Protocol Title: A Randomized Controlled Clinical Trial of Thymoglobulin® and Extended Delay of Calcineurin Inhibitor Therapy for Renal Protection after Liver Transplantation: A Multi-Center Study

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CLINICAL STUDY PROTOCOL

A Randomized Controlled Clinical Trial of Thymoglobulin® and Extended Delay of Calcineurin Inhibitor Therapy for Renal Protection after Liver Transplantation
A Multi-Center Study

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STUDY SYNOPSIS:

TITLE OF STUDY: A Randomized Controlled Clinical Trial of Thymoglobulin® and Extended Delay of Calcineurin Inhibitor Therapy for Renal Protection after Liver Transplantation: A Multi-Center Study

PROTOCOL NUMBER:

PHASE: 2

STUDY CENTERS: Multicenter Study [Cleveland Clinic, University of Cincinnati, Liver Transplant, Medical College of Wisconsin, Cleveland Clinic Weston]

RESEARCH HYPOTHESIS: Initial treatment with 4.5 mg/kg of thymoglobulin® and delayed introduction of calcineurin inhibitor therapy (CNI) [tacrolimus] for 10 days will result in superior preservation of renal function and similar subject and graft survival in comparison to subjects receiving CNI no later than postoperative Day 2, along with no polyclonal or monoclonal antibody induction therapy.

STUDY DESIGN: This is a phase II study to be conducted at three centers. Subjects will be randomized in equal numbers to receive Thymoglobulin® and delayed CNI (Delay CNI) or standard CNI initiation with no antibody therapy (Early CNI). All subjects will also receive a maintenance immunosuppressive regimen consisting of corticosteroids and mycophenolate mofetil (MMF) according to standards of practice in orthotopic liver transplantation (OLT).

DURATION OF STUDY: 24-30 months

NUMBER OF SUBJECTS: 110

STUDY POPULATION: Subjects receiving a first OLT from a deceased donor (whole organ or split).

INCLUSION/EXCLUSION CRITERIA:

INCLUSION CRITERIA

- Patients undergoing deceased donor solitary liver transplantation
- Age 18-75 years at the time of transplantation
- Willingness and ability to comply with the study procedures
- Signed informed consent form
- For patients with Hepatocellular carcinoma as indication for OLT, (must be within the Milan Criteria)
- Hepatitis C, positive or negative, patients

EXCLUSION CRITERIA

- Prior kidney transplantation
- Congenital or iatrogenic absence of one kidney
- Subjects on renal replacement therapy at the time of OLT
- MELD score >34
- HIV positive patient
- Patient with current severe systemic infection
- History of bacterial peritonitis within 30 days prior to OLT
- Active infection or recent infection within 30 days prior to OLT
- Use of a calcineurin inhibitor continuously for more than 90 days within the past 6 months
- History of hypersensitivity to Thymoglobulin®, rabbits or tacrolimus
- Pregnant and/or nursing (lactating) females.
- Women of childbearing age who are unwilling to use effective contraception during the duration of the study, and for 30 days after study participation and/or last dose of Study Drug.

STUDY MEDICATION (DOSE/ROUTE/REGIMEN): Subjects randomized to the (Delay CNI) group will be treated with Thymoglobulin® (total dose of 4.5 mg/kg) administered in three doses; (each dose being 1.5 mg/kg – administered Day 0 [after transplant], Day 2, and Day 4 post transplant), along with CNI delay for 10 days. CNI will be initiated on postoperative (post-transplant) Day 10. Subjects will also receive a maintenance immunosuppression regimen of corticosteroids and MMF in accordance with the standard practice at each clinical center.

REFERENCE MEDICATION (DOSE/ROUTE/REGIMEN): Subjects randomized to the (Early CNI) group (Control group) will receive no antibody therapy for induction and will start CNI therapy on postoperative (post-transplant) Day 2. Subjects will also receive a maintenance immunosuppression regimen of corticosteroids and MMF in accordance with the standard practice at each clinical center.

STUDY SYNOPSIS: (continued)

PRIMARY ENDPOINT(s):

- The incidence of AKI at 30 days post-transplant.
- Serum creatinine and eGFR at 30 days post-transplant

SECONDARY ENDPOINT(s):

- The incidences of acute cellular rejection, (ACR), at 30 days post-transplant.
- Patient survival at 6 months post-transplant.
- Graft survival at 6 months post-transplant.
- Incidence of AEs and SAEs

ABBREVIATIONS AND TERMS:

ACE	angiotensin converting enzyme
ACR	acute cellular rejection
ADQI	acute dialysis quality initiative
AE	adverse event
AKI	acute kidney injury
AKIN	acute kidney injury network
ALT	alanine aminotransferase
ARF	abnormal renal function
AST	aspartate aminotransferase
ATN	acute tubular necrosis
AUC	area under the curve
BCG	Bacillus Calmette-Guerin (vaccine)
B-HCG	Beta Human Chorionic Gonadotropin
BID	twice a day
BP	blood pressure
BPAR	biopsy proven acute rejection
BUN	blood urea nitrogen
Ca	calcium
CBC	complete blood count
CD3+	Cluster of differentiation 3
CFR	Code of Federal Regulations
CKD	Chronic Kidney Disease
Cl	chloride
CMV	cytomegalovirus
CNI	Calcineurin Inhibitor(s)
CO2	carbon dioxide
CO-I	Co Investigator
CrCl	creatinine clearance
CRD	Clinical Research and Development
CRF	Case Report/Record Form
CsA	Cyclosporine
CSR	Clinical Study Report
CSSF	Clinical Supply Shipment Form
CYP2D6	Enzyme (Cytochrome P450, Family 2, Subfamily D, Polypeptide 6)
CYP3A4	Enzyme (Cytochrome P450, Family 3, Subfamily A, Polypeptide 4)
D/C	discontinue
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DOB	date of birth
DS	double strength
DSMB	Data Safety Monitoring Board

EBV	Epstein-Barr Virus
EC	Ethics Committee
ECG	Electrocardiogram
eGFR	estimated Glomerular Filtration rate
EMR	electronic medical record
ESRD	end stage renal disease
FDA	United States Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HAT	hepatic artery thrombosis
HBcAb	hepatitis core antibody
HBV	hepatitis B Virus
HCC	Hepatocellular Carcinoma
Hct	Hematocrit
HCV	hepatitis C Virus
HDL	high-density lipoprotein
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA reductase
HUS	Hemolytic Uremic Syndrome
ICU	Intensive Care Unit
IDMS	Integrated Database Management System
IEC	Independent Ethics Committee
ILD	Interstitial lung disease
IL2	interleukin 2
INH	Inhalation
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	intent-to-treat
IV	Intravenous(ly)
K	potassium
Kg	Kilogram
LDL	low-density lipoprotein
LT	Liver Transplant (ation)
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MDRD	Modification of diet in renal disease
MCV	mean corpuscular volume
MELD	Model for End-Stage Liver Disease
mg	Milligram
mIU	milli-international units
mL	Milliliter
MMF	mycophenolate mofetil
MPV	mean platelet volume

MR#	medical record number
MRI	magnetic resonance imaging
Na	sodium
NG	Nasogastric
ng	nanogram
OLT	Orthotopic Liver Transplantation
P	pulse
PCP	Pneumocystis carinii pneumonia
PCR	polymerase chain reaction
P-gp	P-glycoprotein
PI	Principal Investigator
PK	Pharmacokinetics
PML	progressive multiple leukoencephalopathy
PNF	primary non function
PO	Oral(ly)
PRCA	pure red cell aplasia
PRES	posterior reversible encephalopathy syndrome
PTLD	post transplant lymphoproliferative disorder
PVAN	polyoma virus-associated nephropathy
PVI	polyoma virus infections
R	respiration(s)
r-ATG	Rabbit Anti-thymocyte Globulin
RBC	red blood cell
RDW-CV	red cell distribution width – coefficient variation
REB	Research Ethics Board
RF	Renal Function
RIFLE	Risk, Injury, Failure, Loss, and End Stage Kidney Disease
RRT	renal replacement therapy
SAE	Serious Adverse Event
SBP	Spontaneous Bacterial Peritonitis
SOC	Standard of Care
SOP	Standard of Practice/Standard Operating Procedure
sCr	serum creatinine
T	temperature
TAC	tacrolimus
Thymo	Thymoglobulin®
TMA	Thrombotic Microangiopathy
TTP	Thrombotic Thrombocytopenic Purpura
UNOS	United Network for Organ Sharing
U/S	Ultrasound
US	United States
UTI	urinary tract infection
VLDL	very-low-density lipoprotein
WBC	white blood cell
WOCBP	women of child-bearing potential

INTRODUCTION:

A. Overview

Immunosuppression therapy has significantly improved the success rate of liver transplantation. Tacrolimus (TAC) is the most widely used calcineurin inhibitor in liver transplantation. TAC suppresses the immune responses by inhibiting the activity of calcineurin. However, calcineurin inhibitors are associated with the long-term side effect of nephrotoxicity, which is a leading cause of the morbidity and mortality after liver transplantation (*Chinnakotla et al., 2003; Donovan et al., 2003*). The late withdrawal of calcineurin inhibitors may not improve renal function, as early damage is irreversible after several months. A delayed introduction of calcineurin inhibitors has been proposed as an alternative strategy to reduce the rate of renal dysfunction immediately following liver transplantation. Alternative immunosuppressive therapies, such as interleukin 2 (IL2) inhibitors and agents that block the proliferation of T- and B-lymphocytes have shown limited success, but they are all associated with increased graft rejection, failure, and eventually increased mortality.[5, 6] An alternative strategy designed to protect early renal function via induction immunosuppression with Thymoglobulin® may avoid these risks associated with alternative maintenance or induction immunosuppression strategies. (anti-thymocyte globulin [rabbit]; Sanofi; Cambridge, MA) is a polyclonal gamma immunoglobulin, obtained by immunization of rabbits with human thymocytes (*Thymoglobulin® Prescribing Information*).

Thymoglobulin® mediates T-cell suppressive effects via inhibition of proliferative responses to several mitogens, with post-transcription blockade of interferon gamma and CD25 synthesis.[7-10] Possible mechanisms by which Thymoglobulin® induces immunosuppression *in vivo* include T-cell clearance from the circulation and modulation of T-cell activation, homing and cytotoxic activities.[11] T-lymphocyte depletion is observed in transplant patients shortly after administration of Thymoglobulin®.[12] This may result from the complement-dependent lysis in the intravascular space or the opsonization and subsequent phagocytosis by macrophages of antibody-coated T-cells (*Bonnefoy et al., op cit*). T-lymphocyte depletion in peripheral blood persists for several days to several months following cessation of Thymoglobulin® therapy, without associated morbidity.[13]

Thymoglobulin® is currently indicated for the treatment of acute renal transplant rejection in the United States (US). Thymoglobulin® has been widely prescribed in Europe since 1985, and is registered in 51 countries. Clinical trials conducted outside the US have demonstrated that Thymoglobulin® has been used successfully in various immunosuppressive regimens to prevent or treat acute graft rejection following kidney, heart, liver, and pancreas transplantation. The reported rates of acute rejection reversal ranged from 78% to 100% for all types of acute rejection, and from 74% to 94% for corticosteroid-resistant renal rejections.[14-20] When used as an induction (prophylactic) agent, Thymoglobulin® was reported equivalent to, or better than, other immunosuppressive regimens, such as triple therapy or quadruple therapy using Atgam® (lymphocyte immune globulin, anti-thymocyte globulin [equine] sterile solution), OKT3 (Orthoclone OKT®3 [muromonab-CD3]), or IL2 inhibitors for the prevention of graft rejection in various types of solid organ transplantations. Thymoglobulin® demonstrated a consistent adverse event (AE) profile across published controlled clinical studies (*Thymoglobulin® Investigator Brochure*).

