CLINICAL RESEARCH PROJECT

Protocol #15-H-0015
IND # Exempt
Product: Eculizumab

Date: January 5, 2018

To: Richard Cannon, MD, Chair, NHLBI, IRB

Title: Complement Inhibition Using Eculizumab to Overcome Platelet Transfusion Refractoriness in Patients with Severe Thrombocytopenia

Other identifying words: platelet refractoriness, complement inhibition, eculizumab

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Note: **Investigators designated to obtain informed consent from research subjects.*

Subjects in Study:	Number	Sex	Age range
Subjects:	up to 15	either	Recipients: 18-75 inclusive

Number of enrollments for each subject: up to 3

Project involves ionizing radiation? No
Off site project? No

Multi-institutional project?	No
DSMB Involved?	Yes
Tech Transfer?	Yes (CRADA)

PRECIS:

Platelet transfusion can be a life-saving procedure in preventing or treating serious bleeding in patients who have low and/or dysfunctional platelets. Treatment of blood cancer and other blood diseases as well as bone marrow transplantation is not possible without platelet transfusion support. Unfortunately, 20-60% of chronically transfused patients will stop responding to these transfusions putting them at risk for serious bleeding complications. Data support the concept that in many patients, platelet counts fail to increase after a platelet transfusion because the transfused platelets are destroyed by the body's complement. In order to overcome this problem, we will inhibit complement activity with the medication eculizumab that specifically binds and suppresses complement. We hypothesize that when we treat patients who have platelet refractoriness with eculizumab, the platelet counts will increase to higher numbers after platelet transfusions, decreasing the risk of bleeding complications associated with having a low platelet count.

Contents

1.0 OBJECTIVES

1.1. PRIMARY OBJECTIVE:

To evaluate safety and efficacy of eculizumab to overcome platelet transfusion refractoriness in patients with severe thrombocytopenia.

1.2 SECONDARY OBJECTIVE:

1.2.1 To evaluate safety and efficacy of second and third time treatment using eculizumab to overcome platelet transfusion refractoriness in patients with severe thrombocytopenia who responded to eculizumab treatment previously.

1.2.2 To evaluate activation of the classical and alternative complement pathways in plasma, including but not limited to plasma total complement CH50, C3, C4, C5, C5a (PD), Ba and sC5b-9.

2.0 BACKGROUND AND SCIENTIFIC JUSTIFICATION

RATIONALE:

Platelet transfusion can be a life-saving procedure in preventing or treating serious complications from hemorrhage in patients who have disorders manifesting as thrombocytopenia and/or platelet dysfunction. Treatment of hematological and non-hematological diseases as well as bone marrow transplantation is not possible without allogeneic platelet support. Transfusing platelets prophylactically to prevent bleeding rather than waiting to treat active bleeding is now a common practice (Blajchman *et al.*, 2008; Meyer *et al.*, 2013; Slichter, 2007a; Slichter, 2007b). In 2007, 1.5 million platelet products were transfused in the USA and 2.9 million in Europe. Depending on the patient, and disease characteristics, 20-60% of chronically transfused patients will ultimately develop platelet refractoriness, making this a major world-wide health problem (Stroncek *et al.*, 2007).

Platelet refractoriness is defined by a lack of an adequate post transfusion platelet count increment. Platelet refractoriness is associated with bleeding complications, reduced overall survival and longer hospital stays and higher hospital costs(Rebulla, 2005; Slichter, 1998). The National Institutes of Health is a major referral center for hematologic disorders, including severe aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), congenital thrombocytopenia, and a long list of premalignant and malignant hematological disorders. These patients are characterized by pancytopenia, a hypocellular bone marrow, and suppression of hematopoiesis. One treatment modality that we utilize is bone marrow stem cell transplantation that results in recovery of the hematopoesis from engrafting donor stem cells. However, until patients recover hematopoesis they remain pancytopenic. Thromobocytopenia leads to petechiae of the skin and mucous membranes, epistaxis, menses that last more than a month, and gum bleeding. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis

and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage which has a high degree of mortality. The only modality to prevent life threatening hemorrhage is platelet transfusion. However, patients who develop platelet refractoriness do not respond to platelet transfusions. Any treatment that prevents platelet destruction in these patients until recovery of hematopoiesis and endogenous platelet recovery occurs would potentially be lifesaving.

As with other centers, our institute performs bone marrow transplantation which is not possible without adequate platelet support. Management of platelet refractoriness in our center focuses on using HLA-matched platelets (Banaji *et al.*, 1986; Nambiar *et al.*, 2006; Pavenski *et al.*, 2013; Schiffer *et al.*, 1983). However, HLA-matched platelets are not always available, and incur significant expense to support an infrastructure for the accrual of HLA matched donors, limiting their collection to tertiary medical centers. Attempts to reduce the production of HLA antibodies using immunosuppressive agents (e. g. IVIG, cyclosporine, steroid) typically do not achieve a sustained resolution of platelet refractoriness(Blanchette, 1985; Christie *et al.*, 1993; Reiner *et al.*, 1995; Schiffer *et al.*, 1984; Slichter *et al.*, 1987; Tilly *et al.*, 1990; Zeigler *et al.*, 1991).

