10 years) pediatric survivors of liver transplantation.
- The substantial potential benefit (physical, psychosocial, and financial) resulting from immunosuppressive withdrawal that participants can enjoy over a long lifetime.
- The availability of donor blood and recipient blood and tissue for mechanistic testing.
- The minimal maintenance immunosuppression currently utilized in these patients (a single agent) may increase the likelihood of successful withdrawal.

Patients for whom the indication for transplantation was hepatitis B, hepatitis C, autoimmune hepatitis, or primary sclerosing cholangitis are excluded because these diseases may recur after transplantation and their posttransplant course may be affected by withdrawal or intensification of immunosuppression as necessitated by a precipitated episode of rejection.

3.5 STOPPING RULES

3.5.1 Ongoing Review

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 participants in the trial and withdrawal of immunosuppression in current trial participants will be suspended at any time if any of these reviews concludes that there are significant safety concerns.

3.5.2 Specific Adverse Events Independent of Participant Enrollment

Enrollment and immunosuppression withdrawal in trial participants will be suspended if any of the following occurs:

- Any death
- Any graft loss

- Two participants requiring antibody therapy for graft rejection
- Two participants diagnosed with chronic rejection

3.5.3 Specific Adverse Events as a Proportion of Enrolled Participants

An undue incidence of the following qualifying events will be grounds for suspending enrollment and immunosuppression withdrawal in trial participants:

- Severe graft rejection by Banff criteria.
- Rejection that requires more than one course of bolus corticosteroids.

Table 5 illustrates the thresholds for the number of qualifying events that would trigger suspension of enrollment and further withdrawal of immunosuppression in participants depending on the number of evaluable participants. The incidence rate is the number of participants with a qualifying event observed at any time after enrollment divided by the number of evaluable participants enrolled. An evaluable participant is defined as a participant who has been enrolled for at least 4 weeks or a participant who has had a qualifying event. These thresholds reflect the assumption that qualifying events should not occur in significantly more than 15% of participants in the trial. The basis for the stopping rule is that if there is reasonable evidence that the true event rate exceeds that value, suspension should occur. Each time a qualifying event occurs, the current number of evaluable participants can be compared with the number in Table 5 for the corresponding threshold. The trial will continue if the current number of evaluable participants for the threshold is greater than the number in Table 5 and will be suspended otherwise.

An 80% confidence interval is chosen, instead of a larger one, to reduce the chance of not suspending soon enough. Assessments begin with the second event.

Table 5. Qualifying event thresholds triggering suspension of enrollment and immunosuppression withdrawal based on the number of evaluable participants

Threshold for Number of Qualifying Events	Evaluable Participant Number	Observed Event Rate	80% Confidence Interval	
			Lower Bound	Upper Bound
2	3	0.67	0.20	0.97
3	7	0.43	0.17	0.72
4	11	0.36	0.17	0.60
5	16	0.31	0.16	0.50
6	20	0.30	0.17	0.47

3.6 PACE OF ENROLLMENT AND STUDY DURATION

Enrollment is defined as starting on the day that immunosuppression withdrawal is initiated. Enrollment is planned for 2 years and will be limited to a maximum of two participants every 4 weeks. Maximum patient participation is up to 11 years; therefore, the total study duration is projected to be a maximum of 13 years.

4. ELIGIBILITY

4.1 STUDY POPULATION

Pediatric recipients of parental living related donor hepatic allografts with adequate and stable graft function and no evidence of rejection or significant allograft dysfunction.

4.2 INCLUSION CRITERIA

1. Living-donor liver transplantation from a parental donor.
2. Age less than 18 years at the time of transplantation.
3. At least 4 years since transplantation.
4. Availability and willingness of parental liver donor to participate in the trial.
5. Liver biopsy at screening demonstrating no evidence of acute or chronic rejection and a less than stage 2 fibrosis on the Ishak scale.
6. 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.
7. Negative purified protein derivative (PPD) test results or history of appropriate treatment.

4.3 EXCLUSION CRITERIA

1. Indication for transplantation liver failure due to autoimmune disease, such as autoimmune hepatitis, primary sclerosing cholangitis, or primary biliary cirrhosis.
2. Hepatitis B infection as defined by the presence of HB_SAg or active treatment for hepatitis B.
3. Hepatitis C infection as defined by the presence of antibody against hepatitis C.
4. 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.
5. Transplantation of a second organ before, simultaneously with, or after liver transplantation; or liver retransplantation.
6. Aspartate or alanine aminotransferase (AST or ALT) greater than 2 times the upper limit of normal.
7. Total bilirubin and direct bilirubin, and either alkaline phosphatase or gamma-glutamyl transferase (GGT) greater than 2 times the upper limit of normal.
8. Clinically significant change in hepatic function in the past 26 weeks.
9. GFR less than 40 mL/min/1.73 m^2.
10. Immunosuppression with
    a. a 50% dose increase in a current agent within 26 weeks of screening, *or*
    b. more than one agent within 52 weeks of screening.
11. Any systemic illness requiring or likely to require immunosuppressive drug use.

12. Human immunodeficiency virus (HIV) infection.
13. Pregnancy or breastfeeding.
14. Unwillingness or inability to comply with study requirements and procedures.

4.4 PREMATURE TERMINATION

4.4.1 Premature Termination of Immunosuppression Withdrawal

Section 5.3 outlines parameters for discontinuation of immunosuppression withdrawal. All participants who fail to withdraw will be followed as specified.

4.4.2 Premature Termination from the Trial

Withdrawal of consent. Participants who withdraw consent will undergo all mechanistic assessments scheduled for visit 12 (see Appendices 1–3) at the time that consent is withdrawn. Such participants will be followed but will not be replaced.

Failure to return. Participants who do not return for visits and do not respond to repeated attempts by the site staff to attend study visits will be considered *lost to follow-up*. Such participants will not be replaced.

5. STUDY INTERVENTION

5.1 IMMUNOSUPPRESSION 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. Immunosuppression withdrawal. (*See section 5.1 for definition of *high dose* and *low dose*.)

