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Protocol Title: Early Intervention with Eculizumab to Treat Thrombotic Microangiopathy/atypical Hemolytic Uremic Syndrome (TMA/aHUS)-associated Multiple Organ Dysfunction Syndrome (MODS) in Hematopoietic Stem Cell Transplant (HCT) Recipients

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Early Intervention with Eculizumab to Treat Thrombotic Microangiopathy/atypical Hemolytic Uremic Syndrome (TMA/aHUS)-associated Multiple Organ Dysfunction Syndrome (MODS) in Hematopoietic Stem Cell Transplant (HCT) Recipients

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STATEMENT OF COMPLIANCE

[redacted]

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the applicable laws and regulations.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

I agree to conduct this trial in accordance with the ethical principles outlined in the Declaration of Helsinki.

Site Name (type/print): _____

Site Principal Investigator Name (type/print): _____

Site Principal Investigator Signature: _____ Date: _____

ABBREVIATIONS

CCHMC	Cincinnati Children's Hospital Medical Center
CHLA	Children's Hospital Los Angeles
HCT	Hematopoietic cell transplant
aHUS	atypical hemolytic uremic syndrome
TMA	thrombotic microangiopathy in hematopoietic cell transplant recipients
MODS	Multi-organ dysfunction syndrome
Ur pr/cr ratio	Random urine protein/random urine creatinine ratio
PK/PD	pharmacokinetic/pharmacodynamics testing
PRES	Posterior reversible encephalopathy syndrome
NRM	Non-relapse mortality
autoHCT	Autologous hematopoietic cell transplant
alloHCT	Allogeneic hematopoietic cell transplant
sC5b-9	Soluble membrane attack complex
TTP	Thrombotic thrombocytopenic purpura
ADAMTS13	a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
CH50	Total hemolytic complement activity
DSMB	data and safety monitoring board
IND	Investigational new drug
Cyst C GFR	Cystatin C based glomerular filtration rate
AKI	Acute kidney injury
CKD	Chronic kidney injury
PH	Pulmonary hypertension
GI	Gastrointestinal
BSI	Blood stream infection
PICU	Pediatric intensive care unit
REMS	Risk Evaluation and Mitigation Strategy
FDA	Food and Drug administration
C5	Complement Factor 5
LDH	Lactate dehydrogenase
PNH	Paroxysmal nocturnal hemoglobinuria
ID	Identification
FFP	Fresh frozen plasma
TPE	Therapeutic plasma exchange
IVIG	Intravenous immunoglobulin
PI	Principal investigator
AE	Adverse event
SAE	Severe adverse event
CRC	Clinical research coordinator

PROTOCOL SYNOPSIS

Title	Early intervention with eculizumab to treat thrombotic microangiopathy/atypical hemolytic uremic syndrome (TMA/aHUS)-associated multiple organ dysfunction syndrome (MODS) in hematopoietic stem cell transplant (HCT) recipients
Abbreviation	*TMA/aHUS in HCT recipients will be abbreviated as "TMA".*
Study type	Single arm multi-institutional prospective study
Population	HCT recipients with clinical diagnosis of high risk TMA
Enrollment	21 patients (plus replacements if needed for early death or relapse)
Participating Institutions	Cincinnati Children's Hospital Medical Center (CCHMC) Children's Hospital Los Angeles (CHLA) Children's Hospital of Philadelphia (CHOP) Dana-Farber Cancer Institute (DFCI)/Boston Children's Hospital (BCH)
Expected study duration	4 years: anticipate enrollment of 7-10 patients annually for 2-3 years with 1 year post-transplant follow-up.
Purpose and rationale	The large majority of the published clinical experience with treatment of TMA with complement blockade has been performed in a single center (Cincinnati), where the biology and treatment of TMA is a key research focus. In this protocol we seek to establish a treatment regimen that can be generalizable to multiple sites and does not require time-intensive individual PK and PD monitoring. In preparation for this study, we established prospective screening for TMA, determined high-risk disease markers, and performed eculizumab detailed pharmacokinetic/pharmacodynamics (PK/PD) studies in children receiving HCT. We will perform the current study at multiple centers to establish exportability; the regimen is expected to provide satisfactory outcomes for a large majority of children without highly specialized monitoring.
Hypotheses	1. Early intervention with complement blocker eculizumab will double survival in HCT recipients with high risk TMA, as compared to historical untreated controls. 2. An optimal eculizumab dosing schedule can be determined for this population through eculizumab pharmacokinetic and pharmacodynamics (PK/PD) testing.
Primary endpoint	Survival at 6 months from TMA diagnosis.
Statistical considerations for primary endpoint	This prospective single arm study will test the null hypothesis of 18% survival versus a projected increase in survival to 45% at 24 weeks from TMA diagnosis in this high risk population by using early therapy with eculizumab. The primary endpoint will be assessed using a 1-sided exact binomial distribution test. The sample size of 21 has a greater than 80% power of yielding a significant p-value. The primary analysis will be intent-to-treat. A secondary "successfully treated patient analysis" (defined as >4 doses of eculizumab administered) will also be performed using a non-relapse mortality endpoint. Children who receive 4 or fewer doses of therapy, or who relapse with malignancy prior to 24 weeks on the study will be replaced.
Secondary endpoints	• Cumulative incidence of organ dysfunction at 6 months after TMA diagnosis and 1 year after HCT, compared with historical controls with high risk TMA using MODS definition is shown in APPENDIX II. Organ systems evaluated will be: o Renal ▪ Includes hypertension evaluation o Cardiac o Pulmonary o Gastrointestinal o CNS function o Pericardial Effusion

	• Timing and extent of resolution of organ dysfunction in the first year after therapy. • 1 year non-relapse mortality (NRM) compared with historical controls. • Characterization of eculizumab PK/PD.
Study design	This is prospective single arm multi-institution study in patients undergoing allogeneic (alloHCT) or autologous hematopoietic stem cell transplantation (autoHCT). We will enroll 21 consecutive HCT recipients (plus replacements if needed for early death due to inability to receive a minimal level of therapy, or relapse; see statistical section for details) diagnosed with high risk TMA using our published criteria (Jodele et al, Blood 2014). The eculizumab dosing regimen for this study was derived from our clinical and PK/PD data in 50 eculizumab treated HCT recipients with a goal to identify drug administration schedule that would be effective in the majority of HCT recipients with high risk TMA due to high mortality seen in untreated and undertreated disease. All patients will receive eculizumab loading doses at the beginning of therapy followed by induction dosing due to high drug clearance that occurs during the first five weeks of therapy. This allows rapid disease control, which is essential to avoid organ injury. Maintenance dosing will continue until all patients complete a total of 24 weeks of therapy (Table 1). Survival will be assessed at 6 months from TMA diagnosis as the primary study endpoint. With high mortality associated with TMA after HCT, it is important to avoid undertreatment of these patients in order to improve chances of preventing MODS and death. This dosing regimen should be feasible at institutions that don't have the availability to monitor drug PK/PD in real time. PK/PD testing obtained during this study will allow us further optimize the eculizumab dosing schedule and to determine patients in whom shorter therapy might be possible. All study patients will receive antimicrobial prophylaxis adequate to cover N. Meningitides for the duration of the eculizumab therapy plus until the CH50 and eculizumab serum level is unmeasurable (<25 μg/ml), when complement should no longer be blocked. Eculizumab PK/PD studies will be performed on all study patients and will be used for secondary endpoints. After the primary study endpoint is met, patients will be treated and monitored as clinically indicated, with final follow-up at 1 year after HCT.

Inclusion criteria	Patients of any age undergoing allogeneic or autologous HCT with:
	• Histologic TMA diagnosis
	• OR clinical TMA diagnosis and presenting with high risk disease features including:
	  • elevated plasma sC5b-9 above laboratory normal value (≥244ng/ml). AND
	  • proteinuria: measured as ≥30mg/dL of protein on random urinalysis x2 **or** protein/creatinine ratio ≥1mg/mg **or** patient receiving renal replacement therapy.
	TMA diagnostic criteria are shown in APPENDIX I
	• Minimum weight of ≥ 5kg.
	• ADAMTS13 Activity lab sample must be drawn during or prior to screening (results can be pending and are not required for initial eligibility). Development of TMA can be a medical emergency and thus if patient meets all other eligibility criteria, patient may proceed with enrollment and study treatment even if ADAMTS13 results are not available. If test results confirm diagnosis of TTP defined by ADAMTS13 activity test <10%, then study treatment will be immediately halted and patient will be removed from study.

Exclusion criteria	• Known hypersensitivity to any constituent of the study medication. • Subjects with unresolved serious Neisseria meningitides infection or • Progressive severe infection.

	• Patients previously treated with eculizumab or other complement blockers within the 60 days prior to first dose of study treatment.
Laboratory monitoring	TMA activity markers and PK/PD studies are listed in Table 3. PK/PD data will be used to evaluate the efficacy of complement blockade with the eculizumab regimen administered.
Eculizumab dosing	See Table 1
Study drug availability	Eculizumab (Soliris) will be provided by Alexion Pharmaceuticals at no cost to study subjects.

1. INTRODUCTION

1.1 Purpose and rationale of the study

Thrombotic microangiopathy or transplant associated atypical hemolytic uremic syndrome (aHUS) is an important cause of multi-organ dysfunction syndrome (MODS) and death after HCT (further in the protocol referred as "TMA"). The large majority of the published clinical experience with treatment of TMA with complement blocking agent eculizumab has been performed at a single center (Cincinnati), where the biology and treatment of TMA is a key research focus. In this protocol we seek to establish a treatment regimen that can be generalizable to multiple sites and does not require time-intensive individual pharmacokinetic and pharmacodynamics (PK/PD) monitoring. In preparation for this study, we established prospective screening for TMA, determined high-risk disease markers, and performed detailed eculizumab PK/PD studies in children receiving HCT. We will perform the current study at multiple centers to establish exportability; with that established, the approach should be able to be adopted more broadly. The regimen is expected to provide satisfactory outcomes for a large majority of children without highly specialized monitoring. Due to the high mortality observed among patients with high-risk TMA who don't receive targeted anti-complement therapy, a randomized study including untreated control arm is not considered to be ethically feasible.

1.2 Significance of the study in relation to human health

Hematopoietic stem cell transplantation (HCT)-associated thrombotic microangiopathy (TMA) is an understudied complication of HCT that significantly affects transplant related morbidity and mortality. Over the past several decades, the cause of TMA was unknown, limiting treatment options to non-specific therapies adapted from other diseases. Our recent prospective studies dedicated to the study of TMA have provided new insights into the pathogenesis of, and genetic susceptibility to TMA, raising awareness of this important transplant complication and allowing for the identification of novel therapeutic targets. Specifically, many patients with TMA develop multiple organ dysfunction syndrome (MODS) due to tissue injury through endothelial damage mediated by activation of the complement pathway. This knowledge has led to complement blockade using eculizumab as a therapy directly targeting the cause of the disease. This new strategy has the potential to favorably influence clinical practice and change the standard of care for how HCT recipients with TMA are managed. Preliminary data and experience to date suggest that early TMA therapy will reduce incidence of MODS after HCT and improve long-term outcomes in transplant survivors. The data this study will generate will have importance beyond the field of stem cell transplantation in helping to understand mechanisms of TMA induced endothelial injury that are relevant to other microangiopathies in whom populations are smaller and prospective systematic study is not possible.

