A Phase I/II Drug Withdrawal Study of Alloantigen-Specific Tregs in Liver Transplantation

Protocol ITN074ST

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IND Sponsor: DAIT NIAID

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Protocol Chair
Sandy Feng, MD, PhD
Professor, Department of Surgery
University of California, San Francisco
505 Parnassus Ave., Box 0780, M-896
San Francisco, CA 94143
Tel: 415- 353-8725 Fax: 415- 353-8709
Email: Sandy.Feng@ucsfmedctr.org

Protocol Manufacturing Chair
Qizhi Tang, PhD
Professor, Department of Surgery
Director, UCSF Transplantation Research Lab
University of California, San Francisco
513 Parnassus Ave., Box 0780, HSE-520
San Francisco, CA 94143-0780
Tel: 415-476-1739 Fax: 415-502-8326
Email: qizhi.tang@ucsfmedctr.org

ITN Clinical Trial Physician
Sindhu Chandran, MBBS
Associate Director, Clinical and Translational Medicine
Clinical Trials Group,
Immune Tolerance Network
University of California, San Francisco
Diabetes Center
513 Parnassus Avenue, HSW 11, Box 0534
San Francisco, CA 94143-0534
Tel: 415-610-4284
Email: schandran@immunetolerance.org

NIAID Medical Monitor
Nancy D. Bridges, MD
Chief, Transplantation Branch, Division of Allergy, Immunology and Transplantation, NIAID
5601 Fisher Lane, Room 6B31
Rockville, MD 20852-6601
Tel: 240-627-3535
Email: nbridges@niaid.nih.gov

NIAID Regulatory Affairs
Julia Goldstein, MD
Senior Regulatory Affairs Officer
Division of Allergy, Immunology and Transplantation, NIAID
5601 Fisher Lane, Room 7B29
Rockville, MD 20852-6601
Tel: 240-627-3509
Email: goldsteinj@niaid.nih.gov

ITN Lead Biologist
Cynthia Breeden, MPH
Emory University School of Medicine
Department of Surgery, Division of Transplantation
Larsen Lab
Woodruff Memorial Research Building
101 Woodruff Circle, Room 5314
Atlanta, GA 30322
Tel: 404-379-5811
Email: cbreeden@immunetolerance.org

ITN Manager of Clinical Operations
Geo Gaile
Manager, Clinical Operations
Clinical Trials Group
Immune Tolerance Network
University of California, San Francisco
Diabetes Center
513 Parnassus Avenue, HSW 11, Box 0534
San Francisco, CA 94143-0534
Phone: 415-854-7485 Fax: 415-353-4404
Email: ggaile@immunetolerance.org

NIAID Project Manager
Natasha Watson, BSN
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane
Rockville, MD 20852
Tel: 301-335-8476 Fax: 240-627-3115
Email: nwatson@niaid.nih.gov

Rho Scientist
Karen Kesler, PhD
Senior Statistical Scientist
Rho Inc.
2635 East NC Highway 54
Durham, NC 27713
Tel: 919-595-6244 Fax: 919-408-0999
Email: karen.kesler@rhoworld.com

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SYNOPSIS

Title	A Phase I/II Drug Withdrawal Study of Alloantigen-Specific Tregs in Liver Transplantation
Short Title	Liver Transplantation with Tregs at UCSF
IND Sponsor	DAIT NIAID
Conducted by	Immune Tolerance Network
Protocol Chair(s)	Sandy Feng, MD, PhD
Accrual Objective	9 participants who receive the target Treg product (arTreg) dose
Study Treatment	The investigational product is donor alloantigen-reactive T regulatory cells (arTregs). The supportive regimen for this product includes everolimus (EVR), leukapheresis, cyclophosphamide (CTX), and mesna.
Study Design	This is a single-center, prospective, open-label, non-randomized clinical trial exploring cellular therapy to facilitate immunosuppression (IS) withdrawal in liver transplant recipients. The primary investigational product is arTreg. Study interventions include: 1) EVR conversion; 2) administration of CTX and mesna prior to a single intravenous (IV) dose of the arTregs, and 3) tacrolimus (TAC) discontinuation followed by 4) complete IS withdrawal. We plan to accrue 9 participants who will receive the target arTreg dose of at least 90 x 10^6 cells (section 5.2.1.4). This will comprise the PP sample. Participants who receive ≥30 x 10^6 cells but <90 x 10^6 cells as a result of low cell yield will not count towards the planned accrual, and no more than 4 participants will be allowed in this group. This group will be included in the ITT sample. See section 9.1 for additional details on the analysis samples. The plan for IS, cellular therapy and IS withdrawal is described in sections 5.1 to 5.3.5. Participants who successfully withdraw from all IS will undergo research biopsies as outlined in section 1.3.1.9. The research biopsy at 52 weeks following IS discontinuation will be used to determine whether they meet the primary efficacy endpoint of operational tolerance. Participants determined to be operationally tolerant will be followed until 104 weeks following IS discontinuation. Participants who fail IS withdrawal after 52 weeks but before 104 weeks will be followed until week 104 or 12 weeks after resuming IS, whichever is longer (Appendix 5). Participants who do not successfully withdraw from all IS will complete 104 weeks of High Intensity Safety Follow-up (Appendix 6) after failing IS withdrawal. All other participants will be followed per section 4.4.

Study Duration

Accrual duration (section 3.1) will be 113 weeks (2.2 years) from first enrollment.

Total duration of study participation will be influenced by the participant's course during the study.

- Participants who participate in all phases of the study, i.e. who receive arTregs, achieve complete IS withdrawal, and are operationally tolerant (Study Definitions) at 52 weeks off IS will be followed for 2 years after the last IS dose (Appendix 5).
- Participants who receive arTregs, achieve complete IS withdrawal, and then resume IS (sections 5.4.2 and 5.4.3.2) will be followed for 2 years after first completing IS withdrawal or 12 weeks after resuming IS, whichever is longer (Appendix 5).
- Participants who receive arTregs, but are not eligible to initiate or do not complete IS withdrawal will be followed for 2 years from the date they were deemed ineligible or failed IS withdrawal (Appendix 6).
- Participants who receive CTX but do not receive arTregs will be followed for 1 year post CTX administration (Appendix 7).
- Participants who initiate EVR conversion but do not receive CTX will be followed for up to 30 days as outlined in 4.4 (RFU visit in Appendix 2).

Primary Objective

The primary objectives are to evaluate the safety and efficacy of a single IV dose of autologous, donor alloantigen-specific T regulatory cell product, arTreg, to induce operational tolerance early after adult living or deceased donor liver transplantation.

Primary Endpoints

The primary safety endpoints are defined as:

1. Number and severity of adverse events (AEs) attributed to arTreg.
2. Number and severity of AEs attributed to a) leukapheresis, b) CTX, or c) mesna.

The primary efficacy endpoint is operational tolerance. Operational tolerance is defined as:

1. Discontinuation of all IS for 52 weeks;
2. ALT $\leq$ 50 U/L* for $\geq$ 2 measurements separated by $\geq$ 1 week in the 6 weeks prior to the liver biopsy at 52 weeks after the last IS dose; and
3. Liver biopsy at 52 weeks ($\pm$ 4 weeks) after the last IS dose that meets the biopsy criteria for operational tolerance (Table 5) as assessed by central pathology.

*If the investigator determines that abnormal laboratory results are related to an intercurrent illness, the investigator may request that the participant's data be reviewed by an adjudication committee. If the adjudication

committee agrees, they may grant an extension of the primary endpoint visit window (+ 4 weeks). This adjudication committee will consist of the NIAID Medical Monitor, the ITN Clinical Trial Physician, and a Protocol Chair from another LITTMUS protocol (section 1.1.1).

Secondary Endpoints

1. Incidence of grade 3 or greater infections following arTreg infusion.
2. Number and severity of biopsy-proven acute rejection (AR) and/or clinical rejection events at any time after arTreg infusion.
3. Number of chronic rejection (CR) events at any time after arTreg infusion.
4. Number of participants who develop any malignancy.
5. The proportion of participants who discontinue TAC ≥ 12 weeks with ALT ≤ 50 U/L and a liver biopsy 12 - 26 weeks after the last TAC dose that meets biopsy criteria for IS minimization (Table 9) as assessed by central pathology.
6. Durability of operational tolerance defined as the time from achieving the primary endpoint to IS reinitiation or to the end of trial participation.

Exploratory Mechanistic Endpoints

Mechanistic studies to evaluate the effects of the arTreg on the immunological profiles of liver transplant recipients. These endpoints include the number of participants with:

- evidence of a tolerance-associated gene profile[1] as described by A. Sanchez-Fueyo et al;
- a loss or change of alloresponses following arTreg infusion;
- an increase in peripheral blood Treg numbers after arTreg infusion;
- an increase in intragraft Treg numbers after arTreg infusion.

The hypotheses and associated assays for these endpoints are described in sections 7.2 and 7.3.

Inclusion Criteria

RECIPIENT

Individuals must meet all of the following criteria to be eligible:

1. Able to understand and provide informed consent.
2. Male or female, 18 to 70 years of age at the time of enrollment.
3. End-stage liver disease and listed for a living or deceased-donor primary solitary liver transplant.
4. Agreement to use contraception; section 5.2.4.4.
5. Removed in protocol version 3.0.
6. Removed in protocol version 4.0.
7. Positive EBV antibody test.

8. In the absence of contraindication, vaccinations must be up to date per the DAIT Guidance for Patients in Transplant Trials (Refer to the Manual of Procedures).

LIVING DONOR

Living donors must meet all of the following criteria to be eligible:

1. Able to understand and provide informed consent.
2. Male or female, 18 to 70 years of age at the time of enrollment.
3. Meets site-specific clinical donor eligibility requirements.
4. Meets donor eligibility manufacturing requirements within 7 days before or after the blood collection for manufacturing.
5. Willingness to donate appropriate biologic samples.

DECEASED DONOR

Deceased donors must meet the following criteria for their recipients to remain eligible:

1. Meets site-specific clinical donor eligibility requirements.
2. Meets donor eligibility manufacturing requirements.

Exclusion Criteria

RECIPIENT

Individuals who meet any of the following criteria will not be eligible:

1. History of previous organ, tissue, or cell transplant.
2. For CMV antibody negative recipients, a CMV antibody positive donor.
3. Known contraindication to CTX or mesna.
4. Serologic evidence of HIV-1/2 infection.
5. The need for chronic anti-coagulation or anti-platelet agents other than aspirin that cannot be safely discontinued for a minimum of 1 week to safely perform a liver biopsy.
6. End stage liver disease secondary to autoimmune etiology (such as autoimmune hepatitis, primary biliary cirrhosis, or primary sclerosing cholangitis), or other contraindications to drug withdrawal.
7. Psychological, familial, sociological, or geographical factors potentially hampering compliance with the study protocol and follow-up visit schedule.
8. Any condition that, in the opinion of the investigator, may interfere with study compliance.
9. History of cardiac disease (ischemic heart disease requiring revascularization, history of or current treatment for dysrhythmia, or evidence of congestive heart failure) unless cleared by a cardiologist.

10. Any past or current medical problems, treatments or findings which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the candidate's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study. This includes past, present, or future enrollment in studies that affect eligibility at the time of EVR conversion.
11. History of malignancy or any concomitant malignancy, except HCC, completely treated in-situ cervical carcinoma, or completely treated basal cell carcinoma (Study Definitions).
12. Chronic use of systemic glucocorticoids or other immunosuppressives, or biologic immunomodulators.

LIVING DONOR

There are no exclusion criteria for living donors.

DECEASED DONOR

There are no exclusion criteria for deceased donors.

ABBREVIATIONS

ADL	activities of daily living
AE	adverse event
AFP	A-fetoprotein
ALT	alanine aminotransferase
APCs	antigen presenting cells
AR	acute rejection
arTreg	alloantigen-reactive Tregs
CBC	complete blood count
CFR	Code of Federal Regulations
CKD	chronic kidney disease
CMV	Cytomegalovirus
CNI	calcineurin inhibitor
COVID-19	coronavirus disease 2019
CR	Chronic rejection
CRF	chronic renal failure
CRS	cytokine release syndrome
CTX	cyclophosphamide
DAIT	Division of Allergy, Immunology, and Transplantation
DFCI	Dana-Farber Cancer Institute
DLTs	dose limiting toxicities
DMF	drug master file
DSA	donor specific antibodies
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
eCRF	electronic case report form
ES	enrolled sample
EVR	everolimus
FACS	Fluorescence-activated cell sorting
FDA	US Food and Drug Administration
GCP	good clinical practice
GGT	gamma-glutamyl transpeptidase
GMP	good manufacturing practice

GVHD	graft verse host disease
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HICTF	Human Islet and Cellular Transplantation Facility
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
IBW	Ideal body weight
ICH	International Council for Harmonisation
IRB	institutional review board
IS	immunosuppression
ISW	immunosuppression withdrawal
ITN	Immune Tolerance Network
ITT	intent to treat
IV	intravenous
MELD	Model for End-Stage Liver Disease
MGH	Massachusetts General Hospital
MMF	mycophenolate mofetil
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events* (version 5.0, November 27, 2017)
nCoV	novel coronavirus
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NINV	non-immune, non-viral
PBMC	peripheral blood mononuclear cells
PCP	pneumocystis pneumonia
PP	per protocol
Pred	prednisone
PTLD	post-transplant lymphoproliferative disorder
RFU	reduced follow-up
RT-PCR	reverse transcriptase- polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan

SAR	suspected adverse reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
sBc	stimulator B cell
SDCC	Statistical and Data Coordinating Center
SOC	standard of care
SS	safety sample
T1D	type 1 diabetes
TAC	tacrolimus
Tmem	T memory cells
Tr1	Type 1 regulatory T cells
Tregs	regulatory T cells
UCSF	University of California, San Francisco
ULN	upper limit of normal
UTI	urinary tract infection
VOD	Veno-occlusive liver disease
WOCBP	women of childbearing potential

STUDY DEFINITIONS

Acute rejection	Diagnosed in accordance with Banff global assessment criteria (section 5.4.3.2.1).
Allograft dysfunction	When ALT ≥ 100 U/L and/or GGT is elevated relative to baseline. Elevation of GGT sufficient to meet allograft dysfunction is defined as ≥ 2-fold the ULN if the baseline value was < than the ULN; or, ≥ 2-fold the baseline value if the baseline value was ≥ than the ULN (section 5.4.3.1).
Baseline GGT	The average of two GGTs taken at the following times: GGT taken at EVR Conversion Eligibility (V101[R]); and GGT taken at the initiation of EVR conversion (V101S) (section 5.3.4.1).
Chronic rejection	Diagnosed in accordance with Banff global assessment criteria (section 5.4.3.2.2).
Clinical rejection	Participants who are treated empirically based on investigator clinical suspicion in cases where a biopsy is indeterminate or in rare cases where a biopsy cannot be performed (section 5.4.3.2.3).
Completely treated in-situ cervical or basal cell carcinoma	Cleared by an appropriate provider.
Enrolled sample	All participants who sign consent (section 9.1).
Failed IS withdrawal	All participants who do not complete IS withdrawal, or resume IS unless determined otherwise by an adjudication committee (sections 4.4, 5.4.2, and 5.4.3.2.3).
Failure to initiate study therapy	Participants who do not initiate study therapy for any of the following reasons will be terminated: • Failure to meet study continuation parameters post-transplant (section 5.3.1.1) • Inability to tolerate TAC during **Study Stage 1**, leading to discontinuation of TAC or conversion to cyclosporine (section 5.3.1.2) • Failure to meet criteria for Eligibility 2: EVR Conversion (section 5.3.2) • Failure to initiate EVR conversion (section 5.3.2.4). See section 4.5.
Initiation of EVR conversion	EVR conversion is initiated once the participant receives their first dose of EVR (section 5.3.2.4).
Intent to treat sample	All participants who receive at least the minimum arTreg dose (section 9.1).

Manufacturing failure	Onset of arTreg manufacture which does not result in a product meeting release criteria for infusion.
Minimum arTreg dose	30 to <90 x 10^6 cells (section 5.2.1.4).
Onset of arTreg manufacture	Initiation of first leukapheresis procedure
Operational tolerance	Discontinuation of IS for 52 weeks; ALT ≤ 50 U/L* for ≥ 2 measurements separated by ≥ 1 week in the 6 weeks prior to the liver biopsy at 52 weeks after the last IS dose; and Liver biopsy at 52 weeks (± 4 weeks) after the last IS dose that meets the biopsy criteria for tolerance. *If the investigator determines that abnormal laboratory results are related to an intercurrent illness, the investigator may request that the participant's data be reviewed by an adjudication committee. If the adjudication committee agrees, they may grant an extension of the primary endpoint visit window (+4 weeks). This adjudication committee will consist of the NIAID Medical Monitor, the ITN Clinical Trial Physician, and the Protocol Chair from another LITTMUS protocol (Table 5; sections 1.1.1 and 3.3.1).
Per protocol sample	All participants who receive at least the target arTreg dose (section 9.1).
Resolution of rejection episode	An episode of biopsy-confirmed or clinical rejection will be considered resolved when ALT (with or without elevated GGT) ≤ 50 U/L. For these cases, it is recognized that GGT levels decline very slowly following rejection and therefore will not be used to define resolution. If an episode of biopsy-confirmed or clinical rejection involves elevated GGT alone, it will be considered resolved when GGT is ≤ 1.5 x baseline levels. For participants with biopsy-confirmed rejection, a repeat biopsy to further document resolution of rejection may be performed at the discretion of the investigator (section 5.4.3.3).
Safety sample	All participants who initiate EVR conversion (section 9.1).
Screen failure	Participants who do not meet criteria for Eligibility 1: Pre-transplant (section 4.1) are failures and will be terminated (section 4.5).
Steroid refractory acute rejection	AR that has failed to resolve with steroid treatment (section 5.4.3.2.1).
Study therapy	The investigational product is donor alloantigen-reactive T regulatory cells (arTregs). The supportive regimen for this product includes EVR, leukapheresis, CTX, and mesna (section 4.4).
Successful EVR conversion	Target EVR and TAC levels are maintained over two consecutive measurements with ALT ≤ 50 U/L and GGT ≤ the ULN or ≤ 1.5 x the baseline GGT (section 5.3.2.4).
Target arTreg dose	90 to 500 x 10^6 cells (section 5.2.1.4).

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND INFORMATION

1.1.1 Study Summary

The success of liver transplantation is limited by the complications of chronic IS, including medication-related organ toxicities and the risks of cancer, infection, and cardiovascular disease. Transplantation tolerance, defined as the ongoing acceptance of the transplanted graft in the absence of IS, but with otherwise normal immunologic function, would be of great potential benefit. One proposed approach to tolerance is to modify the host immune response through infusion of autologous, functionally immunosuppressive regulatory cells. As a demonstration that exogenous cells can favorably modify the host immune response, animal models show that immunological tolerance can be adoptively transferred to organ recipients using cells from another recipient tolerized to the same donor.[2] Transfer of tolerance has been achieved in a rodent model with purified preparations of regulatory immune cells.[3] Such adoptively transferred T cells can also lead to infectious tolerance by generating an ongoing regulatory response among the recipient's T cells, which have not been manipulated ex vivo.[4] Infectious tolerance remains even after the disappearance of the original tolerance-inducing cells. In humans, T cells with regulatory and immunosuppressive characteristics can be purified and expanded from peripheral blood mononuclear cells (PBMCs) or other cell sources. Several groups have explored the use of such cells for their potential utility to suppress allogeneic responses. Thus, controlling an organ recipient's alloimmune response against a donor organ using cellular therapy is seen as a promising approach that might ultimately supplement current pharmacologic approaches, or allow patients to minimize IS. Several clinically relevant approaches using Tregs, Type 1 regulatory T cells (Tr1), mesenchymal stromal cells, tolerance-inducing dendritic cells and macrophages with regulatory potential have been proposed and are being studied.[5-9] Among these, the use of Tregs is among the most promising.[10,11]

To advance our understanding of the use of different Treg products in organ transplantation, the Immune Tolerance Network and NIAID have created a consortium, called LITTMUS (**Li**ver **T**ransplantation with **T**regs at **M**GH and **U**C**S**F) to carry out independent studies of regulatory cell therapy to induce immunologic tolerance in liver transplantation. This consortium is modeled on the precepts guiding other cooperative efforts exploring cellular therapy in solid organ transplantation such as the ONE Study consortium (http://www.onestudy.org/). Initial studies in this consortium will be carried out by investigators at the University of San Francisco (UCSF) and the Massachusetts General Hospital (MGH) who will conduct single-center, single-arm, phase I/II drug withdrawal studies of alloreactive Treg products in living- or deceased-donor liver transplant recipients. The studies will use a common clinical protocol (identical inclusion/ exclusion criteria, study design, endpoints, etc.), and site-specific, locally manufactured autologous Treg products, each of which has been used in previous clinical studies. Early after liver transplantation, infusion of the Treg product will be preceded by lymphodepletion using CTX, and followed by IS minimization and complete IS withdrawal within 30 months after liver transplantation. This study, protocol ITN074ST, will be conducted at UCSF and will use an alloantigen-reactive Treg cellular product called arTregs, manufactured at UCSF. arTregs are produced by culturing purified recipient Tregs with activated and irradiated donor stimulated B cells (sBc).

The consortium is open to future participation by other institutions with appropriate regulatory cell manufacturing and clinical trial infrastructure.

1.1.2 IS Withdrawal in Liver Transplantation

Liver transplantation is the only effective treatment for end stage liver disease.[12] However, as with any organ transplant, most recipients require lifetime IS; this carries with it a significant burden of associated costs as well as on-target and off-target side effects. The long-term goal of this research is to develop novel approaches that will facilitate reduction, and potentially elimination, of IS.

Liver allografts have long been thought to have natural tolerogenic potential. In small animal models, liver allografts are often accepted spontaneously and in large animals, "prope" tolerance was reported by Calne.[13] There is also evidence from older clinical studies that the likelihood of spontaneous tolerance post liver transplant may exceed 50% in appropriately selected patients, compared with only 5% with other organs such as the kidney.[14-18] These observations provided the foundation for recent clinical trials of IS withdrawal in stable pediatric and adult liver transplant recipients. Previous experiences with IS withdrawal in liver transplantation patients not only provide evidence for the safety of drug withdrawal in this population, but also establish the baseline of spontaneous tolerance for the proposed trial.

That IS withdrawal in adult liver transplant recipients can be performed with an acceptably low risk of permanent graft injury or graft loss is supported by a number of recent trials. For example, Benitez et al. found that of 98 patients who attempted withdrawal, 57 (58%) had AR, which in all cases was readily reversed with treatment.[16] In 21 of 57 cases, rejection was reversed merely by increasing calcineurin inhibitor (CNI) doses and levels. In the remaining patients, escalation of CNIs was combined with low-dose steroids (20 mg over 4-6 weeks; 30 patients), moderate-dose steroids (40-60 mg over 4-6 weeks; 4 patients), and steroid boluses (1 patient). Ultimately, normal graft function (based on liver function tests) was restored in all cases. The likelihood of successful drug withdrawal was strongly correlated with time after transplant: operational tolerance was achieved in only 13% (3 of 24) of those 3-5.7 years post-transplant, compared with 38% (19 of 50) if 5.7-10.6 years and 80% (19 of 24) if >10.6 years post-transplant.

Similar results were observed in DAIT-sponsored Protocol ITN030ST (NCT00135694), an ITN-supported withdrawal trial in liver transplant recipients that enrolled both a hepatitis C virus (HCV) arm and a non-immune, non-viral (NINV) arm.[19] Of 30 HCV participants, five were found to be tolerant (17%). In the NINV arm, 5 of 47 were tolerant (11%). Of note, in this trial, IS withdrawal began as early as one year post-transplant, which may in part explain the comparatively lower rate of tolerance observed versus that seen in the European trials, although differences in cohorts may also be contributory.[16] All rejections were successfully treated with reinstitution of maintenance IS and in only 5 cases was a steroid bolus required.[19] Together, these two studies suggest that IS withdrawal can be attempted with a low likelihood of irreversible graft injury or graft loss if rejection occurs.

1.1.3 Current Trial

The objectives of the current trial are to: (1) Perform an open label phase I/II pilot study to assess the safety and preliminary efficacy of arTreg administration followed by IS withdrawal in recipients of deceased or living donor liver transplants, and (2) Conduct detailed immunological monitoring of the arTreg recipients.

A trial in which Treg infusion is delayed until several weeks to months post-transplant has a number of advantages compared to earlier administration of Tregs, including: 1) avoiding the

immediate perioperative period in which surgery-associated inflammation may induce a milieu hostile to Treg survival and function; 2) allowing transition to a Treg-supportive IS regimen (EVR) after the initial perioperative period, once that drug's risks, such as impaired wound healing, have decreased; 3) allowing the proposed use of leukapheresis and CTX after the patient has recovered from the transplant surgery; and 4) avoiding enrollment of the subset of patients who have poor outcomes post-liver transplant. In the proposed study, patients will be treated with a lymphodepleting agent, CTX, prior to Treg infusion to maximize the ability of the delivered cells to undergo expansion post-infusion and to shift the balance of Treg to Teffector cells in favor of Tregs.

1.2 RATIONALE

1.2.1 Rationale for Treg Therapy in Allogeneic Transplantation

Based on numerous animal and clinical studies, our working hypothesis is that long-term drug-free allograft acceptance requires a combination of deletion of a large fraction of alloreactive cells and the development or delivery of regulatory cells capable of inhibiting the specific immune response to the allograft. Within this framework, the focus of the current protocol is the clinical development of donor alloantigen-specific Tregs as a cellular therapeutic that can be used in conjunction with conventional IS drugs that spare Tregs; i.e., mTOR inhibitors.

It is clearly established from mouse models (*scurfy*) and patients with IPEX syndrome that Tregs (as defined by their expression of the transcription factor Foxp3) are required for the maintenance of self-tolerance.[1-3] Increasing data from human and animal models suggests that maintaining tolerance to autologous antigens is not the sole role of Tregs, and that Tregs may also play a crucial role in fostering tolerance to alloantigens.[4-10] Stable graft function on low dose IS is closely correlated with high numbers of Tregs, both in blood and in the grafts themselves.[20] Moreover, many experimental protocols to induce tolerance or to allow IS minimization are based on strategies that promote Treg induction or function, or, more recently, that use Treg-based cellular therapies.[8] Even the induction of mixed chimerism, initially thought to induce tolerance solely via deletion, is very likely to depend on regulation as well.[11-13] While cellular therapies have not been demonstrated to lead to tolerance to date, further investigation, as described in the current protocol, is warranted.