5.2 ASSESSMENT OF COMPLIANCE WITH STUDY INTERVENTION

Compliance will be assessed at each scheduled visit or telephone contact in accordance with the schedule of events (Appendices 1–4).

5.3 INTERRUPTION OR DISCONTINUATION OF STUDY INTERVENTION

5.3.1 Interruption of Study Intervention

Immunosuppression withdrawal may be temporarily interrupted for up to 4 weeks for the following reasons:

As clinically indicated.

To accommodate the post-immunosuppression-withdrawal liver biopsy visit window. The duration of the lowest taper level before completing immunosuppression withdrawal may be extended for up to 4 weeks in order for the liver biopsy to be performed within the allowed visit window (see section 6.1).

During this time the participant will be followed per the withdrawal schedule (see Appendix 1).

Immunosuppression withdrawal may be resumed at any time during the 4-week period as indicated. Participants whose immunosuppression withdrawal has been suspended for more than 4 weeks and for whom a biopsy is not indicated will be considered to have failed immunosuppression withdrawal.

Participants who fail immunosuppression withdrawal will move into the medium-intensity follow-up schedule (see Appendix 2) for 52 weeks and then will be discharged from the study.

5.3.2 Discontinuation of Study Intervention

Immunosuppression withdrawal will be discontinued for a participant if he or she experiences any of the following:

- A second episode of mild rejection not requiring treatment.
- Any episode of rejection (mild, moderate, or severe) requiring treatment.
- Chronic rejection.
- Abnormal LFTs in the absence of biopsy-proven rejection that do not substantially improve (see section 5.5.2) within the maximum interval specified in section 5.3.1, Interruption of Study Intervention.

Such participants will be considered to have failed immunosuppression withdrawal.

Participants who fail immunosuppression withdrawal will move into the medium-intensity follow-up schedule (see Appendix 2) for 52 weeks after their LFTs have improved (see section 5.5.2) and then will be discharged from the study.

5.4 CONCOMITANT MEDICATIONS

Participants may receive all required concomitant medications as clinically indicated.

5.5 ASSESSMENT OF ALLOGRAFT DYSFUNCTION AND TREATMENT OF REJECTION

5.5.1 Definition of Allograft Dysfunction and Indication for an Allograft Biopsy

Allograft dysfunction occurs when liver function test (LFT) values are elevated compared with baseline, as defined below. LFTs comprise ALT, alkaline phosphatase, and GGT assessments. Baseline LFTs are the mean of the screening value, the prebiopsy value, and—if available—the most recent home laboratory value. Allograft dysfunction occurs when either ALT *or* both alkaline phosphatase and GGT are elevated compared with baseline, as defined below:

If normal or below normal at baseline: Allograft dysfunction occurs when the value reaches twice the upper limit of normal.

If above normal at baseline: Allograft dysfunction occurs when the value reaches twice the baseline value.

If allograft dysfunction is unexplained, a liver biopsy must be performed. Liver tests can be repeated once for confirmation of allograft dysfunction before biopsy.

5.5.2 Diagnosis, Grading, and Monitoring of Rejection

Allograft biopsy will be used to diagnose and grade rejection. Biopsies will be read locally for clinical decision-making, according to the Banff global assessment criteria.[68] The liver biopsy will then be reviewed centrally at the ITN Liver Pathology Core Laboratory at which time protocol grading will be determined. If the biopsy does not demonstrate rejection, an evaluation for other causes of liver dysfunction will be performed. A diagnosis of rejection will necessitate monitoring of LFTs according to clinical standards. Changes in LFTs will be evaluated as follows:

- **If normal or below normal at baseline:**
  - *Substantially improved* when the value is less than or equal to the upper limit of normal.
  - *Improved* when the value is less than 1.5 times the upper limit of normal.
  - *Not improved* when the value is greater than or equal to 1.5 times the upper limit of normal.
- **If above normal at baseline:**
  - *Substantially improved* when the value is less than or equal to 1.2 times baseline.
  - *Improved* when the value is less than 1.5 times baseline.
  - *Not improved* when the value is greater than or equal to 1.5 times baseline.

An episode of rejection is deemed resolved when LFTs are substantially improved per definitions provided.

5.5.3 Treatment of Acute Rejection

5.5.3.1 Additional Assessments Performed During a Rejection Episode

During a period of diagnosed rejection, additional assessments will be performed according to clinical standard of care:

LFTs. LFTs will be performed upon diagnosis of allograft dysfunction or rejection and at the resolution of rejection.

CMV and EBV. During rejection, collection will continue as scheduled in the SOE (see Appendices 1–3).

Quantitative IgGs and autoantibodies. Blood samples will be drawn upon diagnosis of rejection unless these have been drawn within the previous 4 weeks.

5.5.3.2 Mild Rejection Not Requiring Treatment

Mild rejection will be treated as clinically indicated at the discretion of the investigator; management will depend on the results of additional follow-up as, outlined in Figure 2.

If this is the first episode of mild rejection and it resolves without treatment, the participant may resume immunosuppression withdrawal at the same dose and level at which withdrawal was interrupted.

If this is the second episode of mild rejection not requiring treatment, the participant will not be permitted to resume tapering and will be considered to have failed immunosuppression withdrawal (see section 5.3).

5.5.3.3 Any Rejection Requiring Treatment

If a participant experiences a single episode of mild rejection requiring treatment, or moderate or severe rejection, immunosuppression withdrawal will be discontinued. These participants will be considered to have failed immunosuppression withdrawal (see section 5.3). The algorithm for treatment of rejection is outlined in Figure 2. These definitions apply to the use of immunosuppressive agents for treatment of rejection:

Reinstitution. Returning to the regimen used before beginning immunosuppression withdrawal.

Intensification. Increasing the dose of immunosuppressant compared with that used before beginning immunosuppression withdrawal.

Addition. Initiating another nonsteroid immunosuppressant.

Conversion. Changing from one immunosuppressant to another.

Steroid boluses. Use of high-dose corticosteroids for a defined course, which may or may not be followed by an addition of corticosteroids to the maintenance immunosuppression regimen.

