

STUDY PROTOCOL

The De-novo Use of Eculizumab Alongside Conventional Therapy in Presensitized Patients Receiving Cardiac Transplantation: An Open-Label, Investigator-Initiated Pilot Trial: [The DUET Cardiac Trial]

Protocol Version	
Date of Protocol	
Proposal	July 9, 2015
Reference Number	TBD

Study Sponsor	
Patel, Jignesh K., MD	
Address	8536 Wilshire Blvd #302 Beverly Hills, CA 90211
Phone	310-248-8300
FAX	310-248-8333

Investigational Agent Supplier	
Alexion Pharmaceuticals	
Address	352 Knotter Drive Cheshire, CT 06410
Phone	203-272-2596
FAX	203-271-8198

This study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

1.1 TABLE OF CONTENTS:

1.0 General Information.....	1
1.1 Table of Contents.....	2
2.0 Background	4
2.1 Schematic: The Complement Cascade and the Mechanism of Action of Eculizumab.....	6
2.2 References.....	7
3.0 Trial Objectives & Purpose.....	8
3.1 Primary Outcome Measure.....	8
3.2 Secondary Outcome Measure.....	8
4.0 Trial Design.....	8
4.1 Immunologic Risk Factor Surveillance.....	8
4.2 Treatment Success.....	9
4.3 Sample Size and Power.....	9
4.4 Statistical Methods.....	9
5.0 Patient Selection	10
5.1 Inclusion Criteria.....	10
5.2 Exclusion Criteria.....	11
5.3 Subject Identification.....	11
5.4 Demographic Data.....	11
5.5 Medical History.....	11
5.6 Withdrawal of Subjects from the Study.....	11
6.0 Therapeutic Protocol Design.....	11
6.1 Dosage, Administration, and Pharmacokinetics.....	12
6.2 Treatment Modification.....	13
6.3 Investigational Product Identity.....	13
6.4 Adverse Events.....	14
7.0 Immunosuppression+ Desensitization Pre + Post Transplant.....	15
8.0 Treatment of Rejection.....	18
9.0 Prophylactic Medication.....	19
10. Safety Monitoring and Evaluation Schedule	20
11.0 Clinical and Laboratory Assessment.....	24

12.0 Drug Accountability.....24

13.0 Data Handling and Quality Assurance.....24

14.0 Ethics..... 25

15.0 Confidentiality.....26

Appendix A: Eculizumab Constitutional Symptoms ADE Evaluation.....27

Appendix B: Eculizumab: Investigational Medication Brochure.....28

Appendix C: ISHLT Grading of Acute Cellular and Antibody Mediated Rejection.31

Appendix D: Eculizumab Safety Monitoring & Evaluation Schedule:Attachment

2.0 BACKGROUND

There is a missing link in the present armamentarium to prevent antibody mediated rejection (AMR) in solid organ transplantation. In cardiac transplantation, it is associated with more hemodynamic compromise, cardiac allograft vasculopathy and a higher mortality even when asymptomatic^{1,2,3,4} With the unavailability of better treatment options, present day maintenance therapies remain little changed to treatment strategies used over thirty years ago. In the early eighties, with the introduction of cyclosporine and later tacrolimus, the one year actuarial survival rate in cardiac transplantation escalated significantly from 40% to over 90%.^{1,3} Today, however, despite the identification of the deleterious effects of both cellular and antibody mediated rejection, cardiac transplantation long-term survival rates remain stagnant possibly due to the inability of present regimens to effectively target the humoral pathway. By preferentially blocking interleukin II production, contemporary doses of calcineurin inhibitors have less of an effect on humoral rejection. Although tacrolimus possesses the ability to decrease MHC expression and modulate B cell responses through T cell down-regulation, its efficacy on antibody mediated rejection is limited due to dose related side effects (i.e. namely, renal dysfunction, diabetes, anemia, neutropenia, hyperkalemia, and hypomagnesiemia). Likewise, despite the ability of selective medications to abrogate the potentiation of either antibody production (i.e.: IVIG, bortezomib, rituximab) or cytokine expansion (i.e. sirolimus, everolimus, mycophenolate), the mortality rate of AMR, accounting for up to 20% of all long-term cardiac transplantation deaths, continues to remain high^{1,3,4}.

The growing proportion of sensitized cardiac recipients presents an additional challenge to the transplant practitioner attempting to minimize the occurrence of antibody mediated rejection. Patients pre-exposed or “sensitized” to antigen exposing events (i.e.: blood transfusions, multiple pregnancies, prior organ transplantations, ventricular support devices) are more likely to both possess preformed and develop de-novo antibodies. Sensitized patients with panel reactive antibodies > 25% are at risk for increased mortality after heart transplantation^{5,6}. Present desensitization strategies aim to decrease antibodies in the hope of facilitating the likelihood of transplantation.^{7,8} However, such strategies may in turn be increasing the recipient’s likelihood for AMR and subsequent graft failure due to B-cell memory and post-transplant antibody rebound. The search for newer therapeutic strategies in solid organ transplantation should therefore not only aim to increase the number of sensitized patients eligible for transplantation, but to also prolong post-transplant survival and reduce morbidity.