We hypothesize that the function of Tregs in maintaining immune tolerance in transplant patients can be harnessed through administration of ex-vivo expanded autologous Treg products. In solid organ and hematopoietic stem cell transplantation (HSCT), Tregs are necessary and, under defined experimental conditions in animal models,[21] sufficient to establish transplantation tolerance. Tregs have a unique and robust therapeutic profile that is distinct from small-molecule and biological IS and anti-inflammatory drugs. Tregs can migrate to sites of inflammation and have multiple distinct immune regulatory mechanisms to respond to a spectrum of inflammatory conditions at different sites.[22,23] Tregs are long-lived and, in addition, can exhibit linked suppression and infectious tolerance by inducing new Tregs.[24] Therefore, tolerance induced by a single infusion of Tregs may persist even beyond the survival of the original therapeutic cells.

1.2.2 Why Explore Treg Efficacy in Liver Transplantation?

As noted elsewhere in this background section, several groups, including at UCSF and MGH, have embarked on safety studies of Treg products in renal transplantation; those experiences lead us to believe that exploring the potential efficacy of arTregs to induce transplantation

tolerance after liver transplantation might be medically important to improve current practices in clinical immunotherapy.

Liver transplantation is an ideal platform for testing the safety and efficacy of Treg for four reasons. First, because of its inherent relatively low immunogenicity, liver transplant recipients are usually maintained on lower IS doses than recipients of other organs. Second, surveillance for rejection uses simple blood tests, where transaminase levels can give early indications of cellular injury representing a potential rejection event. Third, possibly in part as a result of the ability to detect rejection early, rejection is relatively easier to reverse in the liver recipient compared to other organ transplants. Finally, because of the inherent regenerative capacity of the liver, the long-term sequelae of rejection, when rapidly detected and treated, are minimal.[15,16]

Developing safe approaches to minimize or eliminate the need for long-term IS in liver transplant recipients could have a significant impact on the health of thousands of patients. Furthermore, it is possible that such approaches might be extended to the very large numbers of recipients of other organ and tissue transplants, making a significant impact on many lives.

1.2.3 Antigen-Specific vs. Polyclonal Tregs

Activation of the immune suppressive function of Tregs requires engagement of the cells' antigen-specific T-cell receptor (TCR), and thus Treg-mediated suppression is antigen specific. Experimental models of Treg therapy in transplantation demonstrate that donor-reactive Tregs are more effective in inducing tolerance than polyclonal Tregs, because anti-donor, allo-reactive Tregs preferentially migrate and accumulate in graft tissues, and suppress the activation of anti-graft immune responses, locally and in graft-draining lymph nodes.[21,25]

1.2.4 T-regulatory Cell Product

1.2.4.1 *Summary of Clinical Experience for arTregs: MGH-DFCI and UCSF*

Several approaches have been developed to utilize ex vivo selected or expanded Tregs for clinical use. Additional sections will discuss these approaches in more detail. Features of Treg products developed at different centers are illustrated in the following table.

Table 1. arTreg Products at MGH-DFCI and UCSF

	Trial/Protocol	Responder	Stimulator	IND (DMF)	Indication	N	Costim blockade
MGH-DFCI							
Product 1	Guinan, NEJM 1999[26]	Donor bone marrow	Irradiated recipient PBMC		Haplo-identical BMT	NA	CTLA4-Ig (Repligen)
Product 1	Davies Blood 2008[27]	Donor bone marrow	Irradiated recipient PBMC		Haplo-identical BMT	19	CTLA4-Ig (Repligen)
Product 1a	Davies Blood 2008[27]	Donor bone marrow	Irradiated recipient PBMC		Haplo-identical BMT	5	Anti-B7.1 and anti-B7.2
Product 2	Multi-center PBSCT (NCT00376480 or NCT00475384). 12 are described ASH abstract 2008.[28]	Donor PBMC	Second haploidentical donor or irradiated recipient PBMC	12085	Haplo-identical PBSCT	16	Anti-B7.1 and anti-B7.2
Product 3	ONE Study (NCT02091232)[29]	Recipient PBMC	Irradiated living organ donor PBMC	15907	Kidney	3	belatacept
Product 3	LITTMUS/ITN073ST[∞]	Recipient PBMC	Irradiated living or deceased organ donor PBMC	18123	Liver	Proposed	belatacept
UCSF							
Product 1	TASK/CTOT-21[∞] (NCT02711826)[25]	Recipient PBMC	Irradiated activated living donor B cells	16626 (15431)	Kidney	1	Not applicable
Product 1	DART (ONE Study) (NCT02244801)	Recipient PBMC	Irradiated activated living donor B cells	16043 (15431)	Kidney	2	Not applicable
Product 1	DELTA/RTB-002[∞] (NCT02188719)	Recipient PBMC	Irradiated activated deceased donor splenocytes	15479 (15431)	Liver	1	Not applicable
Product 1	ARTEMIS/CTOTc-12[∞] (NCT02474199)	Recipient PBMC	Irradiated activated living donor B cells	16345 (15431)	Liver	5	Not applicable
Product 1[*]	LITTMUS/ITN074ST[∞]	Recipient PBMC	Irradiated activated living donor B cells or deceased donor splenocytes	18329 (15431)	Liver	Proposed	Not applicable

[*] See section 5.2.1 for a summary of arTreg manufacture.
[∞] On-going, DAIT NIAID-sponsored clinical trials.

1.2.4.2 *UCSF Treg Product*

1.2.4.2.1 Overview

The Treg product (arTreg) that will be used in this trial, is being used in several DAIT NIAID-sponsored solid organ transplantation clinical trials (Table 1).

1.2.4.2.2 Rationale for the Treg Product Proposed Dose

The arTreg dose selection is based on three factors: prior human experience, estimated effective dose, and current manufacturing capacity.

Prior Human Experience The safety and efficacy of Treg therapy in humans have been evaluated in several clinical studies in HSCT and autoimmune diabetes using polyclonal Tregs.[30-35] Data obtained in these studies show promising safety and efficacy profiles.

There are 8 published reports of Treg therapy in humans, five in GVHD,[30,31,33,35,36] two in type 1 diabetes (T1D),[32,34] and one in liver transplantation.[10] All these studies administered polyclonal Tregs, except the liver transplantation study in which a non-selected preparation of allo-reactive Tregs was infused. As of August 2020, 9 patients have received UCSF manufactured arTregs, 3 in kidney transplantation and 6 in liver transplantation. The highest dose of polyTreg infused into GVHD patients was ~7 x 10^9 cells (100 x 10^6/kg) and the highest dose in T1D patients was 2.6 x 10^9 cells (~38 x 10^6/kg). The highest dose of arTregs infused into a liver transplant recipient was 400 x 10^6. All studies reported that all Treg infusions have been well tolerated with no infusion reactions and no increase infection or cancer relapse in GVHD patients.

Estimated Effective Dose

In the liver transplant trial most similar to protocol ITN074ST[10], 610 - 2,590 x 10^6 autologous PBMCs stimulated for 13 days with irradiated donor PBMCs in the presence of antibodies to CD80 and CD86 were infused.[10] The product lots were administered after CTX for lymphodepletion and contained an average of 28% FOXP3+ cells: the lowest dose infused contained 31 x 10^6 and the highest dose infused contained 466 x 10^6 $Foxp3^+$ Tregs. The results showed that Treg doses of 30 to <90 x 10^6 Tregs have suboptimal efficacy to induce operational tolerance (2 successes out of 4). However, doses between 90 and 500 x 10^6 Tregs appeared to be more efficacious (5 successes out of 6) to support successful IS withdrawal. Based on these published studies, we estimate that a dose of 90 - 500 x 10^6 will likely be well tolerated by patients and support successful IS discontinuation.

Manufacturing Capacity

Current experiences of the UCSF manufacturing facility support the feasibility of producing the selected dose of arTregs proposed in this trial. Manufacturing yield for the arTreg lots vary depending on the frequency of Tregs in peripheral blood and the ability of the arTregs to expand using the established processes. Some end-stage liver disease patients have less peripheral Tregs due to fewer $CD4^+$ T cells in the circulation compared to normal control subjects. This deficit can be partially corrected using leukapheresis to ensure isolation of 5 x 10^6 Tregs in the starting material. In the UCSF experience (unpublished data), clinical arTreg expansion ranges between 7 to 390 fold (average: 101 fold). The referenced UCSF data support the feasibility of manufacturing a minimum dose of 30 to <90 x 10^6 arTreg cells and the planned target dose of 90 to 500 x 10^6 arTreg cells proposed in protocol ITN074ST.

1.2.4.2.3 Clinical Studies with the arTreg UCSF Product

Overview

The UCSF group has developed a GMP-compliant process to manufacture autologous donor arTregs using activated and irradiated sBc for selective expansion of alloantigen-reactive Tregs.[25] There are currently several DAIT-sponsored INDs using the arTreg product

manufactured at UCSF. The manufacturing process is described in detail in UCSF-sponsored DMF 15431 (Table 1).

Kidney Trials

As of April 2018, a total of seven subjects have been enrolled in the TASK/CTOT-21 study (IND #15431, NCT02711826). Three subjects were randomized to receive arTregs. Of these three subjects, 2 did not receive arTregs due to 1) possible EBV reactivation in the donor B cells 2) failure to meet the target arTreg dose. One kidney transplant recipient received 432.5 x 10^6 arTregs at approximately 14 months post-transplant. There were no infusion reactions. The subject experienced 2 SAEs, which included CMV infection and antibody mediated rejection, which were both deemed unrelated to the arTreg infusion (*unpublished results*).

As of the end of 2017, 2 kidney transplant recipients have received 300 x 10^6 arTregs on day 3 after transplant in the DART study (IND #16043, NCT02244801). One subject experienced a urinary tract infection (UTI) SAE was a pre-existing condition in this patient and deemed not related to arTreg infusion. The other subject experienced a SAE of elevated serum creatinine 3 days after arTreg infusion that was treated and responded to steroid pulse. The biopsy did not meet the criteria of rejection. It was deemed possibly related to arTreg infusion (*unpublished results*).

Liver Trials

As of April 2018, a total of six subjects have thus far been enrolled in the ARTEMIS/CTOTc-12 study (IND #16345, NCT02474199); all subjects were eligible by biopsy to proceed with IS withdrawal and all subjects tolerated the first two steps of IS withdrawal and were eligible to receive arTreg infusion. For two of the six, an infusible dose of arTregs could not be made so no further IS withdrawal was undertaken and the subjects were moved to safety follow-up. The other four subjects received arTregs: 2 received the full dose (300 – 500 million) while 2 received a "suboptimal" dose (100 – 300 million). One subject who received the full dose has achieved the primary endpoint of 75% CNI reduction. The other three subjects experienced rejection as detailed in Table 2. The only other reported adverse event was "bronchitis" (AE Grade 2) that occurred prior to arTreg infusion (*unpublished results*).

Table 2. Summary of Rejection Events following arTreg Infusion in ARTEMIS/CTOCc012

Subject	Days after arTreg infusion	Central pathology	AE Grade	SAE	Comments
8001	83	Mild to moderate	2 = Moderate	No	Subject discontinued all IS the day after arTreg infusion
6001	167	Indeterminate to mild	3 = Severe	Yes – hospitalization	
6003	33	Mild	2 = Moderate	No	Subject had recent diarrheal illness with mild liver tests abnormalities at time of arTreg infusion

Comparison with ongoing trials

Table 1 notes ongoing DAIT NIAID-sponsored clinical trials testing the UCSF manufactured product, arTreg, in adult solid organ transplantation recipients. The deLTa and ARTEMIS trials evaluate arTregs in liver transplantation. As shown in Table 3, protocol ITN074ST differs from the above referenced protocols in many key aspects, including patient population (living versus deceased donor recipients), time after transplantation, lymphocyte depletion agent, backbone IS, and IS management/withdrawal. These differences may ultimately provide insights as to the optimal context for arTreg administration with respect to both safety and efficacy.

Table 3. Comparison of Liver Transplant Trials at UCSF Evaluating arTreg

	LITTMUS (proposed)	deLTa	ARTEMIS
Lead site(s)	***UCSF and MGH***	UCSF	UCSF
Living vs deceased donor	***Living or Deceased***	Deceased	Living
Time after liver transplantation	***Early / de novo***	Early / de novo	2 - 7 yrs post-transplant
Lymphodepletion	***Cytoxan***	ATG	None
Backbone IS	***Full dose mTOR***	Full dose mTOR or CNI	Low dose CNI
Treg product	***Allo-reactive***	Allo-reactive	Allo-reactive
Immunosuppression withdrawal	***Yes***	No	Yes

1.2.5 Rationale for Conditioning with CTX Prior to Treg Infusion

1.2.5.1 *Role of in vivo homeostatic expansion*

Use of Treg therapy in organ transplantation is limited by multiple practical constraints on cell collection and timely and effective ex vivo manipulation. An additional consideration is the capacity for in vivo expansion. In general, all lymphocyte subclasses expand under conditions of lymphopenia ("homeostatic expansion"), although both memory T cells and Tregs are favored (i.e., proliferate more vigorously) compared with naïve T cells.[37]

Bolton HA et al. have demonstrated in an allogeneic murine HSCT model that co-administration of Treg and conventional CD4+ T cells was not an effective means of GVHD prophylaxis, whereas administration of Treg to lymphopenic mice followed by slower homeostatic expansion of all subsets resulted in better Treg reconstitution and decreased evidence of alloreactivity, emphasizing a probable role of Tregs in constraining T memory cell expansion.[38] In a non-human primate model of kidney transplantation in which ex vivo costimulatory blockade was used to generate anergic and allospecific Treg, administration of CTX to the non-human primates prior to anergic Treg infusion was essential to enable tolerance induction.[39]

In HSCT studies, significant expansion of Treg, generated ex vivo using costimulatory blockade and administered as part of the allograft to highly lymphopenic patients after lymphomyeloablation, was evident from 60 days to at least 1 year post-transplant.[40]

1.2.5.2 *Approaches to achieve lymphopenia*

The optimal mechanism by which to achieve lymphopenia in the solid organ transplant setting is not known. Anti-lymphocyte or anti-T cell antibodies have relatively long half-lives, which may make them inappropriate to be administered prior to a T cell-based therapy. CTX, a nitrogen mustard-derived DNA alkylating agent, has a long history of use in autoimmune and

malignant lymphoid disease as a lymphodepleting and immunosuppressive agent. In addition, dose-related specific effects on T-cell subsets, and perhaps on innate immune cell populations, have been postulated.[41]

1.2.5.3 *Best timing for CTX*

CTX has been administered in the first week after allogeneic HSCT in large numbers of published animal and human settings.[42] In these studies, the goal has not been lymphodepletion (as all such recipients are already profoundly lymphoablated using both myeloablative and reduced conditioning regimens), but rather selective eradication of alloreactive donor cells proliferating in response to the hematopoietic recipient/stimulator. This early CTX treatment window, which capitalizes on both lymphodepletion and selective allodepletion, would raise significant concerns and likely not be appropriate in the proposed liver transplant setting. CTX requires activation in the liver and rapid renal clearance to achieve efficacy with minimal toxicity. The high Model for End-Stage Liver Disease (MELD) score and the related medical issues observed during the pre-transplantation period in liver transplant candidates, as well as the complex status of liver transplant patients shortly after the surgery, make potential morbidity from CTX administration more likely. This is the case because the hepatic CTX metabolic pathway required to activate CTX prodrug to the active metabolites is frequently compromised.

The goal of delayed CTX administration will be to capitalize on the clinical improvement and improved hepatic function of patients later after transplant. Rather than focusing on allodepletion, we would be harnessing the capacity of CTX to produce generalized lymphodepletion.[43]

1.2.5.4 *Effect of CTX on non-proliferating Teffector cells in vivo*

Although not well studied, a theoretical concern for the use of CTX is its potential lack of efficacy in depleting non-proliferating Tmemory cells. However, the use of standard induction and maintenance IS in the patients prior to CTX is designed to minimize the number of such cells. Persistent and proliferating alloreactive cells should be susceptible to CTX. Indeed, in mouse HSCT models, CTX administration has ameliorated ongoing graft versus host disease.[44] Such relative alloselectivity may be abrogated by CNI, perhaps by inhibition of IL-2-mediated proliferation.[45]

1.2.5.5 *Effect of CTX on Tregs in vivo*

CTX has also been shown, in a dose-dependent manner, to reduce the number of Tregs in vivo. In protocol ITN074ST, arTregs will be generated exogenously, rendering the effects of CTX on in vivo Tregs relatively unimportant. This has been confirmed in mouse studies of combining CTX preconditioning with donor-specific Treg infusion to induce islet transplant tolerance.[46]

1.3 POTENTIAL RISKS AND BENEFITS

1.3.1 Potential Risks

1.3.1.1 *CTX (Cytoxan®) Risks*

Myelosuppression, IS, Bone Marrow Failure and Infections

CTX can cause myelosuppression (leukopenia, neutropenia, thrombocytopenia and anemia), bone marrow failure, and severe IS which may lead to serious and sometimes fatal infections, including sepsis and septic shock. Latent infections can be reactivated.

Urinary Tract and Renal Toxicity

Hemorrhagic cystitis, pyelitis, ureteritis, and hematuria have been reported with CTX. Medical and/or surgical supportive treatment may be required to treat protracted cases of severe hemorrhagic cystitis. Urotoxicity (bladder ulceration, necrosis, fibrosis, contracture and secondary cancer) may require interruption of CTX or cystectomy. Urotoxicity can be fatal. Urotoxicity can occur with short-term or long-term use of CTX.

Cardiotoxicity

Myocarditis, myopericarditis, pericardial effusion including cardiac tamponade, and congestive heart failure, which may be fatal, have been reported with CTX therapy. Supraventricular arrhythmias (including atrial fibrillation and flutter) and ventricular arrhythmias (including severe QT prolongation associated with ventricular tachyarrhythmia) have also been reported after treatment with regimens that included CTX.

The risk of cardiotoxicity may be increased with high doses of CTX (in the range planned in the current trial), in patients with advanced age, and in patients with previous radiation treatment to the cardiac region and/or previous or concomitant treatment with other cardiotoxic agents. Particular caution is necessary in patients with risk factors for cardiotoxicity and in patients with pre-existing cardiac disease.

In post-marketing reporting, the following have been reported in patients taking CTX: cardiac arrest, ventricular fibrillation, ventricular tachycardia, cardiogenic shock, pericardial effusion, myocardial hemorrhage, myocardial infarction, cardiac failure (including fatal outcomes), cardiomyopathy, myocarditis, pericarditis, carditis, atrial fibrillation, supraventricular arrhythmia, ventricular arrhythmia, bradycardia, tachycardia, palpitations, QT prolongation.

Pulmonary Toxicity

Pneumonitis, pulmonary fibrosis, pulmonary veno-occlusive disease and other forms of pulmonary toxicity leading to respiratory failure have been reported during and following CTX treatment. Late onset (greater than 6 months after start of CTX) pneumonitis appears to be associated with increased mortality. Pneumonitis may develop years after treatment with CTX.

Secondary Malignancies

CTX is genotoxic. Secondary malignancies (urinary tract cancer, myelodysplasia, acute leukemias, lymphomas, thyroid cancer, and sarcomas) have been reported in patients treated with CTX-containing regimens. The risk of bladder cancer may be reduced by prevention of hemorrhagic cystitis.

Veno-occlusive Liver Disease

Veno-occlusive liver disease (VOD) including fatal outcome has been reported in patients receiving CTX-containing regimens. A cytoreductive regimen in preparation for bone marrow transplantation that consists of CTX in combination with whole-body irradiation, busulfan, or

other agents has been identified as a major risk factor. VOD has also been reported to develop gradually in patients receiving long-term low-dose immunosuppressive doses of CTX. Other risk factors predisposing to the development of VOD include preexisting disturbances of hepatic function, previous radiation therapy of the abdomen, and a low performance status.

Infertility

Male and female reproductive function and fertility may be impaired in patients being treated with CTX. CTX interferes with oogenesis and spermatogenesis. It may cause sterility in both sexes. Development of sterility appears to depend on the dose of CTX, duration of therapy, and the state of gonadal function at the time of treatment. CTX-induced sterility may be irreversible.

Embryo-Fetal Toxicity

CTX can cause fetal harm when administered to a pregnant woman. Exposure to CTX during pregnancy may cause birth defects, miscarriage, fetal growth retardation, and fetotoxic effects in the newborn. CTX is teratogenic and embryo-fetal toxic in mice, rats, rabbits and monkeys. Female patients of reproductive potential should avoid becoming pregnant and should use highly effective contraception during treatment and for 1 year after completion of therapy.

Pregnancy Category D Risk Summary

CTX can cause fetal harm when administered to a pregnant woman based on its mechanism of action and published reports of effects in pregnant patients or animals. Exposure to CTX during pregnancy may cause fetal malformations, miscarriage, fetal growth retardation, and toxic effects in the newborn.

Malformations of the skeleton, palate, limbs and eyes as well as miscarriage have been reported in humans after exposure to CTX in the first trimester. Fetal growth retardation and toxic effects manifesting in the newborn, including leukopenia, anemia, pancytopenia, severe bone marrow hypoplasia, and gastroenteritis have been reported after exposure to CTX.

Nursing Mothers

CTX crosses into breast milk. Neutropenia, thrombocytopenia, low hemoglobin, and diarrhea have been reported in infants breast fed by women treated with CTX.

Common Adverse Reactions

More common side effects include; neutropenia, fever or chills, nausea, vomiting, anorexia, abdominal discomfort, gastrointestinal pain and diarrhea, alopecia, skin rash, and pigmentation of the skin and changes in nails.

1.3.1.2 *Mesna (MESNEX®) Risks*

Hypersensitivity Reactions

MESNEX may cause systemic hypersensitivity reactions, including anaphylaxis. These reactions may include fever, cardiovascular symptoms (hypotension, tachycardia), acute renal impairment, hypoxia, respiratory distress, urticaria, angioedema, laboratory signs of disseminated intravascular coagulation, hematological abnormalities, increased liver enzymes,

nausea, vomiting, arthralgia, and myalgia. These reactions may occur with the first exposure or after several months of exposure.

Dermatologic Toxicity

Drug rash with eosinophilia and systemic symptoms and bullous and ulcerative skin and mucosal reactions, consistent with Stevens-Johnson syndrome or toxic epidermal necrolysis have occurred. MESNEX may cause skin and mucosal reactions characterized by urticaria, rash, erythema, pruritus, burning sensation, angioedema, periorbital edema, flushing and stomatitis. These reactions may occur with the first exposure or after several months of exposure.

The most frequently reported side effects of Mesna when given without chemotherapy include headache, injection site reactions, flushing, dizziness, nausea, vomiting, abdominal pain/colic, pain, constipation, decreased white blood cell count, decreased platelet count, decreased red blood cell count, somnolence, diarrhea, anorexia, fever, pharyngitis, hyperesthesia, influenza-like symptoms, hair loss, and coughing. A false positive test for urinary ketones may occur in patients treated with Mesna.

An uncommon side effect of Mesna is allergic reaction. Usually it is mild in the form of a skin rash or itching. This reaction rarely becomes severe. **Mesna is contraindicated in patients with a known hypersensitivity to Mesna or other thiol compounds such as captopril and penicillamine.**

Pregnancy Category B

There are no studies of Mesna in pregnant women. Reproduction studies performed in rats and rabbits at oral doses approximately 10 times the maximum recommended total daily human dose on a body surface area basis (1000 mg/kg in rabbits and 2000 mg/kg in rats) revealed no evidence of harm to the fetus due to Mesna. The incidence of malformations in human pregnancies has not been established for Mesna. No long-term studies in animals have been performed to evaluate the carcinogenic potential of Mesna.

Nursing Mothers

It is not known whether mesna or dimesna is excreted in human milk. Benzyl alcohol present in maternal serum is likely to cross into human milk and may be orally absorbed by a nursing infants.

1.3.1.3 *UCSF Treg Product Infusion Risks*

Although Treg infusion has been innocuous in animal models, there is relatively scant experience in humans. Three Treg therapy trials in GVHD have been reported. The first-in-man trial by Trzonkowski et al involved two patients.[36] The first patient had chronic GVHD two years after transplantation. After receiving 0.1 x 10^6/Kg of flow-sorted ex-vivo expanded Tregs from the donor, the symptoms subsided and the patient was successfully withdrawn from IS. The second patient had acute GVHD that progressed despite three infusions with an accumulative dose of 3 x 10^6/Kg expanded donor Tregs. A larger scale phase I trial led by Brunstein et al[47] enrolled twenty-three patients with advanced hematologic malignancy. The patients were treated with two units of umbilical cord blood as a source of stem cells and effector T cells. Tregs were isolated using anti-CD25 immunomagnetic bead selection from

third-party cord blood samples that had 4- to 6- HLA match with the recipient. Up to 6 x 10^6/Kg Tregs were infused after ex-vivo expansion using anti-CD3 and anti-CD28 conjugated beads. The infused Tregs were detectable in circulation for up to 7 days. During the one-year period after Treg infusion, the investigators observed no dose-limiting toxicities (DLTs) or increase in AEs compared to historical controls. Incidences of acute and chronic GVHD were reduced in patients received Treg therapy. This group later reported a third trial that enrolled 28 patients with high-risk hematological malignancies.[31] Patients received anti-CD25 immunomagnetic bead enriched donor Tregs without ex vivo expansion four days before receiving one-haplo-mismatched hematopoietic stem cell and Tconv transplants from the same donors. A majority of the patients received 2 x 10^6/kg Tregs with 1 x 10^6/kg Tconvs, and no adjunct IS was given after transplant. Patients demonstrated accelerated immune reconstitution, reduced CMV reactivation, and low incidence of tumor relapse and GVHD. Collectively, these studies show that Treg therapy has minimal toxicity in the setting of GVHD.