B. Kidney Dysfunction in Liver Transplantation

Acute Kidney Injury (AKI): Rapid decline in renal function with or without the need for renal replacement therapy is common in the period immediately before and after liver transplantation.[1-4, 21-24] In a series of 105 OLT recipients, McCauley et al showed that 94.3% of recipients had acute renal failure defined as a 50% increase from the baseline preoperative serum creatinine.[4] In addition, 10% of recipients in the McCauley series from Pittsburgh required renal replacement therapy (RRT) in the form of CVVHD or intermittent hemodialysis.[4] In a Spanish series, Gainza et al found acute renal failure, (serum creatinine >2.0 mg/dL or need for RRT), in 46% of OLT recipients in the postoperative period.[23] Series from Mount Sinai Medical Center, New York and Baylor College of Medicine, Dallas found a postoperative RRT rate of 22% and 12% respectively in relatively large cohorts, (n = 1602 and 724), of OLT recipients.[22, 24] Large differences in the reported rates of postoperative acute renal failure is partly due to the lack of use of a standard definition of acute renal failure of which there was none until 2004.[25, 26]

O'Riordan employed the RIFLE criteria[25] for the definition of acute renal failure in a study of 359 OLT recipients in Ireland, and found an 11.1% postoperative incidence of 2-fold increase in serum creatinine and a 25.7% incidence of 3-fold postoperative increase in serum creatinine, or the need for RRT.[2] Most studies consistently show that postoperative renal failure had a significant impact on post transplant outcomes, including mortality.[1-4, 21-24]

McCauley reported an association between postoperative peak serum creatinine and mortality.[4] Mortality was 6.7%, 33.3%, 40% and 67% for peak serum creatinine of 1.7-3.0mg/dL, 3.1-4.3mg/dL, 4.4-5.6mg/dL and 7.7-8.9mg/dL, respectively.[4] In the study by O'Riordan, 30-day survival was 95.7%, 91.2% and 76.3% for OLT recipients with postoperative normal renal function, 2-fold increase in serum creatinine and 3-fold increase in serum creatinine or need for RRT, respectively ($P<0.001$ normal renal function vs. 3-fold increase in serum creatinine or RRT).[2] The study also found a 1-year survival of 47.5% in recipients with 3-fold increase in serum creatinine or RRT compared to 78.4% in those with normal postoperative renal function ($p<0.001$).[2] Postoperative acute renal failure that was severe enough to warrant RRT was associated with a 2-fold increase in hospital stay (39 days vs. 73 days)[2] and a five-fold increase in the duration of postoperative intensive care unit stay (2 days vs. 10.5 days).[24] Finally, postoperative acute renal failure is a potent risk factor for chronic kidney disease (CKD) in the late post-transplant period.[27-29]

Chronic Kidney Disease (CKD): Chronic kidney disease of varying severity is even more common than postoperative acute renal failure in OLT recipients.[22, 28-33] McCauley found CKD, (defined as serum creatinine ≥1.7mg/dL), in 77.3% of OLT recipients.[4] In contrast, when CKD was defined as serum creatinine >2.8mg/dL, Fisher et al found CKD in only 4% of 883 recipients in the United Kingdom. Gonwa et al found CKD, (defined as serum creatinine >2.5mg/dL), in 4.9% and end stage renal disease (ESRD) in 5.4% among 834 OLT recipients.[29] In a study of 1602 OLT recipients, Paramesh et al found an incidence of ESRD of 23% at a median time of 46 months after liver transplantation.[22] Out of 1173 OLT recipients studied by Schmitz from Germany, CKD, (defined as serum creatinine ≥1.8mg/dL for ≥2 weeks), was observed in 11.7% of recipients.[30] In a French series of 204 OLT recipients, Neau-Cransac et al reported a CKD incidence of 10.7%,

(CKD was defined as serum creatinine >2.3 mg/dL).[31] The wide range in reported incidence is part due to the different thresholds used to define CKD.
In a registry study of 36,849 OLT recipients in the US in which the NKF KDQOI definition of CKD[34] was applied, investigators found a 5-year CKD stage IV-V, (stage IV CKD = eGFR 15-29min/min/1.73m^2; stage V CKD = ESRD), incident rate of 18.1%.[33] Registry data in the U.S. indicate that 3-6% of deceased donor kidney transplantations that are currently performed in the U.S. go to previous recipients of orthotopic liver transplantation (OLT) which further worsens the critical shortage of transplantable organs for the overall ESRD population.[33] Chronic kidney disease in the liver transplant population is associated with a dramatically increased predisposition to cardiovascular events, increased risk of hospitalization and a four-fold excess mortality when compared to recipients with preserved renal function.[28, 35-37]

Risk factors known to predispose OLT recipients to CKD include pre-transplant elevated serum creatinine, perioperative acute renal failure, pre and post-transplant diabetes mellitus and hypertension.[29, 33, 38] A renal biopsy and precise assessment of renal function is often not feasible in OLT recipients, in the immediate pre-transplant period. Thus, it is unclear what level of renal function and structural renal histology predispose OLT recipients to an increased risk of CKD.
In the OLT recipients with CKD, the underlying renal disease is often presumed to be chronic CNI nephropathy, but a small histologic series showed a myriad of underlying renal diseases as the cause of CKD in this population.[29] Moreover, presumably normal pre-transplant serum creatinine or serum creatinine-based formulaic estimation of GFR may mask significant underlying pre-transplant renal disease, as serum creatinine itself is a late and imprecise measure of GFR decrement, particularly in patients with advanced liver disease.[39]

An indication of the high prevalence of occult kidney disease in liver transplant candidates is a finding from a study of 30 OLT recipients from the University of Alabama in Birmingham, by McGuire et al, which showed significant histologic evidence of glomerulonephritis in 25 of the 30 subjects who underwent intraoperative kidney biopsy at the time of OLT. A small Australian series from the early 1990s also demonstrated that de novo glomerular disease was uniformly present in the pre-transplant kidney biopsy in 23 hepatitis C negative OLT recipients with normal serum creatinine and benign urinary sediment.[40] These studies suggest that significant, but clinically occult glomerular disease, may be present in a sizable fraction of OLT recipients prior to liver transplantation. Further insults related to the hemodynamic perturbation of the implantation surgery or CNI-based immunosuppressive therapy may unmask and accelerate the course of such underlying renal diseases. Worse yet, immediate post-transplant exposure to CNI therapy is a major physiologic insult that may tip the balance to severe acute renal failure in the early post-transplant period in the recipients with underlying renal disease.

Serum creatinine (sCr) has been included in the new Model for End-Stage Liver Disease (MELD)-based organ allocation system employed in the US by the United Network of Organ Sharing (*UNOS, 2003*).

The MELD calculation is: MELD Score = 0.957 × Loge (creatinine mg/dL) + 0.378 × Loge (bilirubin mg/dL) + 1.120 ×Loge (International Normalized Ratio [INR]) + 0.643

While originally intended as a predictor for pre-transplant mortality, MELD, and in particular serum creatinine, has been shown to be a strong predictor not only of post-transplant renal function, but also of graft and patient survival. Among 14,447 US and Canada liver transplants, performed between July 1, 1997 and March 1, 2001, there were 1,557 deaths within 90 days of transplant and 1,484 thereafter. Bilirubin and sCr were significantly associated with early post transplant death, and of the MELD factors or albumin, only sCr predicted late mortality (all p <0.001) (*Wolfe, et al., 2003*). For long-term liver graft survivors, renal failure is the leading cause of death after10 years (*Chinnakotla, et al., op cit.*). Renal dysfunction also contributes independently to neurological complication after liver transplantation (*Donovan, et al., op cit*).

C. Thymoglobulin® in Liver Transplantation

Preliminary data from a single-center experience, using Thymoglobulin® induction in liver transplantation, to delay calcineurin inhibitor use and spare renal function suggests that such a strategy is worth further evaluation in a prospectively designed clinical trial. This retrospective analysis of 298 consecutive adult primary liver transplant recipients (66.8% male, mean age 54.8 ± 11.7 years), transplanted between 1991and 2002 has recently been presented. A pre-transplant baseline sCr of 132 mmol/L, (1.5 mg/dL), was arbitrarily set as abnormal renal function (ARF). Fifty-nine patients (Group 1) had ARF, and 239 (Group 2) had normal renal function. In 215 patients, Thymoglobulin® induction was initiated on the day of surgery, at 1.5 mg/kg, for a median of 5.4 doses over the initial 2 weeks. In the other 83 patients, no induction with Thymoglobulin® or other antibodies was used. All patients received cyclosporine or tacrolimus (TAC) and corticosteroid maintenance immunosuppression. Also, 77% of the patients received either mycophenolate mofetil (MMF) or azathioprine (Imuran). Indications for orthotopic liver transplantation and demographics were similar between the two groups.

Freedom from rejection was statistically significantly higher in the Thymoglobulin® group at one year post-transplant, (72% vs 50%; p = 0.001). Group 1 (ARF) had lower patient and graft survival at 1, 6, and 12 months compared to Group 2 (normal RF); [(81, 73, 61% vs. 96, 91, 87%) for patient survival, and (78, 66, 54% vs. 93, 84, 79%) for graft survival, (p <0.001)]. In Group 1, the use of Thymoglobulin® improved early patient survival, (85% vs. 67%; p = 0.078) and early rejection- free graft survival, (75% vs. 42%; p = 0.018).

Initiation of calcineurin inhibitor therapy was significantly delayed in the Thymoglobulin® group, (median Day 6 vs. Day 1; p = 0.0074). The sCr recovered significantly for those in Group 1 who received Thymoglobulin® induction, by one month post-transplant. The authors concluded that Thymoglobulin® induction provided an event-free early post-liver transplant course resulting in better early patient and graft survival, as well as a better rejection free primary graft survival.[41]

D. Study Rationale

Functional recovery of renal function from acute renal failure occurs in 75% of patients at approximately 14 days after onset of the disease (Schrier, Diseases of the Kidney). In liver transplantation, intraoperative hemodynamic insults typically lead to acute renal failure which may be further worsened by exposure to CNI therapy in the early postoperative period. In practice, patients who demonstrate early evidence of acute renal failure often have their CNI therapy delayed for 4-5 days. This duration of CNI delay is too short to have any salutary effect on the

course or severity of acute kidney injury as less than 20% of patients experience any functional recovery by day 5.

Thymoglobulin® (Sanofi, Cambridge, MA) is a polyclonal immunosuppressive agent that is derived from rabbits immunized with pediatric thymocytes. It contains antibodies to a wide variety of T-cell antigens and MHC antigens and is approved for the treatment of kidney rejection by the Food and Drug Administration (FDA) in the United States. Thymoglobulin® has been shown to be a safe and efficacious induction therapy that permits delayed exposure to CNI therapy while preventing the occurrence of acute rejection in kidney transplantation.[42, 43] We hypothesize that any perioperative insult leading to AKI in OLT recipients is unlikely to be beneficially impacted by a short delay of CNI introduction. We further hypothesize that avoidance of CNI for 10 days will have a beneficial effect on the course and severity of perioperative AKI. Since perioperative AKI is a potent risk factor for CKD in the late post-transplant period, we hypothesize that minimizing the risk and severity of AKI with prolonged delayed exposure to CNI will have a beneficial effect on renal function late after liver transplantation.

INVESTIGATIONAL AGENT – [ANTI-THYMOCYTE GLOBULIN (RABBIT)] – r-ATG THYMOGLOBULIN®:

A. Chemistry

Thymoglobulin® (anti-thymocyte globulin [rabbit]) is a purified, pasteurized, gamma immunoglobulin, obtained by immunization of rabbits with human thymocytes (*Thymoglobulin® Prescribing Information*). This immunosuppressive product, a polyclonal antibody, contains cytotoxic antibodies of defined specificity against functional molecules on T-lymphocytes. Thymoglobulin® is supplied as a sterile, freeze-dried product for intravenous administration after reconstitution with a diluent, (Sterile Water for Injection, United States Pharmacopeia [USP]).

Reconstituted preparation contains approximately 5 mg/mL of Thymoglobulin®, of which >90% is gamma immunoglobulin. The reconstituted solution has a hydrogen ion concentration (pH) of 7.0 ± 0.4. To minimize the risk of transmission of infective agents, viral testing is performed during production, and a viral inactivation step, (pasteurization, [i.e. heat treatment of the active ingredient at 60°C for 10 hours]) is an integral part of the Thymoglobulin® manufacturing process. The potency of the product is determined by lymphocytotoxicity assay. Each lot is analyzed and determined to meet quality standards before release.

B. Immunopharmacology

The *in vitro* mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. Thymoglobulin® includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and β2 microglobulin (*Bonnefoy et al., op cit),*[44] as well as dendritic cells[45] and adhesion molecules.[46, 47] Thymoglobulin® (>0.1 mg/mL) mediates T-cell suppressive effects via inhibition of proliferative responses to several mitogens with post-transcription blockade of interferon gamma and CD25 synthesis (*Bonnefoy et al., op cit*). The *in vivo* mechanism of action of Thymoglobulin® is also not fully understood. Possible mechanisms by which Thymoglobulin® may induce immunosuppression *in vivo* include T-cell clearance from the circulation and modulation of T-cell activation, homing and cytotoxic activities (*Clark et al., opcit*). T-lymphocyte depletion is promptly observed in transplant patients following administration of Thymoglobulin®

(*Guttmann et al., op cit*). This may result from the complement-dependent lysis in the intravascular space or the opsonization and subsequent phagocytosis by macrophages of antibody-coated T cells (*Bonnefoy et al., op cit*). T-lymphocyte depletion in peripheral blood persists for several days to several months following cessation of Thymoglobulin® therapy (*Gaber et al., op cit*).