5.5.3.4 Rejection after Successful Completion of Immunosuppression Withdrawal

Participants who experience any episode of rejection after successful completion of immunosuppression withdrawal will not be eligible for further attempts at withdrawal. These participants will undergo reinstitution of maintenance immunosuppression after resolution of the rejection episode as defined in section 5.5.2. They will undergo safety follow up as described in section 6.6.

5.5.4 Treatment of Chronic Rejection

A diagnosis of chronic rejection is indicated when a biopsy fulfills the Banff criteria and total and direct bilirubin are above normal. Chronic rejection will be treated per the standard of care.

Participants who experience chronic rejection will be considered to have failed immunosuppression withdrawal (see section 5.3).

5.6 PROPHYLACTIC MEDICATIONS

5.6.1 For Participants Receiving Corticosteroids

Participants who receive corticosteroid bolus for treatment of allograft dysfunction will receive nystatin to prevent fungal infection and trimethoprim/sulfamethoxazole ([TMP/SMX] Bactrim™ or Septra®), pentamidine inhalation therapy, or dapsone to prevent *Pneumocystis carinii* pneumonia (PCP).

5.6.2 For Participants Receiving Antibody Therapy

Participants who receive antibody therapy for the treatment of allograft dysfunction will receive prophylaxis for a minimum of 12 weeks with nystatin to prevent fungal infections; with trimethoprim/ sulfamethoxazole ([TMP/SMX] Bactrim™ or Septra®), pentamidine inhalation therapy, or dapsone to prevent PCP; and with valganciclovir to prevent cytomegalovirus (CMV).

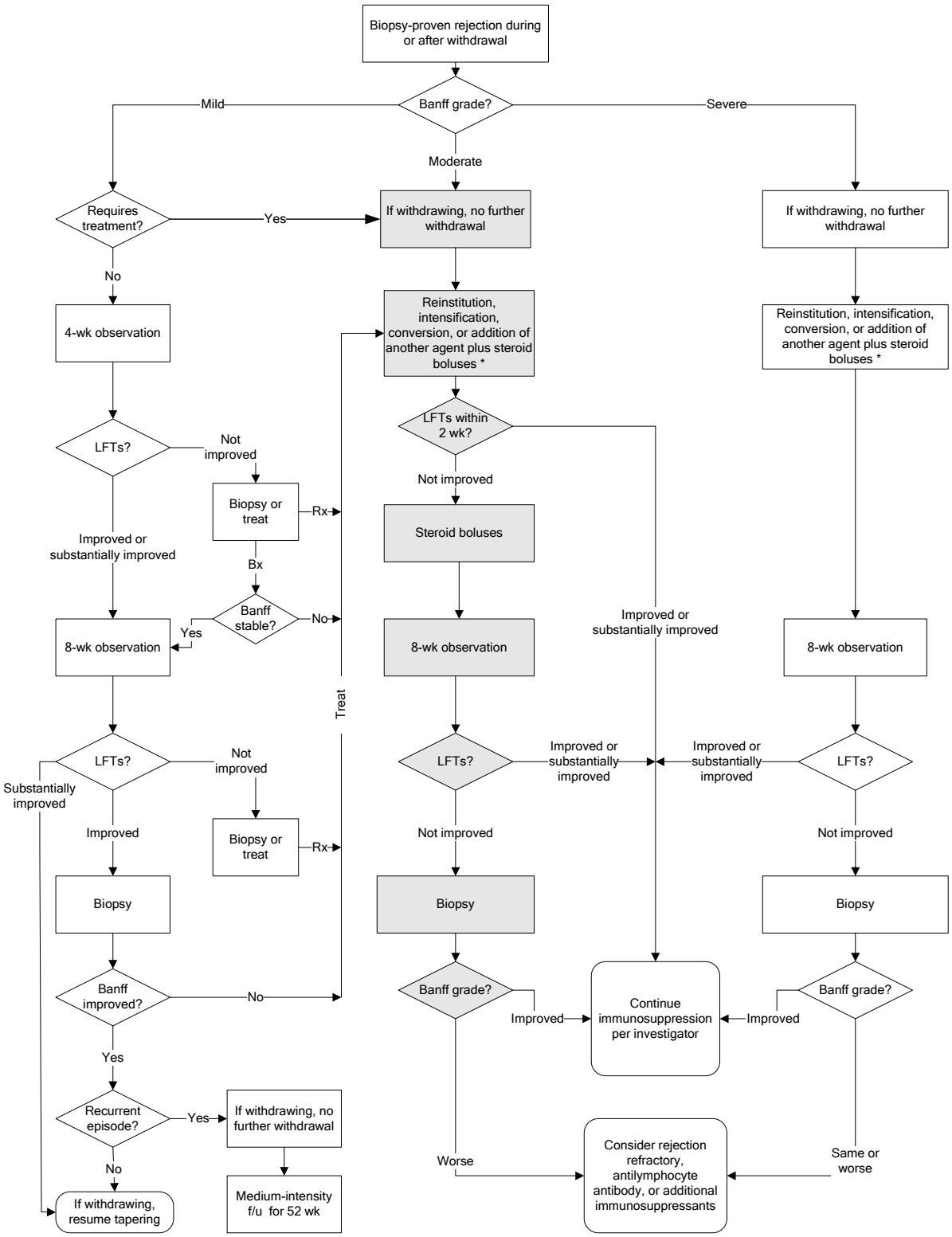

Figure 2. Management of acute rejection. (*See section 5.5.3.3 for definitions.)

6. STUDY PROCEDURES

6.1 VISIT WINDOWS

All visits, except as noted below, should be completed within ±2 weeks of the scheduled time points in the SOE (see Appendices 1–4):

- 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 participants 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.

