A Trial of Crizanlizumab for the Treatment of Retinal Vasculopathy with Cerebral Leukoencephalopathy (RVCL)

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CONFIDENTIAL

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1.0 BACKGROUND AND STUDY RATIONALE

1.1 Retinal Vasculopathy with Cerebral Leukoencephalopathy (RVCL)

Retinal vasculopathy with cerebral leukoencephalopathy (RVCL) is a very rare and uniformly fatal genetic condition that affects the microvasculature especially of the brain and eye. Symptoms begin in adulthood (usually in the mid-30s to early 40s) and include loss of vision, mini-strokes, and dementia [1]. Other patients have suffered from microvascular disease involving the kidneys, osteonecrosis, and gut ischemia. Some of these features of microvascular occlusive disease resemble ischemic events that occur during sickle cell disease, although RVCL patients do not experience pain crises. RVCL includes three conditions which were previously thought to be distinct: hereditary endotheliopathy, retinopathy, nephropathy, and stroke (HERNS); cerebroretinal vasculopathy (CRV); and hereditary vascular retinopathy (HVR) [2]. RVCL is inherited in an autosomal dominant manner and is caused by C-terminal heterozygous frameshift (fs) mutations in TREX1, a DNase gene [2]. There is no treatment for RVCL, only symptomatic management.

1.2 TREX1 [Three prime repair exonuclease 1]

TREX1 is an endoplasmic reticulum (ER)-associated enzyme that, in addition to its DNA enzymatic activities, may also regulate sugar metabolism in the ER [3]. TREX1 mutations have also been associated with several autoimmune and autoinflammatory diseases. In RVCL, frameshift (fs) mutations of TREX1 result in C-terminal truncation and this dysregulates the oligosaccharyltransferase (OST) complex leading to free glycan release from dolichol carriers. Since microvascular ischemia is the major driver of disease and organ damage in RVCL, we hypothesize that preventing leukocyte adhesion to the vascular endothelium may protect against microvascular occlusive disease in RVCL. Indeed, selectin-mediated leukocyte adhesion plays a major role in microvascular occlusion in multiple microvascular diseases including sickle cell disease.

1.3 Crizanlizumab

Crizanlizumab produced by Novartis is the first and only P-selectin antagonist to receive the FDA breakthrough designation for prevention of sickle cell pain crisis. Crizanlizumab is a humanized monoclonal P-selectin antibody that prevents leukocyte adhesion to the vascular endothelium, thereby limiting risk of microvascular occlusion. Crizanlizumab has undergone Phase I and II trials for sickle cell disease at 2.5 mg/kg and 5 mg/kg doses. Crizanlizumab resulted in fewer hospitalizations and fewer pain crises in these patients, likely as a consequence of its ability to prevent microvascular occlusion.

1.4 Study Rationale

Mutations in TREX1 cause RVCL. The disease-causing mutation was discovered at Washington University in Saint Louis. P-selectin is mobilized to the surface of activated vascular endothelial cells and promotes leukocyte adhesion to the blood vessel wall. Dr. Miner did his PhD training in the McEver laboratory, where crizanlizumab was first generated, and therefore has significant expertise in P-selectin, crizanlizumab, and related pathophysiology. Now, Dr. Miner cares for the largest cohort of RVCL patients in the world, and leads a research laboratory that has a major focus on rare vasculopathies including RVCL. The Miner laboratory has preliminarily observed a correlation with levels of soluble P-selectin and the number of brain lesions in patients with RVCL. Since microvascular occlusive disease can result from leukocyte adhesion to the vascular endothelium, we hypothesize that crizanlizumab will help to limit ischemia and brain lesions in patients with RVCL. This may lead to the development of fewer ischemic brain and eye lesions.

There is no approved or effective therapy for RVCL, which is 100% penetrant and causes blindness and early-onset dementia in 100% of patients. In some patients with RVCL, anti-inflammatory treatment (e.g., prednisone) has reduced the size of edematous brain lesions. **Unfortunately, prednisone is too toxic for long-term continuous therapy, and we do not treat these patients with maintenance steroids. Furthermore, other immunosuppressive medications have not been effective, and cytokine levels are not elevated in RVCL. Collectively, our findings indicate that RVCL is not an autoinflammatory or autoimmune disease. Instead, our histological analysis and cytokine assays suggest that RVCL is a disease of endothelial dysfunction, leading to microvascular occlusion and ischemia.** Since crizanlizumab has less toxicity than prednisone and can prevent vasoocclusion, we expect that crizanlizumab will limit the progression of brain and eye lesions in patients with RVCL.

RVCL typically results in new brain and eye lesions that are readily apparent on imaging at 6- or 12-month intervals. Numbers and the size of new ischemic brain and eye lesions will be monitored at visits to assess disease progression while on crizanlizumab.

2.0 OBJECTIVES AND ENDPOINTS

2.1 Primary Endpoint

The primary endpoint is rate of progression in cerebral lesions in the year after initiation of crizanlizumab compared to the year before initiation.

