

A Single Center, Open-Label Study to
Determine the Safety and Efficacy of a
Dosing Regimen of Eculizumab Added to
Conventional Treatment in the Prevention
of Antibody-Mediated Rejection (AMR) in
Positive Crossmatch Patients Undergoing
Living Donor (LD) Kidney Transplantation

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Title:

A single center, open-label study to determine the safety and efficacy of a dosing regimen of eculizumab added to conventional treatment in the prevention of antibody-mediated rejection (AMR) in positive crossmatch living donor kidney transplantation (+XMatch LDKTx).

Principal Investigator:

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Investigational Product, Dosage and Route of Administration:

Soliris™ (eculizumab) 900 mg and 1200 mg, administered intravenously (IV)

Study Duration:

Up to 15 months, including 3 months follow-up after last dose of drug.

Objectives:

The primary objective of this study is to evaluate the safety and efficacy of eculizumab in preventing AMR in positive crossmatch patients undergoing +XMatch LDKTx.

Background:

Antibody against Class I and II Human Leukocyte Antigens (HLA) is an increasingly common finding in renal transplant candidates.¹ When the anti-HLA antibody is directed against the kidney donor and is present at high levels at the time of transplant, the antibody binds to the renal allograft endothelium activating the complement cascade leading to graft thrombosis, termed hyperacute rejection.² Anti-donor HLA antibody is measured using a crossmatch assay in which recipient serum is mixed with peripheral blood lymphocytes of the donor. A strongly positive crossmatch was long considered an absolute contraindication to kidney transplantation and most patients with anti-HLA antibody never were able to receive a kidney transplant.³ Unfortunately, these patients lives were shortened by chronic dialysis and their quality of life severely suffered.

Over the past decade, significant progress has been made in overcoming early antibody-mediated renal allograft injury. Our group here at Mayo Clinic, Rochester has been one of the leaders in this area of “desensitization”.^{2,4} Our protocols have primarily employed multiple pretransplant plasmaphereses (PP) in order to reduce anti-HLA antibody to “safe” levels at the time of transplant. Our group has performed more than 200 such transplants in the past decade providing the possibility of transplant to previously untransplantable patients. With improvements in patient selection and management, the one-year graft survival has now reached almost 90%, however, several significant problems remain.

Despite our best efforts, transplantation in these patients is still complicated by a high rate of acute humoral rejection (AHR).² AHR occurs later than hyperacute rejection (usually from 7-21 days after transplantation), but is otherwise similar to hyperacute rejection in that it involves activation of the complement cascade leading to severe allograft damage. It occurs in greater than 40% of all transplants in which anti-HLA antibody is present at high levels (defined as a positive B cell flow crossmatch channel shift >200). AHR can be quite difficult to control leading to early graft loss in approximately 10% of cases. The long-term prognosis of successfully-treated AHR is poor with more than half of the patients losing their allograft in the next 3 years to accelerated endothelial cell damage termed transplant glomerulopathy.⁵ Thus, a major focus of our efforts is to develop new protocols to prevent AHR.

Eculizumab:

Eculizumab is a humanized IgG_{2/4K} antibody produced by murine myeloma cell culture and purified by standard bioprocess technology. It is approved by the FDA for the treatment of Paroxysmal Nocturnal Hemoglobinuria (PNH). Eculizumab binds specifically to the 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. PNH is a genetic disease in which patients generate abnormal RBCs that are deficient in terminal complement inhibitors, rendering these RBCs sensitive to terminal complement-mediated hemolysis.

It is our hypothesis that blockade of terminal complement activation with eculizumab at the time of transplant in combination with our current protocols will reduce the incidence of AHR in patients with anti-donor HLA antibody.

Patients included in this study will be those who have demonstrable anti-HLA antibody specific for their living donor. This is defined as a positive B cell crossmatch with a channel shift of >200 and anti-donor specificities as determined using HLA coated microbeads. These standard assays are performed by the Histocompatibility Laboratory here at Mayo Clinic, Rochester.

Historical Control Group:

A major limitation of studies in +Xmatch kidney transplants is that relatively few patients are transplanted each year. For example, we only perform approximately 20 +Xmatch transplants/year and thus do not have large enough numbers to perform a randomized trial.