1.3 Background

TMA occurs in 30% of children undergoing HCT, and about half of these children develop MODS that is generally lethal if not treated early. TMA affects the endothelium of small vessels, leading to MODS

presenting as renal failure, pulmonary hypertension with cardiac failure, polyserositis, and bowel ischemia and necrosis. Retrospective literature consistently reports over 80% mortality with TMA/MODS, with the large majority of patients dying within 6 months of TMA diagnosis.[1-3] To date there is no effective, generally adopted standard of care therapy for TMA in HCT recipients. In our earlier study, we showed 9% overall survival in patients with high risk TMA and MODS who did not receive TMA targeted therapy with 82% of patients dying by week 24 after TMA diagnosis (*Jodele et al, Blood 2014*).[4]

Laboratory investigations we performed demonstrated that patients with TMA develop MODS through endothelial damage mediated by complement system activation, allowing us to propose a novel therapeutic strategy using the complement blocker eculizumab. We identified that activated terminal complement and proteinuria are early prognostic markers of poor survival in TMA and complement blockade with eculizumab can significantly improve survival in these patients who historically have dismal outcomes. In contrast to this, in a single center prospective study we showed that eculizumab therapy given for TMA and MODS improved survival to 62% compared to 9% (p=0.0007) in patients with same risk TMA not receiving eculizumab (Jodele et al, BBMT 2013[5] and 2015[6]). We have extended this experience using complement blockade for TMA/MODS, having treated more than 50 patients, optimizing therapy through pharmacokinetic and pharmacodynamic (PK/PD) testing. Our experience and publications indicate that the main reason for failure in treating TMA after HCT is late initiation of eculizumab intervention. Once TMA/MODS is advanced, severely impaired organ function can't be salvaged. In our clinical cohort, patients were started on eculizumab therapy on average 43 days from TMA diagnosis; those at end stages of their disease more often having poor outcome. Our data to date also indicate that eculizumab pharmacokinetics in HCT recipients differ significantly from patients reported with atypical hemolytic uremic syndrome (aHUS)(Jodele et al, BBMT 2015).[6] HCT recipients with TMA have much higher disease activity and much faster eculizumab clearance indicating the need to determine an optimal eculizumab dosing schedule that will be effective in HCT patients through PK/PD based eculizumab dosing.

There is a significant need for a prospective, multicenter study that examines eculizumab efficacy in TMA after HCT in consistent manner, initiating therapy early in patients at high risk for TMA associated MODS. Because of the high mortality observed among patients with high-risk disease who did not receive targeted anti-complement therapy, a randomized study with untreated control group is not considered to be ethically feasible. Also, we have demonstrated that the HCT population has a much more severe TMA phenotype and much higher eculizumab clearance than reported in patients with aHUS and PNH,[7,8] so we need to determine optimal dosing regimen in HCT recipients with TMA to be used as standard of care in the future. This prospective clinical study will result in novel eculizumab dosing regimen for HCT recipients with high risk TMA that is effective in preventing TMA-associated MODS.

1.4 Previous work done in this area

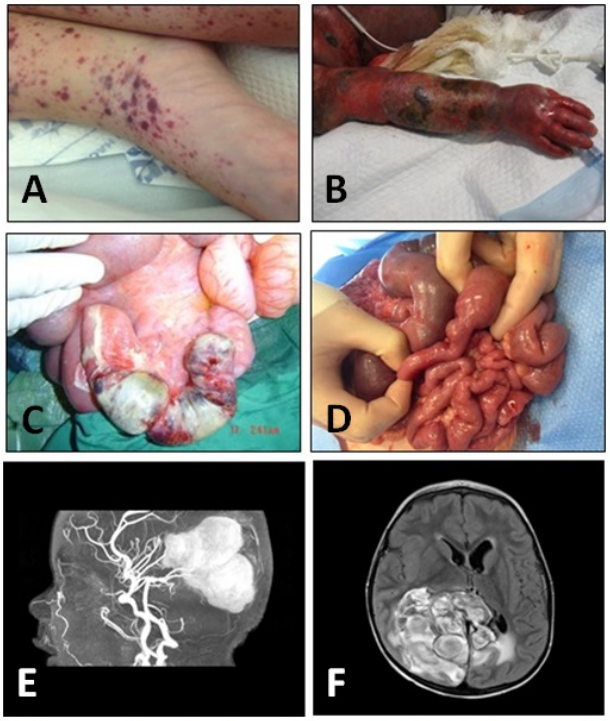
TMA-associated MODS in HCT recipients

In our prospective study we documented overall TMA incidence in 39% (39/100) of children undergoing HCT and identified TMA risk criteria. HCT recipients with TMA who had proteinuria ≥30 mg/dL and elevated sC5b-9 at TMA diagnosis had worse 1-year post-transplant survival (<20%, high risk patients). Patients with TMA, but without proteinuria and without elevated sC5b-9, all survived without targeted intervention (low risk group). Having one risk factor, proteinuria or elevated sC5b-9 placed patients in moderate risk group. We now confirmed our data in 500 prospectively monitored HCT recipients and continue to observe total TMA incidence at 34% (mild to severe phenotype). Severe TMA phenotype TMA with MODS occurred in 11% of all transplanted patients. All these patients had evidence of terminal complement activation and required therapy with eculizumab.

We also showed that pediatric intensive care unit (PICU) admissions for MODS were much higher in children with TMA as compared to those without TMA (46% vs 15%, p=0.01)(Jodele et al Blood 2015).[9] Overall non-relapse mortality in children with TMA and MODS was over 90% without TMA targeted

therapy. The historical rate from our prospective study had survival documented in 2/11 (18%) patients with high risk TMA with MODS without relapse who did not receive any targeted TMA therapy within 24 weeks from TMA diagnosis and overall survival was with 10 of 11 patients dying within one year post HCT. Similar rates are consistently reported in retrospective studies showing over 80% non-relapse mortality (NRM) with TMA and MODS with patients dying within 6 months of TMA diagnosis.[2,3,10-12] Cho et al reported 29% incidence of TMA (n=148) with <20% survival in patient meeting hematologic and renal diagnostic criteria ("definite TMA"), and concluded that TMA should be treated as soon as it is suspected, to avoid irreversible organ damage since therapeutic interventions like plasma exchange, defibrotide or discontinuation of calcineurin inhibitors had the greatest benefit if therapy was initiated early, but there are no clinical trials evaluating any TMA targeted treatment methods. Oran et al reported 55 of 66 patients (83%) dying at a median of 3 months after TMA diagnosis. Median survivals from the date of the first TMA episode for non-responders to plasmapheresis was 1.07 months vs 7.5 months in responders (p=0.0001). Survival at 6 months was 0% and 50.7% for TMA non-responders and responders to plasmapheresis, respectively.

Fig.1 Organ injury in HCT recipients with TMA



Skin vasculopathy (A) and necrosis (B); Small bowel necrosis (C) and stricture (D); CNS bleed (E,F)

Genetic predisposition to TMA after HCT: We performed a hypothesis-driven analysis of 17 candidate genes known to play a role in complement activation as part of a prospective study (n=100) of TMA in HCT recipients. We examined the functional significance of gene variants by using gene expression profiling. Sixty-five percent of patients with TMA had genetic variants in at least one gene compared with 9% of patients without TMA ($p < 0.0001$) **(Fig.1)**. Gene variants were increased in patients of all races with TMA, but nonwhites had more variants than whites (2.5 [range, 0-7] vs 0 [range, 0-2]; $p < 0.0001$). Variants in ≥3 genes were identified only in nonwhites with TMA and were associated with high mortality (71%). RNA sequencing analysis of pre-transplantation samples showed upregulation of multiple complement pathways in patients with TMA who had gene variants, including variants predicted as possibly benign by computer algorithm, compared with those without TMA and without gene variants.

Kidney injury: Consistent with our previous studies, we observed that proteinuria and hypertension were the earliest signs of TMA in HCT recipients, along with an elevation in the lactate dehydrogenase (LDH). In contrast, kidney dysfunction assessed by serum creatinine was a late marker, highlighting its limitations as it remains an insensitive marker to detect impaired renal function in pediatric HCT recipients who generally have low muscle mass and thus low creatinine generation rates.[4] In a prospective 94 patient cohort we showed that pediatric equations including Cystatin C (Cyst C) performed better than those including only creatinine,[13] therefore we have adopted Cystatin C based glomerular filtration rate (GFR) monitoring of acute kidney injury (AKI) for children undergoing HCT. In the group of 30 children treated with eculizumab for high risk TMA in Cincinnati, the median decline in CycC GFR due to TMA was 75% from pre-transplant baseline, indicating severe kidney injury occurs with the microangiopathic process. Thirty six percent of these patients progressed to ≥ stage 3 chronic kidney disease. By reports in the literature, patients with TMA as compared with HSCT patients without TMA are 4 times more likely to develop chronic kidney disease (CKD) and 9 times more likely to have hypertension with kidney function declining to as low as 40% of normal 2 years after HCT.[14,15]

Cardiac and pulmonary injury: We showed in autopsy specimens that HCT-TMA can be associated with significant pulmonary vascular injury. This presents clinically as hypoxemic respiratory failure with acute pulmonary hypertension (PH). Of note, echocardiography can identify vascular injury associated with TMA as early as 7 days after HCT.[16] In our prospective TMA study (n=100) pulmonary hypertension was diagnosed exclusively in the TMA group, and respiratory failure and pericardial effusions were strongly associated with TMA ($p<0.01$).[4] A separate prospective cardiac screening study of 227 patients at our institution supported our initial observations, confirming that clinically significant pericardial effusions were associated with TMA ($p<0.01$).

Gastrointestinal (GI) injury: In our prospective study severe gastrointestinal bleeding occurred only in subjects with TMA; notably only those who received TMA targeted therapy survived.[4] Patients with intestinal TMA presenting as ischemic colitis who were treated with eculizumab sometimes had intestinal strictures requiring surgical resection. A retrospective blinded review of pathology samples we performed showed that histologic evidence of TMA can be identified in the bowel of subjects with systemic high risk TMA features.[17]

Blood stream infections (BSI): In a prospective analysis of 374 children who underwent HCT at our institution to determine the incidence, risk factors, and outcomes of patients that developed a bloodstream infection (BSI) we showed that nearly 50% of all patients with a BSI developed sepsis, 25% were transferred to the PICU, and 10% died within 10 days of the positive culture. One-year non-relapse mortality (NRM) was significantly increased in patients with one BSI (34%) and more than one BSI (56%) in the first year post-HCT compared with those who did not develop BSIs (14%) ($p \leq 0.0001$). TMA was significantly associated with development of a BSI in HCT recipients (OR, 2.94; $p < 0.0001$) likely due to impaired intestinal vascular barriers due to microangiopathy.

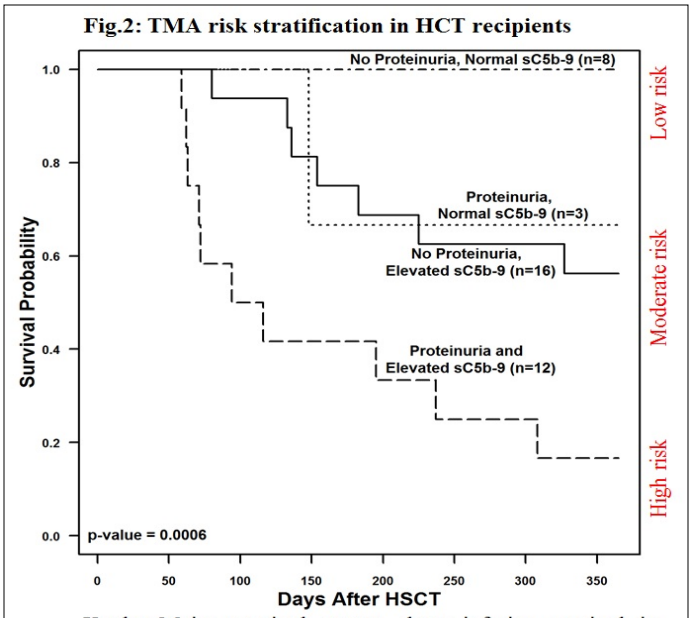


Fig.2: TMA risk stratification in HCT recipients

Kaplan-Meier survival curves show inferior survival in HCT patients with proteinuria and elevated serum sC5b-9 at TMA diagnosis (Jodele et al, Blood 2014)

TMA risk stratification

Our prospective monitoring study showed that children with both proteinuria (≥30mg/dL) and terminal complement activation as measured by elevated plasma sC5b-9 level (>244ng/mL) in addition to hematologic TMA markers (elevated LDH, de novo anemia and thrombocytopenia, low haptoglobin and schistocytes) at TMA diagnosis had very poor a 1-year HCT survival (<20%), whereas subjects without complement activation (normal plasma sC5b-9) and no proteinuria all survived without any interventions (20% vs 100%, $p<0.0006$).[4] These data again suggest that complement activation plays a significant role in the pathogenesis and severity of TMA after HCT and allowed us to propose risk stratification criteria to identify subjects who may benefit from complement blocking therapy. HCT recipients with TMA who have elevated sC5b-9 and proteinuria now are stratified as "high risk TMA" due to high risk for MODS and death and are offered eculizumab therapy, those with normal sC5b-9 and no proteinuria are "low risk TMA" and are closely monitored without interventions. Interventions for patients with one high risk feature are offered case by case based on TMA-associated organ injury **Fig.2**.