Recently, a phase I/II study applying polyclonally expanded fluorescence-activated cell sorting (FACS) purified Tregs to type 1 diabetic patients has been reported by the Trzonkowski group.[32] The study enrolled 10 type 1 diabetic children (aged 8-16 years) within 2 months after diagnosis. Four patients received 10 x 10^6 Tregs/kg body wt. and the remaining 6 patients received 20 x 10^6 Tregs/kg body wt. The patients were followed for 4-5 months after Treg infusion and no toxicity of the therapy was noted. The authors concluded that Treg therapy was safe and well tolerated in children.

At UCSF, a phase I Treg dose-escalating trial evaluating the safety of Treg therapy in type 1 diabetic patients has been performed.[34] This was a dose escalation study in 14 subject in 4 cohorts receiving doses of polyclonal autologous Tregs from 0.05 to 26 x 10^8 cells. This study demonstrated that infused Tregs were phenotypically stable. A proportion of cells were long-lived and detectable in vivo up to 1 year after infusion. There were no infusion reactions reported and no grade 3 or higher events attributed to the cell therapy.

In transplantation, one Treg therapy trial has been published.[10] Another 12 trials are active and registered on ClinicalTrials.gov (Table 4) in liver and kidney transplantation.[11]

Table 4. Reported or Registered Clinical Trials of Treg Cell Therapy

Targeted Enrollment	Type of Tregs	Type of Trials	Trial ID
Liver Trials			
10	Autologous Tregs stimulated with irradiated donor PBMCs with costimulation blockade	Phase 2	Japan[10]
24	Autologous donor antigen–expanded Tregs	Phase 1	deLTa-UCSF, NCT02188719
10	Autologous polyclonally expanded Tregs	Phase 1 & 2	Nanjing, China, NCT01624077
18	Autologous donor antigen–expanded Tregs	Phase 1 & 2	ARTEMIS-UCSF, NCT02474199
26	Autologous polyclonally expanded Tregs	Phase 1 & 2	ThRIL-KCL, NCT02166177
Kidney Trials			
3	Autologous polyclonally expanded Tregs	Phase 1	TASKp-UCSF, NCT02088931
45	Autologous polyclonally or donor antigen–expanded Tregs	Phase 1 & 2	TASK-UCSF, NCT02711826
10	Autologous polyclonally expanded Tregs	Phase 1	TRACT-Northwestern, NCT02145325
8	Autologous Tregs stimulated with irradiated donor PBMCs with costimulation blockade	Phase 1	One Study–MGH, NCT02091232
12	Autologous polyclonally expanded Tregs	Phase 1	One Study–UK, NCT02129881
9	Autologous polyclonally expanded Tregs	Phase 1 & 2	One Study–Charité, NCT02371434
8	Autologous donor antigen–expanded Tregs	Phase 1	One Study–UCSF, NCT02244801

1.3.1.4 *Liver Allograft Rejection Due to IS Withdrawal Risks*

The primary risk of IS withdrawal is that of graft rejection. Graft rejection carries three attendant risks: risk of irreversible graft injury or failure, the need for liver biopsy to verify the diagnosis, and the risk accompanying treatment(s) needed to reverse the rejection once the diagnosis has been secured.

Rejection-associated graft injury or graft loss

Given the risk of rejection, the trial will be designed to allow early detection of rejection through frequent monitoring during the period of IS withdrawal and in the period early after IS cessation. Based on the experience of other withdrawal trials, with this approach it is expected that rejection episodes will be detected early and readily reversed. While theoretically possible, severe rejection leading to graft loss, re-transplantation or patient death is very unlikely and has been only rarely observed with IS withdrawal in liver allograft recipients. In 4 separate studies of IS withdrawal on a total of 267 adult liver allograft recipients published between 1997 and 2013[16,18,48,49] only a single graft loss was reported.[48,49]

For-cause liver biopsy risks

Liver biopsy to permit histopathological assessment remains the gold standard in diagnosis of rejection and will be employed in all suspected cases of rejection in this trial unless clinically contraindicated or logistically infeasible. The procedure is usually performed percutaneously under ultrasound guidance and local anesthetic. It is usually associated with mild pain lasting only a few hours (rarely a few days). The risk of significant bleeding requiring transfusion is 0.5-1% and the risk of bile leak or injury to adjacent organs (pneumothorax, bowel perforation etc.) is <0.5%; the risk of one of these complications leading to death is estimated at 0.1-0.01%.[50]

Risk associated with treatment of rejection

The experience reported to date in liver IS withdrawal trials suggests that rejection episodes that occur during staged weaning and serial monitoring every 2-3 weeks are readily reversed by reinstitution of CNI-based IS alone, or CNI in combination with low dose steroids. This may be associated with transient worsening of certain co-morbidities (diabetes, hypertension, hyperlipidemia, etc.), but is very unlikely to result in irreversible damage. The need to use strong IS regimens to reverse rejection (e.g. repeated steroid boluses, T cell depleting antibodies) may lead to increased risk of infection (e.g. CMV reactivation), malignancy and renal dysfunction. Within a carefully monitored clinical trial, the development of rejection episodes of such severity is extremely unlikely. In 4 separate studies of IS withdrawal on a total of 267 adult liver allograft recipients published between 1997 and 2013[16,18,48,49] only 2 episodes of severe rejection were reported.[48,49] One episode resulted in graft loss and the second reversed with steroids alone. In these 267 patients, all other rejection episodes were graded as mild or moderate and were treated successfully with reinstitution of maintenance IS with or without steroids.

1.3.1.5 ***Maintenance IS Risks***

Administration of all IS therapies used presently to prevent rejection of transplanted tissues carry general risks of opportunistic infection and malignancy, including lymphoma, and skin cancers. These agents are not recommended for nursing mothers, and it is recommended (and mandated in the current protocol) that women of childbearing potential (WOCBP) use effective contraception before, during and for at least 4 months following administration of these agents.

The use of mycophenolate acid, TAC, and steroid IS is well established in liver transplantation. EVR is FDA approved for use in liver transplantation. The risks for dose-related toxicities and side effects from these IS medications are not different for participants in this trial. The reduced dosing in conjunction with other agents in the Treg-supportive IS regimen may expose the participant to an increased risk of rejection.

1.3.1.6 ***Over Immunosuppression Risks***

Chronic IS is associated with a variety of side effects including infection, malignancy, hypertension, diabetes and increased risk of cardiovascular disease. Chronic CNI use is also associated with nephrotoxicity, which is responsible for a significant rate of chronic renal failure (CRF) at 5 years post orthotopic liver transplantation (OLT). CRF confers a significant mortality risk and many patients ultimately require renal replacement therapy.[51-56] Patients demonstrating progressive loss in functional nephron mass are at greatest risk of progressing to

chronic kidney disease (CKD) which is known to confer a more than 4 fold increase in mortality risk.

1.3.1.7 *Venipuncture Risks*

Frequent blood draws to allow close monitoring of liver function and to assess for safety is essential for the trials safe conduct. Risks of blood draw or venipuncture are typically minimal with temporary local discomfort. Rare, but more serious risks would include ecchymosis, thrombophlebitis, and infection.

The amount of blood that may be drawn from adult participants for research purposes will not be more than 550 mL over an eight-week period. The additional amount of blood could contribute to the development of anemia. The participant's clinical condition will be taken into consideration to determine if research blood tests can be performed.

1.3.1.8 *Leukapheresis Risks*

Leukapheresis will be performed on the recipient prior to initiating the CTX conditioning regimen. Complications that may occur during leukapheresis include changes (increase or decrease) in blood pressure or pulse, bleeding, or hypocalcemia. Bleeding, fever, and infection from leukapheresis can also occasionally occur. On very rare occasions, these complications may be life-threatening.

1.3.1.9 *Liver Biopsy Risks*

Participants who meet eligibility for all phases of the study and successfully complete IS withdrawal will undergo a minimum of 3 biopsies per protocol. This is in addition to a pre-implant biopsy performed on the liver prior to transplantation into the recipient. These biopsies will occur at the following time points:

1. 5-15 days after the arTreg infusion, to evaluate the effect of arTreg infusion and eligibility to proceed in the study;
2. Prior to withdrawing prednisone and EVR, to determine eligibility; and
3. 52 weeks after successful IS withdrawal, to assess the primary endpoint of the study.

Mild AEs resulting from a liver biopsy include local pain during and for a short period of time (hours or at most days) after the procedure that will be experienced to some degree by every participant. The second adverse event that is typically of mild to moderate severity is bleeding. Although some bleeding likely occurs with every biopsy, it typically does not result in any symptoms; the only sign might be a small decrement in hemoglobin / hematocrit. More serious bleeding after a liver biopsy is typically diagnosed by a significant drop in the hemoglobin / hematocrit that does not cause any symptoms. The risk of requiring a transfusion secondary to excessive bleeding is 0.5 to 1%.[50] Symptomatic hemorrhage and/or the requirement for operative or other procedural intervention to stop bleeding occurs in <0.5% of procedures.

Other potentially serious risks associated with liver biopsy include pneumothorax or colonic perforation. If either were to occur, hospitalization, procedural or operative intervention may be necessary. Finally, there is a very small risk of death, estimated at 0.1 to 0.01%.[50]

1.3.2 Potential Benefits

This study provides no direct or immediate benefit to the participants. However, the results of the study could improve future care of transplant patients.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objectives are to evaluate the safety and efficacy of a single IV dose of autologous, donor alloantigen-specific T regulatory cell product, arTreg, to induce operational tolerance early after adult living or deceased donor liver transplantation.

2.2 SECONDARY OBJECTIVES

The secondary objective is to evaluate the safety and tolerability of cytoreduction in combination with the arTregs to facilitate IS withdrawal in liver transplant recipients.

2.3 MECHANISTIC OBJECTIVES

The mechanistic objectives are to evaluate the effects of the infused arTregs on the immunological profiles of liver transplant recipients.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a single-center, prospective, open-label, non-randomized clinical trial exploring cellular therapy to facilitate IS withdrawal in liver transplant recipients. The primary investigational product is arTreg. Study interventions include: 1) EVR conversion; 2) administration of CTX and mesna prior to a single IV dose of the arTregs; and 3) TAC discontinuation followed by 4) complete IS withdrawal.

We plan to accrue 9 participants who will receive the target arTreg dose of at least 90 x 10^6 cells (section 5.2.1.4). This will comprise the PP sample. Participants who receive ≥ 30 x 10^6 cells but <90 x 10^6 cells as a result of low cell yield will not count towards the planned accrual, and no more than 4 participants will be allowed in this group. This group will be included in the ITT sample. See section 9.1 for additional details on the analysis samples.

The plan for IS, cellular therapy and IS withdrawal is described in sections 5.1 to 5.3.5.

Participants who successfully withdraw from all IS will undergo research biopsies as outlined in section 1.3.1.9. The research biopsy at 52 weeks following IS discontinuation will be used to determine whether they meet the primary efficacy endpoint of operational tolerance. Participants determined to be operationally tolerant will be followed until 104 weeks following IS discontinuation. Participants who fail IS withdrawal after 52 weeks but before 104 weeks will be followed until week 104 or 12 weeks after resuming IS, whichever is longer (Appendix 5).

Participants who do not successfully withdraw from all IS will complete 104 weeks of High Intensity Safety Follow-up (Appendix 6) after failing IS withdrawal.

All other participants will be followed per section 4.4.

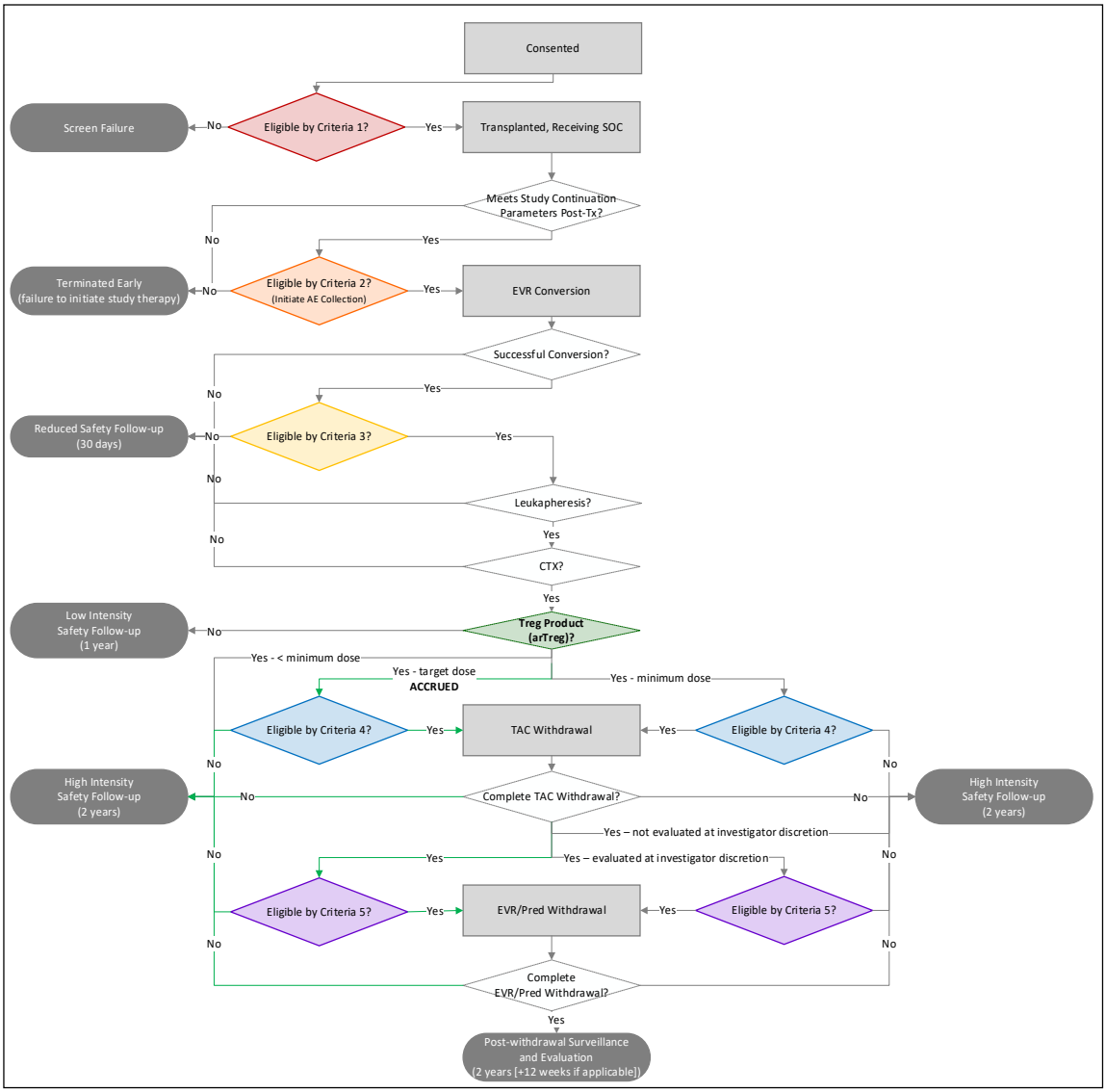

Figure 1. Study Design

3.2 STUDY DURATION

Accrual duration (section 3.1) will be 113 weeks (2.2 years) from first enrollment.

Total duration of study participation will be influenced by the participant's course during the study.

- Participants who participate in all phases of the study, i.e. who receive arTregs, achieve complete IS withdrawal, and are operationally tolerant (Study Definitions) at 52 weeks off IS will be followed for 2 years after the last IS dose (Appendix 5).

- Participants who receive arTregs, achieve complete IS withdrawal, and then resume IS (sections 5.4.2 and 5.4.3.2) will be followed for 2 years after first completing IS withdrawal or 12 weeks after resuming IS, whichever is longer (Appendix 5).
- Participants who receive arTregs, but are not eligible to initiate or do not complete IS withdrawal will be followed for 2 years from the date they were deemed ineligible or failed IS withdrawal (Appendix 6).
- Participants who receive CTX but do not receive arTregs will be followed for 1 year post CTX administration (Appendix 7).
- Participants who initiate EVR conversion but do not receive CTX will be followed for up to 30 days as outlined in 4.4 (RFU visit in Appendix 2).

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoints

3.3.1.1 *Primary Safety Endpoints*

The primary safety endpoints are defined as:

1. Number and severity of AEs attributed to arTregs.
2. Number and severity of AEs attributed to a) leukapheresis, b) CTX, or c) mesna.

3.3.1.2 *Primary Efficacy Endpoint*

The primary efficacy endpoint is operational tolerance. Operational tolerance is defined as:

1. Discontinuation of all IS for 52 weeks;
2. ALT ≤ 50 U/L* for ≥ 2 measurements separated by ≥ 1 week in the 6 weeks prior to the liver biopsy at 52 weeks after the last IS dose; and
3. Liver biopsy at 52 weeks (± 4 weeks) after the last IS dose that meets the biopsy criteria for operational tolerance (Table 5) as assessed by central pathology.

*If the investigator determines that abnormal laboratory results are related to an intercurrent illness, the investigator may request that the participant's data be reviewed by an adjudication committee. If the adjudication committee agrees, they may grant an extension of the primary endpoint visit window (+ 4 weeks). This adjudication committee will consist of the NIAID Medical Monitor, the ITN Clinical Trial Physician, and the Protocol Chair from another LITTMUS protocol (section 1.1.1).

Table 5. Biopsy Criteria for Operational Tolerance: Comparison of Biopsy Obtained 52 Weeks after the Last Dose of Immunosuppression to the Pre-Weaning (Visit 300) Biopsy[57]

Compartment	Findings
Portal inflammation and interface activity	Similar to pre-weaning (Visit 300) biopsy with no more than minimal or focal mild, portal mononuclear inflammation. No more than minimal interface necro-inflammatory activity, limited to a minority of tracts.
Centrizonal/peri-venular inflammation	Similar to pre-weaning (Visit 300) biopsy with no more than minimal perivenular necro-inflammatory activity.

Bile duct changes	Similar to pre-weaning (Visit 300) biopsy, without new onset biliary epithelial cell senescence changes or ductopenia involving > 10% of portal tracts.
Fibrosis	Similar to pre-weaning (Visit 300) biopsy, without progressive portal/periportal or perivenular fibrosis or architectural distortion (≤1 point increase on the Ishak scale).
Arteries	Negative for obliterative or foam cell arteriopathy.

3.3.2 Secondary Endpoints

1. Incidence of grade 3 or greater infections following arTreg infusion.
2. Number and severity of biopsy-proven AR and/or clinical rejection events at any time after arTreg infusion.
3. Number of CR events at any time after arTreg infusion.
4. Number of participants who develop any malignancy.
5. The proportion of participants who discontinue TAC ≥ 12 weeks with ALT ≤ 50 U/L and a liver biopsy 12 - 26 weeks after the last TAC dose that meets the biopsy criteria for IS minimization (Table 9) as assessed by central pathology.
6. Durability of operational tolerance defined as the time from achieving the primary endpoint to IS reinitiation or to the end of trial participation.

3.3.3 Exploratory Mechanistic Endpoints

Mechanistic studies to evaluate the effects of arTregs on the immunological profiles of liver transplant recipients. These endpoints include the number of participants with:

- evidence of a tolerance associated gene profile[1] as described by A. Sanchez-Fueyo et al;
- a loss or change of alloresponses following arTreg infusion;
- an increase in peripheral blood Treg numbers following arTreg infusion;
- an increase in intragraft Treg numbers following arTreg infusion.

The hypotheses and associated assays for these endpoints are described in sections 7.2 and 7.3.

3.4 PREMATURE TERMINATION OR SUSPENSION OF THE TRIAL / STOPPING RULES

3.4.1 Ongoing Review

The Data Safety Monitoring Board (DSMB) will review safety data not less often than yearly.

3.4.2 Study Stopping Rules Guidance

3.4.2.1 *Study-Related AEs Contributing to Stopping Rules*

If any one of the criteria listed below are met, study enrollment will be suspended and arTreg infusion will be held pending review of all pertinent data by the NIAID Transplant DSMB, DAIT NIAID, and the ITN. In the event that, at the time that the study is paused, there is a subject who has received CTX conditioning, the study PI and Medical Monitor will determine whether it is in the patient's best interest to receive arTreg infusion, and the subject will be informed of the circumstances and the decision.

- Any CTCAE grade 4 or higher infusion reaction
- Any study-defined grade 4 or higher infection (section 8.4.1) after initiation of EVR conversion
- Any diagnosis of malignancy, except recurrent HCC and skin cancer after initiation of EVR conversion
- Any death or graft loss after initiation of EVR conversion
- Any grade 4 or higher hemorrhagic cystitis event related to CTX
- Rejection after arTreg infusion:
  - Severe AR in two or more participants (Study Definitions)
  - Steroid resistant AR (section 5.4.3.3)
  - CR (Study Definitions)
- AR or clinical rejection (Study Definitions) in ≥ 3 participants within 6 weeks after arTreg infusion

3.4.2.2 *Manufacturing Failure(s) Contributing to Stopping Rules*

- Four patients receive less than the target dose of arTregs (90 to 500 x 10^6 cells)
- ≥20% failure rate to manufacture a minimum dose of arTregs (see Study definition for manufacturing failure and minimum dose). The lower limit of the 95% confidence interval for the rate of manufacturing failure will be used for this calculation (see Table 6 below). This manufacturing stopping rule will not go into effect till after the 4th patient undergoes an attempt to manufacture arTregs.

Table 6. Stopping Rule Table for a 20% Manufacturing Failure Threshold

Number of subjects with manufacturing failure	Number of subjects in whom manufacturing was attempted	Cumulative incidence rate (%)	Lower 95% exact confidence limit (%)
3	4	75	24.86
4	5	80	34.26
4	6	66.67	27.13
4	7	57.14	22.53
5	8	62.5	28.92
5	9	55.56	25.14

4. ELIGIBILITY

4.1 RECIPIENT (ELIGIBILITY 1 – PRE-TRANSPLANT)

4.1.1 Inclusion Criteria

Individuals must meet all of the following criteria to be eligible:

1. Able to understand and provide informed consent.
2. Male or female, 18 to 70 years of age at the time of enrollment.
3. End-stage liver disease and listed for a living or deceased-donor primary solitary liver transplant.
4. Agreement to use contraception; section 5.2.4.4.
5. Removed in protocol version 3.0.
6. Removed in protocol version 4.0.
7. Positive EBV antibody test.
8. In the absence of contraindication, vaccinations must be up to date per the DAIT Guidance for Patients in Transplant Trials (refer to the Manual of Procedures).

4.1.2 Exclusion Criteria

Individuals who meet any of the following criteria will not be eligible:

1. History of previous organ, tissue, or cell transplant.
2. For CMV antibody negative recipients, a CMV antibody positive donor.
3. Known contraindication to CTX or mesna.
4. Serologic evidence of HIV-1/2 infection.
5. The need for chronic anti-coagulation or anti-platelet agents other than aspirin that cannot be safely discontinued for a minimum of 1 week to safely perform a liver biopsy.
6. End stage liver disease secondary to autoimmune etiology (such as autoimmune hepatitis, primary biliary cirrhosis, or primary sclerosing cholangitis), or other contraindications to drug withdrawal.
7. Psychological, familial, sociological, or geographical factors potentially hampering compliance with the study protocol and follow-up visit schedule.
8. Any condition that, in the opinion of the investigator, may interfere with study compliance.
9. History of cardiac disease (ischemic heart disease requiring revascularization, history of or current treatment for dysrhythmia, or evidence of congestive heart failure) unless cleared by a cardiologist.
10. Any past or current medical problems, treatments or findings which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the candidate's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study. This includes past, present, or future enrollment in studies that affect eligibility at the time of EVR conversion.

11. History of malignancy or any concomitant malignancy, except HCC, completely treated in-situ cervical carcinoma, or completely treated basal cell carcinoma (Study Definitions).
12. Chronic use of systemic glucocorticoids or other immunosuppressives, or biologic immunomodulators.

4.2 LIVING DONOR

4.2.1 Inclusion Criteria

Living donors must meet all of the following criteria to be eligible:

1. Able to understand and provide informed consent.
2. Male or female, 18 to 70 years of age at the time of enrollment.
3. Meets site-specific clinical donor eligibility requirements.
4. Meets donor eligibility manufacturing requirements within 7 days before or after the blood collection for manufacturing.
5. Willingness to donate appropriate biologic samples.

4.2.2 Exclusion Criteria

There are no exclusion criteria for living donors.

4.3 DECEASED DONOR

4.3.1 Inclusion Criteria

Deceased donors must meet the following criteria for their recipients to remain eligible:

1. Meets site-specific clinical donor eligibility requirements.
2. Meets donor eligibility manufacturing requirements.

4.3.2 Exclusion Criteria

There are no exclusion criteria for deceased donors.

4.4 PREMATURE DISCONTINUATION OF STUDY THERAPY

Study therapy: The investigational product is arTreg. The supportive regimen for this product includes EVR, leukapheresis, CTX, and mesna.

Study therapy will be prematurely discontinued for the following events:

- Hypersensitivity: A participant develops a persistent, life threatening hypersensitivity reaction to mesna or CTX, despite the measures described in sections 5.2.2.1 and 5.2.2.2.
- The arTreg infusion will be stopped and will not be restarted if there is a hypersensitivity reaction, CTCAE grade ≥3 infusion related reaction, or any infusion-related serious AE (SAE).
- AE: A participant experiences an AE attributable to the study therapy that, in the judgment of the principal investigator or the medical monitor, presents an unacceptable consequence or risk to the participant.

- Intercurrent illness or infection: A participant develops an illness or infection that requires treatment not consistent with protocol requirements; or, a participant develops an intercurrent illness that, in the judgment of the principal investigator, makes further participation in the study not in the patient's best interest.
- Protocol violation. If a participant cannot comply with the study protocol and the protocol deviation is sufficient to jeopardize his or her well-being or the integrity of the study.
- Pregnancy.
- The principal investigator or medical monitor determines that the study therapy is no longer in the best interests of the participant.
- The participant is unwilling or unable to continue on study therapy.

Participants who do not initiate any study therapy will be prematurely terminated.

Participants who do not complete EVR conversion will complete 30 days of Reduced Safety Follow-up (RFU) Visit in Appendix 2.