We have retrospectively studied the incidence of AHR in our +Xmatch kidney transplants and this experience will serve as our historical control group (non-eculizumab treated). We currently stratify +Xmatch patients by their baseline anti-donor antibody levels as follows:

B cell flow crossmatch channel shift:

Group	Treatment
>300	high risk, perform pretransplant PP to safe levels (<300) or 10 PP (whichever endpoint is reached first)
200-300	moderate risk, no pretransplant PP, but close post –transplant monitoring
<200	low risk, not included in this study

Almost all episodes of AHR occur in the first 28 days after transplant. During this time frame, antibody levels in +Xmatch Kidney Transplant recipients usually follow one of two patterns: 1) anti-donor HLA levels remain low and AHR does not develop, or 2) anti-donor HLA antibody levels increase in the first few weeks and AHR develops. AHR is always proven by kidney biopsy and is almost always accompanied by an increase in serum creatinine.

In a recent published analysis of 60 +Xmatch KTx from 1/05 to 1/07, we found that the incidence of AHR was 39% (16/41) in patients with DSA levels >300 at baseline and 31% (9/29) in patients with DSA levels <300 at baseline.⁶ This study also demonstrated a good correlation between DSA levels post-transplant and histological findings on protocol renal allograft biopsies obtained at the same time point (see Figure below). Specifically, when the B cell FXM channel shift was >359, AHR was noted on 92% (923/25) of biopsies. AHR was not found on any biopsies with a B cell FXM <300. Thus, this study forms the basis for the historical control group for the current study.

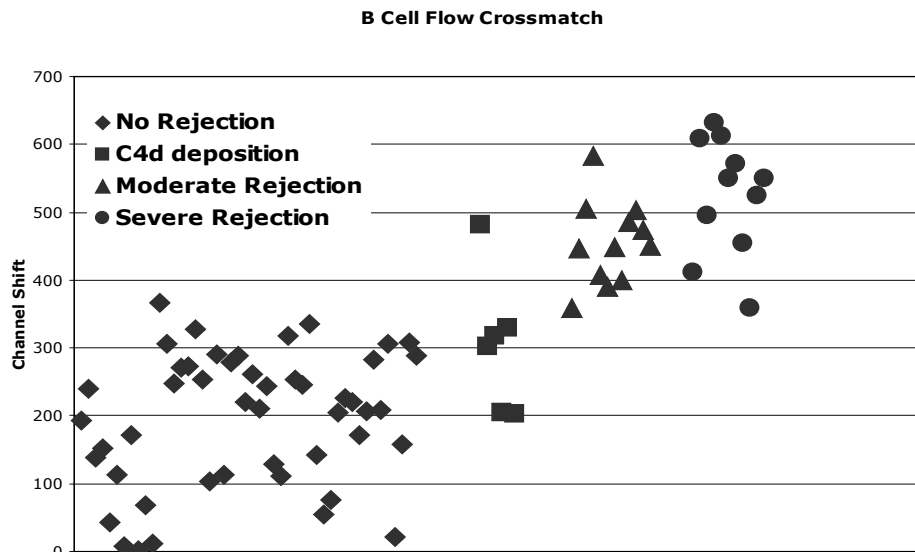


Figure: Association of donor specific alloantibody level to histologic evidence of acute humoral rejection in +Xmatch KTx

Statistical Analysis:

The primary endpoint of this study is the incidence of AHR in the first 28 days after +XMatch KTx.

We plan to enroll 40 patients in the trial. The overall expected AHR rate in these forty patients is 40% (16 patients should develop AHR). 20 patients are powered to detect a 50% reduction in AHR with a p value <0.05, one-sided t test (actual number of AHR patients =4).

We also will examine the AHR in patients with B cell flow crossmatch channel shifts > 400. This should be an additional method to determine the efficacy of eculizumab. Historically, we have shown that almost all patients with channel shifts >400 have AHR, but hypothesize that eculizumab should prevent AHR at this level.