Eculizumab therapy and pharmacokinetics/pharmacodynamics (PK/PD) in HCT recipients:

As we were studying complement blocking therapy in HCT patients with high-risk TMA, we discovered that eculizumab pharmacokinetics in HCT recipients differ significantly from those reported for other diseases (aHUS and PNH). We measured eculizumab serum concentrations, total hemolytic complement activity (CH50), and plasma sC5b-9 concentrations in 18 HCT recipients with high-risk TMA presenting with complement activation. Population PK/PD analyses correlated eculizumab concentrations with complement blockade and clinical response and determined inter individual differences in PK parameters. Our first cohort included 6 HCT recipients that were treated using an aHUS dosing regimen.[5] Two critically ill patients died before ever achieving therapeutic drug levels. Eculizumab and total complement activity (CH50) levels that were measured at the same time points strongly correlated with each other. Specifically, a CH50 level of <10% normal corresponded with an eculizumab concentration >100 mg/mL, considered to be the therapeutic target concentration for complete complement blockade. Subsequently, 12 patients were treated using PK/PD dose guided regimen.[6] In the PK analysis, we found significant interpatient variability in eculizumab clearance, ranging from 16 to 237 mL/hr/70 kg in the induction phase. The degree of complement activation measured by sC5b-9 concentrations at the start of therapy, in addition to actual body weight, was a significant determinant of eculizumab clearance and disease response.

All subjects with elevated sC5b-9 above normal (>244ng/mL) cleared eculizumab below therapeutic of 100µg/mL in less than 72 hours during first 2 weeks of therapy while sC5b-9 was elevated, indicating that HCT recipients with TMA require loading doses at least Q72h to sustain adequate drug level to control complement activation, so currently recommended weekly dosing given for aHUS is not adequate for HCT recipients with TMA. The clearance progressively declined as the disease was controlled and became similar to what is reported in aHUS after the 5th week of therapy. Drug trough levels ≥100µg/mL were required to sustain TMA control in maintenance therapy. We validated a more frequent early-dosing algorithm in another 11 patients. We also showed that therapeutic eculizumab serum concentrations of ≥100µg/mL correlated well with total complement activity (CH50) suppression below detectable, <10% of normal lab value allowing us to use CH50 as accurate marker for eculizumab dose adjustments. In a publication of first 30 treated patients, we reported that 64% of patients had complete resolution of TMA and were able to safely discontinue eculizumab without disease recurrence. Overall survival was significantly higher in treated subjects compared with untreated patients (62% versus 9%, p =0.0007).[6] To date we have treated 50 patients with severe TMA using eculizumab therapy at CCHMC with an overall survival of 72% (36 survivors, 14 non-survivors). Median treatment time was 10 weeks (range 2-32, including survivors and non-survivors). Out of 50 Eculizumab treated patients 94.3% of responders were free of TMA with less than or equal to 24 weeks of treatment. We developed an eculizumab clearance prediction algorithm using pre-therapy sC5b-9 value, actual body weight and first eculizumab dose (mg). This algorithm was used to develop the dosing schedule for this study.

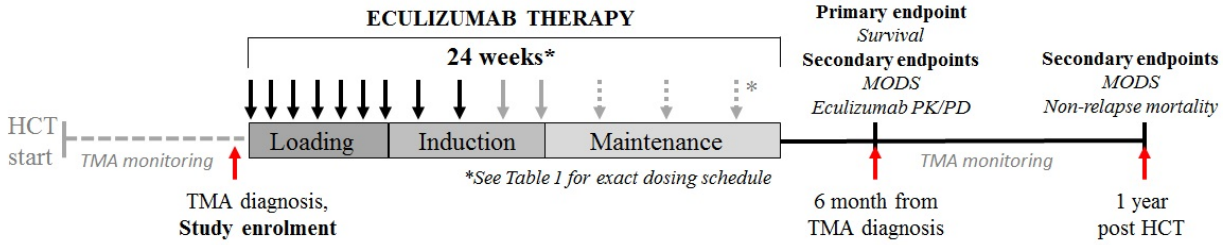
Eculizumab use for treatment of TMA in HCT recipients includes dealing with the issue of the FDA Risk Evaluation Mitigation Strategy (REMS) requirement of meningococcal vaccination. TMA after HCT usually occurs within the first 100 days after transplantation when patients are severely immunocompromised and are not able to mount a response to vaccines. We evaluated 30 HCT recipients treated with eculizumab for high-risk TMA without meningococcal vaccine. All patients received antimicrobial prophylaxis adequate for Neisseria meningitides during eculizumab therapy and for 8 weeks after discontinuation of the drug. Median time to TMA diagnosis was 28 days after transplant (range, 13.8 to 48.5). Study subjects received a median of 14 eculizumab doses (range, 2 to 38 doses) for HCT-associated TMA therapy. There were no episodes of meningococcal infection. The incidences of bacterial and fungal bloodstream infections were similar in patients treated with eculizumab (n=30) as compared with those with HSCT-associated TMA who did not receive any complement blocking therapy (n=39). Our data indicate that terminal complement blockade in the early post-transplant period can be performed without

meningococcal vaccination while using appropriate antimicrobial prophylaxis until complement function is restored after therapy completion.[18]

2. STUDY PLAN

2.1 Study synopsis

We propose a prospective single arm multi-institution study in patients undergoing allogeneic or autologous HCT. The study will be performed at Cincinnati Children's (CCHMC), Children's Hospital Los Angeles (CHLA), Children's Hospital of Philadelphia (CHOP), and Dana-Farber Cancer Institute (DFCI)/Boston Children's Hospital (BCH), all large pediatric transplant centers (~400 transplant/year combined). All patients are prospectively monitored for TMA after starting HCT as part of clinical care. Patients with high risk TMA[2] will be enrolled on this study after meeting eligibility criteria. The eculizumab dosing regimen for this study was derived from our clinical and PK/PD data in 50 eculizumab treated HCT recipients and was designed with a goal of being effective in majority of HCT recipients with high risk TMA, avoiding under treatment that allows ongoing organ damage. Loading doses are given ≤72h intervals based on ultra-high clearance during the first 2 weeks of therapy until sC5b-9 normalizes. Induction doses are given weekly to sustain therapeutic eculizumab trough level of ≥100µg/mL, since eculizumab clearance in HCT patients remains much higher than in aHUS during first 5 weeks of therapy. Maintenance dosing for HCT recipients will be identical to the regimen used for aHUS, since eculizumab clearance is similar in both populations when complement activation is controlled. All patients will receive total of 24 weeks of therapy before eculizumab is stopped and patients continue close clinical monitoring (Table 1). Survival will be assessed at 6 months from TMA diagnosis as the primary study endpoint. We hypothesize that this dosing regimen should be feasible at institutions that don't have availability of monitoring drug PK/PD in real time. PK/PD data obtained during this study will be used to further optimize eculizumab dosing schedule and to determine patients in whom shorter therapy might be safe. All study patients will receive



antimicrobial prophylaxis adequate to cover N. Meningitides while complement is blocked (refer to Section 6.1).

Figure 1. Overview of the study design

2.2 Hypotheses

1. Early intervention with complement blocker eculizumab will double survival in HCT recipients with high risk TMA, as compared to historical untreated controls. 2. An optimal eculizumab dosing schedule can be determined for this population through eculizumab PK/PD testing.

2.3 Number of subjects

This study will enroll 21 consecutive HCT recipients with high risk TMA (plus replacements if needed for early death or relapse or inability to receive therapy as noted in the statistics section) at participating institutions.

2.4 Selection

This study will be conducted at Cincinnati Children's Hospital (CCHMC), Children's Hospital Los Angeles (CHLA), Children's Hospital of Philadelphia (CHOP), and Dana-Farber Cancer Institute (DFCI)/Boston Children's Hospital (BCH) in patients undergoing allogeneic or autologous hematopoietic stem cell transplant and have a diagnosis of TMA with high risk disease features.

2.5 Recruitment

The study will enroll 21 consecutive patients with high risk TMA (see statistics section for details). Dr. Jodele or one of the other Bone Marrow Transplant Physicians who are co-investigators at CCHMC or investigators at participating sites will consult with the patient and/or the parent/legal guardian to explain the procedures, risks and benefits of the study at the patient/parent's level of understanding. Opportunity will be given to consider the study and have questions answered. Information will be given in a written format in the form of a description of the study, which will include a signature space for consent to be given. Participation is voluntary, and all subjects/parents/guardians will give informed consent to participate.

2.6 Expected duration of the study

Expected study duration is 4 years: we anticipate enrolling 7-10 patients each year for 2-3 years with 1 year post-transplant follow-up.

3. STUDY ENDPOINTS

3.1 Primary study endpoint

The primary study endpoint is survival at 6 months post the date of TMA diagnosis.

In our 100 patient prospective observational study, overall TMA incidence was 39% (39/100) with 11 of these 39 patients having high risk TMA with MODS (Jodele et al, Blood 2014). The historical survival rate for these patients with high risk TMA with MODS from our prospective study who did not receive any TMA targeted therapy was 2/11 (18%) children alive 24 weeks from TMA diagnosis and overall survival of 9% (1/11) at 1 year after HCT. Similar rates are consistently reported in retrospective studies from others showing <20% survival with TMA and MODS with patients dying within 6 months of TMA diagnosis.

3.2 Secondary study endpoints

- Cumulative incidence of organ dysfunction at 6 months after TMA diagnosis and 1 year after HCT, compared with historical controls with high risk TMA using MODS definition is shown in APPENDIX II. Organ systems evaluated will be:
    - Renal
        - Includes hypertension evaluation
    - Cardiac
    - Pulmonary
    - Gastrointestinal
    - CNS function
    - Pericardial effusion
- Timing and extent of resolution of organ dysfunction in the first year after therapy.
- 1 year non-relapse mortality (NRM) compared with historical controls.
- Characterization of eculizumab PK/PD.

4. ELIGIBILITY

The study participants will include patients undergoing allogeneic or autologous HCT at participating institutions. All HCT patients will be assessed for eligibility by their attending bone marrow transplant physician. Eligible patients/parents will be approached for their interest in participation. Identification, screening, and enrollment of patients will be performed by one of the investigators participating in the study. The study is open to all eligible patients regardless of gender or ethnicity.

4.1 Inclusion Criteria

Patients of any age undergoing allogeneic or autologous HCT with:

- Histologic TMA diagnosis
- OR clinical TMA diagnosis and presenting with high risk disease features including:
    - elevated plasma sC5b-9 above laboratory normal value (≥244ng/ml).
      AND
    - proteinuria: measured as ≥30mg/dL of protein on random urinalysis x2 **or** protein/creatinine ratio ≥1mg/mg **or** patient receiving renal replacement therapy.