Participants who complete EVR conversion but do not receive any CTX will complete 30 days of RFU Visit in Appendix 2. This includes participants who initiated leukapheresis.

Participants who receive any CTX or mesna but do not receive the arTreg infusion will complete 104 weeks of Low Intensity Safety Follow-up per Appendix 7.

Participants who receive any infusion of arTregs but either do not attempt or do not complete IS withdrawal will complete 104 weeks of High Intensity Safety Follow-up per Appendix 6. Participants who do not complete IS withdrawal will have failed IS withdrawal.

The DSMB will be provided reports on all cases of premature termination and discontinuation of study therapy.

4.5 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants who withdraw consent for further treatment will be terminated from the study. Participants who initiate EVR conversion will be asked if they would be willing to complete an end-of-study visit to include the assessments in Visit 400 (Appendix 5).

Investigator decision. The principal investigator and/or Medical Monitor may choose to terminate a participant from further participation if they deem it is not in the interest of the participant.

Screen failure. Participants who do not meet criteria for Eligibility 1: Pre-transplant (section 4.1) are failures and will be terminated.

Failure to initiate study therapy. Participants who do not initiate study therapy (Study Definitions) for any of the following reasons will be terminated:

- Failure to meet study continuation parameters post-transplant (section 5.3.1.1)

- Inability to tolerate TAC during **Study Stage 1**, leading to discontinuation of TAC or conversion to cyclosporine (section 5.3.1.2)
- Failure to meet criteria for Eligibility 2: EVR Conversion (section 5.3.2)
- Failure to initiate EVR conversion (section 5.3.2.4).

There is no upper limit on the number of participants who are prematurely terminated due to being screen failures or failing to initiate study therapy.

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

5. STUDY THERAPIES, MEDICATIONS AND PROCEDURES

5.1 OVERVIEW

Figure 2 provides a visual guide to the sequential stages in the trial and an overview of an individual's participation.

This study proceeds through a defined sequence of stages before and after transplantation. Study recruitment and informed consent occurs prior to transplantation, with samples of blood, serum, and PBMC samples collected at any time after informed consent, but prior to the administration of any IS.

After transplantation, participants will initially receive standard IS with TAC, along with a mycophenolate product and/or steroids, as described in section 5.3.1 **(Study Stage 1: Initial post-transplant IS)**.

After at least 30 days, participants will then be evaluated for eligibility to initiate **Study Stage 2: EVR conversion**, as described in section 5.3.2.

Participants completing this stage will be evaluated for **Study Stage 3: arTreg Manufacturing and Investigational Treatment** which includes leukapheresis, cyclophosphamide and arTreg infusion (section 5.3.3). In the case of participants who have received a liver transplant from a living donor, donor PBMCs used in arTreg manufacture will be obtained from the living donor, if not previously collected (section 5.3.3.1); the donor phlebotomy will be performed at least 14 days prior to the recipient leukapheresis.

Following arTreg infusion, participants will be evaluated for eligibility to initiate **Study Stage 4: TAC withdrawal** (section 5.3.4).

After completion of **Study Stage 4**, participants will be evaluated for eligibility to withdraw from the remaining maintenance IS (**Study Stage 5: EVR/Prednisone withdrawal**, section 5.3.5). Participants who discontinue all IS according to protocol schedule will then enter **Study Stage 6: Post-IS Withdrawal Surveillance and Evaluation**. The primary endpoint of operational tolerance will be evaluated 52 weeks after scheduled complete IS withdrawal, and final study clinical evaluation will be evaluated 104 weeks after scheduled complete IS withdrawal.

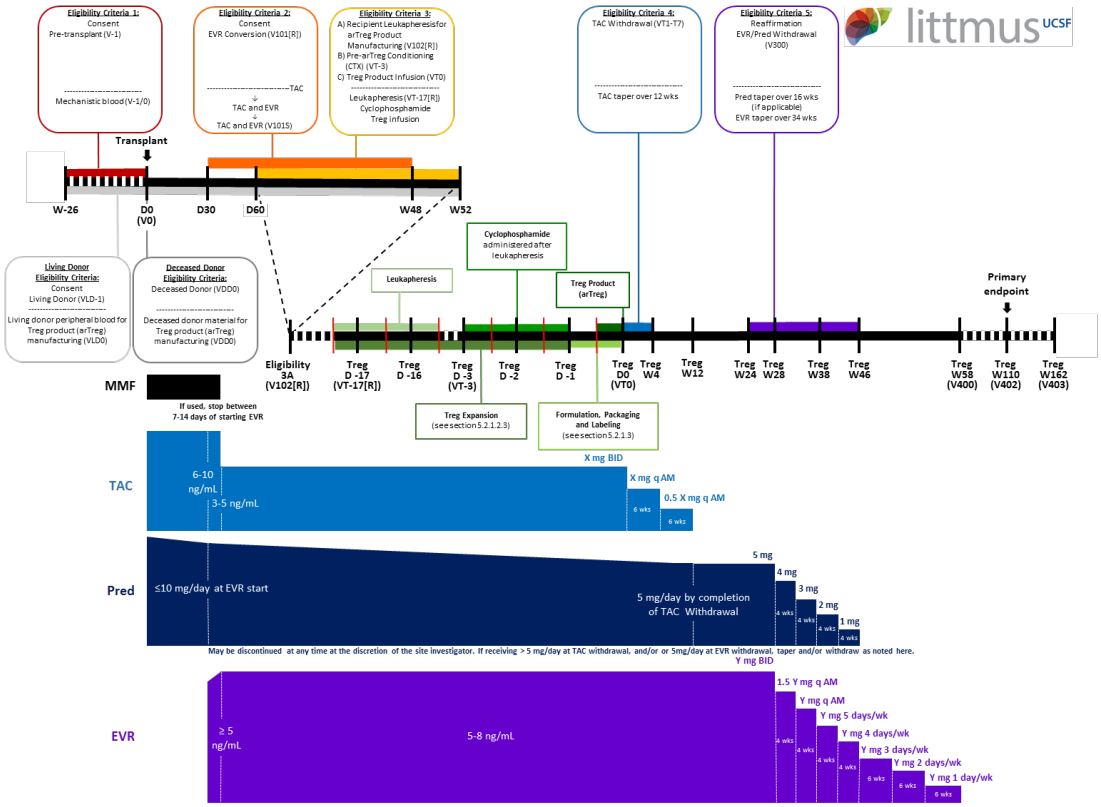

Figure 2. Overview of an Individual's Participation (Study Stage 1 to 6)

Participation comprises pre-transplant, transplant and EVR conversion (upper left); investigational treatment (middle left and section 5.3.3); and IS withdrawal (middle right and sections 5.3.4 and 5.3.5).

5.2 STUDY MEDICATIONS

5.2.1 Investigational Agent: Donor Alloantigen-Reactive Regulatory T Cells (arTreg)

5.2.1.1 *Overview*

The arTreg product is manufactured at the Human Islet and Cellular Transplantation Facility (HICTF) and GMP Facility, an FDA-registered facility located at UCSF. arTregs are autologous cells that have been collected from the transplant recipient by leukapheresis, purified to obtain the $CD4^{+}CD127^{lo/-}CD25^{+}$ population and cultured for 16 days to selectively expand those that are reactive to alloantigens. The expanded cells are then concentrated and resuspended in a defined sterile solution at the required cell concentration for infusion.

5.2.1.2 *Manufacturing*

5.2.1.2.1 Recipient Material

Leukapheresis from the liver transplant recipient per section 5.3.3.2.4 will be transported to the UCSF manufacturing facility for isolating PBMCs and further processing. Onset of arTreg manufacture for a participant is defined as the initiation of the first leukapheresis procedure.

5.2.1.2.2 Donor Material (Manufacture of sBcs)

Deceased Donor

Donor spleen and/or blood are recovered at the time of liver procurement and transported to the manufacturing facility for processing and manufacturing of sBcs. If donor tissue cannot be collected or cannot be used, the liver transplant recipient will not be able to participate further in this clinical trial, and will undergo applicable follow-up as detailed in section 4.4.

Living Donor

PBMC for manufacture of sBcs from living donors will be collected via phlebotomy at least 14 days prior to the recipient leukapheresis. If the living donor becomes unable or unwilling to donate the required cells, the liver transplant recipient will not be able to participate further in this clinical trial, and will complete safety follow-up as detailed in section 4.4.

Expanded sBcs are irradiated (25-35 Gy), banked and cryopreserved at 1-10 x 10^6 cells/mL prior to use in manufacture of the arTregs.

5.2.1.2.3 Treg Expansion

arTregs are manufactured at UCSF's HICTF by expansion in co-culture with donor sBc. First, CD4+127lo/-25+ Tregs are purified from recipient source cell material. This population is cultured at 1:4 ratio with irradiated donor sBcs in the presence of IL-2 for 11 days in medium supplemented with deuterated glucose to label the expanding cells. Cells are then restimulated with anti-CD3/anti-CD28 magnetic beads for 5 additional days. After restimulation, arTreg cells are harvested, washed and the beads removed by magnetic separation. Cells are then re-suspended in 100 ml infusion solution, as described below.

5.2.1.3 *Formulation, Packaging, and Labeling*

arTreg is a sterile, single-cell suspension containing 30 to 500 x 10^6 donor allo-antigen-reactive regulatory T cells in 100 ml of a defined infusion solution containing 49.02% (v/v) PlasmaLyte-A, 49.02% (v/v) Dextrose 5% (v/v), 0.45% NaCl (v/v), and 1.96% (v/v) 25% human serum albumin (HSA), USP for IV administration. The arTreg product does not contain antibiotics or preservatives.

After product labelling, the lot is quarantined at 4°C until release. Each lot is released for clinical use with an interim Certificate of Analysis (CoA), pending completion of the sterility test. A copy of the CoA will be included with each product lot delivered to the clinical site. The final CoA is issued when the sterility test result is available and a copy is also provided to the clinical center. Released product is transported to the clinical site refrigerated at 2-15°C in containers validated to maintain the set temperature for >30 hours after lot release.

5.2.1.4 *Dosage, Preparation, and Administration*

Eligible participants will receive a single dose of arTregs containing 30 to 500 x 10^6 total cells. The target dose is 90 to 500 x 10^6 total cells. However, if a minimum arTreg dose of 30 to <90 x 10^6 cells is manufactured (sections 3.1 and 9.1), the product will be infused. If the dose obtained after product manufacture is < 30 x 10^6 cells, the product will not be infused (section 1.2.4.2.2). If the dose obtained after product manufacture is > 500 x 10^6 cells, a dose aliquot will be prepared so that the administered dose will be ≤ 500 x 10^6 cells, and ≥ 30 to 500 x 10^6

total cells. Manufacturing failure for a participant is defined as the onset of arTreg manufacture which does not result in a product that meets release criteria for infusion.

arTregs will be administered as a single infusion via a peripheral IV primed with saline by gravity over approximately 20 to 30 minutes. The product will be infused only after verification of the product label and the recipient ID by clinical study personnel to ensure the lot is administered to the correct recipient. Following administration of the arTreg, the infusion bag, tubing and peripheral IV line is flushed with up to 250 ml normal saline to ensure that the complete dose is infused.

Pre-medications will be administered 30 - 60 minutes prior to arTreg infusion. Pre-medications are 650 mg acetaminophen and 25 - 50 mg diphenhydramine IV or by mouth.

Vital signs will be taken just prior to, 5 minutes into, and at the end of the infusion, as well as 30 minutes, 1 hour, and 2 hours after the infusion. Emergency medical equipment will be available during the infusion in case the participant has an allergic response or an infusion reaction that can result in a cytokine release syndrome (CRS).

The IV line will be maintained after the infusion and the participant will remain in the clinical research unit for a minimum of 24 hours, which will allow ongoing monitoring for any infusion-related signs and symptoms.

5.2.1.5 *Product Accountability*

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator will maintain adequate records of the disposition of the products, including the date and quantity of the biologic received, to whom the biologic was dispensed (participant-by-participant accounting), and a detailed accounting of any biologic that is accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity dispensed.

All records regarding the disposition of the investigational product will be available for inspection.

5.2.2 arTreg Preparative Agents

5.2.2.1 *Cyclophosphamide (Cytoxan®)*

5.2.2.1.1 Formulation, Packaging and Labeling

CTX for Injection, USP, is a sterile, white cake available as 500 mg, 1 g, and 2 g strength vials.

- 500 mg vial contains 534.5 mg CTX monohydrate equivalent to 500 mg CTX and 375 mg mannitol
- 1 g vial contains 1069.0 mg CTX monohydrate equivalent to 1 g CTX and 750 mg mannitol
- 2 g vial contains 2138.0 mg CTX monohydrate equivalent to 2 g CTX and 1500 mg mannitol

Store vials at or below 25°C (77°F). During transport or storage of CTX vials, temperature influences can lead to melting of the active ingredient, CTX.

CTX will be supplied by the hospital pharmacy.

5.2.2.1.2 Dosing

CTX is administered prior to arTreg.

CTX 40 mg/kg will be administered as described in section 5.3.3. It is intended as a strong lymphodepleting agent to allow homeostatic expansion of the infused arTregs (section 1.2.5).

5.2.2.1.3 Preparation and Handling

Handle and dispose of CTX in a manner consistent with other cytotoxic drugs. Caution should be exercised when handling and preparing CTX for Injection, USP (lyophilized powder), or bottles containing CTX tablets. To minimize the risk of dermal exposure, always wear gloves when handling vials containing CTX for Injection, USP (lyophilized powder).

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use CTX vials if there are signs of melting. Melted CTX is a clear or yellowish viscous liquid usually found as a connected phase or in droplets in the affected vials. Do not use CTX if it contains particles, is cloudy or discolored, or if the vial is cracked or damaged.

CTX does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions. Use aseptic technique.

Reconstitution of CTX: reconstitute CTX using 0.9% Sodium Chloride Injection, USP or Sterile Water for Injection, USP with the volume of diluent listed below in Table 7. Add the diluent to the vial and gently swirl to dissolve the drug completely.

Table 7. Reconstitution in Preparation for IV Infusion

Strength	Volume of Diluent	CTX Concentration
500 mg	25 mL	20 mg per mL
1 G	50 mL	
2 G	100 mL	

Dilution of reconstituted CTX: Further dilute the reconstituted CTX solution to a minimum concentration of 2 mg per mL with any of the following diluents:

- 5% Dextrose Injection, USP
- 5% Dextrose and 0.9% Sodium Chloride Injection, USP
- 0.45% Sodium Chloride Injection, USP

5.2.2.1.4 Administration

Participants will receive CTX, 40 mg/kg (based on lesser of ideal or actual body weight); CTX will be infused per institutional practice.

Adults (18 years and older)[58]:

- IBW (male) = 50 kg + (2.3 kg x height in inches over 5 ft.)
- IBW (female) = 45.5 kg + (2.3 kg x height in inches over 5 ft.)

CTX should not be administered to patients who are pregnant or breast-feeding, or patients taking a tumor necrosis factor blocking medicine (e.g., etanercept).

To reduce the likelihood of adverse reactions that appear to be administration rate-dependent (e.g., facial swelling, headache, nasal congestion, scalp burning), CTX should be infused very slowly. Duration of the infusion should be appropriate for the volume and type of carrier fluid.

5.2.2.1.5 Pretreatment

Approximately 60 minutes prior to administration of CTX, the participant should be pre-medicated with the following or managed per institutional standards to prevent nausea:

- Decadron® (dexamethasone), 20 mg IV dose
- Benadryl® (Diphenhydramine), 25 mg IV dose
- Ativan® (Lorazepam) Injection, 1 mg IV dose
- Zofran® (Ondansetron), 24 mg IV dose

5.2.2.2 *Mesna (MESNEX®) Injection*

5.2.2.2.1 Formulation, Packaging and Labeling

Mesna is an agent to inhibit hemorrhagic cystitis induced by CTX. The active ingredient is a synthetic sulfhydryl compound designated as sodium-2-mercaptoethane sulfonate. MESNEX® Injection is a sterile, nonpyrogenic, aqueous solution of clear and colorless appearance in clear glass multidose vials for IV administration. MESNEX® Injection contains 100 mg/mL mesna, 0.25 mg/mL edetate disodium and sodium hydroxide for pH adjustment. MESNEX® Injection multidose vials also contain 10.4 mg of benzyl alcohol as a preservative. The solution has a pH range of 7.5-8.5.

Mesna is converted to an inactive form in the blood. Then, as it is circulated through the kidneys, it is reactivated. The reactivated mesna works by interacting with metabolites (substances produced by breakdown of a drug in the body). Two specific chemotherapies, ifosfamide and CTX, when broken down, produce a metabolite acrolein. This metabolite is toxic to the bladder. Mesna binds to and inactivates acrolein thereby preventing or reducing bladder problems.

Mesna will be supplied by the hospital pharmacy in the form of MESNEX® for injection.

5.2.2.2.2 Dosing

Mesna is administered in conjunction with CTX.

MESNEX® is dosed and given per institutional practice.

5.2.2.2.3 Preparation and Handling

Determine the volume of MESNEX® injection for the intended dose.

Dilute the volume of MESNEX® injection for the dose in any of the following fluids to obtain a final concentration of 20 mg/mL:

- 5% Dextrose Injection, USP
- 5% Dextrose and 0.2% Sodium Chloride Injection, USP
- 5% Dextrose and 0.33% Sodium Chloride Injection, USP
- 5% Dextrose and 0.45% Sodium Chloride Injection, USP
- 0.9% Sodium Chloride Injection, USP
- Lactated Ringer's Injection, USP

The MESNEX® injection multidose vials may be stored and used for up to 8 days after initial puncture.

Store diluted solutions at 25°C (77°F). Use diluted solutions within 24 hours. Do not mix MESNEX® injection with epirubicin, CTX, cisplatin, carboplatin, and nitrogen mustard.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Any solutions which are discolored, hazy, or contain visible particulate matter should not be used.

5.2.2.2.4 Administration

MESNEX® is administered per institutional practice with CTX.

5.2.2.2.5 Pretreatment

Participants will receive pre-hydration in order to establish good urine flow with dilute urine specific gravity. They must meet standards of their institutional protocol for CTX administration at the prescribed dose and frequency.

5.2.3 Maintenance Immunosuppressive Agents

5.2.3.1 *Overview*

5.2.3.2 *Tacrolimus (Prograf®)*

TAC is approved in the U.S for the prophylaxis of organ rejection in liver, kidney and heart transplantation. Generic TAC may be substituted at the discretion of the site investigator.

For further information on TAC, please refer to the package insert for Prograf® at: http://www.astellas.us/docs/prograf.pdf.

TAC will be administered post-transplant based on the center standard of care. Standard of care will be continued until participants are evaluated for EVR conversion and administered as described in section 5.3.1.2. TAC will later be withdrawn as described in section 5.3.4.4.

TAC will be supplied by the hospital or local pharmacy.

5.2.3.3 *Prednisone*

For further information on prednisone, please refer to the drug label at: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=3115aef0-fd50-4ec8-a064-3effb695f3f2#druglabelcontent.

Prednisone will be administered post-transplant based on the center standard of care. Standard of care will be continued until participants are evaluated for EVR conversion and administered as described in section 5.3.1.2.

Prednisone may be discontinued at any time at the discretion of the site investigator.

Participants receiving > 5 mg/day of prednisone at the time of TAC withdrawal will taper prednisone as described in section 5.3.4.4. Participants receiving 5mg/day of prednisone at the time of EVR withdrawal will withdraw prednisone as described in section 5.3.5.4.

Prednisone will be supplied by the hospital or local pharmacy.

5.2.3.4 *Everolimus (Zortress®)*

EVR is indicated for the prophylaxis of organ rejection in liver and kidney transplantation. EVR is to be administered no earlier than 30 days post-transplant concurrently in combination with reduced doses of TAC and corticosteroids.

For further information on EVR, please refer to the package insert for Zortress® at: https://www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/zortress.pdf.

EVR will be administered as described in section 5.3.2.4 and later withdrawn as described in section 5.3.5.4.

EVR will be supplied by the hospital or local pharmacy.

5.2.3.5 *Mycophenolate products*

MMF and mycophenolic acid are Inosine Monophosphate Dehydrogenase Inhibitors. MMF is the 2-morpholinoethyl ester of the active metabolite mycophenolic acid. MMF is approved in the U.S. for use in the prophylaxis of organ rejection in patients receiving allogeneic renal, cardiac or hepatic transplants. Mycophenolic acid is approved in the U.S. for use in the prophylaxis of organ rejection in patients receiving allogeneic renal transplants.

For further information on MMF, please refer to the package insert for Cellcept® at: http://www.gene.com/gene/products/information/cellcept/pdf/pi.pdf.

For further information on mycophenolic acid please refer to the package insert for Myfortic® at: http://www.pharma.us.novartis.com/product/pi/pdf/myfortic.pdf.

Azathioprine may be used if participants are unable to tolerate MMF.

A mycophenolate compound may be administered post-transplant based on the center standard of care. Standard of care will be continued until participants are evaluated for EVR conversion (section 5.3.1.2).

Mycophenolate products will be supplied by the hospital or local pharmacy.

5.2.4 Concomitant Medications

5.2.4.1 *Prophylactic medications*

5.2.4.1.1 Viral Prophylaxis

IV ganciclovir (Cytovene®) and/or oral valganciclovir (Valcyte®) will be administered for prophylaxis of CMV and Epstein-Barr virus (EBV) post-transplant and following CTX and arTreg infusion according to the center's standard of care.

In addition, if not already receiving viral prophylaxis, five days following CTX administration, participants will receive a 7-day course of famciclovir (Famvir®) 500 mg PO daily for prophylaxis against herpes simplex virus (HSV).

5.2.4.1.2 Fungal and Yeast Prophylaxis

Participants will receive oral fluconazole for fungal prophylaxis post-transplant according to the center's standard of care.

Participants will receive center standard of care prophylaxis for candida infection post-transplant.

5.2.4.1.3 Pneumocystis Pneumonia (PCP) and Bacterial Infection Prophylaxis

Participants will receive standard of care prophylaxis for PCP post-transplant until at least 52 weeks after CTX and arTreg infusion.

Five days following CTX administration, participants will receive prophylaxis against bacterial infections in the setting of neutropenia per institutional practice.

5.2.4.1.4 Other Prophylaxis

Participants will receive Granulocyte-Colony Stimulating Factor (G-CSF) after CTX per institutional standard of care.

5.2.4.2 *Vaccinations*

Participants should receive seasonal influenza vaccination at some point during the influenza vaccination season as standard of care. However, participants must not receive any vaccination within 28 days prior to leukapheresis for arTreg manufacture, and for a period of 28 days after the arTreg infusion.

Live vaccines are prohibited for the duration of the study.

5.2.4.3 *Prohibited medications*

Use of any IS medications other than those specified in this protocol, including oral courses of corticosteroids for >7 days for any reason, necessitates withdrawal from receiving further study therapy (Study Definitions) or protocol directed IS withdrawal. Topical or inhaled corticosteroids or steroid mouthwashes will not be considered IS medications.

Other medications that in the opinion of the investigator may interfere or adversely interact with the study therapy specified in this protocol are prohibited.

5.2.4.4 *Contraception*

WOCBP must use contraception with more than 80% effectiveness according to the FDA's Office of Women's Health (http://fda.gov/birthcontrol) from the time of enrollment until 52 weeks after completion of the last study therapy due to the unknown effects of this protocol on the developing fetus. These participants must consult with their physician and determine the most suitable method(s) from this list to be used during this time. Abstinence is also an option.

5.2.4.5 *Reporting Concomitant Medications*

All prophylactic medications, vaccines, IS medications, anticoagulants and any antibiotics, antimicrobials, and antifungals taken by or administered to liver transplant recipients starting from transplant and continuing throughout their study participation will be reported.

5.2.5 Drug Accountability

Under federal regulations (21CFR 312.62) an investigator is required to maintain adequate records of the disposition of the investigational product, including the date and quantity of drug that was received, the participants to whom drug was dispensed (participant by participant accounting), and an account of any drug accidentally or deliberately destroyed. The investigator will ensure that the investigational product supplies are stored as specified in the protocol and pharmacy manual in a secured area, with access limited to authorized study personnel as described in the clinical study agreement.

Records for receipt, storage, use, and disposition of the study drug will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed. All remaining unused investigational product will be returned to the sponsor or sponsor's representative after study termination, or destroyed with the permission of the sponsor in accordance with applicable law and study site procedures. If investigational product is to be destroyed locally, the investigator will provide documentation in accordance with sponsor's specifications.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.2.6 Assessment of Compliance with Study Medications

All study medications will be administered at sites by trained medical staff; compliance, therefore, will be monitored by the site and documented on the electronic case report forms (eCRFs).

5.3 POST-TRANSPLANT STUDY PROCEDURES

As noted in Figure 2, this study proceeds through a defined sequence of stages post-transplantation. Study recruitment and informed consent occurs prior to transplantation, with samples of blood, serum, and PBMC samples collected at any time after informed consent, but prior to the administration of any IS.

5.3.1 Study Stage 1: Initial Post-transplant IS

5.3.1.1 *Study Stage 1: Initial Post-Transplant IS – Study Continuation Parameters*

In the interim between transplant and Eligibility 2: EVR Conversion (section 5.3.2), confirmation of the following (tested at the time of transplant, with results available after transplant) must occur. If any of these requirements are not confirmed, the participant will be prematurely terminated from the study (section 4.5).

1. Removed in protocol version 4.0.
2. Participants must have a negative HCV PCR RNA test at transplantation.
3. For participants with HCC, the last AFP obtained within 3 months prior to liver transplantation must be < 400 μg/L.
4. The recipients' explanted liver must not have evidence of increased risk of recurrent cancer: explant must be within the Milan criteria, with no vascular invasion, and with no cholangiocarcinoma morphology.
5. For deceased donor recipients, there must be sufficient donor material according to arTreg manufacturing specifications.
6. The transplanted donor liver must not have any unexpected histopathology discovered upon the pre-implant liver biopsy that contraindicates the initiation of EVR conversion.
7. For CMV antibody-negative recipients, the donor must be CMV antibody-negative.
8. The donor and recipient must be ABO blood type compatible.
9. The donor and recipient must not be HLA-DR matched at both loci.
10. For deceased donor recipients, the donor must be HCV antibody and NAT negative.
11. Participants must not have received any investigational (unlicensed) drugs within 8 weeks prior to pre-transplant mechanistic sample collections or any time post-transplant.
12. Participants must not have any new diagnosis of malignancy since transplant.