6.2 GENERAL ASSESSMENTS

These general assessments will be performed (see Appendices 1– 4):

- Informed consent. Written informed consent and subject assent (if applicable) will be obtained before performing any study assessments or procedures.
- Demographic history. A history will be taken to obtain the participant's date of birth, sex, race, and ethnicity.
- General medical history. A history will be taken to document any present or past diseases, any past or planned medical/surgical procedures, and information on the condition under study. Additionally, the most recent results of any previous testing of ALT or alkaline phosphatase and GGT will be documented.
- Liver transplant specific medical history. Rejection episodes, history of any cancer, CMV, infectious episodes, medication change histories, indication for liver transplantation, and results from any liver biopsy performed less than 1 year before screening. (If results for that period are not available, results of the most recent liver biopsy before screening will be collected).

- Physical examination.
- Vital signs. Temperature, blood pressure, pulse, respiration, weight, and height will be recorded.
- Review of inclusion and exclusion criteria.
- PPD skin test. A purified protein derivative (PPD) test to assess infection with tuberculosis will be performed at screening. Results of a prior test performed within 1 year prior to screening will be accepted in lieu of the test at screening and will be recorded on the case report forms (CRFs)
- Telephone consultation. Telephone consultations will be performed as scheduled to assess adverse events, changes in concomitant medications, and compliance with study therapy and laboratory assessments.
- Adverse events. Participants will be assessed for adverse events at all site visits. All adverse events will be recorded on the CRFs.
- Concomitant medications. All reported concomitant medications will be recorded on the CRFs.

6.3 STUDY SITE AND LOCAL LABORATORY ASSESSMENTS

These laboratory assessments will be performed at study sites or at local laboratories where each participant resides:

- Hematology includes CBC with differential and platelets, INR, and PTT.
- Comprehensive chemistry includes Na, K, Cl, CO_2, Mg, Ca, PO4, BUN, Cr, glucose AST, ALT, GGT, alkaline phosphatase, LDH, albumin, total protein, total bilirubin, direct bilirubin, uric acid, and fasting cholesterol panel (total cholesterol, HDL, LDL, and triglycerides).
- Basic chemistry includes Na, K, Cl, CO_2, BUN, Cr, and glucose.
- Liver panel includes AST, ALT, GGT, alkaline phosphatase, total bilirubin, and direct bilirubin.
- Autoantibodies include AMA, ASMA, ANA, and ALKMA.
- Quantitative immunoglobulin (IgG).
- Viral serology includes evaluation of antibodies to CMV, EBV, hepatitis A, hepatitis B, hepatitis C, and HIV.
- Urine hCG.
- GFR (i.e., measurement of serum creatinine and child's height).
- Hemoglobin A_{1C}.
- Tacrolimus or cyclosporine serum levels.

6.4 CENTRAL LABORATORY ASSESSMENTS

Whole blood–quantitative PCR for CMV and EBV reactivation will be performed for all participants.

6.5 LIVER BIOPSIES

Liver biopsies will be performed as follows:

1. At screening.
2. For those participants who successfully withdraw from immunosuppression:

a. Upon successful completion of immunosuppression withdrawal.
b. Upon completion of 104 weeks of medium-intensity follow-up, if still immunosuppression free.
c. Upon completion of 104 weeks of low-intensity follow-up, if still immunosuppression free.
d. Upon completion of 156 weeks of Extended Participant follow-up, if still immunosuppression free.

3. For any unexplained allograft dysfunction (see section 5.5.1).

6.5.1 Biopsy Technique

A consent specific for this procedure will be obtained according to guidelines at the investigative sites. Liver biopsies will be performed with an 18-gauge or larger needle using a percutaneous technique. Investigators may use ultrasound guidance at their discretion. A minimum of 4 cm of core tissue will be obtained:

- 1 cm in RPMI with fetal calf serum will be sent to the protocol chair for intrahepatic lymphocyte studies; however, if this laboratory is not receiving samples, than the tissue will be divided equally among the three core labs below.
- 1 cm will be formalin fixed and paraffin imbedded at screening (visit −1). Local laboratory will keep two slides for local diagnosis and the rest of the block will be sent to the ITN Pathology Core Laboratory for routine histology and immunohistochemical analysis. For all other visits the full 1 cm in formalin will be sent to the ITN Pathology Core Laboratory for routine histology and immunohistochemical analysis.
- 1 cm placed in RNAlater® will be sent on dry ice to the ITN RT-PCR Core Laboratory for intrahepatic gene expression profiling.
- 1 cm embedded in OCT media. Cryomold will be frozen and sent to the ITN Core Laboratory for immunohistochemical analysis.

6.5.2 Tissue Handling and Disposition During Rejection Episodes

Biopsies to rule out rejection will be handled similarly, except that 1 cm in saline will be sent in formalin to the local pathology laboratory for analysis instead of to the protocol chair. Any extra tissue beyond 4 cm, however, will be placed in RPMI with 20% fetal calf serum and sent to the protocol chair for intrahepatic lymphocyte studies.

6.6 FOLLOW-UP ASSESSMENTS

Frequency of visits and the number of assessments performed will depend on whether the patient is undergoing high-, medium-, or low-intensity follow-up (see Figure 3 and Appendices 1–3).

All participants who successfully withdraw from immunosuppression must complete 12 weeks of high-intensity follow-up, 104 weeks of medium-intensity follow-up, and 104 weeks of low-intensity follow-up (see Appendices 1–3). The 12 weeks of high-intensity follow-up will begin immediately after the last dose of immunosuppressant medication is taken.

All participants who remain off immunosuppression after successful completion of low- intensity follow-up will undergo 208 weeks (4 years) of Extended Follow-up as described in Appendix 4.

Participants who experience any episode of rejection after successful complete of immunosuppression withdrawal will move into the medium-intensity follow-up schedule (see Appendix 2) for 52 weeks after their LFTs have improved (see section 5.5.2). They will then be discharged from the study.

Participants who fail immunosuppression withdrawal will move into the medium-intensity follow-up schedule (see Appendix 2) for 52 weeks and then will be discharged from the study.