Thymoglobulin® is a potent immunosuppressive agent that demonstrates a rapid and profound effect in lowering white blood cells (WBC), primarily by decreasing the T-cell counts. The magnitude and duration of lymphopenia have been studied. Reductions of circulating lymphocytes by 83% to 92% from pretreatment values were seen after a single dose of Thymoglobulin®, and were sustained throughout the daily dosing period in 4 clinical pharmacology studies (*data on file, Sanofi*). The recovery from Thymoglobulin®-induced lymphocyte depletion was gradual, beginning soon after cessation of therapy, with most recovered by 3 months. Recovery may take longer than 3 months, but persistent lymphocyte depletion has not resulted in any morbidity. T-cell subsets determined by flow cytometry also demonstrate similar dramatic decreases. In a Phase III randomized trial of 26 patients, T-cell depletion was greater in the Thymoglobulin®-treated patients as compared with the Atgam® (Pharmacia-Upjohn, Kalamazoo, MI)-treated patients. T-cell depletion lasted for at least 90 days after the cessation of therapy. At that time, T-cell counts remained 40% lower in Thymoglobulin®-treated patients than in Atgam®-treated patients (*Gaber et al., op cit*).

After intravenous (IV) infusion of Thymoglobulin®, (1.25 to 1.5 mg/kg/day, each infusion administered over 4 hours for 7-11 days), Thymoglobulin® levels were on average 21.5 μg/mL (range 10-40 μg/mL), 4-8 hours post-infusion, with a half-life of 2-3 days after the first dose, and 87 μg/mL (range 23-170 μg/mL), with a half-life of 1-3 months after the last dose (*Bourdage and Hamlin, op cit*). During a Thymoglobulin® Phase III randomized trial, of the 108 patients evaluated, anti-rabbit antibodies developed in 68% of the Thymoglobulin®-treated patients, and anti-horse antibodies developed in 78% of the Atgam-treated patients. These antibodies appear to be non- blocking, anti-isotypic antibodies.[48,49]

C. Overview of Clinical Efficacy and Safety

Treatment of Acute Rejection

A controlled, double-blind, multi-center, randomized clinical trial comparing Thymoglobulin® and Atgam for the reversal of acute renal allograft rejection was conducted in 163 renal transplant patients with biopsy-confirmed Banff Grade II (moderate), Grade III (severe), or corticosteroid-resistant Grade I (mild) acute rejection, while receiving maintenance immunosuppressive therapy (*Gaber et al., op cit*). Patients were randomized on a 1:1 basis to receive 7-14 days of Thymoglobulin® 1.5 mg/kg/day, or Atgam 15 mg/kg/day. Thymoglobulin® was better than or at least as successful as Atgam in reversing acute rejection episodes.

Based on intent-to-treat analysis, 72 of 82 Thymoglobulin®-treated patients (87.8%) and 61 of 80 Atgam-treated patients (76.3%) had a successful response to study treatment. Ten Thymoglobulin®-treated patients and 21 Atgam-treated patients failed the original therapies. They were all rescued with subsequent center-specific treatments. One year after treatment, 83% of Thymoglobulin®-treated patients compared with 75% Atgam-treated patients still had a functioning allograft ($p = 0.194$). The 1-year patient survival rate was 93% for Thymoglobulin®-treated patients and 96% for Atgam-treated patients ($p = 0.26$).

Efficacy of Thymoglobulin® in Liver Transplantation

In previous eras of transplant immunosuppression, induction with anti-lymphocyte antibodies has been used to reduce acute rejection. With the advent of calcineurin inhibitor-based immunosuppression, particularly TAC, antibody has been used less frequently in liver transplantation. However, a number of transplant centers have reported single-center experiences with the use of Thymoglobulin® for prophylaxis of rejection in liver transplantation.

The University of Indiana studied 109 adult liver transplant patients over 15 months. Six patients who died in the perioperative period, prior to receiving Thymoglobulin®, were excluded. All patients had at least two months post-transplant follow-up. Postoperatively, patients received 3 doses of Thymoglobulin®, (2 mg/kg/dose), administered every other day, steroids tapered to 20 mg/day by postoperative Day 7, and TAC initiated on postoperative Day 3-4, (trough levels 10-12). Elevations in liver function test numbers (LFTs) in the first 4 weeks were investigated with doppler ultrasound, followed by endoscopic retrograde cholangiopancreatography (ERCP). If these tests were non-diagnostic, biopsy was performed to evaluate rejection. The report noted 96% of patients were alive. No graft was lost due to rejection. Ten percent of patients had episodes of rejection, and a cumulative total of 6%, 8% and 10% of patients had biopsy proven rejection at 4, 8 and >8 weeks, respectively. All rejection episodes were reversed with a steroid pulse, and no patient had steroid resistant rejection. No patient had more than 1 episode of rejection. Three of the 10 patients with rejection had autoimmune hepatitis and were placed on mycophenolic acid (Myfortic) in addition to TAC and steroids. Two patients experienced vascular complications, [(i.e. hepatic artery thrombosis (HAT)]. Thymoglobulin®-related AEs were limited to fever, chills, tachycardia, and worsening oxygen saturation. No patient required reintubation as a result of Thymoglobulin®. No patients had post-transplant lymphoproliferative disorder (PTLD).

In a subset analysis, 42% of patients had hepatitis C Virus (HCV). Rejection occurred in 12% of HCV patients and 9% of non-HCV patients, (not statistically significant). At 10 months post-transplant, 31% of HCV patients had biopsy-proven hepatitis. The authors concluded that Thymoglobulin® induction therapy increased patient and graft survival, lowered incidence of rejection, and was associated with minimal detrimental side effects (*Mangus et al. 2003*).

In an earlier published retrospective review of 63 liver transplant recipients involving 73 OLT procedures, the use of Thymoglobulin® compared with a group not using Thymoglobulin® as part of induction therapy was evaluated.[50] Standard triple therapy included cyclosporine, azathioprine and prednisone. The data analyzed included patient survival, graft survival, rejection episodes, time to first rejection episode, hematological parameters, and infectious complications. Fifty-seven OLT procedures were induced with Thymoglobulin® plus triple therapy and 16 received triple therapy alone. All retransplants were performed under Thymoglobulin® induction. Three to 6 vials (25 mg/vial) of Thymoglobulin® were administered via IV infusion each day. Dose adjustments were based on WBC and platelet counts. Doses were held when platelets were below 30,000 X 10^6/mL, and/or WBC count was below 2500 x 10^3/mL. Fever (61%), thrombocytopenia, anemia, and leucopenia were the most common AEs reported with Thymoglobulin® induction therapy. These AEs were managed by dose reduction or drug administration on alternate days. Infectious complications were similar in both groups. Rejection episodes occurred less frequently in the Thymoglobulin® group (n = 33, 58%) when compared with the triple therapy control group

(n = 12, 75%). Rejection in the Thymoglobulin® group occurred later after transplantation, at a median of 51 days, compared to 11.5 days in the control group ($p < 0.05$). Occurrence of rejection while patients were on Thymoglobulin® was low, (7%), compared with the control group, (50%). However, patient and allograft survival at one year was slightly lower in the Thymoglobulin® group, (84% and 68%, respectively), compared with the control group, (94% and 81%, respectively). Although, in general, patients who ultimately received Thymoglobulin® were more ill prior to transplant, based on their need for ventilator support (21% vs. 0%), ICU status (37% vs. 12%), and renal dysfunction (11.8% vs. 8.1%), prior to transplantation. This retrospective study demonstrated that Thymoglobulin® as part of induction therapy resulted in a lower incidence of graft rejection during the first 30 days post-transplant and a longer graft survival time before first rejection. In addition, Thymoglobulin® allowed for the delay of initiation of cyclosporine therapy for up to 3 weeks post-transplant, without an increase in infectious complications.

Experience with the elimination of steroid use in OLT through induction with Thymoglobulin® was recently reported.[51, 52] In an initial prospective, randomized study, 36 patients received Thymoglobulin® 1.5 mg/kg via IV infusion during the anhepatic phase of liver transplantation, followed by a second dose on postoperative Day 1, compared with 35 liver transplant recipients who received methylprednisolone, 1000 mg IV during the anhepatic phase, followed by steroid tapering and conversion to oral prednisone. Both groups received TAC and MMF as maintenance immunosuppression. Steroids and MMF maintenance were discontinued by 3 months post LT. Patient survival was 91% in both groups. Graft survival rate was 89% in both groups. Biopsy-proven acute rejection rate was 20.5% (n = 7/33) in the Thymoglobulin® group, all responsive to increased doses of TAC, compared with 32% (n = 11/33, not statistically significant) in the group receiving corticosteroids, of whom 7 patients required additional corticosteroids, and only 4 were responsive to increased doses of TAC. The incidence rate of hepatitis C was 50% in the Thymoglobulin® group, and 71% in the corticosteroid group, (not statistically significant). Infectious complications were less in the Thymoglobulin® group, primarily due to higher cytomegalovirus (CMV) infections in the corticosteroid group. In summary, the authors concluded that Thymoglobulin® induction accompanied by TAC and MMF maintenance was effective in eliminating the need for concomitant corticosteroid therapy.

Eason has reported follow-up on 140 OLT recipients using the prospectively randomized steroid induction versus the steroid-free protocol, using two doses of Thymoglobulin®, 1.5 mg/kg/dose (*Eason 2002, op cit*). Maintenance immunosuppression consisted of TAC and MMF, with TAC monotherapy at 2 weeks in the Thymoglobulin® induction, steroid-free group, and at 3 months in the steroid group. Patients receiving Thymoglobulin® induction had a lower incidence of steroid-requiring rejection, (3% vs. 62%, $p = 0.01$). The incidence of post-transplant diabetes was 5% in the Thymoglobulin® induction group, compared with 16% in steroid-treated patients ($p < 0.05$). The incidence of CMV infection was lower in the Thymoglobulin® induction patients, (9%), compared with steroid-treated patients, (19%). The incidence of recurrent hepatitis C was 58% in Thymoglobulin® induction patients, compared with 72% in steroid-treated patients, with more steroid-treated patients progressing to fibrosis. The authors concluded that steroid-free immunosuppression using Thymoglobulin® and TAC monotherapy, "is effective in preventing rejection and minimizing severity of rejection with a decreased incidence of immunosuppression-related adverse effects."

Another recent preliminary report of a retrospective study compared two immunosuppressive therapies for adult living donor liver transplantation (LDLT) recipients with respect to prevention of acute cellular rejection (ACR), TAC dose and toxicities, and graft and patient survival. Between May, 2001 and December, 2002, 29 LDLT were performed. The first 17 patients, (Group 1), were treated with TAC and steroids. The additional 12 patients, (Group 2), were treated with a steroid-sparing protocol using low dose TAC and Thymoglobulin® administered as a 5 mg/kg dose via continuous infusion, over 4 hours, prior to surgery. Clinical records were reviewed to assess the following variables: mean daily TAC dose at 2 months after transplant, Thymoglobulin® and calcineurin inhibitor side effects, ACR, infections, and graft and patient survival. There was no notable difference in demographics between the 2 groups, (age, sex, diagnosis, and UNOS status). The mean follow-up was 7 months. There were no reported complications from the Thymoglobulin® infusion. Five of the 17 patients (30%) in Group 1 experienced early, (<30 days), biopsy-proven rejection episodes; two of the 12 patients (17%) in Group 2, experienced early rejection. All the rejection episodes in both groups were mild and responded to 2 steroid boluses, and no grafts were lost to rejection. Mean daily TAC dose at 2 months was 5.2 mg/day in Group 1, and 2.1 mg/day in Group 2 (p <0.05). The number of viral infectious episodes was similar in both groups. No TAC-related neurotoxicity was seen in Group 2, while two patients in Group 1 were switched to sirolimus monotherapy due to TAC neurotoxicity. No change from TAC to another drug was made in any patient in Group 2. Patient survival was as follows: three patients in Group 1 died, all from sepsis. All 12 patients in Group 2 were alive and well. The authors concluded that these very preliminary results of Thymoglobulin® induction, followed by postoperative steroid-free low dose TAC in the controlled setting of LDLT, showed that Thymoglobulin® induction was safe and provided adequate immunosuppression, with minimal risk of rejection, less risk of CNI toxicities, and improved graft and patient survival, when compared to historic controls with standard TAC and steroid immunosuppression (*Masetti et al., 2003*).

In summary, induction with Thymoglobulin® has been reported to lower acute rejection rates. It has been used in protocols to spare the use of steroids and calcineurin inhibitors. The rate of recurrence of hepatitis C infection in some series was lower, but was not statistically significant.

Overview of Thymoglobulin® Safety

Total patient exposure to Thymoglobulin® worldwide, since 1984, when Thymoglobulin® was first licensed in France, is approximately 2.1 million vials, equivalent to over 54,000 courses. Thymoglobulin® induces T-cell depletion and modulation. The lymphocyte count is reduced by >85% after the first dose, with reductions sustained throughout a course of treatment. T-cell depletion in peripheral blood persists for several days to several months following cessation of treatment. Approximately 60% of patients are expected to experience AEs with the use of Thymoglobulin®. Thymoglobulin® presents a consistent AE profile, with AEs that are generally manageable or reversible (*Granjard et al., 1989*). AEs with Thymoglobulin® occur with similar or less frequency than those reported with other polyclonal, anti-T-cell agents. The most frequently reported AEs include fever, chills, leukopenia, thrombocytopenia, headache, diarrhea, and abdominal pain. These usually occur after the first infusion. The cause of some of these AEs is probably related to cytokine release. Premedication with corticosteroids and antihistamines, and a decrease in the infusion rate may reduce the incidence and severity of certain AEs. While

anaphylactic hypersensitivity reactions are rare, delayed allergic responses in the form of serum sickness have been reported.