5.3.1.2 *Study Stage 1: Initial Post-Transplant Immunosuppression*

All participants will be placed on an initial post-transplant IS regimen based on the center's standard of care (sections 5.2.3.2, 5.2.3.3, and 5.2.3.5).

For this study, TAC is a required medication during the initial stages; inability to tolerate TAC during **Study Stage 1**, leading to discontinuation of TAC or conversion to cyclosporine, will result in early termination from the clinical trial. Please see Table 8 for additional details.

Table 8. Post-Transplant Immunosuppression

Medication	Parameter
Prograf® (or generic equivalent)	Participants must enter the EVR conversion with a Prograf® trough level of 6-10 ng/mL. Target trough levels will decrease to 3-5 ng/mL once the participant achieves a therapeutic EVR trough between 5-8 ng/mL.
Mycophenolate Compound (MMF or equivalent)	Participants may be taking mycophenolate mofetil (MMF, Cellcept®) or mycophenolic acid (Myfortic®). Azathioprine may be used if participants are unable to tolerate MMF. This medication should be discontinued approximately 7-14 days after starting EVR. If MMF, Myfortic, or azathioprine is not part of the participant's immunosuppression regimen, the participant may continue with EVR conversion.
Prednisone	Participants must enter EVR conversion with daily dose ≤ 10 mg. In the interest of patient safety, clinical judgment should be exercised when tapering steroid doses, particularly if graft rejection has occurred.

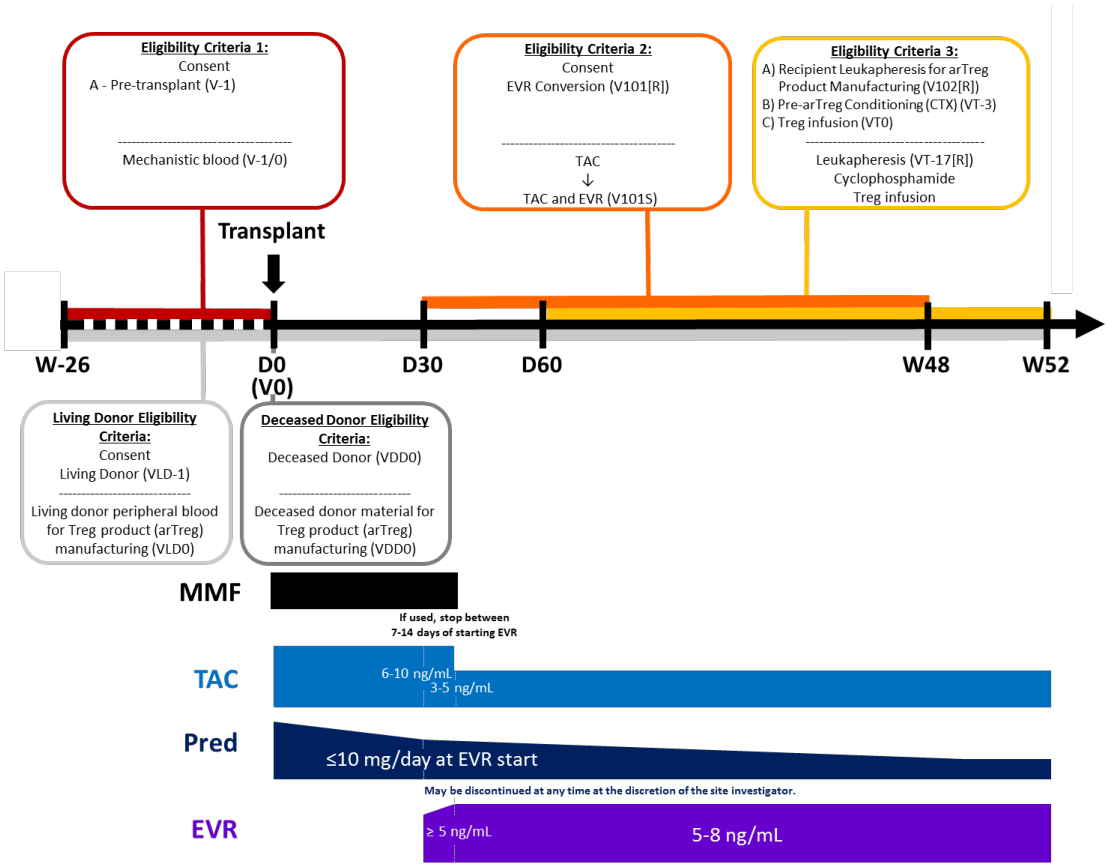

Figure 3. Post-Transplant IS and EVR Conversion Regimen (Study Stage 1, 2, and 3A)

5.3.2 Study Stage 2: EVR Conversion

5.3.2.1 *Study Stage 2: Timing to Apply Eligibility Criteria 2 for EVR Conversion*

Between day 30 and week 48 post liver transplant, participants will be evaluated for eligibility to be converted to an EVR-based IS regimen. Participants not meeting eligibility on initial evaluation may be re-evaluated throughout this period for eligibility, but must meet all eligibility requirements and begin EVR conversion prior to week 48 post-liver transplant. Laboratory assessments required to confirm eligibility must be completed within 7 days prior to initiating EVR conversion.

Participants ineligible for conversion to an EVR-based IS regimen will be terminated from the study (section 4.5).

5.3.2.2 *Study Stage 2: Inclusion Criteria for EVR Conversion*

Participants must meet the following criteria to be eligible for conversion to EVR-based IS:

1. Able to understand and provide informed consent.
2. Negative result for 2019-nCoV by RT-PCR. Participants with a history of positive test result since enrollment will need two consecutively negative results to be eligible for EVR conversion.
3. For participants with a history of HCV, completion of treatment for HCV and maintenance of a sustained viral response of ≥ 24 weeks duration at the time that eligibility for EVR conversion is assessed.

5.3.2.3 *Study Stage 2: Exclusion Criteria for EVR Conversion*

Participants who meet any of the following criteria will not be eligible for conversion to EVR-based IS:

1. ALT > 50 U/L or alkaline phosphatase > 200 U/L.
2. Evidence of hepatic artery stenosis or thrombosis by Doppler or angiography.
3. Urine protein/creatinine ratio > 1.0.
4. Estimated GFR < 40 ml/min per 1.73m² as calculated by CKD-EPI method.
5. Abnormal wound healing or uncontrolled wound infection per physical examination.
6. Hemoglobin < 8.0 g/dL.
7. Absolute neutrophil count < 1,200/µL.
8. Platelets < 50,000/µL.
9. AR episode according to Banff criteria or clinical rejection (Study Definitions) based on local assessment. Participants may be re-evaluated for eligibility ≥ 4 weeks following resolution of the AR episode (Study Definitions).
10. Any new diagnosis of malignancy since transplant.

5.3.2.4 *Study Stage 2: EVR Conversion Schedule and Dosing*

EVR conversion must be initiated (Visit 101S) within 7 days of the eligibility visit (Visit 101/101R).

At the start of conversion from TAC to EVR, the latter will be started at 1.5 mg BID with the dose adjusted to achieve a trough concentration of 5-8 ng/mL (Figure 3). EVR conversion is initiated once the participant receives their first dose of EVR.

Seven to 14 days after initiation of EVR, the mycophenolate compound will be discontinued, if used.

Once EVR trough concentration is ≥ 5 ng/mL, the baseline TAC dose will be cut approximately in half to achieve a trough concentration of 3-5 ng/mL.

Once the target EVR and TAC levels are achieved and maintained over two consecutive measurements with ALT ≤ 50 U/L and GGT ≤ the upper limit of normal (ULN) or ≤ 1.5 x the baseline GGT (Study Definitions), participants will be considered successfully converted to EVR-based IS.

5.3.3 Study Stage 3: arTreg Manufacturing and Investigational Treatment

5.3.3.1 *Overview of Study Stage 3*

Study Stage 3 involves a sequence of steps required for collection, manufacturing and administration of the investigational product. First, source cell material is collected from the transplant recipient in **Stage 3A: Recipient Leukapheresis** (section 5.3.3.2) for use in arTreg manufacturing. Next, in **Stage 3B: pre-arTreg Conditioning** (section 5.3.3.3) the conditioning regimen is administered, consisting of CTX with mesna. **Stage 3C: arTreg infusion** (section 5.3.3.4) is the final step in stage 3.

Donor phlebotomy (only if the recipient received a liver graft from a living donor)

If the transplant recipient has received a liver from a live donor, the transplant donor will undergo phlebotomy at least 14 days prior to the recipient leukapheresis. The donor phlebotomy is performed in order to provide donor-derived stimulator cells to be used in the arTreg manufacturing process, as noted in 5.2.1.2.3. The donor must have negative result for 2019-nCoV by RT-PCR within 7 days prior to blood collection for manufacturing. If the donor is unable or unwilling to successfully complete phlebotomy, the recipient will not be able to participate further in this clinical trial, and the transplant recipient will complete safety follow-up as detailed in section 4.4.

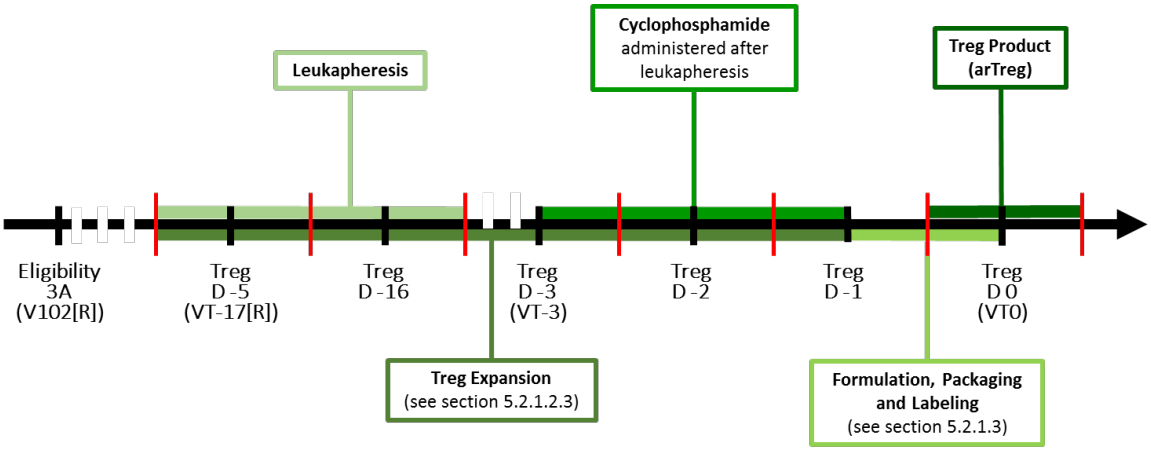

Figure 4. Investigational Treatment Study Regimen (Study Stage 3)

Recipient leukapheresis (**Study Stage 3A**) will be performed 16-17 days before arTreg infusion (**Study Stage 3C**). CTX conditioning (**Study Stage 3B**) will be administered after leukapheresis. CTX will be administered 24-72 hours before arTreg infusion **(Study Stage 3C**).

5.3.3.2 *Study Stage 3A: Recipient Leukapheresis for arTreg Product Manufacturing*

5.3.3.2.1 Study Stage 3A: Timing to Apply Eligibility Criteria 3A – Recipient Leukapheresis for arTreg Product Manufacturing

Following EVR conversion (**Study Stage 2**), between day 60 and week 52 post liver transplant, participants will be evaluated for eligibility for leukapheresis for arTreg manufacturing.

Participants determined not eligible due to any of the criteria below may be re-evaluated, up to 52 weeks post liver transplant.

Leukapheresis must be performed no more than 28 days after establishing eligibility. Assessments requiring results within a shorter period prior to leukapheresis, as described in sections 5.3.3.2.2 and 5.3.3.2.3, may need to be repeated within this timeframe to confirm eligibility.

If the liver allograft was from a living donor, donor phlebotomy will be performed at least 14 days prior to the recipient's leukapheresis. If the liver allograft was from a deceased donor, donor spleen and/or blood recovered at the time of liver procurement are used.

5.3.3.2.2 Study Stage 3A: Inclusion Criteria for Recipient Leukapheresis for arTreg Product Manufacturing

Participants must meet all of the following criteria to be eligible for leukapheresis:

1. Negative test for hepatitis C infection by HCV PCR RNA.
2. Negative test for hepatitis B infection by HBV PCR DNA.
3. Most recent 12-hour TAC trough levels < 8 µg/L measured within 10 days prior to leukapheresis.
4. Most recent 24-hour EVR trough levels > 5 µg/L measured within 10 days prior to leukapheresis.

5. Negative test for 2019-nCoV by RT-PCR within 7 days prior to leukapheresis (may be repeated on the day of leukapheresis at the discretion of the site investigator). Participants with a history of positive test result since EVR conversion will need two consecutively negative results to be eligible for leukapheresis.
6. Meets vaccination requirements per section 5.2.4.2.

5.3.3.2.3 Study Stage 3A: Exclusion Criteria for Recipient Leukapheresis for arTreg Product Manufacturing

Participants who meet any of the following criteria will not be eligible for leukapheresis:

1. Detectable circulating CMV DNA.
2. Detectable circulating EBV DNA.
3. Active bacterial infection, including but not limited to pneumonia, urinary tract infection (UTI), or sepsis.
4. Estimated GFR < 40 ml/min per 1.73m² as calculated by CKD-EPI equation.
5. Development of a condition requiring chronic anti-coagulation or anti-platelet agents other than aspirin that cannot be safely discontinued for a minimum of one week to safely perform a liver biopsy.
6. History of rejection since initiating EVR conversion; evidence of AR or CR according to Banff criteria based on local assessment or clinical rejection (Study Definitions).
7. ALT > 50 U/L.
8. For living donor recipients, insufficient donor material according to arTreg manufacturing specifications.
9. Hemoglobin < 8.0 g/dL.
10. Absolute neutrophil count < 1,500/μL.
11. Platelets < 50,000/μL.
12. Any condition that, in the opinion of the investigator, may pose additional risks from continued participation in the study, may interfere with the participant's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study.
13. MMF or equivalent administered within 10 days prior to leukapheresis.
14. For females of child bearing potential, a positive pregnancy test.
15. GGT > the ULN or > 1.5 x the baseline GGT (Study Definitions).

5.3.3.2.4 Study Stage 3A: Recipient Leukapheresis

Leukapheresis will take place in an apheresis center with qualified staff. The time requirement for the procedure is usually between 1 and 1.5 hours to process 6 liters of blood, which will provide a minimum of 1 x 10^9 mononuclear cells. Participants will be monitored throughout the procedure and supportive measures such as oral calcium supplementation will be provided.

If the leukapheresis product does not contain sufficient cell numbers for arTreg manufacturing, a 2nd leukapheresis can be performed if the participant continues to meet eligibility (section 5.3.3.2). Eligibility for a 2nd leukapheresis must be established by week 52 post liver transplant.

Leukapheresis will be performed exactly 16-17 days prior to arTreg infusion.

5.3.3.3 *Study Stage 3B: pre-arTreg Conditioning (CTX)*

5.3.3.3.1 Study Stage 3B: Timing to Apply Eligibility Criteria 3B – Pre-arTreg Conditioning

Following the leukapheresis procedure, participants will be evaluated for eligibility to receive CTX. Blood counts collected prior to leukapheresis will be used to determine whether a participant may receive CTX, however, physician review prior to CTX administration is required to confirm eligibility.

Participants who receive CTX cannot receive a second dose of CTX.

5.3.3.3.2 Study Stage 3B: Inclusion Criteria for Pre-arTreg Conditioning

Participants must meet the following criterion to receive CTX:

1. Confirmation from the manufacturing facility that arTreg product is expanding appropriately to yield an infusible product.

5.3.3.3.3 Study Stage 3B: Exclusion Criteria for Pre-arTreg Conditioning

Participants who meet the following criteria will be ineligible to receive CTX:

1. Active and untreated bacterial infection, including but not limited to pneumonia, UTI, or sepsis.
2. Development of ischemic heart disease requiring revascularization, development of dysrhythmia requiring treatment, or clinical evidence of congestive heart failure.
3. Any condition that, in the opinion of the investigator, may pose additional risks from continued participation in the study, may interfere with the participant's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study.

5.3.3.3.4 Study Stage 3B: CTX Conditioning Administration

CTX will be administered after leukapheresis; the timing may vary from approximately 13-16 days after leukapheresis. Information about dosing, preparation/handing, and administration of CTX, mesna, and pretreatment medications are described in sections 5.2.2.1 and 5.2.2.2.

5.3.3.4 *Study Stage 3C: arTreg Infusion*

5.3.3.4.1 Study Stage 3C: Timing for Eligibility Criteria 3C – arTreg Infusion

At least 24 hours after CTX administration and no more than 72 hours after CTX administration, participants will be evaluated for eligibility to receive arTreg infusion.

5.3.3.4.2 Study Stage 3C: Inclusion Criteria for arTreg Infusion

Participants must meet the following criterion to be eligible to receive arTreg infusion:

1. arTreg product which meets release criteria for infusion.

5.3.3.4.3 Study Stage 3C: Exclusion Criteria for arTreg Infusion

There are no exclusion criteria at this step.

5.3.3.4.4 Study Stage 3C: arTreg Infusion

The arTreg product will be infused as described in section 5.2.1.4.

5.3.3.4.5 Study Stage 3C: IS Management and Treatment of Elevated Liver Tests after arTreg Infusion and Prior to TAC Withdrawal

TAC dosing may be adjusted to maintain trough concentration of 3-7 ng/mL per investigator discretion for the treatment of elevated LFTs that occur after arTreg infusion and prior to initiating TAC withdrawal. EVR dosing should be continued to maintain a trough concentration of 5-8 ng/mL.

5.3.4 Study Stage 4: TAC Withdrawal

5.3.4.1 *Study Stage 4: Timing to Apply Eligibility Criteria 4 – TAC Withdrawal*

Within 15 days after receiving the minimum or target arTreg dose (Study Definitions), participants will be evaluated for eligibility for TAC withdrawal.

5.3.4.2 *Study Stage 4: Inclusion Criteria for TAC Withdrawal*

Participants must meet the following criteria to be eligible for TAC withdrawal:

1A. ALT < 75 U/L and GGT < 75 or < 1.5 x the baseline GGT (Study Definitions)

OR

1B. ALT ≥ 75 U/L or GGT ≥ 75 or ≥ 1.5 x the baseline GGT (Study Definitions): participants may not begin TAC withdrawal until the liver biopsy 5 to 10 days after receiving the arTregs is performed and assessed to have a Rejection Activity Index (RAI) of 0-3 per Banff Global Criteria based on Local Pathology[50,59-61].

5.3.4.3 *Study Stage 4: Exclusion Criteria for TAC Withdrawal*

Participants who meet any of the following criteria will not be eligible for TAC withdrawal:

1. History of rejection since leukapheresis; evidence of AR or CR according to Banff criteria on protocol allograft biopsy based on local assessment or clinical rejection (Study Definitions).
2. Estimated GFR < 40 ml/min per 1.73m² as calculated by CKD-EPI equation.
3. Development of a condition requiring chronic anti-coagulation or anti-platelet agents other than aspirin that cannot be safely discontinued for a minimum of 1 week to safely perform a liver biopsy.
4. Any condition that, in the opinion of the investigator, may pose additional risks from continued participation in the study, may interfere with the participant's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study.

5.3.4.4 *Study Stage 4: IS Management during TAC withdrawal*

Withdrawal may be initiated within 14 days of TAC withdrawal eligibility confirmation. Participants who meet eligibility by inclusion criteria #1A will undergo a liver biopsy 5 to 15 days after receiving the arTregs and may continue with TAC withdrawal if the biopsy is assessed to have a RAI of 0-3 per Banff Global Criteria based on Local Pathology.[50,59-61]

Withdrawal will occur over two 6-week intervals, for a total of 12 weeks in the absence of a pause. Withdrawal will proceed as indicated in Figure 5. Dose reductions can occur within a +7 day window for each taper level.

If the participant is receiving prednisone, the prednisone dose will be tapered to a maximum of 5 mg/day by the completion of TAC withdrawal. Prednisone may have been discontinued at an earlier time point at the discretion of the investigator. The exact schedule for prednisone withdrawal may be varied at the discretion of the investigator based on the clinical and immunologic status of the participant.

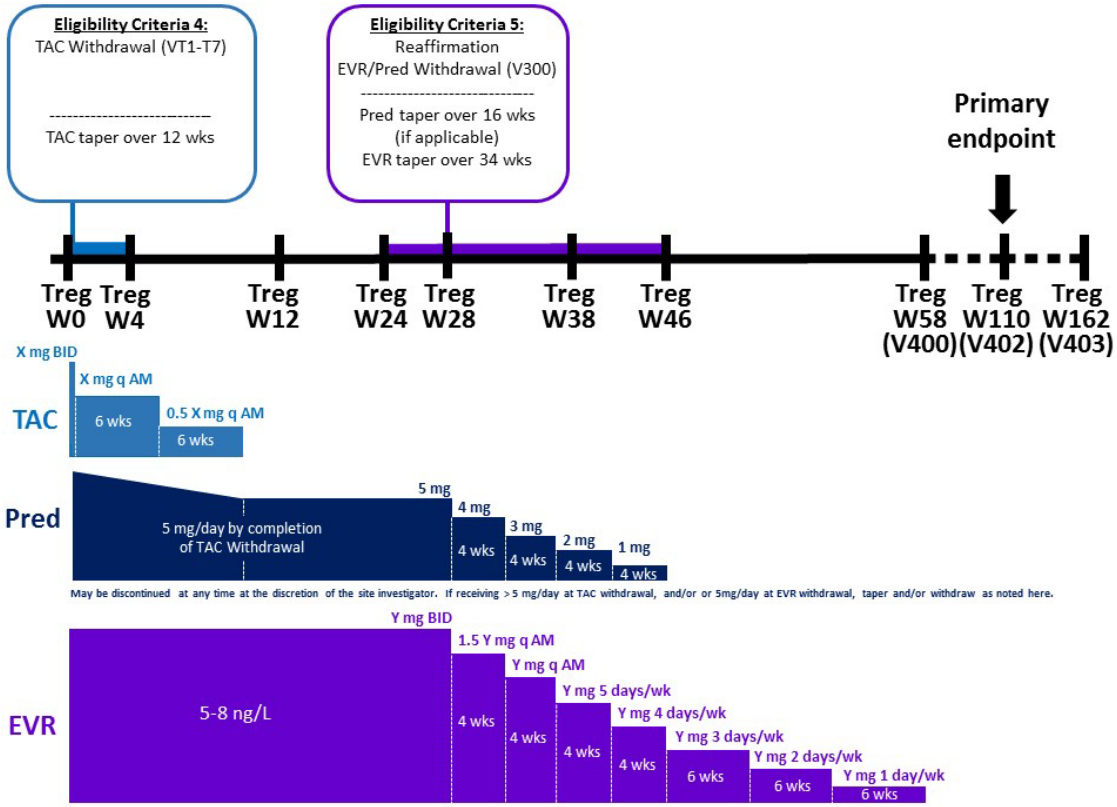

Figure 5. TAC Withdrawal and EVR/Prednisone Withdrawal Regimen (Study Stage 4, 5 and 6)

5.3.5 Study Stage 5: EVR/Prednisone Withdrawal

Participants who successfully complete **Study Stage 4** will remain on EVR. In addition, these participants may optionally remain on ≤ 5 mg/day of prednisone; prednisone may have been discontinued at an earlier time point at the discretion of the investigator.

5.3.5.1 *Study Stage 5: Timing to Apply Eligibility Criteria 5 – EVR/Prednisone Withdrawal*

Between 12 to 26 weeks of receiving EVR ± prednisone alone, participants who received ≥ 90 x 10^6 arTregs will be evaluated for eligibility for EVR/prednisone withdrawal.

Participants who received a dose of arTregs ≥ 30 x 10^6 cells but < 90 x 10^6 cells may be considered for EVR/prednisone withdrawal at the discretion of the investigator.

5.3.5.2 *Study Stage 5: Inclusion Criteria for EVR/Prednisone Withdrawal*

Participants must meet all of the following criteria to be eligible for EVR/prednisone withdrawal:

1. Able to provide reaffirmation of informed consent.
2. Received a minimum of 12 weeks of EVR following complete TAC withdrawal.
3. Had a liver biopsy that fulfills the criteria in Table 9 based on central pathology.

5.3.5.3 *Study Stage 5: Exclusion Criteria for EVR/Prednisone Withdrawal*

Participants who meet any of the following criteria will not be eligible for EVR/prednisone withdrawal:

1. History of rejection since initiating EVR conversion (**Study Stage 2**); evidence of AR or CR according to Banff criteria on protocol allograft biopsy based on local assessment or clinical rejection (Study Definitions).
2. ALT > 50 U/L.
3. Estimated GFR < 40 ml/min per 1.73m² as calculated by CKD-EPI method.
4. Development of a condition requiring chronic anti-coagulation or anti-platelet agents other than aspirin that cannot be safely discontinued for a minimum of 1 week to safely perform a liver biopsy.
5. Any condition that, in the opinion of the investigator, may pose additional risks from continued participation in the study, may interfere with the participant's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study.
6. GGT > the ULN or > 1.5 x the baseline GGT (Study Definitions)

Table 9. Study Stage 5: EVR/Prednisone Withdrawal. Eligibility 5 Biopsy Findings for IS Minimization[57]

Compartment	Finding(s)
Portal inflammation and interface activity	No more than minimal or focal mild portal mononuclear inflammation. No more than minimal interface necro-inflammatory activity, limited to a minority of portal tracts.
Centrizonal / perivenular inflammation†	Negative for perivenular inflammation.
Bile duct changes	Absence of lymphocytic bile duct damage, ductopenia, and biliary epithelial senescence changes, unless there is an alternative, non-immunological explanation (e.g. biliary strictures).
Fibrosis	No more than mild fibrosis, with rare or no portal-to-portal bridging.
Arteries+	Negative for isolated "v" lesions (lymphocytic arteritis), or obliterative or foam arteriopathy.
† Modified from Banff 2012 recommendations[62] because of widespread recognition that the lesion represents a rejection reaction and has the potential to progress and cause perivenular fibrosis after weaning. + Isolated "v" lesions (lymphocytic arteritis) was added to the Banff 2012 because of evidence of similar lesions in renal allografts leading to a suboptimal outcome even in patients maintained on immunosuppression.[63]	

5.3.5.4 *Study Stage 5: EVR/Prednisone Withdrawal*

The Schedule of Events (Appendix 4) specifies the study visits for **Study Stage 5**. EVR/Prednisone withdrawal will be initiated within 8 weeks of the eligibility biopsy.

