Official Title:

Phase III Multicenter Open-label Randomized Clinical Trial Comparing Everolimus and Low Dose Tacrolimus to Tacrolimus and Mycophenolate Mofetil at 6 mo Post-Transplant to Prevent Long-term Complications After Pediatric Heart Transplantation

Brief Title:

Tacrolimus/Everolimus vs. Tacrolimus/MMF in Pediatric Heart Transplant Recipients Using the MATE Score (TEAMMATE)

NCT Number:

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CLINICAL RESEARCH PROTOCOL

PHASE III MULTI-CENTER OPEN LABEL RANDOMIZED CLINICAL TRIAL COMPARING EVEROLIMUS AND LOW DOSE TACROLIMUS TO TACROLIMUS AND MYCOPHENOLATE MOFETIL AT 6 MONTHS POST HEART TRANSPLANT TO PREVENT LONG-TERM COMPLICATIONS AFTER PEDIATRIC HEART TRANSPLANTATION

Clinical Co-PI, Study Co-Chair & Regulatory PI
Christopher S.D. Almond, MD, MPH
Stanford University (IND Holder)
Department of Pediatrics - Cardiology
Lucile Packard Children's Hospital
750 Welch Road, Suite 325
Palo Alto, California 94304

Data Coordinating Center and Statistical PI
Lynn A. Sleeper, ScD
Department of Cardiology
Boston Children's Hospital
300 Longwood Avenue
Boston, MA 02115

Clinical Co-PI, Study Co-Chair
Kevin P. Daly, MD
Department of Cardiology
Boston Children's Hospital
300 Longwood Avenue
Boston, MA 02115

Program Officer:
Rahul Thakar, PhD
Senior Science Officer, Congressionally Directed Medical Research Programs
1077 Patchell Street, Bldg 1054
Fort Detrick, MD 21702-5024

Central Institutional Review Board
Boston Children's Hospital
300 Longwood Avenue
Boston, MA 02116

Funding Agency
United States of America Department of Defense, U.S. Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014

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List of Abbreviations

AE	Adverse event
AMR	Antibody-mediated rejection
ATG	Anti-thymocyte globulin
BMP	Basic metabolic panel
BP-ACR	Biopsy-proven acute cellular rejection
BSA	Body surface area
C_0	Concentration at time 0 (immediately prior to next dose)
CAV	Cardiac allograft vasculopathy
CBC	Complete blood count
CDER	Center for Drug Evaluation Research
CK	Creatine kinase
CKD	Chronic kidney disease
CMV	Cytomegalovirus
CRA	Clinical research associate
CRF	Case report form
DCC	Data coordinating center
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
eCRF	Electronic case report form
EMB	Endomyocardial biopsy
EQ-5D-Y	European Quality of Life Scale, 5 dimensions, youth version
EVL	Everolimus
FDA	Food and Drug Administration
GFR	Glomerular filtration rate
HDL	High-density lipoprotein
HSV	Herpes simplex virus
HT	Heart transplantation
ICF	Informed consent form
IRB	Institutional Review Board
ISHLT	International Society of Heart and Lung Transplantation
ITT	Intention-to-Treat
LAR	Legally appointed representative
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LDTAC	Low-dose tacrolimus
LFT	Liver function tests
LVEF	Left ventricular ejection fraction
MATE	Major Adverse Transplant Event
MG	Milligram
mL	Milliliter
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
mTOR	Mammalian target of rapamycin

NG	Nanogram
OOPD	Office of Orphan Product Development
pAMR	Pathologic diagnosis of cardiac antibody-mediated rejection
PCR	Polymerase chain reaction
PCWP	Pulmonary capillary wedge pressure
PG	Per gastrum (by stomach)
PHTS	Pediatric Heart Transplant Study
PI	Principal investigator
PJP	*Pneumocystis jirovecii*
PO	Per orem (by mouth)
PTLD	Post-transplant lymphoproliferative disease
REB	Research Ethics Board/Committee
SAE	Serious adverse event
SAP	Statistical analysis plan
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAC	Tacrolimus
TG	Trigylcerides
UNOS	United Network for Organ Sharing
UP	Unanticipated problem
US	United States of America

Study Summary

FDA Title	Phase III Multi-Center Open Label Randomized Clinical Trial Comparing Everolimus and Low Dose Tacrolimus to Tacrolimus and Mycophenolate Mofetil Initiated at 6 Months Post Heart Transplant To Prevent Long-term Complications After Pediatric Heart Transplantation
Short Title	Multicenter Randomized Trial of Everolimus in Pediatric Heart Transplantation
Alternative Title	**T**acrolimus/**E**verolimus **A**gainst Tacrolimus/**M**ycophenolate mofetil in Pediatric Heart Transplant Recipients using the **M**ajor **A**dverse **T**ransplant **E**vents Score (**TEAMMATE**)
Protocol Number	TEAMMATE-009
Phase	Phase III
Methodology	Multi-Center Open Label Randomized Clinical Trial
Study Duration	5 years
Study Center(s)	25 sites
Objectives	*Efficacy:* To test the hypothesis that among children who survive to 6 months post-heart transplantation (HT), those randomized to everolimus and low-dose tacrolimus (EVL/LDTAC) for 30 months will have a lower total burden of three major adverse transplant events: cardiac allograft vasculopathy (CAV), chronic kidney disease (CKD), and biopsy-proven acute cellular rejection (BP-ACR), as defined by the MATE-3 score, compared to children randomized to standard therapy with tacrolimus and mycophenolate mofetil (TAC/MMF). *Safety:* To test the hypothesis that among children who survive to 6 months post-HT, those randomized EVL/LDTAC for 30 months will not have a higher risk of graft loss due to all causes as defined by the MATE-6 score, compared to children randomized to standard therapy with TAC/MMF. *Medication Tolerability:* To determine the tolerability of EVL/LDTAC compared to TAC/MMF for prevention of major post-transplant complications by characterizing the frequency and incidence of AEs that are at least possibly related to everolimus and low-dose tacrolimus or TAC/MMF.
Number of Subjects	210 subjects

Diagnosis and Main Inclusion Criteria	Children who survive 6 months post-HT are trial eligible if all criteria below are met: Orthotopic heart transplantation Age < 21 years at time of transplant Stable immunosuppression at the time of randomization with no contraindication to any of the study or control group immunosuppressants Planned follow-up at a study site through 36 months post-HT Subject or legal adult representative capable of providing informed consent (in general assent will be sought for children ≥12 years)
Study Product, Dose, Route, Regimen	Everolimus (Marketed as Zortress; Everolimus) Initial pediatric dose based on Body Surface Area (BSA) as outlined in the current everolimus package insert: BSA <0.6 m^2 0.25 mg po q12h BSA 0.6-0.9 m^2 0.5 mg po q12h BSA 0.9-1.2 m^2 0.75 mg po q12h BSA 1.2-1.5 m^2 1 mg po q12h BSA >1.5 m^2 1.25 mg po q12h The dose will be adjusted based on trough level drawn immediately prior to dose administration of the next dose to maintain the serum concentration of everolimus between 3 and 8 nanograms per milliliter (ng/mL)
Duration of administration	30 months
Reference therapy	Tacrolimus and Mycophenolate Mofetil
Statistical Methodology	Assumptions include: 90% power, two-sided $\alpha = 0.05$, standard deviation (SD) of MATE-3 = 2.4; 15% crossover from TAC/MMF to EVL/LDTAC; 5% crossover from EVL/LDTAC to TAC/; within-subject correlation R=0.5; 5% loss to follow-up; 2% inflation for interim look. Based on these assumptions, the required sample size is 105 per group (total 210 subjects). The primary efficacy analysis will be conducted according to the intention-to-treat principle and will be a Zero-Truncated Wilcoxon test comparing the MATE-3 scores for EVL/LDTAC vs. TAC/MMF at 30 months post-randomization. The primary safety analysis will be a 95% two-sided CI for the 2.5-year treatment group difference (EVL/LDTAC minus TAC/MMF) in MATE-6 score. If the upper limit of the confidence interval (CI) is within the pre-specified clinically acceptable difference of less than 3 points, then we will conclude that EVL/LDTAC has no higher burden of death/graft loss than does TAC/MMF.

Safety evaluation	An Independent Data and Safety Monitoring Board (DSMB) will review safety data throughout the trial. After the first 10 patients randomized to EVL reach the 180-day mark (Visit 3), a DSMB safety review will be performed. Two interim looks for efficacy are also planned, at 50% and 80% information. All adverse events, including renal function and infection-related adverse events, will be monitored closely throughout the trial.

Summary of Protocol Changes

Version 010

Section	Changes
3.1-3.2, 3.4	Clarified wording regarding calculation of MATE-3 and MATE-6 in the case of death/allograft loss
3.3	Addition of secondary endpoints prior to data analysis
Table 6-1	Clarified specimen types for Ancillary Studies
7.6	Clarified that study site transplant volume was dichotomized as ≤12 transplants per year and > 12 transplants per year.
7.6.3.3	Per recommendation of the DSMB, changed the statistical test to be used for comparison of the primary efficacy endpoint to the Zero-Truncated Wilcoxon test; Clarified the secondary analytic approaches and referred to the SAP for further details.

Version 009

Section	Changes
6.5-6.11 Visits 2-8	Clarified that submission of all creatinine, BUN, tacrolimus, and everolimus levels collected during the post-transplant/study period will be provided to allow for adjudication of the CKD endpoint.
6.11 Visit 8	Clarified that if key MATE diagnostic assessments (creatinine, biopsy, coronary angiography) were not collected at Visit 8, the results of the next clinical assessment should be reported (even if out of window) to maintain trial integrity and allow for MATE score calculation.

Version 008

Section	Changes
Table 6-1: Schedule of Study Visits & 6.12 Visit 8A	Addition of Visit 8A to collect Visit 8 Biobank samples in consented patients who were unable to have samples collected at Visit 8.

Version 007

Section	Changes
Title Page	Clarified that everolimus or Zortress is the study product.
Summary of Protocol Changes	A summary table including changes in this protocol version and prior protocol versions has been added.

5.1 Study Drug: Description; 5.3 Treatment Regimens; 5.5 Preparation and Administration of Study Drug; 5.6 Subject Compliance Monitoring	Clarified that Zortress® or generic forms of everolimus determined by the FDA to be bioequivalent to Zortress® Tablets may be used under this protocol. The use of the brand name Zortress® has been removed from most sections to further clarify that we are not specifically referring to the Zortress® brand.
5.4 Method for Assigning Subjects to Treatment Groups	Clarified that a short grace period will be added to allow patients already in the screening phase at the time of the 210th randomization to complete randomization.
5.9 Packaging	Added a description of the packaging for FDA approved everolimus tablets manufactured by West-Ward Pharmaceuticals Corp.

Version 006

Section	**Changes**
5.1 Study Drug: Description; 5.5 Preparation and Administration; 5.9 Packaging	Everolimus 1 mg tablets may be used under this protocol.
5.3.2 Tacrolimus with mycophenolate mofetil (TAC/MMF) and Figure 6-1: Main Study Diagram	Changed units for mycophenolic acid (MPA) C_0 levels from ng/mL to mcg/mL. MPA levels are measured in mcg/mL and the goal range of 2-4 based on published literature uses mcg/mL.
Figure 6-1: Main Study Diagram	Clarification that non-invasive assessment for rejection at Visit 1/1A can only be performed in infants less than 18 months old.
Table 6-1: Schedule of Study Visits	Urine protein and urine creatinine measurements were duplicated in this table under the headings of 'Chronic Kidney Disease' and 'Full Laboratory Assessment.' The frequency of measurement of urine protein and urine creatinine was clarified.
Table 6-1: Schedule of Study Visits	A footnote has been added which clarifies when urine protein and urine creatinine will be collected at Visits 1, 1A, 3, 6, and 8. The footnote states, "Attempts will be made to obtain a non-invasive urine sample, however urine testing is not required in young children who are unable to provide such a sample."
Table 6-1: Schedule of Study Visits	Clarification that non-invasive assessment for rejection at Visit 1/1A can only be performed in infants less than 18 months old.
6.1 Visit 1	Clarification that non-invasive assessment for rejection at Visit 1/1A can only be performed in infants less than 18 months old.
6.13.2 At randomization	Clarification that non-invasive assessment for rejection at Visit 1/1A can only be performed in infants less than 18 months old.

6.14 Coronary Angiography Protocol	Clarification that invasive coronary angiography at 18 months post-randomization is optional (as previously reflected in Table 6-1) but that if coronary angiography is performed, the center should submit angiograms to the angiography core laboratory for review.
14 Appendix 1: Lansky and Karnofsky Functional Status Scale	Clarification that the Lansky and Karnofsky scales range from 10 to 100 and are only assigned in 10 point increments.

Version 005

Section	**Changes**
4.2 Exclusion Criteria	1. Added "or waitlisted for a non-heart organ transplant" to #1 2. Separated "Moderate or severe proteinuria" exclusion criterion from GFR for clarity, creating two separate criteria. 3. Clarified that moderate or severe proteinuria is the exclusion 4. Deleted parenthetical about urine protein to urine creatinine ratio >0.5 mg/mg. This single value is insufficient for categorizing the degree of proteinuria, particularly in infants less than 2 years old.
5.3.2 Tacrolimus with mycophenolate mofetil	Added: "If the subject was on enteric-coated mycophenolic acid due to a prior history of gastrointestinal side effects with MMF, the subject can continue on enteric-coated mycophenolic acid without trialing MMF a second time (see Section 6.29)."
Table 6-1: Schedule of Study Visits	Added approximate blood draw amounts.
Section 6.3-6.11 (Visits 1-8)	Added: "MMF/MPA levels should be checked if consistent with clinical routine at the local center."
Section 6.3, 6.4 (Visits 1, 1A)	Added: "*If a urine sample cannot be obtained in a child at Visit 1/1A, then the site PI is allowed to assign a proteinuria status based on historical testing and clinical history.*" No invasive collection will be performed for urine as part of the study. This allows the clinician to assess the risk of proteinuria in the case that a sample cannot be collected.
Section 6.6, 6.9, 6.11 (Visits 3, 6, 8)	Added: "Urine testing will be performed only in children able to provide a urine sample." No invasive collection will be performed for urine as part of the study.

Section 6.25 Management of Proteinuria	1. Corrected that the correct ratio for assessment of proteinuria in children is the urine protein to **urine creatinine** ratio. **Serum creatinine** had been inadvertently used in this section. 2. Clarified that the guidelines for urine protein/urine creatinine ratios apply to children above the age of 2 years old and that clinical judgment and/or pediatric nephrology consultation should be considered for younger children with concern for proteinuria.
7.3.2 Expedited Reporting Requirement for Serious Adverse Events	Added Health Canada as a relevant regulatory agency for the study.
Table 7-3-2. Reporting Timeline for the DCC to Regulatory Bodies	Confirmed that serious, unexpected, drug related adverse events would be reported to Health Canada in an expedited timeframe. In addition clarified which agencies/bodies would receive reports of various types of adverse events.
Section 7.3.2.2 Reporting to Regulatory Agencies	Added Health Canada as a relevant regulatory agency for the study. Added: "Adverse drug reactions that are serious, unexpected, and related to the investigational drug will be reported to Health Canada as outlined in Table 7-3-2 in compliance with the "Guidance Document for Clinical Trial Sponsors: Clinical Trial Applications.""
Section 7.6.1 Randomization Scheme	Clarified stratification factor #1 is: "Sensitization status (presence vs. absence of donor specific antibody)" Deleted "History of treated rejection" **This change was made prior to randomization of the first patient but mistakenly omitted from Protocol Number TEAMMATE-004.**
Section 8.4 Records Retention	At request of Health Canada added: "The Sponsor and/or the designated Data Coordinating Center, will maintain study records for a period of 25 years."

Version 004

Section	**Changes**
Safety Evaluation	After the first 10 patients are randomized to EVL reach the 180-day mark (visit 3), a DSMB safety review will be performed.
3.3 Secondary Study Endpoints – Efficacy	2 endpoints were clarified
3.5 Secondary Study Endpoints – Safety	Safety endpoints were clarified
Table 6-1: Schedule of Study Visits	In-person screen for Endpoint Events updated

Basic Laboratory Assessment and Full Laboratory Assessment	Lab frequency updated. EBV PCR, CMV PCR, Urine protein, Urine creatinine, triglycerides, HDL, LDL are now performed at 4 of the 8 study visits instead of all study visits.
Visit sections	Language updated in each visit section to be consistent with Table 6-1: Schedule of Study Visits. Clarification that triglycerides and total cholesterol should be obtained fasting for at least 4 hours.
7.4.1 Independent Data and Safety Monitoring Board	Initial DSMB review to take place once the 10th subject is randomized to EVL. This was requested by the members of the DSMB.
7.6.3.3 Aims 1 and 2 7.6.3.3 Interim Analyses	Sentences updated to clarify the analysis plan.

Version 003 of the protocol represents the first protocol version approved by the IRB and under which subjects were enrolled in the TEAMMATE Trial. Additional details of changes between Version 001 and 003 are available upon request.

1 General Investigational Plan

Median survival after pediatric heart transplantation is 15 years in the current era. This means that a substantial fraction of patients transplanted during childhood fail to survive to adulthood, or require heart re-transplantation, because of complications related to heart transplant. These complications include heart transplant rejection, infection, coronary artery disease, post-transplant lymphoproliferative disorder (PTLD; a form of lymphoma seen in transplant recipients), and kidney failure. Most complications stem not from the heart transplant itself, but from the drugs commonly used to suppress the immune system in order to prevent rejection. In the US, tacrolimus (TAC) and mycophenolate mofetil (MMF), have emerged over the past decade as the standard of care for pediatric heart transplant immunosuppression. While pediatric survival has improved significantly in the era of TAC and MMF, post-HT complications remain a major problem that limits median survival to 15 years. Recently, everolimus (EVL) has emerged as a potential alternative immunosuppressant that may prevent rejection, coronary artery disease and kidney failure more effectively than TAC/MMF when administered in combination with low-dose tacrolimus (LDTAC). Preliminary studies suggest that EVL, and its first-generation analog sirolimus, are well tolerated in children after HT, regardless of whether it is started in response to coronary artery disease, in response to chronic kidney disease, or empirically 4-6 months after transplant in an effort to prevent the development of these complications[1]. However, studies are generally limited to single-center experiences using historical controls and have inadequate statistical power to demonstrate treatment differences. Thus, the investigators propose to conduct the first multicenter randomized clinical trial of maintenance immunosuppression in pediatric heart transplantation to systematically evaluate the safety and efficacy of EVL with LDTAC vs. TAC/MMF to prevent long-term complications which lead to death/graft loss. Because pediatric HT is a rare/orphan disease (fewer than 500 performed annually in the US and fewer than 7,000 prevalent cases), we propose to use a recently validated surrogate endpoint, the major adverse transplant event (MATE) score, as the primary endpoint to power the trial. Because no Food & Drug Administration (FDA)-approved immunosuppressants currently exist for children after heart transplant (all prescriptions are off-label) and market incentives to support a trial are limited, the investigators have funded the trial through a Fiscal Year 2016 Peer Reviewed Medical Research Program Clinical Trial Award sponsored by the Department of Defense office of the Congressionally Directed Medical Research Programs. It is worth noting that in contrast to adults, children have a substantially longer *potential* life expectancy if post-transplant complications can be minimized, making the prevention of late complications an urgent priority for the pediatric heart transplant community.

2 Study Objectives

The primary purpose of this study is to improve the long-term survival of children undergoing heart transplantation in the US by reducing transplant- and immunosuppression- related major adverse transplant events. Specifically, we wish to determine whether the combination of everolimus and low-dose tacrolimus (EVL/LDTAC) reduces the risk of coronary artery vasculopathy (CAV), chronic kidney disease (CKD) and biopsy proven-acute cellular rejection (BP-ACR) without an increase in graft loss due to all causes (e.g. infection).

Primary Objectives

Efficacy: To test the hypothesis that among children who survive to 6 months post-HT, those randomized to EVL/LDTAC for 30 months will have a lower total burden of three major adverse transplant events: CAV, CKD and BP-ACR, as defined by the MATE-3 score, compared to children randomized to standard therapy with TAC/MMF.

Safety: To test the hypothesis that among children who survive to 6 months post-HT, those randomized to EVL/LDTAC for 30 months will not have a higher risk of graft loss due to all causes as defined by the MATE-6 score, compared to children randomized to standard therapy with TAC/MMF.

Medication Tolerability: To determine the tolerability of EVL/LDTAC compared to TAC/MMF for prevention of major post-transplant complications by characterizing the frequency and incidence of AEs that are at least possibly related to EVL/LDTAC or TAC/MMF.