In a US Phase III controlled clinical trial (N = 163), comparing the efficacy and safety of Thymoglobulin® and Atgam for the treatment of acute renal allograft rejection, the AEs were similar in both treatment groups and were generally manageable or reversible (*Gaber et al, op cit*). Infection and PTLD are associated with use of immunosuppressive agents, including Thymoglobulin®. Some transient abnormalities of liver function tests have been described in patients with aplastic anemia, treated with Thymoglobulin® or other anti-thymocyte globulins. It is not clear whether these rarely reported abnormalities are related to the disease and pre-existing liver dysfunction, or to the treatment.[53]

In a more recent retrospective, single center, comparative study *(Bajjoka et al)* 118 patients received Thymoglobulin® with delayed initiation of CNI at 4.6 $\pm$ 2.3 days, compared to 80 patients treated with early initiation of CNI at 1.9 $\pm$ 2.5 days after liver transplantation. The Thymoglobulin® group had a significantly higher baseline serum creatinine (2.8 vs. 2.2, P=0.005) and lower baseline eGFR (30.4 vs. 37.4, P<0.001). At one year post transplant the thymoglobulin® group had a significantly lower serum creatinine (1.4 vs. 1.7, P<0.001), and higher eGFR (57.3 vs. 43.7, P<0.001). A significantly lower number of patients in the thymoglobulin® group stayed on dialysis at one year (0.8% vs. 13%, P<0.001). Graft and patient survival at one year were the same, and there was less incidence of rejection in the Thymoglobulin® group[54].

In a recent phase I/II randomized, controlled trial of 2 different doses of Thymoglobulin® and extended delay of CNI initiation, compared to early CNI therapy after liver transplantation, serum creatinine and eGFR in patients induced with Thymoglobulin® was significantly better at one year, (1.0 vs. 1.1 vs. 1.5mg/dL), and (87.8 vs. 72.0 vs. 51.0 mL/min) respectively. The study was designed in three groups, 10 patients per group. In one group the Thymoglobulin® dose was 3 mg/Kg (2 doses of 1.5 mg/Kg); in the second group the Thymoglobulin® dose was 4.5 mg/Kg (3 doses of 1.5 mg/Kg). Patients in these two groups received CNI by Day 10 after transplantation. The third group received early CNI, within two days after liver transplantation. Rejection rate was higher in the low-dose Thymoglobulin® group, but there was no difference in patient and graft survival at one year. [55]

An overview of the safety profile of Thymoglobulin® is presented. The most common systemic AEs during and after Thymoglobulin® infusion are: fever (63%), chills (57%), pain (46%), headache (40%), abdominal pain (38%), diarrhea (37%), hypertension (37%), nausea (37%), peripheral edema (34%), dyspnea (28%), asthenia (27%), hyperkalemia (27%), and tachycardia (27%), (*Thymoglobulin® Prescribing Information*). Hypotension, vomiting, and malaise have also been commonly reported. The likely mechanism of action for these events is the release of cytokines during the first dose administration, particularly if appropriate premedications were not administered. Localized AEs, such as pain at the infusion site and peripheral thrombophlebitis, have also been reported. Rare delayed allergic reactions such as serum sickness, (fever, pruritus, and rash associated with joint and muscle pain), may occur 7 to 15 days post-treatment initiation. Immediate serious allergic reactions and/or anaphylaxis are very rare.

AEs associated with the presence of antibodies inducing cross-reactions such as leucopenia (57%) and thrombocytopenia (37%) have been reported during and after treatment with Thymoglobulin®, (*Thymoglobulin® Prescribing Information*). These reactions may emerge during the first 2 days of treatment or after treatment. The mechanism of action likely causing these effects involves the presence of antibodies cross-reacting with shared antigens of lymphocytes, neutrophils, and platelets, or additive effects from concomitant medication. Monitoring of the WBC and platelet counts enables the severity and frequency of such reactions to be reduced.

AEs associated with over-immunosuppression, including infectious complications (bacterial, fungal, viral, and/or protozoal), and malignancies, (particularly lymphoproliferative syndrome), have been reported. It is important to note that concomitant or previous immunosuppressive treatments may contribute to over-immunosuppression. Appropriate anti-infective prophylaxis will significantly reduce the incidence of infections and possibly the incidence of viral-associated malignancies.

STUDY OBJECTIVES:

A. Primary Objective:

- To determine if a 10 day delayed introduction of CNI reduces the risk/incidence of perioperative acute kidney injury, (AKI), and long-term renal dysfunction in patients undergoing liver transplantation.

B. Secondary Objective(s):

- To determine if a 10 day delayed introduction of CNI affects the risk of graft rejection.
- Determine patient survival at 6 months post-transplant and compare survival rates between the two groups.
- Determine graft survival at 6 months post-transplant and compare graft survival rates between the two groups.
- Determine the incidence of AEs and SAEs in each group.

INVESTIGATIONAL PLAN:

A. Overall Study Design

This is a 24-month, Phase II, multi-center, two-arm, randomized study of adult patients receiving a single organ liver transplant from a deceased donor; the purpose being to determine the efficacy of Thymoglobulin® induction and delayed initiation of CNI in the long-term preservation of renal function after liver transplantation. This study is based on the outcomes of an earlier phase I pilot study which was performed at the Cleveland Clinic.

Study subjects will be asked to sign the Informed Consent at the time of admission for liver transplantation at Cleveland Clinic, and three other transplant centers, (University of Cincinnati, Medical College of Wisconsin, and Cleveland Clinic Weston, Florida), prior to OLT and prior to any study procedures, (that are not SOC), being performed. All subjects should be familiar with the Informed Consent and its content, and have been given opportunity to have their questions answered about the study, prior to signing the Informed Consent.

The length of the study will be approximately 24-36 months to allow for recruitment, and approximately 12 months of ongoing follow-up after the last patient is enrolled. A total of 110

subjects will be randomized (1:1) into one of two groups, 55 subjects per group. Subjects randomized to Group 1 will receive Thymoglobulin® induction, (4.5 mg/Kg, in 3 doses of 1.5 mg/Kg/dose), and delayed initiation of CNI, (to begin on Day 10 post LT). Subjects randomized to Group 2 will receive will receive early CNI initiation, (to be started no later than Day 2 post LT), and no Thymoglobulin® induction, **(or any other antibody)**. In addition, all subjects will receive Mycophenolate Mofetile (MMF) and Corticosteroid immunosuppression as stated in Table 1.

Table 1: Immunosuppression Protocol

PHASE II RANDOMIZED 2-ARM MULTI-CENTER STUDY		
Treatment	**GROUP/ARM 1 Thymoglobulin® + Delayed CNI**	**GROUP/ARM 2 Early CNI Group**
Thymoglobulin®	Thymoglobulin® 1.5 mg/kg/dose Day 0, 2, and 4 (Total 4.5 mg/kg)	None
Tacrolimus	Start on post-transplant Day 10	Start no later than 48 hours post-transplant
Mycophenolate mofetile	1000 mg BID start on day one post-transplant	1000 mg BID start on day one post-transplant
Corticosteroids	As per Transplant Center Immunosuppression Protocol - Taper off between 21 days-6 months post LT	As per Transplant Center Immunosuppression Protocol - Taper off between 21 days-6 months post LT

The Data Safety Monitoring Board (DSMB) will review the data on the first 30 subjects who have completed 30 days study enrollment.

Primary Endpoints:

- The incidence of AKI at 30 days post-transplant.
- Serum creatinine and eGFR at 30 days post-transplant.

Secondary Endpoints:

- The incidences of acute cellular rejection, (ACR), at 30 days post-transplant.
- Patient survival at 6 months post-transplant.
- Graft survival at 6 months post-transplant.
- The incidences of AEs and SAEs.

The primary endpoint for the study is a composite renal endpoint of Acute Kidney Injury, **(stages 2 to 3),** at 30 days post OLT. The staging of AKI will follow the classification system proposed by the Acute Kidney Injury Network (AKIN) depicted in **Table 2**.[26]

TABLE 2: Classification / Staging for Acute Kidney Injury (AKI)

Classification/Staging System for Acute Kidney Injury[26]		
Stage:	**Serum Creatinine Criteria**	**Urine Output Criteria:**
1	Increase in serum creatinine of ≥ 0.3 mg/dL, (≥ 26.4 μmol/L), or an increase of ≥ 150% to 200%, (1.5 to 2-fold), from baseline.	Less than 0.5 mL/kg per hour for more than 6 hours
2	Increase in serum creatinine of > 200% to 300%, (>2 to 3-fold), from baseline.	Less than 0.5 mL/kg per hour for more than 12 hours
3[a]	Increase in serum creatinine to more than 300%, (>3-fold), from baseline, (or serum creatinine of ≥ 4 mg/dL, [≥354 μmol], with an acute increase of ≥ 0.5 mg/dL, [44 μmol/L].	Less than 0.3 mL/kg per hour for 24 hours, or anuria for 12 hours.

[a]Only **one criterion** (creatinine or urine output) must be fulfilled to qualify for a stage. Individuals receiving renal replacement therapy (RRT) are considered to have met the criteria for **Stage 3** irrespective of the stage they were in at the time of RRT.

B. Patient Population

Study participants will be recruited from the Liver Transplant Programs at Cleveland Clinic, Cleveland, Ohio; University of Cincinnati, Cincinnati, Ohio; Medical College of Wisconsin Milwaukee, Wisconsin, and Cleveland Clinic Weston, Florida

C. Number of Patients: One hundred and ten (110) liver transplant recipients will be enrolled in this study, **approximately** 40-50 patients at Cleveland Clinic, (the lead center), and 15-25 patients at each of the three additional participating centers (number of enrollment may vary at different centers).

Sample size estimates for study of liver dysfunction

Sample sizes were computed on the bases of using Welch's two-sample t-tests to compare changes in creatinine between the control and the thymo 4.5 groups. Assumptions regarding differences in means and standard deviations for each group were based on the results of preliminary data and are given in Table 3. The smaller of the two standard deviations was varied along a range from that observed in the preliminary data to being equal to the larger standard deviation. The former would tend to yield smaller sample sizes, while the latter larger. Assuming that 10% of the patients that are initially enrolled will drop-out before 12 months values are measured, the sample sizes indicated should be increased by 10% to account for this drop-out.

These calculations were done using SAS software (version 9.3, Cary, NC). A significance level of 5% and two-tailed tests were assumed for all testing.

For delta creatinine, we are assuming a difference in means between groups of 0.32. In the pilot data delta creatininte in the control group had a standard deviation of 0.51, while the thymo 4.5 group a standard deviation of 0.12. Therefore, three levels of standard deviation—0.12, 0.32, and 0.51. Based on these calculations, enrolling enough patients to meet the sample size requirements for detecting differences in delta creatinine (within 2 SD, 0.32) will mean having ample sample size for detecting differences in month 12 GFR. Accordingly, we noticed that a sample size of 110 (55 in each group) would give us 90% power with the safest margin for 10% dropouts throughout the study.

Table 3. Minimum sample sizes need to achieve desired levels of power

				Power	
Comparison	Difference	SD1	SD2	80%	90%
Delta Creatinine	0.32	0.51	0.12	46	60
			0.32	58	78
			0.51	82	110
Month 12 GFR	36.8	26.6	20.5	16	20
			23	18	22
			26.6	20	26

D. Selection of Study Population:

INCLUSION CRITERIA

- Patients undergoing deceased donor solitary liver transplantation.
- Age 18 – 75 years at the time of transplantation.
- Willingness and ability to comply with the study procedures.
- Signed Informed Consent Form.
- For patients with Hepatocellular carcinoma as indication for OLT, (must be within the Milan Criteria)
- Hepatitis C positive or negative patients.

EXCLUSION CRITERIA

- Prior kidney transplantation.
- Congenital or iatrogenic absence of one kidney.
- Subjects on renal replacement therapy at the time of OLT.
- MELD score >34.
- HIV positive patient.
- Patient with current severe systemic infection.
- History of bacterial peritonitis within 30 days prior to OLT.
- Active infection or recent infection within 30 days prior to OLT.

- Use of a calcineurin inhibitors continuously, for more than 90 days, within the past 6 months.
- History of hypersensitivity to thymoglobulin®, rabbits, tacrolimus or iohexol.
- Pregnant and/or nursing (lactating) females**.**
- Women of childbearing potential who are unwilling to use effective contraceptive methods during the duration of the study.