Patient Population:

- 40 patients on eculizumab plus conventional therapy
- Patients recruited for this study will be recipients of a LD kidney transplant from two identified risk groups:
 - Group A-High Risk: Patients have a positive crossmatch (+XM) to the donor allograft (defined as a positive T-cell flow cytometry [FCXM] of greater than or equal to 300 prior to desensitization, or as a positive B-cell FCXM of greater than or equal to 300 prior to desensitization with demonstrable Class II DSA on solid-phase assays), or
 - Group B-Moderate Risk: Patients have a positive B cell FCXM in the range of 200 – 299
- All transplant recipients must undergo desensitization with multiple pretransplant plasmapheresis treatments prior to transplantation to either achieve a FCXM of <300 or until the patient has undergone 10 PP.
- All patients must have all appropriate vaccinations administered at least two weeks prior to the time that desensitization is begun consistent with local practice

Study Design:

There are three Phases in this study:

Enrollment Phase

Screening Period: Informed consent signed; inclusion/exclusion criteria obtained and evaluated. Patient will be vaccinated against *Neisseria meningitides*, *Pneumococcus* or *Hemophilus influenzae*. Patients will be desensitized according to the standard of care (local Desensitization Protocol).

Treatment Phase (Table 1)

- Patients will be given 1200 mg of eculizumab intravenously over 30 minutes, one hour prior to surgery.
- Patients will be given 900 mg of eculizumab on Day 1 post-transplant.
- Patients will not undergo post-transplant plasmapheresis unless they demonstrate AHR on biopsy.
- Patients will then be given 900 mg of eculizumab weekly through four weeks post-transplant
- At week 4, patients will be assessed for B cell FCXM. Patients with B cell FCXM less than 200 will stop eculizumab treatment. Patients with B cell FCXM greater than or equal to 200 will continue eculizumab treatment every 14 days from week 5 through week 9. The dose will be increased and dosing will now be every 2 weeks instead of weekly. Similar “discontinuation assessments” will be performed at week 9, 26 and 52. If the BFXM is <200, then the eculizuamb will be stopped. If the BFXM ≥ 200 , then the eculizumab will be continued as outlined below.
- In addition, eculizumab 600 mg will be administered immediately after each plasmapheresis (PP) and immediately after any fresh frozen plasma (FFP) that is given post-transplant during the treatment period
- Between 5 and 8 doses of eculizumab will be administered over the course of the study (plus additional doses based on individual PP and/or FFP requirements)
- Blood for serum levels of eculizumab will be drawn prior to each dose and one hour after each infusion to determine the pharmacokinetic-pharmacodynamic (PK-PD) relationship. Blood serum levels will also be drawn prior and post plasmapheresis.

Table 1: Eculizumab Dosing Schedule

Day/Week	Eculizumab Dose
Day 0 (1 hr pre-transplant)	1200mg
Day 1	900mg
Week 1	900mg
Week 2	900mg
Week 3	900mg
Week 4 (1 st discontinuation assessment)	900mg
	-If FCXM ≥ 200, continue Eculizumab as follows
Week 5	1200mg
Week 7	1200mg
Week 9 (2 nd discontinuation assessment)	1200mg
Week 26 (3 rd discontinuation assessment)	1200mg every 2 weeks
Week 39	1200mg every 2 weeks

(4 th discontinuation assessment)	
Week 52 (Last discontinuation assessment)	Discontinue drug

Follow-up Phase:

Following the completion of dosing, patients will return for follow-up visits at 3 and 6 months post-transplant to obtain biopsies and collect follow-up data. Patients who continue the drug for 12 months will receive their final assessment at 15 months.

Biopsy Protocol: All patients will undergo protocol renal allograft biopsy at 30 minutes, days 4, 7, 14 and months 1, 3, ~~and 6~~ and 12 to rule out AMR. We also will perform biopsies for increased serum creatinine >0.2 mg/dl over baseline not attributable to other causes (increased serum tacrolimus level, urine leak, urinary obstruction, etc). Biopsies will be processed for light microscopy, electron microscopy and C4d immediately in order to rule out AMR. Biopsies will be banked and stained for membrane attack complex in batches.

The following criteria must be met for the diagnosis of AMR:

Serological Criteria:

Evidence of circulating DSA by channel shift (T-cell or B-cell of greater than 300)

Plus

Immunological Criteria:

Positive C4d staining in peri-tubular capillaries (PTC)

Plus

Histological Criteria (has one or more of the following):

- Neutrophil infiltration in the PTC
- Arteriole fibrinoid necrosis
- Acute tubular injury pattern
- Mesangiolysis
- Glomerular microthrombi
- Evidence of endothelial damage based on electron microscopy

Efficacy Assessments:

Major endpoints for this study are:

1. The incidence of AMR during the first 4 weeks and the first 9 weeks post transplant. Diagnosis of AMR will be based histological findings using Banff '05 criteria.⁷
2. In patients who have serum creatinine increased by 0.2 mg/dL or greater above nadir, a second endpoint will be the number of days of serum creatinine is \geq 0.2 mg/dL above nadir through 9 weeks.