Secondary Objectives

- To determine whether EVL/LDTAC is superior to TAC/MMF at delaying time to patient death
- To determine whether EVL/LDTAC is superior to TAC/MMF at delaying time to cardiac allograft loss
- To determine whether EVL/LDTAC is superior to TAC/MMF at preserving kidney function (eGFR)
- To determine whether EVL/LDTAC is superior to TAC/MMF at preventing CAV
- To determine whether EVL/LDTAC is superior to TAC/MMF at preventing BP-ACR
- To compare quality of life between subjects taking EVL/LDTAC and TAC/MMF as assessed by the EuroQOL EQ-5D-Y (Youth version)
- To compare functional status between subjects taking EVL/LDTAC and TAC/MMF as assessed by Lansky (<16 years) or Karnovsky scores (>16 years)
- To compare the frequency and incidence of medication related AEs in subjects taking EVL/LDTAC and TAC/MMF

3 Study Design

3.1 General Design

This is a Phase 3 multi-center open label randomized clinical trial. The primary aim of the study is to test the hypothesis that, among children who survive to 6 months post-HT, children randomized to EVL/LDTAC will have a lower total burden of three major adverse transplant events —CAV, CKD and BP-ACR as defined by MATE-3—compared to children randomized to TAC/MMF, without a higher predicted risk of death/graft loss due to all causes as defined by MATE-6. The rationale for this approach is that while we wish to focus on reducing the three transplant complications that EVL has been shown to affect, we would not want to omit from consideration death/graft loss events caused by other major adverse transplant events, such as infection, as was found in an adult heart transplant trial of EVL and cyclosporine (reference population cyclosporine/MMF).[2] Thus MATE-3 is the primary efficacy endpoint whereas MATE-6, which is predictive of all-cause graft loss, is the primary safety endpoint. A schematic diagram of the trial can be found in Section 6 (**Figure 6-1**). Patients will be randomized at 6 months post-HT if they meet the study eligibility criteria and provide informed consent. Subjects will be followed for a total of 30 months post-randomization.

Table 3-1. The MATE Score[3]

	0 (No Disease)	1 (Mild)	2 (Moderate)	3 (Severe)	4* Death or Graft Loss	MATE	
						3	6
Chronic Kidney Disease	**Stage 1-2** ≥60 ml/min/ 1.73 m^2	**Stage 3A** 45-59 ml/min/ 1.73 m^2	**Stage 3B** 30-44 ml/min/1.73 m^2	**Stage 4-5** ≤29 ml/min/1.73 m^2, dialysis or renal transplant	**Death or Graft Loss**	**X**	**X**
Coronary Allograft Vasculopathy	**ISHLT CAV_0**	**ISHLT CAV_1**	**ISHLT CAV_2**	**ISHLT CAV_3**	**Death or Graft Loss**	**X**	**X**
Treated Biopsy Proven-Acute Cellular Rejection	**ISHLT Grade 0**	**ISHLT Grade 1R**	**ISHLT Grade 2R**	**ISHLT Grade 3R**	**Death or Graft Loss**	**X**	**X**
Antibody Mediated Rejection (AMR)	**ISHLT Grade pAMR0**	**ISHLT Grade pAMR1**	**ISHLT Grade pAMR2**	**ISHLT Grade pAMR3**	**Death or Graft Loss**		**X**
Infection	**None**	**Low-risk** PO/IM Antibiotics	**Moderate-Risk** Not life threatening, IV antibiotics	**High-Risk** Life threatening, IV antibiotics	**Death or Graft Loss**		**X**
PTLD	**None**	**Low-risk** Polymorphic PLTD	**Moderate-risk** Monomorphic no "high-risk" features	**High-risk** CNS or Marrow Involvement	**Death or Graft Loss**		**X**

Additional details on categorical classification can be found in the published manuscript.[3]
*Patients experiencing death or graft loss will be assigned a score of 4 in each category resulting in a MATE-3 score of 12 (if death/graft loss is due to a MATE-3 event) or a MATE-6 score of 24 (if death/graft loss is due to a MATE-6 event).
pAMR: Pathologic diagnosis of cardiac antibody-mediated rejection

3.2 Primary Study Endpoint - Efficacy

The primary efficacy endpoint of the trial is a validated score ranging from 0 to 12, denoted by the MATE-3 score.[3] The score represents the cumulative burden between 0 and 30 months post-randomization (6 and 36 months post-transplant) of three major adverse transplant events (see **Table 3-1**): CAV, CKD, and BP-ACR, as defined by published international guidelines/consensus statements.[4-10]

The MATE-3 score is a composite ordinal score that captures elements of both major adverse transplant event frequency and severity for CAV, CKD and BP-ACR, major adverse transplant events that may be reduced by everolimus based on previous studies. To avoid several mild complications summing to a value greater than graft loss, graft loss due to any MATE-3 cause is automatically assigned a MATE-3 score of 12.

3.3 Secondary Study Endpoints - Efficacy

- Overall patient survival from randomization to 30 months post-randomization (36 months post-HT)
- Overall graft survival (freedom from death or re-transplant) through 30 months post-randomization (36 months post-HT)
- Change in kidney function after start of study drug as assessed by change in estimated glomerular filtration rate (eGFR) using the modified Schwartz equation from month 0 to 6, month 0 to 12 and month 0 to 30 post-randomization (i.e., 12, 18, and 36 months post-HT).
- Freedom from event for each of the 3 MATE-3 components: CKD, CAV and BP-ACR.
- Freedom from composite efficacy failure, defined as freedom from first occurrence of death, repeat HT, 2R/3R ACR rejection or rejection with HD compromise.
- The composite score consisting of the following elements: MATE CAV, MATE CKD, MATE BP-ACR, and CMV infection (as defined by the MATE infection sub-score and where any CMV infection is assigned a value of at least 1).
- Freedom from CAV, CKD, BP-ACR, or any CMV infection.
- The change in CKD stage from prior to randomization to 30 months post-randomization. The rationale for this endpoint is that change in CKD stage can take on a negative value to reflect the scenario where renal function improves if the CKD stage at trial completion is lower than the CKD stage at enrollment.
- The MATE-3 score where the MATE CKD score component is calculated by the change in the MATE CKD score from prior to randomization to 30 months post-randomization. The MATE CKD score may take on a negative value to reflect the scenario where renal function can improve if the MATE CKD score at trial completion is lower than the MATE CKD score at enrollment.
- The MATE-3 score where the CKD MATE score component is replaced by the change in the CKD stage from prior to randomization to 30 months post-randomization. The change in CKD stage may take on a negative value to reflect the scenario where renal function can improve if the CKD stage at trial completion is lower than the CKD stage at enrollment.
- The composite score consisting of the following four elements: MATE CAV, MATE BP-ACR, MATE ΔCKD, and CMV infection (as defined by the MATE infection sub-score and

where any CMV infection is assigned a value of at least 1); The MATE ΔCKD score is calculated by the change in the CKD MATE score from prior to randomization to 30 months post-randomization. The CKD MATE score may take on a negative value to reflect the scenario where renal function can improve if the CKD stage at trial completion is lower than the CKD stage at enrollment.

- The composite score consisting of the following four elements: MATE CAV, MATE BP-ACR, ΔCKD Stage, and CMV infection (as defined by the MATE infection sub-score and where any CMV infection is assigned a value of at least 1); The ΔCKD score is calculated by the change in the CKD stage from prior to randomization to 30 months post randomization. The change in CKD stage may take on a negative value to reflect the scenario where renal function can improve if the CKD stage at trial completion is lower than the CKD stage at enrollment.
- Lansky (< 16 years at randomization) and Karnofsky (≥ 16 years at randomization) Scores at 18 and 30 months post-randomization (6, 24, 36 months post-HT); these scores are validated functional performance scores originally developed for cancer patients.[11-14] Both scores have been used to describe the functional status of heart transplant recipients.[11, 15, 16] The instruments are found in Appendix 1.
- EuroQOL EQ-5D-Y (Youth version)[17, 18] measured at 18 and 30 months post-randomization (24 and 36 months post-HT) for subjects ages ≥ 8 years at randomization; EQ-5D-Y proxy version 1 will be used for children ≥ 4 years but less than 8 years at randomization or children ≥ 8 years who are unable to complete the EQ-5D-Y at randomization. These validated quality of life instruments use a simple two-page questionnaire involving a simple ordinal scale across 5 domains plus a visual analog scale. The youth versions are tailored to an appropriate developmental level so that quality of life can be reproducibly assessed in children. Country and language specific versions will be used. The instruments are found in Appendix 2.

3.4 Primary Study Endpoint - Safety

The primary safety endpoint of the trial is a validated score ranging from 0 to 24, denoted the MATE-6 score. The score represents the predicted risk of graft loss based on the cumulative burden between 0 and 30 months post-randomization (6 to 36 months post-HT) of all six major adverse transplant events as defined published international guidelines/consensus statements[4-10] (**Table 3-1**).

The MATE-6 score is a composite ordinal score that captures elements of both major adverse transplant event frequency and severity for six major adverse transplant events and has been shown to predict long-term graft loss (CPE-statistic 0.74)[3]. To avoid several mild complications summing to a value greater than graft loss, graft loss due to any MATE-6 cause is automatically assigned a MATE-6 score of 24.

3.5 Secondary Study Endpoints – Safety

The purpose of the secondary safety endpoints is to examine individually the MATE-6 score endpoints that are not included in the MATE-3 efficacy endpoint, and to complete the safety profile of the drugs under study, and include:

- Freedom from event for each of the 3 MATE-6 components not captured in MATE-3: pAMR, infection, and PTLD.
- The frequency and incidence of AEs including, but not limited to, hyperlipidemia, anemia, thrombocytopenia, interstitial lung disease, aphthous stomatitis, proteinuria, and rash that are unlikely related, possibly related, or related to EVL/LDTAC compared to TAC/MMF. These AEs will be reported as individual endpoints as well as a composite.
- Freedom from composite of any major adverse transplant event (CKD, CAV, BP-ACR, pAMR, PTLD and infection) as defined in the MATE score.[3]
- Freedom from first occurrence of any level 2 severity CKD major adverse transplant event as defined in the MATE score.[3]
- Freedom from first occurrence of any level 2 severity CAV major adverse transplant event as defined in the MATE score.[3]
- Freedom from first occurrence of any level 2 severity BP-ACR major adverse transplant event as defined in the MATE score.[3]
- Freedom from first occurrence of any level 2 severity pAMR major adverse transplant event as defined in the MATE score.[3]
- Freedom from first occurrence of any level 2 severity PTLD major adverse transplant event as defined in the MATE score.[3]
- Freedom from first occurrence of any level 2 severity infection major adverse transplant event as defined in the MATE score.[3]

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Orthotopic heart transplantation performed 5-7 months prior to randomization
2. Age < 21 years at time of transplant
3. Stable immunosuppression at the time of randomization with no contraindication to everolimus, tacrolimus, or mycophenolate mofetil
4. Planned follow-up at a study site for the 30 month duration of the study.
5. Subject or legal adult representative capable of providing informed consent (in general, assent will be sought for children aged 12 years or older).

4.2 Exclusion Criteria

1. Multi-organ transplant (e.g. heart-lung or heart-liver) or waitlisted for a non-heart organ transplant.

2. Known hypersensitivity to everolimus, sirolimus, tacrolimus or mycophenolate mofetil (MMF), or to components of the drug products.
3. Patients on maintenance corticosteroid therapy exceeding a dose equivalent of prednisone 0.1 mg/kg/day at randomization.
4. High-risk for rejection defined as active rejection, recurrent (≥ 2 episodes of grade 2R rejection) cellular rejection, recurrent rejection (≥ 2 episodes of any grade) with hemodynamic compromise, steroid-resistant rejection or unresolved antibody-mediated rejection during the first 6 months post-HT.
5. Graft dysfunction (LVEF <40% or Wedge >22 mmHg or CI <2.2 L/min/m2)
6. Stage 4 or 5 CKD (eGFR <30 ml/min/1.73m^2)
7. Moderate or severe proteinuria.
8. Active infection requiring hospitalization or treatment dose medical therapy.
9. Patients with ongoing wound healing problems, clinically significant wound infection requiring continued therapy or other severe surgical complication in the opinion of the Site Principal Investigator.
10. Fasting Serum Cholesterol ≥300 mg/dL OR greater than or equal to 7.75 mmol/L AND fasting triglycerides ≥2.5x the upper limit of normal (ULN). NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication, and reduction of serum cholesterol and triglyceride levels to below exclusion ranges are confirmed.
11. Uncontrolled diabetes mellitus.
12. Diagnosis of PTLD during the first 6 months post-HT.
13. History of non-adherence to medical regimens.
14. Patients who are treated with drugs that are strong inducers or inhibitors of cytochrome P450 3A4 (CYP3A4) and cannot discontinue the treatment (see Table 5-7).
15. Patients who are pregnant or breast-feeding or intend to get pregnant during the study period.

4.3 Subject Recruitment and Screening

All children who receive post-HT clinical care at participating study sites will be eligible for participation in the study. A physician or study coordinator involved with the study will screen subjects between 4 and 7 months post-HT. Subjects deemed eligible for inclusion based on screening will be approached by a physician or study coordinator involved with the study to inform them of the study and determine the patient, parent, guardian, and/or legally appointed representative's (LAR's) interest in learning more about the study and participating. Subjects expressing interest in participating in the study after a detailed review of the potential risks and benefits will be invited to participate. After completing the informed consent process, informed consent will be signed by the patient, parent, guardian, and/or LAR (as appropriate) prior to randomization. At each stage of the recruitment, screening, and study follow-up, it will be emphasized that participation in the study is entirely voluntary and the patient/family can withdraw consent to participate any time. Assent will be obtained from all developmentally able patients who are of an appropriate age. In general we expect patients greater than 12 years of age to be capable of assent. If patients reach the age of majority (18 years old in most locations) during the course of the study, patients will be asked to sign consent themselves for continued participation in the study. Ages for assent and consent may vary slightly by study site according to local

practices and customs but will be standardized at a given site by the Institutional Review Board (IRB) or Research Ethics Board/Committee (REB).

4.4 Early Withdrawal of Subjects

4.4.1 Permanent Withdrawal from Study Drug

Withdrawal from study drug is the permanent discontinuation of study drug before the last scheduled data collection time point is met. This may occur if there is a clinical indication or physician preference for withdrawal from the assigned study drug. However, the subject will remain in the trial and complete all study visits and requirements, with the exception of drug levels. Withdrawal from study drug with continued participation in the trial is preferable to withdrawal from the trial (see Section 4.4.2), in order to ensure complete data for the primary, intention-to-treat, analysis of the trial. There are instances where study drug may need to be withdrawn on only a temporary (less than two months duration) basis (see Section 5.6). In this instance, all study visits will also be completed. For cases where the patient, parent(s) or LAR(s) request that the study drug be permanently discontinued, it will be up to the treating physician to make a clinical decision on care subsequent to the formal withdrawal request. It is likely that the patient would be transitioned back to standard care with TAC and MMF.

4.4.2 Withdrawal from Trial

Withdrawal from the trial is the discontinuation of study participation before the last scheduled data collection time point is met. Every effort will be made to minimize the number of subjects who withdraw from the trial. However, if such withdrawal occurs at the request of the subject or physician, all data collected prior to withdrawal from the study will remain in the study database for use in analysis; however, data collection will not continue after the withdrawal date. It should be noted that study withdrawal is not required if the patient, parent(s) or LAR(s) requests drug discontinuation (see Section 4.4.1).

Reasons for withdrawal from the study may include: (1) the subject, parent, guardian, or LAR elect to withdraw consent from all future study activities, including follow-up; (2) the subject reaches the age of majority and declines to provide consent for continuation in the study; (3) the Clinical Site Principal Investigator (PI) determines that it is in the best interest of the patient to be withdrawn or (4) the Research Monitor withdraws the subject from the trial (**see Section 7.4.2**). Every effort will be made to retain all subjects in the trial and have all study visits completed, even if withdrawal from study treatment is indicated. The patient is considered to be lost to follow-up if attempts to reestablish contact with the participant have failed for more than one year following the last successful contact. Because acute withdrawal of the investigational drug without initiation of a replacement drug is unsafe, drug withdrawal will be performed in accordance with the maintenance immunosuppression conversion protocol at each site.

4.4.3 Data Collection and Follow-up for Withdrawn Subjects

For subjects who withdraw prematurely from the study, every reasonable effort will be made to consent the patient to obtain basic survival data throughout the protocol's defined follow-up

period. For subjects/LAR who decline further follow-up, every effort will be made to consent patients to track outcomes through data reported to UNOS and the PHTS, if eligible.

5 Study Drug

5.1 Description

Everolimus (Marketed as Zortress®) is a proliferation signal inhibitor used in immunosuppressive therapies for prevention of acute and chronic rejection. It selectively inhibits mammalian target of rapamycin (specifically mTORC1) and blocks proliferative signals of growth factor, thereby preventing cells from entering the S phase. Everolimus is currently approved for use in prophylaxis of organ rejection in adult kidney and adult liver transplant patients, and approved in Europe for heart transplantation.

Everolimus will be prescribed as 0.25-, 0.5-, 0.75-, and 1-mg tablets. Zortress®, is a trade name for everolimus, and is available as 0.25-, 0.5-, 0.75-, and 1-mg tablets. In addition, 0.25-, 0.5-, 0.75-mg Everolimus Tablets are available from West-Ward Pharmaceuticals and have been determined to be bioequivalent to Zortress Tablets by the FDA. Any form of everolimus that has been FDA approved and determined by the FDA to be bioequivalent to Zortress Tablets may be used under this protocol. Dosing strengths must be able to adhere to the dosing strengths specified in **Sections 5.3 and 5.4**. If suspension form is required, individual tablets will be reconstituted in 5 mL of water just prior to administration (**see section 5.4** for detailed procedure).

5.2 Treatment Arms

Subjects will be assigned to one of two treatment arms in a ratio of 1:1 at Visit 1 or 1A after they pass screening procedures.

- Everolimus with low-dose tacrolimus (EVL/LDTAC)
- Tacrolimus with mycophenolate mofetil (TAC/MMF)

5.3 Treatment Regimens

5.3.1 Everolimus with low-dose Tacrolimus (EVL/LDTAC)

Dose Initiation

At 6 months post-transplant, subjects who are eligible and have been randomized to treatment arm (EVL/LDTAC) will receive EVL according to Table 5-1 Everolimus Starting Dose and Treatment Schedule.

Table 5-1 Everolimus Starting Dose and Treatment Schedule

BSA (m^2)	Everolimus Starting Dose	Route of administration	Frequency
< 0.6	0.25 mg	PO/PG	Every 12 hours

0.6 – 0.9	0.5 mg	PO/PG	Every 12 hours
0.9 – 1.2	0.75 mg	PO/PG	Every 12 hours
1.2 – 1.5	1 mg	PO/PG	Every 12 hours
> 1.5	1.25 mg	PO/PG	Every 12 hours

Everolimus will be administered by mouth (per orem; PO) or by stomach (per gastrum; PG) twice daily (12 hours apart) and adjusted per goal levels (C_0 3-8 ng/mL). Patients will receive the initial starting dose of EVL based on BSA (m^2) at time of study drug initiation. Treatment duration will be for the duration of the study (30 months after randomization). EVL will be administered consistently approximately 12 hours apart (i.e. 9 AM and 9 PM or 10 AM and 10 PM) with or without food to minimize variability in absorption and at the same time as TAC. Initial dosing (approximately 0.8 mg/m^2/dose BID) is based on previously published studies in pediatric renal transplant patients that demonstrated pharmacokinetic parameters of EVL[19-22]. These studies are briefly summarized below.

Van Damme-Lombaerts and colleagues[21] used an initial single-dose of EVL 1.2 mg/m^2 and established that the half-life of EVL was similar in pediatric and adult patients, whereas the volume of distribution was smaller in pediatric than in adult patients. Results of this study showed rapid absorption of EVL and positive linear correlation of clearance with age, weight and body surface area. Conclusions of this initial pharmacokinetic study in pediatric patients were that dosing would require a dose scaled down to body size (secondary to smaller volume of distributions), but that pediatric patients would be able to maintain the same twice-daily dosing regimen as adults (secondary to similarities in half-life). In another multicenter trial, Vester and colleagues[22] similarly concluded that body-surface-area-adjusted dosing regimens of EVL resulted in predictable drug exposure and trough levels.

Subsequent clinical trials have been conducted to further determine safety and efficacy of EVL in pediatric renal transplant patients. In a multicenter, open-label, single-arm trial of EVL in combination with cyclosporine and corticosteroids, Hoyer and colleagues[20] characterized longitudinal pharmacokinetics of EVL by obtaining trough blood samples on days 3, 5, 6, and 7 and at months 1, 2, 3, and 6. Pharmacokinetic parameters were derived including pre-dose trough concentration (C_0), the peak concentration, time to reach peak, area under the concentration-time curve (AUC), and apparent oral clearance. Results support the use of body surface area adjusted (mg/m^2) dosing regimens and correlation between EVL C_0 and AUC in pediatric patients. Furthermore, Hoyer et al. concluded that EVL dosage of 0.8 mg/m^2 (max 1.5 mg) twice-daily yielded adequate EVL exposure.

Additionally, Ettenger and colleagues[19] published long-term prospective data on pediatric renal transplant patients who were initiated on EVL (0.8 mg/m^2 per dose orally twice daily) within 48 hours of transplantation. The mean daily dose of EVL during the first 36 months post-transplant was 1.53 mg/m^2, and remained relatively constant throughout the study. Pharmacokinetic analysis of six-month data supports the use of EVL dosing adjusted according to BSA, and showed that with EVL 0.8 mg/m^2 (maximum 1.5 mg/m^2) twice daily, three-quarters of patients achieved a steady-state EVL trough level above 3 ng/mL.

Dose Modification

Everolimus dose titrations can occur once daily and will be adjusted to achieve therapeutic goal levels (C_0 3-8 ng/mL). Dose titrations will occur only in whole tablet size increments to avoid splitting or manipulating tablets. EVL levels will be checked prior to the sixth dose after initiation. EVL dose will be adjusted as per "Table 5-2 Everolimus Maintenance Adjustment Recommendations."

If EVL dose is modified it is important to check TAC level in 2-5 days as TAC levels may vary secondary to EVL dose change.