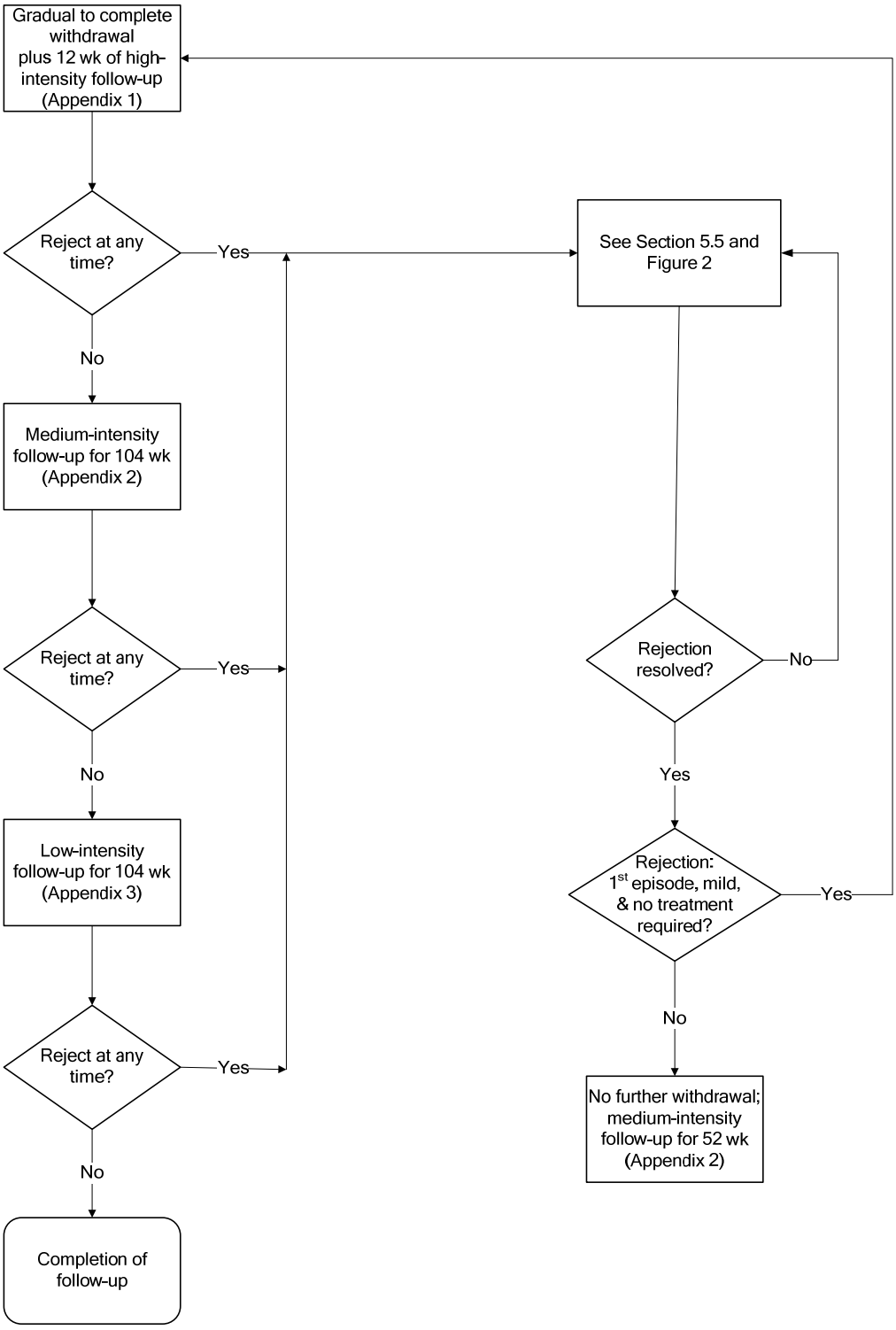

Figure 3. Participant follow-up

7. TOLERANCE ASSAYS

7.1 CELL-BASED ASSAYS

7.1.1 Whole Blood–Flow Cytometry Panel Staining

Process and achievement of tolerance may be closely related to the types and relative frequencies of different immune cell populations. Five-color flow cytometry for multiparameter cell visualization will be used to monitor the dynamic changes in T-cell subsets, naïve vs. memory T cells, regulatory T cells, activated cytotoxic T cells, and NK cells. We will test the hypothesis that changes in the proportion of plasmacytoid, as compared with myeloid dendritic, cells have an effect on, and/or reflect, tolerance. In addition, the presence and frequency of regulatory T cells will be monitored to see if there are differences between tolerant and nontolerant participants. Functionality of such populations will be tested in donor-specific T-cell assays as described in section 7.1.2.

7.1.2 Frozen PBMC–T-cell Assays

There are two pathways that contribute to MHC allorecognition: the direct and indirect pathways. The direct pathway of T-cell activation is mediated by recognition of intact donor MHC alloantigens presented on the surface of donor cells, whereas the indirect pathway involves presentation of allopeptides by MHC molecules on recipient cells. Monitoring antidonor responses mounted by recipient cells is critical since high frequency of donor-specific IFNγ-producing cells appear to correlate with the risk of renal allograft rejection.

Relative contributions of direct and indirect pathways of antigen presentation to allograft rejection can be measured by the ELISPOT assay. The number of recipient T cells stimulated by whole donor PBMC to produce IFNγ can be used to assess direct T-cell allorecognition. Similarly, frequencies of IFNγ-producing recipient T cells can also be measured by stimulated self-APC pulsed with sonicated donor cells or donor class I or class II peptides. With the use of these assays, it should be possible to determine the relative number of donor-specific recipient T cells primed via the direct or indirect pathways.

In this study, we will focus on the direct pathway measures by looking at changes in the nature of T-cell response to alloantigen. In particular, we will look for a shift in the profile of cytokines produced in response to alloantigen from T_H1 to T_H2 type cytokines. The ELISPOT assay can be used in this context to measure frequencies of alloreactive T cells that produce IL-4 or IL-5 vs. IFN-γ. This immune response transition of cytokine production will be measured by ELISPOT assay or intracellular staining.

Whole blood will be collected from parental living donors. The donor blood cells obtained will be cryopreserved and used in the flow cytometry lymphocytic cross match and donor-specific cell-based assays.