  TMA diagnostic criteria are shown in APPENDIX I.
- Minimum weight of ≥ 5kg.
- ADAMTS13 Activity lab sample must be drawn during or prior to screening (results can be pending and are not required for initial eligibility). Development of TMA can be a medical emergency and thus if patient meets all other eligibility criteria, patient may proceed with enrollment and study treatment even if ADAMTS13 results are not available. If test results confirm diagnosis of TTP defined by ADAMTS13 activity test <10%, then study treatment will be immediately halted and patient will be removed from study.

4.2 Exclusion Criteria

- Known hypersensitivity to any constituent of the study medication.
- Subjects with unresolved serious Neisseria meningitides infection or
- Progressive severe infection.
- Patients previously treated with eculizumab or other complement blocker for TMA within the 60 days prior to first dose of study treatment.

5. STUDY DRUG

5.1 Eculizumab availability for study

Eculizumab (Soliris) will be provided by Alexion Pharmaceuticals at no cost to study subjects.

5.2 Eculizumab mechanism of action

Eculizumab, the active ingredient in Soliris, (Alexion Pharmaceuticals) is a monoclonal antibody that specifically binds 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. Eculizumab inhibits terminal complement mediated intravascular hemolysis in patients with PNH and blocks complement-mediated TMA in patients with aHUS. It has received regulatory approval in multiple countries for these two diseases. At CCHMC eculizumab has been used in 50 pediatric HCT recipients using PK/PD based dosing regimen and was shown to be safe in HCT population when antimicrobial prophylaxis is provided for the duration of complement blockade (Jodele et al, BBMT 2015).[6]

5.3 Study drug management

Please refer to the Investigator's Brochure for additional information as follows:

- **Study Drug Storage and Handling:** Please see Section 3.2.4 and 3.2.5 of the Investigator's Brochure.
- **Preparation and Administration:** Please see Section 6.2 of the Investigator's Brochure.
- **Pregnancy:** Please see Section 6.3.1 of the Investigator's Brochure.

5.4 Eculizumab safety profile

Eculizumab Expected Adverse Reactions

MedDRA System Organ Class	Very Common (>1/10)	Common (≥1/100 to <1/10)	Uncommon (≥1/1,000 to <1/100)
Blood and lymphatic system disorders		Thrombocytopenia, Leukopenia, Haemolysis	Coagulopathy, Red blood cell agglutination, Abnormal clotting factor, Anaemia, Lymphopenia
Cardiac disorders			Palpitation
Ear and labyrinth disorders			Tinnitus, Vertigo
Eye disorders			Vision blurred, Conjunctival irritation
Endocrine disorders			Basedow's disease
Gastrointestinal disorders		Diarrhoea, Vomiting, Nausea, Abdominal pain, Constipation, Dyspepsia	Peritonitis, Gastroesophagal reflux disease, Abdominal distension, Gingival pain
General disorders and administration site condition		Oedema, Chest discomfort, Pyrexia, Chills, Fatigue, Asthenia, Influenza like illness	Chest pain, Infusion site paraesthesia, Infusion site pain, Extravasation, Feeling hot,
Hepatobiliary disorders			Jaundice
Immune system disorders		Anaphylactic reaction	Hypersensitivity
Infection and infestations		Meningococcal sepsis, Arthritis bacterial, Upper respiratory tract infection, Nasopharyngitis, Bronchitis, Oral Herpes, Urinary tract infection, Viral infection, Aspergillus Infection	Meningococcal meningitis, Neisseria infection, Sepsis, Septic shock, Pneumonia, Gastrointestinal infection, Cystitis, Lower respiratory tract infection, Fungal infection, *Haemophilus influenzae* infection, Abscess, Cellulitis, Influenza, Gingival infection, Infection, Sinusitis, Tooth infection, Impetigo
Investigations		Coombs test positive	Alanine Aminotransferase increased, Aspartate Aminotransferase increased, Gammaglutamyltransferase increased, Haematocrit decreased, Haemoglobin decreased

MedDRA System Organ Class	Very Common (>1/10)	Common (≥1/100 to <1/10)	Uncommon (≥1/1,000 to <1/100)
Injury, poisoning and procedural complication			Infusion related reaction
Metabolism and Nutrition disorders		Decreased appetite	Anorexia
Musculoskeletal and connective tissue disorders		Arthralgia, Myalgia, Muscle spasms, Bone pain, Back pain, Neck pain, Pain in extremity	Trismus, Joint swelling,
Neoplasms benign, malignant and unspecified (including cysts and polyps)			Malignant melanoma, Myelodysplastic syndrome
Nervous system disorders	Headache	Dizziness, Dysgeusia	Syncope, Tremor, Paraesthesia
Psychiatric disorders			Depression, Anxiety, Insomnia, Sleep disorder, Abnormal dreams, Mood swings
Renal and urinary disorders			Renal impairment, Haematuria, Dysuria
Reproductive system and breast disorders			Spontaneous penile erection, Menstrual disorder
Respiratory, thoracic and mediastinal disorders		Dyspnoea, Cough, Nasal congestion, Pharyngolaryngeal pain, Rhinorrhoea	Epistaxis, Throat irritation
Skin and subcutaneous tissue disorders		Rash, Alopecia, Pruritus,	Urticaria, Dermatitis, Erythema, Petechiae, Skin depigmentation, Hyperhidrosis, Dry skin
Vascular disorders		Hypotension	Accelerated hypertension Hypertension, Haematoma, Hot flush, Vein disorder

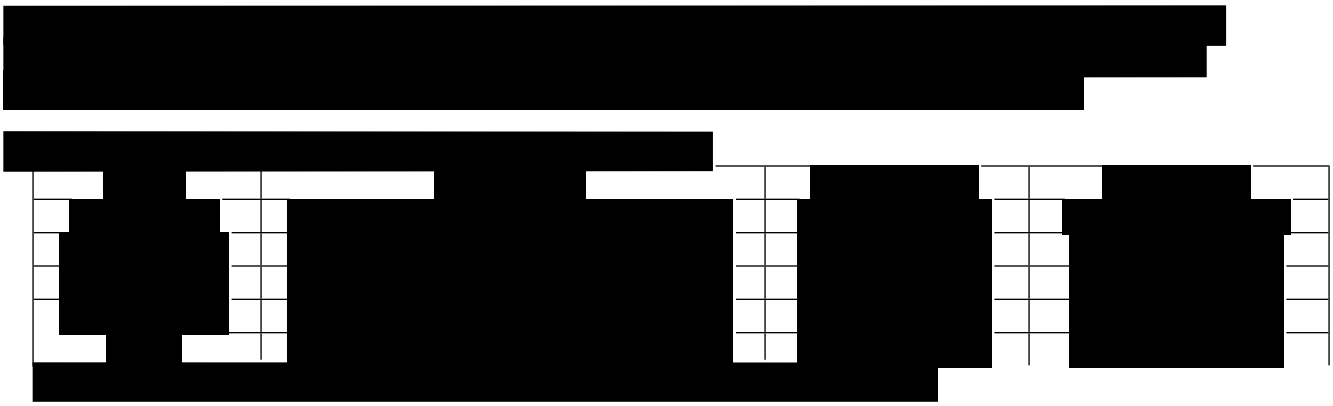
6. TREATMENT PLAN

6.1 Eculizumab dosing and administration for HCT recipients with TMA

- Patients who meet all of the inclusion criteria and none of the exclusion criteria are recommended to be treated as early as possible with eculizumab.
- Healthcare professionals who prescribe eculizumab (Soliris) must enroll in the Soliris REMS program.
- Vaccination of study subjects with meningococcal vaccine according to current ACIP guidelines will be considered if immune response to vaccine is expected based on timing after-HCT. Decision to vaccinate study subjects will be based on the primary treating physician's assessment.

- All study subjects will be counseled for risk of meningococcal infection due to terminal complement blockade.
- Beginning with the first dose of eculizumab, all study subjects will be started on antimicrobial prophylaxis using an agent with activity against *Neisseria meningitides,* if not already receiving systemic antibiotics providing adequate coverage.
    - Recommended agents for patients not receiving broad spectrum antibiotics for other clinical reasons that would cover N. meningitis:
        - Tier 1: Age/weight based therapeutic dosing of ciprofloxacin (PO and IV).
        - Tier 2: Age/weight based therapeutic dosing of oral amoxicillin.
        - If local strains of N. meningitis bacteria are resistant to either of these antibiotics or the patient is allergic or cannot otherwise tolerate the standard prophylactic treatment, the choice of antibiotic will be made upon local guidelines or the recommendation of the local Infectious Disease (ID) consultant.
- Antibiotic prophylaxis will be continued until eculizumab clearance is documented (serum drug concentration <25 µg/ml) and CH50 recovery to normal or above normal levels (based on performing laboratory value) is documented.
- Eculizumab will be stored and dispensed by the pharmacy as listed in medication insert information (Soliris USPI Jan 2016 NH).
- Eculizumab will be administered as intravenous infusion (IV) over 60 minutes using dosing schedule outlined in Table 1.
- No pre-medications are required, but may be given based on the primary treating physician's decision.
- If an adverse reaction occurs during the administration of eculizumab, the infusion may be slowed or stopped at the discretion of the study investigator and institute any appropriate measures. If the infusion is slowed, the total infusion time should not exceed four hours. The adverse reaction must be recorded.
- Patients may remain on current immunosuppressive therapy, including steroids.
- Fresh frozen plasma (FFP) or therapeutic plasma exchange (TPE) should NOT be used during eculizumab therapy, unless there is a strong clinical indication that may affect patient safety. Study subjects who require FFP or TPE for clinical indications will require supplemental doses of eculizumab as listed in Table 2.
- Patients may receive fibrinogen, albumin and IVIG and other monoclonal antibodies as clinically indicated without restrictions.

6.2 Eculizumab dosing schedule for HCT recipients:



6.2.1 Eculizumab dosing schedule modifications from listed in Table 1

Eculizumab therapy may be extended past the number of doses listed in Table 1 or dose (mg) and dosing intervals may be modified if deemed by the treating physician that the listed regimen is placing the patient at risk for TMA or MODS progression due to drug under dosing as evidenced by increased drug clearance (for example in patients with intestinal bleeding). **Dose modification must be discussed with the Lead PI and the reason to modify doses must be documented.**

Eculizumab therapy may be shortened to less than 24 weeks of total therapy, **with prior discussion and approval by the Lead PI**, if deemed by treating physician to be in the best interest to the patient under the following conditions:

- where TMA is adequately controlled or resolved and additional eculizumab doses will not be of benefit,
- or if continuation of eculizumab would interfere with other clinical interventions deemed more essential for patient's wellbeing.

This does not include study drug discontinuation for safety reasons or therapy intolerance. These patients will remain on study and should complete assessments as per Early Termination visit at the time of discontinuation of eculizumab. Refer to the Schedule of Assessments Table for the list of required assessments. Additionally, V37 (Day 180) and V38 (Day 365 post-transplant) visits should be complete according to original protocol schedule.

If eculizumab was held because of other therapeutic interventions and there is no clinical concern for active TMA, then eculizumab can be resumed at the dosing regimen used prior to holding/stopping the drug.

In the event eculizumab is held/stopped early according to the above criteria and the patient has a reactivation or progression of TMA, eculizumab study treatment may be resumed at a more intensified dosing regimen. The dosing regimen to be resumed should be discussed with the Lead PI.