Table 5-2 Everolimus Maintenance Adjustment Recommendations:

Measured trough (C_0) level	**Dose Adjustment to Maintain Everolimus Goal C_0 (trough) level 3-8 ng/mL**
< 3 ng/mL	Increase dose by a minimum of 10–20% (round to nearest 0.25 mg increment); Recheck level in 4–8 doses
3-8 ng/mL	No change; If first dose in range, repeat level in 1-3 weeks; For subsequent doses, repeat level at next clinical transplant visit, no less frequent than every 6 weeks for months 6-12 and every 3 months for months 12-36.
8-12 ng/mL	Decrease dose by a minimum of 10–20% (round to nearest 0.25 mg increment); Recheck level in 4–8 doses
> 12 ng/mL	Hold one dose, decrease dose by 20–40% (round to nearest 0.25 mg increment); Recheck level prior to fourth dose

Dose Monitoring
If the initial EVL level is in range, repeat level in 1-3 weeks; For subsequent doses, repeat level at next clinical transplant visit, no less frequent than every 6 weeks for months 6-12 and every 3 months for months 12-36.

Low-Dose Tacrolimus

Patients randomized to EVL/LDTAC will continue to receive TAC but at a significantly lower dose where it functions as an adjunctive rather than primary immunosuppressant. To avoid worsening renal function, it is important to not run tacrolimus levels higher than the specified target range, one of the most important lessons learned from previous EVL studies in children and adults. A modest rise in creatinine may occur initially until TAC levels have stabilized within the target range.

For subjects receiving a TAC-based immunosuppression immediately prior to randomization:

The TAC trough level should be checked at the randomization study visit. After the subject has received two doses of EVL, the TAC dose should be adjusted to achieve a C_0 (trough level) of 3-5 ng/mL. This will be done based on the clinical experience of the clinical site investigator. All other oral immunosuppression medications (with the exception of prednisone) should be discontinued after the first dose of EVL has been administered. Levels of EVL and TAC should be checked 3-7 days after starting therapy or as suggested above. TAC will be administered PO/PG

for the 30 month duration of the study. Whenever possible, NG administration should be avoided because TAC adheres to the NG tubing leading to inconsistent dose administration.

For subjects not receiving a TAC-based immunosuppression immediately prior to randomization:

EVL should be started as above. TAC should be started concomitant with starting of EVL. The TAC starting dose should be 0.025 mg/kg/day divided in two doses. The doses should be administered every 12 hours. All other oral immunosuppression medications (with the exception of prednisone) should be discontinued after the 1-2 doses of EVL have been administered. Levels of EVL and TAC should be checked 3-7 days after starting therapy. The TAC dose should be adjusted to achieve a C_0 (trough level) of 3-5 ng/mL. TAC will be administered PO/PG for the 30-month duration of the study. Whenever possible, NG administration should be avoided because TAC adheres to the NG tubing leading to inconsistent dose administration.

Prohibited Immunosuppressive Medications for Subjects on EVL/LDTAC

Once subjects have achieved therapeutic levels of EVL and LDTAC, the following immunosuppressive medications are prohibited (except as allowed in Section 6 of the protocol): sirolimus, mycophenolate mofetil, and cyclosporine. If the clinical site investigator feels that one of these medications is clinically indicated, please contact the Clinical Coordinating Center (CCC) to discuss.

Change in LDTAC Goal at 1 Year Post-Transplant

When study subjects randomized to EVL/LDTAC reach 1 year post transplant, the TAC C_0 goal will decrease to 2.5-4.5 ng/mL. Investigators should adjust tacrolimus dosing accordingly. This change is intentionally indexed to the time of transplantation and not to month post-randomization.

Dose Monitoring
If the initial LDTAC level is in range, repeat level in 1-3 weeks; For subsequent doses, repeat level at next clinical transplant visit, no less frequent than every 6 weeks for months 6-12 and every 3 months for months 12-36.

5.3.2 Tacrolimus with mycophenolate mofetil (TAC/MMF)

For subjects receiving TAC/MMF immediately prior to randomization:

The TAC C_0 (trough level) should be checked at the randomization study visit. The TAC dose should be adjusted to achieve a C_0 (trough level) of 7-10 ng/mL. This will be done based on the clinical experience of the clinical site investigator. For sites which use mycophenolic acid (MPA) levels to adjust dosing of MMF, a MPA C_0 should be checked and the dose should be adjusted based on the clinical experience of the clinical site investigator. For sites which do not check MPA levels, MMF should be dosed at 600 mg/m2/dose administered every 12 hours. MMF dose can be adjusted based on side effects according to procedures outlined in Section 6. All other oral immunosuppression medications (with the exception of prednisone) should be discontinued after

therapeutic levels of TAC and/or MMF have been achieved. Levels of TAC and MMF should be checked at the discretion of the clinical site investigator. TAC and MMF will be administered PO/PG for the duration of the study.

For subjects not receiving TAC/MMF immediately prior to randomization:

TAC should be initiated at a dose of 0.1 mg/kg/day divided into two doses administered PO/PG every 12 hours. The TAC C_0 (trough level) should be checked 3-7 days after initiation. The TAC dose should be adjusted to achieve a C_0 (trough level) of 7-10 ng/mL. This will be done based on the clinical experience of the clinical site investigator. If therapeutic trough levels cannot be achieved with every 12 hour administration, TAC may be administered every 8 hours with the same C_0 (trough level) goals.

MMF should be dosed at 600 mg/m^2/dose administered PO/PG every 12 hours. For sites which use MPA levels to adjust dosing of MMF, a MPA C_0 should be checked 3-7 days after initiation and the dose should be adjusted based on the clinical experience of the clinical site investigator to achieve a C_0 MPA level of 2-4 mcg/mL. MMF dose can be adjusted based on side effects according to procedures outlined in Section 6. If the subject was on enteric-coated mycophenolic acid due to a prior history of gastrointestinal side effects with MMF, the subject can continue on enteric-coated mycophenolic acid without trialing MMF a second time (see Section 6.29). All other oral immunosuppression medications (with the exception of prednisone) should be discontinued after therapeutic levels of TAC and/or MMF have been achieved. Levels of TAC and MMF should be checked at the discretion of the clinical site investigator. If TAC dosing administration is adjusted to every 8 hours, MMF can be administered on a similar schedule maintaining the same total daily dose.

Prohibited Immunosuppressive Medications for Subjects on TAC/MMF

Once subjects have achieved therapeutic levels of TAC and/or MPA, the following immusuppressive medications are prohibited (except as allowed in Section 6 of the protocol): everolimus, sirolimus, and cyclosporine. If the clinical site investigator feels that one of these medications is clinically indicated, please contact the CCC to discuss.

Change in TAC Goal at 1 Year Post-Transplant

When study subjects randomized to TAC/MMF reach 1 year post transplant, the TAC C_0 goal will decrease to 5-8 ng/mL. Investigators should adjust tacrolimus dosing accordingly. This change is intentionally indexed to the time of transplantation and not to month post-randomization.

Dose Monitoring

If the initial TAC level is in range, repeat level in 1-3 weeks; For subsequent doses, repeat level at next clinical transplant visit, no less frequent than every 6 weeks for months 6-12 and every 3 months for months 12-36.

5.4 Method for Assigning Subjects to Treatment Groups

Treatment assignment will not be blinded. After written informed consent is obtained, subjects will be randomly assigned to the EVL/LDTAC or TAC/MMF group using secure web-based randomization that is integrated into the database management system. A total of 210 subjects will be randomized to one the two treatment regimens (105 subjects per group). In order to allow patients already consented and undergoing screening for the trial to participate, randomization of subjects will continue to occur for up to 4 weeks after the 210th randomization or for a total of 220 randomizations, whichever comes first. Analysis of treatment groups will proceed according to the Statistical Analysis Plan. The randomization assignments will be generated by Boston Children's Hospital's proprietary randomization software, SciRan. Subject randomization will require prior entry of eligibility data and confirmation that written informed consent has been obtained. Randomization will be performed within 4 strata defined by center transplant volume and history of treated rejection. Allocation will be in a 1:1 ratio using permuted blocks, to ensure balanced allocations across the two treatment groups within each stratum. Each participant will be informed of his/her assignment by the study coordinator at the end of the baseline visit.

5.5 Preparation and Administration of Study Drug

Everolimus will be prescribed as 0.25-, 0.5-, 0.75-, and 1-mg tablets. EVL will be dispensed to the patient by an outpatient clinical pharmacy.

If liquid preparation is required the following steps must occur just prior to administration. Anyone who prepares the suspension for another person should wear gloves to avoid possible contact with the medication.

Using an oral syringe:

1. Obtain correct tablet size for oral suspension (maximum of 1 tablet per 5 mL volume) and place in a 10 mL oral syringe (syringe A). The plunger should be inserted and pushed inward to make contact with the tablet. Attach a syringe cap to the oral syringe. Crush single tablet within the syringe (using the plunger). Once tablet has been crushed, remove the syringe cap and draw up syringe to 5 mL marking (syringe will contain air + contents of crushed tablet).
2. In a separate syringe (syringe B) draw up 5 mL of water and add to oral syringe (syringe A) containing the EVL tablet.
3. Prior to administration, the oral syringe should be inverted gently several times. The full contents of the oral syringe should be immediately dispensed into the mouth of the patient.
4. The same volume of water (5 mL) should be drawn up into the oral syringe, and the contents should be swirled to suspend any remaining particles. The full contents of the syringe should be dispensed into the mouth of the patient.
5. For each tablet, repeat steps 1-4.

For administration of EVL via a gastrostomy tube:

1. Flush the tube with a small amount of sterile water to ensure patency of tube (amount of water flushed should be adequate to clear tube of formula).
2. Attach the medication syringe to the port of the feeding tube and gently push on the plunger until the medication has been completely administered.

3. Flush the tube again with a small amount of sterile water (amount of water flushed should be adequate to clear tube of formula).
4. If multiple medications are being administered, flush the tube between each medication (amount of water flushed should be adequate to clear tube of formula).

The Investigator or responsible site personnel must instruct the patient and/or caregiver to take the study drugs as per protocol. Subjects/caregiver must be instructed to take the appropriate amount of 0.25-, 0.5-, 0.75-, and/or 1-mg tablets based upon their prescribed dose.

5.6 Subject Compliance Monitoring

Compliance with everolimus will be assessed by monitoring of therapeutic goal levels (C_0 3-8 ng/mL). After initial EVL level is in goal range, a repeat level will be checked in 1-3 weeks. For subsequent doses, repeat level(s) will be checked at each clinical transplant visit, no less frequent than every 6 weeks for months 6-12 and every 3 months for months 12-36. Each subject/caregiver will also be asked at each visit whether he or she has been compliant with dosing instructions. Continued non-compliance may lead to withdrawal of the subject from the study, after consultation between the Investigator and Regulatory Sponsor.

Clinical sites will contact outpatient clinical pharmacies to determine the number of refills dispensed for each study medication over the length of the study. Finally, subjects and/or parents will be asked to estimate the number of missed doses of study medication over the (1) interval since the last study visit and (2) 7 days prior to the study visit.

5.7 Indications for Temporary Discontinuation of Assigned Study Drug

The following clinical scenarios represent acceptable reasons for temporary (defined as any duration <2 months) discontinuation of study drug:

1. Life threatening illness or infection
2. Acute renal failure
3. Any non-minor surgical procedure (**see Section 6.24**)

Temporarily holding drug in the case of supratherapeutic levels with resumption of drug when levels are in the therapeutic range does not qualify as temporary discontinuation of study drug and will be interpreted that the subject is on treatment.

5.8 Prior and Concomitant Therapy

Subjects randomized to the EVL/LDTAC arm will receive LDTAC as concomitant therapy and may receive corticosteroids intermittently for treatment of rejection or at lower chronic doses as per institution protocol. All concomitant medications will be recorded at each study visit in the patient's source documentation and electronic case report form (CRF).

G-CSF may be used in the management of acute toxicity such as febrile neutropenia when clinically indicated or at the discretion of the Clinical Site Investigator; however G-CSF may not be substituted for a required dose-reduction. Patients taking chronic erythropoietin are permitted

provided they are on a stable dose prior to study drug administration. Erythropoietin may not be substituted for a required dose reduction.

Inhibitors of CYP3A4 and/or PgP

Patients who are taking strong CYP3A4 inhibitors or inducers at the time of screening who are unable to discontinue treatment are not eligible for enrollment in the study (**Section 4.2 Exclusion Criterion #13**). A list of medications considered strong CYP3A4 inhibitors (≥ 5-fold increase in exposure or > 80% clearance of substrate) and inducers is found in the table below:

Table 5-7 List of Strong CYP3A4 Inhibitors and Inducers

CYP3A4 Strong Inhibitors[23]	CYP3A4 Strong Inducers[24]
boceprevir	carbamazepine
clarithromycin	fosphenytoin (as phenytoin)
Holkira Pak *(dasabuvir + ombitasvir, paritaprevir, and ritonavir)*	phenobarbital
indinavir	phenytoin
itraconazole	primidone
ketoconazole	rifabutin
idelalisib	rifampin
lopinavir/ritonavir	rifapentine
nefazodone	
nelfinavir	
posaconazole	
ritonavir	
saquinavir/ritonavir	
Technivie (*ombitasvir, paritaprevir, and ritonavir)*	
telithromycin	
Viekira Pak *(dasabuvir + ombitasvir, paritaprevir, and ritonavir)*	
voriconazole	

Co-administration with strong CYP3A4 inhibitors and strong CYP3A4 inducers is not recommended during the course of the study. If no therapeutic alternative exists and a strong CYP3A4 inhibitor or inducer is clinically necessary, contact the Trial Co-Chairs to discuss plans for close monitoring of EVL and/or TAC whole blood trough concentrations throughout the duration of therapy. [See Drug Interaction Section in Section 2 above]. If a patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, consider reducing the dose of EVL and/or TAC dose and monitor levels closely. If the inhibitor is discontinued the EVL or TAC dose should be returned to the dose used prior to the initiation of the moderate CYP3A4/PgP inhibitor. Grapefruit and their juices affect P450 and PgP activity. Concomitant use should be avoided.

Inducers of the CYP3A4 and PgP

Patients who are taking strong CYP3A4 inducers at the time of screening who are unable to discontinue treatment are not eligible for enrollment in the study (Section 4.2 Exclusion Criteria #13). A list of strong CYP3A4 inducers can be found in Table 5-7 above.

Co-administration of strong CYP3A4 inducers is not recommended during the course of the study. If no therapeutic alternative exists and a strong CYP3A4 inducer is clinically necessary, contact one of the Trial Co-Chairs to discuss plans for close monitoring of EVL and/or TAC whole blood trough concentrations at the beginning and throughout the duration of therapy. An increase in the dose of EVL and/or TAC as high as twice the currently used daily dose may be necessary in order to maintain adequate trough levels. Enzyme induction usually occurs within 7-10 days, therefore the EVL level should be monitored accordingly to maintain safe levels during the period of treatment with a CYP3A4 inducer and following its discontinuation. Moderate CYP3A4 inducers may be used if clinically indicated. It is recommended that doses of EVL and/or TAC be increased in order to maintain therapeutic drug trough levels. Monitoring of EVL and/or TAC drug trough levels should be performed frequently during initiation and discontinuation of moderate CYP3A4 inducers.

EVL has a number of other drug interactions to be aware of that are summarized in section 5.2.

5.9 Packaging

Everolimus (Zortress®) is currently commercially available and will be supplied as 0.25-, 0.5-, 0.75-, and 1-mg tablets. Each bottle from the manufacturer (Novartis) comes with 60 tablets.

Further information regarding the preparation and packaging of the study drug, everolimus (Zortress®) can be found in the attached everolimus (Zortress®) Packaged Insert. (see section 2)
Everolimus tablets are also available in the following forms:
0.25 mg tablets are supplied as white to off white, round standard convex tablets, plain on one side and “54” over “414” debossed on the other side. NDC 0054-0470-21: Bottles of 60 Tablets

0.5 mg tablets are supplied as white to off white, round standard convex tablets , plain on one side and “54” over “761” debossed on the other side. NDC 0054-0471-21: Bottles of 60 Tablets

0.75 mg tablets are supplied as white to off white, round standard convex tablets , plain on one side and “54” over “044” debossed on the other side. NDC 0054-0472-21: Bottles of 60 Tablets

Further information regarding the preparation and packaging of the study drug, everolimus tablet can be found in the package label from West-Ward Pharmaceuticals Corp.

Patients receiving EVL as outpatient will be prescribed a 30- or 90-day supply with the number of refills based on the interval between visits. Each bottle will be labeled by the outpatient clinical pharmacy in accordance with all applicable national regulations, to include at least the following information: medication name (everolimus), strength, quantity, manufacture date, lot number, expiratory date, instructions for use, route of administration, appropriate precautionary statements, and storage conditions. For dosing suspensions, syringes and bottle adaptors will be made available to the sites.

5.10 Blinding of Study Drug

This is an open-label study so no blinding procedure is required.

5.11 Receiving, Storage, Dispensing and Return

5.11.1 Receipt of Drug Supplies

EVL will be obtained from outpatient clinical pharmacies or the hospital inpatient clinical pharmacy if the subject is admitted to the hospital during the course of the study. As EVL will be obtained per clinical routine, study personnel will not be responsible for receipt, storage, dispensing, or destruction of study drug.

5.11.2 Storage

EVL will be obtained from outpatient clinical pharmacies or the hospital inpatient clinical pharmacy if the subject is admitted to the hospital during the course of the study. As EVL will be obtained per clinical routine study personnel will not be responsible for receipt, storage, dispensing, or destruction of study drug.

5.11.3 Dispensing of Study Drug

EVL will be obtained from outpatient clinical pharmacies or the hospital inpatient clinical pharmacy if the subject is admitted to the hospital during the course of the study. As EVL will be obtained per clinical routine study personnel will not be responsible for receipt, storage, dispensing, or destruction of study drug.

5.11.4 Return or Destruction of Study Drug

EVL will be obtained from outpatient clinical pharmacies or the hospital inpatient clinical pharmacy if the subject is admitted to the hospital during the course of the study. As EVL will be obtained per clinical routine study personnel will not be responsible for receipt, storage, dispensing, or destruction of study drug.

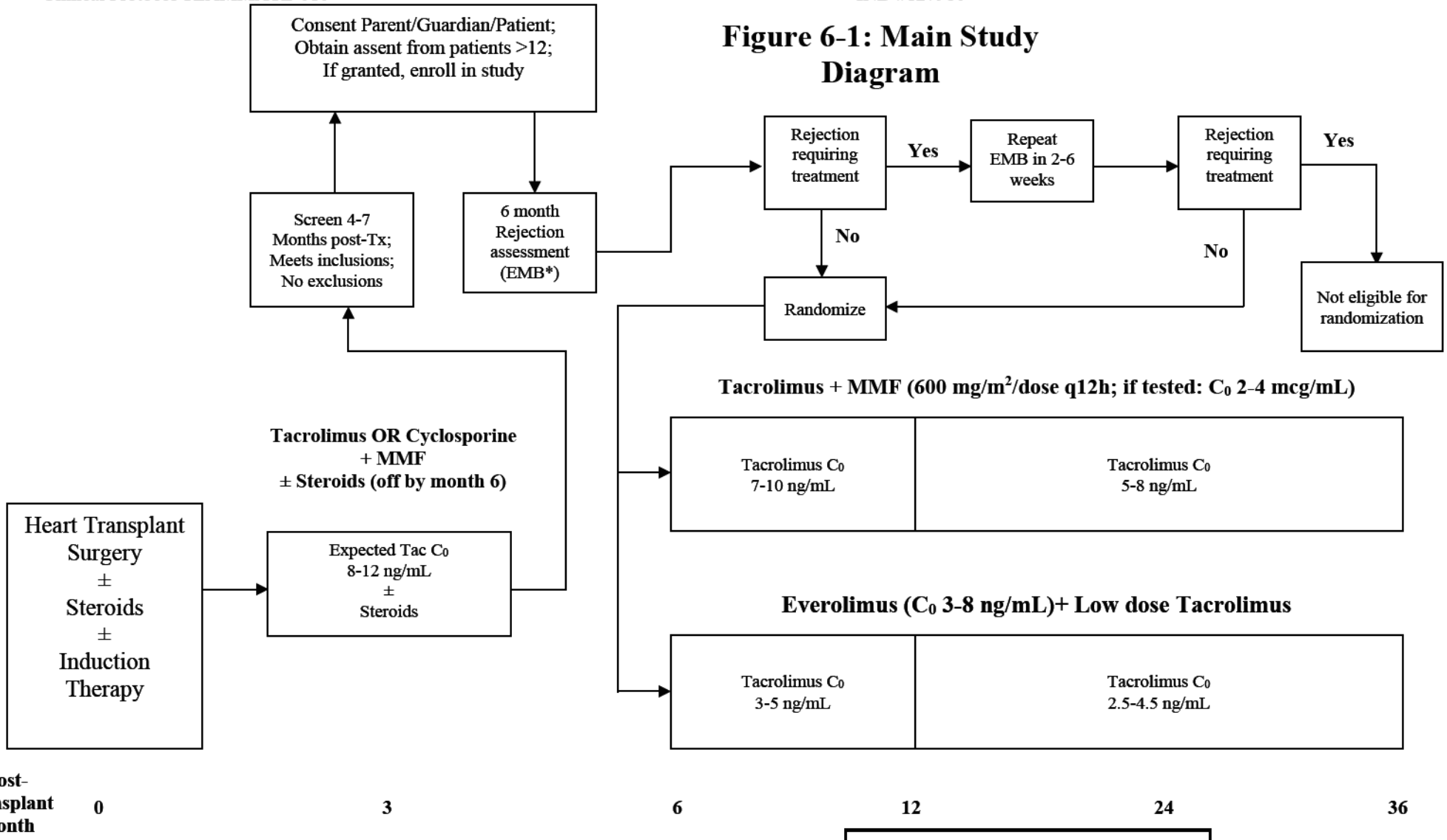
6 Study Procedures

6.1 Overall Study Design

The overall study flow is outlined in the **Figure 6-1: Main Study Diagram** and study visits are outlined in **Table 6-1: Schedule of Study Visits** at the end of this section. Patients are closely followed by the clinical heart transplant teams after they have been accepted and listed for heart transplant. Patients will follow standard clinical protocols at the time of transplant. In general, maintenance immunosuppression involves TAC or cyclosporine and MMF. Corticosteroids will be used per center-specific clinical routine. No treatment is specified by this protocol prior to subject enrollment, however treatment during the first six months may influence qualification for the study based on the inclusion/exclusion criteria.