For participants receiving prednisone, prednisone withdrawal will occur over four 4-week steps, for a total of 16 weeks in the absence of a pause. EVR withdrawal will occur over four 4-week steps followed by three 6-week steps, for a total of 34 weeks in the absence of a pause. Withdrawal will proceed as indicated in Figure 5. Dose reductions can occur within a +7 day window for each taper level.

5.3.6 Study Stage 6: Post-IS Withdrawal Surveillance and Evaluation

See section 5.4.2 for study procedures for the resumption of IS, and section 5.4.3 for the study procedures for assessment and treatment of allograft dysfunction. All participants who achieve complete IS withdrawal according to the withdrawal schedule will complete 104 weeks of Post-withdrawal Surveillance and Evaluation per Appendix 5, Schedule of Events.

If a participant has biopsy-proven rejection or resumes IS for clinical rejection during **Study Stage 6**, they will complete follow-up per section 5.4.2 and subsequent study-mandated biopsies are optional.

5.4 OTHER STANDARD STUDY PROCEDURES

5.4.1 Procedure for Temporary Pause in IS Withdrawal

IS withdrawal may be temporarily paused for up to 4 weeks. During this time interval, the participant will remain at the current dose. If the participant is withdrawing from two medications at the time of a pause, both medications will remain at the current dose. If the participant cannot proceed to the next dose reduction after 4 weeks, they will move to High Intensity Safety Follow-up (Appendix 6) and will considered to have failed IS withdrawal. Participants are allowed up to 3 non-consecutive pauses or a cumulative total of 12 weeks.

Cases in which extenuating circumstances lead to longer pauses will be considered by a committee consisting of the NIAID Medical Monitor, the ITN Clinical Trial Physician, and the Protocol Chair. This committee will adjudicate whether the participant may proceed with IS withdrawal even if the 12-week total has been exceeded.

5.4.2 Procedure for IS Resumption

Participants who meet the following criteria will receive treatment or be restarted on IS per section 5.4.3.3.

1. Participants undergoing IS withdrawal who have biopsy-proven rejection or clinical rejection will discontinue further withdrawal and receive treatment.
2. Participants who have completed IS withdrawal who have biopsy-proven rejection or clinical rejection will be restarted on IS.

All participants who resume IS will have failed IS withdrawal unless determined otherwise by an adjudication committee (section 5.4.3.2.3). Participants who fail IS withdrawal will not be allowed a second attempt.

Participants who fail IS withdrawal while undergoing IS withdrawal will complete 104 weeks of High Intensity Safety Follow-up per Appendix 6. If the participant fails IS withdrawal after completing IS withdrawal, the participant will continue on **Study Stage 6: Post-withdrawal Surveillance and Evaluation** per Appendix 5. These participants will be followed for 104 weeks after completing **Study Stage 5** (section 5.3.5) or 12 weeks after resuming IS, whichever is longer.

5.4.3 Assessment and Treatment of Allograft Dysfunction

5.4.3.1 *Definition of Allograft Dysfunction and Indication for Allograft Biopsy*

Allograft dysfunction occurs when ALT ≥ 100 U/L and/or GGT is elevated relative to baseline.

The baseline GGT for each participant is defined as the average of two GGT values taken at the following time points:

- The GGT taken at EVR Conversion Eligibility (V101[R])
- The GGT taken at the initiation of EVR conversion (V101S)

Elevation of GGT sufficient to meet the definition of allograft dysfunction is measured in relation to the baseline value as noted below:

- If the baseline GGT was < the ULN and the current value is ≥ 2-fold the ULN

  Or,

- If the baseline GGT was ≥ the ULN and the current value is ≥ 2-fold the baseline value.

When allograft dysfunction occurs, participants should be thoroughly assessed for any concurrent illness or alternative diagnosis according to standard of care. Liver tests may be repeated for both verification and re-assessment.

If allograft dysfunction persists for > 7 days and no etiology is identified, a for-cause biopsy must be performed, unless medically contraindicated. If a for-cause biopsy has been performed and is non-diagnostic, further biopsies may be performed at the discretion of the investigator.

At the discretion of the investigator, a for-cause biopsy may be triggered by elevated liver tests below the threshold that defines allograft dysfunction but above the individual participant's baseline.

5.4.3.2 *Diagnosis of Rejection*

5.4.3.2.1 Acute Rejection

AR will be diagnosed in accordance with Banff global assessment criteria.[50,59-61] Severity will be determined by Table 10.[64]

Steroid refractory AR refers to AR that has failed to resolve with steroid treatment.

Table 10. Banff Schema: Grading of Acute Liver Allograft Rejection

Global Assessment	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of AR
Mild	Rejection infiltrate in a minority of triads that is generally mild and confined within the portal spaces with RAI ≤4.
Moderate	Rejection infiltrate expanding most of all of the triads.
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis.
NOTE: Global assessment of rejection grade made on a review of the biopsy specimen and after the diagnosis has been established.	

5.4.3.2.2 Chronic Rejection

CR will be diagnosed in accordance with Banff global assessment criteria.[59-61]

5.4.3.2.3 Clinical Rejection

For cases where a biopsy is indeterminate or in rare cases where a biopsy cannot be performed, participants may be treated empirically based on the investigator's judgment as to the likelihood of rejection based on clinical parameters. In these cases, participants will be considered to have clinical rejection. If a participant is treated for clinical rejection at the study site without undergoing a biopsy, all other visit assessments done at a for-cause biopsy visit (Appendix 8) will be done prior to initiating treatment.

Participants with clinical rejection and no biopsy must undergo a biopsy within a week of initiating treatment for allograft dysfunction, unless medically contraindicated.

In the event an investigator determines that a participant did not experience rejection, but was treated for rejection or received increased IS, the investigator may request that the participant's data is reviewed by an adjudication committee. This adjudication committee will consist of the NIAID Medical Monitor, the ITN Clinical Trial Physician, and a Protocol Chair from an alternate consortium protocol (section 1.1.1). The central pathologist may also be consulted as needed. This committee will adjudicate whether the participant has experienced clinical

rejection. If the committee is unanimous in determining that the participant has not experienced clinical rejection, then the participant can continue with IS withdrawal, or if already off all IS, can remain off. If it is the opinion of any or all committee members that the participant has experienced clinical rejection, the participant has failed IS withdrawal, or if already off all IS, will have failed the primary endpoint.

5.4.3.3 *Guidelines for Management of Allograft Dysfunction or Rejection*

IS will be escalated or reinstituted for all participants who experience biopsy-confirmed or clinical rejection. Investigators may treat rejection according to center standard of care.

The following, however, are recommended guidelines based on previous experience in liver allograft IS withdrawal trials for participant's experiencing allograft dysfunction following initiation or completion of IS withdrawal:

- *Allograft dysfunction with no AR or indeterminate for AR*, and without other explanatory diagnosis should be treated at the discretion of the site investigator with increased or reinstitution of IS. If liver tests do not improve within 4 weeks, repeat biopsy should be considered prior to further escalation of treatment.
- *Mild AR* should be treated initially with increased or reinstitution of IS. If liver tests do not improve within 2 weeks, conversion or addition of another agent should be considered prior to administration of corticosteroids. If corticosteroids are administered, a dose of 20 mg oral prednisone (or equivalent) should be considered. Corticosteroids will be tapered down over a 4-week period. A second biopsy can be performed at any time at the investigator's discretion.
- *Moderate AR with mild biochemical abnormalities and no jaundice* should be treated with increased or reinstitution of IS and 20 mg oral prednisone (or equivalent) with taper down of steroid doses over a 4-week period. If liver tests do not improve within 2 weeks, conversion or addition of another agent should be considered prior to additional corticosteroids. A second biopsy can be performed at any time at the investigator's discretion.
- Moderate AR with marked biochemical abnormalities, severe AR, or CR, should be treated according to center standard of care.
- Antibody treatment should be reserved for steroid-resistant rejection proven by repeat liver biopsy.

An episode of biopsy-confirmed or clinical rejection will be considered resolved when ALT (with or without elevated GGT) is ≤ 50 U/L. For these cases, it is recognized that GGT levels decline very slowly following rejection and therefore will not be used to define resolution. If an episode of biopsy-confirmed or clinical rejection involves elevated GGT alone, it will be considered resolved when GGT is ≤ 1.5 x baseline levels.

For participants with biopsy-confirmed rejection, a repeat biopsy to further document resolution of rejection may be performed at the discretion of the investigator.

6. STUDY ASSESSMENTS AND WINDOWS

6.1 OVERVIEW

Appendices 1-8 present the schedule of events for this trial. The visit windows are outlined in section 6.2, while sections 6.3-6.7 detail the study assessments that are performed. The frequency of the study assessments are noted in the schedule of events.

6.2 VISIT WINDOWS

6.2.1.1 *Recipient*

All scheduled study visits and assessments must occur within the time limits specified below:

- Visits as noted in Table 11.
- Laboratory evaluations (LFTs and IS levels) done locally, should be completed within ±5 days of the scheduled time points.
- Up to 3 non-consecutive pauses (up to 4 weeks each) may occur during Visits 201-203 and Visits 301-307 combined (if needed) as outlined in section 5.4.1.
- If a participant needs a for-cause biopsy within 8 weeks prior to an Appendix 1A to Appendix 4 visit, the mechanistic assessments collected during the For-cause Biopsy Visit will take the place of any mechanistic assessments to be collected during that upcoming Appendix 1A to Appendix 4 visit.
- If a participant needs a for-cause biopsy within 8 weeks prior to an Appendix 5 to Appendix 7 visit, the For-cause Biopsy Visit will take the place of that upcoming Appendix 5 to Appendix 7 visit.

Table 11. Recipient Visit Windows

	Step	Visit	Time Point	Visit Window
Appendix 1A	Eligibility 1: Pre-Transplant	-1	Within 26 weeks prior to Visit 0	
	Transplant	0	Day 0	
	Eligibility 2: EVR Conversion	101/101R	Day 30 to Week 48 post-transplant	
	EVR Conversion Initiation	101S	Within 7 days of V101[R]	
	Eligibility 3A: Recipient Leukapheresis for arTreg Manufacturing	102/102R	Day 60 to Week 52 post-transplant	

	Step	Visit	Time Point	Visit Window
Appendix 2	Recipient Leukapheresis	T-17/T-17R	Within 28 days of V102[R]	
	Eligibility 3B: pre-arTreg Conditioning (CTX)	T-3	CTX administration must occur no more than 72 hours and no less than 24 hours before Treg infusion	
	Eligibility 3C: arTreg Infusion	T0	Treg Day 0	
	Post arTreg Infusion Visits	T1	Treg Day 1	+24 hours
		T7	Treg Day 7	-2/+8 days
		T14	Treg Day 14	-2/+3 days
		T28	Treg Day 28	±5 days
		T84	Treg Day 84	±5 days
	Reduced Safety Follow-up	RFU	Within 30 days after unsuccessful EVR conversion, Visit T-17/T-17R, or Visit T-3	
Appendix 3	TAC Taper Level 1	201	Within 14 days after confirming TAC withdrawal eligibility (Visit T1-T7) (section 5.3.4.4)	
	TAC Taper Level 2 - Off	202-203	Every 6 weeks	+7 days
	TAC Withdrawal Pause Visits	201P	During Taper Level 1 – up to 4 weeks (section 5.4.1)	
		202P	During Taper Level 2 – up to 4 weeks (section 5.4.1)	
	Post TAC Withdrawal Week 24	204	24 weeks after Visit 201	±7 days
Appendix 4	Eligibility 5: EVR/Pred Withdrawal	300	Week 12 to 26 post TAC Withdrawal (section 5.3.5.1 for participants who receive the minimum/target arTreg dose [Study Definitions])	
	EVR/Pred Taper Level 1	301	Within 8 weeks after Visit 300 Biopsy	
	EVR/Pred Taper Levels 2-4	302-304	Every 4 weeks	+7 days
	EVR Taper Levels 5-7	305-307	Every 6 weeks	+7 days
	EVR/Pred Withdrawal Pause Visits	301P-307P	During Taper Level 1 to 7 – up to 4 weeks each (section 5.4.1)	
Appendix 5	Post-IS Withdrawal Surveillance and Evaluation Follow-up Week 0	400	Within 7 days after the last IS dose	
	Post-IS Withdrawal Surveillance and Evaluation Follow-up Week 26	401	26 weeks after the last IS dose	±2 weeks
	Post-IS Withdrawal Surveillance and Evaluation Follow-up Week 52 *(Primary Endpoint Visit)*	402	52 weeks after the last IS dose	±4 weeks
	Post-IS Withdrawal Surveillance and Evaluation Follow-up Week 104	403	104 weeks after the last IS dose	±4 weeks
	Post-IS Withdrawal Surveillance and Evaluation Follow-up Week 105-116	404	12 weeks after restarting IS (participants for who resume IS at the end of study follow-up to ensure they are followed for ≥12 weeks after restarting IS [section 5.4.2])	±2 weeks

	Step	Visit	Time Point	Visit Window
Appendix 6	High Intensity Follow-up Week 0	500	Within 4 weeks after Visit T0 (participants who don't receive the minimum/target arTreg dose [Study Definitions] or attempt TAC withdrawal), Visit 300 (participants who do not attempt EVR/Pred withdrawal), or ISW failure	
	High Intensity Follow-up Week 26	501	26 weeks after Visit T0 (participants who don't receive the minimum/target arTreg dose [Study Definitions] or attempt TAC withdrawal), Visit 300 (participants who do not attempt EVR/Pred withdrawal), or ISW failure	±2 weeks
	High Intensity Follow-up Week 52	502	52 weeks after Visit T0 (participants who don't receive the minimum/target arTreg dose [Study Definitions]) or attempt TAC withdrawal), Visit 300 (participants who do not attempt EVR/Pred withdrawal), or ISW failure	±4 weeks
	High Intensity Follow-up Week 104	503	104 weeks after Visit T0 (participants who don't receive the minimum/target arTreg dose [Study Definitions] or attempt TAC withdrawal), Visit 300 (participants who do not attempt EVR/Pred withdrawal), or ISW failure	±8 weeks
Appendix 7	Low Intensity Follow-up Week 0	600	7 to 10 days after Visit T-3	
	Low Intensity Follow-up Week 4	601	4 weeks after Visit T-3	±5 days
	Low Intensity Follow-up Week 52	602	52 weeks after Visit T-3	±8 weeks
Appendix 8	For-cause Biopsy	FCB	As needed	

6.2.1.2 *Living/Deceased Donor*

All scheduled study visits and assessments must occur within the time limits specified below:

- Visits as noted in Table 12.

Table 12. Living/Deceased Donor Visit Windows

	Step	Visit	Time Point	Visit Window
Appendix 1B	Eligibility: Living Donor Transplant	LD-1	Within 26 weeks prior to Visit 0 in Appendix 1A	
		LD0	Day 0 to Week 26 post-transplant, but prior to Visit 102/102R in Appendix 1A	
	Eligibility: Deceased Donor Transplant	DD0	Day 0	±7 days

6.3 GENERAL ASSESSMENTS

6.3.1 Recipient

Recipients will undergo the following general assessments during the course of their study participation:

- Informed consent
- Eligibility criteria
- Study parameters for continuation (section 5.3.1.1)
- Demographics – date of birth, sex, ethnicity, and race
- Medical history – (within the last 12 months) includes liver disease history to determine time of diagnosis and ABO typing
- Complete physical exam – includes height only at Visit 101
- Limited physical exam – includes body systems: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary
- Vital signs – weight, temperature, blood pressure, respiratory rate, pulse
- Adverse Events – participants will be assessed for AEs and recorded per section 8.3
- Concomitant medications – all concomitant medications will be recorded per section 5.2.4.5
- Remote consultation – e.g. by telephone, email, or a combination of methods
- Telemedicine consultation – requires investigator assessment of the participant by telephone or video conference
- Events of interest – includes the following:
  - Rejection (section 5.4.3.2)
  - Infections: includes wound infection, bacteremia, cholangitis, pneumonia, meningitis, CMV viremia, EBV/PTLD, fungal infection, Cryptococcus, and others
  - Surgical complications or procedures, including biliary obstruction
  - Cytopenia
  - Evidence of CKD

- Recurrent HCC or any cancer

- HCV treatment history – includes last date of treatment for participants with a history of HCV only (section 5.3.1.1)
- Milan criteria, with no vascular invasion, and with no cholangiocarcinoma morphology – criteria apply to the recipient's explanted liver (section 5.3.1.1) for participants with HCC only

6.3.2 Living Donor

Living donors will undergo the following general assessments during the course of their study participation:

- Informed consent
- Eligibility criteria
- Demographics – date of birth, sex, ethnicity, and race
- ABO typing (from the medical record)
- AEs – participants will be assessed for AEs and recorded per section 8.3

6.3.3 Deceased Donor

Data to be collected from medical or UNOS records.

- Eligibility criteria
- Demographics – date of birth, sex, ethnicity, and race
- ABO typing (from the medical record)

6.4 CLINICAL ASSESSMENTS AND PROCEDURES

6.4.1 Recipient

Recipients will undergo the following clinical assessments and procedures during the course of their study participation:

- Doppler or angiography of the liver
- Liver transplant – standard of care
- Liver biopsy (section 6.7)

6.5 CLINICAL LABORATORY ASSESSMENTS

6.5.1 Recipient

These laboratory assessments may be performed at the study site or other qualified local laboratories:

- Hematology – CBC with differential and platelets
- Basic chemistry – BUN, creatinine, total bilirubin, direct bilirubin, GGT, AST, ALT, alkaline phosphatase

- Urine or blood hCG – pregnancy test for WOCBP
- Liver Function Tests – ALT, GGT
- CD4 count – in peripheral blood
- HIV-1/2 –antibody immunoassay
- HCV RNA – hepatitis C viral copy number by quantitative PCR
- HBV DNA – hepatitis B viral copy number by quantitative PCR
- EBV Antibody – EBV IgG (the assessment does not need to be repeated if the participant has a documented positive result)
- EBV DNA – quantitative by PCR
- CMV antibody – CMV IgG (the assessment does not need to be repeated if the participant has a documented positive result)
- CMV DNA – quantitative by PCR
- Estimated GFR – as determined by calculated CKD-EPI[65]
- HLA typing – includes HLA-DR (retrospective data collection from the medical record – repeat testing is not required if already completed as standard of care)
- AFP – section 5.3.1.1
- Urinalysis – urine protein/creatinine ratio
- TAC trough levels
- EVR trough levels
- 2019-nCoV RT-PCR – see sections 5.3.2.2 and 5.3.3.2.2

6.5.2 Living Donor

These laboratory assessments may be performed at the study site or other qualified local laboratories:

- HLA typing – includes HLA-DR (retrospective data collection from the medical record – repeat testing is not required if already completed as standard of care)
- CMV antibody – CMV IgG (the assessment does not need to be repeated if the living donor has a documented positive result)
- EBV antibody – EBV IgG (the assessment does not need to be repeated if the living donor has a documented positive result)
- 2019-nCoV RT-PCR within 7 days prior to blood collection for manufacturing

6.5.3 Deceased Donor

For all assessments, data to be collected from medical or UNOS records. If tests are not available, they should be ordered.

- HLA typing – includes HLA-DR

- CMV antibody – CMV IgG
- EBV antibody – EBV IgG
- HCV testing – antibody and NAT

6.6 ARTREG MANUFACTURE

6.6.1 Recipient

- Leukapheresis (section 5.3.3.2.4)
- 2019-nCoV RT-PCR within 7 days prior to leukapheresis (may be repeated on the day of leukapheresis at the discretion of the site investigator)

6.6.2 Living Donor

- Blood collection for infectious diseases testing – compliant with the current local and federal regulations (21CFR 1271) and requirements
- Blood collection by phlebotomy for manufacture of sBc using KT64.CD40L stimulation

6.6.3 Deceased Donor

- Blood collection for infectious diseases testing – compliant with the current local and federal regulations (21CFR 1271) and requirements
- Donor spleen and/or blood collection for manufacture of sBc using KT64.CD40L stimulation

6.7 LIVER BIOPSY

6.7.1 Types of Biopsies

Research Biopsies

Participants will undergo liver biopsies at times specified in the schedule of events (Appendices 1-5). These biopsies will be used to monitor liver histology, to screen for subclinical rejection, and for mechanistic analyses.

For–cause Biopsies

For-cause biopsies (FCB) will be obtained to confirm suspected rejection and all mechanistic assessments listed in Appendix 8 should be performed.

6.7.2 Central and Local Biopsy Assessments

The guidelines for the use of local and central liver biopsy pathology assessments are outlined below. All will be analyzed per the Banff global assessment criteria.[50,59-61,64]

For the purpose of determining **Study Stage 4: TAC withdrawal** eligibility, the local assessment will be used (section 5.3.4).

For the purpose of determining **Study Stage 5: EVR/Prednisone withdrawal** eligibility (section 5.3.5), and the primary and secondary endpoint evaluations (sections 3.3.1 and 3.3.2), the central assessment will be used.

For the purpose of clinical management, the local pathology assessment will be used. The central pathology assessment will be available to the investigators for consideration at their discretion.

For the purpose of evaluating the study stopping rules (section 3.4.2.1), the local pathology assessment will be used.

6.7.3 Biopsy Technique and Handling

The day 0 pre-implant research biopsy will be performed prior to transplantation of the liver into the recipient. Otherwise, research and for-cause biopsies will be performed per institutional practice guidance. Use of a 16- gauge needle is preferred. A minimum of two core biopsies should be obtained with a minimum of 1 cm of tissue per core biopsy. A consent specific for this procedure will be obtained according to the guidelines at the study site.

6.7.4 Recording of Biopsy Assessments

The local pathology assessment will be recorded on the eCRF by the site. The central pathology assessment will be recorded by the ITN central pathology core laboratory and the data will be transferred at regular intervals to the clinical database.

6.8 MECHANISTIC ASSESSMENTS

- HLA typing – molecular typing may include both Class I (HLA-A, B, C) and Class II (HLA-DRB1, DRB3, DRB4, DRB5, DQA, DQB1, DPB1)
- arTreg collection – archived cell product prior to infusion
- PBMC collection – includes arTreg detection and characterization and PBMC assays. arTreg detection and characterization assessments performed on days 14, 28, and 84 following arTreg infusion may be performed at the study site or remotely.
- Serum collection – includes donor-specific HLA antibody testing and serum assays
- Whole blood collection – gene expression
- Liver biopsy collection – graft histology, including H&E, Trichrome, and immunohistochemistry

See section 7 for detailed discussion of additional mechanistic assays.

7. MECHANISTIC ASSAYS

Although potential markers and technologies are listed, utilized markers and technologies will be selected and finalized based on literature and the state-of-the-art at the time of experimentation. Experiments may be performed in real-time, and/or in batches at (an) agreed upon point(s) in the trial to decrease the potential for variability between experiments.

7.1 RATIONALE FOR IMMUNE STUDIES

A key hypothesis for this study is that adoptive transfer of alloantigen specific Tregs will allow for successful IS withdrawal and allow for operational tolerance. We hypothesize that these cells will dampen the anti-donor response, potentially by a variety of mediators. The current ITN073ST and ITN074ST trials and potential future alloantigen specific Treg trials, are/will be

similar in their design, with the major difference being the generation of and intervention surrounding the infusion of different Treg products. By limiting the differences between these trials we will be able to compare the phenotype, stability, and function of alloantigen specific Tregs produced by at least two different methods. Further, these studies may identify or further confirm characteristics that describe operational tolerance, or lack of rejection, regardless of method of induction. We propose the following immune studies to address the mechanistic hypotheses outlined in section 7.2.

7.2 MECHANISTIC HYPOTHESES

Overall, we hypothesize that successful IS withdrawal will correlate with:

1. Increased arTreg cells in the circulation and graft,
2. Suppression of donor-reactive Teffector cells,
3. Increased intragraft expression and/or presence of tolerance associated genes, and
4. Decreased intragraft expression and/or presence of rejection associated genes.

Functional assay hypotheses are:

1. Tolerant participants will have a loss or altered direct and indirect alloresponses following arTreg infusion.
2. Increased presence of arTreg cells will suppress the expansion of donor-reactive effector T cells (and potentially lead to their deletion or deviation from a pathogenic phenotype), and promote T cell exhaustion and senescence.

Graft-related hypotheses are:

1. Following arTreg infusion, transferred cells will be detectable within the graft.
2. The change of effector and regulatory T cell balance will be reflected within the graft, along with increased intra-graft expression of tolerance-associated genes and decreased intra-graft expression of rejection-associated genes.

Regulatory T cell product characterization hypotheses are:

1. arTreg infusion will lead to a persistent increase of alloantigen-reactive Tregs in both the circulation and graft.

7.3 PROPOSED MECHANISTIC ASSAYS

7.3.1 HLA Typing

HLA typing for both the donor and recipient may be performed, in addition to the standard of care HLA typing performed for enrollment eligibility. Samples provided by the donors and recipients may be used to perform molecular typing for both Class I (HLA-A, B, C) and Class II (HLA-DRB1, DRB3, DRB4, DRB5, DQA, DQB1, DPB1).

7.3.2 Functional Assays

7.3.2.1 *Control of Anti-Donor Responses: DSA*

Following liver transplant, development of DSA by the recipient may portend damage to the liver allograft. In addition to possibly being evident by clinical measures, this damage may also be evident upon histological investigation of the allograft. We will first test liver transplant

recipient serum for PRA (panel reactive antibody) to determine if the recipient has generated anti-HLA reactive antibodies. If this test is positive, we will then test these sera for DSA. Results from donor and recipient HLA typing (section 7.3.1) will be used in conjunction with solid phase, single antigen bead assays to determine if the recipients' anti-HLA antibody is reactive with a donor-type HLA. To further describe and attribute function to these DSA, the IgG subclass (IgG1/2/3/4) and/or ability to bind C1q may be assessed.