E. Patient Numbering

Enrollment will take place after completion of OLT, (within 3 hours post completion of liver transplantation). At the time of **enrollment,** each subject will be assigned a unique subject identifier, beginning with the Site Specific Acronym, [Cleveland Clinic = CC; University of Cincinnati = UC; Medical College of Wisconsin = MW, and Cleveland Clinic Weston = WE]. The Site Acronym will be followed by hyphen [-], and a sequential three digit subject number, [i.e. 001, 002, 003, etc.], for identification. Assigned, (three digit), subject numbers will be sequential, and represent a subject within each clinical site.

Once the subject is enrolled into the study, he/she will be assigned the lowest available subject number at that Study Site. If the subject fails to be randomized, for whatever reason, the subject's number and reason for not progressing into the trial will be entered on the Study Enrollment Log. Once assigned to a subject, a subject number will not be reused. All subjects who fulfill all the inclusion criteria and do not meet any of the exclusion criteria will be assigned a Randomization Number, at the time of Randomization. This number will be used to link the subject to his/her respective treatment group.

F. Treatment Group Assignment

A randomization schedule will be generated and kept by each transplant center, after approval of the protocol by the Cleveland Clinic IRB, and each participating center's respective IRB, and prior to Study Start-up. Randomization numbers will be assigned in the order in which subjects qualify for treatment, not in the order of study subject enrollment.

Subject randomization will be performed provided the patient has signed the Informed Consent AND met all Study Entry Criteria, (Inclusion and Exclusion), **AND is "Enrolled" into the Study. Randomization will be performed within 6 hours of the patient's arrival to the Intensive Care Unit, (ICU),** after the transplant procedure. Subjects will be randomized in a 1:1 fashion to receive delayed CNI with 4.5 mg/Kg Thymoglobulin® [Group 1], or early CNI therapy [Group 2]. All subjects will receive maintenance therapy with MMF and corticosteroids.

G. Early Patient Withdrawal/Study Treatment Discontinuation

Subjects may voluntarily withdraw from the study or be withdrawn from the study, at the discretion of the investigator, at any time. A patient should be withdrawn from the study if the investigator believes that continuation would be detrimental to the patient's well-being. Reasons for **Study Treatment Discontinuation / Early Patient Withdrawal** are:

- Death after consent, but prior to randomization.
- Graft loss and/or re-transplantation.
- Pregnancy.
- Withdrawal of informed consent, (subject's decision to withdraw for any reason).

- Any clinically significant AE/SAE(s), laboratory abnormality, and/or incurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy and/or further participation in the study, (including obtaining vital status of the subject and allograft), is not in the best interest of the subject.
- Unsatisfactory therapeutic effect (e.g. repeated acute rejection).
- Termination of the study by the investigating center or withdrawal of funds by Sanofi.
- Significant protocol violation.
- Protocol deviation resulting in significant patient safety risk.
- Subjects who for any reason cannot be followed and/or are unable to meet the requirements of the protocol.

If Early Patient Withdrawal occurs, or if the subject fails to return for scheduled study visits, the investigator must determine and document which of the above reasons led to the patient's premature withdrawal. For subjects who are lost to follow-up, the investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient, [e.g. date(s) along with documentation of telephone calls, registered letter(s), etc]. Subjects who are prematurely withdrawn from study medication or from the study will not be replaced. Withdrawal of a subject from study treatment does not imply that the subject is withdrawn from the study.

H. Discontinued Subjects

The investigator should accept only those subjects who give a reasonable indication that they will complete the study and comply with study procedures. An evaluation that reflects the status of the subject at premature termination, with a final assessment and reason(s) for termination, must be documented. No replacements will be allowed for treated subjects who do not complete the study.

STUDY TREATMENTS:

A. Investigational and Control Drugs

The administration of Thymoglobulin®, to allow for delayed initiation of CNI, is the Investigational Treatment of the study. It will be administered only to those subjects randomized to Group 1/Arm 1, the delayed CNI group/arm. Subjects randomized to Group 2, the early CNI treatment group/arm will not receive any placebo therapy. The dosing of Thymoglobulin® will be a total of 4.5 mg/Kg, administered as 1.5 mg/Kg/dose, in 3 divided doses.

All Thymoglobulin® infusions will be administered through **a central IV line, via a volumetric infusion pump, through a 0.22 micron filter.** The first dose of Thymoglobulin® will be infused over 6-12 hours, on Day 0, after liver transplantation**, (within 12 hours after arrival to the Intensive Care Unit).** The subsequent two (2) additional doses of Thymoglobulin® will be infused over 4-6 hours (each dose), on Day 2 and Day 4 post LT.

Subjects are to receive pre-medications, (according to the antibody infusion protocols of each investigative center), [e.g. corticosteroids, anti-histamine, and acetaminophen], to minimize infusion related events.

A total of 110 subjects will be randomly assigned to one of two Treatment Groups/Arms, in a 1:1 ratio, (55 subjects per Group/Arm).

Group 1/Arm 1: [Thymoglobulin® Induction (4.5mg/Kg – Total Dose – administered in 3 Infusions of 1.5mg/Kg per Infusion **{Each dose of Thymoglobulin® to be corrected (rounded up or down to the nearest 25mg dose) – the maximum single dose not to exceed 150mg}**, on Day(s) 0, 2, and 4 post OLT)], ***plus*** Delay CNI initiation – CNI start on Day 10 post OLT, ***plus*** MMF and Corticosteroids.

Group 2/Arm 2: [Early CNI – CNI initiation by Day 2 post OLT], ***plus*** MMF and Corticosteroids. No Thymoglobulin®/or any other Antibody induction.

B. Dose Adjustments / Dose Holds / Discontinuation:
Thymoglobulin® dose adjustments should be based on AEs / SAEs.

The Following will Necessitate a Dose Reduction, and/or Discontinuation of Thymoglobulin® Infusion [Dose 2 and/or Dose 3]:

- **Major/Severe Allergic Reaction or Anaphylactic Reaction** – all further Thymoglobulin® Infusions are to be **DISCONTINUED** – patient will be taken **"Off Study".**
- **ANC less than 1200 cells/µL -** Reduce dose by 50%
- **ANC less than 800 cells/µL -** Hold dose and re-evaluate the following day
- **Platelet count: <20000** give half of the dose or delay the dose for 24h and re-check. Infuse platelets if platelet count is still <20000 after 24h of delay before Thymo infusion. **Do not infuse if platelet count is <10000.**

INFUSION REACTION:

- **All patients are to be pre-medicated with [corticosteroids, antihistamine, acetaminophen (per institutional standard)], prior to each Thymoglobulin® Infusion, (in order to minimize the incidence of "infusion reaction").**

- **In the event of Thymoglobulin® Infusion Reaction [fatigue, headache, nausea, vomiting, abdominal pain, fever, chills, pruritus, urticaria] – INTERRUPT THE INFUSION AND INFORM THE PHYSICIAN BEFORE RESUMING THE INFUSION.**

- **The infusion may be resumed at a 50% decreased flow rate (with a maximum length of infusion of 8 hours):**

  AS CLINICALLY INDICATED, BASED ON:
  - **The patient's reaction**
  - **The clinical decision of the Physician/Investigator at each Center**
  - **Each center's "Institutional Standard"**

Vital Signs should be checked and the patient assessed every 15 minutes until the infusion is completed. The Infusion Reaction is to be documented on the Infusion Record and the AE CRF.

SEVERE INFUSION REACTION:
[Hypotension / Shortness of Breath / Bronchospasm / Anaphylaxis]

- **STOP THE INFUSION AND NOTIFY THE PHYSICIAN**
- **FOLLOW INSTITUTIONAL PROTOCOL FOR SEVERE DRUG REACTION**

C. Treatment Blinding

This is an open-label study.

D. Study Drug Supply, Storage, and Tracking:

Thymoglobulin® is the Study Drug. Sanofi will supply the Study Drug, [Thymoglobulin® (r-ATG)] directly to the study sites (Cleveland Clinic, University Cincinnati, Medical College of Wisconsin, and Cleveland Clinic Weston). . Drug accountability and monitoring, will be on these individual study sites.

Study drug will be received by designated person(s) at each Study Site. Study drug will be handled and stored safely and properly, and kept in a secure location. Upon receipt, all study drug will be stored according to instructions specified on the drug label. Study drug and supplies are to be dispensed in accordance with the manufacturer's guidelines and clinical trial protocol.

The Study Pharmacy personnel at each Study Site will maintain accurate records of the study drug shipment(s) received and the dispensing of study drug in a drug accountability ledger. Monitoring of drug accountability will be performed by the Study Pharmacy personnel at each Study Site. At the conclusion of the study all unused drug will be disposed of as per the guidelines set forth by Sanofi and each Study Site Policy/Standard of Practice/Standard Operating Procedure (SOP). The drug accountability ledger will be kept at the Study Site, in the Site Pharmacy, in accordance with each Study Site's Policies.

E. Dispensing of Investigational Drug:

Patients randomized to Group 1/Arm 1 will receive Thymoglobulin®, to be dispensed from each Study Site's respective pharmacy. The preparation and methods of administration of Thymoglobulin® will be according to the recommended dosing guidelines of the manufacturer. Prior to dispensing study drug to the patient, a Drug label will be affixed to each dose of Thymoglobulin®. Each drug label will identify the patient, in accordance with each Study Site's Policy and Standard of Practice/Standard Operating Procedure (SOP). Drug labels will comply

with all legal requirements. Proper storage conditions and drug administration instructions are to be printed on the drug label.

OTHER MEDICATIONS/THERAPIES USED IN THE STUDY:

A. CNI Treatment:

Tacrolimus will be the only CNI used in this study, **[with the exception of change over to cyclosporine in the event of neurotoxicity due to tacrolimus].**

Tacrolimus will be administered to all subjects according to the protocol-specified dosing regimen, [to maintain Tacrolimus Blood Trough (C-0h) Levels/Concentrations] shown in **Table 4**. Tacrolimus to be administered by mouth or through the NG tube [suspension] if not able to take by mouth. In rare cases, better if not, could be administered as sublingual. We should try not to use tacrolimus as intravenous unless absolutely necessary.

Table 4:

Tacrolimus Blood Trough (C-0h) Levels / Concentration-Guided Dosing Regimen		
	Group 2 / Arm 1 Thymoglobulin® / Delayed CNI Group	**Group 2 / Arm 2 Early CNI Group**
Day(s) Post OLT	**Blood Trough C-0h Level**	**Blood Trough C-0h Level**
Day(s) 0-10	**N/A – No CNI permitted**	**≥ 8 ng/mL and ≤ 12 ng/mL**
Day(s) 10-30	**≥ 6 ng/mL and ≤ 12 ng/mL**	**≥ 6 ng/mL and ≤ 12 ng/mL**
Day(s) 31-60	**≥ 6 ng/mL and ≤ 10 ng/mL**	**≥ 6ng/mL and ≤ 10ng/mL**
Day(s) 61-90	**≥ 5 ng/mL and ≤ 8 ng/mL**	**≥ 5 ng/mL and ≤ 8 ng/mL**
Day(s) 91-179	**≥ 5 ng/mL and ≤ 8 ng/mL**	**≥ 5 ng/mL and ≤ 8 ng/mL**
Day(s) 180+	**≥ 3 ng/mL and ≤ 8 ng/mL**	**≥ 3 ng/mL and ≤ 8 ng/mL**

In the event of CNI intolerance, **(e.g. nephrotoxicity)**, dose reduction of tacrolimus may be necessary. The tacrolimus dose may be adjusted as needed by Investigator. All dose adjustments/modifications will be recorded on the appropriate CRF page, at each Study Visit.

In the event of **neurotoxicity, due to tacrolimus,** the patient will be switched over to **cyclosporine** as the **CNI alternative.** In this event the patient will not need to be removed from the study. This change in CNI, (from tacrolimus to cyclosporine), will be recorded on the appropriate CRF.