Patients will be screened for meeting study inclusion criteria at 4-7 months post-transplant. This requires being on stable maintenance immunosuppression at enrollment without contraindication to EVL, TAC, or MMF. If the patient meets inclusion criteria, and does not have any exclusion criteria, the patient, parent, guardian and/or legally authorized representative will be approached for participation in the study. If, after learning about the study, the family elects to participate, consent will be obtained from the patient or parent/legal guardian as appropriate. If the patient is of a certain age and developmentally capable of providing assent, written assent will be obtained from the patient for participation in the study. Ages for assent and consent vary by study site according to local practices and customs but will be standardized at a given site by the IRB or REB. If consent occurs prior to the 6±1 month post-transplant window, eligibility will be reconfirmed prior to randomization.

Figure 6-1: Main Study Diagram



***Non-invasive assessment allowed in young infants (defined as less than 18 months old) and assessment is not concerning for rejection. If non-invasive assessment suggests possible rejection, EMB is performed.**

Everolimus Initial Starting Dose

BSA (m^2)	Dose
< 0.6	0.25 mg PO q12h
0.6 - 0.9	0.5 mg PO q12h
0.9 – 1.2	0.75 mg PO q12h
1.2 – 1.5	1 mg PO q12h
> 1.5	1.25 mg PO q12h

Table 6-1: Schedule of Study Visits

	Visit Number	**0***	**1**	**1A#**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**8A@**	
	Months Post-Randomization (Approximate Months Post-Transplant)	**-1 (5)**	**0 (6)**	**0# (7)**	**3 (9)**	**6 (12)**	**9 (15)**	**12 (18)**	**18 (24)**	**24 (30)**	**30 (36)**	**30-45 (36-51)**	**Event-Driven**
	Visit Window (months)	± 2	± 1	± 1	± 1	± 1	± 1	± 1	± 2	± 2	± 2	NA	
Patient Characteristics at Transplant	Age, UNOS Status, Blood Type, Waitlist duration, HD Support, Labs	X											
Donor Characteristics	Cause of death, ischemic time, EBV IgG, CMV IgG, Toxoplasma IgG	X											
Histocompatibility	T- and B- cell crossmatch results, HLA Antibody screen	X											
Treatment Summary Form	Induction therapy, Tx Hospitalization Length of stay, Maintenance IS, Infectious Prophylaxis, Hospitalizations, Rejection summary, DSA		X										
Randomization Eligibility Form	Inclusion/exclusion criteria, results of 6 month EMB and treatment		X										
In-person screen for Endpoint Events	CKD, BP-ACR, pAMR, CAV, PTLD, Infection		X	X	X	X	X	X	X	X	X		
In-person screen for Adverse Events (AE) and Serious Adverse Events (SAE)	SAE, Hospitalizations, Pregnancy, Re-transplant, Death, Lost to follow up, New onset diabetes, aphthous stomatitis, lung disease		X	X	X	X	X	X	X	X	X		
Immunosuppression Drug Trough Levels	Tacrolimus AND Everolimus OR Mycophenolate Mofetil **(1 mL)**	X	X	X	X	X	X	X	X	X	X		X
Chronic Kidney Disease	Height, weight, creatinine		X	X	X	X	X	X	X	X	X		

		Visit Number	0*	1	1A#	2	3	4	5	6	7	8	8A@	
		Months Post-Randomization (Approximate Months Post-Transplant)	-1 (5)	0 (6)	0# (7)	3 (9)	6 (12)	9 (15)	12 (18)	18 (24)	24 (30)	30 (36)	30-45 (36-51)	Event-Driven
		Visit Window (months)	± 2	± 1	± 1	± 1	± 1	± 1	± 1	± 2	± 2	± 2	NA	
Endomyocardial Biopsy/Rejection Screen	Indication, ACR grade, pAMR grade, DSA (if performed), RHC, Echo, Rhythm, Treatment (yes + type OR no)			X$	X		X			X		X		X
Selective Coronary Angiography	CAV Grade, Affected vessels, Stents, Angiograms for Core Lab Review						X			O		X		X
Study Medication Compliance Measures	Medication refill history, Patient self-report					X	X	X	X	X	X	X		X
Quality of Life Assessment	Administer EuroQOL EQ-5D-Y (Youth Version), EQ-5D-Y proxy version, Lansky OR Karnofsky scores			X	X					X		X		
PTLD	Pathologic description, imaging results, physical exam, EBV serologies, EBV PCR, LDH, treatment													X
Infection	Type of infection, diagnostic testing, treatment, length of hospitalization, complications of infection or treatment													X
General Assessment	Age, Height, Weight, Blood Pressure, Heart Rate, Medications			X	X	X	X	X	X	X	X	X		
Basic Laboratory Assessment	CBC, BMP, LFTs **(2 mL)**			X	X	X	X	X	X	X	X	X		
Echocardiogram	EF, SF, EDV, MR, TR, RVp, pericardial effusion			X	X	X	X	X	X	X	X	X		
Full Laboratory Assessment	anti-HLA antibody (donor specific) with titer and/or C1q,			X	X		X			X		X		

Visit Number		**0***	**1**	**1A#**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**8A@**	
Months Post-Randomization (Approximate Months Post-Transplant)		**-1 (5)**	**0 (6)**	**0# (7)**	**3 (9)**	**6 (12)**	**9 (15)**	**12 (18)**	**18 (24)**	**24 (30)**	**30 (36)**	**30-45 (36-51)**	**Event-Driven**
Visit Window (months)		± 2	± 1	± 1	± 1	± 1	± 1	± 1	± 2	± 2	± 2	NA	
	anti-HLA antibody (non-DSA), EBV PCR (qualitative), CMV PCR (qualitative), urine protein%, urine creatinine%, fasting triglycerides, fasting HDL, fasting LDL **(9 mL)**												
IVUS, *Optional*&	Maximal intimal thickness, atheroma volume, luminal area, intimal index, total intimal volume		O			O					O		
Coronary Artery Pressure Wire for CFR, *Optional*&	CFR before and during adenosine induced hyperemia					O			O		O		
Blood/Urine for Biobank/Ancillary Studies	Plasma, peripheral blood mononuclear cells, and urine%		O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL	O 5-10 mL
Total Blood Draw Volume for Routine Clinical Care	See above categories: • Immunosuppression Drug Trough Levels • Basic Laboratory Assessment • Full Laboratory Assessment	1 mL	12 mL	12 mL	3 mL	12 mL	3 mL	3 mL	12 mL	3 mL	12 mL	5-10 mL	0 mL

*Visit 0 may be combined with Visit 1.
#Visit 1A is only performed in patients who have rejection at their initial study visit. Patients who continue to have rejection at this visit are not eligible for randomization or continuation in the study.$Non-invasive assessment allowed in young infants (defined as less than 18 months old) and assessment is not concerning for rejection. If non-invasive assessment suggests possible rejection an endomyocardial biopsy should be performed.&At centers which routinely perform IVUS and CFR per clinical protocol. Limited to patients in whom examination is technically feasible. Generally this is patients weighing at least 25 kg. %Attempts will be made to obtain a non-invasive urine sample, however urine testing is not required in young children who are unable to provide such a sample.
@Visit 8A collection only in consented patients who were unable to have Biobank samples collected at Visit 8

6.2 Visit 0

Visit Timing: 4-7 months post-heart transplant; May be combined with Visit 1

After consent has been obtained, the medical record will be reviewed to collect subject and donor specific characteristics. This will include subject age at transplant, height, weight, UNOS Status at transplant, patient blood type, total waitlist time, active waitlist time, hemodynamic support at time of transplant, and laboratory studies on the day of transplant (Cytomegalovirus (CMV) IgG, Epstein-Barr virus (EBV) IgG, CBC, etc.). Donor records will be reviewed and specified information will be included including donor age, donor blood type, donor cause of death, graft ischemic time, EBV IgG, CMV IgG, and left ventricular ejection fraction. Histocompatibility records will be reviewed. Results of the T-cell and B-cell donor specific crossmatch will be recorded and pre-transplant anti-HLA antibody results will be recorded along with MFI and titer of any detected antibodies.

6.3 Visit 1

Visit Timing: 6 months post-transplant ± 1 month; Randomization to occur at this visit if eligible

At Visit 1 all subjects will be screened for rejection with endomyocardial biopsy per clinical routine. If a subject is a young infant (defined as less than 18 months of age), a non-invasive assessment including an echocardiogram, electrocardiogram, and physical examination can be performed as a rejection screen. If that screen suggests possible rejection, the subject will undergo an endomyocardial biopsy per clinical routine. If the screen is negative, the subject will be considered to not have rejection. At Visit 1, a treatment summary form will be collected from medical record review, consultation with the patient/family, and consultation with the transplant clinician. This summary will collect information on induction therapy, information about the transplant hospitalization (length of intubation, length of ICU stay, length of stay, complications), information about maintenance immunosuppression including when corticosteroids were weaned, information about CMV, EBV, herpes simplex virus (HSV), *pneumocystis jirovecii* pneumonia (PJP) prophylaxis, hospitalizations (numbers and indications), rejection history, and donor specific antibody history.

A general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, aphthous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including complete blood count (CBC), basic metabolic panel (BMP), liver function tests (LFT), drug trough levels (TAC, EVL), EBV polymerase chain reaction (PCR), CMV PCR, urine protein, urine creatinine, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). *If a urine sample cannot be obtained in a child at Visit 1/1A, then the site PI is allowed to assign a proteinuria status based on historical testing and clinical history.* MMF/MPA levels should be checked if consistent

with clinical routine at the local center. Triglycerides, HDL, and LDL should be obtained fasting for at least 4 hours, and preferably 8 hours, prior to the blood draw. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion.

HLA antibody screening will be performed per clinical routine. If donor specific antibody is detected, serum can be titered. We recommend the following dilutions: neat, 1:8, 1:32. If clinically indicated and available, C1q testing will be performed. The results of the HLA testing will be recorded in the study database.

The results of the endomyocardial biopsy will be recorded along with the pathologists ACR grade, pAMR grade, CD68 results, and C4d results. The pathology report will be deidentified and entered into the study database for adjudication. The invasive hemodynamic measurements will be recorded including mixed venous saturation, right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Any medical or surgical treatment administered in response to the biopsy will be recorded.

At centers which perform IVUS evaluation of the coronary arteries per clinical routine, a baseline examination will be performed and the results recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication.

A trial eligibility form will be completed to ensure that the patient meets inclusion criteria and does not have any exclusion criteria. If the subject does not have rejection requiring treatment, the patient will be randomized per protocol to TAC/MMF or EVL/LDTAC. If the patient does have rejection *requiring treatment, the patient will return for repeat biopsy in 2-6 weeks for rescreening at Visit 1A.*

If randomized:
EuroQOL EQ-5D-Y (Youth version) will be administered for subjects ≥ 8 years at randomization; EQ-5D-Y proxy version for children ≥ 4 years but less than 8 years old at randomization OR for children ≥ 8 years who are unable to complete the EQ-5D-Y at randomization. The transplant clinician will be asked to assign a Lansky score for subjects < 16 years old at randomization and a Karnofsky score for subjects ≥ 16 years old at randomization. Scores will be recorded in the study database. The same instruments/scores administered at baseline will be used throughout the study for a given subject.

6.4 Visit 1A (Only performed if rejection is present at Visit 1)

Visit Timing: 2-6 weeks following Visit 1 up to a maximum of 8 months post-transplant; Randomization to occur at this visit if eligible.

At Visit 1A all subjects who had rejection at Visit 1 will be screened for rejection with endomyocardial biopsy per clinical routine. At Visit 1A, the Visit 1 treatment summary form will be updated to include any new information. Sources include medical record review, consultation with the patient/family, and consultation with the transplant clinician. This summary will collect information on ATG dosing, information about the transplant hospitalization (length of intubation, length of ICU stay, length of stay, complications), information about maintenance immunosuppression including when corticosteroids were weaned, information about CMV, EBV, HSV, PJP prophylaxis, hospitalizations (numbers and indications), rejection history, and donor specific antibody history.

A general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, drug trough levels (TAC, EVL), EBV PCR, CMV PCR, urine protein, urine creatinine, triglycerides, HDL, and LDL. *If a urine sample cannot be obtained in a child at Visit 1/1A, then the site PI is allowed to assign a proteinuria status based on historical testing and clinical history.* MMF/MPA levels should be checked if consistent with clinical routine at the local center. Triglycerides, HDL, and LDL should be obtained fasting for at least 4 hours, and preferably 8 hours, prior to the blood draw. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion.

HLA antibody screening will be performed per clinical routine. If donor specific antibody is detected, serum can be titered. We recommend the following dilutions: neat, 1:8, 1:32. If clinically indicated and available, C1q testing will be performed. The results of the HLA testing will be recorded in the study database.

EuroQOL EQ-5D-Y (Youth version) will be administered for subjects $\geq$ 8 years at randomization; EQ-5D-Y proxy version for children $\geq$ 4 years but less than 8 years old at randomization OR for children $\geq$ 8 years who are unable to complete the EQ-5D-Y at randomization. The transplant clinician will be asked to assign a Lansky score for subjects $<$ 16 years old at randomization and a Karnofsky score for subjects $\geq$ 16 years old at randomization. Scores will be recorded in the study database. The same instruments/scores administered at baseline will be used throughout the study for a given subject.

The results of the endomyocardial biopsy will be recorded along with the pathologists ACR grade, pAMR grade, CD68 results, and C4d results. The pathology report will be

deidentified and entered into the study database for adjudication. The invasive hemodynamic measurements will be recorded including mixed venous saturation, right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Any medical or surgical treatment administered in response to the biopsy will be recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication.

A trial eligibility form will be completed to ensure that the patient meets inclusion criteria and does not have any exclusion criteria. If the subject does not have rejection requiring treatment, the patient will be randomized per protocol to TAC/MMF or EVL/Low-dose TAC. If the patient does have rejection requiring treatment, the patient is not eligible for randomization and they will not complete additional study visits.

6.5 Visit 2

Visit Timing: 3 months following randomization ± 1 month
(Approximately 9 months post-transplant)

At Visit 2, a general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, and drug trough levels (TAC, EVL). MMF/MPA levels should be checked if consistent with clinical routine at the local center. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

6.6 Visit 3

Visit Timing: 6 months following randomization ± 1 month
(Approximately 12 months post-transplant)

At Visit 3 all subjects will be screened for rejection with endomyocardial biopsy per clinical routine. A general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, drug trough levels (TAC, EVL), EBV PCR, CMV PCR, urine protein, urine creatinine, triglycerides, HDL, and LDL. MMF/MPA levels should be checked if consistent with clinical routine at the local center and urine testing will be performed only in children able to provide a urine sample. Triglycerides, HDL, and LDL should be obtained fasting for at least 4 hours, and preferably 8 hours, prior to the blood draw. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

HLA antibody screening will be performed per clinical routine. If donor specific antibody is detected, serum can be titered. We recommend the following dilutions: neat, 1:8, 1:32. If clinically indicated and available, C1q testing will be performed. The results of the HLA testing will be recorded in the study database.

The results of the endomyocardial biopsy will be recorded along with the pathologists ACR grade, pAMR grade, CD68 results, and C4d results. The pathology report will be deidentified and entered into the study database for adjudication. The invasive hemodynamic measurements will be recorded including mixed venous saturation, right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Any medical or surgical treatment administered in response to the biopsy will be recorded.

Selective coronary angiography will be performed per clinical routine. The local interventional cardiologist or transplant cardiologist assessment of the degree of CAV along with specific affected vessels will be recorded. The images from the coronary angiography will be de-identified, labeled with the subject ID and sent to the core laboratory for review as specified in Section 7.

At centers which perform IVUS evaluation of the coronary arteries per clinical routine, a 1 year examination will be performed and the results recorded. At centers which perform Coronary flow Reserve, the results of this examination will be recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

6.7 Visit 4

Visit Timing: 9 months following randomization ± 1 month
(Approximately 15 months post-transplant)

At Visit 4, a general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, and drug trough levels (TAC, EVL). MMF/MPA levels should be checked if consistent with clinical routine at the local center. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

6.8 Visit 5

Visit Timing: 12 months following randomization ± 1 month
(Approximately 18 months post-transplant)

At Visit 5, a general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An adverse event screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to

follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, and drug trough levels (TAC, EVL). MMF/MPA levels should be checked if consistent with clinical routine at the local center. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

6.9 Visit 6

Visit Timing: 18 months following randomization ± 1 month
(Approximately 24 months post-transplant)

At Visit 6 all subjects will be screened for rejection with endomyocardial biopsy per clinical routine. A general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, drug trough levels (TAC, EVL), EBV PCR, CMV PCR, urine protein, urine creatinine, triglycerides, HDL, and LDL. MMF/MPA levels should be checked if consistent with clinical routine at the local center and urine testing will be performed only in children able to provide a urine sample. Triglycerides, HDL, and LDL should be obtained fasting for at least 4 hours, and preferably 8 hours, prior to the blood draw. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

HLA antibody screening will be performed per clinical routine. If donor specific antibody is detected, serum can be titered. We recommend the following dilutions: neat, 1:8, 1:32. If

clinically indicated and available, C1q testing will be performed. The results of the HLA testing will be recorded in the study database.

EuroQOL EQ-5D-Y (Youth version) OR EQ-5D-Y proxy version will be administered. The same instrument used at the randomization visit will be repeated at this visit. The transplant clinician will be asked to assign either a Lansky score OR Karnofsky score. The same functional status scoring scale used at the randomization visit will be used at this visit. Scores will be recorded in the study database. The same instruments/scores administered at baseline will be used throughout the study for a given subject.

The results of the endomyocardial biopsy will be recorded along with the pathologists ACR grade, pAMR grade, CD68 results, and C4d results. The pathology report will be deidentified and entered into the study database for adjudication. The invasive hemodynamic measurements will be recorded including mixed venous saturation, right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Any medical or surgical treatment administered in response to the biopsy will be recorded.

Results of selective coronary angiography, if performed per local clinical routine, will be recorded. The local interventional cardiologist or transplant cardiologist assessment of the degree of CAV along with specific affected vessels will be recorded. The images from the coronary angiography will be deidentified, labeled with a unique angiogram ID and sent to the core laboratory for review.

At centers which perform Coronary Flow Reserve per clinical routine, the results of this examination will be recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

6.10 Visit 7

Visit Timing: 24 months following randomization ± 1 month
(Approximately 30 months post-transplant)

At Visit 7, a general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, apthuous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, and drug trough levels

(TAC, EVL). MMF/MPA levels should be checked if consistent with clinical routine at the local center. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

6.11 Visit 8

Visit Timing: 30 months following randomization ± 1 month
(Approximately 36 months post-transplant)

At Visit 8 all subjects will be screened for rejection with endomyocardial biopsy per clinical routine. A general assessment will be performed to include age, height, weight, blood pressure, heart rate, and current medications. An AE screen will be performed including hospitalizations since last study visit, pregnancy, re-transplant, death, lost to follow up, new onset diabetes, aphthous stomatitis, and lung disease. Laboratory studies will be performed per clinical routine and results recorded including CBC, BMP, LFTs, drug trough levels (TAC, EVL), EBV PCR, CMV PCR, Urine protein, Urine creatinine, triglycerides, HDL, and LDL. MMF/MPA levels should be checked if consistent with clinical routine at the local center and urine testing will be performed only in children able to provide a urine sample. Triglycerides, HDL, and LDL should be obtained fasting for at least 4 hours, and preferably 8 hours, prior to the blood draw. An echocardiogram will be performed per clinical routine and the following parameters will be recorded: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

HLA antibody screening will be performed per clinical routine. If donor specific antibody is detected, serum can be titered. We recommend the following dilutions: neat, 1:8, 1:32. If clinically indicated and available, C1q testing will be performed. The results of the HLA testing will be recorded in the study database.

EuroQOL EQ-5D-Y (Youth version at baseline (6 months post-HT), 24 and 36 months post-transplant for subjects between the ages of 8 and 15 years; EQ-5D-Y proxy version for children 4 -7 years or children ≥ 8 years who are unable to complete the EQ-5D-Y. The EQ-5D-3L will be used for subjects ≥ 16 years and older at enrollment. The transplant clinician will be asked to give a Lansky score for subjects < 16 years old and a Karnofsky score for subjects ≥ 16 years old. Scores will be recorded in the study database.

The results of the endomyocardial biopsy will be recorded along with the pathologists ACR grade, pAMR grade, CD68 results, and C4d results. The pathology report will be deidentified and entered into the study database for adjudication. The invasive hemodynamic measurements will be recorded including mixed venous saturation, right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Any medical or surgical treatment administered in response to the biopsy will be recorded.

Selective coronary angiography will be performed per clinical routine. The local interventional cardiologist or transplant cardiologist assessment of the degree of CAV along with specific affected vessels will be recorded. The images from the coronary angiography will be deidentified, labeled with a unique angiogram ID and sent to the core laboratory for review.