3. Once AMR criteria are met initially – a third endpoint will be the duration and severity of biopsy characteristics consistent with AMR through three months. Duration is measured as number of days with biopsy Grade 2 or greater; severity is measured by number of days at Grade 3 vs. Grade 2.
4. Once AMR criteria are met initially - serum creatinine (renal function) at one, three, and six months.

NOTE: Biopsies will be performed after AMR is diagnosed in the following manner:

After the initial biopsy, PPs are done daily for 3 days, followed by another biopsy. If the second biopsy also demonstrates AMR, the process is repeated until there is resolution of the AMR. For patients with recalcitrant AMR (persists beyond 2 weeks), splenectomy will be considered.

Other endpoints include:

1. Incidence of AMR at 30 minutes, ~~4 days~~, 7 days, 14 days, 1 month, 3 and 6 months post-transplant
2. Presence of C5b-9 in all biopsies at any time point in the first 12 months after transplant
3. The incidence of AMR in patients with B-cell FXM >350 at any time point in the first 12 months after transplant. In the control group, 92% of these patients showed AMR
4. In patients with B-cell FXM >350 at any time point in the first 12 months after transplant, the percentage of patients demonstrating an increase in serum creatinine ≥ 0.2 mg/dL from nadir determined within the first week post-transplant throughout the duration of the 3 month dosing period
5. Incidence of delayed graft function (DGF) post-transplant (defined as the requirement of dialysis within the first week post transplant)
6. Incidence of dialysis post-transplant through 9 weeks (number of days)
7. Number of PP post-transplant through 9 weeks
8. Percentage of patients requiring splenectomy
9. Effect on renal function
 - Creatinine clearance (calculated)
 - Iothalamate clearance (at 1 month and 2 months post-transplant)
10. Graft survival at 3, 6, 9, and 12 months
11. Incidence of transplant glomerulopathy within 12 months of transplant
12. Change in serum creatinine from just prior to transplant to the nadir
13. Serum creatinine at 1, 3, 6, 9 and 12 months post-transplant
14. Percentage of patients developing acute cellular rejection (ACR) at 1 and 2, 3 and 6 months
15. Histologic evidence of damage by electron microscopy even in the absence of light microscopic damage

16. Number of patients whose FXM remained elevated (≥ 200) through 12 months of treatment.
17. Number of patients whose FXM decreased sufficiently (< 200) to warrant discontinuation of the drug prior to the 12 month mandatory drug discontinuation.

Follow-up (post-treatment) biopsies will be performed at 3 and 6 months post-transplant in all patients and at 3 months after the last dose of eculizumab in those that continue drug after 6 months to look for histological evidence of complement-mediated damage and cell infiltration.

Safety Assessments:

Adverse events will be collected and recorded at each patient visit.

Inclusion Criteria:

1. 18 years of age
2. Has end stage renal disease (ESRD) and is to receive a kidney transplant from a LD to whom he/she has either:
 - a. a positive crossmatch requiring pretransplant desensitization (defined as a positive T-cell FCXM of greater than or equal to 300 prior to desensitization, or as a positive B-cell FCXM of ≥ 300 prior to desensitization with demonstrable Class II DSA on solid-phase assays) or
 - b. a positive crossmatch not requiring desensitization (defined as FCXM between 200 and 299)
3. Willing to comply with the protocol
4. Females of child-bearing potential must have a negative pregnancy test (serum β -HCG) and sexually active females must agree to use a reliable and medically approved method of contraception
5. Willing and able to give written informed consent
6. Vaccinated against *Neisseria meningitides* (quadrivalent vaccine), *Pneumococcus* or *H. influenzae* at least two weeks prior to beginning desensitization

Exclusion criteria:

1. Unstable cardiovascular condition
2. Previous splenectomy
3. Active bacterial or other infection which is clinically significant in the opinion of the investigator
4. Known or suspected hereditary complement deficiency
5. Participation in any other investigational drug study or was exposed to an investigational drug or device within 30 days of randomization
6. Pregnant, breast-feeding, or intending to conceive during the course of the study, including the three month follow-up period after drug discontinuation
7. Known hypersensitivity to the treatment drug or any of its excipients
8. History of illicit drug use or alcohol abuse within the previous year