Cyclosporine (CsA) will be administered to maintain Cyclosporine Blood Trough (C-0h) Levels/Concentrations shown in **Table 5.**

Table5:

Cyclosporine (CsA) Blood Trough (C-0h) Levels / Concentration-Guided Dosing Regimen		
	Group 2 / Arm 1 Thymoglobulin®/Delayed CNI Group	**Group 2 / Arm 2 Early CNI Group**
Day(s) Post OLT	**Blood Trough C-0h Level**	**Blood Trough C-0h Level**
Day(s) 0-10	**N/A – No CNI permitted**	**≥ 200 ng/mL and ≤ 300 ng/mL**
Day(s) 10-30	**≥ 150 ng/mL and ≤ 250 ng/mL**	**≥ 150 ng/mL and ≤ 250 ng/mL**
Day(s) 31-60	**≥ 150 ng/mL and ≤ 200 ng/mL**	**≥ 150 ng/mL and ≤ 200 ng/mL**
Day(s) 61-90	**≥ 100 ng/mL and ≤ 200 ng/mL**	**≥ 100 ng/mL and ≤ 200 ng/mL**
Day(s) 91-179	**≥ 100 ng/mL and ≤ 150 ng/mL**	**≥ 100 ng/mL and ≤ 150 ng/mL**
Day(s) 180+	**≥ 100 ng/mL and ≤ 150 ng/mL**	**≥ 100 ng/mL and ≤ 150 ng/mL**

B. Corticosteroids:

All subjects in the study will be treated with daily corticosteroids based on each Study Site's Liver Transplant Corticosteroid taper protocol. Study subjects are to be tapered off corticosteroids according to the respective center's tapering protocol, **and by 6 months post LT, [with the exception of those patients who require low dose corticosteroid maintenance for primary hepatic disease for which OLT was performed].**

The following Prednisone taper schedule to be used at Cleveland Clinic. Prednisone – 1000mg IV intra-operative – Day 0. Day 1 – 50mg Q 6 hrs. X 4 doses. Day 2 – 40mg Q 6 hrs. X 4 doses. Day 3 – 30mg Q 6 hrs. X 4 doses. Day 4 – 20mg Q 6 hrs. X 4 doses. Day 5 – 20mg Q 12 hrs. X 2 doses. Then 20mg daily X 4 days; 15mg daily X 4 days; 10mg daily X 4 days; 5mg daily X 4 days; Then D/C. Patient to be off Prednisone after Day 21 post LT. **Prednisone doses may be administered IV or NG if patient is unable to take PO/orally.**

C. Mycophenolate Mofetil (MMF) Therapy:

All subjects in the study will be treated with MMF for a minimum of 6 months. The **initial dosing of MMF is to be 2 Grams daily, (1 Gram twice daily – every 12 Hours).** The dose can be subsequently adjusted at the Investigator's discretion, **based on subject tolerance and AEs.** Mycophenolate Mofetil should be administered orally, (PO/NG). However, intravenous (IV) dosing is permitted, if needed, due to intercurrent illness, postoperative ileus, or other causes, at the Investigator's discretion. **Initial dose of MMF to be administered IV or NG on Day 0, after OLT**. Subsequent doses should be administered PO or NG, as soon as the subject is able to tolerate medications by mouth. The dose and schedule may be adjusted on the basis of laboratory values, (e.g. decreased WBCs), and subject tolerability, (as stated below). For full prescribing information, see the package insert.

For subjects who develop nausea, diarrhea, or other MMF-related gastrointestinal side effects, the MMF dose may be decreased to the maximally tolerated dose. For subjects who develop neutropenia, dosing with MMF should be interrupted, or dose reduced as per the package insert.

D. Prophylaxis Therapies:

Antibacterial, Antifungal, and Anti-Pneumocystis jirovesi (carinii) Prophylaxis:

Standard antifungal, antibacterial, and anti-***Pneumocystis jirovesi (carinii)*** pneumonia **prophylaxis** therapies will be administered based on Institutional standards. All p**rophylaxis dosing should be consistent for all patients at the same institution.**

CMV Prophylaxis:

CMV reactivation is common in the transplant population. Therefore, **antiviral prophylaxis for CMV will be as follows:**

Required CMV prophylaxis therapy is presented in Table 6 below.

Table 6: Required CMV Prophylaxis Therapy or similar coverage per center protocol:

Status	**Required Prophylaxis Therapy**
Donor (–)/Recipient (–)	Acyclovir 400mg PO BID x 3 months
Donor (–)/Recipient (+)	Valganciclovir 900 mg PO, BID x 14 days OR Ganciclovir 5mg/kg IV Q12 hr x 14days [for those patients unable to take PO meds] Then Acyclovir 400mg PO BID x 3 months.
Donor (+)/Recipient (–)	Same as Donor (–)/Recipient (+)
Donor (+)/Recipient (+)	Same as Donor (–)/Recipient (+)

Adjustment of Ganciclovir / Valganciclovir for Renal Dysfunction:

Ganciclovir:

CrCl (>80) 5mg/kg Q12 hrs

CrCl (50-79) 2.5mg/kg Q12 hrs

CrCl (25-49) 2.5mg/kg Q24hrs

CrCl (<25) 1.25mg/kg Q24hrs

Valganciclovir:

CrCl (>60) 900 mg BID

CrCl (40-59) 450 mg BID

CrCl (25-39) 450 mg Q24hrs

CrCl (10-24) 450 mg Q48hrs

E. Liver Biopsies

There are no **"protocol-driven"** Liver Biopsies in the Study. However, a Liver Biopsy will be necessary in the following instances for confirmatory diagnosis:

- For "suspected" Acute Cellular Rejection (ACR)
- For Hepatitis C (HCV) recurrence (for HCV positive patients), or as per institutional standard for HCV-positive patients.
- F. Diagnosis and Treatment of Acute Rejection Episodes:

In the event of suspected acute liver allograft rejection, **an allograft biopsy MUST be performed PRIOR to the initiation of anti-rejection therapy.** Whenever possible, anti-rejection therapy should be postponed until a histological diagnosis of rejection is confirmed. These biopsies will be read locally at each clinical center and the histological lesions will be graded according to the **Grading of Acute Liver Allograft Rejection, (Banff).** Biopsy results from the local pathologist are to be recorded on the Liver Allograft Biopsy CRF. Patients experiencing a Biopsy Proven Acute Rejection (BPAR) episode should be treated in accordance with the Study Site standard practice. Treatment is to be consistent for all subjects at each respective Study Site. Details of treatment of rejection will be recorded on the appropriate CRF. A biopsy for suspected rejection will be performed based on clinical/biochemical information and judgment of the Investigator(s).

G. Diagnosis and Treatment of HCV:

For suspected HCV infection, an allograft biopsy will be performed prior to initiation of any anti-HCV viral therapy. The biopsy will be read locally, at each Study Site, and the histological lesion(s) will be graded. The biopsy results, as reported by the Study Site local pathologist, will be recorded on the Liver Allograft Biopsy CRF. Patients experiencing a biopsy proven recurrence of HCV infection are to be managed following the local standard practice in a consistent manner within the center for all subjects. Details of treatment of HCV will be recorded on the appropriate CRF.

H. Study Drug (Thymoglobulin®) Discontinuation

In the absence of medical contraindication or significant protocol violation, every effort will be made to continue Thymoglobulin® dosing. Reasons for Thymoglobulin® (Study Drug) discontinuation will be recorded on the appropriate CRF. Reasons for Thymoglobulin® (Study Drug) discontinuation are:

- Death
- Graft Loss
- Withdrawal of Informed Consent
- Clinically significant AEs/SAEs
- Unsatisfactory therapeutic effect
- Significant protocol violation
- Protocol deviation resulting in significant patient safety risk

All subjects discontinuing Thymoglobulin® prior to completing the target total dose will continue to be followed in order to obtain follow-up information, and should not be considered withdrawn

from the study. Information will be collected on serum creatinine levels, urea, eGFR, rejection episodes, graft loss/re-transplant, malignancies, opportunistic infections, patient survival and immunosuppressive therapy.

Subjects who did not complete the planned Thymoglobulin® course will be followed for any SAE(s) occurring within 30 days following the last dose of Thymoglobulin®. **These SAE(s) will be reported on the (MedWatch SAE Form) and UPLOADED to the Sanofi PV Portal, and faxed to Cleveland Clinic PI – "Attention: Bijan Eghtesad, MD" at (216) 444-9375, within 24 hours of Study Site discovery/notification.** Since subjects will be followed even after discontinuation of the Study Drug, the **Study Completion CRF** should only be completed at 30 days, and Month 12 or earlier if the subject can no longer be followed. The following reasons are potential causes for premature discontinuation of study: graft loss, death, lost to follow-up and withdrawal of consent.

I. Emergency Un-Blinding of Treatment Assignment

This is an open-label study.

J. Study Completion and Post-Study Treatment

A Study Completion CRF should be completed at Month 12. The Investigator also must provide follow-up medical care for all patients who are prematurely withdrawn from the study, or must refer them for appropriate ongoing care. In addition, investigators will be asked to provide, for each randomized patient, a one-year follow-up data to describe patient survival, graft survival, acute rejection, current immunosuppressive regimen, recurrence of hepatitis C and renal function.

EFFICACY AND SAFETY ASSESSMENTS:

A. Renal function

Renal function will be assessed by estimated Glomerular Filtration Rate (eGFR), using the MDRD 4-variable equation. Serum creatinine will also be used as a parameter for determination of renal function at each Study Visit. Subjects will be classified for each occurrence of AKI according to the criteria in **Table 2**. Serum creatinine and weight must be recorded on the Study Visit CRF. All subjects will have their eGFR assessed at baseline, **Month 1, 3, 6, 9 and Month 12 post-transplant.**

B. Acute Cellular Rejection

Acute cellular rejection (ACR) will be assessed using the **Banff Criteria** for rejection. All acute rejection episodes will require biopsy confirmation. Acute rejection will be defined as any biopsy-proven **Grade 1 (mild) or higher rejection using the Banff Criteria.** Biopsy results, (as graded by the local pathologist at each respective Study Site), will be recorded on the Liver Allograft Biopsy CRF using a standard liver biopsy grading procedure, for both Acute and Chronic Rejection. **In addition, all episodes of ACR will be considered an Adverse Event, (AE), and an AE (MedWatch) Form must be completed.**

Therapy for biopsy-proven acute rejection (BPAR) will be administered according to Study Site Institutional Standards.

C. Graft loss

The allograft will be presumed to be lost if a patient has a liver re-transplant, or in the event of patient death. In the event of Graft Loss, an **SAE (MedWatch) Form must be completed and UPLOADED to the Sanofi PV Portal, and faxed to Cleveland Clinic PI – "Attention: Bijan Eghtesad, MD" at (216) 444-9375,** within 24 hours of Study Site discovery/notification. The reason for graft loss will be recorded on the Graft Loss CRF and on the Study Completion CRF.

D. Death

In the event of patient death, an **SAE (MedWatch) Form must be completed and UPLOADED to the Sanofi PV Portal, and faxed to Cleveland Clinic PI – "Attention: Bijan Eghtesad, MD" at (216) 444-9375,** within 24 hours of Study Site discovery/notification. The events leading to the death must be entered on the AE/SAE CRF and on the Study Completion CRF.

E. Safety

Adverse Events and Serious Adverse Events (AEs/SAEs)

THYMOGLOBULIN® – Rabbit Thymocyte Globulin (r-ATG):
The most common systemic Adverse Events occurring during and after Thymoglobulin® Infusion are:

- Fever (63%)
- Chills (57%)
- Pain (46%)
- Headache (40%)
- Abdominal pain (38%)
- Diarrhea (37%)
- Hypertension (37%)
- Nausea (37%)
- Peripheral Edema (34%)
- Dyspnea (28%)
- Asthenia (27%)
- Hyperkalemia (27%)
- Tachycardia (27%)

Hypotension, vomiting and malaise have also been commonly reported. The likely mechanism of action for these events is the release of cytokines during the first dose administration. Localized AEs, such as pain at the infusion site and peripheral thrombophlebitis, have also been reported. Rare delayed allergic reactions such as serum sickness, (fever, pruritus, and rash associated with joint and muscle pain), may occur 7 to 15 days post-treatment initiation. Immediate serious allergic reactions and/or anaphylaxis are very rare.

The following Adverse Events, associated with the presence of antibodies inducing cross-reactions, have been reported during and after treatment with Thymoglobulin®:

- Leucopenia (57%)
- Thrombocytopenia (37%)

These reactions may emerge during the first 2 days of treatment or after treatment.

Adverse Events associated with over-immunosuppression, including infectious complications (bacterial, fungal, viral, and/or protozoal), and malignancies, (particularly lymphoproliferative syndrome), have also been reported.

TACROLIMUS:
The following Adverse Reactions have been associated with Tacrolimus:

- **Lymphomas and Other Malignancies, particularly of the skin.** Post transplant lymphoproliferative disorder (PTLD) has been reported.
- **Serious Infections:** Bacterial, viral, fungal, and protozoal infections, including opportunistic infections. These infections may lead to serious, including fatal, outcomes.
- **Polyoma Virus Infections:** Including polyoma virus-associated nephropathy (PVAN), mostly due to BK virus infection, and JC virus-associated progressive multifocal leukoencephalopathy (PML).
- **Cytomegalovirus (CMV) Infections**
- **New Onset Diabetes**
- **Nephrotoxicity**
- **Neurotoxicity** (including posterior reversible encephalopathy syndrome (PRES), delirium, and coma. Seizures and less severe neurotoxicities, including paresthesias, and other changes in motor function, mental status, and sensory function, tremor and headache have been reported.
- **Hyperkalemia**
- **Hypertension**
- **Anaphylactic reactions** have occurred with injectables containing castor oil derivatives.
- **Pure Red Cell Aplasia (PRCA)**

The most common adverse reactions, (≥ 40%), in Liver Transplant patients were: tremor, headache, diarrhea, hypertension, nausea, abnormal renal function, abdominal pain, insomnia, paresthesia, anemia, pain, fever, asthenia, hyperkalemia, hypomagnesemia, and hyperglycemia.