6.2.2 Supplemental eculizumab dosing for patients receiving FFP and TPE

Study subjects requiring fresh frozen plasma (FFP) or therapeutic plasma exchange (TPE) will receive supplemental eculizumab dosing as listed in Table 2. Subjects receiving FFP or TPE will continue to have study assessments performed as per the Schedule of Assessment table. Eculizumab serum concentration (drug level) and CH50 should be performed prior to each supplement eculizumab dose if clinically feasible, for at least first 5 FFP/TPE sessions to assure that drug supplementation is adequate. Research Biomarker Panel is not required before supplement eculizumab doses.

6.2.3 Patient monitoring during eculizumab therapy

Laboratory monitoring is summarized in Tables 3 and 4. Dose changes will **NOT** be made in real time from those listed in Table 1 based on the PK/PD data with exception of patients meeting dosing

modification criteria as listed in section 6.2.1. PK/PD data will be used to establish optimal fixed dosing schedule required for HCT recipients with high risk TMA to prevent MODS in future clinical use.

Table 3: Schedule of Assessments (Baseline, Loading and Induction Phases)

	Screening **	Base Line	Loading												Induction•				
Study Visits*&		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V15**	**V16**	**V17**	**V18**	**UNS+ visit**
Days	**Prior to First Eculizumab Infusion**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 19**	**Day 26**	**Day 33**	**Day 40**	
Informed Consent	X																		
Inclusion/ Exclusion laboratory and clinical assessments[a]	X																		
Medical history/Demographics	X																		
ADAMTS13 activity	X~																		
Complete Complement Profile (CCHMC Lab)		X																	
CFH auto-antibody [b] (CCHMC Lab)		X																	
CH50 [b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Plasma sC5b-9[b,c] (CCHMC Lab)	X		X	X	X	X	X	X	X	X	X	X	X	X	X [c]	X [c]	X [c]	X [c]	X
Haptoglobin		X																	
Urine protein or random urine protein creatinine ratio^	X																		
Eculizumab infusion[d,±]			X		X			X			X			X	X	X	X	X	X
Eculizumab serum concentration[b] (CCHMC Lab)				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Research Biomarker Panel[e]		X	X		X			X			X			X	X	X	X	X	X
Safety Assessments[f]	X	Performed as per institutional standard of care.																	
AEs/SAEs[g]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC/Diff, LDH, Schistocytes	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Other Laboratory Tests[h]	X		X						X					X	X	X	X	X	X
MODS Assessments[i]		X							X					X	X	X	X	X	X
Repository Samples[j]		X							X					X	X	X	X	X	X

	Screening **	Base Line	Loading												Induction•				
Study Visits*&		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V15**	**V16**	**V17**	**V18**	**UNS+ visit**
Days	**Prior to First Eculizumab Infusion**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 19**	**Day 26**	**Day 33**	**Day 40**	
PE/PI· Dialysis, RBC/Platelet Transfusion	Procedures to be completed as deemed medically necessary by treating physician. If completed, data will be captured.																		X

Abbreviations: AE=Adverse Event; EOS=End-of-Study; ET=Early Termination; PE/PI=Plasma Exchange/Plasma Infusion; UNS=Unscheduled Visit

** Screening and Baseline may occur on the same day. All pre-therapy laboratory assessments should be completed within 7 days prior to initiating therapy with Eculizumab. Any labs that were completed as part of standard clinical care prior to consent may be used and will not be repeated.

~ ADAMTS13 Activity lab sample must be drawn during or prior to screening. If patient meets all other eligibility criteria, the patient may proceed with enrollment and study treatment even if ADAMTS13 results are not available. If test results confirm diagnosis of TTP defined by ADAMTS13 activity test <10%, then study treatment will be immediately halted and patient will be removed from study.

• **A +/- 48 hours window will be added to accommodate scheduling changes during the induction phase.**

* If a patient completes all the Study Visits (Visits 1-38), Visit 38, the last Study Visit, will be the end-of-study (EOS) visit, at which time the patient completes the study.

& **If a patient withdraws early from the study, <u>prior</u> to completing Visit 36, an Early Termination (ET) Visit will be performed within one week of withdrawal (see Table 4).** Any assessments not performed within 7 days of ET visit will need to be completed – any assessments performed within 7 days do <u>not</u> need to be repeated or recollected.

+ Unscheduled visit and procedures will be performed at the Investigator's discretion and results will be recorded on the CRF.

^ Please note urine protein ≥30 mg/dL x2 is required for eligibility. Urine protein or random urine protein creatinine ratio are not required if the patient is already receiving renal replacement therapy (RRT). RRT requirement is sufficient to meet study eligibility criteria.

[a] Up to 7 days prior to the baseline visit (Screening), laboratory and clinical assessments demonstrating fulfillment of inclusion criteria are performed

[b] TMA Test Panel includes CFH autoantibody, plasma sC5b-9 and eculizumab pharmacokinetic panel (eculizumab serum concentration, CH50) and is to be performed at CCHMC, as feasible. (Refer to Appendix III for link for test requisition and additional information.) For patients requiring eculizumab serum level and CH50 on the same day, testing for both may be ordered as Eculizumab Pharmacokinetic Panel on the CCHMC Test Requisition and performed from the same blood sample (1 mL blood in serum tube). Blood may be collected during routine draws. On days of eculizumab administration, the eculizumab serum concentration sample is to be collected **PRIOR** to administration of eculizumab, preferably within 90 minutes before eculizumab infusion. Among patients receiving plasma exchange/plasma infusion (PE/PI), dialysis or transfusion, blood will be collected prior to initiation of PE/PI, dialysis or transfusion, as feasible. Eculizumab pharmacokinetic panel (eculizumab serum concentration and CH50) should be performed prior to each supplement eculizumab dose if clinically feasible, for at least first 5 FFP/TPE sessions to assure that drug supplementation is adequate. Additional blood samples may be collected at scheduled clinical visits or unscheduled visits, at the discretion of the Treating Physician and/or Principal Investigator. **Following discontinuation of eculizumab treatment, eculizumab serum concentration and CH50 will be monitored weekly until normalized or above normal as per protocol section 6.2.4**.

[c] sC5b-9 will be evaluated twice weekly from Day 19 through Day 40 while patient is an inpatient; once discharged, sC5b-9 will be collected weekly with each visit, as feasible. Following discontinuation of eculizumab treatment, sC5b-9 will be monitored weekly until eculizumab serum concentration and CH50 have normalized or above normal as per protocol section 6.2.4.

[d] Eculizumab will be administered by IV infusion over 60 minutes according to the dosing information on Table 1. For patients receiving PE/PI, supplemental eculizumab doses should be administered according to Table 2. If supplemental eculizumab doses are given on a day in which eculizumab is not scheduled, they will be recorded on the CRF as an unscheduled visit. **Following discontinuation of eculizumab treatment, eculizumab serum concentration and CH50 will be monitored weekly until normalized or above normal as per protocol section 6.2.4**.

[e] Research Biomarker Panel: plasma and serum samples for measures as outlined in the research laboratory manual will be collected for shipment to an Alexion designated laboratory. (Refer to lab manual for biomarker collection and shipping information.) Among patients receiving PE/PI, dialysis or transfusion, blood will be collected prior to initiation of PE/PI, dialysis or transfusion, as feasible. On days when eculizumab is administered, blood samples should be collected **PRIOR** to administration of eculizumab, preferably within 90 minutes before eculizumab administration. Research Biomarker panel samples are not required before supplemental eculizumab doses. If a pre-transplant DNA sample is available, it may be requested for a TMA-associated genetic panel.

[f] Safety assessments (i.e., vital signs, physical examination) will be performed as per SOC. Weight and height (height may be pre-transplant value) will be collected for all patients at Screening and at the end of study. Use vital signs collected at the start of day shift, i.e., at approximately 8 AM.

[g] AEs will be collected for 60 days (5 half-lives) after the last eculizumab infusion and all SAEs will be collected.

[h] Other laboratory tests will include, but are not limited to full panel blood chemistry, serum creatinine, cystatin C GFR, haptoglobin, urinalysis and random urine protein creatinine ratio, as per institutional SOC.

[i] Clinical assessments of renal, cardiac, gastrointestinal, pulmonary and neurological signs and symptoms will be assessed as part of SOC, approximately once a week using MODS Form in Appendix II.

[j] Patients will be consented to enable the collection of blood, urine and tissue research samples for the tissue repository as listed in APPENDIX IV. Blood samples collected from the tissue repository will include testing to assess the immunogenicity of eculizumab. Patients who are already enrolled in an HCT tissue repository at participating institutions will not be approached for this study to avoid duplicate specimen collection. **Baseline repository samples must be <u>drawn within 24 hours prior</u> to first dose of eculizumab.**

[k] PE/PI may include fresh frozen plasma (FFP), plasma exchange or plasma infusion.

[±] Eculizumab therapy course may be shortened according to criteria in Section 6.2.1. At the time of discontinuation of eculizumab, complete all study assessments as listed for the Early Termination visit. Any assessments not performed within 7 days of stopping therapy will need to be completed – any assessments performed within 7 days do not need to be repeated or recollected. Patient should be monitored for adverse events and MODS assessments should be completed for 60 days after the last eculizumab infusion. **Following discontinuation of eculizumab treatment, eculizumab serum concentration and CH50 will be monitored weekly until normalized or above normal as per protocol section 6.2.4**. Patient will remain on study and should complete V37 (Day 180) and V38 (Day 365 post-transplant) visit according to original protocol schedule.

Table 4: Schedule of Assessments (Maintenance Phase and End of Study/Early Termination)

	Maintenance[#]																			EOS	UNS[+] Visit
Study Visits*	**V19**	**V20**	**V21**	**V22**	**V23**	**V24**	**V25**	**V26**	**V27**	**V28**	**V29**	**V30**	**V31**	**V32**	**V33**	**V34**	**V35**	**V36**	**V37**	**ET[&] [±]**	**V38**	
Days	**Day 47**	**Day 54**	**Day 61**	**Day 68**	**Day 75**	**Day 82**	**Day 89**	**Day 96**	**Day 103**	**Day 110**	**Day 117**	**Day 124**	**Day 131**	**Day 138**	**Day 145**	**Day 152**	**Day 159**	**Day 166**	**Day 180**	**--**	**Day 365[§]**	
ADAMTS13 activity																		X[◦]		X[◦]		
Complete Complement panel[a] (CCHMC Lab)																		X[◦]		X[◦]		
CFH auto-antibody[a] (CCHMC Lab)																		X[◦]		X[◦]		
CH50[a]		X		X		X		X		X		X		X		X		X		X		X
Plasma sC5b-9[a] (CCHMC Lab)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Eculizumab infusion[b,±]		X		X		X		X		X		X		X		X		X		X		X
Eculizumab serum concentration[a] (CCHMC Lab)		X		X		X		X		X		X		X		X		X		X		X
Research Biomarker Panel[c]		X		X		X		X		X		X		X		X		X	X	X		X
Safety Assessments[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs/SAEs[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

CBC/Diff, LDH and Schistocytes	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Other Laboratory Tests[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MODS Assessments[g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[j]	X	X
Repository Samples[h]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PE/PI[i], Dialysis, RBC/Platelet Transfusions		Procedures to be performed as deemed medically necessary by treating physician. If completed, data will be captured.																				X

Abbreviations: AE=Adverse Event; EOS=End-of-Study; PE/PI=Plasma Exchange/Plasma Infusion; ET=Early Termination; UNS=Unscheduled Visit

[#] During Maintenance phase, study assessments will be performed at a minimum of every 2 weeks, a maximum of every week. Study visit windows during Maintenance phase will be +/- 48 hours for study infusions, eculizumab serum concentration, CH50, and research biomarker panel to accommodate patient scheduling. All other labs can be collected the same day or any day within the same week of the scheduled study visit.

***** If a patient completes all the Study Visits (Visits 1-38), Visit 38, the last Study Visit, will be the end-of-study (EOS) visit, at which time the patient completes the study.