Platelet refractoriness is calculated based on pre- and post-transfusion platelet counts, the number of transfused platelets, and the patient's weight. The most commonly used definition to establish platelet refractoriness is a post-transfusion corrected count increment (CCI) less than 7500/uL 10-60 min following the transfusion, and less than 5000/uL at the 18-24 hrs time points(Novotny, 1999). CCI = (absolute count increment x body surface area)/number of platelet transfused. Immune and non-immune causes have been implicated in the mechanism of platelet refractoriness. Immune causes involve alloimmunization against human leukocyte antigens (HLA), and human platelet antigens (HPA) following patient exposure to lymphocytes through transfusion, and pregnancy (Rebulla, 2005; Slichter, 1998).

While clinical studies have described the phenomenon of platelet alloimmunization in detail, a full elucidation of its pathophysiology remains elusive. The majority of clinically relevant HLA antibodies have been shown to be immunoglobulins G and M (IgG and IgM) which are directed against HLA class I epitopes, namely, HLA-A and B antigens. When patients generate IgG and IgM to allogeneic platelets, it is assumed the destruction of platelets occurs by one of the known antibody dependent mechanisms, namely eradication via the reticuloendothelial system (RES), ADCC, and complement activation. However, the inability to overcome platelet refractoriness by multiple sequential infusions of platelets, high dose infusions of IVIG, and splenectomy have established that full saturation of the RES and FcRIIIA (resulting in full inhibition of NK-cell mediated ADCC) alone is insufficient to prevent platelet destruction, highlighting the important role complement plays in the pathophysiology of this process (Kickler *et al.*, 1990; Pavenski *et al.*, 2012).

Several assays are available to detect the presence of anti-HLA class I antibodies in the serum of alloimmunized patients, including solid-phase enzyme-linked immunosorbent assays and flow

cytometric detection of antibody binding to beads coated with purified HLA antigens. Notwithstanding the convenience of these assays, a complement fixation technique remains more sensitive and reliable for measuring anti-platelet antibodies. Earlier work at NIH and other centers have been shown that sera from platelet alloimmunized patients were able to fix complement on reactive cells leading to their destruction. Heat inactivation of the serum, which results in complement inactivation, completely overcomes this property (Cines *et al.*, 1979; Heal *et al.*, 1996; Heinrich *et al.*, 1971; Lehman *et al.*, 1987; Shulman *et al.*, 1962; Shulman *et al.*, 1964).Similarly, allogeneic kidney graft rejection/destruction occurs due to the development of allogeneicanti-HLA antibodies. Recently, Loupy et.al demonstrated that anti-HLA antibodies activate complement pathways leading to kidney graft destruction(Loupy *et al.*, 2013). Measuring complement levels C3, C4 and CH50 are widely available. These assays have low sensitivity, as complement system is very dynamic and replenishes itself quickly. However, in our preliminary investigation we observed decrease in all three components of complement, C3, C4 and CH50, after platelet transfusion in two heavily HLA-alloimmunized patients (Table 1, Figure 1). This difference was not detected in a non HLA-alloimmunized patient, who received platelet transfusion (Table 1). The difference in complement levels before and after platelet transfusion was detected when measured within 10 to 60 min after transfusion ended, however these differences were undetectable after 1 hour.

Taken altogether, these data overwhelmingly support the concept that platelet destruction in alloimmunized platelet transfusion recipients is dependent on complement pathways. Although the destruction of platelets by complement leading to platelet refractoriness is known to occur, inhibiting complement activity to overcome platelet destruction and platelet transfusion refractoriness has not yet been studied. Eculizumab,is a monoclonal antibody that binds and inhibits C5 complement, therefore blocking both classic and alternative pathways of complement. Eculizumab is effective in blocking complement-mediated RBC destruction associated with paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS), and has been approved by the FDA for this use. Pharmacodynamic assays demonstrate that inhibition of C5 sufficient to prevent hemolysis occurs at eculizumab plasma concentrations in excess of 35 ug/ml. The maximum plasma concentrations of eculizumab with therapeutic doses of the drug are achieved within 1 hourof infusion with the half-life of this agent being 272 ± 82 hrs (mean ± SD)(Dmytrijuk *et al.*, 2008).

This research protocol is designed to evaluate the safety and effectiveness of eculizumab to overcome platelet refractoriness which occurs as a consequence of complement activation. To avoid the risk of bacteremia with encapsulated organisms associated with complement blockade, we will vaccinate all patients with meningococcal vaccine and monitor them closely. Subjects in this study are unable to wait 2 weeks to develop immunity, therefore they will also take antibiotics to prevent meningococcal infection for 14 days. Should eculizumab be effective in overcoming platelet refractoriness in these patients then we will have established that the benefits of this agent surpass the risks associated with complement blockade.