ADVERSE EVENT AND SERIOUS ADVERSE EVENT (AE/SAE) REPORTING:

A. Adverse Events (AEs):

An Adverse Event is any untoward or unfavorable medical occurrence, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom(s) or disease, (even if the event is not considered to be related to the study intervention). Adverse Events encompass both physical and psychological harms. For this study, the study intervention consists of the administration of low dose Thymoglobulin® Infusion/Induction, (on Day 0, Day 2 and Day 4 post LT), along with a delayed initiation of CNI, (tacrolimus), on Day 10 post LT.

Medical conditions/diseases present before study participation will be considered Adverse Events (AEs), only if they worsen after randomization, or any procedures or treatments specified in the protocol. Abnormal laboratory values or test results will constitute an AE only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Information regarding all Adverse Events, [whether volunteered by the subject, discovered by Investigator inquiry, or detected through physical examination, laboratory test or other means], will be collected and recorded on the AE CRF, and followed as is appropriate. Adverse Events will be described as follows:

- Duration (with start and end dates)
- Severity Grade (mild, moderate, severe)
- Relationship to the study drug/intervention (suspected / not suspected) [Related/Possibly Related or Not Related]
- Action(s) taken
- Outcome

B. Serious Adverse Events (SAEs):

A Serious Adverse Event (SAE) is any adverse experience that results in any of the following:

- Death
- A life-threatening experience
- An inpatient hospitalization or prolongation of an existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Important medical event that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening experience: Any AE that places the patient, in the view of the Investigator, at immediate risk of death from the AE as it occurred. Does not include an AE that had it occurred in a more severe form, might have caused death.

Persistent or significant disability/incapacity: A substantial disruption of a person's ability to conduct normal life functions.

Important medical events: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations such as important medical events which may not be immediately life-threatening or result in death or hospitalization, but might jeopardize the patient or may require intervention to prevent any of the other outcomes listed in the definition of a Serious Adverse Event. These should also be considered Serious Adverse Events.

In addition, all biopsy confirmed reports of acute cellular rejection (ACR) will be submitted to Sanofi PV as an SAE whether or not the event has met the SAE criteria. Since acute cellular rejection (ACR) is a secondary endpoint of the study, all BPAR episodes are to be reported through the Sanofi PV procedures, for consistency and accuracy in event reporting and data analysis. The event is to be noted with an SAE criterion of **"Other Important Medical Event".**

Any SAE occurring after patient randomization, until 30 days after the patient has stopped study participation, must be reported. In addition, for patients who are withdrawn from

study participation "early", SAEs are to be reported for up to 12 months after the date of patient randomization, if a relationship to the study is "suspected".
Information about all SAEs will be collected and recorded on the SAE (MedWatch) Form. **To ensure patient safety, the SAE (MedWatch) Form must be completed and UPLOADED to the Sanofi PV Portal, and faxed to Cleveland Clinic PI – "Attention: Bijan Eghtesad, MD" at (216) 444-9375, within 24 hours of Study Site discovery/notification.**

C. Pregnancies
Any female subject who becomes pregnant after the initiation of study treatment will be discontinued from further study treatment and participation. The patient will continue to be treated per standard treatment as per the Study Site standard practice. Any pregnancy occurring while the patient is participating in the study, (and for up to 30 days after study participation), is to be reported to Sanofi PV within 24 hours of learning of the occurrence. **For all pregnancies, a MedWatch SAE Form will be completed and UPLOADED to the Sanofi PV Portal, and faxed to Cleveland Clinic PI – "Attention: Bijan Eghtesad, MD" at (216) 444-9375, within 24 hours of discovery/notification.**

Each Study Site will report Adverse Events / Serious Adverse Events to the respective Study Site IRB in accordance with each Study Site's, IRB Reporting Policy.

STUDY ASSESSMENTS:

A. Visit Schedule and Assessments
Study subjects will be seen and evaluated according to the **Study Visit/Assessment Schedule, [Appendix A].** A Visit Window of **[± 3 days]** should be maintained for **[Visits 11 and 12 (Months 1 and 3)]**. A Visit Window of **[± 7 days]** should be maintained for **[Visits 13-15 (Months 6, 9, and 12)]**. Whenever possible, effort should be made to ensure that all Study Visits are timed to coincide with regularly scheduled post-transplant follow-up visits. Additional clinic visits may be made according to the subject's status and the routine practice at the Investigative Study Site.

B. Physical Examination:
A limited physical examination/assessment will be performed at all study visits. Information about the physical examination must be present in the source documentation at the study site. Significant findings that are present prior to the start of study drug are to be recorded as part of the patient's Relevant Medical History/Current Medical Conditions, and recorded on the appropriate CRF. Significant findings made after randomization, which meet the definition of an Adverse Event, are to be recorded as an AE or SAE, and recorded on the appropriate CRF.

C. Vital Signs:
Vital signs include measurements of Height (only at baseline); Body Weight; Pulse (while sitting or lying); and Systolic/Diastolic Blood Pressure(s). The results will be recorded on the appropriate CRF.

D. Laboratory Evaluations:

The following laboratory studies are to be performed at the time points as stated below and on the Study Visit Calendar. In addition, laboratory studies should be performed consistent with Standard of Care for the Liver Transplant Program at each respective Study Site. Blood samples will be collected in a fasting state, **(if possible).** All patients with laboratory tests containing clinically significant abnormal values are to be followed until the values return to normal ranges or until a valid reason, (other than drug related AE), is identified.
Patients who are withdrawn "early" from the study will continue to have labs monitored as part of their routine post liver transplant follow-up per each study site post liver transplant protocol.

Hematology:
To include: Hemoglobin, WBC, differential count and platelet count.**[Day (-1) or 0 [Pre-OLT]; Month(s) 1, 3, 6, 9, and 12.**

Blood Chemistry:
Albumin, sodium, potassium, calcium, inorganic phosphate, chloride, urea, creatinine, glucose, AST, ALT, alkaline phosphatase, total bilirubin. **[Day (-1) or 0 [Pre-OLT]; Month(s) 1, 3, 6, 9, and 12. eGFR will be measured on all subjects Day 10, Month(s) 1, 3, 6, 9, and 12.**

Lipid Panel – [Triglycerides, Total Cholesterol, HDL Cholesterol, calculated VLDL Cholesterol, calculated LDL Cholesterol, calculated Total Cholesterol to HDL ratio, calculated LDL to HDL ratio, calculated Non HDL Cholesterol]. **Month(s) 1, 3, 6, 9, and 12.**

Viral Serology:
Viral Serology Testing is to be performed at **Baseline [Day (-1) or Day 0],** (if **NOT** previously performed): HIV 1 and 2 antibody screen; Hepatitis B Surface Antigen, Hepatitis B Core Antibody Total, Hepatitis B Surface Antibody Qualitative; Hepatitis C Antibody. **Prior results for Viral Serology Testing may be used.** Date(s) of results are to be documented on the CRF.

Viral Load / Viral Infections (HCV, EBV, CMV, HBV):
Hepatitis C – [Hep C RNA by PCR, Quantitative]; **EBV –** [EBV by PCR Quantitative]; **CMV –** [CMV DNA detection]; **Hepatitis B Virus –** [Hep B DNA, Ultra Quantitative]. **[HCV levels (for HCV negative patients), EBV, CMV, and HBV levels to be performed per Standard of Care for the Liver Transplant Program at each respective Study Site]. Patients who are HCV positive will require HCV RNA (quantitative levels) drawn at Week 1, Month(s) 1, 3, 6, 9, and 12 in order to follow the patient's HCV response to Thymoglobulin®.**

CNI – Trough Levels:
Tacrolimus – C-0h blood trough level(s). Therapeutic drug monitoring will include Tacrolimus C-0h blood trough levels, which will be performed per Study Site Institutional Standards. Trough levels for Tacrolimus are to be performed approximately 12 hours after the previous dose of drug. **[Month(s) 1, 3, 6, 9, and 12, and per Investigative Site Liver Transplant Protocol]**

Pregnancy Tests:
A **serum or Urine pregnancy test [β-HCG] will be performed locally, at each Study Site,** on all female patients who are considered physically capable of becoming pregnant – Women of Child-Bearing Potential (WOCBP), **on Day 0 or (-1), (prior to rATG infusion).** In addition, a **Urine Pregnancy Test** will be performed at **Day 10, Month 3, and Month 12 post LT Visits.** Women are considered post-menopausal and NOT of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms), or six months of spontaneous amenorrhea with [serum FSH levels > 40 mIU/mL and estradiol < 20 pg/mL] OR have had surgical bilateral oophorectomy (with or without hysterectomy) at least 6 weeks prior. In the case of oophorectomy alone, follow up hormone level assessment must be completed to establish reproductive status and child bearing potential.

All women of child bearing potential (WOCBP), defined as women physiologically capable of becoming pregnant must agree to the use of **TWO birth control methods, [a double barrier method], OR a singular birth control method may be used in the following instances, [Intra Uterine Devices (IUDs, Tubal Sterilization, the patient's partner had a Vasectomy] throughout study participation, and for 30 days after study participation and/or last dose of Study Drug.**

Adequate barrier methods of contraception include: diaphragm with spermicide, cervical cap with spermicide, male condom, female condom, contraceptive sponge.

E. Tolerability/Acceptability:
Tolerability will be evaluated as part of AE and SAE analyses.

F. Pharmacokinetics:
No pharmacokinetics evaluations will be performed in the study.

G. Pharmacogenetics/pharmacogenomics:
No pharmacogenetic or pharmacogenomic samples will be collected in this study.

DATA COLLECTION, QUALITY ASSURANCE, AND MANAGEMENT

A. Data Safety Monitoring Board (DSMB):

A DSMB will assess the incidence of AKI, death, graft loss, acute cellular rejection (ACR), and SAEs in this study. In addition, other predefined safety variables, (to be defined in the DSMB charter), will be monitored, in order to minimize potential risks to study participants. The DSMB will monitor emerging efficacy and overall safety data to ensure that the benefits and risks of study participation remain acceptable. The DSMB will have access to fully un-blinded data for primary and secondary efficacy variables, all SAEs, and other selected AEs of special interest. The DSMB will have the authority to recommend, **(to Sanofi and/or the Cleveland Clinic [the Lead Study Site],** the alteration and/or termination of the trial or a treatment group, or cessation of further enrollment into one of the treatment groups.

Procedures for the operations, meeting schedule, and pre-specified rules for trial modification will be determined in consultation with the DSMB members and detailed in a separate charter.

The DSMB will be an independent board comprised of three physicians with experience in liver transplantation and/or nephrology, and one independent statistician. A physician is not allowed to participate in this clinical trial while serving on the Board. Selected efficacy and safety variables collected in the study, and detailed in a separate charter will be reviewed on a monthly basis by the DSMB. The collection and summary of these data points will be prepared in a semi-blinded fashion (using dummy treatment codes) by the biostatistician. The final run of the analysis programs will be run by the biostatistician using the real treatment codes. The results of the analyses will be delivered by the independent statistician to the DSMB. The members of the study team will be blinded from the results. The members of the Board will be primarily responsible for the clinical interpretation of the results.

The board members will also be responsible for advising the investigative team as to whether or not any changes need to be made to the conduct of the study. The stopping rules are specified in a separate DSMB charter. Decisions, based on the recommendations of the Board, will take into account the potential risks and benefits associated with continuing enrolment of patients into the study. Such information and recommendations will be used in the best interest of the patients already enrolled in the trial. The final decision, with respect to modification of any protocol, will be made by the investigative team and the Sanofi Scientific Review Committee. In the event of study termination, all health authorities and investigators will be notified of the termination, within **two (2) business days.**

B. Data Entry and Review

During the study, a representative from the primary Study Site will visit each site regularly to monitor the progress of enrollment or through monthly conference calls. The Investigator at each Study Site must maintain a Study Binder/File for each patient enrolled in the study. The "original" signed Informed Consent Form (ICF) will be kept in each subject's Study Binder/File. In addition, source documents, consisting of hospital and other medical records, (containing demographic and medical information, laboratory data, biopsy reports, visit reports, and other procedure and test results and assessments), will be kept in each subject's Study Binder/File. All information collected on the Case Report Forms (CRFs) must be traceable to the source documents. Data not requiring a written or electronic record will be defined before study start and will be recorded directly on the CRFs, which will be documented as being the source data. Data will be monitored centrally, as entered into the study database. If it is deemed necessary, a monitor from the primary site may visit individual sites for on-site monitoring. Each Study Site Principal Investigator must give the primary site monitor access to all relevant source documents, to confirm their consistency with the CRF entries.