[&] **If a patient withdraws early from the study, prior to completing Visit 36, an Early Termination (ET) Visit will be performed within one week of withdrawal (see Table 4).** Any assessments not performed within 7 days of ET visit will need to be completed – any assessments performed within 7 days do not need to be repeated or recollected.

[+] Unscheduled visits and procedures will be performed at the Investigator's discretion and results will be recorded on the CRF.

[◦] Obtain only at Visit 36 or Early Termination Visit if abnormal prior to starting therapy.

[§] EOS/Day 365 study visit is to be performed approximately one year, ± 60 days, **from the date of transplant**.

[a] TMA Test Panel includes CFH autoantibody, plasma sC5b-9 and eculizumab pharmacokinetic panel (eculizumab serum concentration, CH50) and is to be performed at CCHMC, as feasible. (Refer to Appendix III for link for test requisition and additional information.) For patients requiring eculizumab serum level and CH50 on the same day, testing for both may be ordered as Eculizumab Pharmacokinetic Panel on the CCHMC Test Requisition and performed from the same blood sample (1 mL blood in serum tube). Blood may be collected during any routine draws before eculizumab administration. On days of eculizumab administration, the eculizumab serum concentration sample is to be collected **PRIOR** to administration of eculizumab, preferably within 90 minutes before eculizumab infusion. Among patients receiving plasma exchange/plasma infusion (PE/PI), dialysis or transfusion, blood will be collected prior to initiation of PE/PI, dialysis or transfusion, as feasible. Eculizumab pharmacokinetic panel (eculizumab serum concentration and CH50) should be performed prior to each supplement eculizumab dose if clinically feasible, for at least first 5 FFP/TPE sessions to assure that drug supplementation is adequate. Additional blood samples may be collected at scheduled clinical visits or unscheduled visits, at the discretion of the Treating Physician and/or Principal Investigator. **Following discontinuation of eculizumab treatment, eculizumab serum concentration and CH50 will be monitored weekly until normalized or above normal as per protocol section 6.2.4**.

[b] Eculizumab will be administered by IV infusion over 60 minutes according to the dosing information on Table 1. For patients receiving PE/PI, supplemental eculizumab doses should be administered according to Table 2. If supplemental eculizumab doses are given on a day in which eculizumab is not scheduled to

be they will be recorded on the CRF as an unscheduled visit. **Following discontinuation of eculizumab treatment, eculizumab serum concentration and CH50 will be monitored weekly until normalized or above normal as per protocol section 6.2.4.**

[c] Research Biomarker Panel: plasma and serum samples for measures as outlined in the research laboratory manual will be collected for shipment to an Alexion designated laboratory. (Refer to lab manual for biomarker collection and shipping information.) Among patients receiving PE/PI, dialysis or transfusion, blood will be collected prior to initiation of PE/PI, dialysis or transfusion, as feasible. On days when eculizumab is administered, blood samples should be collected prior to administration of eculizumab, preferably within 90 minutes before eculizumab administration. Research Biomarker panel samples are not required before supplemental eculizumab doses.

[d] Safety assessments (i.e., vital signs, physical examinations) will be performed as per SOC. Weight and height (height for baseline may be pre-transplant value) will be collected for all patients at Screening and at the end of study. Use vital signs collected at the start of day shift, i.e., at approximately 8 AM.

[e] AEs will be collected for 60 days (5 half-lives) after the last eculizumab infusion and all SAEs will be collected (see protocol section 8.3 for additional details).

[f] Other laboratory tests will include, but are not limited to full panel blood chemistry, serum creatinine, cystatin C GFR, haptoglobin, urinalysis and random urine protein creatinine ratio, as per institutional SOC.

[g] Clinical assessments of renal, cardiac, gastrointestinal, pulmonary and neurological signs and symptoms will be assessed as part of SOC, approximately once a week using the MODS form in Appendix II. MODS Assessments must be completed for 60 days following the last eculizumab infusion, either weekly as inpatient or if outpatient, then with each clinic visit but not more than once a week.

[h] Patients will be consented to enable the collection of blood, urine and tissue research samples for the tissue repository as listed in APPENDIX IV. Blood samples collected from the tissue repository will include testing to assess the immunogenicity of eculizumab. Patients who are already enrolled in an HCT tissue repository at participating institutions will not be approached for this study to avoid duplicate specimen collection. **Baseline repository samples must be <u>drawn within 24 hours prior</u> to first dose of eculizumab.**

[i] PE/PI may include fresh frozen plasma (FFP), plasma exchange or plasma infusion

[j] MODS Assessments must be completed for 60 days following the last eculizumab infusion, either weekly as inpatient or if outpatient, then with each clinic visit but not more than once a week.

[±] Eculizumab therapy course may be shortened according to criteria in Section 6.2.1. At the time of discontinuation of eculizumab, complete all study assessments as listed for the Early Termination visit. Any assessments not performed within 7 days of stopping therapy will need to be completed – any assessments performed within 7 days do not need to be repeated or recollected. Patient should be monitored for adverse events and MODS assessments should be completed for 60 days after the last eculizumab infusion. **Following discontinuation of eculizumab treatment, eculizumab serum concentration and CH50 will be monitored weekly until normalized or above normal as per protocol section 6.2.4**. Patient will remain on study and should complete V37 (Day 180) and V38 (Day 365 post-transplant) visit according to original protocol schedule.

6.2.4 Patient monitoring after eculizumab therapy completion

The procedures listed below apply for patients who complete the therapy as prescribed by the protocol or who have received a shortened course of study therapy as per the criteria listed in section 6.2.1.

- Weekly CH50 levels will be checked until they return to normal or above normal values (see normal CH50 values for performing laboratory) to document that patients have recovered from complement suppression.
- Weekly serum eculizumab serum concentration will be checked until it is documented to be below measurable (<25 μg/mL) to document that drug is completely cleared from the blood.
- Weekly sC5b-9 will be monitored until normalization of CH50 levels (levels may also be above normal) and eculizumab drug clearance is documented (eculizumab serum concentration is <25 μg/mL) to assure that terminal complement does not get activated again after the eculizumab is cleared, then as clinically indicated.
- Anti-meningococcal prophylaxis will continue until the CH50 level is normal and eculizumab serum concentration is unmeasurable (<25 μg/mL).
- MODS assessments are to be completed weekly through 60 days following the last eculizumab infusion, either weekly as inpatient or if outpatient, then with each clinic visit, but not more than once a week.
- Patients are to be monitored for adverse events through 60 days following the last eculizumab infusion.

6.3 Concomitant HCT medication and supportive care guidelines

All study subjects will follow routine HCT monitoring and supportive care procedures as per standard institutional practices.

6.4 Post-transplant follow-up

After study therapy and required monitoring is completed, patients will be followed per institutional standards through one year post-transplant.

7. TOXICITY MONITORING

7.1 Eculizumab safety

Eculizumab is generally well tolerated in HCT recipients (section 1.4).[18] Because of the risk of meningococcal infections (black box warning), eculizumab is available only through a restricted program under a Risk Evaluation and Mitigation Strategy (REMS). Under the Soliris REMS, all eculizumab prescribers will be enrolled in the REMS program.

7.2 Risk for meningococcal infections during eculizumab therapy

Life-threatening and fatal meningococcal infections have occurred in patients with aHUS treated with eculizumab and may be fatal if not recognized and treated early. The Advisory Committee on Immunization Practices (ACIP) recommends meningococcal vaccination in patients with complement deficiencies. It is recommended that patients are immunized with a meningococcal vaccine at least 2 weeks prior to administering the first dose of eculizumab, unless the risks of delaying eculizumab therapy outweigh the risks of developing a meningococcal infection. Patients on this trial will not be able to respond to vaccine, and will instead receive antimicrobial prophylaxis (section 7.3). Patients should be monitored for early signs of meningococcal infections, and evaluated immediately if infection is suspected. For this reason,

eculizumab is available only through a restricted program under a Risk Evaluation and Mitigation Strategy. (REMS). Under the eculizumab (Soliris) REMS, prescribers must enroll in the program.

7.3 Prevention of meningococcal infections in HSCT recipients receiving eculizumab

TMA after HCT usually occurs within the first 100 days after transplantation when patients are severely immunocompromised and are not able to mount a response to vaccines. We evaluated 30 HCT recipients treated with eculizumab for high-risk TMA without meningococcal vaccine. All patients received antimicrobial prophylaxis adequate for Neisseria meningitides during eculizumab therapy and for 8 weeks after discontinuation of the drug. Median time to TMA diagnosis was 28 days after transplant (range, 13.8 to 48.5). Study subjects received a median of 14 eculizumab doses (range, 2 to 38 doses) for HCT-associated TMA therapy. There were no episodes of meningococcal infection. The incidences of bacterial and fungal bloodstream infections were similar in patients treated with eculizumab as compared with those with HCT-associated TMA who did not receive any complement blocking therapy. There were no other clinically significant side effects attributed to eculizumab. Our data indicate that terminal complement blockade in the early post-transplant period can be safely performed without meningococcal vaccination while using appropriate antimicrobial prophylaxis until complement function is restored after therapy completion (section 1.4).

- All study subjects will be counseled regarding risk of meningococcal infection due to terminal complement blockade.
- All study subjects will be started on antimicrobial prophylaxis using an agent with activity against *Neisseria meningitides* if not already receiving systemic antibiotics providing adequate coverage beginning with the first dose of eculizumab (refer to Section 6.1).
- Anti-meningococcal prophylaxis will continue until the CH50 level is normal and the eculizumab serum concentration is unmeasurable (<25 μg/mL).

7.4 Risk to an unborn fetus

While eculizumab had been used in pregnant women with preeclampsia, PNH, and aHUS, this drug is listed as Category C (not enough research has been done to determine if these drugs are safe).

8. ASSESSMENT OF SAFETY

8.1 Study monitoring and auditing

Monitoring and auditing procedures will be followed to ensure that the study is conducted, documented, and reported in accordance with the IRB approved protocol, all applicable federal regulations and guidelines, and applicable regulatory requirements at participating institutions.

Verification of eligibility will be performed and appropriate documentation of informed consent will be documented for all subjects enrolled into the study. The timeliness of Adverse Event and Serious Adverse Event reporting will be monitored to ensure regulatory compliance. All case report forms (CRF) for the first subject enrolled into the study will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents. Thereafter, a minimum of 10% of enrolled subjects' CRFs will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents.

The extent of monitoring activities will be subject to change based on enrollment, the degree of risk or significance of monitoring findings, and other study management issues.

Each participating site will be responsible for maintaining all source documents, research records, all IRB approval documents, Drug Accountability Record forms, patient registration lists, response assessments reports, etc. for their site's participation in this study.

A study monitor will audit study documents to ensure compliance.

8.2 Data safety monitoring plan

A Data and Safety Monitoring Board (DSMB) will be convened for this study. The Board will be comprised of qualified physicians, not associated with this protocol. The DSMB will meet with the Lead PI or designees and review study data/progress a minimum of approximately every 6 months or more often at the discretion of the Lead PI and/or DSMB.

The DSMB will be notified immediately and will review all unanticipated problems involving risk to subjects or others, unanticipated serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the review. Based on the review of these events, the DSMB should make a recommendation regarding study continuation.

8.3 Adverse events (AE)

8.3.1 Adverse event definitions

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Serious Adverse Event (SAE)
A serious adverse event is an undesirable experience that results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization. Hospitalizations for the following reasons should not be reported as serious adverse events:
    - Non-life-threatening hospitalization due to clinical problems associated with stem cell transplantation process, initiation or adjustments of care of transplant-associated complications, diagnostic evaluations, or social reasons.
    - Non-life-threatening hospitalization due to clinical problems associated with study subjects' primary medical condition, therapy targeting primary medical condition, or for initiation or adjustments of care associated with primary condition management, diagnostic evaluations or social reasons.
    - Hospital admission for a planned diagnostic workup/procedure/disease treatment.
    - Admission for known complications of TMA will be recorded on the MODS assessments and not upgraded to a serious adverse event solely on the basis of hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- In a congenital anomaly or occurrence of malignancy

- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above

Note: Events that may not meet criteria as serious should be reviewed and captured as a non-serious adverse event according to the requirements for reporting AEs (see section 8.3.2).