Subject	HLA antibody	Transfusion frequency	Platelet transfusion							
			pre transfison				post transfusion (10-60 min)			
			PLT count	C3	C4	CH50	PLT count	C3	C4	CH50
1	A31,30,66,25,1,26,32	Daily	9,000/uL	96.6	24.8	256	27,000/uL	88.4	22.3	222
2	A 31,30,68,25,29,80 B82,45,44 weak57,58	Weekly	9,000/uL	124.4	26.4	208	45,000/uL	120.2	25.2	180
4	none	Monthly	19,000/uL	107.6	17.5	251	90,000/uL	107.8	16.8	249

Table 1: Patient characteristics and complements level before and after platelet transfusion. Normal values: C3 (90-180 mg/dL), C4 (10-40 mg/dL), CH50 (55-45 CAE U)

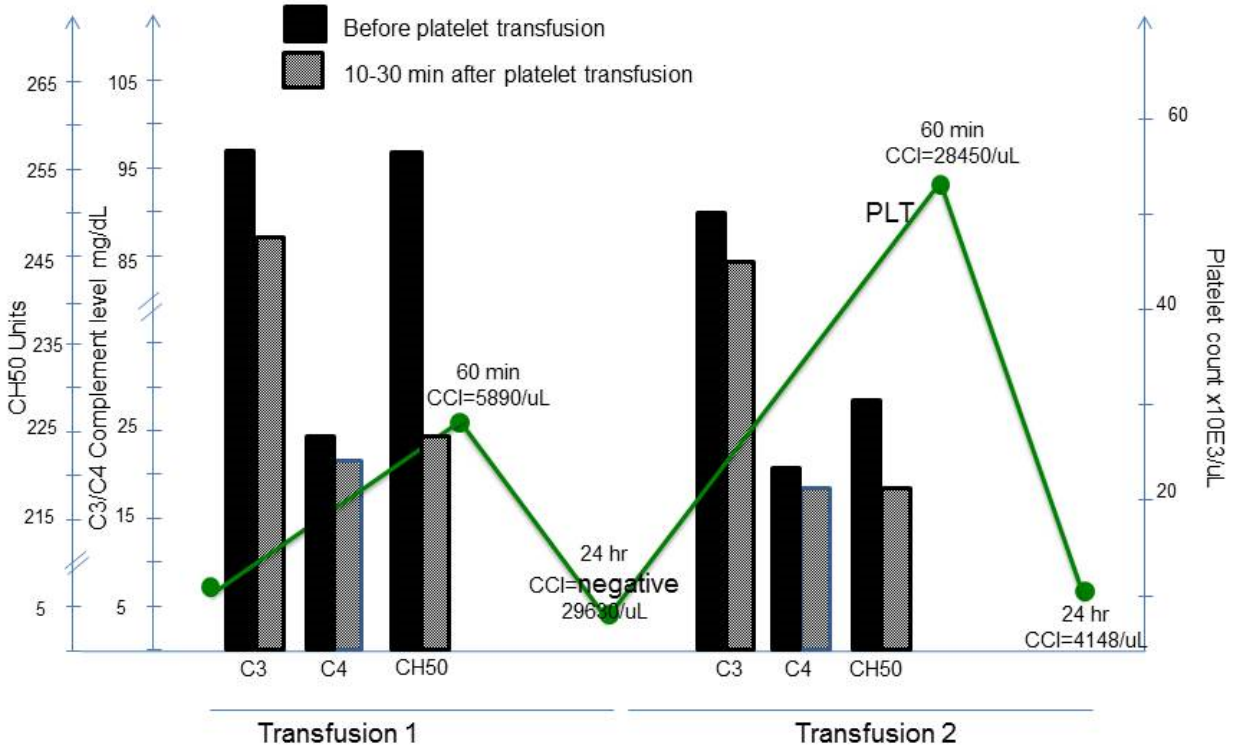


Figure 1: Graphical representation of complement difference in one subject before and after two independent platelet transfusions. C3, C4, CH50 level was measured in the plasma prior and 10-60 mins after the platelet transfusion in HLA alloimmunized subjects 1. (•) indicates platelet counts before, 10-60 mins, and 24hr after the platelet transfusion. Corrected count increments (CCI), are indicated at 60 mins, and 24hr time points after each transfusion. This patient lacked

an adequate post-transfusion platelet count increment at 60 mins and 24hrs after transfusion 1, and at 24hrs after transfusion 2.
CCI = (absolute count increment per ul) x (body surface area in m^2)/number of platelet transfused ($x10^{11}$). For examples, assuming each unit of random donor platelets contains 5.5 x 10^{10} platelets, a 5 unit-pack contains 2.75 x 10^{11}, a platelet increment of 20,000/ul in a person of 1.8 m^2 after 5 unit-pack would represent a CCI of (20,000 x 1.8/2.75) = 13,090/ul.

3.0 STUDY DESIGN

INDICATION:
Immune mediated platelet refractoriness.

SUBJECT POPULATION:
Subjects with thrombocytopenia (due to congenital causes, bone marrow failure, hematologic malignancies, and treatment related) requiring platelet transfusion in patients who demonstrate immune platelet refractoriness diagnosed by the following:

a) Lack of an adequate post-transfusion platelet count increment, defined by, CCI <7500/ul at 10-60 min, together with CCI <5000/ul at 18-24 hrs (in those who had a CCI at 10-60 min ≥ 5000/ul) after at least 2 consecutive platelet transfusions.
b) Presence of anti-HLA class A and/or B antibodies.