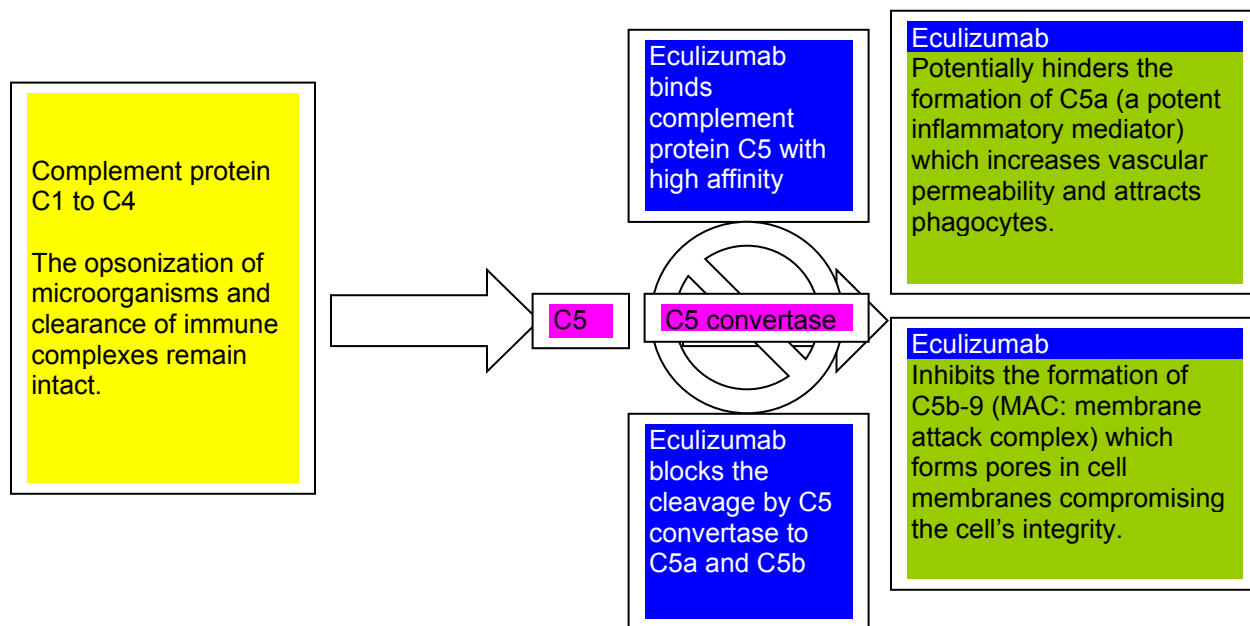
A central component of antibody-mediated cell injury is complement activation. Histologically, evidence of complement activation with the presence of complement c3d and c4d deposition and evidence for endothelial cell injury, is highly supportive of the diagnosis of AMR and is now an important component in the pathological classification of AMR in cardiac transplantation^{6,7,8}. Clearly the best approach to treating AMR is by blocking the cytolytic effects of alloantibody at its initial presentation. One strategy would involve prevention of complement activation.

Eculizumab is a monoclonal antibody that specifically binds to complement protein C5 with high affinity, thereby inhibiting its cleavage to C5a and C5b and preventing the generation of the terminal complement complex C5b-9^{9,10}. (See Schematic 2.1: Description of the Complement Cascade and the Mechanism of Action of Eculizumab) By this mechanism, eculizumab (Soliris®) inhibits terminal complement mediated intravascular hemolysis in paroxysmal nocturnal hemoglobinuria patients.⁹

In cardiac transplant recipients, the inhibition of terminal complement activation may be the missing link to decreasing possibly both complement-mediated AMR and cellular rejection (CR) by inhibiting both the inflammatory effects of both circulating antibodies and cytokine induced cell death. A recent clinical trial in renal transplantation with terminal complement inhibition using eculizumab has shown very promising results, significantly reducing the incidence of AMR in the first 3 months after transplant from 41.2% to 7.7% compared to a historical control cohort⁵. At one year post renal transplantation, there was a significant decrease in transplant glomerulopathy, a process of chronic rejection analogous to chronic allograft vasculopathy in cardiac transplantation^{5,6}.

Furthermore, the use of terminal complement inhibition (at complement protein C5) and not earlier in the immune cascade, allows the opsonization of bacteria and the clearance of immune complexes to remain intact, potentially minimizing the likelihood for the adverse event of infection.⁹⁻¹² Carrying a limited side effect profile (of headache, naso-pharyngitis, back pain, nausea, and fatigue⁸) and the feasibility of administering a thirty minute infusion, eculizumab may be easily given at the time of a normally scheduled post-operative clinical follow-up. Additionally, by using eculizumab immediately post transplant and then continuing use for the first eight weeks post transplantation¹⁻⁵ (when the risk of AMR is the greatest), the devastating repercussions of the complement cascade are potentially obviated. Thus, eculizumab may have the potential to minimize the occurrence of AMR, limit graft dysfunction, limit development of cardiac allograft vasculopathy and prolong long-term cardiac transplantation survival. In cardiac transplantation, the development of accelerated cardiac allograft vasculopathy as defined by an increase in maximal intimal thickness at matched sites of $\geq 0.5\text{mm}$ has been shown to be a surrogate marker for increased mortality, non-fatal major adverse cardiac events and development of angiographic vasculopathy at 5 years¹⁶.

**2.1 SCHEMATIC:
THE COMPLEMENT CASCADE & THE MECHANISM OF ACTION OF ECULIZUMAB**



2.2 REFERENCES

1. Kfoury AG, Hammond ME, Snow GL, et al. Cardiovascular mortality among heart transplant recipients with asymptomatic antibody mediated or stable mixed cellular and antibody-mediated rejection. *J Heart Lung Transplant* 2009;28:781-84.
2. Uber WE, Self SE, Van Bakel AB, et al. Acute antibody-mediated rejection following heart transplantation. *Am J Transplant* 2007;7: 2064-74.
3. Wu GW, Kobashigawa JA, Fishbein MC, et al. Asymptomatic antibody-mediated rejection after heart transplantation predicts poor outcomes. *J Heart Lung Transplant* 2009;28:417-22.
4. Michaels PJ, Espejo MI, Kobashigawa J et al.: Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. *J Heart Lung Transplant* 22(1), 58-69 (2003).
5. Stegall MD, Diwan T, Raghavaiah S et al. Terminal Complement Inhibition Decreases Antibody-Mediated Rejection in Sensitized Renal Transplant Recipients. *Am J of Transplant* 2011 Sept 22.
6. Nwakanma L, Williams J, Weiss E, Russell S et al. Influence of pretransplant panel-reactive antibody on outcomes in 8,160 heart transplant recipients in recent era. *Ann Thor Surg* 84(5), 1556-1562.
7. Kobashigawa, K; Crespo-Leiro, MG; Ensminger, SM et al. Hermann Reichenspurner, Annalisa Angelini, Gerald Berry, Margaret Burke, Lawrence Czer, Nicola Hiemann, Abdallah G. Kfoury, Donna Mancini, Paul Mohacsi, Jignesh Patel, Naveen Pereira, Jeffrey L. Platt, Elaine F. Reed, Nancy Reinsmoen, E. Rene Rodriguez, Marlene L. Rose, Stuart D. Russell, et al. Report from a consensus conference on antibody-mediated rejection in heart transplantation. *JHLT* 2011; **30**(3):252-69.
8. Gerald J. Berry, Annalisa Angelini, Margaret M. Burke, Patrick Bruneval, Michael C. Fishbein, Elizabeth Hammond, Dylan Miller, Desley Neil, Monica P. Revelo, E. Rene Rodriguez, Susan Stewart, Carmela D. Tan, Gayle L. Winters, Jon Kobashigawa, Mandeep R. Mehra; The ISHLT working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: Evolution and current status (2005–2011) *The Journal of Heart and Lung Transplantation*, Volume 30, Issue 6, June 2011, Pages 601-611
9. Eculizumab: Soliris® [package insert]. Cheshire, CT: Alexion Pharm Inc; 2009
10. Locke JE, Magro CM, Singer AL, Segev DL, Haas M, Hillel AT, et al. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am J Transplant* 2009 Jan;9(1):231-5.
11. Wang H, Arp J, Liu W, Faas J, et al. Inhibition of Terminal Complement Components in Presensitized Transplant Recipients Prevents Antibody-Mediated Rejection Leading to Long-Term Graft Survival and Accommodation. *Journal of Immunology* 2007, 179, 4451-4463.
12. Thomas TC, Rollins SA, Rother RP et al. Inhibition of Complement by Humanized Single Chain Fv. *Molecular Immunology* 1996; 33: 1389-1401.
13. Rinder CS, Rinder HM, Smith BR et al. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. *J Clin Invest* 1995; 96: 1564-1572.