At centers which perform IVUS evaluation of the coronary arteries per clinical routine, a 1 year examination will be performed and the results recorded. At centers which perform Coronary Flow Reserve per clinical routine, the results of this examination will be recorded.

A screen for endpoint events will be performed. The events include graft rejection (both ACR and pAMR), PTLD, infection, CAV, and CKD. The screen will derive information from at least the following three sources: (1) interview of the subject and/or parent/guardian, (2) interview with the transplant clinician, (3) medical record review. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. To allow for proper classification of the CKD endpoint, all clinical creatinine values, blood urea nitrogen (BUN) values, tacrolimus levels and/or everolimus levels that are available in the electronic medical record prior to the end of study visit will be extracted and uploaded for adjudication.

The final MATE score is determined based on diagnostic assessments performed at Visit 8 (End of Study Visit) and its final determination is of critical importance to trial integrity. The key Visit 8 diagnostic assessments needed to determine the MATE score are:

1. Creatinine
2. Endomyocardial Biopsy Pathology Report and Right Heart Catheterization
3. Selective Coronary Angiography Images for Core lab Review and Local CAV Grade

If any of these key diagnostic assessments are not performed at Visit 8, the results of the next clinical assessment performed at the study site should be reported as a Visit 8 assessment. If the testing cannot be performed by the earlier of: (1) **12 months** from Visit 8 or (2) by the final date for study data entry, then the testing will be reported as not done. A protocol deviation will be entered for any out of window assessment.

6.12 Visit 8A

Visit Timing: 30-45 months following randomization
(Approximately 36-51 months' post-transplant)

For subjects who had consented to participation in the TEAMMATE Biobank **AND** were unable to have TEAMMATE Biobank blood and urine samples collected at Visit 8 for any reason, a supplemental TEAMMATE Biobank collection can take place at any clinical visit up to 15 months following completion of Visit 8. The only research specific procedures that will occur at this visit are collection of blood and urine specimens for the TEAMMATE Biobank. This will involve collection of up to 10 mL of blood (if deemed safe by the site investigator) and approximately 5 mL of urine.

6.13 Event Driven Endpoint Assessment

If the endpoint event screen reveals that a possible endpoint event has occurred, and endpoint event form will be completed and clinical information about the event will be recorded. Pathology reports, clinic notes, and/or laboratory testing relevant to the endpoint events will be deidentified and entered into the study database for endpoint event adjudication. This information will be used for adjudication of endpoint events.

Rejection

If a subject has suspected rejection and undergoes any additional endomyocardial biopsy, the results of the endomyocardial biopsy will be recorded along with the pathologists ACR grade, pAMR grade, CD68 results, and C4d results. The pathology report will be deidentified and entered into the study database for adjudication. The invasive hemodynamic measurements will be recorded including mixed venous saturation, right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Any medical or surgical treatment administered in response to the biopsy will be recorded.

In the case where a subject is diagnosed with clinical rejection and treated, but the treating clinician deems performance of a biopsy unsafe, all available clinical information will be recorded.

Results of HLA antibody screening, if performed per clinical routine, will be recorded. If donor specific antibody is detected, serum can be titered. We recommend the following dilutions: neat, 1:8, 1:32. If clinically indicated and available, C1q testing will be performed. The results of the HLA testing will be recorded in the study database.

Results of the echocardiogram will at the time of the rejection event will be recorded. Parameters include: left ventricular ejection fraction, left ventricular shortening fraction, left ventricular end diastolic volume, degree of mitral regurgitation, qualitative right ventricular

function, degree of tricuspid regurgitation, right ventricular pressure, presence of pericardial effusion. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

Cardiac Allograft Vasculopathy

If additional selective coronary angiography procedures are performed due to clinical concern, results will be reported. The local interventional cardiologist or transplant cardiologist assessment of the degree of CAV along with specific affected vessels will be recorded. The images from the coronary angiography will be de-identified, labeled with a unique angiogram ID and sent to the core laboratory for review. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

Post-Transplant Lymphoproliferative Disease

If a diagnosis of PTLD is made at the local center, the following information will be recorded: clinical description of location of the PTLD, results of any imaging studies used to identify the PTLD (CT scan, ultrasound, PET scan, MRI, etc.), physical examination, pathology report from any biopsies of the PTLD, EBV serologies, EBV PCR, lactate dehydrogenase (LDH), treatment plan for PTLD. In general PTLD is a diagnosis made based on pathology and attempts should be made to biopsy affected organ(s) and/or lymph organs. If tissue for pathologic diagnosis cannot be safely obtained in the opinion of the investigator, please contact one of the Trial Co-Chairs to discuss the clinical scenario. This will ensure that adequate diagnostic information is available to the endpoint adjudication committee. Subsequent follow up information regarding the resolution of the PTLD, including results of imaging, treatment course, and complications will be recorded. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

Infection

If a patient is diagnosed with or treated for an infection (exception of a non-specific viral syndrome), the following information will be recorded: type of infection, how diagnosis was made, results of all infectious diagnostic testing used to make the decision, treatment prescribed, treatment completed, length of hospitalization (if any), complications of infection, and complications of treatment. A patient self-report medication compliance screen will be performed. Pharmacy refill records for EVL, TAC, and/or MMF will be requested and recorded.

6.14 Endomyocardial Biopsy Protocol

6.14.1 Prior to randomization

Endomyocardial biopsies shall be performed per individual center protocol. There will be no suggested or required biopsy procedures prior to randomization.

6.14.2 At randomization

It is part of the clinical routine of all enrolling centers to perform a per clinical protocol endomyocardial biopsy and right heart catheterization in all heart transplant patients greater than 1 year of age at 6 months post-transplantation. For young infants (defined as less than 18 months of age), clinical protocols at some centers require an endomyocardial biopsy while others use non-invasive screening to determine if a endomyocardial biopsy. Either option is acceptable in the context of the study. For those centers choosing non-invasive screening for young infants, testing will include, at a minimum, vital signs (heart rate, respiratory rate, blood pressure), weight, electrocardiogram, echocardiogram, and laboratory studies (CBC, Chem-7 plus Ca, TAC trough level ± mycophenolate mofetil level). For patients with a reassuring evaluation, the patient will deemed to have no rejection, the endomyocardial biopsy can be skipped, and the patient will be eligible for enrollment. If the evaluation is not reassuring, a right heart catheterization and endomyocardial biopsy must be performed prior to randomization. Patients become eligible for randomization based on the results of the endomyocardial biopsy and clinical evaluation as outlined in **Figure 6-1**.

6.14.3 After randomization

Per clinical protocol endomyocardial biopsies and right heart catheterizations will be performed at 6, 18, and 30 months post-randomization (approximately 12, 24, and 36 months post-transplantation) at all centers for all enrolled subjects. In addition, per clinical protocol invasive cardiac allograft vasculopathy screening will take place at 12, 24, and 36 months post-transplantation at all centers for all enrolled subjects. The details of what information will be collected, and how that data is collected, are outlined below.

Additional per clinical protocol endomyocardial biopsies are allowed under the study protocol and the frequency will not be standardized across centers. However, within a given center, all subjects should follow the same per clinical protocol biopsy frequency regardless of treatment arm. In order to ensure that this is the case, each center will be asked to provide their usual risk clinical biopsy protocol. All biopsy procedures should include a right heart catheterization and the results reported on an event driven biopsy form.

For cause endomyocardial biopsies are allowed at any point during the study. A right heart catheterization should be performed along with every biopsy procedure and the results reported on an event driven biopsy form. Any biopsy which results in treatment of rejection (beyond adjustment of oral immunosuppression medications), should be followed up with a repeat biopsy 1 to 6 weeks later in order to confirm adequate treatment of rejection.

With every endomyocardial biopsy procedure performed during the 30-month period following randomization (6-36 months post-transplant), the following should occur:

6.14.3.1 Right Heart Catheterization

A balloon directed end-hole catheter will be used to perform a right heart catheterization in the standard fashion to obtain right atrial, right ventricular, pulmonary artery and pulmonary capillary wedge pressures. Venous pressure measurements should include an

a-wave, v-wave and mean. Arterial pressure measurements should include a systolic, diastolic, and mean pressure. Ventricular pressure measurements should include a peak systolic pressure and an end-diastolic pressure. A mixed venous saturation from the pulmonary artery (MPA, RPA, or LPA) will be obtained. This will allow for standard calculation of cardiac index by Fick. For centers which routinely perform thermodilution measurements of cardiac output, the average value of three bolus thermodilution measurements will be recorded in addition to the mixed venous saturation.

6.14.3.2 Endomyocardial Biopsy

Endomyocardial biopsy will then be performed per institutional protocol. A minimum of three samples of non-fibrotic endomyocardium will be obtained at a given biopsy procedure for hematoxylin and eosin staining as this is the minimum requirement to rule out cellular rejection. An additional sample of endomyocardium will be sent for C4d staining. In addition, a CD68 stain should be performed on one of the biopsy specimens as this is now a recommended part of the pathologic assessment for antibody mediated rejection[8].

6.15 Coronary Angiography Protocol

Invasive coronary angiography will be performed in all patients at 6 and 30 months post-randomization (approximately 12 and 36 months post-heart transplantation) per clinical protocol. If invasive coronary angiography is performed by local center routine at 18 months post-randomization (24 months post-transplant), results of that testing will be reported and angiograms provided for core laboratory review. Patients weighing greater than 25 kg who are cared for at centers who perform intravascular ultrasound (IVUS) should also have baseline IVUS performed at 6, 12 and 36 months post-transplant. The left anterior descending coronary artery (LAD) or left circumflex coronary (LCx) artery should be imaged as described below. Serial IVUS recordings and measurements should be performed in the same vessel at each assessment.

Functional assessments such as dobutamine stress echo and cardiac MRI perfusion stress imaging will be left up to clinicians to determine timing and indications, particularly in setting of abnormal coronary angiograms, or abnormal systolic function on echocardiogram. Similarly, routine 24 hour Holter monitor and cardiopulmonary exercise stress testing may be performed per heart center's standard clinical protocol.

6.15.1 Contraindications to Selective Coronary Angiography

Allergic reactions to iodinated contrast, lack of vascular access, chronic kidney disease stage 4 or 5 (GFR < 30 ml/min/1.73m2).

In patients with severe chronic kidney disease in whom contrast administration is felt to be clinically necessary, prehydration with normal saline or sodium bicarbonate should be considered.

Patients with contraindications to invasive screening should undergo a clinically appropriate alternative test.

6.15.2 Vascular Access

Vascular access will be obtained per institutional protocol. Access sites will be dependent on the available access for an individual patient. Venous access will be obtained in the femoral vein, internal jugular or subclavian vein. Arterial access will be via the femoral artery. Radial artery access may be used if there is no femoral artery to access. The decision to administer heparin will be per institutional protocol and may depend upon whether a left ventricular angiogram is performed.

Sheath size and coronary artery catheter sizes will be size dependent and determined by the operator.

If IVUS is being performed, 5F arterial sheath and a JL3 guide catheter or larger will be required. The choice of guide catheter will be patient size dependent.

6.15.3 Left Heart Catheterization (optional)

A pigtail catheter will be advanced via the femoral artery over a wire, retrograde up the descending aorta, around the aortic arch and across the aortic valve into the left ventricle. The wire will be removed. Left ventricular pressures will be measured and a left ventricular angiogram will be performed (Left ventricle angiogram, 30º RAO / 60º LAO - 20º cranial tilt biplane, 1ml/kg up to 25 ml of contrast at 1ml/second up to 25 ml/second.) The left ventricular angiogram may be deferred if an echocardiogram is performed on the day of catheterization. Pullback pressure measurements will be made into the descending aorta and the pigtail catheter will be removed over a wire. For patients who undergo diagnostic echocardiogram on the day of heart catheterization, an echo EF may be substituted for the LV angiogram.

6.15.4 Left Coronary Artery Evaluation

The left coronary artery will be selectively cannulated with a coronary angiographic catheter. Catheter selection is dependent upon patient size and coronary ostial location[25]. Selective left coronary arteriograms will be performed by hand injections. Operators may choose to administer intracoronary nitroglycerin prior to injection (2-5 mcg/kg up to a maximum of 200 mcg). At least two orthogonal views of the left coronary artery should be obtained. Recommended projections are as follows[26]:

30º RAO – 20º CAU – Best visualization of left main bifurcation, proximal LAD, LCx

50-60º LAO – 20-30º cranial – Best for visualization of proximal and mid-LAD, septals, and diagnoals

0º PA – 30º cranial – Best for left main, proximal and mid LAD

50º LAO – 30º caudal (Spider view) – Best visualization of left main bifurcation and proximal LAD and LCx

6.15.5 Right Coronary Artery Evaluation

The right coronary artery will selectively cannulated with a coronary angiographic catheter. Catheter selection is dependent upon patient size and coronary ostial location[25]. Selective right coronary arteriograms will be performed by hand injections. Operators may choose to administer intracoronary nitroglycerin prior to injection (2-5 mcg/kg up to a maximum of 200 mcg). At least two views of the right coronary artery should be obtained. Recommended projections are as follows:

PA 0 – 30º Cranial: Best for visualization of the mid and distal RCA

Lateral: 90º (straight lateral): Best for visualization of the proximal RCA

6.15.6 Coronary Flow Reserve (Optional Participation, Center Dependent)

A 014" coronary flow wire will be inserted into the left coronary catheter and manipulated into the distal left anterior descending coronary artery or left circumflex coronary artery. Coronary Flow Reserve will be performed in the distal LAD/LCx after administration of intracoronary adenosine (dose range 3-12 mcg based on patient size). Repeat administration will be performed until a maximal response is achieved with a maximum adenosine dose of 12 mcg. Repeat measurements can be made in the mid and proximal vessel if the response in the distal vessel is blunted or seems submaximal. A normal study will be defined as a doubling of coronary flow during adenosine induced hyperemia.

6.15.7 IVUS (Optional Participation, Center Dependent)

The IVUS catheter will then be advanced over the flow wire into the mid-LAD/LCx or distal LAD/LCx, using the ultrasound to guide advancement. IVUS catheter advancement will only occur over the flow wire only and the advancement will stop prior to occlusion of the lumen of the vessel. Using the track-back device the IVUS catheter will be pulled back at 1.0mm/s along the course of the coronary artery (temporal resolution of 30 frames per second). A suitable length of vessel will imaged to allow analysis of at least 15 cross-sectional images evenly spaced (at ≈1.5-mm intervals in mid-diastole) The IVUS catheter and left coronary artery guider catheter will be removed.

We will use semi-automatic interactive edge detection software (QIVUS) which has been shown to improve reproducibility of measurements, with a very low inter-observer variability[27]. Maximal intimal thickness, mean intimal index, eccentricity index, and Stanford grade will be recorded. Mean intimal index is the ratio of the mean intimal area to the sum. The eccentricity index is the difference between maximal and minimal intimal thickness divided by the maximal intimal thickness.

6.15.8 Grading of Cardiac Allograft Vasculopathy (CAV)

Classification of coronary disease will be based on angiograms, as angiographic assessment of CAV was used for the development of the MATE score. We will use the ISHLT classification[5] with modification of restrictive physiology as described by Kindel et al.[28].

Grade	Description

CAV_0 (Not significant)	No detectable angiographic lesion
CAV_1 (Mild)	Angiographic left main (LM) < 50%, or primary vessel with maximum lesion of < 70%, or any branch stenosis < 70% (including diffuse narrowing) without allograft dysfunction
CAV_2 (Moderate)	Angiographic LM < 50%; a single primary vessel ≥ 70%, or isolated branch stenosis ≥ 70% in branches of 2 systems, without allograft dysfunction
CAV_3 (Severe)	Angiographic LM ≥ 50%, or two or more primary vessels ≥ 70% stenosis, or isolated branch stenosis ≥ 70% in all 3 systems
Functional Upgrading	
Upgraded to CAV_3	Angiographic evidence of CAV_1 or CAV_2 **PLUS** Evidence of systolic dysfunction with LVEF ≤ 45% **OR** Restrictive hemodynamics (RAP > 12 mmHg or PCWP > 15 mmHg) **OR** Cardiac index < 2.1 mL/min/m2

Definitions

a). A "Primary Vessel" denotes the proximal and Middle 33% of the left anterior descending artery, the left circumflex, the ramus and the dominant or co-dominant right coronary artery with the posterior descending and posterolateral branches.

b). A "Secondary Branch Vessel" includes the distal 33% of the primary vessels or any segment within a large septal perforator, diagonals and obtuse marginal branches or any portion of a non-dominant right coronary artery.

6.16 Treatment of Acute Cellular Rejection

Treatment for ACR is at the ultimate discretion of the individual transplant clinicians. However, the following treatment guidelines should be followed when possible:

ACR Biopsy Grade (1990/2004[4, 29])	Initial Treatment Recommendation
0	None
1A/1R	None; Optimize IS trough levels
1B/1R	None; Optimize IS trough levels
2/1R	None; Optimize IS trough levels
2R	Steroids; Repeat biopsy in 2-6 weeks
3R	IV Steroids; Consider ATG; Repeat biopsy in 2-6 weeks

Hemodynamically Significant Rejection Definition	Need for IV inotropes, MCS;
HD Treatment	IV Steroids; Consider ATG, plasmapheresis; Repeat biopsy in 1-6 weeks

6.17 Treatment of Antibody Mediated Rejection

Treatment for acute cellular rejection is at the ultimate discretion of the individual transplant clinicians. However, the following guidelines for when to treat should be followed when possible:

AMR Biopsy Grade[8]	Treatment Specification
0	None
pAMR 1 (H+ or I+)	No treatment recommended; if DSA present or evidence of allograft dysfunction, treatment can be considered
pAMR 2	Recommend treatment, see table for options
pAMR 3	Recommend treatment, see table for options
Any pAMR wiith HD Significant Rejection*	Recommend treatment, see table for options

*Definition: Need for inotropes or mechanical circulatory support

Treatment Options for Antibody Mediated Rejection (Adapted from Colvin *et al*[30])

Therapeutic Modality	Dose	Frequency	Duration
Corticosteroids	Oral: 1–3 mg/kg IV: 250–1000 mg	Daily	3 d
IVIg	1–2 g/kg in 2–4 divided doses	1–3 Times weekly	Variable
Tissue plasma exchange (plasmapheresis)	1–7 Sessions/wk, 1–4 weekly cycles Exchange 1–2 times the blood volume with FFP or albumin as replacement	1–7 Sessions	Variable
Photopheresis	Oral: 0.6 mg/kg (target level ≥50 ng/mL 2 h after ingestion) or 25 mg/m^2	Variable	Up to 6 mo
Monomurab (OKT3)	5 mg (or 2.5 mg for ≤30 kg) IV	Once daily	10–14 d
Rabbit ATG (thymoglobulin)	0.75–1.5 mg/kg IV	Daily	5–7 d
Equine anti-thymocyte globulin (ATGAM)	10–15 mg·kg^{-1}·d^{-1} IV	Daily	5–7 d

Rituximab	375 mg/m^2 weekly	Weekly	1–4 wk
Alemtuzumab	Prophylaxis: 30 mg IV on day 1 and 4, 20 mg IV postoperatively within 24 h of transplantation, or 30 mg IV Treatment: 20 mg IV or 30 mg SQ	Once	…
Bortezomib	1.3–1.5 mg/m^2	Daily	4 doses
Eculizumab	600–900 mg IV	Every 7–14 d	Until desired response
Mycophenolate	Adult: 1.5 g PO or IV BID Pediatric: 600 mg/m^2 to maximum 2 g or 10 mL	Twice daily	Indefinite
Cyclophosphamide	0.5–1 g/m^2 every 3 wk for 4–6 mo (with rituximab and plasmapheresis) 2 mg/kg IV for 1 mo, then monthly for 2 mo	Every 3 wk Monthly	4–6 mo 2 mo
Total lymphoid irradiation	80 cGy (1 cGy=1 rad) twice weekly over 5 wk to achieve a cumulative dose of 8 Gy	Twice weekly	Over 5 wk (cumulative dose of 8 Gy)

6.18 Event Driven Endpoint Assessment

If the endpoint event screen reveals that a possible endpoint event has occurred, and endpoint event form will be completed and clinical information about the event will be recorded. This information will be used for adjudication of endpoint events.

6.19 Treatment of Isolated Donor Specific Antibody without Evidence of Antibody Mediated Rejection

Clinical screening for donor-specific antibody should take place at the time of transplant and at 6, 12, 24 and 36 months post-transplantation. If donor-specific antibody appears as part of this screening, clinicians should determine if the patients have any evidence of antibody mediated rejection. This may be accomplished by endomyocardial biopsy and invasive hemodynamic assessment. Alternatively, if the patient is asymptomatic, a non-invasive assessment can be performed (ECG, echocardiogram, laboratory studies, physical examination). The strength of any detected donor-specific antibody should be determined by performing serial dilutions (titers) to determine the detection limit. Recommended titers are NEAT, 1:8, and 1:32. In addition, if C1q testing is available at your local histocompatibility lab, it is recommended that this test be run to help risk stratify the antibody. In patients with DSA but no pAMR (DSA+; pAMR-), we would recommend closely following clinical status and retesting for the presence of DSA within 3 months of initial positive testing. No treatment is recommended for isolated DSA, nor is it precluded by protocol.

If the endpoint event screen reveals that a possible endpoint event has occurred, and endpoint event form will be completed and clinical information about the event will be recorded. This information will be used for adjudication of endpoint events.