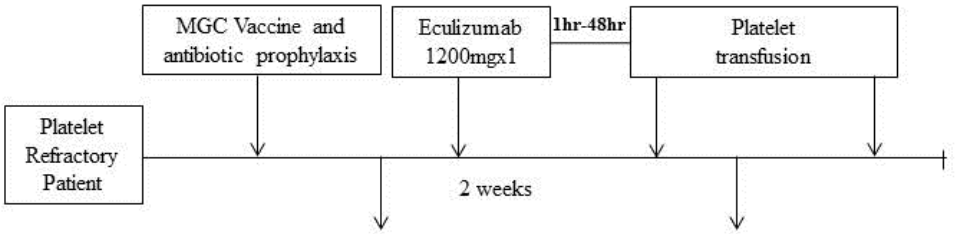
Subjects who meet criteria for platelet refractoriness will be enrolled on study and will receive an infusion of eculizumab once (1200 mg IV infusion over 30-40 min) followed by the first platelet transfusion administered immediately following and up to 48 hours after eculizumab is given .

To monitor response, platelet count immediately before the transfusion as well as serial platelet count 10-60 min, and 18-24 hr after transfusion will be obtained for the first 2 platelet transfusions administered following the eculizumab infusion.

Subjects who met response criteria to initial treatment, and who meet re-enrollment criteria outlined in 4.3 will have an option to re-enroll a second time on study. Subjects who were re-enrolled on study a second time and who met response criteria to this second treatment and meet re-enrollment criteria outlined in 4.3 will have an option for re-enrollment a third and final time on study. All subjects re-enrolled on study (for either a 2^{nd} treatment or a 3^{rd} treatment) will not count as new study subjects. Also, subjects who are re-enrolled a 2^{nd} or 3^{rd} time on study will only be formally evaluated for the defined secondary study outcomes.

Shall this modality (eculizumab) be efficient for treating platelet refractoriness; it will be the first effective treatment for a world-wide growing population of immune mediated platelet refractory patients.

SCHEMA:



Serial platelet count before as well as 10-60 min, and 18-24hr after transfusion. Each blood draw will include total complement, C3, C4, CH50, C5, C5a, Ba, sC5b-9

4.0 ELIGIBILITY ASSESSMENT

4.1 Inclusion Criteria

4.1.1 Ages 18-75 years inclusive.

4.1.2 Ability to comprehend the investigational nature of the study and provide informed consent.

4.1.3 Thrombocytopenia (due to congenital causes, bone marrow failure, hematologic malignancies, and treatment related), defined as <10k/uL without bleeding or <30K/uL with evidence of life threatening bleeding (intracranial hemorrhage, GI bleeding, pulmonary hemorrhage, uncontrolled epistaxis, hematuria).

4.1.4 Diagnosed with immune platelet refractoriness, characterized by all of the following:

a) Lack of adequate post-transfusion platelet count increment, defined by, CCI <7500/ul at 10-60 min, and CCI <5000/ul at 18-24 hrs (in those who had a CCI at 10-60 min ≥ 5000/ul) after at least 2 consecutive transfusions.

b) Presence of anti-HLA class A and/or B antibody.

4.2 Exclusion Criteria

4.2.1 Active meningococcal infection.

4.2.2 Severe psychiatric illness. Mental deficiency sufficiently severe as to make making informed consent impossible.

4.2.3 Positive pregnancy test for women of childbearing age within 1 week.

4.2.4 HIV positive test within 3 months.

4.2.5 Paroxysmal Nocturnal Hemoglobinuria (PNH) disease with evidence of intravascular hemolysis.

4.2.6 Presence ITP/autoimmune thrombocytopenia

4.2.7 Immune platelet refractoriness responsive to treatment with IVIG

4.3 **Re-enrollment Criteria**

4.3.1 Meet response criteria to the initial treatment or second treatment
4.3.2 Meets all initial inclusion/exclusion criteria outlined in 4.1; 4.2

5.0 RESPONSE CRITERIA

An adequate post-transfusion platelet count increment defined by CCI ≥7500/ul at 10-60 min, together with CCI ≥5000/ul at 18-24 hrs after the platelet transfusion. Patients will be deemed to meet criteria for an adequate post transfusion platelet count if they meet the above criteria for one of the first 2 platelet transfusions given following the eculizumab infusion.

6.0 TREATMENT PLAN

6.1 Pre-Study Evaluation (screening)

Subjects will be screened on the 97-H-0041 screening protocol.

- History and physical exam including
- Temperature, pulse, blood pressure, respiratory rate, and weight
- CBC or CBC with differential
- Antibody screen for HLA alloantibodies
- Red cell ABO, Rh antibody screen, DAT (direct antiglobulin test)
- Pregnancy test (serum or urine)

6.2 Treatment with Eculizumab

Eligible patients may receive polyvalent conjugated meningococcal vaccination (Menactra) once before receiving eculizumab. Participants that have previously received a meningococcal vaccination will not be re-vaccinated. Patients in this study are unable to wait 2 weeks to develop immunity, therefore will take antibiotics to prevent meningococcal infection for 14 days.

Subjects who meet re-enrollment criteria outlined in 4.3 will have an option to re-enroll two additional times up to a maximum of 3 times.