14. Kobashigawa JA, Katznelson S, Laks H et al. Effect of pravastatin on outcomes after cardiac transplantation. *NEJM* 1995; 333: 621 –627.

15. Fang et al. Effect of vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. *Lancet*. 2002 Mar 30;359(9312):1108-13.

16. Kobashigawa JA, Tobis JM, Starling RC, et al.: Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* 2005;45:1532-7.

3.0 TRIAL OBJECTIVES AND PURPOSE

This investigational pilot trial is being conducted to determine the safety, tolerability and efficacy of the de-novo use of eculizumab to prevent AMR Grade ≥ 2 in cardiac transplantation recipients in the first year post transplantation.

3.1 PRIMARY OUTCOME MEASURE

Assess the safety, tolerability and efficacy of eculizumab after heart transplantation.

A. Safety: Incidence of hospitalization due to any intravenously treated infections in the 1st year

B. Tolerability: Incidence of patient withdrawal or loss to followup

C. Efficacy: Assess a composite end-point of:

i) Incidence of pathologic AMR Grade ≥ 2 in the first 26 weeks post heart transplantation

ii) Incidence of left ventricular dysfunction in the first 26 weeks defined as LVEF $\leq 40\%$ or a decrease of $>15\%$ from baseline prior to initiation of treatment with eculizumab.

3.2 SECONDARY OUTCOME MEASURE.

- Incidence of HDC: hemodynamic compromise (defined as a decrease in left ventricular ejection fraction (LVEF) by 20% from baseline or a LVEF $<40\%$, a 25% decrease in cardiac index from baseline or a cardiac index < 2.0 , and the need for inotropic support) at 6 months and 1 year after transplantation
- Incidence of ACR Grade $\geq 2R$ in the first year
- Patient survival at 12 months post heart transplantation
- Development of cardiac allograft vasculopathy at 1 year determined by intravascular ultrasound defined as change in site-matched maximal intimal thickness $\geq 0.5\text{mm}$ from baseline to 1 year.
- Presence of inflammatory and complement biomarkers at the time of routine endomyocardial biopsy for the first year post transplantation.
- Assess change in PRA from baseline to 1 year after transplantation
- Assess presence and strength of DSA: donor specific antibody post transplantation

- First year incidence of any treated rejection
- Number and duration of hospitalizations

4.0 TRIAL DESIGN

This study is a non-randomized, open-label, investigator-initiated safety and efficacy trial investigating the de-novo use of eculizumab alongside conventional therapy to prevent antibody mediated rejection. The duration of the study will include an open enrollment period and at least 12 months of follow-up (post-transplant). The trial will treat a total of 20 sensitized patients with a panel reactive antibody score of $\geq 70\%$ and not previously or currently enrolled in another ongoing trial. At the time of initial baseline screening prior to transplantation, patients willing to consent to the investigational use of eculizumab will receive the drug.

4.1 IMMUNOLOGIC RISK FACTOR SURVEILLANCE

Surveillance during the first year will consist of a minimum of ten right ventricular endomyocardial biopsies; performed weekly for the first month after transplantation, then biweekly until the third month, monthly until the sixth month, and bimonthly, thereafter, until completion of the first year. If a patient is treated for rejection, a follow-up biopsy will be performed 2 weeks following treatment to document regression or resolution of rejection. All biopsy specimens will be graded according to the International Society for Heart and Lung Transplantation (ISHLT) 2011 grading schemes for both humoral and cellular rejection. Angiograms will be performed at baseline (4-8 weeks after transplant and at one year and will include assessment by intravascular ultrasound to assess and quantitate intimal thickening. At the time of transplantation and prior to the initiation of any cytolytic therapy a retrospective cross match will be sent. Please refer to Appendix D: **Eculizumab Safety Monitoring and Evaluation Schedule** for additional procedure and trial laboratories.

Alloantibody Serology Assessment

Alloantibody will be measured using single antigen flow beads (SAFBs, LABscreen, One Lambda, Canoga Park, CA) each coated with a specific HLA type (Class I and Class II types). Samples will be collected per standard of care.

4.2 TREATMENT SUCCESS

Success of treatment will occur if safety, tolerability, and efficacy (a decreased incidence of AMR Grade ≥ 2 in eculizumab treated patients) is demonstrated.

4.3 SAMPLE SIZE AND POWER

This is a small scale preliminary trial which will enroll a total of up to 20 patients. This pilot trial is not meant to be nor will it be expected to achieve sample population power. The necessary sample size to achieve power and test the hypothesis that eculizumab reduces the incidence of rejection is not feasible as a single center study. Analyses from this single center study will be descriptive.

4.4 Demographic Data

Demographic data will include age (donor and recipient), race, sex, cytomegalovirus (CMV) seropositivity, whether the etiology of the recipients heart failure was due to ischemic cardiomyopathy or not, positive peri-operative T or B cell flow cytometry and complement dependent cytotoxicity crossmatch, and ischemic time; these data will be presented in the following manner: continuous data (i.e., age, ischemic time) will be summarized descriptively by mean, standard deviation, median, and range. Categorical data (i.e., sex, CMV seropositivity, positive peri-operative T or B cell flow cytometry and complement dependent cytotoxicity positive crossmatch, whether the etiology of the recipients heart failure was ischemic or not, and race) will be presented as enumerations and percentages.