7.3.2.2 *Detection of Donor Reactivity*

7.3.2.2.1 Serum Assays

We propose to collect serum samples to test for the presence of regulatory proteins and/or the absence of proteins indicative of graft rejection. This testing may use multiplex technology to assay cytokine and/or other protein amounts.

7.3.2.2.2 Allospecific T Cells

We propose to test liver recipients for allospecific responses to their donor. We hypothesize that tolerant recipients will have a loss of both direct and indirect alloresponses following arTreg infusion. Donor reactive cells may be deleted, become exhausted, and/or become senescent.

To this end, we will collect PBMCs from both donor and recipients. Splenic or lymph node cells may be substituted in the case of deceased donors. To detect direct responses, recipient antigen presenting cells may be cultured in the presence of donor PBMCs. To detect indirect responses, recipient dendritic cells may be "fed" donor antigen before culture with recipient PBMCs. Recipient anti-donor reactivity may be assessed by flow cytometry (cellular activation, proliferation, and/or cytokine production), cytokine secretion, and/or other methods. Suitable controls would be included.

We may also identify donor-reactive T cell clones in recipients pre-transplant, and investigate their persistence/presence post-transplant. This experiment may include a direct recognition mixed lymphocyte reaction to identify recipient cells that proliferate in the presence of donor antigen. These cells may be sorted into CD8+, CD4+ Treg and CD4+ Tconv populations. Doing so would allow us to determine if the donor-reactive TCRs present were in the CD8+, CD4+ or Treg lymphocyte subsets. The TCR sequences of these cells would then be determined by deep sequencing. At specified times following transplant, recipient cells would again be sorted into subsets and their TCR sequences assessed by deep sequencing. By comparing pre-transplant donor-reactive TCRs to TCRs present post-transplant, we may be able to assess their persistence. This method has been previously published by Morris, et al.[66] Detection of exhausted, senescent, and/or anergic cells may include addition of antibodies and/or cytokines to cell cultures before assaying for anti-donor responses.

7.3.2.2.3 Immunophenotyping

We have hypothesized that development of tolerance in liver transplant recipients is a dynamic process that can in part be monitored by changes in cell phenotype and populations as assessed by immunophenotyping.

To this end, we may perform flow cytometry/immunophenotyping on both fresh and/or frozen PBMC to determine PBMC phenotype and proliferative status. Potential cell populations include, but are not limited to, T and B cells, and their subsets, co-stained with phenotypic

markers suggestive of anergy, senescence, exhaustion, activation, regulation, and recent thymic emigration. Such markers may include CD3, CD4, CD8, CD19, CD45RA, CD45RO, CCR7, CD57, PD-1, KLRG-1, CD28, CD80/86, HLA-DR, Foxp3, CD25, and CD31.

7.3.3 Graft-Related Assays

7.3.3.1 *Histological Assessment*

Changes that occur within the graft, both in terms of structure and cellular content, may help detail interactions between the recipient's immune system and the liver allograft, and be informative as to whether these interactions are beneficial or detrimental to the graft. To this end, we may use both paraffin-embedded and/or OCT-mounted frozen liver biopsy tissue to capture a snapshot of the state of the allograft. Stains such as hematoxylin and eosin (H&E) and Trichrome staining may be used to capture structural and gross changes within the graft. To further describe and/or detect the presence of a recipient anti-donor reaction, staining for bound Class II antibodies and/or C4d may be performed. To characterize the presence/identity of any cellular infiltrate within the graft, tissue may be examined for the presence/location of Tregs (Foxp3), Th1/2/17 cells (Tbet/GATA-3/IL-17), T cells (CD3/4/8), NK cells (CD56), activated cells (HLA-DR), and/or B cells (CD19/20). Other markers of interest may identify endothelial cells (CD31) and smooth muscle actin. Markers in addition to the examples listed above may be included, and their presence may be detected by fluorescence, enzyme reaction, mass spectrometry, or another method as appropriate.

7.3.3.2 *Gene Expression*

Gene expression of PBMCs and within the liver allograft itself may be informative as to the status of the recipient's immune response to the allograft. If the recipient is experiencing, or about to experience, a rejection event, a signature of inflammatory mediators and/or of non-tolerance may be present. In contrast, if the recipient is tolerant, or to become tolerant, of the allograft a signature of tolerance may be present.

A gene signature of operational liver tolerance has been described in the blood and within the graft.[1,67] Potential genes associated with tolerance include genes indicative of an increase in γδ T cells, and genes involved with iron homeostasis. We hypothesize that potential genes associated with rejection, as described by Bonaccorsi-Riani et al.[68] will not be upregulated in tissue from tolerant participants.

The methylation status of different genes may also indicate whether these genes are turned "on" or "off", and how permanent the on/off may be. We may examine gene expression, miRNAs, and/or gene methylation in peripheral blood (whole blood, PBMC, and/or sorted PBMC cell subsets), and/or liver allograft biopsy tissue. DNA, total RNA, and/or miRNA would be extracted from these samples. cDNA would be reverse transcribed from RNA. Methods for testing may include microarray and/or RT-PCR technology to identify and/or confirm the presence/absence of genes of interest.

7.3.3.3 *Treg Product Detection within the Graft*

We hypothesize that we will be able to detect the infused arTreg cells within the graft using deuterium labelling of the cell product. While this labeling will detect the persistence of transferred cells, it may not necessarily detect the proliferation of these cells. Interrogation of biopsy specimens for these cells may occur at 5-15 days post arTreg infusion, eligibility for EVR/prednisone withdrawal, and 52 weeks after the last IS dose (Appendices 2-5).

Additionally, TCR sequences of arTreg cells may be determined, and biopsy samples interrogated for the presence of these clones.

7.3.4 Treg Cell Product Characterization

7.3.4.1 *Detection of Transferred Cells Post-Transfer*

We hypothesize that we will be able to detect arTreg post transfer, and that the total number of Tregs detected in the blood will increase relative to pre-transfer numbers. To facilitate specific detection of the arTreg, deuterium labelling may be used. While this labeling will detect the persistence of transferred cells, it may not detect the proliferation of these cells. Historically, the circulating arTreg cell concentration increases immediately post-transfusion, peaks in the first two weeks, and gradually declines, remaining detectible for even 1 year after infusion. Interrogation of blood samples will occur prior to arTreg infusion, 1 day, and 1, 2, 4, and 12 weeks after arTreg infusion.

Additionally, TCR sequences of arTregs may be determined, and blood and/or biopsy samples interrogated for the presence of these sequences.

7.3.4.2 *Comparison of Treg products*

We propose to characterize and compare the different allo-reactive Tregs tested within the consortium. For example, RNA-seq and/or flow cytometry may be used to identify and describe their phenotype and potential regulatory mechanisms. We also may characterize the stability of the regulatory phenotype by epigenetic analysis of whole genome methylation.

7.4 QUALITY CHECK/PRELIMINARY STUDIES

Samples acquired may be used for either quality checks (if deemed necessary) or preliminary studies that could potentially be applied to the entire cohort of participants. Examples of when a quality check may be requested include issues that may arise from shipping/storage, to ensure sites are collecting samples correctly, or to compare data generated longitudinally within a core lab. Preliminary studies may include a pilot experiment when changing to a new core or piloting an assay/technique that may be of interest to this, and/or other, studies.

7.5 FUTURE / UNPLANNED STUDIES

Specimens stored during the trial may be used in future assays to reevaluate biological responses as research tests are developed over time. Additionally, samples may be used for assays/ experiments outside the scope of this proposal, such investigation of miRNA expression, differences in the TCR repertoire as evaluated by sequencing, proteomics or other explorations that may emerge and be compelling during and/or after the trial period. Re-evaluations or new assays will only be performed on samples of participants who have consented for future research. Blood, tissue, and other biologic samples will be collected and saved to allow for the possible use in later gene association studies. Specific consent will be obtained for the storage and use of participant DNA. The ITN sample sharing policy will apply for the provision of samples to study or outside investigators. Investigators may request use of specimens for tolerance related and unrelated studies. Only specimens from participants that have consented to future and/or future genetic studies, as appropriate, will be shared.

8. ADVERSE EVENTS

8.1 OVERVIEW

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as described in section 8.2. All AEs and SAEs will be recorded in the source documents and on the appropriate eCRF(s). All data will be reviewed periodically by the NIAID Transplant DSMB, which may provide recommendations to DAIT NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

AEs 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, institutional review boards (IRBs), and health authorities. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A*: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*; and ICH Guideline E-6: *Guidelines for Good Clinical Practice*; and applies the standards set forth in the National Cancer Institute (NCI)s *Common Terminology Criteria for Adverse Events Version 5.0* (published November 27, 2017). This document is referred to herein as the "NCI-CTCAE manual."

8.2 DEFINITIONS

8.2.1 Adverse Event

An AE is any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (ICH E-6 Guidelines for GCP).

8.2.2 Suspected Adverse Reaction and Adverse Reaction

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the study drug caused the AE. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse reaction. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug (21 CFR 312.32(a)).

8.2.3 Unexpected AE

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure or package insert or is not listed at the specificity, severity or rate of occurrence that has been observed; or, is not consistent with the risk information described in the general investigational plan or elsewhere in the IND.

"Unexpected" also refers to AEs or suspected adverse reactions that are mentioned in the Investigator Brochure or package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a).

8.2.4 Serious Adverse Event

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. This would also include confirmed cases of COVID-19.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol-mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

8.3 COLLECTING AND RECORDING ADVERSE EVENTS

8.3.1 Collection Period

Recipient

AEs will be collected from the time informed consent is obtained during Study Stage 2: EVR Conversion (section 5.3.2) until the participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

Living Donor

AEs grade 3 and higher that occur within 48 hours of phlebotomy for manufacturing will be collected for the study unless the blood for manufacturing is collected within 24 hours of the donor hepatectomy.

8.3.2 Collecting Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

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

- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an AE, as defined in section 8.3.4.1.

8.3.3 Recording AEs

Throughout the study, the investigator will record AEs and SAEs as described previously (section 8.2) on the appropriate eCRF regardless of the relationship to study therapy or study procedure.

8.3.4 Severity of Adverse Events to be Recorded

8.3.4.1 *Adverse Events*

All AEs, regardless of seriousness or severity, will be collected during the collection period specified in section 8.3.1.

AE grades will be defined per CTCAE criteria. Where the CTCAE relies on site normal ranges to assess grades, the normal ranges listed in Harrison's Principles of Internal Medicine, 18th edition (McGraw-Hill, 2011) will be used.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

8.3.5 Methods of Recording

8.3.5.1 *Recording AEs*

Throughout the study, the investigator will record all AEs per section 8.3.4.1 on the appropriate eCRF. The investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.5.2 *Recording SAEs*

SAEs will be recorded on the SAE eCRF and health authorities will be notified as outlined in section 8.5.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the NCI-CTCAE National Cancer Institute's *Common Terminology Criteria for Adverse Events 5.0* (published November 27, 2017). This manual provides a common language to describe level of severity, analyze and interpret data, and articulate the clinical significance of all AEs.

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

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

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

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent AE is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an AE if changes in therapy or monitoring are implemented as a result of the event/result.

It is acceptable for a protocol to use additional and/or alternative grading scale(s) for all AEs or only for drug or procedure-specific AEs; such scales need to be clearly described herein.

8.4.1.1 *AEs Related to the IP/Study Medications*

Recipient

Attribution assessment for the following study drugs will be made when an AE is reported:

- CTX and Mesna
- Treg supportive IS regimen (TAC, Mycophenolate product, EVR, and Prednisone)
- IS withdrawal (TAC, EVR, and Prednisone)

8.4.1.2 *AEs Related to Study Procedures*

Recipient

Attribution assessment for the following study procedures will be made when an AE is reported:

- Leukapheresis
- arTreg Infusion
- Liver biopsies
- Research blood draws

Living Donor

Attribution assessment for the following study procedures will be made when an AE is reported:

- Phlebotomy for manufacturing

8.4.2 Attribution

AEs will be categorized for their relation to the study therapy or procedure. The principal investigator will make the initial determination of the relation, or attribution, of an AE to the study and will record the initial determination on the appropriate eCRF and/or SAE reporting form. The relation of an AE to the study will be determined using definitions in Table 13 below. Final determination of attribution for safety reporting will be decided by DAIT/NIAID.

Table 13. Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
Unrelated Category		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
Related Categories		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The adverse event is clearly related.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting SAEs to the IND Sponsor

This section describes the responsibilities of the site investigator to report SAEs to the sponsor via the SDCC eCRF. Timely reporting of AEs is required by 21CFR 312 and ICH guidelines.

Site investigators will report all SAEs (section 8.2), regardless of relationship or expectedness to study procedures within 24 hours of discovering the event. All confirmed cases of COVID-19 will also be reported as SAEs, regardless of severity.

For SAEs, all requested information on the AE/SAE eCRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF will be updated and submitted.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or sub investigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
2635 East NC Highway 54
Durham, NC 27713 Toll-free - (888) 746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.5.1.1 *Reporting AEs of Study Specific Relevance (Non-Serious AE) to the IND Sponsor*

Allograft dysfunction, clinical rejection, and biopsy proven rejection NOT meeting serious criteria will be reported as AEs of Study Specific Relevance and will be recorded on the AE/SAE eCRF within 5 business days of discovering the event.

8.5.2 Reporting SAEs to Health Authorities

After an AE requiring 24 hour reporting (per section 8.2) is submitted by the site investigator and assessed by DAIT/NIAID, there are two options for DAIT/NIAID to report the AE to the appropriate health authorities:

8.5.2.1 *Annual Reporting*

DAIT/NIAID will include in the annual study report to the health authorities all AEs classified as:

- Serious, expected, suspected adverse reactions described in section 8.2.
- Serious and not a suspected adverse reaction described in section 8.2.
- Pregnancies

Note that all AEs (not just those requiring 24-hour reporting) will be reported to the health authorities in the Annual IND report.

8.5.2.2 *Expedited Safety Reporting*

This option, with 2 possible categories, applies if the AE is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction [SUSAR]** (section 8.2.2 and section 8.2.3 and 21 CFR 312.32(c)(1)(i))

The sponsor shall report any suspected adverse reaction that is both serious and unexpected. The sponsor shall report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the AE, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Category 2: **Any findings from studies that suggests a significant human risk**

The sponsor shall report any findings from other epidemiological studies, analyses of AEs within the current study or pooled analysis across clinical studies or animal or in vitro testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed

consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

The sponsor shall notify the appropriate health authorities and all participating investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

All principal investigators must report SAEs to their respective IRBs as mandated by them.

8.5.3 Reporting SAEs to the DSMB

The DAIT NIAID and ITN will provide the NIAID Transplant DSMB with data of all SAEs on an ongoing basis, including quarterly reports of all SAEs.

8.5.4 Reporting of AEs to IRBs/IECs

All investigators shall report AEs, including expedited reports, in a timely fashion to their respective IRBs/IECs in accordance with applicable regulations and guidelines. All Safety Reports to the FDA shall be distributed by DAIT/NIAID or designee to all participating institutions for site IRB/IEC submission.

8.6 REPORTING PREGNANCY

The investigator shall be informed immediately of any pregnancy in a study subject or a partner of a study subject. A pregnant subject shall be instructed to stop taking study medication. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the Statistical and Data Coordinating Center (SDCC) all pregnancies within 1 business day of becoming aware of the event using the Pregnancy eCRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SDCC when details about the outcome are available. When possible, similar information shall be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to SDCC using the SAE reporting procedures described above.

8.7 REPORTING OF OTHER SAFETY INFORMATION

An investigator shall promptly notify the site IRB as well as the SDCC when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an AE.

8.8 REVIEW OF SAFETY INFORMATION

8.8.1 Medical Monitor Review

The PI, the NIAID Medical Monitor, and the NIAID DSMB will review safety data on an ongoing basis. Enrollment and initiation of study treatment may be suspended at any time if any of these reviews conclude that there are significant safety concerns.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the SDCC.

8.8.2 DSMB Review

8.8.2.1 *Planned DSMB Reviews*

The DSMB shall review safety data at least yearly during planned Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

The DSMB will be informed of an Expedited Safety Report in a timely manner.

8.8.2.2 *Ad hoc DSMB Reviews*

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, an *ad hoc* comprehensive DSMB Safety Review will convene if any of the stopping guidelines listed in section 3.4.2 are met. After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

The analysis samples are described beginning with the most inclusive.

Enrolled sample (ES): All participants who sign consent.

Safety sample (SS): All participants who initiate EVR conversion.

Intent to treat (ITT) sample: All participants who receive at least the minimum arTreg dose.

Per protocol (PP) sample: All participants who receive at least the target arTreg dose.

The minimum and target doses are described in section 5.2.1.4.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Overview

The principal features of the plan for statistical data analysis are outlined in this protocol and will be described in greater detail in the statistical analysis plan (SAP). Analysis of study data will be conducted to address all objectives of the trial and other interrelationships among all data elements of interest to the Investigators and of relevance to the objectives of the study.

9.2.2 Primary Endpoints

9.2.2.1 *Primary Safety Endpoints*

The primary safety endpoints, defined in section 3.3.1.1, will be descriptively summarized with counts and percentages. Frequency tables by the investigational product and investigational regimen and by CTCAE grade will be presented.

AEs will be considered attributed to arTregs if the AE is reported with possible or related attribution to arTreg. AEs will be considered attributed to the investigational regimen if the AE is reported with possible or related attribution to a) leukapheresis, b) CTX, or c) mesna. The severity will be determined by the CTCAE grade.

The primary safety endpoints will be analyzed using the safety, ITT and PP samples.

9.2.2.2 *Primary Efficacy Endpoint*

The primary efficacy endpoint, defined in section 3.3.1.2, will be descriptively summarized with a point estimate and two-sided, 95% exact binomial confidence interval. The numerator will be the number of participants that meet the primary efficacy endpoint.

The primary efficacy endpoint will be analyzed using the ITT and PP samples.

9.2.3 Secondary Endpoints

The secondary endpoints are defined in 3.3.2. Table 14 describes the secondary endpoints and their corresponding analysis parameters to be estimated in the study. The table also lists the analysis samples in the order of importance. Secondary endpoints will be listed or summarized, as appropriate, using standard descriptive statistics for continuous and categorical data.

Table 14. Analysis of Secondary Endpoints

Endpoint	Analysis Parameter	Analysis Sample
Incidence of infections grade 3 or greater following arTreg infusion	Proportion of participants with an infectious AEs of grade ≥3 (defined in 8.4.1) after arTreg infusion with a two-sided, 95% exact binomial confidence interval	ITT, PP, SS
Number and severity of biopsy-proven AR or clinical rejection events any time after arTreg infusion	Number and the categorization of severity (per Banff) of biopsy-proven AR or clinical rejection events after arTreg infusion	ITT, PP, SS
Number of CR events any time after arTreg infusion	Number of CR events after arTreg infusion	ITT, PP, SS
Number of participants who develop any malignancy	Number of participants with any malignancy	ITT, PP, SS

Endpoint	Analysis Parameter	Analysis Sample
Proportion of participants who discontinue TAC ≥12 weeks with ALT ≤ 50 U/L and a liver biopsy 12 - 26 weeks after the last TAC dose that meets the biopsy criteria for IS minimization (Table 9) as assessed by central pathology	Proportion of participants who successfully discontinue TAC for ≥12 weeks (regardless of other IS), have ALT ≤50 U/L, and liver biopsy 12 - 26 weeks after the last TAC dose that meets the biopsy criteria for IS minimization (Table 9), with a two-sided, 95% exact binomial confidence interval	ITT, PP
Time from achievement of the primary endpoint to IS reinitiation or to the end of trial participation	The number of days from the week 52 primary endpoint biopsy to the earliest of either IS reinitiation or end of trial participation (only calculated for those deemed operationally tolerant for the primary endpoint)	ITT, PP

9.2.4 Safety Analysis

All analyses will be repeated using the SS as labeled in Table 14. All AEs will be classified by body system and preferred term according to the MedDRA dictionary. Frequency tables by category of event (e.g. serious, related) and by NCI-CTCAE v.5.0 grade will be presented. Selected laboratory values will be summarized and displayed graphically.

9.2.5 Exploratory Analysis

Participants who are in the ES but are not in the SS will be utilized to explore relationships between their baseline characteristics and events of interest (section 6.3.1) that differentiate them from participants who go on to the SS.

9.2.6 Medical History

Medical history within the past 12 months—including the existence of current signs and symptoms—will be collected for each body system.

9.2.7 Use of Medications

All medications defined in section 55 taken by or administered to liver transplant recipients starting from transplant and continuing throughout their study participation will be collected. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

9.3 SAMPLE SIZE

This pilot study is designed to evaluate 9 participants. Therefore, no formal power and sample size analyses have been performed. However, with 9 evaluable participants receiving at least the target arTreg dose (section 5.2.1.4), incidence rates of operational tolerance and corresponding two-sided, 95% exact binomial confidence interval are shown in Table 15.

Table 15. Incidence Rates and Confidence Intervals for 9 Participants Attempting Complete IS Withdrawal following an Infusion of Treg Product (arTreg)

Number of Participants able to Achieve Operational Tolerance	Incidence Rate (%)	95% Confidence Interval (%)
0	0	(0, 33.63)
1	11.1	(0.28, 48.25)
2	22.2	(2.81, 60.01)

Number of Participants able to Achieve Operational Tolerance	Incidence Rate (%)	95% Confidence Interval (%)
3	33.3	(7.49, 70.07)
4	44.4	(13.70, 78.80)
5	55.6	(21.20, 86.30)
6	66.7	(29.93, 92.51)
7	77.8	(39.99, 97.19)
8	88.9	(51.75, 99.72)
9	100	(66.37, 100)

9.4 MISSING DATA

Steps will be taken to avoid missing data. For all secondary analyses, no imputations for missing data will be performed.

9.5 POOLED ANALYSIS WITH OTHER LITTMUS TRIALS

LITTMUS protocols were developed collectively for the explicit purpose of harmonizing study design, data collection, and analysis endpoints.

The analyses will be repeated as specified above with all available data from other LITTMUS trials.

9.6 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the SAP. Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the DSMB, the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants 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 ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11. QUALITY CONTROL AND QUALITY ASSURANCE

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

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

The eCRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Some data requirements will be addressed outside the EDC using SAS® software. Data queries will be issued and resolved within the EDC system or SAS®.

Study staff at the site will enter information into the eCRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. 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 changes in the database to be monitored and maintained in accordance with federal regulations.

Study staff will enter data from a study visit on the relevant eCRFs within 3 days following the visit or the time when data become available.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved by the Sponsor, the IRB and submitted to FDA 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 participating in the study, taking the study drug, and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or 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 study investigator, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, 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. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

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

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Appendix 1A. Study Stage 1, 2, and 3A: Schedule of Events – Recipient Enrollment and EVR Conversion

	-	Study Stage 1	Study Stage 2		Study Stage 3A
	Eligibility 1: Pre-Transplant	**Transplant**	**Eligibility 2: EVR Conversion**	**EVR Conversion Initiation**	**Eligibility 3A: Recipient Leukapheresis for arTreg Manufacturing**
Window	-26 Wks	-	D30 - Wk48	Within 7 days of V101[R]	D60 - Wk52
Visit Number	**-1**[1]	**0**[2]	**101/101R**[2,3,4]	**101S**	**102/102R**[5,6]
General Assessments[7]					
Informed consent	X[8]		X		
Eligibility	X		X		X
Study parameters for continuation		X			
Demographics	X				
Medical History	X				
Complete physical exam			X[9]		
Limited physical exam					X
Vital signs			X		X
Adverse Events			X[10]		X
Concomitant medications		X	X		X
Events of interest		X[11]			
HCV treatment history, if applicable			X		
Milan criteria, with no vascular invasion, and with no cholangiocarcinoma morphology, if applicable		X			
Clinical Assessments and Procedures					
Doppler or angiography			X		
Liver transplant		X[12]			

[1] The local laboratory and mechanistic assessments do not need to be repeated if Visit -1 becomes out of window of Visit 0, as participants will undergo additional eligibility steps prior to initiating any study therapy (Study Definitions).

[2] Participants who fail to initiate study therapy (section 4.5) will be terminated from further study participation.

[3] Participants experiencing an AR episode may be re-evaluated for *Eligibility 2: EVR Conversion* at least 4 weeks following resolution of AR (section 5.3.2.3) (Visit 101R). If Visit 101R occurs within 8 weeks of Visit 101, do not collect repeat mechanistic assessments.

[4] Laboratory assessments needed to verify eligibility for EVR conversion must occur within 30 days after Visit 101/101R. EVR conversion must be initiated within 7 days after these laboratory assessments.

[5] Participants ineligible due to any of the *Eligibility 3A: Recipient Leukapheresis for arTreg Product Manufacturing* criteria may be re-evaluated up to 52 weeks post liver transplant (Visit 102R). If Visit 102R occurs within 8 weeks of Visit 102, do not collect repeat mechanistic assessments.

[6] For participants who either failed EVR conversion or successfully completed EVR conversion but did not meet *Eligibility 3A: Recipient Leukapheresis for arTreg Product Manufacturing*, complete the Reduced Follow-up Visit (RFU) in Appendix 2.

[7] Reference section 6.3.1 for specific assessment details.

[8] Consent will be obtained prior to notification of organ offer by transplant center to the participant and separately from obtaining consent for transplant.

[9] Includes height.

[10] Adverse events will be collected once informed consent is obtained during Study Stage 2: EVR conversion.

[11] Collected from time of transplant until *Eligibility 2: EVR Conversion* (Visit 101).

[12] See section 5.2.4.1 for prophylactic medications per the center's standard of care post-transplant.