Antibiotic Selection

First choice	Ciprofloxacin 500 mg PO every 12 hours (400 mg IV every 12 hours if unable to take oral medication)
Second choice	Ceftriaxone 1 gram IV every 24 hours
Allergy to cephalosporin and quinolones	Consult Infectious disease consult service
Subject is on antibiotics	If the antibiotic regimen in place covers Neisseria Meningitidis (e.g ceftazidime, meropenem) no additional antimicrobial agent needs to be added. Ciprofloxacin or ceftriaxone will only be added if current antibiotics are discontinued before the 2 week prophylaxis period is completed. If the current antibiotic regimen does not cover Neisseria Meningitidis, the

	regimen will require adjustment to include this coverage. Consult with Infectious Disease consult service for guidance if unsure about appropriate Neisseria meningitidis coverage

DOSING REGIMEN:
Eculizumab 1200 mg IV x 1 based on a dose adopted from study "terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipient" (Stegall *et al.*, 2011).

6.3 Treatment monitoring (day 0 to day +14)

- Weekly clinical assessment: temperature, pulse, blood pressure, respiratory rate
- All patients will have:
    - Serial platelet count before transfusion as well as after 10-60 min, and 18-24 hr after the first 2 platelet transfusions that are given after the eculizumab infusion. The 18-24 platelet count post transfusion will only need to be obtained if the CCI at the 10-60 min blood draw was ≥7500/ul.
    - At each blood draw assays to evaluate activation of the classical and alternative complement pathways in plasma
        - Plasma total complement CH50, C3, C4 will be analyzed at NIH clinical center
        - C5, C5a (PD), Ba and sC5b-9 will be collected as research samples and will be shipped to Alexion for analysis.
- Bi-weekly CBC or CBC with differential
- Admission on discretion of PI

Orders and results will be tracked through the electronic medical record (CRIS) screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

7.0 BIOSTATISTICAL CONSIDERATIONS

This is a single arm, non-randomized, pilot study.

7.1 Sample Size and Study Design

A response probability (platelet increase, defined as CCI>7500/uL at 10-60 min together with CCI>5000/uL at 18-24 hrs post transfusion) of 20% or less during the first enrollment would warrant terminating the treatment on this subject population, and we hypothesize that the actual response probability using this treatment would reach 60% or more. We will determine the sample size using the Two-Stage design. At the first stage, 6 subjects will be accrued, and we will stop the study if there is no response in the first 6 subjects. If one or more subjects respond to the treatment at the first stage, then an additional 4 subjects will be accrued, bringing the total number of subjects to n=10. To account for dropouts, up to 15 subjects will be enrolled.

Anticipated drop outs are due to inability to tolerate blood draws. We will reject the null hypothesis and find the treatment successful if there are at least 5 subjects with platelet increment as defined. The type I error is 0.033 and the power is 83% based on this two-staged design.

7.2 Endpoints

7.2.1 Primary endpoint

To evaluate the safety and efficacy of eculizumab to increase the platelet increment, defined as CCI>7500/uL at 10-60 min together with CCI>5000/uL at 18-24 hrs post transfusion in patients with platelet refractoriness following treatment with eculizumab and platelet transfusion.

7.2.2 Secondary end points

- To evaluate safety and efficacy of second and third eculizumab infusion to overcome platelet transfusion refractoriness in patients with severe thrombocytopenia who responded eculizumab treatment previously.
- To compare the platelet increment and complement activation (between post-transfusion and pre-transfusion), after treatment with eculizumab with the platelet increment and complement activation at the study enrollment when the subjects are diagnosed with platelet refractoriness.
- To evaluate hemorrhagic complications.

7.3 Study Analysis

All subjects who receive initial treatment will be included in the primary analyses. Every effort will be made to reach and evaluate the subjects before transfusion as well as at 10-60 min, and 18-24 hr after transfusion of the first 2 platelet transfusions given after the eculizumab infusion. Adverse events will be tabulated by severity and attribution to treatment and disease.

Final statistical analysis will be performed once all subjects have completed the end of study assessments and all data have been archived for analysis. The planned analyses will include descriptive statistics on the proportions of platelet response and time to response. The response probabilities will be estimated using the sample proportions and their inferences including confidence intervals and hypotheses testing will be evaluated using Binomial distributions. Secondary analysis based on mixed effect regression, logistic regression and survival analysis will also be employed if deemed appropriate.

Subjects who met response criteria to initial treatment, and who meet re-enrollment criteria outlined in 4.3 will have an option to re-enroll a second time on study. Subjects who were re-enrolled on study a second time and who met response criteria to this second treatment and meet re-enrollment criteria outlined in 4.3 will have an option for re-enrollment a third and final time on study. All subjects re-enrolled on study (for either a 2nd treatment or a 3rd treatment) will not count as new study subjects. Also, subjects who are re-enrolled a 2nd or 3rd time on study will only be formally evaluated for the defined secondary study outcomes.

7.4 Stopping rules
Should initial 6/6 subjects have NO increased platelet increment following treatment with eculizumab and platelet transfusion, the study will stop.

7.5 Off treatment criteria
Patients will receive one dose of eculizumab, no more doses are scheduled for each enrollment.
7.6 Off study criteria (and subject replacement)
Patients will be taken off study for the following:

7.6.1 Death
Patients dying of unrelated causes before the study period is over may be replaced.

7.6.2 Withdrawal per subject choice
Subjects will be given ample opportunity to withdraw from the study. If subjects withdraw we will replace to reach total of 10 patients.