5.0 PATIENT SELECTION

All patients worked up for a heart transplant at the Heart Institute at Cedars-Sinai Medical Center (CSMC) with a panel reactive antibody (PRA) $\geq 70\%$ at any time prior to screening.

5.1 INCLUSION CRITERIA

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

- Patient is greater than or equal to 18 years of age
- Patient with a panel reactive antibody (PRA) $\geq 70\%$ at any time prior to screening
- Patient is considered compliant and intends to be available for at a minimum follow-up study period of 1 year
- Patient must be vaccinated against *Neisseria meningitides* at least 2 weeks prior to receiving treatment therapy
- Voluntary written informed consent is obtained before performance of any study-related procedure not considered routine medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care
- Female subject is either post-menopausal or surgically sterilized or willing to use two acceptable methods of birth control (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study and for up to 2 months after the last dose of study medication

5.2 EXCLUSION CRITERIA

Patients meeting any of the following exclusion criteria are not to be enrolled in the study:

- Donor or recipient age < 18 years or > 75 years
- Cold ischemia time > 6 hours
- Patient at risk for tuberculosis (TB):
 - Current clinical, radiographic, or laboratory evidence of active or latent TB as determined by local standard of care
 - History of active TB:
 - Within the last 2 years, even if treated
 - Greater than 2 years ago, unless there is documentation of adequate treatment according to locally accepted clinical practice
 - Patient at risk of reactivation of TB precludes administration of conventional immunosuppression (as determined by investigator and based upon appropriate evaluation)
- Receipt of desensitization treatment with rituximab less than 1 week prior to therapy
- Receipt of a live vaccine within 4 weeks prior to study entry
- Patients with current or recent severe systemic infections within the 2 weeks prior to transplantation. Patients must be cleared by the heart transplant team in conjunction with the infectious diseases specialists
- Prior history of splenectomy

5.3 SUBJECT IDENTIFICATION

After signing the informed consent form, the subject will be identified by a subject identification number.

5.4 DEMOGRAPHIC

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner: continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range. Categorical data (i.e., sex and race) will be presented as enumerations and percentages.

5.5 MEDICAL HISTORY

Medical history will be collected, including the existence of current signs and symptoms and clinical significance for each body system. The etiology of each recipient's pre-transplant heart failure will be further enumerated by statistical analysis.

5.6 WITHDRAWAL OF SUBJECTS FROM STUDY

Subjects may be withdrawn from the study for the following reasons (At their own request or at the request of their legally acceptable representative):

- At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result
- If, in the investigator's opinion, continuation of the study would be harmful to the subject's well-being

- Occurrence of adverse drug reactions that from the investigator's point of view have a negative impact on the subject's health or well-being
- If the subject becomes pregnant
- If the subject is noncompliant with the protocol or instructions given by the investigator
- If the sponsor stops the study

If a study subject decides to discontinue participation prematurely for any reason the subject will not require a study related termination visit and will no longer be seen for any study-related follow-up or procedure; including study clinical assessments, study laboratory assessments, or study therapy. No data will be further submitted on any study subject after the date of termination with the exception of pregnancy. If the reason for discontinuation is pregnancy, then additional data regarding outcome of pregnancy and status of newborn data will be collected

Additionally, subjects maybe terminated due to one of the following:

1. The subject elects to withdraw consent from all future study activities, including follow-up.
2. The subject is lost to follow-up.
3. The subject dies.

6.0 THERAPEUTIC PROTOCOL DESIGN

This study is a non-randomized, open-label, investigator-initiated safety and efficacy trial investigating the de-novo use of eculizumab alongside conventional maintenance therapy to prevent rejection. The duration of the study will include an open enrollment period and at least 12 months of follow-up (post-transplant). The trial will enroll a total of up to 20 sensitized patients with a panel reactive antibody score of $\geq 70\%$ and not previously or currently enrolled in another ongoing trial. At the time of initial baseline screening prior to transplantation, patient's willing to consent to the investigational use of eculizumab will be enrolled..

6.1 DOSAGE, ADMINISTRATION, AND PHARMACOKINETICS

At the time of transplantation the patient will receive 1200 mg of eculizumab via a 35 minute peripheral IV(IVBP) infusion 35 minutes prior to release of the recipient's cross clamp (immediately prior to reperfusion) followed by thymoglobulin 1.5 mg/kg IVPB. The thymoglobulin will be repeated (if blood counts permit) for a total of five doses. On Day 1, 900 mg of eculizumab will be repeated. On Day 5, the patient will receive IVIG 1 gram/kg daily x 2 Days. On post-operative day 7, 14, 21 +/- 2 days eculizumab (900 mg) will be repeated. On days 28, 42, and 56 +/- 2 days the patient will receive 1200 mg of eculizumab as a 35 minute infusion at each scheduled visit.

Terminal

Complement
Inhibition
Protocol
(N=20)

Assigned Medication Interventions

Vaccination:

Meningococcal Vaccine 0.5 mg SQ x1 (2 weeks to 5 years prior to transplant)

Initial Therapy:

Each dose of eculizumab as an IVPB over 35 minutes.

Day 0:	1200 mg (Administered prior to cross-clamp (or reperfusion) at the time of cardiac transplantation)
Day 1:	900 mg
Day 7 +/- 2 days:	900 mg
Day 14 +/- 2 days:	900 mg
Day 21 +/- 2 days:	900 mg

Maintenance Therapy:

Each dose of eculizumab as an IVPB over 35 minutes.

Day 28 +/- 2 days:	1200 mg
Day 42 +/- 2 days:	1200 mg
Day 56 +/- 2 days:	1200 mg
Day 60-360	900 mg (Treatment Modification: defined below*)

The dosage, administration, and pharmacokinetics as described above is part of standard of care and serves as a guideline only.