9. History of meningococcal disease
10. Medical condition that, in the opinion of the investigator, might interfere with the patient's participation in the study, pose an added risk for the patient, or confound the assessment of the patient (e.g. severe cardiovascular or pulmonary disease)
11. Previously been enrolled in this trial

Safety Information in Patients with PNH:

Eculizumab generally is well-tolerated by patients with PNH. Safety data is provided from 3 parent trials in which 195 patients with PNH were treated with eculizumab for a median of 22 months and had infection related adverse events and serious adverse events (SAEs) similar to placebo treated controls in the first 6 months.⁸⁻¹⁰

Eculizumab blocks terminal complement therefore patients may have increased susceptibility to infections, especially with encapsulated bacteria. There appears to be an increased incidence of bacterial meningitis with eculizumab treatment. All patients were vaccinated against *Neisseria meningitides* and during 382 patient years of treatment, there were 2 cases of meningococcal septicemia (0.52 per 100 patient years) which were promptly treated and recovered without sequelae. The current recommendation is to administer meningococcal vaccine at least 2 weeks prior to receiving the first dose of eculizumab and this recommendation will be followed in our trial. Patients will be monitored for early signs of meningococcal infections (headache, neck stiffness, fever, mental status changes) and will be treated with antibiotics if necessary.⁸⁻¹⁰

As with all protein products, eculizumab administration may result in infusion reactions including anaphylaxis or other hypersensitivity reactions. In previous clinical trials, no patients experienced transfusion reactions that required discontinuation of drug. However, if signs of a drug reaction occur, the infusion will be stopped and appropriate medical therapy will be administered.⁸⁻¹⁰

Adverse Reactions:

The most frequently reported adverse reactions (>10% overall and greater than placebo) are: headache, nasopharyngitis, back pain and nausea.

Contraindications:

1. Patients not currently vaccinated against *Neisseria meningitides*
2. Pregnancy
3. Nursing mothers
4. Age <18 years

The safety of eculizumab in pediatric populations, in pregnancy and in nursing mothers has not been determined.

Eculizumab in Transplant Patients:

Two kidney transplant recipients have received eculizumab for the treatment of acute humoral rejection (AHR). One of the patients was here at Mayo (under an FDA-approved compassionate use protocol in 2006) and one at Johns Hopkins. Neither developed infection nor any other side effect related to eculizumab despite receiving multiple other immunosuppressive agents. The patient here at Mayo still has a functioning graft two years after treatment.

In addition, 6 patients have received eculizumab in the current trial with no infections or complications that can be directly linked to the drug. Importantly, 3 of the 6 patients have developed high levels of DSA that should have resulted in AHR, but none of the 3 had AHR on biopsy and all grafts are functioning normally.

Safety Monitoring Protocol for Eculizumab:

As stated previously, abundant data suggests that eculizumab is safe when given as monotherapy. However, we will be giving the drug as part of a multi-drug immunosuppressive therapy. Only two patients have received eculizumab in a situation that is similar, but not identical, to that of the study.

The primary perceived risk for patients in this study will be infection. Patients will be followed closely while treated with eculizumab. The patient's will receive the drug both as an inpatient and as an outpatient. They will be monitored at least every other day during the first month of the study and will remain in Rochester when receiving the drug. As part of our routine post-transplant care, the patient will be examined by a nephrologist or physician assistant who is a member of the study team. They will be examined for clinical signs of infection. In addition, they will have vital signs (temperature), screening for opportunistic infection (CMV and Polyoma virus copies) and CBC as per our protocol.

Data Safety Monitoring Protocol:

Given the small size of the study (40 patients) and the relative short accrual period (likely to be 2 years), we plan to employ a data safety monitoring protocol with specific stopping rules instead of a Data Safety Monitoring Board.

The stopping rules include:

1. Any infection with *Neisseria meningitides*, *Pneumococcus* or *H. influenzae* at any time
2. Any patient death for any cause
3. Systemic bacterial infection in two patients (including urosepsis or line sepsis)
4. Severe reaction to eculizumab infusion

If these occur, we will stop enrollment, notify the IRB and reassess the study.

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