7.6.3 Withdrawal by physician decision
Subject will be withdrawn from the study if subject cannot tolerate blood draws, or if subject no longer will require platelet transfusions.

7.6.4 Completion of the study.

8.0 DATA AND SAFETY MONITORING

8.1 Safety Monitoring

Principal Investigator (PI): Accrual and safety data will be monitored by the PI.

NHLBI IRB**:** Accrual and safety data will be monitored reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed subject consent and assent forms will be reviewed and approved by the properly constituted IRB operating according to Protection of Human Subjects Research Title 45 CFR Part 46 of the Code of Federal Regulations (45 CFR 46). This committee must approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

DSMB**:** The NHLBI Data Safety and Monitoring Board (DSMB) will review the protocol at the regularly scheduled six or twelve month intervals as applicable. A progress report will be forwarded to the DSMB at these times. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

8.2 Definitions

Adverse Event (AE): Established adverse events from administration of eculizumab, including, headache, nasopharyngitis, back pain, nausea, vomiting, hypertension, diarrhea, upper respiratory tract infection, myalgia, anemia, leukopenia will be monitored.
Any untoward medical occurrence in a human subject, including grade 3 or 4 (e.g., abnormal physical exam or below mentioned laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.
An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious Adverse Event (SAE): A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Suspected adverse reaction: Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in

the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets all of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. **related or possibly related** to participation in the research; and
3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.
Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:
   a. Increases risks, or causes harm, to participants.
   b. Decreases potential benefits to participants.
   c. Compromises the integrity of the NIH HRPP.
   d. Invalidates the study data.

2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. Minor (non-serious) non-compliance: Non-compliance that, is neither serious nor continuing.

8.3 NHLBI-IRB and (Clinical Director) (CD) reporting

8.3.1 Serious Events

Reports to the IRB and CD:

The PI must report Serious UPs, Serious PDs, and UADEs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event using the NIH Problem Form.

For device research, the PI must report to the IRB any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency as soon as possible, but no later than 5 working days.

Reports to the IRB Chair and CD

The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event, using the NIH Problem Form.

8.3.2 Non-serious Events

Reports to the IRB and CD:

The PI must report all UPs that are not Serious to the IRB and CD, and PDs that are not Serious to the IRB, not more than 14 days after the PI first learns of the event using the NIH Problem Form.

8.3.3 Deaths

The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event.

8.3.4 Reports at the time of continuing IRB review

At continuing review, the PI will provide to the IRB a summary of:

- All UPs
- All PDs
- All AEs
- If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

8.4 Adverse Events Management

The AEs will be graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html. The category that overall best "fits" the relationship between the adverse event and the study drug should be chosen and recorded on the CRF and NIH Problem Form, if appropriate.

The investigator is responsible for assessing the causal relationship between any events and the study treatment. AEs will be attributed as unrelated, unlikely, possibly, probably, or definitely related to study medication or procedures. Additionally, the investigator is responsible for providing appropriate treatment for the event and for adequately following the event until resolution.

8.5 Reporting of Serious Adverse Events

SAE reporting to Alexion Pharmaceuticals
All unexpected and possibly, probably or definitely related SAEs occurring during the study will be reported to Alexion Pharmaceuticals within 7 days of investigator awareness if serious and within 14 days of investigator awareness in not serious.

SAEs will be sent to:
ClinicalSAE@alxn.com
Fax: + 1-203-439-9347

DSMB
NHLBI Data Safety Monitoring Board (DSMB): Reports of serious AEs that are unexpected and suspected will also be forwarded as soon as possible, but no later than seven (7) days in the case of death or life-threatening serious AEs or within fifteen (15) days after the occurrence of all other forms of serious adverse events to the DSMB. All serious AEs will be included for review by the DSMB.

8.7 Data Management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. Laboratory values from referring home physicians will be entered into the system. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable.

Research data will be prospectively collected by authorized Investigator personnel and entered into an NHLBI-approvedDatabase.

The database will maintain complete data records on each research subject. Subjective and objective patient experiences during the duration of the study will be documented in the patient medical record notes.. Any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records will be used as additional source for data collection.

Neither individual personal identifiers nor the key linking coded data to individuals will be released to Alexion Pharmaceuticals without prior IRB approval and an executed MTA or CTA.

With amendment M, Dr. Phuong Vo, who was the original Principal Investigator of the study, will assume the role of Associate Investigator. Dr. Vo has taken a clinical position at Fred Hutchinson Cancer Center, Seattle, WA and will be analyzing identifiable data. Dr. Vo's role in the research will be limited to data analysis. An FWA coverage agreement to cover this activity will be executed upon approval of Amendment M.

End of study procedures:
Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

Loss or destruction of data:
Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Publication policy:
Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research (OHSR).