6.2 TREATMENT MODIFICATION

*If the patient receives any additional plasmapheresis or fresh frozen plasma within the first year post-transplantation, 900 mg of eculizumab will be provided as a infusion immediately afterwards. (Note: this contingent dose was placed on the Eculizumab Safety Monitoring and Evaluation Schedule: Appendix D) on Day 180 but applies to day 60-360 post transplant in a contingent fashion)

6.3 INVESTIGATIONAL PRODUCT IDENTITY

Eculizumab is a monoclonal antibody that binds with high affinity to compliment protein C5, which inhibits its cleavage to C5a and C5b and prevents the generation of the terminal complement complex C5b-9. In patients with paroxysmal nocturnal hemoglobinuria (PNH), eculizumab is FDA approved to inhibit terminal complement mediated intravascular hemolysis. Eculizumab is a FDA Pregnancy Category C agent. Patients must be administered a meningococcal vaccine at least two weeks prior to

initiation of eculizumab therapy. It is a contraindication to provide any patient eculizumab with an unresolved *Neisseria meningitidis* or pneumococcus infection.

Eculizumab is supplied as an intravenous solution supplied as 300 mg in a 30 ml vial. The dose will require dilution to a final concentration of 5 mg/mL by adding appropriate amounts D5W to a final infusion volume is 120 mL for 600 mg doses and 180 mL for 900 mg doses. Admixed solutions are stable for 24 hours at 2 to 8 degrees Celsius (36 to 46 degrees Fahrenheit) and at room temperature. Each dose will be administered via IV infusion over 35 minutes via gravity feed, a single-type pump, or an infusion pump; total infusion time should not exceed 2 hours. Doses should not be administered by IV push or IV bolus injection.

6.4 ADVERSE EVENTS

Possible adverse events with eculizumab include: gastrointestinal: nausea (16%, musculoskeletal: Backache (19%), neurologic: headache (44%), respiratory: cough (12%), nasopharyngitis (23%), other: fatigue (12%), immunologic: viral disease (2%)⁹. Patients will be instructed to report signs/symptoms of meningococcal infection (e.g.: fever of 103 degrees F (39.4 degrees C) or higher; severe muscle aches with flu-like symptoms, and eyes sensitive to light; fever and a rash; confusion; or moderate to severe headache with nausea and vomiting, fever, or stiff neck or back) with the transplant study investigators.

7.0 IMMUNOSUPPRESSION+ DESENSITIZATION PRE + POST TRANSPLANT

Records of all desensitization will be recorded.

Pre-Transplantation:

Patients may receive antibody reduction therapies (with high dose IVIG and rituximab, and/ or bortezomib desensitization) prior to transplantation in an attempt to decrease the PRA. While awaiting transplantation a PRA and subsequent cPRA are performed on a monthly basis. Patients will not be eligible for eculizumab therapy less than one week of completing of rituximab desensitization therapy and CD20 count >2%.

Sandwiched Desensitization: HD (high dose) IVIG-Rituxan-Rituxan- HD IVIG:

(Reference: Jordan, S et al. Current approaches to treatment of antibody-mediated rejection. Pediatric Transplantation Volume 9, Issue 3, pages 408–415, June 2005) Pre-transplant each patient with a calculated PRA \geq 70% and stable outside the hospital awaiting or being worked up for transplantation will receive up to 3 cycles of IVIG-Rituxan-IVIG. Human polyclonal intravenous immune globulin (Gamunex 10% 2 g per kilogram of ideal body weight (up to a maximum of 140 grams) will be administered over 2 days on day 0, 1 and day 30,31 of therapy. On day 7 and 22, rituximab 1gram or 375 mg/m² (if the patient is \leq 50 kg) will be administered. To reduce the frequency of infusion-related side effects, 30 to 60 minutes before scheduled infusions of intravenous

immune globulin or rituximab, all patients were given intravenous methylprednisolone (40-100 mg), oral acetaminophen (650 mg), and oral diphenhydramine (50 mg). Flow PRAs are sent at baseline, day 22 and day 44 of therapy.

Bortezomib Desensitization: (Reference: Patel J, Everly M, Chang D, Kittleson M, Reed E, Kobashigawa J: Reduction of alloantibodies via proteasome inhibition in cardiac transplantation. J Heart Lung Transplant 2011;30:1320-6.) Pre-transplant each patient with a calculated PRA > 70% and in the hospital awaiting listing or transplantation (ie: UNOS listed 1A status) will receive up to 3 cycles of bortezomib 1.3 mg/m² via IV push over 3-5 seconds on a day 2, 5, 8, and 11 schedule with a 10 day rest period. Plasmapheresis will be given on days 1 and 2, 4 and 5, 7 and 8 and 10 and 11 of each cycle pre-transplant to speed up antibody removal from circulation. On plasmapheresis days the bortezomib dose is to be administered after plasmapheresis. If not taken for transplantation sooner, a maximum of 3 pre-transplant cycles of bortezomib/plasmapheresis may be given to achieve a PRA <50% If platelet count ≤30 ×10⁹/L or ANC ≤0.75 x 10⁹/L on a bortezomib dosing day (other than day 1) the dose will be held until If platelet count >30 ×10⁹/L or ANC >0.75. Flow PRA are sent at baseline and day 18 of therapy. Patients refractory to IVIG-Rituximab or UNOS listed Status 1A patients with a calculated PRA ≥ 50% requiring desensitization will be considered for bortezomib therapy.

Post-Transplantation:

Induction therapy will include up to five days of rabbit antithymocyte globulin (Thymoglobulin®). Maintenance immunosuppression therapy will include tacrolimus, mycophenolate mofetil, and prednisone. Patients with evidence of post-transplant rejection (ACR≥2R or AMR≥2 will be transitioned from mycophenolate to either sirolimus or everolimus at 3-6 months after transplant.

Thymoglobulin®

Doses of Thymoglobulin® will be given as part of the post-transplant protocol. Specifically, a dose of 1.5 mg/kg/dose intravenously over 6 hours will be given on post-operative days 0, 1, 2, 3 and 4,. If the patient develops hematologic toxicity, dose adjustments will occur as below.

Dose Adjustment for Thymoglobulin®

WBC> 3000 /mm ³ or Platelets > 75,000 cells/μL	NO Change
WBC 2000 - 3000/mm ³ or Platelets ≤ 75,000 cells/μL	Reduce dose of Thymoglobulin® by 50%
WBC ≤ 2000/mm ³ or Platelets ≤ 50,000 cells/μL	Hold dose

Tacrolimus

Tacrolimus will be initiated immediately postoperatively at 0.5 mg naso-gastrically or by mouth (if tolerating) twice a day and titrated upwards to achieve the following trough

goals.