6.20 CMV Prophylaxis and Treatment Algorithm

CMV is a common cause of morbidity and mortality following heart transplantation. In particular, CMV disease is associated with development of cardiac allograft vasculopathy (CAV). Serologic status has also been associated with CAV, mostly in CMV naïve recipients. There is suggestion that EVL may allow for better immune control of CMV in transplant recipients. Valganciclovir and ganciclovir are well tolerated and effective antiviral treatments against CMV. We recommend risk stratification of patients based on donor/recipient serologic testing and prophylactic treatment of CMV as outlined above. Serologic testing in young infants can be unreliable as testing may reflect maternal CMV exposure. For young infant donors and recipients, we recommend that recipients be stratified into one of the three groups with the help of an infectious disease consultant.

6.20.1 CMV Definitions:

Adapted from Kotton 2013[31], Humar 2006[32]

- *CMV infection:* CMV antibody positivity (IgG) and/or viral detection by viral culture, urine or saliva shell vial assay, PCR testing/ antigenemia. Any CMV-negative recipient who is transplanted from a CMV-positive donor by definition will become CMV-infected through the donor organ.
- *CMV replication:* Asymptomatic detection of CMV viremia as measured by quantitative PCR/antigenemia.
- *CMV disease:* Detection of virus and observation of clinical signs and symptoms
    - *CMV Syndrome:* CMV infection with systemic symptoms such as fever, leukopenia, thrombocytopenia, muscle pain, malaise
    - *CMV Disease with Organ/Tissue Involvement:* CMV infection and/or CMV syndrome with the addition of organ involvement (hepatitis, pneumonitis, pancreatitis, colitis, retinitis, or meningoencephalitis).

Abbreviations for CMV IgG Serologic Status before Transplant

- D-: donor CMV negative
- D+: donor CMV positive
- R-: recipient CMV negative
- R+: recipient CMV positive

We recommend that recipient serologic testing be repeated immediately prior to transplantation.

6.20.2 CMV Risk Stratification

Risk Group	Serologic Results	Recommended Length of Prophylaxis post-ATG
Low	D-/R-	None
Intermediate	D+ OR D-/R+	3-6 months
High	D+/R-	6 months*

*Recommend continuation of prophylaxis until subjects have been randomized, are on treatment, and have achieved therapeutic drug levels.

6.20.3 Valganciclovir Dosing:

CrCl (ml/min/1.73m2)	<30 kg	30-50 kg	>50 kg
>60	15 mg/kg/dose PO daily	450 mg PO daily	900 mg PO daily
40-59	7.5 mg/kg/dose PO daily	225 mg PO daily	450 mg PO daily
25-39	7.5 mg/kg/dose PO every other day	225 mg PO every other day	450 mg PO every other day
10-24	7.5 mg/kg/dose PO twice a week	225 mg PO twice a week	450 mg PO twice a week

It is recommended that high and intermediate risk patients should receive IV ganciclovir 5 mg/kg IV daily until they are tolerating enteral medications, at which point they should be switched to PO valganciclovir. Dosing may be delayed for up to 24 hours to determine level of renal function/dysfunction following heart transplant surgery.
*If patients receive a second course of **ATG** for refractory rejection, it is recommended prophylaxis be restarted. Duration should be guided by donor/recipient serologic status as specified above.*

6.20.4 Neutropenia

Full dosing of antiviral agents in patients with neutropenia should be continued. Discontinuing or changing other bone marrow-suppressive medications may be helpful. Antiviral dose should not be reduced in patients with neutropenia as this will lead to subtherapeutic dosing and associated resistance. In patients with significant neutropenia thought to be associated with antiviral agent, consider discontinuation of antiviral and CMV PCR surveillance.

Patients who require discontinuation of antiviral prophylaxis due to toxicities should have quantitative CMV PCR monitoring every 1-2 weeks so that preemptive therapy may be initiated in the event of rising CMV viral loads.

6.20.5 Monitoring after prophylaxis is completed

For **intermediate** and **high risk** patients, it is recommended that CMV PCR be monitored monthly for 6 months following completion of prophylaxis.

For **any** patient, CMV PCR and/or serologic testing should be considered with any febrile illness or if considering an additional course of ATG.

6.21 HMG Co-A Reductase Inhibitors

Pravastatin has been demonstrated to reduce the incidence of CAV in adult heart transplant recipients[33] and several studies have suggested benefit of statin therapy in children[34, 35]. Given the hyperlipidemia that can be seen with EVL, we recommend that all patients should be on pravastatin. All subjects should be placed on pravastatin or atorvastatin once they turn 8 years old and we recommend starting all patients greater than 2 years of age on pravastatin. Monitoring with creatine kinase (CK) prior to, and 6 weeks after, initiation of statin therapy is recommended. However, each center should adhere to a common protocol with regard to statin management. Dosing is at the discretion of the individual clinician.

6.22 Management of EBV Viremia

No recommendations.

6.23 PTLD Management

There is no recommended treatment protocol for PTLD. Treatment will be at the discretion of the treating clinician. If discontinuation of TAC, EVL, or MMF will be part of the treatment plan, please contact one of the Trial Co-Chairs to discuss plans. When safe and feasible, adherence to the study maintenance immunosuppression protocol is recommended.

6.24 Guideline for Converting Children to Everolimus/Low-Dose Tacrolimus

The following steps are recommended for conversion of subjects to EVL/LDTAC:

Everolimus Initial Starting Dose	
BSA (m^2)	Dose
< 0.6	0.25 mg PO Q12h
0.6 – 0.9	0.5 mg PO Q12h
0.9 – 1.2	0.75 mg PO Q12h
1.2 – 1.5	1 mg PO Q12h
> 1.5	1.25 mg PO Q12h

After first dose of EVL, stop MMF.

Check level of EVL prior to sixth dose. If EVL level is greater than 3 ng/mL, adjust TAC to achieve a target trough level of 3-5 ng/mL per maintenance protocol below. If EVL level is > 8 ng/mL, adjust dose per maintenance adjustment recommendations. If EVL level is less than 3 ng/mL, increase the EVL dose, do not change the TAC dose, and repeat an EVL level and TAC level after six additional doses.

If EVL dose is changed it is important to check TAC level in 1-4 days as levels may vary secondary to EVL dose change.

6.25 Surgery Clinical Guideline

EVL is known to impair wound healing. If a subject assigned to the EVL arm requires elective surgery, it is recommended that EVL be discontinued in the perioperative period according to the following clinical guideline. EVL trough levels should be checked to ensure that they remain in the target range.

The planned surgery should be discussed directly with the surgeon. If, in the judgment of the surgeon, the expected surgical wounds are small and likely to heal well despite the known anti-proliferative effects of EVL, consideration may be given to continuing EVL throughout the perioperative period. Surgical techniques which may improve wound healing include placing closed suction drains, use of subcutaneous sutures, sealing or ligating lymphatic ducts, performing prophylactic peritoneal fenestration and avoiding extensive dissection. The surgeon should consider suture selection carefully in order to maximize apposition of the wound and minimize the risk of wound dehiscence. Surgical staples may be left in place for 3-4 weeks to prevent dehiscence. Depending upon its performance characteristics, absorbable suture may or may not maintain adequate tension.

For all other elective surgical procedures:

1. Discontinue EVL 7-10 days prior to the date of the planned procedure.
2. If most recent TAC trough level was within the goal range of 2.5-5 ng/mL, increase the TAC dose by 50-100% one dosing interval after the EVL is discontinued in order

to achieve a goal TAC trough level of 5-8 ng/mL (after 1 year post-transplant) or 7-10 ng/mL (before 1 year post-transplant).

3. Start a secondary immunosuppression drug 24-48 hours after EVL has been discontinued. Mycophenolate mofetil dosed at 600 mg/m2/dose q12h is the recommended secondary immunosuppression. However, the investigator may elect to use any secondary immunosuppression drug (i.e. azathioprine, enteric coated mycophenolic acid, etc.), or elect to use TAC monotherapy for the perioperative period.
4. TAC drug trough levels should be checked 3-7 days after EVL discontinuation and the dose adjusted to achieve the goal trough level specified in step #2. Levels should be repeated over this interval based on the investigator's discretion.
5. If the subject must be NPO prior to the surgical procedure, the subject, parent, or investigator should discuss whether it is safe to take the immunosuppression medications with a small sip of water during the NPO period. If this is not allowed, then the immunosuppressive medications should be taken with a small sip of water once the subject is clinically able following anesthesia.
6. The surgical wounds should be assessed 7-14 days following the surgical procedure. If there are no concerns with wound healing, the secondary immunosuppression agent should be discontinued on post-operative day 14 and the TAC dose should be reduced to the prior dose. EVL should be resumed at the prior dose 1-2 dosing interval(s) after the secondary immunosuppression medication is discontinued and/or the TAC is dose reduced. If the surgeon is concerned about inadequate wound healing, resumption of EVL therapy can delayed for up to an additional 7 days.
7. Trough levels of TAC and EVL should be checked at the investigator's discretion after prior dose has been resumed.

If a patient who is being treated with EVL therapy requires urgent or emergent surgery, EVL should be held immediately. Emergency surgery should not be delayed in order to allow EVL concentrations to decline. The investigator should alert the surgeon that patients taking EVL are known to have issues with impaired wound healing as it may affect the procedural approach. The above clinical guideline can be followed in order to minimize EVL exposure during the post-operative recovery period following an urgent/emergent surgical procedure.

If the investigator has any questions regarding peri-operative immunosuppression, they should contact one of the Trial Co-Chairs.

6.26 Management of Proteinuria

Patients with moderate range proteinuria during screening are not eligible for enrollment in the study (see **Section 4.2 Exclusion Criteria**). All patients will have a urine protein to urine creatinine ratio measured at screening (Visit 1) and at Visits 3, 6, and 8 if able to provide a sample. Patients who have a urine protein to urine creatinine ratio above normal (> 0.2 mg protein per mg creatinine in children at least 2 years of age and > 0.5 in children below 2 years of age) should have a urinalysis with microscopic exam performed and a repeat urine protein to urine creatinine ratio should be repeated on a first morning void.

Subjects with a first morning urine protein to urine creatinine ratio above normal should have a repeat first morning sample obtained. If the second sample confirms proteinuria the following steps should occur (The guidelines below apply to children ≥ 2 years old, younger children have more physiologic proteinuria, please use clinical judgment and/or consult a pediatric nephrologist for younger children.):

If the repeat protein/creatinine ratio is ≥2 mg/mg, the patient should be referred to pediatric nephrologist, a SAE form should be completed, and the investigator should contact one of the Trial Co-Chairs. If the subject is taking EVL, strong consideration should be given to discontinuation of EVL and crossover to the TAC/MMF treatment.

If the repeat protein/creatinine ratio is ≥1 mg/mg but <2 mg/mg, the investigator should contact one of the Trial Co-Chairs and referral to a pediatric nephrologist is recommended if feasible. It is recommended that the first morning urine samples should be obtained monthly and sent for protein/creatinine ratios. The following management strategy should be considered (in collaboration with a pediatric nephrologist if referred):

1. Treating proteinuria with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, particularly if hypertensive. The goal protein/creatinine ratio should be <0.5 mg/mg.
2. Lifestyle interventions to treat obesity (diet and exercise).
3. Initiation of statin therapy (i.e. pravastatin) in all subjects with dyslipidemia. (The study protocol recommends that all study subjects over the age of 8 years old be placed on a statin, regardless of lipid profile.)
4. Dietary sodium and protein restriction.

If the repeat protein/creatinine ratio is <1 mg/mg, the following management strategy should be considered:

1. Treating proteinuria with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, particularly if hypertensive. The goal protein/creatinine ration should be < 0.5 mg/mg.
2. Lifestyle interventions to treat obesity (diet and exercise).
3. Initiation of statin therapy (i.e. pravastatin) in all subjects with dyslipidemia. (The study protocol recommends that all study subjects over the age of 8 years old be placed on a statin, regardless of lipid profile.)
4. Dietary sodium and protein restriction.

After appropriate management has been initiated, the urine protein to urine creatinine ratio should be repeated. If the degree of proteinuria continues to increase and the urine protein to urine creatinine ratio exceeds 1 mg/mg then referral to a pediatric nephrologist should be considered and consultation with the Trial Co-Chairs is recommended. Subjects who are taking EVL who have nephrotic range proteinuria or persistent urine protein to creatinine ratios above 1 may need to withdraw EVL treatment after consultation with the Trial Co-Chairs. As in all cases where EVL must be withdrawn, an appropriate change to the immunosuppression regimen is required so as to maintain an equivalent level of immunosuppression. The most likely recommendation will be conversion to standard dose TAC and MMF.

6.27 Management of Stomatitis

The development of oral ulcerations is an expected complication of EVL therapy and occurs in 5-20% of patients (though some reports suggest that as many of 60% of patients are affected at one time or another) receiving the medication at the doses that will be used in this study. The incidence of stomatitis may be slightly higher with oral suspensions of EVL as they can be directly absorbed by the oral mucosa. The ulcers are generally painful, ovoid, and superficial with a greyish-white center and erythematous margin. They generally do not form on the gingiva or dorsum of the tongue but tend to affect the inner lip, soft palate, and ventral/lateral tongue. Somatitis is generally not severe and it is important not to discontinue therapy prematurely when patients experience this side effect. The recommended management of stomatitis is as follows:

1. Maintain good oral hygiene with gentle brushing and a soft toothbrush. Use a mild toothpaste (without sodium lauryl sulfate). Use salt and/or baking soda rinses to sooth the ulcers.
2. Avoid spicy, acidic, hot, or hard foods which can increase pain of ulcers.
3. Avoid harsh oral agents such as peroxide, antifungals, alcohol, and iodine.
4. Topical high-potency steroids such as clobetasol and dexamethasone can be used to promote healing and for symptom relief.
5. Topical anesthetics such as viscous lidocaine can be used to reduce pain.
6. Topical magnesium hydroxide/aluminum hydroxide (i.e. Maalox) can be used to promote symptom relief.
7. Multiple agents may be combined in a “magic mouthwash” for ease of administration.
8. Check EVL levels to ensure that trough level is not above the goal range of 3-8 ng/mL.

If stomatitis remains severe despite topical treatment and EVL trough levels remain within goal range, consideration can be given to EVL dose reduction in order to target the lower end of the goal range (3-5.5 ng/mL). If stomatitis persists, contact the trial principal investigator to discuss the next step in treatment. In severe, resistant cases, this may involve EVL discontinuation and conversion to standard dose TAC and mycophenolate mofetil in consultation with the PI. If lesions persist despite EVL withdrawal, referral to otolaryngology to evaluate for PTLD and/or other forms of cancer is warranted.

6.28 Management of Hypertriglyceridemia

Hypertriglyceridemia is a known side effect of EVL therapy and occurs in approximately 20% of patients on EVL and low dose calcineurin inhibitor. Hypertriglyceridemia is defined as TG $\geq$ 100 mg/dL (1.1 mmol/L) if under 10 years old or TG $\geq$ 130 mg/dL (1.5 mmol/L) in children above 10 years old. It is recommended that all subjects with hypertriglyceridemia be counseled regarding lifestyle modifications including:

1. Encouraging physical activity to include 30 minutes of aerobic exercise 5 days per week.
2. A low saturated fat diet with reduced intake of simple carbohydrates.

3. If overweight or obese, participation in a structured nutrition/wellness program designed to facilitate healthy eating habits, physical activity, and weight loss.
4. High dose omega-3 fatty acid supplementation (dose 2 to 4 g per day). Store in the refrigerator to help with taste/smell.

Subjects with persistently elevated TG levels greater than 500 mg/dL (5.6 mmol/L) or single TG levels greater than 1000 mg/dL (11.3 mmol/L) are at risk for acute pancreatitis and treatment with fibrates or niacin should be initiated. If a lipid specialist is available, subjects should be referred for consultation. Fibric acid medications interact with statin medications. In addition there is a higher risk of myopathy and rhabdomyolysis in patients with chronic kidney disease. All transplant patients on these medications should have CK levels monitored. A transplant pharmacist should be consulted if these interactions exist.

If severely elevated TG levels greater than 1000 mg/dL (11.3 mmol/L) persist despite treatment, contact a trial Co-Chair to discuss the next step in treatment. In cases of severe hypertiglyderidemia, EVL discontinuation and conversion to standard dose TAC and mycophenolate mofetil may need to be considered in consultation with the PI.

6.29 Management of Dyslipidemia

The study protocol recommends starting pravastatin in all patients greater than 2 years of age and pravastatin or atorvastatin in all patients greater than 8 years of age. Monitoring of CK prior to, and 6 weeks after, initiation of pravastatin or atorvastatin is recommended for all patients. If patients develop hypercholesterolemia during the study and they are not on a statin therapy, initiation of statin therapy is recommended. In addition the patient/family should be counseled on weight reduction, increased physical activity, smoking cessation for all occupants of the household, and promoting a healthful diet. In cases of severe dyslipidemia, EVL discontinuation and conversion to standard dose TAC and MMF may need to be considered in consultation with one of the Trial Co-Chairs.

6.30 Management of Gastrointestinal Symptoms Related to MMF

MMF is known to cause an enteropathy which can lead to nausea, vomiting, abdominal pain, diarrhea, and/or weight loss. At times this requires dose reduction or discontinuation of MMF. If MMF-related enteropathy is confirmed or suspected by the investigator, MMF should first be either dose reduced and/or held for 1-3 days. MMF can then be resumed at a lower dose. If symptoms persist despite MMF dose reduction(s), then conversion to enteric coated mycophenolic acid can be performed. For the purposes of the study this will be considered an equivalent drug and a per protocol treatment for patients assigned to TAC/MMF. If symptoms persist, referral to a gastroenterologist is recommended. In cases of severe gastrointestinal symptoms, MMF discontinuation and conversion to an alternate secondary immunosuppression (i.e. azathioprine) or conversion to EVL/LDTAC may need to be considered in consultation with one of the Trial Co-Chairs.

7 Safety and Adverse Events

Subjects must be carefully monitored for AE's both to protect individual human subjects enrolled in the trial and to provide a complete and accurate assessment of the safety profile of the investigational drug. All AE's will be collected in this study from the time of consent until 30 days after the subject's participation has ended (i.e. completion of study or withdrawal of consent). The definition of AEs and reporting procedures are summarized below.

7.1 Definitions

7.1.1 Adverse event

An AE is any untoward medical occurrence (symptom, sign, illness or experience) associated with the use of a drug in humans, whether or not it is considered drug-related. An AE may be a new occurrence of a medical condition or worsening of a pre-existing medical condition.

7.1.2 Serious Adverse Event

A serious adverse event (SAE) includes any event that results in any of the following outcomes

- Death
- Life-threatening i.e., the subject was at immediate risk of death from the event as it occurred in the opinion of the Investigator. It does not refer to an event, which hypothetically might have caused death if more severe.
- Persistent or significant incapacity/disability
- Requires hospitalization or prolongs hospitalization (Note: a pre-scheduled hospitalization is not considered an SAE nor are hospital stays less than 12 hours in duration). Hospitalization unrelated to the AE is excluded (e.g. social hospitalization such as a parent/family unable to find transportation during the night).
- Congenital anomaly/birth defect
- Any other medically important event, in the opinion of the clinical site PI, that jeopardizes the subject and may require medical or surgical intervention to prevent one the outcomes listed above. Examples of other medically significant events include allergic bronchospasm requiring intensive treatment in an emergency department or home, laboratory abnormalities that do not result in hospitalization.

The following are not considered SAEs in this study

- A visit to the emergency department or other hospital area for <24 hours duration that does not result in admission (unless it meets criteria for a medically important or life-threatening event).
- Admissions per routine clinical routine at the study site for a planned medical/surgical procedure (e.g. if a study site routinely admits to the hospital after routine cardiac catheterization post-HT that would not qualify as a SAE nor would admissions for

intravenous gamma globulin (IVIG) infusions for sensitized patients unless the IVIG is being given to treat a drug related SAE).
- Elective surgery planned prior to signing study consent (e.g. gastrostomy or tracheostomy tube removal, routine dental procedure).
- Admission for social circumstance that carries no bearing on health status and requires no medical or surgical intervention (e.g. lack of transportation, unable to safely transport child/family because of inclement weather, family situation or administrative circumstances).

7.1.3 Unanticipated Problems (UPs) Involving Risk to Subjects or Others

All AEs will be evaluated as to whether their occurrence was or whether it was not anticipated to occur. The purpose of this reporting is to provide new, important information on serious reactions or events previously unobserved or undocumented.

7.1.3.1 Unanticipated Events

Any incident, experience, or outcome is considered unanticipated if it meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB/REB approved protocol or consent/assent form, the Investigators brochure, the Physician's Desk Reference, the FDA approved drug label, or commonly used drug databases such as Micromedex)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

7.1.3.2 Expected Events

An event is considered expected if it is known to be associated with the underlying disease process (heart transplantation) or with a study drug that the subject is taking. For this protocol, some of the drug-related expected events are listed in **Table 7-1-3-2** below. Table 7-1-3-2 should not be considered exhaustive. If an AE is listed for the study drug in the FDA-approved drug label or a commonly used drug database (Micromedex, Lexi-Comp) then it is considered expected.