9.0 HUMAN SUBJECTS PROTECTIONS

The investigators will protect the rights and welfare of human research subjects set forth in 45 C.F.R. Part 46 and 21 C.F.R Part 50, *Protection of Human Subjects.*

Rationale for Subject Selection: Thromobocytopenia leads to petechiae of the skin and mucous membranes, epistaxis, menses that last more than a month, and gum bleeding. The most feared complication of thrombocytopenia is intracranial hemorrhage which has a high degree of mortality. The only modality to prevent life threatening hemorrhage is platelet transfusion. However, patients who develop platelet refractoriness do not respond to platelet transfusions. Data support the concept that in many patients, platelet counts fail to increase after a platelet transfusion because the transfused platelets are destroyed by the body's complement. Therefore, we hypothesize that when we treat patients who have platelet refractoriness with eculizumab that inhibits complement, the platelet counts will increase to higher numbers after platelet transfusions, decreasing the risk of bleeding complications.

Pregnant women are excluded from accrual to eliminate the possibility of drug effects on a developing fetus. Subjects who are unable to provide informed consent will be excluded from the study due to possibly adverse events may not be comprehensive to the subject.
Historically Hematology Branch have not seen patients above 75 years old, therefore 75 was determine to be upper age limit.

Recruitment effort: Strategies for recruitment will include announcement to the medical team during rounds. The study will be listed on the clinicaltrials.gov, and Clinical Center research studies.

9.1 Competition between Branch Protocols: none.

9.2 Reimbursement

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NHLBI policy.

Payment for participation: $0

9.3 Participation of children

Pediatric subjects are excluded, due to insufficient data on pharmacokinetics in addition to variation in complement activity.

9.4 Risks and Discomforts

Related to eculizumab

Eculizumab may cause: life-threatening and fatal meningococcal infections. Therefore, subjects will be immunized with a meningococcal vaccine at least 2 weeks prior to administering the first dose of eculizumab.

It may also cause Suppressed immune system, Headache, Back pain, Nausea, Diarrhea, Vomiting, Abdominal discomfort, Hypertension, Fatigue, Pyrexia, Anemia, Leukopenia, Fatigue, Cough, Constipation, Myalgia.

Related to Blood Tests

Some subjects may experience localized bruising at the site of venipuncture. Some subjects may experience a vasovagal response. In order to minimize the potential for fall injuries after a blood draw, subjects will be closely monitored for dizziness or lightheadedness before they are allowed to stand.

9.5 Risks in Relation to Benefit

The investigational nature and objectives of this study, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, and potential alternative therapies

will be carefully explained to the patient or the patient's surrogate, and a signed informed consent document will be obtained.

There is no prospect of direct benefit for the subjects on this study, but is likely to yield generalizable knowledge about the subject's disorder or condition.

The potential risk for subjects receiving eculizumab in this study is that it may be toxic, ineffective, or both. However, eculizumab is utilized for FDA-approved indication (Paroxysmal Nocturnal Hematuria, PNH) and this study will attempt to minimize any significant variation from standard clinical care.

The risks of this study are reasonable in relation to the indirect benefits that will be provided. For adult research subjects, the level of risk is greater than minimal risk. (45 CFR 46.102)

9.6 Informed Consent

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study.

9.6.1 Informed consent for non-English speaking subjects.

We anticipate the enrollment of non-English speaking research participants into our study. The IRB approved full consent document will be translated into the subject's native language in accordance with the Clinical MAS Policy M77-2.

If there is an unexpected enrollment of a research participant for which there is no translated IRB approved consent document, the PI and/or those authorized to obtain informed consent will use the short form oral consent process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2) and 21 CFR 50.27 (b) (a). The English version of the IRB approved consent document will be used as the summary of the text to be provided.

We prospectively request the IRB approve the use of the short form consent process in up to five of enrollees who are non-English speaking. We will notify the IRB at the time of continuing review of the frequency of the use of the short form process (number per language). Should we reach the threshold of five of participants, we will notify the IRB of the need for an additional use of the short form and that we will have the consent document translated into the given inherent language.

9.7 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation will be provided a copy of the document when they were

added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial members of the research team reported a potential conflict of interest.

This protocol has a CRADA with Alexion Pharmaceuticals.

10.0 PHARMACEUTICALS

10.1 Eculizumab

We will use the ECULIZUMAB (h5G1.1-mAb) Investigator's Brochure, Edition 21.0, dated July 11, 2015 and Soliris® (eculizumab) Package Insert as a reference.

10.1.1 Availability

Soliris® (eculizumab) will be provided by Alexion Pharmaceuticals and will be dispensed by the NIH pharmacy.

10.1.2 Formulation

The product is formulated at pH 7 and each vial contains 300 mg of eculizumab, 13.8 mg sodium phosphate monobasic, 53.4 mg sodium phosphate dibasic, 263.1 mg sodium chloride, 6.6 mg polysorbate 80 (vegetable origin) and water for Injection, USP.

10.1.3 Storage and Stability

Eculizumab is supplied as 300 mg single-use vials containing 30 mL of 10 mg/mL sterile, preservative-freeeculizumab solution per vial. Vials must be stored in the original carton until time of use under refrigerated conditions at 2°C to 8°C (36°F-46°F) and protected from light. Do not use beyond the expiration date stamped on the carton. Eculizumab should not be frozen or shaken. A single unit 300 mg carton contains one 30 mL vial of eculizumab (10 mg/mL).