	-	Study Stage 1	Study Stage 2		Study Stage 3A
	Eligibility 1: Pre-Transplant	**Transplant**	**Eligibility 2: EVR Conversion**	**EVR Conversion Initiation**	**Eligibility 3A: Recipient Leukapheresis for arTreg Manufacturing**
Window	-26 Wks	-	D30 - Wk48	Within 7 days of V101[R]	D60 - Wk52
Visit Number	**-1**[1]	**0**[2]	**101/101R**[2,3,4]	**101S**	**102/102R**[5,6]
Liver biopsy		X[13]			
Local Laboratory Assessments[14]					
HLA typing[15]	X				
Urine or blood hCG					X
Hematology	X[16]		X		X
Basic chemistry	X[16]		X		X
Liver function tests				X	
CD4 count					X
Urinalysis			X		X
Estimated GFR			X		X
HIV-1/2	X				
2019-nCoV RT-PCR			X		X
HCV RNA		X			X
HBV DNA					X
EBV Antibody	X[17]				
EBV DNA					X
CMV Antibody	X[17]				
CMV DNA					X
AFP[18]		X			
TAC trough levels			X		X
EVR trough levels					X

[13] Liver allograft biopsy should be obtained on the pre-implant liver prior to transplantation into the recipient. Local pathology assessment must be obtained.
[14] Reference section 6.5.1 for specific assessment details.
[15] Retrospective data collection from the medical record. Repeat testing not required if already completed as standard of care.
[16] Must be performed within 24 hours of the mechanistic blood collections (PBMC, serum, and whole blood collections) and can occur any time after consent until transplant. If the PBMC collection occurs on two separate dates, complete this assessment on each collection date.
[17] The assessment does not need to be repeated if the participant has a documented positive result.
[18] Last assessment within 3 months prior to transplant for participants with HCC only.

	-	Study Stage 1	Study Stage 2		Study Stage 3A
	Eligibility 1: Pre-Transplant	**Transplant**	**Eligibility 2: EVR Conversion**	**EVR Conversion Initiation**	**Eligibility 3A: Recipient Leukapheresis for arTreg Manufacturing**
Window	-26 Wks	-	D30 - Wk48	Within 7 days of V101[R]	D60 - Wk52
Visit Number	**-1**[1]	**0**[2]	**101/101R**[2,3,4]	**101S**	**102/102R**[5,6]
Study Medications					
Mycophenolate Compound (MMF or equivalent)[19]		SOC		X[20]	
Prednisone[21]		SOC		X (≤10 mg daily)	X
Tacrolimus		SOC		X (trough 6-10 ng/mL)	X (target trough 3-5 ng/mL)
Everolimus				X	X (target trough 5-8 ng/mL)
Mechanistic Laboratory Assessments					
HLA Typing[22]	X[23]				
PBMC collection (for UCSF lab)					X
PBMC collection	X[23,24]		X		
Serum collection	X[23]		X		
Whole blood collection	X[23]		X		
Liver biopsy collection		X			

[19] If MMF, Myfortic, or azathioprine is not part of the participant's IS regimen, the participant may continue with EVR conversion (Table 8).

[20] Discontinued approximately 7-14 days after starting EVR (Table 8).

[21] Prednisone may be discontinued at any time at the discretion of the investigator (section 5.2.3.3).

[22] Involves a buccal swab collection, however, blood/PBMCs will be used if needed.

[23] Mechanistic assessments can be collected any time after informed consent on Visit -1 but prior to the administration of any IS or blood products at the time of the transplant.

[24] Specimen will be collected using two mechanistic specimen collections kits. Both kits can be collected on the same day or on different dates.

Appendix 1B. Schedule of Events – Donor Enrollment and Participation

	Living Donor		Deceased Donor
	Eligibility: Living Donor		Eligibility: Deceased Donor
Window	-26 Wks	D0 - Wk26	±7 days
Visit Number	**LD-1**	**LD0**	**DD0[1]**
General Assessments			
Informed consent	X		
Eligibility	X	X[2]	X
Demographics	X		X
ABO Typing (from medical record)	X		X
Adverse Events		X[3]	
Local Laboratory Assessments[4]			
HLA typing[5]	X		X
CMV Antibody	X[6]		X
EBV Antibody	X[6]		X
HCV testing			X
arTreg Manufacture			
Blood collection for infectious diseases donor testing		X[2]	X
Blood collection by phlebotomy for manufacture of stimulated irradiated donor B cells (KT64.CD40L stimulation)		X[7]	
Spleen and/or blood collection for manufacture of stimulated irradiated donor B cells (KT64.CD40L stimulation)			X
Mechanistic Laboratory Assessments[8]			
HLA Typing[9]		X[10]	X[11]
PBMC collection		X[10]	
Blood and/or spleen collection			X

[1] For all assessments other than peripheral blood and/or spleen collection for arTreg and mechanistic collections, data to be collected from medical and/or UNOS records. If tests are not available, they should be ordered.

[2] Living donor must meet donor eligibility manufacturing requirements within 7 days prior to the blood collection for manufacturing.

[3] Adverse events ≥ grade 3 that occur within 48 hours of research blood draw for arTreg manufacturing will be collected for the study unless the blood for manufacturing is collected within 24 hours of the donor hepatectomy.

[4] For specific assessment details, reference section 6.5.2 for living donors and section 6.5.3 for deceased donors.

[5] Retrospective data collection from the medical record. Repeat testing not required if already completed as standard of care.

[6] The assessment does not need to be repeated if the living donor has a documented positive result.

[7] Must occur at least 14 days prior to the Recipient's Leukapheresis Visit (Visit T-17/T-17R) in Appendix 2 (section 5.3.3.1).

[8] For deceased donors, mechanistic collections will occur if material is available.

[9] Involves a buccal swab collection, however, blood/PBMCs will be used if needed.

[10] May be collected prior to transplant if the donor has consented and is eligible, the donor's recipient is found eligible per *Eligibility 1: Pre-Transplant,* and the transplant date has been confirmed.

[11] Can be performed on any appropriate sample type collected for mechanistic purposes.

Appendix 2. Study Stage 3 (A, B, C): Schedule of Events – Investigational Treatment

	Study Stage 3A	Study Stage 3B	Study Stage 3C						-
	Recipient Leukapheresis	Eligibility 3B: pre-arTreg Conditioning (CTX)	Eligibility 3C: arTreg Infusion	Eligibility 4: TAC Withdrawal	-				Reduced Safety Follow-up
				Post arTreg Infusion Visits[1]					
arTreg Infusion Day	-17	-3	0	1	7	14	28	84	
Window	≤28 days of V102[R]	≤72 hours & ≥24 hours before arTreg infusion (VT0)	-	+24 hours	-2/+8 days	-2/+3 days	±5 days	±5 days	≤30 days after unsuccessful EVR conversion, VT-17[R], or VT-3
Visit Number	**T-17/T-17R[2,3]**	**T-3[3,4]**	**T0**	**T1**	**T7[5]**	**T14[5,6]**	**T28[5,6]**	**T84[5, 6]**	**RFU**
General Assessments[7]									
Eligibility		X	X	X[8]					
Complete physical exam			X						
Limited physical exam		X		X	X				X
Vital signs		X	X[9]	X	X				X
Adverse Events	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
Remote consultation						X	X	X	
Clinical Assessments and Procedures									
Liver biopsy					X[10]				
Local Laboratory Assessments[11]									
Hematology	X	X	X	X	X	X	X	X	X
Basic chemistry	X	X	X	X	X				X
CD4 count	X		X	X	X	X	X	X	
Estimated GFR				X					

[1] For participants who receive any arTregs but are ineligible for or do not attempt TAC withdrawal, move to Appendix 6 (section 4.4) while completing these visits concurrently.

[2] If the leukapheresis product does not contain a sufficient number of cells for Treg manufacturing, a second leukapheresis can be performed (Visit T-17R) provided the participant continues to meet *Eligibility 3A: Recipient Leukapheresis for arTreg Product Manufacturing* (section 5.3.3.2). Visit 102R must be completed if more than 28 days have elapsed since the prior Visit 102 assessments. Visit 102R to confirm eligibility for a second leukapheresis must be established by week 26 post liver transplant.

[3] For participants who do not receive any CTX or mesna (VT-3), complete the Reduced Follow-up Visit (RFU) (section 4.4).

[4] For participants who receive any CTX or mesna but do not receive any arTregs (VT0), move to Appendix 7 (section 4.4).

[5] This visit may occur concurrently with Appendix 3 for participants who initiate TAC withdrawal (V201) or Appendix 6 for participants who fail ISW (section 4.4).

[6] Participants who are unable or unwilling to travel to the study site will have the option of completing this study visit remotely.

[7] Reference section 6.3.1 for specific assessment details.

[8] For participants who receive the minimum or target arTreg dose, confirm *Eligibility 4: TAC Withdrawal* (section 5.3.4). For participants who meet eligibility, initiate TAC withdrawal (V201) in Appendix 3 within 14 days of eligibility determination. For participants who do not meet eligibility, complete visits in Appendix 6 (section 4.4). Complete remaining post arTreg Infusion Visits (T7-T84) concurrently with either Appendix 3 or Appendix 6 visits.

[9] Vital signs will be taken prior to, 5 minutes into, and at the end of the infusion, as well as 30 minutes, 1 hour, and 2 hours after the infusion (section 5.2.1.4).

[10] Local pathology assessment must be obtained to evaluate participants either for *Eligibility 4: TAC Withdrawal* or to continue TAC withdrawal (section 5.3.4). Assessment to be done 5-15 days after receiving the arTreg.

[11] Reference section 6.5.1 for specific assessment details.

	Study Stage 3A	Study Stage 3B	Study Stage 3C						-
	Recipient Leukapheresis	Eligibility 3B: pre-arTreg Conditioning (CTX)	Eligibility 3C: arTreg Infusion	Eligibility 4: TAC Withdrawal	-				Reduced Safety Follow-up
				Post arTreg Infusion Visits[1]					
arTreg Infusion Day	-17	-3	0	1	7	14	28	84	
Window	≤28 days of V102[R]	≤72 hours & ≥24 hours before arTreg infusion (VT0)	-	+24 hours	-2/+8 days	-2/+3 days	±5 days	±5 days	≤30 days after unsuccessful EVR conversion, VT-17[R], or VT-3
Visit Number	**T-17/T-17R[2,3]**	**T-3[3,4]**	**T0**	**T1**	**T7[5]**	**T14[5,6]**	**T28[5,6]**	**T84[5, 6]**	**RFU**
TAC trough levels		X	X	X	X[12]				X[13]
EVR trough levels		X	X	X	X				X[13]
arTreg Manufacture									
Leukapheresis	X[14,15]								
Study Medications									
Prednisone		X[16,17]				X[18]			SOC
Tacrolimus		X (target trough 3-5 ng/mL)[17]				X[18]			SOC
Everolimus		X (target trough 5-8 ng/mL)							SOC
Mesna[19]		X							
Cyclophosphamide[20]		X[21]							
Famciclovir		X[22]							
Bacterial Prophylaxis		X[23]							
G-CSF		X[24]							
arTreg Infusion[25]			X[21]						
Mechanistic Laboratory Assessments									
arTreg archiving			X[26]						
PBMC collection (for UCSF lab)	X		X[27]	X	X	X	X	X	

[12] Complete only if the participant hasn't initiated TAC withdrawal.
[13] Complete if the participant is receiving the IS medication that is relevant for the trough level.
[14] Performed exactly 16-17 days prior to arTreg infusion. See section 5.3.3.2.4 for leukapheresis details.
[15] PBMCs will be collected for mechanistic purposes from any leftover material.
[16] Prednisone may be discontinued at any time at the discretion of the investigator (section 5.2.3.3).
[17] For participants who have initiated TAC withdrawal (following Visit T1/T7), please see Appendix 3 for dosing instructions.
[18] See Appendix 3 for dosing instructions for participants who are undergoing TAC withdrawal, or Appendix 6 for participants who did not attempt TAC withdrawal or failed ISW.
[19] See section 5.2.2.2 for mesna dosing and administration details.
[20] Administered approximately 13-16 days after leukapheresis (VT-17/T-17R) (section 5.3.3.3.4). See section 5.2.2.1 for CTX dosing and administration details.5.2.2
[21] See section 5.2.4.1 for prophylactic medications per the center's standard of care following CTX and arTreg infusion.
[22] If not already receiving viral prophylaxis, five days following CTX administration, participants will receive a 7-day course of famciclovir (Famvir®) 500 mg PO daily for prophylaxis against herpes simplex virus (HSV) (section 5.2.4.1.1).
[23] Five days following CTX administration, participants will receive prophylaxis against bacterial infections in the setting of neutropenia per institutional practice (section 5.2.4.1.3).
[24] After cyclophosphamide per section 5.2.4.1.4.
[25] See section 5.2.1.4 for arTreg dosing and administration details.
[26] May be collected <1 day prior to arTreg infusion.
[27] Mechanistic blood samples to be collected prior (-24 hours) to arTreg infusion.

	Study Stage 3A	Study Stage 3B	Study Stage 3C						-
	Recipient Leukapheresis	Eligibility 3B: pre-arTreg Conditioning (CTX)	Eligibility 3C: arTreg Infusion	Eligibility 4: TAC Withdrawal	-				Reduced Safety Follow-up
				Post arTreg Infusion Visits[1]					
arTreg Infusion Day	-17	-3	0	1	7	14	28	84	
Window	≤28 days of V102[R]	≤72 hours & ≥24 hours before arTreg infusion (VT0)	-	+24 hours	-2/+8 days	-2/+3 days	±5 days	±5 days	≤30 days after unsuccessful EVR conversion, VT-17[R], or VT-3
Visit Number	**T-17/T-17R[2,3]**	**T-3[3,4]**	**T0**	**T1**	**T7[5]**	**T14[5,6]**	**T28[5,6]**	**T84[5, 6]**	**RFU**
PBMC collection	X[28]								X[29]
Serum collection	X		X[27]	X					X[29]
Whole blood collection	X		X[27]	X					X[29]
Liver biopsy collection					X				

[28] Collected from any leftover material from the leukapheresis bag used for arTreg manufacturing.

[29] Only for participants who successfully completed EVR conversion but do not receive any CTX, and/or mesna. Participants may have undergone leukapheresis.

Appendix 3. Study Stage 4: Schedule of Events – TAC Withdrawal

		Study Stage 4					
		TAC Taper Level			TAC Withdrawal Pause Visits[1]		Post TAC Withdrawal
		1	2	Off			
After Initiating TAC Withdrawal Week		0	6 *(up to 10[2])*	12 *(up to 20[2])*	During Taper Level 1	During Taper Level 2	24
Window		≤14 days after TAC Withdrawal Eligibility Confirmation (Visit T1/T7)	+7 days		-		±7 days
Visit Number[3]		**201**	**202**	**203[4]**	**201P**	**202P**	**204[5]**
General Assessments[6]							
Limited physical exam							
Vital signs							
Adverse Events		X	X	X	X	X	X
Concomitant medications		X	X	X	X	X	X
Telemedicine consultation							X
Remote consultation		X	X	X	X	X	
Local Laboratory Assessments[7]							
Hematology				X			X
Basic chemistry				X			X
LFTs *(±5 day window)*		Every 2 Weeks[8]			Every 2 Weeks[8]		X[9]
Estimated GFR							
TAC trough levels							
EVR trough levels				X			X
EVR trough levels *(±5 day window)*		Every 2 Weeks[8]			Every 2 Weeks[8]		X[9]
Study Medications							
Prednisone		tapered to 5 mg/day[10,11]			tapered to 5 mg/day[10,11]		X[10]
Tacrolimus		tapered per section 5.3.4.4			tapered per section 5.3.4.4		
Everolimus		X (target trough 5-8 ng/mL)			X (target trough 5-8 ng/mL)		X[12]

[1] Only complete additional visits as needed if TAC withdrawal is paused (section 5.4.1).
[2] In the event TAC withdrawal is paused (section 5.4.1).
[3] For participants who receive the minimum or target arTreg dose but either do not attempt TAC withdrawal (V201) or fail ISW, move to Appendix 6 (section 4.4).
[4] Complete 12 weeks after initiating TAC withdrawal.
[5] Complete 24 weeks after initiating TAC withdrawal unless the participant has moved to Appendix 4.
[6] Reference section 6.3.1 for specific assessment details.
[7] Reference section 6.5.1 for specific assessment details.
[8] Until V204 or until the participant is evaluated for *Eligibility 5: EVR/Pred Withdrawal* (V300 in Appendix 4), whichever is sooner.
[9] Complete every 2 weeks until the participant has been off TAC for 12 weeks. Once the participant has been off TAC for 12 weeks, complete every 4 weeks until the participant is evaluated for *Eligibility 5: EVR/Pred Withdrawal* (V300 in Appendix 4).
[10] Prednisone may be discontinued at any time at the discretion of the investigator (section 5.2.3.3).
[11] For participants receiving > 5 mg/day of prednisone at the time of TAC withdrawal, prednisone will be tapered as described in section 5.3.4.4.
[12] Target trough 5-8 ng/mL.

Appendix 4. Study Stage 5: Schedule of Events – EVR/Prednisone Withdrawal

	Study Stage 5								
	Eligibility 5: EVR/Pred Withdrawal	EVR/Pred Taper Level				EVR Taper Level			EVR/Pred Withdrawal Pause Visits[1]
		1	2	3	4	5	6	7	
After Initiating EVR/Pred Withdrawal Week[2]	-	0	4	8	12	16	22	28	Up to 3 4-week Pauses[3]
Window	Wk 12-26 Post TAC Withdrawal	≤8 Wks after V300 Biopsy	+7 days						-
Visit Number[4]	**300[5]**	**301**	**302**	**303**	**304**	**305**	**306**	**307[6]**	**301P-307P**
General Assessments[7]									
Informed consent	X								
Eligibility	X								
Limited physical exam	X								
Vital signs	X								
Adverse Events	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
Telemedicine consultation						X			
Remote consultation		X	X	X	X		X	X	X
Clinical Assessments and Procedures									
Liver biopsy	X								
Local Laboratory Assessments[8]									
Hematology	X					X			
Basic chemistry	X					X			
LFTs *(±5 day window)*	Every 4 Weeks	Every 2 Weeks							Every 2 Weeks
Estimated GFR	X								
EVR trough levels	X								
Study Medications									
Prednisone[9]	X	tapered per section 5.3.5.4							
Everolimus	X	tapered per section 5.3.5.4							X[10]
Mechanistic Laboratory Assessments									
PBMC collection	X								
Serum collection	X								

[1] Only complete additional visits as needed if EVR/Pred withdrawal was paused (section 5.4.1). Complete visit number that corresponds to the taper level that is paused, i.e. taper level 4 is paused, complete Visit 304P.

[2] Weeks reflect time after initiating EVR/Pred Withdrawal in the absence of any pauses (section 5.4.1).

[3] Three non-consecutive pauses (up to 4 weeks each) during IS (TAC [Appendix 3] and EVR/Pred) withdrawal (section 5.4.1).

[4] For participants who fail ISW or do not attempt EVR/Pred withdrawal, move to Appendix 6.

[5] Complete for all participants who successfully complete TAC withdrawal. For participants who received the minimum arTreg dose (Study Definitions), withdrawal will be at the discretion of the investigator (section 5.3.5.1). If the participant remains off TAC but does not initiate EVR/Pred withdrawal, move to Appendix 6.

[6] For participants who successfully complete ISW, move to Appendix 5.

[7] Reference section 6.3.1 for specific assessment details.

[8] Reference section 6.5.1 for specific assessment details.

[9] Prednisone may be discontinued at any time at the discretion of the investigator (section 5.2.3.3). For participants receiving 5 mg/day of prednisone at the time of EVR withdrawal, prednisone will be withdrawn as described in section 5.3.5.4.

[10] Tapered per section 5.3.5.4.

	Study Stage 5								
	Eligibility 5: EVR/Pred Withdrawal	EVR/Pred Taper Level				EVR Taper Level			EVR/Pred Withdrawal Pause Visits[1]
		1	2	3	4	5	6	7	
After Initiating EVR/Pred Withdrawal Week[2]	-	0	4	8	12	16	22	28	Up to 3 4-week Pauses[3]
Window	Wk 12-26 Post TAC Withdrawal	≤8 Wks after V300 Biopsy	+7 days						-
Visit Number[4]	**300[5]**	**301**	**302**	**303**	**304**	**305**	**306**	**307[6]**	**301P-307P**
Whole blood collection	X								
Liver biopsy collection	X								

Appendix 5. Study Stage 6: Schedule of Events – Post-withdrawal Surveillance and Evaluation

For participants who successfully complete ISW.

	Study Stage 6				
Follow-Up Week	**0**	**26**	**52**	**104**	**105-116**
Window	≤7 days after last IS dose	±2 wks	±4 wks[1]	±4 wks	±2 wks
Visit Number	**400**	**401**	**402**	**403**	**404[2]**
General Assessments[3]					
Limited physical exam	X	X	X	X	
Vital signs	X	X	X	X	
Adverse Events	X	X	X	X	X
Concomitant medications	X	X	X	X	X
Remote consultation					X
Clinical Assessments and Procedures					
Liver biopsy			X		
Local Laboratory Assessments[4]					
Hematology	X	X	X	X	X
Basic chemistry	X	X	X[5]	X	X
LFTs *(±5 day window)*[6]	Every 2 Weeks[7]	Every 4 Weeks		Every 8 Weeks	Per standard of care[8]
Mechanistic Laboratory Assessments					
PBMC collection	X	X	X	X	
Serum collection	X	X	X	X	
Whole blood collection	X	X	X	X	
Liver biopsy collection			X		

[1] If the investigator determines that abnormal laboratory results prior to the Visit 402 liver biopsy are related to an intercurrent illness, the investigator may request that the participant's data be reviewed by an adjudication committee, who may grant an extension of the primary endpoint visit window (3.3.1.2)

[2] Complete for participants who resume IS at the end of study follow-up to ensure they are followed for at least 12 weeks after restarting IS (section 5.4.2). Complete V404 12 weeks after restarting IS.

[3] Reference section 6.3.1 for specific assessment details.

[4] Reference section 6.5.1 for specific assessment details.

[5] Must be done within the two weeks prior to the biopsy.

[6] For participants who experience rejection (section 5.4.3.2), record results of all assessments collected from standard of care into the clinical database.

[7] Continue LFT schedule every 2 weeks from EVR Taper Level 7 Visit in Appendix 4. Once the participant has been off all IS for 14 weeks, complete LFTs every 4 weeks until the participant's next visit.

[8] Record results of all assessments collected from standard of care into the clinical database.

Appendix 6. Schedule of Events – High Intensity Safety Follow-up

For participants who receive any arTreg but either do not attempt or fail ISW.

Follow-Up Week	0	26	52	104
Window	≤4 wks after VT0, V300, or ISW Failure[1]	±2 wks	±4 wks	±8 wks
Visit Number	500	501	502	503
General Assessments[2]				
Limited physical exam	X	X	X	X
Vital signs	X	X	X	X
Adverse Events	X	X	X	X
Concomitant medications	X	X	X	X
Local Laboratory Assessments[3]				
Hematology	X	X	X	X
Basic chemistry	X	X	X	X
LFTs *(±5 day window)*[4]	Every 8 Weeks			
TAC trough levels[5]	X[6]	X[6]	X[6]	X[6]
TAC trough levels *(±5 day window)*[5]	Every 8 Weeks			
EVR trough levels[5]	X[6]	X[6]	X[6]	X[6]
EVR trough levels *(±5 day window)*[5]	Every 8 Weeks			
Mechanistic Laboratory Assessments				
PBMC collection	X[7]	X	X	
Serum collection	X[7]	X	X	
Whole blood collection	X[7]	X	X	

[1] Visit T0 for participants who don't receive at least the minimum arTreg dose (Study Definitions) or attempt TAC withdrawal, Visit 300 for participants who do not attempt EVR/Pred withdrawal, or ISW failure.
[2] Reference section 6.3.1 for specific assessment details.
[3] Reference section 6.5.1 for specific assessment details.
[4] Record results of all assessments collected from standard of care into the clinical database.
[5] If the participant is receiving the IS medication that is relevant for the trough level, record results of all assessments collected from standard of care into the clinical database.
[6] Complete if the participant is receiving the IS medication that is relevant for the trough level.
[7] If Visit 500 occurs within 8 weeks of an Appendix 2, Appendix 3 or Appendix 4 visit that included collection of this mechanistic assessment, do not repeat this mechanistic assessment during V500.

Appendix 7. Schedule of Events – Low Intensity Safety Follow-up

For participants who receive any CTX and/or mesna but do not receive any arTreg

Follow-Up Week	0	4	52
Window	7-10 days after VT-3	±5 days	±8 wks
Visit Number	600	601	602
General Assessments [1]			
Limited physical exam	X		X
Vital signs	X		X
Adverse Events	X	X	X
Concomitant medications	X	X	X
Remote consultation		X	
Local Laboratory Assessments[2]			
Hematology	X	X[3]	X
Basic chemistry	X	X	X

[1] Reference section 6.3.1 for specific assessment details.
[2] Reference section 6.5.1 for specific assessment details.
[3] Complete assessment every 4 weeks until absolute neutrophil count ≥ 1,500/µL.

Appendix 8. Schedule of Events – For-Cause Biopsy

Visit Number	FCB[1,2,3]
General Assessments[4]	
Limited physical exam	X
Vital signs	X
Adverse Events	X
Concomitant medications	X
Clinical Assessments and Procedures	
Liver biopsy	X
Local Laboratory Assessments[5]	
Hematology	X
Basic chemistry	X
Mechanistic Laboratory Assessments	
PBMC collection	X
Serum collection	X
Whole blood collection	X
Liver biopsy collection	X

[1] If a participant needs a for-cause biopsy within 8 weeks prior to an Appendix 1A-Appendix 4 visit, the mechanistic assessments collected during the FCB visit will take the place of any mechanistic assessments to be collected during that upcoming Appendix 1A-Appendix 4 visit.

[2] If a participant needs a for-cause biopsy within 8 weeks prior to an Appendix 5-Appendix 7 visit, the FCB visit will take the place of that upcoming Appendix 5-Appendix 7 visit.

[3] If a participant is treated for clinical rejection at the study site without a biopsy (section 5.4.3.2.3), complete all other visit assessments done at a for-cause biopsy visit prior to initiating treatment.

[4] Reference section 6.3.1 for specific assessment details.

[5] Reference section 6.5.1 for specific assessment details.