7.1.3 Liver Biopsy–Histology

Tissue obtained by liver biopsy will be stained by hematoxylin-and-eosin and will be assessed for histologic evidence of graft rejection. Immunohistochemistry will be performed to identify the types of recipient cells that infiltrate the allograft and may contribute to rejection. Conversely, tolerant grafts may contain cell infiltrates that are protective, another parameter that can be monitored. These

studies may reveal distinctions in immune cell populations in the liver of individuals with successful immunosuppression withdrawal, as compared with nontolerant individuals.

7.2 WHOLE BLOOD DNA–HLA GENOTYPES

Since the development of tolerance may depend on the degree of HLA-matching between donor and recipient, DNA collected from participants (transplant recipients and living donors) will be used to perform sequence-based HLA typing. A complete class I and class II haplotype will be completed, including fine typing of the DQB and DRB regions. The degree of matching between donor and recipient haplotypes will be correlated to phenotypic tolerance.

Genotyping for single nucleotide polymorphisms (SNPs) in select immune response genes will also be performed. These analyses will be used to correlate clinical tolerance with genotypes of candidate immune response genes.

7.3 GENE EXPRESSION PROFILING

7.3.1 Whole Blood–Gene Expression Profiling

Previous gene profiling studies in biopsy material of transplant recipients indicate that changes in expression of a panel of genes, as measured by quantitative real-time PCR (RT-PCR) or DNA microarrays can be predictive of acute allograft rejection. These genes include perforin, cyclooxygenase, and IL-7, among others. Interestingly, some studies indicate that these changes are also measurable by RT-PCR of RNA made from peripheral blood.

Additionally, peripheral blood RNA samples taken just before withdrawal of immunosuppression, during withdrawal, and post withdrawal will be measured on microarray and RT-PCR to see if we can discover new patterns of genes that indicate tolerance-like state or a predisposition to tolerance vs. rejection before drug withdrawal. Both microarray and high-throughput quantitative RT-PCR will be used to define global changes in gene expression. Monitoring during withdrawal of immunosuppression will allow the detection of changes in gene expression patterns predictive of, or correlated with, rejection. Peripheral blood expression of acute rejection–associated genes will be complemented by profiles of these same genes in protocol-directed or clinically indicated liver biopsies.

7.3.2 Liver Biopsy RNA–Gene Expression Profiling

Transcriptional profiling will be performed at time points described in the schedule of events (see Appendices 1–3). Total RNA will be isolated and analyzed for expression of selected immune response genes related to rejection and immunoregulatory functions, including granzyme B, cyclooxygenase, IFNγ, TGF-β, IL-4, IL-7, IL-10, and IL-15. In addition, we will study genes, such as FoxP3, which may indicate immune regulation in the liver. The success or failure of immunosuppression withdrawal will be correlated with the expression of both rejection-associated genes and immune regulation genes. The analysis will be performed using TaqMan® RT-PCR at the ITN Core Laboratory.

7.4 SERUM ASSAYS

7.4.1 Serum–Secreted Cytokines

Levels of various immune cytokines will be measured in participant's serum to determine if cytokine levels are linked to induction of clinical tolerance or are indicative of allograft rejection. Serum will

be collected at regular intervals during the study as well as at the time of clinically directed biopsies. The cytokines analyzed will include IL-2, IL-5, IL-10, TNF-α, and IFN-γ. Higher levels of IL-2, TNF-α, and IFN-γ are indicative of a T_H1-mediated immune response. Higher levels of IL-5 and IL-10 are indicative of a T_H2-mediated immune response.

By using a multianalytical profiling system, the quality, sensitivity, and throughput of cytokine and chemokine assays can be improved.

7.4.2 Serum–HLA Alloantibodies

Donor-specific alloantibodies have been shown to be less of a risk factor for graft rejection in liver transplant recipients than in recipients of other organ allografts. Their presence, however, may preclude development or signal the absence of functional tolerance. Serum samples collected from participants, therefore, will be evaluated for the presence or absence of donor-specific alloantibodies.

Alloantibody assessments will be performed before immunosuppression withdrawal (baseline) and at selected time points during and after withdrawal. Assessments will include donor-specific cross matching with cryopreserved cells, testing against a panel of cryopreserved lymphocytes or HLA antigen-coated microparticles.

8. ADVERSE EVENTS

8.1 OVERVIEW

Safety data will be recorded on a case report form (CRF) specifically designed for this purpose. All the SAE information will be recorded on the source document and transcribed onto an SAE report; pertinent data will be collected on the appropriate CRF. All data will be reviewed periodically by the Data and Safety Monitoring Board (DSMB). The DSMB has the authority to withdraw any participants and/or terminate the study because of safety concerns.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the NIAID, ITN, principal investigators in the trial, IRBs, and health authorities. This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *ICH Guideline E2A*: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events, Version 3.0 (June 10, 2003).

8.2 DEFINITIONS

8.2.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial. An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first.

8.2.2 Serious Adverse Event

A serious adverse event (SAE) or reaction is defined as "any adverse event that suggests a significant hazard, contraindication, side effect, or precaution." This includes, but is not limited to, any of the following events:

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.
- A life-threatening event: Any adverse experience that, in the view of the investigator, places the participant at immediate risk of death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability.
- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

Regardless of the relation of the adverse event to study participation, the event must be reported as an SAE if it meets any of the above definitions.

8.2.3 Unexpected Adverse Events

An adverse event is considered "unexpected" when its nature (specificity) or severity is not consistent with the usual clinical course of individuals who would qualify for this trial but who were not enrolled.

8.3 COLLECTING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events will be collected from the time consent is obtained from the participant until the time the event resolves or until he/she completes study participation, whichever comes first.

Adverse events may be discovered by any of these methods:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event if it is determined by the investigator to be clinically significant. If this is the case, it must be recorded in the source document and as an adverse event on the appropriate adverse event form(s). The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.3.2 Collecting Serious Adverse Events

Serious adverse events will be collected from the time the participant begins study participation until 30 days after he/she completes or prematurely withdraws from the study.