Attribution to the study drug:

- Unrelated: The AE is clearly NOT related to the intervention
- Unlikely: The AE is doubtfully related to the intervention
- Possible: The AE may be related to the intervention
- Probable: The AE is likely related to the intervention
- Definite: The AE is clearly related to the intervention

Expected and Unexpected Event:

- Expected: Any experience *previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or as specified in the protocol.
- Unexpected: Any experience *not previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or as specified in the protocol.

8.3.2 Adverse event reporting

Safety and tolerability for the study drug will be assessed according to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE published November 27, 2017). The principal investigator or designee at each site will review adverse events closely and assess the event's relationship to study procedures to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study procedures, especially in relation to the underlying disease and baseline lab values for the patient.

All expected and unexpected Grade 3 and higher adverse events possibly, probably or definitely related to the study drug occurring during this study through 60 days following the last dose of eculizumab will be recorded on the case report forms. The site principal investigator will review each event and assess its relationship to study procedures to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study therapy.

Expected events are those that have been previously identified as resulting from administration of the protocol therapy, those that can be attributed to the underlying condition, and/or those associated with transplant. An adverse event is considered unexpected, for reporting purposes only, when either the type of event or the severity of the event is not listed in the Investigator's Brochure, consent and/or protocol.

Additionally for this study, events previously identified as resulting from administration of a stem cell transplant procedure will be considered as expected. The transplant regimen is well known to commonly affect multiple systems such as the hematologic, immunologic and, pulmonary, gastrointestinal systems. The following are known complications of transplant- graft failure, graft versus host disease, disseminated intravascular coagulation, febrile neutropenia, viral, bacterial and fungal infections, hemolysis, septic shock, thrombotic microangiopathy, veno-occlusive disease of liver, adrenal insufficiency, diarrhea, enterocolitis, gastrointestinal pain and bleeding, malabsorption, mucositis, nausea, pancreatitis, vomiting, dyspepsia, fever, pain, cholecystitis, allergic reaction, serum sickness, upper respiratory infection, urinary tract infection, weight gain/loss, electrolyte imbalance, elevation of liver enzymes, acidosis, alkalosis, anorexia, dehydration, glucose intolerance, iron overload, generalized muscle weakness, reversible posterior leukoencephalopathy syndrome, bladder spasms, cystitis, hematuria, urinary frequency, urinary urgency, irregular menstruation, pruritis, hypertension, purpura, and petechiae, polyserositis, seizures, multi-organ failure, death.

Hematologic toxicities of any grade which are attributable to the underlying hematological disease and/or HCT preparative regimen will not be considered adverse events. **In the event that any of these known complications of transplant or hematologic toxicities are thought to be at least possibly attributable to the study drug, the event will be recorded and subsequently reported to the IRB and FDA according to current reporting requirements, as outlined in the preceding paragraphs.**

Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. This excludes TMA since disease progression is anticipated in some patients after initiation of complement blocking therapy and TMA is the subject of this study. Please refer to APPENDIX V for AE reporting requirements for TMA associated conditions. Abnormal laboratory values or test results constitute adverse events only if they are outside expected laboratory abnormalities seen during HCT process, induce clinical signs or symptoms, are considered clinically significant, or require therapy as determined by the PI at participating institutions.

Each site will be responsible for reporting all SAEs to their IRB according to institutional guidelines, current regulations and applicable federal regulations. All SAEs will be reported to the Lead PI/Sponsor or designee via fax or e-mail within 2 business days of the site principal investigator's knowledge of the SAE.

If any serious adverse events occur, current guidelines will be followed for expedited reporting to the IRB, DSMB, and FDA.

All serious and medically significant adverse events considered related to eculizumab by the investigator will be followed until resolved or considered stable.

Serious Adverse Event (SAE) Reporting Guidelines for External Site
Any SAE must be reported to CCHMC as soon as possible but no later than 2 business days of the site principal investigator's knowledge of the SAE. The SAE report (CRF) along with applicable source documentation (de-identified) is required to be submitted via fax or email to Stephanie Edwards (StephanieL.Edwards@cchmc.org) and contain the following information:

- Subject's initials
- Unique study ID
- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (study drug, other drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- The reason the event is serious
- Detailed text that includes the following information:
    - An explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- Relevant supporting source documents

The PI's signature and the date it was signed are required on the completed report.

The Lead PI is responsible for reporting all grade 3-5 unexpected, definite, probable, possible related SAEs on protocol to all participating sites within 5 calendar days of receipt. All serious and medically significant adverse events considered related to eculizumab by the investigator will be followed until resolved or considered stable.

9. RISK/BENEFIT ANALYSIS

This study will be more than minimal risk but with potential for direct benefit to participants.

9.1 Potential benefits

Prevention of or treatment resulting in better outcomes after TMA associated multi-organ dysfunction syndrome (MODS) is a potential direct benefit of the study.

9.2 Potential risks, discomforts, and inconveniencies

Risk associated with study therapy: There are risks to the patients which include acute allergic reaction to the drug and increased risk of meningococcal meningitis. There are single case reports of meningococcal meningitis with long-term use of eculizumab in patients who received meningococcal vaccine. We have reported that eculizumab was safe to use in HSCT patients who are unable to receive vaccines but are on antibiotics prophylaxis for the duration on complement blockade by eculizumab, there were no cases of meningococcal infection in the HCT patients treated with eculizumab at CCHMC.

Potential risks, discomforts, inconveniences, and precautions with study blood draws: Any research samples will be obtained in accordance with institutional standards to ensure patient safety and without compromising needed diagnostic/clinical blood samples or patient health. The total amount of blood drawn will not exceed 5% of the estimated blood volume in any 24 hour period. The total volume of blood in any 24 hour period will include blood drawn for clinical testing, research and waste samples combined. Blood will be drawn through patients' existing central lines whenever possible. Peripheral needle sticks will only be required in the event of no central access. Since patients undergoing HCT have daily laboratory studies performed as routine clinical care, additional study-related tests will be added-on to already collected blood samples whenever possible. Extra blood would only be drawn if specific collection tubes are required. Whenever possible, study blood samples will be obtained at the same time as routine blood draws to eliminate or minimize additional central line access. Urine will be collected by clean catch or cotton balls placed in the diaper as is routinely done during transplantation so catheterization will not be required. However, if a catheter is inserted for clinical indications, urine from the Foley bag will be obtained.

10. STATISTICAL ANALYSIS

Statistical Analysis for primary endpoints

This prospective single arm study will test the null hypothesis of 18% survival against an alternative of increasing survival to 45% at 24 weeks from TMA diagnosis, resulting in a 2.5 times increase in survival rate in patients (18% vs. 45%) in this high risk population by using early therapy with eculizumab. The level of significance will be set to 0.05. The primary endpoint will be assessed using a 1 sided exact binomial distribution test. The sample size of 21 has a greater than 80% power of yielding a significant p-value. The primary analysis will be based upon intent-to-treat. A secondary "successfully treated patient analysis" (defined as > 4 doses of eculizumab administered) will also be performed using a non-relapse mortality endpoint, including all children receiving >4 doses of therapy. Children who receive 4 or fewer doses of therapy, or who relapse with malignancy prior to 24 weeks on the study will be replaced. This is based on preliminary data showing that it takes 11-13 days to control complement activation with therapeutic eculizumab trough level (≥100μg/mL) in patients with severe TMA before clinical response can be anticipated. (Jodele et al, BBMT 2016).

Statistical analysis for secondary endpoints will be descriptive. Eculizumab PK/PD analyses and modeling will be performed as previously published by our group (Jodele et al, BBMT 2015).[6] Interim analysis of eculizumab PK/PD study will be performed after the first 5 subjects complete the loading and induction

phases. Data will be reviewed to determine if target drug levels required for complement blockade are being achieved. Modifications to the dosing regimen may be implemented if interim analysis indicates inadequate target drug levels.

11. PATIENT ENROLLMENT

All patients must be enrolled at the site where they are being treated. Each participating institution will initiate the patient enrollment process and follow the procedures outlined below. Sites may proceed with study treatment following confirmation of eligibility by the site investigators. Sites must notify the Lead PI/Sponsor or designee within 24 hours of eligibility confirmation, and then provide the completed eligibility checklist and de-identified source documents (as listed below) as soon as possible.

Study staff at participating institutions will notify the study staff at Cincinnati Children's Hospital Medical Center (CCHMC) when there is a potential patient for study enrollment. See Section 12 for CRC contact information. All participants are to be registered with CCHMC as soon as possible after signing the study consent document. Study registration documents to be submitted to the CCHMC CRC via fax or email for review include:

1. **De-identified** signed consent (and/or assent documents, as applicable)
2. Completed registration case report form (CRF)
3. Completed eligibility checklist and relevant de-identified source documents

Eligibility documents will be reviewed by the Lead PI/Sponsor or designee. Once eligibility has been established, the patient will be assigned a unique study ID number. This number is unique to the patient and must be written on all data forms and correspondence for the patient. The study ID number will be relayed to the site via email and will serve as the enrollment confirmation.

12. COMMUNICATION BETWEEN PARTICIPATING CENTERS

Prior to opening the study at each center, the Lead PI and research coordinator will conduct a teleconference to orient and train local PIs and research coordinators on study procedures related to data collection and patient monitoring. When the study is open and enrolling at other centers, the Lead PI and/or Sponsor will conduct regularly scheduled calls with the PI/designee and/or study coordinator/nurse from each site. Patient eligibility, subject enrollment, study status and patient safety will be discussed during each call, as applicable.

13. DATA MANAGEMENT

The PI or designee at each site will be responsible for the conduct of the study, monitoring of study progress, and the review of all case report forms at their institution.

Responsibilities of designated site staff will include maintaining documentation of their site's participation in the study. Each site should maintain essential study documentation (including participant case records, IRB approval and annual review, IRB-approved study documents, delegation logs, etc.) according to applicable regulations and institutional practice. Submission of essential documents to CCHMC will be limited as specified in the Study Site Agreement. Supporting source documentation for site participants will be maintained at the originating center and will be submitted with specified CRFs (paper or electronic), or upon request to verify participant CRF data submitted to CCHMC. Note: Each participating site is responsible for upholding their institution's data management guidelines.

This protocol is being conducted as a single research study effort and data from all participating institutions will be included in the analysis of results. All participating sites will follow the same study protocol;

therefore, all protocol amendments will originate from Cincinnati Children's Hospital Medical Center and will be communicated to the participating sites.

CCHMC will be the data coordinating site for this trial. Data collection forms (CRFs) will be drafted in a standard format (paper or electronic) and will be provided to each participating institution by CCHMC. Data collected by sites and submitted to CCHMC will be managed according to CCHMC institutional data management standard practice. Sites will enter data in the study database on a weekly basis. Contact Information:

Stephanie Edwards
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue, ML 11027
Cincinnati, Ohio 45229-3039
Telephone: (513) 636-9292
Fax: (513) 636-6927
Email: StephanieL.Edwards@cchmc.org

Source documentation will be required for verification of study eligibility, for pivotal data points as indicated on the CRFs (including but not limited to, blood counts, complement studies, TMA assessments, organ function studies and results indicating disease recurrence or secondary malignancy or other transplant complications) and for reports of serious adverse events, upon request.

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Regulatory

The protocol and informed consent document for this study will be approved in writing by the Institutional Review Board (IRB) of record for participating institutions prior to any patient being registered on the study. All changes to the protocol as well as any change of study staff will also be approved by the IRB. Records of the Institutional Review Board review and approval of all documents pertaining to this study will be kept on file by the site investigator and/or designee. Informed consent will be obtained prior to the treatment of participants.