Table 7-1-3-2. Drug related AEs

Drug	Expected AEs		
Everolimus	• Acne • Anemia • Angioedema • Asthenia • Constipation • Cough • Decreased appetite • Diabetes mellitus • Diarrhea • Edema • Elevated ALT/SGPT • Elevated AST/SGOT • Fatigue	• Hemolytic uremic syndrome • Hepatitis • Hypercholesterolemia • Hyperlipidemia • Hypertension • Hypertriglyceridemia • Impaired wound healing • Infection • Interstitial lung disease • Kidney dysfunction • Lymphedema • Lymphopenia • Mouth sores	• Nausea • Pericardial effusion • Pleural effusions • Pneumonitis • Proteinuria • PTLD • Renal failure • Seizure • Septic shock • Thrombocytopenia • Thrombotic microangiopathy • Vomiting
Tacrolimus	• Acute renal failure • Acute respiratory distress syndrome • Alopecia • Anaphylaxis • Anemia • Arrhythmia • Constipation • Diabetes mellitis • Diarrhea • Edema • Erythema of the skin • Headache • Hemolytic uremic syndrome	• Hepatitis • Hepatotoxicity • Hyperkalemia • Hypertension • Hypomagnesaemia • Infection • Insomnia • Kidney dysfunction • Lung dysfunction • Lymphopenia • Nausea • Nephrotoxicity	• Paresthesia • Posterior reversible encephalopathy syndrome • Problems with wound healing • Pruritus • PTLD • Rash • Seizure • Septic shock • Thrombocytopenia • Tingling/numbness • Tremor • Vomiting
MMF	• Abdominal pain • Anemia • Anxiety • Asthenia • Backache • Birth defects • Constipation • Cough • Diarrhea • Dizziness • Dyspnea • Edema • Fatigue • Gastric ulcer	• Gastrointestinal hemorrhage • Gastrointestinal perforation • Headache • Hypercholesterolemia • Hyperglycemia • Hypertension • Hypocalcemia • Hypokalemia • Hypomagnesaemia • Infection • Insomnia • Intestinal bleeding • Kidney dysfunction	• Lung disease • Lymphopenia • Nausea • Neutropenia • Paresthesia • Pleural effusion • Progressive multifocal leukoencephalopathy • PTLD • Pulmonary fibrosis • Septic Shock • Thrombocytopenia • Tremor • Vomiting

7.1.4 Non-serious adverse events

All AEs that do not meet any of the criteria for serious should be regarded as *non-serious adverse events*.

7.1.5 Suspected adverse reaction

A suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. Reasonable possibility means there is evidence to suggest a causal relationship (possibly or definitely related) between the drug and the AE for IND safety reporting purposes. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

7.1.6 Adverse reaction

An adverse reaction means any AE for which there is a greater degree of certainty that the drug caused the AE. There is evidence to suggest a definite causal relationship (definitely related) between the drug and the AE for IND safety reporting purposes. Adverse reactions are a subset of suspected adverse reactions.

7.1.7 Adverse Event Reporting Period

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

7.1.8 Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period. At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

7.1.9 Post-Study Adverse Event

The local site PI should follow all unresolved AEs until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the local site PI should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The local site PI

should notify the DCC of any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The DCC should also be notified if the local site PI should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

7.1.10 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

7.1.11 Hospitalization, Prolonged Hospitalization or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow for routine efficacy measurement for the study (i.e. observation overnight after anesthesia for a routine cardiac catheterization).
- Hospitalization or prolonged hospitalization for standard therapy of HT (i.e. admission for rituximab or IVIG infusion), unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical site PI.

For all AEs, including serious and non-serious AEs, the diagnosis of the underlying illness or disorder should be recorded if known, rather than the individual symptoms (e.g. CMV disease rather than fever or diarrhea). If the underlying diagnosis is unknown, the symptom complex should be reported (e.g. fever and diarrhea).

7.1.12 Severity Criteria (Grade 1-5)

All AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0).

Grade (severity)	Definition
Grade 1 (Mild AE)	Experiences which are usually transient, requiring no special treatment, and not interfering with the subject's daily activities
Grade 2 (Moderate AE)	Experiences which introduce some level of inconvenience or concern to the subject, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic measures
Grade 3 (Severe AE)	Experiences which are unacceptable or intolerable, significantly interrupt the subject's usual daily activity, and require systemic drug therapy or other treatment
Grade 4 (Life-threatening or disabling AE)	Experiences which cause the subject to be in imminent danger of death
Grade 5 (Death related to AE)	Experiences which result in subject death

7.1.13 Causality (Attribution)

The site clinical PI is to assess the causal relation (i.e, whether there is a reasonable possibility that the study drug caused the event) using the following definitions:

Relatedness	Definition
Not Related	Another cause of the AE is more plausible; a temporal sequence cannot be established with the onset of the AE and administration of the investigational product; or, a causal relationship is considered biologically implausible
Unlikely Related	The current knowledge or information about the AE indicates that a relationship to the investigational product is unlikely
Possibly Related	There is a clinically plausible time sequence between onset of the AE and administration of the investigational product, but the AE could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the investigational product is one of several biologically plausible AE causes.
Related	The AE is clearly related to use of the investigational product.

7.2 Recording of Adverse Events

All AEs occurring after the subject, parent, guardian, and/or LAR has signed the Informed Consent Form (ICF) must be fully recorded in the subject's hospital record and electronic case report form (eCRF) from the time of ICF signing and dating to 30 days after study completion, termination, or

withdrawal. At each contact with the subject, the local site PI must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document, and also in the appropriate AE module of the eCRF. All clearly related signs, symptoms, laboratory investigations and abnormal diagnostic procedures results should be recorded in the source document, and should be grouped under one diagnosis. Documentation must be supported by an entry in the subject's file.

Each event should be described in detail along with start and stop dates, seriousness, severity, relationship to investigational product, action taken and outcome. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

7.3.1 Local Site PI reporting: notifying the Data Coordinating Center (DCC)

SAEs and UPs must be reported to the DCC at Boston Children's Hospital within 24 hours of the local site PI's awareness of the event and include all SAEs and UPs that occur from the time of ICF signing and dating to 30 days after study completion. To report such events, an AE form denoting the serious and/or unanticipated nature of the event must be completed electronically by the local site PI and submitted to the DCC within 24 hours. The local site PI will keep a copy of this SAE information on file at the study site. SAEs and UPs must also be reported to the study clinical research associate (CRA), medical monitor, designee of the study CRA, or designee of the medical monitor by phone and/or e-mail with confirmation of receipt. Table 7-3-1 summarize reporting timelines for local site PIs.

Table 7-3-1. Reporting Timeline for Clinical Centers to Notify DCC

Classification	Reporting Timeframe
Serious	Within 24 hours of first knowledge of the event
Unanticipated Problem	Within 24 hours of first knowledge of the event
Important Medical Event (Pregnancy)	Within 24 hours of first knowledge of the event
Not Serious	Within 7 calendar days of first knowledge of the event

Within the following 48 hours, the local site PI must provide further information on the SAE or the UP in the form of a written narrative to the DCC. This should include a copy of the completed Serious Adverse Event (SAE) form and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs should be provided promptly to the DCC. The Medical Monitor or his designee will review all SAEs post-randomization within 2 working days of the report receipt in order to assign a final determination on expectedness and relatedness to drug.

All SAEs and UPs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to ≤ CTCAE grade 1
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow up after demonstration of due diligence with follow-up efforts)
- The subjects starts another heart transplant rejection prevention regimen

All deaths should be reported with the primary cause of death as the AE term, rather than death itself, which is typically the outcome of the event. If a death occurs within 30 days of study completion, the death must be reported as an SAE. An isolated laboratory abnormality that is assigned grade 4, according to CTCAE definition, is not reportable as an SAE unless the Investigator assesses that the event meets standard International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH) criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as a SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria. If the local site PI is in doubt about the applicable reporting obligations, he/she should consult with the study CRA, medical monitor, the designee of the CRA, or the designee of the medical monitor.

The medical monitor or designee will assess each AE to determine whether it is unexpected according to the ICF, Protocol Document, medication package insert, or commonly used drug database (i.e. Micromedex, Lexi-Comp).

Pregnancy

Before study enrollment, subjects must agree to take appropriate measures to avoid pregnancy. As MMF, a treatment in the control arm, is a known teratogen, each female subject or their LAR must complete Risk Evaluation and Mitigation Strategies (REMS) training. It is recommended that any sexually active female use two forms of birth control to prevent pregnancy. A female subject must immediately inform the Investigator if she becomes pregnant from the time of consent to 30 days after study completion. However, should a pregnancy occur in female study subject, consent to provide follow-up information regarding the outcome of the pregnancy and the health of the infant until 30 days old will be requested.

Although pregnancy itself is not regarded as an AE, it is considered an Important Medical Event, and the outcomes will need to be documented. Any pregnancy occurring in a subject or subject's partner from the time of consent to 30 days after the last dose of study drug must be reported. Any occurrence of pregnancy must be reported to the IND Sponsor, or designee, per SAE reporting timelines. All known pregnancies will be followed for outcome, which is defined as elective termination of the pregnancy, miscarriage, or deliver of the fetus. For pregnancies with an outcome of liver birth, the newborn infant will be followed until 30 days old and will need to be reported to

the IND Sponsor by SAE reporting timelines. Any congenital anomaly/birth defect noted in the infant must be reported as a SAE.

7.3.2 Expedited Reporting Requirement for Serious Adverse Events

All AEs that are drug-related and either (1) unanticipated (SUSAR) or (2) fatal or life threatening will be reported by the DCC to the IRB/REB, the FDA, Health Canada, the funding agency (U.S. Department of Defense) and the Chair of the Data and Safety Monitoring Board (DSMB) within 7 calendar days of report receipt by the DCC. In addition, important medical events such as pregnancy will be reported to regulatory bodies within 15 calendar days of report receipt. Expedited reporting is not required for other AEs. Table 7-3-2 summarizes the reporting timelines for all events by the DCC to regulatory bodies.

Table 7-3-2. Reporting Timeline for the DCC to Regulatory Bodies

Classification	Unexpectedness	Relatedness*	Reporting Timeframe
Serious: Fatal life-threatening	Unexpected	Related	Within 7 calendar days of report receipt to IRB, FDA, DSMB, and Health Canada
Serious: Fatal life-threatening	Unexpected or Expected	Unrelated	Semi-annually to DSMB and DoD; Annually to FDA and IRB
Serious (Suspected Adverse Reaction)	Unexpected	Related	Within 15 calendar days of report receipt to IRB, FDA, DSMB, and Health Canada
Serious	Unexpected or Expected	Unrelated	Semi-annually to DSMB and DoD; Annually to FDA and IRB
Important Medical Event (Pregnancy)			Within 15 calendar days of report receipt to IRB, FDA, DSMB
Not Serious	Unexpected or Expected	Unrelated or Related	Semi-annually to DSMB and DoD; Annually to FDA and IRB

*Unrelated= Not related or unlikely to be related
Related= Possibly related or definitely related

7.3.2.1 Reporting to Regulatory Agencies – Institutional Review Board

Guidelines for reporting of AEs to the IRB are described in Section 7.3.2 and Table 7-3-2.

If a local site is not ceding authority to the central IRB, then the local site PI is responsible for complying with their local IRB/REB's reporting requirements. Copies of each report and documentation of IRB/REB notification and receipt will be kept in the local site PI's study file. A central IRB located at Boston Children's Hospital will be used for the majority of sites in this trial.

7.3.2.2 Reporting to Regulatory Agencies – Food and Drug Administration and Health Canada

Guidelines for reporting of AEs to the FDA are described in Section 7.3.2 and Table 7-3-2.The IND Sponsor or its designee (such as the DCC) is required to report certain study events in an expedited fashion to the FDA in accordance with 21 CFR§312.32. These written notifications of AEs are referred to as IND safety reports. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease-related.

Adverse drug reactions that are serious, unexpected, and related to the investigational drug will be reported to Health Canada as outlined in Table 7-3-2 in compliance with the "Guidance Document for Clinical Trial Sponsors: Clinical Trial Applications."

7.3.2.3 Reporting to Local Site PIs

For any site that has not ceded authority to the central IRB, the DCC shall submit to the local site PI the determination regarding safety concerns or lack thereof from the DSMB after each DSMB meeting (or at the direction of the DSMB Chair with respect to UPs reviewed on an expedited basis).

The local site PI shall provide to his/her IRB/REB the DSMB summary.

7.4 Medical Monitoring

It is the responsibility of each the local site PI to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of AEs as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious AEs that occur in patients at the local PI's site..

7.4.1 Independent Data and Safety Monitoring Board

An independent DSMB will monitor the data for this study. The DSMB is composed of experts in transplantation, cardiology, biostatistics and clinical trial design, bioethics, and pharmacy. The DSMB will review trial data on a periodic basis, but not less than twice per year. The DSMB report will include information on accrual rate, data quality and data completeness, overall and by center. It will also include safety endpoint data and narratives by treatment arm. In addition to semi-annual meetings, the DSMB will review the trial results for safety when 180-day post-randomization data are available from the 10th patient assigned to the EVL/LDTAC arm. The 180-day initial review will also include the results from all subjects randomized to TAC/MMF who have 180-day post-randomization data available by the time that the 10th EVL/LDTAC 180-day data are complete. The focus of this initial DSMB review will be the primary safety and AE rates up to 180 days. In addition, the DSMB will regularly review serious AEs and protocol deviations associated with the research to ensure the protection of human subjects. The DSMB and IND Sponsor are assisted by a Medical Monitor in reviewing SAEs. The DCC Medical Monitor will serve as the IND Sponsor's designee for determining causality and expectedness of all SAEs. Results of the DSMB deliberations will be communicated to the IRB/REB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, according to the timelines in Table 7-3-2.

7.4.2 Department of Defense Mandated Independent Research Monitor

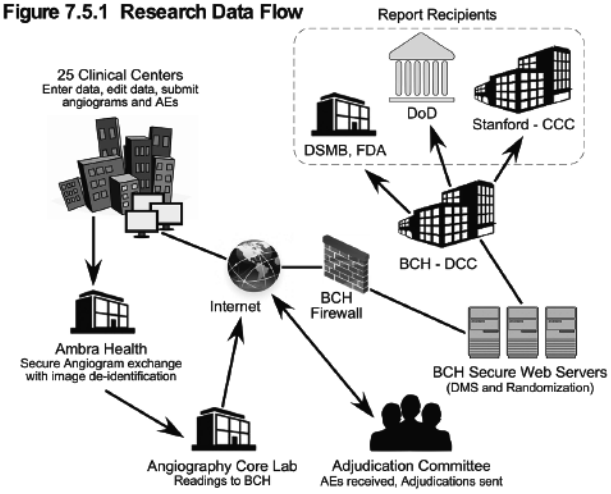
The Research Monitor, Tajinder Pal Singh, MD, is responsible to oversee the safety of the research and report observations/findings to the IRB/REB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB/REB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB/REB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB/REB or other designated official and the U.S. Army Medical Research and Materiel Command Office of Research Protections, Human Research Protection Office.

7.5 Data Management Plan

7.5.1 Database Management System (DMS) Capabilities

All trial data will be entered electronically into an FDA-compliant (per 21CFR11) secure, version-controlled, web-based electronic data capture and DMS that supports regulatory trials to ensure Good Clinical Practice (GCP). This system permits direct, paperless data entry from the study sites, with central data storage at the DCC. It will have a password expiration period of 120 days in order to ensure enhanced security protection of sensitive data.
The data management system will provide real-time reporting to the DoD, the FDA, CCC and study sites, as well information to users on protocol compliance and upcoming study visits. It allows for role-appropriate access, and provides audit trails for data entry, exports, and reports. Trial data will be entered using subject identification number; neither patient name nor medical record number will be submitted to the DCC. The link of name and subject ID number will reside only at the study site. **Figure 7.5.1** depicts research data flow.

Figure 7.5.1 Research Data Flow



7.5.2 Data Recovery

The study database is backed up nightly. At any point in time, BCH retains in storage 7 contiguous days of backup, followed by 4 weekly backups and 13 monthly backups. The servers for long-term storage are located at BCH in a fully power and network-redundant commercial data center. In addition, the database management system retains an audit trail of every action occurring within the system and this audit trail history is captured within each database backup.

7.5.3 Database Lock

For key deliverables requiring analysis of the trial data, an export of the entire database into SAS datasets will be performed at such periodic intervals (e.g., semiannual DSMB reports) in order to have a frozen dataset from which all results will be generated. At trial end, a final lock and export will occur after all data queries are resolved. The final trial results and FDA summary report will be prepared from this locked dataset. The study database will be retained at the DCC for 5 years after trial end. The exported raw SAS datasets and the analytic datasets will be maintained at the DCC indefinitely.

7.6 Statistical Plan

7.6.1 Randomization Scheme

After written informed consent is obtained, subjects will be randomly assigned to the EVL/LDTAC or TAC/MMF group using secure web-based randomization that is integrated into the DMS. The randomization assignments will be generated by Boston Children's Hospital's proprietary randomization software, SciRan. Subject randomization will require prior entry of eligibility data and confirmation that written informed consent has been obtained. A stratified randomization scheme will be used. The stratification factors are:
Sensitization status (presence vs. absence of donor specific antibody) and
Study site transplant volume (Lower vs. Higher, defined as ≤12 per year vs. > 12 per year),
for a total of 4 strata.
Allocation will be in a 1:1 ratio using permuted blocks, to ensure balanced allocations across the two treatment groups within each stratum. Each participant will be informed of his/her assignment by the study coordinator at the end of the baseline visit.

In case of randomization system failure or failure of internet access at the centers, back-up envelope assignments will be provided when a site is classified as qualified to enroll. These back-up randomization assignments will also be generated by the BCH randomization software, SciRan.

7.6.2 Power and Sample Size

Aim 1 (Efficacy): To determine if the cumulative burden of domain-specific MATE scores for CAV, CKD, BP-ACR (MATE-3) is superior for EVL/LDTAC compared to TAC/MMF our null hypothesis is that the mean MATE-3 scores at 30 months are equal for the EVL/LDTAC and TAC/MMF groups (specific aim 1). The alternative hypothesis is that the mean MATE-3 scores at 30 months differ for the EVL/LDTAC and TAC/MMF groups. With the assumptions shown in Table 7.6.2,

Table 7.6.2. Sample Size Assumptions

- Two-sided superiority test, $\alpha = 0.05$
- Comparison of Month 30 means, adjusted for baseline score
- 15% crossover from EVL/LDTAC to TAC/MMF
- 5% crossover from TAC/MMF to EVL/LDTAC
- SD of MATE-3 = 2.4

- Within-subject correlation R=0.5 (per PHTS data)
- 5% loss to follow-up
- 2% inflation for interim look for efficacy

a total of 210 subjects is required to detect with 90% power a mean difference of 1.2 in the 2.5-year change score between the two independent treatment groups. A difference of 1.2 points is one-half SD, which is typically considered to be a minimum clinically important difference.[36, 37] The calculation assumes, however, a reduced mean difference of 0.96 to account for the treatment crossover shown in Table 7.6.2, that will incur dilution of treatment effect in the primary intention-to-treat analysis. The treatment group comparison will be adjusted for the baseline MATE-3 score, thus the required sample size is $N^*=N(1-R^2)$, where N=130 and R=0.5. Hence N*=97. With the inflation for loss to follow-up and an interim look, the required sample size is (97/0.95)*1.02 = **105 per group (total 210 subjects).**

If the treatment crossover from TAC/MMF to EVL/LDTAC is 15% rather than the 5% rate shown in Table 7.6.2, the planned sample size will still yield 80% power to detect the desired mean difference in a baseline-adjusted comparison of Month 30 MATE-3 scores.

Aim 2 (Safety): With 194 analyzable subjects (97x2; see above), a 95% two-sided CI for the 30-month treatment group difference in MATE-6 score assessing safety will have width ± 1.24, thus is able to rule out the pre-specified acceptable difference of less than 3 points with high confidence. Therefore: With a sample size of 210 subjects (105 in each arm) the proposed trial will have more than adequate power to determine if, compared to TAC/MMF, the cumulative burden of domain-specific MATE scores for CAV, CKD and ACR (MATE-3) differs from (is lower than) that of EVL/LDTAC, without a higher cumulative burden of MATE overall (MATE-6) given the assumptions stated above. The assumptions are conservative, employing a relatively small detectable difference between groups and accommodation for treatment crossover, loss to follow-up and inflation for an interim look.

7.6.3 Analysis Plan

7.6.3.1 General Approach

Statistical analyses for this trial will be performed in two stages: Stage 1: Analyses will be conducted during the conduct of the trial, in aggregate (both treatment arms combined), to identify outliers in the clinical data (covariates), patterns of missing data, and to identify any unusual differences by center. Stage 2: These analyses will be performed at trial end, and are described in Sections 7.6.3.3 to 7.6.3.7. The primary analytic approach will be intention-to-treat (ITT). This means that all subjects randomized will be analyzed according to their randomized assignment and included in the analysis regardless of their duration of follow-up.

7.6.3.2 Study Population Definitions

The Study Populations are defined as follows:

(1) Intention-to-Treat (ITT) Population: All patients who are randomized who were assessed at least once after the administration of at least one dose of study medication.

(2) Modified ITT Population: The subset of patients in the ITT Population who were not found post-randomization to have failed any trial eligibility criterion that was measured prior to randomization (i.e., excludes ineligible patients who were randomized in error).

(3) Per-Protocol Population: The subset of patients in the Modified ITT Population who: a) do not have major deviations from the protocol; and b) have at least two post-baseline assessments (i.e., have at least 6 months of data post-randomization). Major protocol deviations will be pre-specified prior to the start of the trial.
(4) As Treated Population: All randomized patients who received at least one dose of study medication and undergo at least one safety assessment.

Primary efficacy analyses (for all endpoints) will be conducted on data from the ITT population, with subjects remaining in their assigned treatment group regardless of actual treatment received.
Secondary efficacy analyses (for the primary efficacy endpoint and its components) will be conducted on data from the modified ITT population and the Per-Protocol population, with subjects remaining in their assigned treatment group regardless of actual treatment received.
Tertiary efficacy analyses (for the primary efficacy endpoint and its components) will be conducted on the As Treated Population, with subjects classified into the group corresponding to treatment actually received (i.e., non-ITT), defined using a compliance measure described in the SAP. This population may exclude some subjects who do not meet the compliance criterion for either of the 2 trial treatments.