Month 1: 10-15 ng/ml

Month 2-3: 8-12 ng/ml

Month 4 and onward: 5-10 ng/ml

Rejection: 8-12 ng/ml

Trough levels will be checked daily in house while hospitalized and routinely with every scheduled clinic and EMB visit for life long management. This frequent monitoring of therapeutic blood levels will be used as a surrogate marker for compliance.

Mycophenolate Mofetil

All patients will be initiated on mycophenolate mofetil (MMF) 1500 mg IVPB Q12H immediately post-operatively. When the patient tolerates orals, the same dose will be provided by mouth. Doses will be adjusted for gastrointestinal tolerance, infection, and bone marrow suppression (see below) if necessary. Goal MPA levels are to be 1.5 to 4 ng/ml.

Dose Adjustment for Mycophenolate

WBC cells/μL	Reduction in current MMF dose
>2000 cells/ μ L	None
< 2000 and ANC > 1000 cells/ μ L	Decrease current dose by 50%
< 2000 with ANC < 1000 cells/ μ L	Hold until ANC > 1000 cells/ μ L, G-CSF may be administered per physician discretion; Once ANC > 1000 cells/ μ L, restart MMF at 30-50% lower dose

Corticosteroids: Prednisone

At cross clamp the patient will receive 500 mg of methylprednisolone. When the patient enters the ICU the patient will receive an additional 500 mg of methylprednisolone as a continuous infusion over 24 hours. Subsequently, the patient will receive 100mg of methylprednisolone daily x 3 days as a pre-medication for Thymoglobulin® and then 40 mg IVP daily as a pre-medication for IVIG. On post-operative day 3, if the patient is tolerating orals, the patient will transition to an oral steroid taper as follows: (Note if the patient does not tolerate orals, an equivalent dose of methylprednisolone will be provided IV.)

Prednisone 40mg bid x 1 day (2 doses)

Prednisone 35mg bid x 1 day (2 doses)

Prednisone 30mg bid x 1 day (2 doses)

Prednisone 25mg bid x 1 day (2 doses)

Prednisone 20mg bid x 1 day (2 doses)

Prednisone 15mg bid x 1 day (2 doses)

Prednisone: 10 mg BID until 1 month post-transplant

At 1 month: taper by 1 mg q2wks so patients go from 10 BID to 10 QD by month 3

At 3 months: taper by 1 mg q2wks so patients go from 10 QD to 5 QD by month 6

The immunosuppression + desensitization pre + post transplant as described above is part of standard of care and serves as a guideline only.

8.0 TREATMENT OF REJECTION

Cellular Rejection:

Grade 1R biopsy results does not require treatment.

Grade 2R-3A rejection: Asymptomatic: Pt. will be treated with a steroid pulse as follows:

Prednisone 50mg bid x 3days
Prednisone 40mg bid x 1 day
Prednisone 30mg bid x 1day
Prednisone 25mg bid x 1day
Prednisone 20mg bid x 1 day
Prednisone 15mg bid x 1day
Prednisone 10mg bid x 1day
Prednisone 10mg qd until further notice
Repeat Biopsy in 2 weeks.

Switch mycophenolate to either sirolimus (target trough 4-8 ng/ml) or everolimus (target trough 3-5 ng/ml)

Grade 2R-3A; Symptomatic Rejection:

Hemodynamic compromise (decreased LVEF <40% or CI<2.0) Start IV solumedrol 500mg QD x 3days.

Start rATG 1.5mg/kg x 5-7days.

Consider plasmapheresis for 3-5 days.

After completion of rATG, patients will begin prednisone bolus and taper as follows:

Prednisone 50mg bid x 1day
Prednisone 40mg bid x 1 day
Prednisone 30mg bid x 1day
Prednisone 25mg bid x 1day
Prednisone 20mg bid x 1 day
Prednisone 15mg bid x 1day
Prednisone 10mg bid x 1day
Prednisone 10mg qd until further notice

Antibody-Mediated Rejection:.

For asymptomatic AMR (AMR \geq 2) with preserved left ventricular function and hemodynamics, patients will receive oral prednisone bolus and taper.

For symptomatic patients (AMR \geq 2) with LVEF \leq 40% patients will be treated as per Grade 2R-3A above with the addition of IVIG after completion of ATG and plasmapheresis (if utilized).

If hemodynamic compromise is present follow above treatment for symptomatic 2R-3A rejection but all patients will receive plasmapheresis followed sucrose-free IVIG (Gammunex® 10%) 1 gm/kg x 2days in a row.

Switch mycophenolate to either sirolimus (target trough 4-8 ng/ml) or everolimus (target trough 3-5 ng/ml)

The treatment of rejection as described above is part of standard of care and serves as a guideline only.

9.0 PROPHYLACTIC MEDICATION

1. Antipneumocystic: PCP Prophylaxis:

Bactrim SS 1 tab Qday on Mon, Wed, Fri for 1 year.

If sulfa allergic: Check G6PD. If no issues, start Dapsone 100mg Qday for 1 year.

If the patient has a G6P deficiency, then Mepron 1500mg Qday will be prescribed for 1 year.

2. Antiviral: CMV Prophylaxis

A. Recipient Negative/Donor Negative

Acyclovir 800mg PO bid for 6months.

B. Recipient Positive/Donor Positive or Recipient Pos/Donor Negative:

Valcyte 450mg PO bid for 6 months

C. Recipient Negative/Donor Positive

Valcyte 450mg PO bid for 12months

3. Anti-fungal: Thrush Prophylaxis

Mycelex troche 10mg PO BID for a 3month duration.

Note: For rejection episodes/steroid bolus, all prophylaxis will be restarted for 3 months.

4. Chronic Allograft Vasculopathy Prophylaxis:

A. **Statin Therapy:** Pravastatin: Pravachol® 20 mg PO QHS will be initiated immediately after transplantation if liver function tests (LFTs) are within

]normal limits. If LFTs are elevated the statin will be held until resolution of liver function is achieved. Treatment will be lifelong. Reference: [Reference (11): Kobashigawa JA, Katznelson S, Laks H et al. Effect of pravastatin on outcomes after cardiac transplantation. *NEJM* 1995; 333: 621 –627]

B. Vitamin Supplementation: Vitamin C 500 mg PO BID and Vitamin E 400 Units PO BID will be initiated one week post transplantation. Treatment will be lifelong. [Reference(12): Fang et al. Effect of vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. *Lancet*. 2002 Mar 30;359(9312):1108-13.]