Investigational New Drug Application (IND): Cincinnati Children's will file an IND to cover the use of eculizumab for the proposed clinical trial at participating institutions.

14.2 Confidentiality

Any copies of research records will be kept in locked files and any information stored on computer will be password protected. The information from the study may be published; however, subjects will not be identified in such publications. The publication will not contain information about the subjects that would enable someone to determine their identity as a participant without the subject's authorization. The Institutional Review Board may have access to these records. The use of clinical information for research purposes and the protection of privacy will be done in compliance with HIPAA requirements at participating institutions.

To further protect the privacy of study participants, a Certificate of Confidentiality is issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help

achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14.3 Future use

Leftover clinical/tissue samples, tissue repository samples and medical information will be stored indefinitely, after subject study participation is complete. Samples and medical information will be retained unless the participant chooses to withdraw. If the participant withdraws from further study participation, previously collected and stored samples and medical information will be either destroyed or stored for future use based on participants request and or permission. Samples and medical information that have already been distributed to researchers prior to withdrawal of consent as well as any data obtained from the distributed sample will not be destroyed.

The samples may be used by researchers at Cincinnati Children's Hospital Medical Center or at other institutions if some are left over after the tests for this study after IRB approval. Only de-identified data and/or samples will be provided to researchers at other institutions.

15. STUDY FUNDING AND COMPENSATION

Alexion Pharmaceutical will provide study drug at no cost to study participants. Additionally, Alexion will receive and analyze the Research Biomarker Panel samples at no cost to study participants. Any other research laboratory tests will be covered by the study. Clinically indicated laboratory studies and supportive care are part of HCT care for patients receiving eculizumab and payment will be requested from the patient's insurance. There will be no compensation to patients.

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17. APPENDIX I: TMA DIAGNOSIS IN HSCT RECIPIENTS

TMA diagnosis in HCT recipients is confirmed by A or B

A. Histologic TMA diagnosis in tissue biopsy
OR
B. Laboratory and clinical markers
- For diagnosis of high risk TMA, patient must have proteinuria (#6) and terminal complement activation as measured by elevated plasma sC5b-9 level (#7).
- Total of 4 out of 7 markers are required to meet TMA diagnosis.

No	TMA marker	Date
1	[ ] LDH above normal value for age	_ _/_ _/_ _ _ _
2	[ ] Schistocytes on peripheral blood smear	_ _/_ _/_ _ _ _
3	[ ] De novo thrombocytopenia or require platelet transfusions	_ _/_ _/_ _ _ _
4	[ ] De novo anemia or require RBC transfusions	_ _/_ _/_ _ _ _
5	[ ] Hypertension >99% for age (<18y of age) or 140/90 (≥18y of age) or receiving antihypertensive therapy[19]	_ _/_ _/_ _ _ _
6	[ ] Proteinuria ≥30mg/dL on random urinalysis x2 or random urine protein creatinine ratio >1mg/mg or patient receiving renal replacement therapy.	_ _/_ _/_ _ _ _
7	[ ] Terminal complement activation: elevated plasma sC5b-9 above normal limit of (≥244ng/ml)	_ _/_ _/_ _ _ _

18. APPENDIX II: DEFINITIONS OF TMA-ASSOCIATED MODS IN HSCT RECIPIENTS

MODS will be defined as: evidence of TMA as listed in APPENDIX 1 and at least one of below listed organ impairment.

Organ Dysfunction	Definition	Start date	Resolution date
Renal	≥50% reduction of Cystatin C GFR from pre-HSCT value or the lowest value during or prior to diagnosis of TMA, whichever is lower	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
Pulmonary	Any need for positive pressure ventilation (non-invasive or invasive) for ≥24 hours	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
Cardiovascular	Pulmonary hypertension (PH) diagnosed by cardiologist using cardiac catheterization or PH criteria on echo (RV pressure ≥50% of systemic pressure, ventricular septal flattening, or right ventricular dysfunction)	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
Pericardial Effusion	Clinically significant pericardial effusion requiring medical therapy (like diuretics) or drainage (like pericardiocentesis)	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
Severe hypertension	Hypertension requiring ≥ 2 antihypertensive medications for > 24 hours or continuous antihypertensive infusion for ≥12 hours or resulting in complications like CNS bleeding or posterior reversible encephalopathy syndrome (PRES).	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
CNS	CNS bleeding or seizures clinically attributable to posterior reversible encephalopathy syndrome (PRES)	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _
Gastro-intestinal	GI Bleeding and/or intestinal strictures attributable to TMA requiring medical or surgical interventions	_ _/_ _/_ _ _ _	_ _/_ _/_ _ _ _

19. APPENDIX III: TMA TEST REQUISITION SHEET

TMA laboratory test requisition for can be downloaded from Cincinnati Children's website by searching "TMA" or using this link:

https://www.cincinnatichildrens.org/service/t/thrombotic-microangiopathy/requisitions

20. APPENDIX IV: STUDY SAMPLE COLLECTION FOR REPOSITORY

Research samples as described below will be collected from study participants who are not currently enrolled onto the Hematopoietic Stem Cell Transplant (HSCT) Tissue Repository (CCHMC Study #: 2012-1156) or a similar repository study at participating sites. Blood samples collected from the tissue repository will include testing to assess the immunogenicity of eculizumab.

Peripheral blood [including plasma, serum and nucleated cells (MNCs or PBMC)], and urine samples will be collected weekly through 100 days post-transplant (blood twice weekly), approximately monthly for the first year or at follow-up visits, or during scheduled clinical visits, if less often, till the first year after transplant, and at the time of any transplant complications. Stool samples will be collected weekly as feasible at the site PI's discretion. Other tissue samples (like tissues from clinically obtained biopsies or bone marrow aspiration samples) will be collected as available. If a pre-transplant DNA sample is available, it may be requested for a TMA-associated genetic panel.

These samples will be processed, frozen, and stored at the site where they were collected. Then samples will be shipped in batches to CCHMC. These samples may be used in current and future research studies.

Procedures

1) Peripheral Blood Draw*

Patients undergoing a peripheral blood draw will undergo venipuncture after an appropriate site has been identified and cleaned per standard procedure. Topical lidocaine or similar topical anesthetic may be applied prior to the venipuncture. A central line would be used in patients who have this access.

*Blood volumes will be based on patient weight and estimated blood volume. No more than 5 ml/kg of the patient's body weight will be taken for both clinically indicated and research purposes (per standard transplant practice). The first priority will be clinically indicated volumes.

2) Urine

Urine is to be collected, stored and shipped in batches to CCHMC.

3) Stool

Stool will be collected as per institutional standard of practice.

4) Leftover Clinical Samples

Blood, tissue and body fluids that are leftover after clinical testing (peripheral blood, urine, stool, bone marrow, tumor specimens, immortalized B-cell lines, oral specimens, DNA), that would otherwise be discarded, may be stored.

5) Database

Access to study database will be limited to the Principal Investigator and her designees via password protected security measures. Each specimen collected will be entered into the specimen database, along with all information collected.

6) Release of Specimens to Investigators

Release of any specimen to investigators must be approved by the Principal Investigator or designee. The procedures for release of specimens are described below.

a. CCHMC investigators
In order for a CCHMC investigator to receive specimens and/or data, the following must be submitted to CCHMC study staff:

1) Completed application

2) Documentation of CCHMC IRB approval (IRB #) for the proposed research project if the use of the samples and/or data released from the repository constitutes human subjects research per 45 CFR 46 or genetic research.

Upon receipt of the above application and approval by the principal investigator or designee, the specimen(s) will be released to the CCHMC investigator.

b. External investigators
As this will become a valuable resource for potential research, it is anticipated that it will serve as a tissue repository for investigators at other centers. The external investigators can apply for de-identified specimens alone, and/or de- identified patient clinical information. No identifying information will be released to external investigators at any point.

In order for an investigator at another center to receive any data or specimens, the following must be submitted to CCHMC study staff:

1) Completed application
2) Local IRB or Ethics Committee (country specific IRB equivalent) approval document if the use of the samples and/or data released from the repository constitutes human subjects research per 45 CFR 46 or genetic research. (If this document is in a language other than English, it must be translated and verified by a CCHMC employee fluent in that language. This can be a member of the CCHMC interpreter team).

Upon receipt of the above documents and approval by the principal investigator or designee, the specimen(s) will be shipped to the external investigator.

Special considerations:

Genetic studies: There is potential for genetic studies involving these specimens, including, but not limited to complement genes and other genetic pathways possibly implicated in TMA or organ dysfunction after HCT.

Other Repositories: Additional samples may be collected from other repositories, if available, in special circumstances (i.e. in the event pre-transplant samples are needed for genetic testing).

21. APPENDIX V: ADVERSE EVENT REPORTING OF TMA ASSOCIATED CONDITIONS

TMA associated conditions that will <u>not</u> be reportable as AEs

Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. **This excludes TMA since disease progression is anticipated in some patients after initiation of complement blocking therapy and TMA is the subject of this study.**

Below is a list of conditions/symptoms that are considered part of TMA for which sites would not have to report as AEs if they worsen after start of eculizumab (i.e., are considered worsening or progressive TMA). Conditions/symptoms, that in and of themselves would be <u>considered serious, as per the criteria in section 8.3.1,</u> will need to be reported immediately as SAEs.

Please contact Lead Site study staff with any questions regarding these requirements.

	Will **not** be reportable as AE*	Requires to be reported as SAE
Renal	Proteinuria Drop in GFR Increase in creatinine Electrolyte imbalance not requiring renal replacement tx Weight gain Tissue edema **not resulting in renal replacement support needs listed under SAE*	CRRT, HD, aqua filtration
Pulmonary	O2 requirement Abnormal radiologic studies Abnormal blood gases and pulmonary function tests **not resulting in ventilatory support needs listed under SAE*	Pulmonary dysfunction and or bleeding requiring positive pressure ventilation (invasive or non-invasive)
Cardiac	Abnormal cardiac echo or cardiac catheterization tests, including elevated RV pressure, RV dysfunction, pulmonary hypertension, effusions requiring monitoring or support and/or medical therapy.	Cardiac function abnormalities resulting in hemodynamic instability requiring inotropic support, invasive or non-invasive ventilatory support or surgical procedures
Serositis	Pleural, pericardiac effusions, ascites requiring support care, monitoring and/or medical therapy	Effusions resulting in impending tamponade and/or hemodynamic, pulmonary instability, or compartment syndrome requiring surgical interventions

Severe hypertension	Hypertension requiring medical management on any number of medications or continues infusions	Hypertension resulting in acute clinically significant complications like PRES, seizures, CNS bleeding
CNS dysfunction	Altered mental status requiring clinical monitoring or medical interventions	Seizures, PRES, CNS bleed
GI dysfunction	Upper and/or lower GI bleed requiring transfusion support and/or medical interventions	GI dysfunction requiring surgical interventions or resulting in hemodynamic/respiratory instability requiring inotropic support and/or invasive or non-invasive respiratory support
Hematologic	Laboratory evidence of hemolysis, anemia, thrombocytopenia requiring transfusion support	Hemolysis resulting in hemodynamic instability or thrombocytopenia resulting in life threatening bleeding requiring inotropic support and/or invasive or non-invasive respiratory support
ID	Documented bacterial infections requiring monitoring and medical treatment	Infections resulting in septic shock, meningitis, and/or requiring surgical procedures
Ophthalmologic	Visual acuity changes attributable to hypertension or TMA-associated vascular injury requiring monitoring and medical interventions	Acute vision loss (blindness)
GU	Hematuria requiring monitoring, medical therapy or bladder irrigation	Hematuria requiring surgical interventions or resulting in hemodynamic instability