Primary safety and tolerability analyses (for all endpoints) will be conducted on data from the ITT population.
Secondary safety and tolerability analyses (for all endpoints) will be conducted on data from the As Treated population, with subjects classified into the group corresponding to treatment actually received, defined using a compliance measure described in the SAP. This population may exclude some subjects who do not meet the compliance criterion for either of the two trial treatments.

7.6.3.3 Aims 1 and 2 (MATE Score for Efficacy & Safety)

We wish to test the superiority hypothesis that among children who survive to 6 months post-HT, children randomized to EVL/LDTAC will have, at 30 months post-randomization, a lower total burden of CAV, CKD and ACR compared to children randomized to TAC/MMF (Aim 1; Efficacy; MATE-3), without a higher burden of death/graft loss (Aim 2; Safety; MATE-6)

Aim 1 (Efficacy): The primary efficacy endpoint analysis, per recommendation of the DSMB, will be a Zero-Truncated Wilcoxon test comparison of EVL/LDTAC vs. TAC/MMF MATE-3 scores at 30 months post-randomization (analysis of covariance). The test of superiority for efficacy will be the test of whether the MATE-3 scores differ according to the Zero-Truncated Wilcoxon test comparison and will be significant if the p-value is smaller than the nominal p-value associated with the final look at the data.

As a secondary analytic approach for the primary efficacy endpoint, we will perform two analyses, although these may not be robust due to the departure from normality of MATE scores. These approaches include (1) a baseline adjusted comparison of means and (2) mixed effects longitudinal regression appropriate for randomized trials[39] (i.e., parameterization that induces adjustment for baseline) will be used to model MATE-3 as a function of: a) continuous time post-randomization and b) interaction terms between the indicator for treatment assignment and time, with the

variance-covariance matrix based on the sequence of the scheduled visits. The test of superiority for efficacy using this secondary analytic approach will be the test of whether the two slopes differ, as it represents the treatment effect on cumulative MATE-3 burden over time. If EVL/LDTAC is more effective, we expect that mean MATE-3 scores in this group will change little with time, while the mean MATE scores from the TAC/MMF group will increase with time (positive slope). If the time effects are not found to be linear hence imposing bias if a linear model is used, then the time effect will be fit using indicators for the study visits (7 degrees of freedom) and the categorical time x treatment assignment interaction will be tested, with the relative superiority of one treatment group over the other will be assessed using a time-weighted contrast of the mean MATE-3 score from the two treatment groups.

Aim 2 (Safety): A 95% two-sided CI for the 2.5-year treatment group difference (EVL/LDTAC minus TAC/MMF) in MATE-6 score will be constructed, based on a baseline-adjusted comparison of the mean EVL/LDTAC vs. TAC/MMF MATE-6 scores at 30 months post-randomization (analysis of covariance; See SAP for method of CI construction). If the upper limit of the CI is within the pre-specified clinically acceptable difference of less than 3 points (i.e., no large difference in MATE-6 score between treatment groups, which ranges from 0 to 24), then we will conclude that EVL/LDTAC has no higher burden of death/graft loss than does TAC/MMF.

For secondary endpoints in both Aim 1 and 2, a variety of methodologies will be utilized and detailed in the full Statistical Analysis Plan (SAP). These include longitudinal generalized linear models for Lanksy, Karnofsky, EuroQOL EQ-5D scores, and change in renal function; and time to event methods, in a competing risks framework (e.g., subdistribution hazard model) where indicated, such as for estimation of freedom from an individual MATE AE with the competing risks of death and re-transplant. These analyses will be detailed in the SAP.

7.6.3.4 Aim 3 (Drug Tolerance)

Fisher exact tests will be used to compare the percentage of subjects who experience at least one occurrence of each type of AE, with particular attention to hyperlipidemia, anemia, thrombocytopenia, interstitial lung disease, aphthous stomatitis, proteinuria, and rash. Incidence rates of AEs will also be calculated to account for multiple AEs of a given type per subject, with Poisson regression utilized to test for differences between treatment groups. Subgroup analyses will also be conducted to estimate the percentage of subjects with events possibly or definitely related to study treatment, as well as the percentage of subjects with an event within each body system class.

7.6.3.5 Interim Analyses

For safety, the adverse and serious AE rates will be estimated by treatment arm and presented to the DSMB at least twice annually. In addition, as an early review of patient safety, although no formal early stopping rule for safety is planned, the DSMB will review the outcomes of the first ten subjects assigned to EVL/LDTAC, at 180 days following the randomization of the tenth subject. The rates by arm will be presented overall for each group as well as by treatment-relatedness. The counts of AEs that lead to study treatment discontinuation as well as those

resulting in death will be presented. Safety laboratory findings by study visit will also be compared by treatment arm.

For efficacy, two interim looks by treatment arm are planned, when there is 50% and 80% statistical information. Statistical information will be calculated as the proportion of follow-up visits completed out of the total expected number of follow-up visits. An O'Brien-Fleming boundary for premature trial termination will be applied, detailed in the SAP and subject to DSMB approval. The 50% and 80% information points will occur between months 19-21 and months 31-33 if the accrual rate is uniform; hence, approximately one year apart.

7.6.3.6 Subgroup Analysis

The following pre-specified subgroups defined at the time of randomization will be utilized in final analyses:

- Center transplant volume (Lower vs. Higher, ≤12 vs. >12 annually)
- History of treated rejection (present vs. absent)
- Age <1 year vs. ≥1 year
- CKD stage (1 vs. 2 vs. 3)
- Sensitization status (presence vs. absence of donor-specific antibody).
- Males vs. Females
- Race (2 comparisons): White, Non-Hispanic vs. All Others; White vs. Black vs. Asian vs. Other
- Ethnicity: Hispanic vs. Non-Hispanic
- Type of induction therapy (3 comparisons):
  - None vs. IL-2 receptor blocker (basiliximab, daclizumab) vs. thymoglobulin (ATG);
  - None vs. IL-2 receptor blocker vs. low-dose ATG (<5 mg/kg total dose) vs. high-dose ATG (≥5 mg/kg total dose)
  - Low-dose ATG (<5 mg/kg total dose) vs. high-dose ATG (≥5 mg/kg total dose) – excludes patients with no induction therapy or IL-2 receptor blocker therapy
- Type of maintenance immunosuppression prior to randomization: TAC/MMF vs. cyclosporine/MMF

For the primary efficacy endpoint, a test of the interaction of Treatment assignment x Subgroup factor will be conducted to determine whether there is differential treatment effectiveness at 30 months according to patient subgroup. Because the trial is not powered to detect small to medium treatment differences within subgroups, any test with $p<0.10$ will be considered to be hypothesis-generating, i.e., potential evidence of interaction.

7.6.3.7 Missing Data and Sensitivity Analyses

Because subjects who die will have a reduced number of study visits, the number of data points contributed will be fewer than those who complete the trial. As a secondary analysis, the maximum MATE score of 12 (MATE-3) and 24 (MATE-6) corresponding to death as of the last visit will be imputed for the remaining study visits if death occurs, to assess whether the primary analysis model

results are robust to the modeling of truncated information. This approach, as well as other sensitivity analyses and additional detail regarding the primary and secondary analytic approaches (e.g., per-protocol; covariate-adjusted; restriction to first transplant recipients) for the key trial endpoints, will be fully described in a SAP finalized prior to the first randomization.

7.7 Publication of Study Results

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor and publications committee. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the Investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

8.1.1 Identifiers

Each patient screened for possible entry to the trial will be identified with a unique 7-digit study identification (ID) number with the following format:

Study Patient Identification Number: ____ ____ ___ - ___ ___ ___ ____
Site Patient

The leading digits will represent the study site, and the remaining digits will serve as the patient sequence number within site. No patient names, initials, addresses, or medical record numbers will leave the enrolling site as part of the research data. (Draft data collection forms will be modeled according to this system, with no initials included in data entry). The angiograms to be analyzed for this trial will be identified by a separate, unique ID number with a link to the patient ID number maintained only at the study site. The angiograms will be transferred to the Angiography Core Laboratory using a secure transfer and de-identification portal hosted by Ambra Health, which has been successfully used for other studies at Boston Children's Hospital that have research images.

Subject medical records may be reviewed and research staff may contact subjects up to 5 years after the study ends for the last patient participating (approximately 2026). To follow trial patients long-term after study completion, patient clinical trial data will be linked to the patient's data in national transplant registries, including the Pediatric Heart Transplant Study Group (if your center participates), and the United Network for Organ Sharing. Once the linkage is complete, all patient identifiers will be removed. If a center participates in the PHTS registry, data from the subject's study visits may be transferred into the PHTS data registry after the clinical trial is complete.

8.1.2 Study Records

The maintenance of confidentiality of study records and data is of paramount importance and the procedures are described in detail in the trial informed consent form. Study data will be kept in locked cabinets or areas at the study site. The link between patient name and study ID number will be stored in a separate location. Published reports will include study data only in aggregate. While identifying information will not leave the local institution, other persons such as members of the DCC to conduct a site visit and data audit and members of the DoD will have permission to review study records. A statement of this access will be present in the informed consent form.

8.1.3 Sharing of Individual Study Results

All clinical data collected in this study are based on routine clinical care following heart transplant in children. Therefore, there are no clinical study results that the patient's physician will not already have access to, to share and to use for the patient's clinical management. The self-reported EQ-5D responses to assess QOL may not be part of routine clinical care; since this is a self-administered instrument, the participant is already aware of the information.

8.1.4 Data Reporting

Data will be reported in aggregate form for annual FDA reports, semi-annual DMSB reports, and at trial end. The FDA report will be prepared in compliance with all formatting and programming requirements that ensure a report that is suitable for a regulatory filing, including adherence to a pre-specified SAP. Listings of individual events, such as AEs, will be identified by study ID number.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, pathology reports, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, digitized imaging data, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Electronic Case Report Forms

The study eCRF is the primary data collection instrument for the study. All data requested on the eCRF must be recorded. All missing data must be explained. eCRFs will be completed electronically for the study and will have automatic data verification in place to decrease the likelihood of incomplete or inaccurate data.

8.4 Records Retention

It is the Investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the Sponsor. In such an instance, it is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained. The Sponsor and/or the designated Data Coordinating Center, will maintain study records for a period of 25 years.

8.5 Quality Assurance/Quality Control (QA/QC) Plan for Study Data

8.5.1 QA/QC of Trial Data

Quality assurance (QA) processes are proactive to ensure, with high likelihood, that data will be collected accurately and consistently. QA of trial data will employ several approaches, including but not limited to: a requirement for sites to perform a test randomization to ensure that real-time randomizations will be executed smoothly; pilot testing of CRFs to identify complex data fields that require adaptation for clinical relevance and accessibility; an online Trial Manual of Operations prepared by the DCC with detailed instructions for data collection by CRF and by data field, where needed; and real-time validations of submitted data. QC of trial data will be achieved through site monitoring, where the DCC CRA will conduct a comparison of source documentation to submitted data; and with ongoing statistical analysis to identify outliers and/or unusual patterns in the data.

8.5.2 Laboratory Evaluations

All the measurements in the proposed study are part of routine clinical care. Blood and/urine samples will be collected for a biobank. Subjects and/or LARs may decide whether or not to participate in the biobank. Such specimens will be labeled with the subject's study ID number and the Biobank Manual of Operations will specify the storage and (if applicable) shipping requirements necessary to ensure viable specimens that maintain the required level of confidentiality.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

This study will be monitored according to standards outlined by the FDA. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

9.2 Auditing and Inspecting

The Investigator will permit study-related monitoring, audits, and inspections by the IRB/REB, the Sponsor, government regulatory bodies, and Medical center compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The Investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as an Investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable medical center compliance and quality assurance offices.

Site performance will be monitored analytically with respect to enrollment, protocol violations, and data quality. Site monitoring via an in-person audit by the DCC CRA will also occur once for each site during the course of the trial, after the site has enrolled at least 4 patients. The audit will include review of regulatory documentation and screening practices as well as a data audit. For-cause site monitoring will also be conducted if warranted, with the DCC PI and/or a trial co-chair traveling to the site to meet with the local study team. A corrective action plan (CAP) will be deployed to facilitate improvement with respect performance issues.

10 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent IRB or REB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB/REB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of this study. The Investigator should provide a list of IRB/REB members and their affiliate to the Sponsor.

All subjects and/or their LAR for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Appendix 3 for a template of the Subject Informed Consent Form. The template consent form will be modified to meet local regulatory requirements and submitted with the protocol for review and approval by the IRB/REB for the study. The formal consent of a subject or their LAR, using the IRB/REB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject, parent, guardian or LAR, and the Investigator-designated research professional obtaining the consent. In general assent will be obtained from all developmentally able patients who are greater than 12 years of age or as required by local regulations. If a subject reaches the age of majority (18 years old at most clinical sites) during the course of the study, the subject will be asked to sign consent him/herself for continued participation in the study if this is in keeping with local custom and regulations.

11 Study Finances

11.1 Funding Source

The study will be funded by a Fiscal Year 2016 Peer Reviewed Medical Research Program Clinical Trial Award sponsored by the Department of Defense office of the Congressionally Directed Medical Research Programs (PR160574).

11.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee and a Committee-sanctioned conflict management plan must be put in place. This plan(s) will be reviewed and approved by the Funding Sponsor prior to participation in the study by the investigator(s).

12 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the IND Sponsor, DCC or DoD for the purposes of performing the study, will be published or passed on to any third party without the consent of the IND Sponsor and publications committee. Any investigator involved with this study is obligated to provide the IND Sponsor (via the DCC) with complete test results and all data derived from the study.

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14 Appendix 1: Lansky and Karnofsky Functional Status Scale

The following standardized rating instruments will be used to assess subject functional status at Baseline, 18 months, and 30 months post-randomization (6, 24, and 36 months post-transplant). The instrument administered will be determined by **age at randomization** and the same instrument will be administered to a given subject throughout the study.

Lansky (< 16 years) and Karnofsky (≥ 16 years) Scores at baseline, 24 and 36 months post-transplant; these scores are validated functional performance scores originally developed for cancer patients [11-14]. Both scores have been used to describe the functional status of heart transplant recipients.[11, 15, 16] Scores range from 10 to 100 and are only assigned in 10 point increments; For example, 40 is an acceptable score while 43 is not.

LANSKY AND KARNOFSKY FUNCTIONAL STATUS SCALE

	Pediatric (Lansky) Scale	Adult (Karnofsky) Scale
100	Fully active, normal	Normal, no complaints, no evidence of disease
90	Minor restrictions in physically strenuous activity	Able to carry on normal activity: minor symptoms of disease
80	Active, but tires more quickly	Normal activity with effort: some symptoms of disease
70	Both greater restriction of and less time spent in play activity	Cares for self: unable to carry on normal activity or active work
60	Up and around, but minimal active play; keeps busy with quieter activities	Requires occasional assistance but is able to care for needs
50	Can dress but lies around much of day; no active play; can take part in quiet play/activities	Requires considerable assistance and frequent medical care
40	Mostly in bed; participates in quiet activities	Disabled: requires special care and assistance
30	In bed; needs assistance even for quiet play	Severely disabled: hospitalization is indicated
20	Often sleeping; play entirely limited to very passive activities	Very sick, hospitalization necessary: active treatment necessary
10	No play; does not get out of bed	Moribund, fatal processes progressing rapidly

15 Appendix 2: EuroQOL

The following standardized rating instruments will be used to assess quality of life at Baseline, 18 months, and 30 months post-randomization (6, 24, and 36 months post-transplant). The instrument administered will be determined by **age at randomization** and the same instrument will be administered to a given subject throughout the study.

EuroQOL EQ-5D-Y (Youth version)[17, 18] at baseline (6 months post-HT), 24 and 36 months post-transplant for subjects ≥ 8 years at randomization; EQ-5D-Y proxy version 1 for children ≥4 years but less than 8 years OR children ≥ 8 years who are unable to complete the EQ-5D-Y. These validated quality of life instruments use a simple two page questionnaire involving a simple ordinal scale across 5 domains plus a visual analog scale. The youth versions are tailored to an appropriate developmental level so that quality of life can be reproducibly assessed in children. Only the US English Language versions are included in this Appendix. Country and language specific versions of the questionnaire will be used and are available upon request.



Health Questionnaire

English version for the USA

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EQ-5D-Y

Describing your health TODAY

Please check the ONE box that best describes your health TODAY.

Mobility *(walking around)*

I have no problems walking around ❑

I have some problems walking around ❑

I have a lot of problems walking around ❑

Taking care of myself

I have no problems taking a bath or shower by myself or getting dressed by myself ❑

I have some problems taking a bath or shower by myself or getting dressed by myself ❑

I have a lot of problems taking a bath or shower by myself or getting dressed by myself ❑

Doing usual activities *(for example, going to school, hobbies, sports, playing, doing things with family or friends)*

I have no problems doing my usual activities ❑

I have some problems doing my usual activities ❑

I have a lot of problems doing my usual activities ❑

Having pain or discomfort

I have no pain or discomfort ❑

I have some pain or discomfort ❑

I have a lot of pain or discomfort ❑

Feeling worried, sad, or unhappy

I am not worried, sad, or unhappy ❑

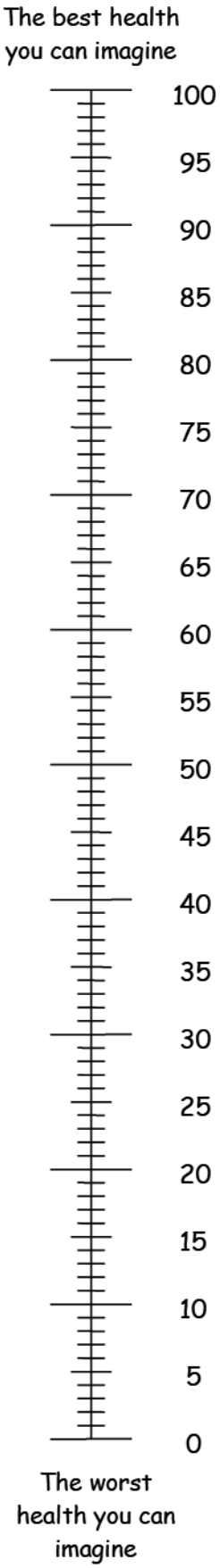
I am a little worried, sad, or unhappy ❑

I am very worried, sad, or unhappy ❑

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How good is your health TODAY

- We would like to know how good or bad your health is TODAY.
- This line is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Please mark an X on the line to show how good or bad your health is TODAY.



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Health Questionnaire

English version for the USA

Proxy version of the EQ-5D-Y: 1

(The purpose of this questionnaire is to explore how a care-giver or someone who knows the child well (proxy), would rate the health of the child. The proxy should not answer on behalf of the child, but rather rate the child's health status as the proxy sees it)

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EQ-5D-Y

Describing the child's health TODAY

Under each heading, please check the ONE box that **you think** best describes the child's health **TODAY**.

Mobility *(walking around)*

He/she has no problems walking around ❑

He/she has some problems walking around ❑

He/she has a lot of problems walking around ❑

Taking care of him/herself

He/she has no problems taking a bath or shower by him/herself or getting dressed by him/herself ❑

He/she has some problems taking a bath or shower by him/herself or getting dressed by him/herself ❑

He/she has a lot of problems taking a bath or shower by him/herself or getting dressed by him/herself ❑

Doing usual activities *(for example, going to school, hobbies, sports, playing, doing things with family or friends)*

He/she has no problems doing his/her usual activities ❑

He/she has some problems doing his/her usual activities ❑

He/she has a lot of problems doing his/her usual activities ❑

Having pain or discomfort

He/she has no pain or discomfort ❑

He/she has some pain or discomfort ❑

He/she has a lot of pain or discomfort ❑

Feeling worried, sad or unhappy

He/she is not worried, sad or unhappy ❑

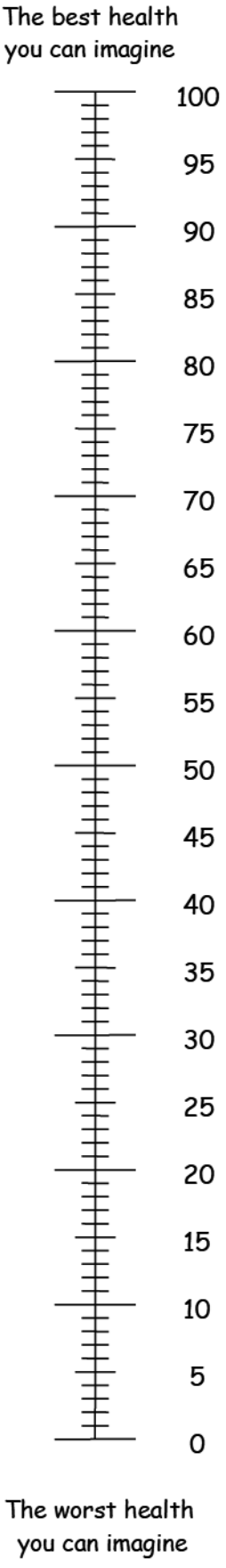
He/she is a little worried, sad or unhappy ❑

He/she is very worried, sad or unhappy ❑

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The child's health TODAY

- We would like to know how good or bad you think the child's health is TODAY.
- This line is numbered 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Please mark an X on the line to show how good or bad **you think** the child's health is **TODAY**.



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