8.3.3 Recording Adverse Events

Throughout the study, the investigator will record all adverse events on the appropriate adverse event case report form (CRF) regardless of their severity or relation to study participation. The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.4 Recording Serious Adverse Events

Serious adverse events will be recorded on the adverse event CRF and on the SAE form, and health authorities will be notified as outlined in section 8.5.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading Criteria

The study site will grade the severity of adverse events experienced by study participants according to the criteria set forth in the National Cancer Institute's Common Toxicity Criteria for Adverse Events Version 3.0 (published June 10, 2003). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

All adverse events will be reported and graded whether they are or are not related to disease progression or study participation.

8.4.2 Attribution Definitions

The relation, or attribution, of an adverse event to study participation will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE reporting form. The relation of an adverse event to study participation will be determined using the descriptors and definitions provided in Table 6.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 6. Attribution of adverse events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related to study participation.

Related Categories		
2	Unlikely	The adverse event is doubtfully related to study participation.
3	Possible	The adverse event may be related to study participation.
4	Probable	The adverse event is likely related to study participation.
5	Definite	The adverse event is clearly related to study participation.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting Timeline

When an investigator identifies a serious adverse event (as defined in section 8.2.2), he or she must notify the Rho Federal Systems Division, Inc. (Rho Fed) Safety Reporting Center within 24 hours of discovering the event using the Rho Fed 24-Hour SAE Reporting Hotline **(1-888-746-3293)**. In addition to being reported by telephone, these events will be entered on the serious adverse event form and the adverse event CRF. Both forms will be faxed to the Rho Fed Safety Reporting Center **(1-888-746-7231)** or **E-MAIL** (rho_productsafety@rhoworld.com). within 24 hours. Rho Fed is responsible for notifying the sponsor within 48 hours of receipt of the event.

8.5.2 Options for Reporting Serious Adverse Events

After the SAE has been assessed by the investigator, there are three options for reporting an event to the appropriate health authorities:

- No requirement to report. This option applies if the adverse event is deemed not serious by the Rho Fed Safety PSS, the ITN clinical physician and the NIAID medical monitor.
- Standard reporting is required. This option applies if the adverse event is classified as one of the following: (a) serious, expected, and study related; (b) serious, expected, and not study related; or (c) serious, unexpected and not study related.
- Expedited reporting is required. This option applies if the adverse event is considered serious, unexpected, and study related. These events must be reported by the sponsor to the appropriate health authorities within 15 days; fatal or life-threatening events must be reported within 7 days. For expedited SAEs, all sites must attach the notification letter and MedWatch/CIOMS to the current investigator's brochure.

All investigators must report serious adverse events to their respective IRBs as mandated by them.

8.5.3 Reporting Serious Adverse Events to the Data Safety Monitoring Board

The DSMB will be provided listings of all SAEs on an ongoing basis. Furthermore, the DSMB will be informed of expedited SAEs by the Regulatory CRO at the same time as health authorities.

8.5.4 Reporting Pregnancy

Any pregnancy that occurs during a clinical study will be reported on an SAE form for tracking purposes only. The investigator should be informed immediately of any pregnancy and should report all pregnancies within 24 hours (as described in section 8.5.1) using the SAE form. The investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible

effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy, and a follow-up SAE reporting form detailing the outcome of the pregnancy should be submitted.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

The following groups of participants will define samples for endpoint analysis:

- Intent-to-treat (ITT) sample: Subjects who have signed informed consent and are enrolled.
- Per-protocol (PP) sample: All participants in whom immunosuppression withdrawal is attempted.

9.2 ANALYSIS PLAN

9.2.1 Analysis of Primary Endpoint

The proportion of participants who are successfully withdrawn from immunosuppression, defined as those who remain off immunosuppression for at least 1 year, will be analyzed using the PP sample and descriptively summarized with a two-sided, 95% CI. Participants with acute rejection occurring after 1 year following complete immunosuppression withdrawal will be considered successfully withdrawn for the purpose of the primary endpoint. This endpoint will also be analyzed using the ITT sample.

9.2.2 Analysis of Secondary Endpoints

9.2.2.1 Safety of Immunosuppression Withdrawal

The following endpoints will be analyzed using the PP and ITT samples:

- The proportion of participants who have graft loss or who die after initiation of immunosuppression withdrawal will be descriptively summarized with a two-sided, 95% CI.
- Median time from initiation of immunosuppression withdrawal to first episode of acute rejection or to first diagnosis of chronic rejection, with a corresponding two-sided 95% CI, will be estimated using the Kaplan-Meier method.
- Distribution of severity among rejection episodes and the incidence of adverse events will be descriptively summarized using frequency tables with enumerations and percentages.
- Descriptive statistics (mean, standard deviation, mean change from baseline) for renal function, blood pressure, cholesterol level, and glucose control over time will be presented.

9.2.2.2 Duration

Median immunosuppression-free duration with a corresponding two-sided 95% CI will be estimated with the Kaplan-Meier method using the PP and ITT samples.

9.2.2.3 Tolerance Predictive Profiles

Immune and genetic profiles will be described and compared between tolerant and nontolerant individuals. Mechanistic endpoints will be analyzed using the PP sample. An objective of the study is to define a predictive profile that differentiates liver transplant recipients who are successfully withdrawn from immunosuppression from those recipients who are not successfully withdrawn, that

is, to see if any one mechanistic assay or combination of mechanistic assays can serve as surrogate markers of tolerance by identifying a subpopulation of liver transplant recipients who subsequently are found to be functionally tolerant. The mechanistic data analysis will involve determining whether each mechanistic assay taken alone or taken in combination with other mechanistic assays can discriminate significantly between tolerant individuals and those at risk of developing alloimmune-mediated graft injury.

9.2.2.4 Rejection Profiles

Rejection profiles will be analyzed in a manner similar to that described above for tolerance predictive profiles.

9.2.2.5 Assessment of Fibrosis Over Time

The change in periportal and perivenular fibrosis will be analyzed using a mixed model repeated measures ANOVA. This endpoint will be analyzed using all subjects who enter Extended Participant Follow up (Appendix 4).