10.1.4 Administration

Eculizumab must be diluted to a final admixture concentration of 5 mg/mL using the following steps:

- Withdraw the required amount of eculizumab from the vial into a sterile syringe
- Transfer the recommended dose to an infusion bag
- Dilute eculizumab to a final concentration of 5 mg/mL by adding the appropriate amount (equal volume of diluent to drug volume) of 0.9% NaCl Injection, USP; 0.45% NaCl Injection, USP; 5% Dextrose in Water Injection, USP; or Ringer's Injection, USP to the infusion bag
- The final admixed eculizumab 5 mg/mL infusion volume is 240 mL for 1200 mg doses

Gently invert the infusion bag containing the diluted eculizumab solution to ensure thorough mixing of the product and diluent. Empty vials and vials with residual materials should be handled per NIH pharmacy standard operating procedures.

Prior to administration, the admixture should be allowed to adjust to room temperature (18-25°C, 64-77°F).The admixture must not be heated in a microwave or with any heat source other than ambient air temperature. The admixture should be inspected visually for particulate matter and discoloration prior to administration.

The product should be administered by IV infusion over 35 minutes (range 25-45 minutes). It is not necessary to protect the infusion bags from light while it is being administered to the subject. The subjects will be monitored for 1 hour following infusion.

Admixed solutions are stable for 24 hours at 2-8°C (36-46°F) and at room temperature. If the admixture is prepared more than 4 hours in advance of a subject's visit, the diluted material should be stored at 2°C to 8°C.

If an AE occurs during the administration of the eculizumab, the infusion may be slowed or stopped at the discretion of the Investigator, depending upon the nature and severity of the event. If the infusion is slowed, the total infusion time should not exceed two hours in adults and adolescents (aged 12 years to 18 years). The adverse event must be captured in the subject's source document and CRF.

10.1.5 Toxicity

As with all protein products, administration of eculizumab may result in infusion reactions, including anaphylaxis or other hypersensitivity reactions. Eculizumab administration should be interrupted in all subjects experiencing severe infusion reactions and appropriate medical therapy administered if signs of cardiovascular instability or respiratory compromise occur. The infusion reaction must be recorded on the AE/Serious AE (SAE) page of the CRF.

The most frequently reported adverse events in the therapeutic trials were suppression of immune system, Headache, Back pain, Nausea, Diarrhea, Vomiting, Abdominal discomfort, Hypertension, Fatigue, Pyrexia, Anemia, Leukopenia, Fatigue, Cough, Constipation, Myalgia.

Subjects who develop AEs of rash, hives, itching, and/or dysphagia of mild to moderate severity during their infusions while receiving may continue to be infused as deemed to be medically appropriate by the Investigator. Medical intervention may include, but is not limited to: slowing of the infusion rate (with or without treatment). Any acute reaction will be treated according to standard medical practice depending upon clinical signs and symptoms. If a subject requires medical intervention measures, this subject should remain at the investigational site for a minimum one-hour observation period after the infusion and until the patient is stable.

Inhibition of the terminal complement complexes predisposes subjects to infections with encapsulated bacteria. In particular, subjects treated with eculizumab are at increased risk for the development of infection caused by N. meningitidis, as infection with this organism is more

frequent in subjects who have a terminal complement deficiency. Because eculizumab can directly inhibit complement activation, an increased susceptibility to infection is a potential adverse effect of eculizumab. Infection with N. meningitidis can be life-threatening or fatal. Therefore, to decrease the risk of such possible infection, all subjects who have not been previously vaccinated for N. meningitidis must be vaccinated with a meningococcal vaccine according to current medical guidelines for vaccination use as soon as possible after signing the informed consent. Tetravalent conjugated vaccination against serotypes A, C, Y, and W135 are required for this study. Subjects with a documented history of vaccination with N. meningitidis will be assessed by the Sponsor-Investigator on a case-by-case basis to determine if re-vaccination will be required prior to IP infusion. There have been reported cases of N. meningitidis in subjects participating in eculizumab clinical studies and in subjects receiving commercial product. The Investigator's Brochure contains the detailed information.

During the study, subjects must carry a detailed card describing the "alert" symptoms for N. meningitidis at all times. Development of the "alert" symptoms card will be the responsibility of Alexion or its designee. The triggers for seeking immediate medical attention are any of the following symptoms:

- Headache with nausea or vomiting
- Headache with fever
- Headache with a stiff neck or back
- Fever of 103°F (39.4°C) or higher with or without rash
- Confusion
- Severe myalgia with flu-like symptoms
- Sensitivity to light

Any subject experiencing any of the above noted symptoms will be seen by a physician as quickly as possible.

10.1.6 Regulatory Status

The FDA issued a letter on September 23, 2014 stating the use of eculizumab in this study is exempt from IND requirements as stated in 21 CFR 312.2 (b) (4). Specifically, the use is exempt because:

1. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use, nor intended to be used to support any other significant change in the labeling for the drug.
2. The investigation is not intended to support a significant change in the advertising for a prescription drug product.
3. The investigation does not involve a change in route of administration, dosage level, or patient population, or other factor that significantly increases the risks (or decreases the acceptability of risks) associated with use of the drug product.
4. The investigation is conducted in compliance with the requirements for institutional review (21 CFR 56) and informed consent (21 CFR 50).

5. The investigation is conducted in compliance with the requirements of 21 CFR 312.7, i.e., the drug may not be represented as safe or effective, nor may it be commercially distributed, for the purposes for which it is under investigation.

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