Anitiplaetet Therapy: Baby aspirin 81 mg PO Qday will be initiated one week post transplantation after the pacing wires have been pulled. Treatment will be lifelong.

The prophylactic medication as described above is part of standard of care and serves as a guideline only.

10.0 SAFETY MONITORING AND EVALUATION SCHEDULE

Eculizumab will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s). Patients may be treated on an out-patient basis, if possible.

The pharmacist will prepare the drug under aseptic conditions. The amount (in mg) of drug to be administered will be determined based time since transplantation.

Day 0:	1200 mg
Day 1:	900 mg
Day 7 +/- 2 days:	900 mg
Day 14 +/- 2 days:	900 mg
Day 21 +/- 2 days:	900 mg
Day 28 +/- 2 days:	1200 mg
Day 42 +/- 2 days:	1200 mg
Day 56 +/- 2 days:	1200 mg

The appropriate amount of Eculizumab will be drawn from the indicated number of injection vial (300 mg/30 ml) and administered as an intravenous infusion over 30 minutes followed by a standard saline flush. Each 300 mg/30 ml of eculizumab are for single use administration.

Before each drug Eculizumab dose, the patient will be evaluated for possible toxicities that may have occurred after the previous dose(s).

Toxicities are to be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0. See Appendix A: Eculizumab Constitutional Symptoms Adverse Drug Event Evaluation
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf

Adverse Event Definitions

An **adverse event** (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (eg, including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the drug, whether or not it is considered to be drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of drug.

Serious Adverse Event Definition

A **serious adverse event** (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in **death**.
- Is **life-threatening**. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires inpatient **hospitalization or prolongation of existing hospitalization**. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in **persistent or significant disability/incapacity**. Disability is defined as a substantial disruption of a persons' ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an **important medical event**. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All AEs will be assessed and documented by the investigator according to the categories detailed below.

Seriousness

For each AE, the seriousness must be determined.

Intensity

The intensity of an AE should be classified as either mild, moderate, or severe.

Casual Relationship

The assessment of the casual relationship between an AE and the administration of treatment is a clinical decision based on all available information at the time of the event. The assessment is based on the question whether there was a “reasonable casual relationship” to the study treatment in question. Possible answers are “yes” or “no.”

The assessment of a possible casual relationship between the AE and protocol-required procedure(s) is based on the question whether there was a “reasonable casual relationship” to protocol-required procedure(s). Possible answers are “yes” or “no.”

Action taken with study treatment

Any action on study treatment (Eculizumab) to resolve the AE is to be documented using the categories listed below.

- Drug interrupted
- Drug discontinued

Outcome

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

Assessments and documentation of adverse events

AEs (including SAEs) observed, volunteered, and solicited will be recorded. AEs will be documented event-based, with change documentation in cases of increases in intensity.

All AEs occurring from the time the subject signed informed consent and 30 days after the subject has completed the study will be documented on the subject's record.

All documented AEs will be followed up until an outcome has been reported or until at least 30 days after the subject has completed the study.

Reporting of serious adverse events

The investigator will be responsible for reporting relevant SAEs to the IRB. Pregnancies will be immediately reported to the IRB although a pregnancy will not be considered an SAE. The pregnancy will be followed and the outcome reported, including the status of the new born. For the pregnancy of a study subject's partner, all efforts should be made to obtain similar information on course and outcome, subject to the partner's consent.

Monitoring of adverse events

Life threatening complications following study drug administration will be reported to the Independent Medical Monitor within 15 calendar days and deaths within 7 calendar days. Serious and unexpected adverse events following study drug administration will be submitted as expedited reports to the monitor on a quarterly basis.

Throughout the study, the Independent Medical Monitor will review safety data on a quarterly basis unless it is an adverse event that requires expedited report. Reports will be submitted to the IRB biannually.

11.0 **CLINICAL AND LABORATORY ASSESSMENTS**

Laboratory assessments

Laboratory evaluations will be performed at certified laboratories according to the study schedule. Required tests are specified in the study flow/budget chart. Please refer to **Appendix D: Eculizumab: Safety Monitoring and Evaluation Schedule** for additional procedure and trial laboratories.

Clinical assessments

All patients will undergo clinical evaluation by the investigator or investigator's designee at times specified on the study flow-charts. Please refer to Appendix D: **Eculizumab Time Schedule and Budget** for additional procedure and trial laboratories.

Clinical evaluations will include assessments of sitting blood pressure, patient weight changes, serious adverse events, study drug doses, allograft rejection, infection, need for dialysis, hospitalizations, re-transplant status, and other immunosuppressive medications.

Serious Infection is defined as any of the following:

- Treatment with an INTRAVENOUS antimicrobial agent for a specific clinical syndrome (not prophylaxis)
- Positive cultures, pathologic identification of microbial agents, or significant serologic changes related to clinical symptoms
- Typical clinical presentation documented by investigator or appropriate consultant.

12.0 **DRUG ACCOUNTABILITY**

No study drug will be administered to subjects before approval is received from the institutional review board (IRB).

All study drugs will be stored at the Research Pharmacy at Cedars-Sinai Medical Center in accordance with applicable regulatory requirements and the instructions given by the manufacturer providing the study drug. Study drugs will be inaccessible to unauthorized personnel. All study medication will be dispensed by the research pharmacist.

13.0 **DATA HANDLING AND QUALITY ASSURANCE**

Data will be recorded onto case report forms using source documentation available at the study site.

Data Processing

Subject data necessary for analysis and reporting will be entered or transmitted into a validated database or data system.

14.0 **ETHICS**

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with application local laws and regulations.

Documented approval from the IRB will be obtained before the start of the study, according to GCP, local laws, and regulations. When necessary, an extension, amendment or renewal of IRB approval must be obtained. The IRB is organized and operates according to GCP and applicable laws and regulations.

Strict adherence to all specifications outlined in this protocol is required for all aspects of study conduct.

The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without IRB approval. As soon as possible, the implemented deviation or change, should be reported to the IRB. Any deviations from the protocol must be explained and documented by the investigator.

Subject information and consent

All relevant information on the study will be summarized in informed consent form. The investigator will explain all relevant aspects of the study to each subject prior to his/her entry into the study (i.e. before any examinations and procedures associated with the selection for the study are performed or any study-specific data is recorded on study-specific forms).