9.3 SAMPLE SIZE

There are no published data available with respect to the success of immunosuppression withdrawal exclusively in living-donor pediatric liver transplant patients 4 years or more post transplant. As described in section 1.2.2, the single source of immunosuppression withdrawal data available in living-donor pediatric liver transplant recipients is from the Kyoto study, which differs from the present study in terms of patient eligibility with respect to time since transplantation (2 years in the Kyoto study vs. 4 years in the present study).[29] The present study will therefore likely include a larger percentage of patients entering on lower relative immunosuppression. 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 patients meeting the primary endpoint in this patient population for which no prior data exist. For example, if 9 of 20 participants 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 patient population.

9.4 PARTICIPANT AND DEMOGRAPHIC DATA

9.4.1 Study Completion

The percentage of participants who complete the study, loss to follow-up, time to loss of follow-up, and reasons for discontinuation (adverse events, etc.) will be presented.

9.4.2 Description of Baseline Characteristics and Demographics

Summary descriptive statistics for demographic, baseline, and transplant-related clinical history characteristics will be provided for all enrolled participants. Demographic characteristics will include age, race, sex, body weight, and height. Continuous data (e.g., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range. Enumerations and percentages will be presented for categorical data (e.g., sex and race).

9.4.3 Medical History

Medical history, including the existence of current signs and symptoms and clinical significance, will be collected for each body system.

9.4.4 Use of Medications

All medications used will be coded using the WHO drug dictionary. The number and percentage of participants receiving concomitant medications/therapies will be presented.

9.4.5 Safety

All adverse events, including infections, malignancies, morbidity, and side effects associated with immunosuppression withdrawal and/or study participation, will be classified by body system and preferred term according to the *Medical Dictionary for Regulatory Activities* (MedDRA).

Frequency tables by category of event (e.g., serious, related to study participation, causing the discontinuation from the study) and by NCI-CTCAE grade will be presented. Laboratory values and vital signs will also be summarized by mean, standard deviation, and change from baseline.

9.5 RANDOMIZATION, STRATIFICATION, AND BLINDING

Individuals who are deemed qualified for the study will be enrolled and assigned a unique participant number within each site. All clinical activities performed in conjunction with this study will be performed in an unblinded manner. However, mechanistic assays will be performed in a blinded fashion because group assignments of tolerant or nontolerant individuals will be unknown until the completion or failure of immunosuppressive withdrawal. Given that this is a one-arm study, there will be no randomization procedure performed.

9.6 PLANNED INTERIM ANALYSES

No interim analyses are planned.

9.7 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol and in the subsequent SAP. Any changes in these principal features will require a protocol or an 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.

10. ACCESS TO SOURCE DATA OR DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants and donors in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational sites must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict

confidentiality of medical and research information that is linked to identified individuals. The investigational sites will normally be notified before auditing visits occur.

11. QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The sponsor is responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

To ensure the reliability of the data recorded in the database, double-data entry will be used for all fields on the CRF. The data will be verified by a series of computerized edit checks, and all relevant data queries will be resolved regularly. When the CRFs are complete, they will be reviewed and signed by the investigator and returned to the sponsor or the CRO. All data from the original signed CRF will be entered in the database, and a comparison program will be run again. All discrepancies will be reviewed, and any resulting queries will be resolved with the investigator and amended in the database. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, with current good clinical practices (GCP), the principles of the Declaration of Helsinki, and with all applicable regulatory requirements.

Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics review committee or institutional review board. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before entering the

study or undergoing any study-specific procedures. The consent process must be recorded in the source document. Consent materials for participants who do not speak or read English must be translated into the participant's appropriate language.

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The attending physician, in the presence of a witness, will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time and for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number, and these numbers rather than names will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on the publication of study results will apply to this study. Authorized participants can find details of the policy statement on the ITN internet website at http://www.immunetolerance.org.

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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
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														

[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**
Urine hCG	X														
Glomerular filtration rate (creatinine and height)	X														
Hemoglobin A_{1C}	X														
Tacrolimus or cyclosporine serum levels		X													
Central Laboratory Assessments															
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

[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.

	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**
Serum–HLA alloantibodies	X[8]								X						X
Whole blood DNA–HLA genotypes[9, 10]	X[8]														

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

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

	Medium-intensity Follow-up [1]											
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[2]	X[3]					X						X
Whole blood–quantitative PCR for EBV reactivation [2]	X[3]					X						X
Liver Biopsies												
Liver biopsies[4, 5]												X
Tolerance Assessments[6,7]												
Whole blood–flow cytometry panel staining						X						X
Frozen PBMC–T-cell assays						X						X

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

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

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

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

[5] 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.

[6] 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.

[7] Do not collect for participants who have failed immunosuppression withdrawal.

	Medium-intensity Follow-up [1]											
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**
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] Participants 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.

Appendix 4. Schedule of Events: Extended Participant Follow-up

Extended Participant Follow-up [1]						
Extended Follow-Up Week:	**8**	**17**	**26**	**35**	**44**	**52**
Visit Number: Year 1	E2	E4	E6	E8	E10	E12
Visit Number: Year 2	E14	E16	E18	E20	E22	E24
Visit Number: Year 3	E26	E28	E30	E32	E34	E36
Visit Number: Year 4	E38	E40	E42	E44	E46	E48
General Assessments						
Physical examination						X
Vital signs						X
Telephone consultation	X	X	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
Comprehensive chemistry						X
Liver panel	X	X	X	X	X	
Autoantibodies						X
Quantitative IgG						X
Glomerular filtration rate (creatinine and height)						X
Hemoglobin A_{1C}						X
Liver Biopsies						
Liver biopsies[2]						X
Tolerance Assessments[3]						
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] Participants enter extended follow-up after completing low-intensity follow-up.
[2] Liver biopsy will be performed as part of the E36 visit. Additional biopsies will be performed according to protocol guidelines or at the discretion of the site investigator.
[3] If a rejection episode occurs, samples for all tolerance assessments that are scheduled for E36 will be collected at this time – including liver biopsy related collections.