C. Data Collection and Quality Control

Designated Study Personnel, (at each Study Site), must enter the information required by the protocol onto the paper Case Report Forms (CRFs). The information from the paper CRFs will then be entered directly into the electronic Database, REDCAP, by designated Study personnel at each Study Site. Edit checks and double data entry with verification upon second entry are built

into specific data items. Text items (e.g. comments) are entered once. Queries are sent to the investigational site using an electronic data query. Designated investigational Study Site staff will be required to respond to all queries and make any necessary changes to the data. Other errors or omissions are entered on Data Query Forms, which are made accessible to the investigational site for resolution.

Concomitant medications entered into the database will be coded using the WHO Drug Reference List which employs the Anatomical Therapeutic Chemical classification system. Coexistent diseases and Adverse Events (AEs) will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

D. Handling of Missing Values/Censoring/Discontinuations

Since the study subjects will be followed for renal function, graft loss, or survival status, the subjects with missing measurements at 30 days will only be those who die, withdraw consent or are lost to follow-up. It is expected there will be very few such patients within 30 days. Missing values at day 30 will be imputed using the last observation carried forward (LOCF) approach. Missing baseline serum creatinine values will be replaced with the most recent available value from the pre-transplant period.

STUDY ENDPOINTS / EFFICACY AND SAFETY

A. Primary Endpoints:

Efficacy and Safety (Primary):

Primary Efficacy and Safety variables to be evaluated at 30 Days Post Liver Transplant are:

- Incidence of AKI
- Serum creatinine and eGFR

B. Secondary Endpoints:

Efficacy and Safety (Secondary):

Secondary Efficacy and Safety variables to be evaluated at 30 Days Post Liver Transplant are:

- The incidences of acute cellular rejection, (ACR), at 30 days post-transplant.
- Patient survival at 6 months post-transplant.
- Graft survival at 6 months post-transplant.
- Incidence of AEs / SAEs

C. Additional Efficacy and Safety Variables for Evaluation:

- Patient survival at 12 months post-transplant
- Graft survival at 12 months post-transplant
- Biopsy-proven acute allograft rejection rates at 6 Months and 12 Months post-transplant.
- AKI (Stage 3 rates) at 3 Months post-transplant
- Renal function measured by eGFR at 12 Months post-transplant
- Microbiologically-proven systemic infection rates at 6 Months and 12 Months post-transplant
- Hepatitis C viral loads (in HCV positive recipients) at 12 Months post-transplant
- Incidence of biopsy proven recurrence of HCV infection at 12 Months post-transplant
- Incidence of AEs and SAEs at 12 months post-transplant

For each efficacy event, simple event rate estimates (proportion of events) at 12 months will be obtained, and event rates between the two treatment groups will be compared using Z-statistic based, two-sided, 95% confidence interval for RC – RE, where RC and RE are the event rates for the Early CNI treated Control Group and the Late CNI Initiated/Thymoglobulin® Study Group, respectively. In addition, for the composite efficacy endpoint and its components, individually, Kaplan-Meier survival methods will be used to estimate probabilities of efficacy events by visit. Greenwood's formula will be used to estimate standard errors and to derive the two-sided, 95% confidence interval of RC – RE, by visit window. HCV replication in HCV positive patients will be compared with summary statistics.

Due to the short duration of study involvement, we expect very few patients to be lost to follow-up, or to withdraw consent. Therefore, for the composite efficacy endpoint, patients who are discontinued from the study for these two reasons, (prior to the incidence of death, graft loss, or biopsy-proven acute cellular rejection), will not be considered as efficacy failures. If there are an adequate number of subjects from each clinical center, a formal test of center by treatment interaction effect will be made.

Adverse Events (AEs) / Infections:

Generally, infections are analyzed together with Adverse Event data. **In addition, infections will be analyzed separately. Infections will be documented on both, the Infection CRF and the AE CRF.** The data collected on both CRFs will be coded with the MedDRA dictionary which gives preferred term and body system information.

Adverse Event and Infection data are to be analyzed as a whole under the heading of "Adverse Events" for each treatment group. The incidence of Adverse Events will be summarized by body system, severity, and causal relationship to Study Drug. All information pertaining to Adverse Events, noted during the study, will be listed by patient. Verbatim details, provided by the Study Site Investigator, are to include:

- **The preferred term**
- **The body system**
- **Start Dates / End Dates**
- **Severity**
- **Relationship to Study Drug**

The "onset" of the Adverse Event" will also be shown, (relative in number of days), to the day of Initial Dose of Study Drug, for those subjects randomized to **Group 1/Arm 1** of the Study. Infection data will be further coded with SNOMED for micro-organism cultured and type of infection, (viral, bacterial, fungal, etc.). The incidence rate of infection **(by type and micro-organism)** will also be tabulated for each treatment group.

Laboratory Data:
Abnormal laboratory values, according to notable criteria, will be identified. A by-patient listing of laboratory data, for patient/lab abnormalities, will be generated. Laboratory values, outside the clinically notable and expanded limits, will be flagged. Appropriate incidence rates of clinically notable abnormalities will be provided by treatment. Further, for each laboratory parameter, a table summarizing the by visit laboratory values, (MDRD eGFR and serum creatinine), will be provided.

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[APPENDIX A.]: STUDY VISIT / ASSESSMENT SCHEDULE

Visit #	1	2	3	4	5	6	7[V]	8[V]	9[V]
Study Day	Day (-1) or 0 Pre- OLT	Day 0 Post OLT	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Obtain Informed Consent Signature[A]	X								
Medical History / Demographics[B]	X								
Prior Medications[B]	X								
Concomitant Medications	X	X	X	X	X	X	X	X	X
Eligibility[C]	X								
Enrollment[D]		X							
Physical Exam[F]	X	X	X	X	X	X	X	X	X
Height[E] / Weight[E]	X		X	X	X	X	X	X	X
VS (T, P, BP)[G]	X	X	X	X	X	X	X	X	X
Donor/Transplant Information		X							
Liver Biopsy[I]						X			
Serology HIV; Hep B; HCV[H]	X								
CMV / EBV Viral Load[R]					X				
HBV Viral Load[R]					X				
HCV Viral Load[S]	X								X
Hematology[L]	X					X			
Chemistry[L]	X					X			
Serum / Urine β-HCG[O]	X								
CNI Blood Trough Level[U]							X Arm 2		
PT / INR[J]	X					X			
r-ATG Induction / Infusion[T]		X Arm 1		X Arm 1		X Arm 1			
Immunosupression (Prednisone)[R]		X	X	X	X	X	X	X	X
Immunosupression (MMF)[R]		X	X	X	X	X	X	X	X
Randomization[K]		X							
Tacrolimus Dosing				X Arm 2	X Arm 2	X Arm 2	X Arm 2	X Arm 2	X Arm 2
Tacrolimus Script									X Arm 2
Patient Instruction re. Tacrolimus dosing + completion of Diary Card									X Arm 2
Distribute Diary Card									X Arm 2
Assess for AEs / SAEs[P]	X	X	X	X	X	X	X	X	X

[APPENDIX A.]: STUDY VISIT / ASSESSMENT SCHEDULE (continued)

Visit #	10	11	12	13	14	15
Study Day	Day 10 Post LT (±2 Days)	Month 1 (± 3 Days)	Month 3 (± 3 Days)	Month 6 (± 7 Days)	Month 9 (± 7 Days)	Month 12 (± 7 Days)
Physical Exam[F]	X	X	X	X	X	X
Height[E] / Weight[E]	X	X	X	X	X	X
VS (T, P, BP)[G]	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X
Hematology[L]	X	X	X	X	X	X
Chemistry[L]	X	X	X	X	X	X
eGFR[M]	X	X	X	X	X	X
Lipid Panel[N]		X	X	X	X	X
Urine β-HCG[O]	X		X			X
CNI Blood Trough Level[U]	X Arm 2	X Arms 1&2	X Arms 1&2	X Arms 1&2	X Arms 1&2	X Arms 1&2
CMV / EBV Viral Load[R]	X					
HBV Viral Load[R]	X					
HCV Viral Load[S]		X	X	X	X	X
Immunosupression (Prednisone)[R]	X	X	X	X		
Immunosupression (MMF)[R]	X	X	X	X	X	X
Initial Tacrolimus Dosing	X Arm 1					
Tacrolimus Script	X Arm 1	X Arms 1 and 2 (as needed)				
Initial Instruction re. Tacrolimus dosing + completion of Diary Card	X Arm 1					
Distribute Diary Card	Arms 1&2	Arms 1&2	Arms 1&2	Arms 1&2	Arms 1&2	
Collection and/or Review of Study Diary	X Arm 2	X Arms 1&2	X Arms 1&2	X Arms 1&2	X Arms 1&2	X Arms 1&2
Assess for AEs / SAEs[P]	X					
Liver Biopsy[I]	X					

A = Signing of Informed Consent to be performed at time of Admission for Liver Transplantation, Day (-1) or Day (0), PRIOR TO OLT AND PRIOR to ANY Study procedures that are not SOC being performed. Patient MUST have previously been presented with the Informed Consent and be familiar with the content prior to signing. Patient to be given opportunity to ask questions and have his/her questions answered about the Study and Study Treatment/Procedures, etc. PRIOR to signing the Informed Consent.

B = Demographics, Significant Medical History [including significant procedures/surgeries/ect. (with approximate start/stop dates or if ongoing)]. Prior relevant and significant medications taken for up to 30 days prior to Transplant.

C = To be reviewed and confirmed prior to Study Enrollment; Eligibility confirmation to be reviewed and signed by 2 Study personnel.

D = Study Enrollment to take place within 3 hours of completion of OLT and arrival in ICU.
E = Obtain Height on Admission for Transplant – Day (-1)/Day 0, only. If unable to obtain height due to patient status on Day (-1)/Day 0, previous (most recent) Height within prior 3 months may be used. Weight to be obtained on Day 0 or (-1), and prior to each r-ATG infusion, and at Study Visits on Day 10, Month 1, Month 3, Month 6, Month 9, and Month 12. If unable to obtain Weight on day of r-ATG infusion (prior to r-ATG infusion), [due to patient's clinical status], most recent weight may be used with approval of the Site Investigator.

F = Targeted Physical Exam to be performed.

G = For patients randomized to Arm 1: [VS to be obtained per Investigative Site Protocol, pre, during and post Thymoglobulin® Infusion], and per Infusion Reaction Protocol – see Infusion Reaction Protocol.

H = To be obtained prior to OLT. Previous results may be used.

I = Liver Biopsy to be performed as clinically indicated, (for confirmation of suspected ACR and/or HCV infection recurrence), and per Investigative Site standard liver transplant protocol. For suspected ACR, Liver Biopsy MUST be performed PRIOR to the initiation of anti-rejection therapy.

J = PT/INR on Day (-1) or Day 0 (prior to liver transplant), and post liver transplant per Investigative Site liver transplant protocol.

K = Randomization to take place within 6 hours of completion of OLT and arrival in ICU.

L = Day (-1) or 0 [Pre OLT]; Day 10, Month(s) 1, 3, 6, 9, and 12 post LT, and per Investigative Site Liver Transplant Protocol.

M = Day 10; Month(s) 1, 3, 6, 9, and 12, and per Investigative Site Liver Transplant Protocol.

N = Month(s) 1, 3, 6, 9, and 12.

O = Serum or Urine pregnancy test to be performed on Day (-1) or Day 0 (prior to OLT), on all Women of Child Bearing Potential (WOCBP). Urine pregnancy test to be performed, (at Day 10, Month 3, and Month 12) post LT Visits, on all Women of Child Bearing Potential (WOCBP).

P = All AE(s)/SAE(s) will be reported from randomization till 30 days after study completion. Any SAE occurring after patient randomization, until 30 days after the patient has stopped study participation, must be reported. In addition, for patients who are withdrawn from study participation "early", SAEs are to be reported for up to 12 months after the date of patient randomization, if a relationship to the study is "suspected".

R = Per Investigative Site Liver Transplant Protocol.

S = For patients who are HCV "Negative" – per Investigative Site Liver Transplant Protocol. For patients who are HCV "Positive" – HCV RNA (quantitative levels) are to be drawn on Day (-1) or Day 0 (pre-OLT), at Week 1, Month(s) 1, 3, 6, 9, and 12 (post OLT), in order to follow the patient's HCV response to Thymoglobulin.

T = r-ATG Infusion will be administered to subjects randomized to Arm 1 ONLY, on Day 0 (after OLT) within 12 hours of arrival in ICU, on Day 2 and Day 4 Post OLT.

U = CNI blood trough levels to be performed beginning day after initial dose of CNI and as per Investigative Site Liver Transplant Protocol, (as per clinically indicated), and at Month(s) 1, 3, 6, 9, and 12 Visits.

V = In case of early discharge after liver transplant these clinic visits and clinical data can be excluded however, immunosuppressive regimen and blood work results should be collected and recorded.