The investigator will also mention that written approval of the IRB has been obtained. Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Only if the subject voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator will personally sign and date the form. The subject will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the subject's research chart. The informed consent form and any other written information provided to subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and/or the written informed consent form. The investigator will inform the subject of changes in a timely manner and will ask

the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IRB's approval prior to use.

15.0 **CONFIDENTIALITY**

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Only the subject number will be recorded. As part of the informed consent process, the subjects will be informed in writing that representatives of the IRB or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects to be identified.

APPENDIX A:

**ECULIZUMAB CONSTITUTIONAL SYMPTOMS /ADVERSE EVENT
QUESTIONNAIRE**

Please circle or mark one number per line to indicate your response as it applies.

The day after my last infusion of eculizumab	Not at all	A little bit	Some-what	Quite a bit	Very much
I had a headache	0	1	2	3	4
I had a runny nose	0	1	2	3	4
I had some nausea	0	1	2	3	4
I was very tired	0	1	2	3	4

Add values for each row for a total score ____/16

APPENDIX B:

INVESTIGATIONAL MEDICATION BROCHURE

eculizumab injection

Alexion Pharmaceuticals

Read the Medication Guide before you start eculizumab and before each dose (infusion). This Medication Guide does not take the place of talking with your doctor about your condition or your treatment. Talk to your doctor if you have any questions about your treatment with eculizumab.

What Is The Most Important Information I Should Know About eculizumab?

Eculizumab is a medicine that affects your immune system. Eculizumab can lower the ability of your immune system to fight infections.

- Eculizumab increases your chance of getting serious and life-threatening meningococcal infections.
 1. You must receive a meningococcal vaccine at least 2 weeks before your first dose of eculizumab
 2. unless you have already had this vaccine.
 3. If you had a meningococcal vaccine in the past, you might need a booster dose before starting eculizumab. Your doctor will decide if you need another dose of a meningococcal vaccine.
 4. A meningococcal vaccine does not prevent all meningococcal infections. You must be aware of the following signs and symptoms of a meningococcal infection:
 - moderate to severe headache with nausea or vomiting
 - moderate to severe headache and a fever
 - moderate to severe headache with a stiff neck or stiff back
 - fever of 103° F (39.4° C) or higher
 - fever and a rash
 - confusion
 - severe muscle aches with flu-like symptoms, and eyes sensitive to light

Call your doctor or get emergency medical care right away if you have any of these symptoms.

What Is Eculizumab?

Eculizumab is a medicine called a monoclonal antibody. Eculizumab is FDA Approved for the treatment of patients with a disease that affects red blood cells called Paroxysmal Nocturnal Hemoglobinuria (PNH). You are receiving this medication as an investigational therapy to prevent organ rejection.

Eculizumab works by blocking part of your immune system. This can possibly reduce the risk of rejection but it can also increase your chance for infection. It is important that you:

Who Should Not Receive Eculizumab?

Do not receive Eculizumab if you:

- have a meningococcal infection
- have not been vaccinated with, or you are not up-to-date with a meningococcal vaccine. Note: Your doctor will vaccinate you before you begin the study.

Tell your doctor if you:

- have an infection or fever
- are pregnant, become pregnant, or are breastfeeding. Eculizumab has not been studied in pregnant or nursing women.

How Do I Receive Eculizumab?

- Eculizumab is given through a vein (I.V. infusion) over 35 minutes.
- You will most likely receive 8 eculizumab infusions on approximately 0,1,7,14,21,28,42,56 days post transplant.
- Following each infusion, you may be monitored for up to one hour afterwards for allergic reactions.

What If I Miss a Dose or Stop Eculizumab Treatment?

- If you forget or miss a Soliris infusion, call your doctor right away.
- Stopping treatment with Soliris may interfere with the medications efficacy to reduce graft rejection.

What Are The Possible Side Effects With Eculizumab?

Serious side effects with Eculizumab include:

- Serious and life-threatening infections. See “What is the most important information I should know about Eculizumab?”

Common side effects with Eculizumab include:

- headaches
- runny nose and colds
- sore throat
- back pain
- nausea

Call your doctor if you have any of these side effects. These are not all the side effects with Soliris. Ask your doctor for more information.

General Information About Eculizumab

Medicines are sometimes prescribed for conditions other than those listed in a Medication Guide. If you have any concerns about Eculizumab, ask your doctor. Your doctor or pharmacist can give you information about Eculizumab that was written for health care professionals.

Medication contains Eculizumab in a solution of water, polysorbate, sodium phosphate and sodium chloride.

Manufactured by Alexion Pharmaceuticals, Inc., 352 Knotter Drive, Cheshire, CT 06410 USA.

APPENDIX C: ISHLT GRADING OF ACUTE CELLULAR AND ANTIBODY MEDIATED REJECTION ON ENDOMYOCARDIAL BIOPSY

ISHLT: Working Formulation classification: ACUTE CELLULAR REJECTION

2004 ISHLT Nomenclature Grade	1990 ISHLT Nomenclature Grade	Histopathological Description after EMB for ACUTE CELLULAR REJECTION
OR	0	No cellular rejection
1R	1A	Mild focal lymphocytic infiltrate without myocyte injury
1R	1B	Mild diffuse but sparse infiltrates without myocyte injury
1R	2	Mild lymphocytic infiltrate with up to one focus of myocyte damage
2R	3A	Moderate multifocal prominent infiltrates with myocyte injury
3R	3B	Diffuse infiltrates with myocyte injury
3R	4	Diffuse lymphocytic infiltrates with myocyte injury and hemorrhage, edema, polymorphonuclear leukocytes and/or vasculitis

ISHLT: Working Formulation classification: ANTIBODY-MEDIATED REJECTION

2011 ISHLT Nomenclature Grade	Histopathological + Immunologic Description after EMB for ANTIBODY-MEDIATED REJECTION
AMR 0: None.	Negative immunologic stain(s) and negative histology.
AMR 1: Suspicious	Positive immunologic stain(s) or histology (but not both).
AMR 2: Definite	Positive immunologic stain(s) and positive histology.
AMR 3: Severe	Positive immunologic stain(s) and positive histology with findings of advanced rejection (interstitial hemorrhage, edema, neutrophils, vasculitis)

AMR Immunopathology:

Immunofluorescence: C3d, C4d, HLA (to assess endothelial capillary integrity)

Immunoperoxidase: C3d, C4d, CD68