2.2 Secondary endpoints include:

1) Rate of progression in white matter atrophy one year after initiation of crizanlizumab compared to one year prior to initiation.
2) Rate of progression in retinal lesions development one year after initiation of crizanlizumab compared to one year prior to initiation.
3) Rate of progression in cognitive decline one year after initiation of crizanlizumab compared to one year prior to initiation.
4) Safety of crizanlizumab will be measured by serious and non-serious adverse events deemed by the study PI to be related to crizanlizumab treatment in any participants
5) Tolerability of crizanlizumab measured by doses held and/or discontinuation of crizanlizumab for each participant throughout the duration of the study.
6) Quality of life while receiving crizanlizumab will be assessed by SF-36 score.

3.0 PATIENT POPULATION

3.1 Inclusion Criteria

1. A diagnosis of RVCL with confirmation by genetic test
2. At least 25 years of age with imaging evidence of RVCL brain or eye disease at the time of study registration
3. Normal hematologic function defined as: WBC > $4x10^9$/L, ANC > $1.5x10^9$/L and Platelets > $100x10^9$/L
4. Females of childbearing potential (FCBP) must agree to refrain from becoming pregnant while on study drug and for 3 months after discontinuation from study drug, and must agree to use adequate contraception including hormonal contraception, (i.e. birth control pills, etc.), barrier method contraception (i.e. condoms), or abstinence during that time-frame
5. Able to understand and willing to sign an IRB-approved written informed consent document (or that of legally authorized representative, if applicable)

3.2 Exclusion Criteria

1. Acute bacterial, fungal, or viral infection
2. Known HIV, untreated latent tuberculosis (TB), or active hepatitis B or C infection or zoster
3. Pregnant and/or breastfeeding. Negative serum pregnancy test required prior to starting study treatment. For FCBP, a negative urine pregnancy test is required before each infusion.
4. Known hypersensitivity to one or more of the study agents
5. Currently receiving or has received any investigational drugs within the 14 days prior to the first dose of study drug
6. Liver function tests (LFTs) higher than 3x the upper limit of normal within last 30 days
7. Treatment with other monoclonal antibody medications within last 30 days
8. Treatment with various forms of anticoagulation within last 30 days, including but not limited to clopidogrel or coumadin or direct thrombin inhibitors

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 STUDY DESIGN / DRUG ADMINISTRATION / TREATMENT

4.1 Study Design and Overview of Treatment Plan

Up to 20 RVCL patients will receive crizanlizumab 5 mg/kg at weeks 1 and 3, and then 5 mg/kg beginning week 7 and every month thereafter for 24 total months. Patients living in the northeast may choose to have their monthly infusions administered at University of Pennsylvania except for month 1, 6, 12, 18 and 24, where they must return to Washington University for follow-up monitoring and infusion. Other than adverse event recording and documentation of infusions, no data collection will occur at the University of Pennsylvania. The University of Pennsylvania will maintain documentation of all infusions, and all adverse events occurring at University of Pennsylvania will be directly communicated to the primary investigator at Washington University in Saint Louis within 24 hours. Monitoring and all other data collection will occur at Washington University in St. Louis, and will include standard-of-care serial MRI as well as standard-of-care eye disease monitoring at pre-defined intervals (see Section 6.0).

4.2 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy will be discontinued and the reason(s) documented in the case report forms.

Patients will be removed from the study for any of the following reasons:

- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of the study drug
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy, confirmed by pregnancy test
- Serious noncompliance with the study protocol

- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- Investigator decides to close the study

4.3 Duration of Follow-up

Following completion of protocol treatment patients will be followed for up to 2 years with standard-of-care imaging and examinations as detailed in section 6.0.

5.0 DRUG ADMINISTRATION AND DOSING GUIDELINES

5.1 Crizanlizumab

Crizanlizumab will be supplied in single use vials containing 10 mL at a concentration of 10 mg/mL for administration by IV infusion. Each patient will receive one dose of crizanlizumab on day 1 of Week 1, day 1 of Week 3, day 1 of Week 7, and then day 1 of every 4-week cycle. On infusion day, the pharmacist or designated personnel will prepare individual doses of crizanlizumab for subjects on a milligram per kilogram basis in a 100 mL infusion bag in accordance with the Pharmacy Manual. Crizanlizumab will be administered over 30 minutes by IV infusion.

Treatments	Pharmaceutical form and route of administration	Dose	Frequency and/or Regimen
SEG101	Intravenous infusion	5.0 mg/kg	Dosing on day 1 of weeks 1, week 3, and week 7, and then every 4 weeks

Vital signs will be taken before and after each infusion. Patients will be monitored for 30 minutes post infusion. Temperature above 100 degrees F at time of infusion will be documented and may delay treatment at the discretion of the PI.

COVID-19 screening questions will be performed prior to the baseline visit and at each subsequent visit during the COVID-19 pandemic. If a patient fails the screening process or has symptoms of upper respiratory infection, loss of taste or loss of smell, then a COVID-19 test will be performed. Treatment will be held until results are confirmed to be negative, and once the patient is no longer having symptoms of infection. If COVID-19 test results are positive, the quarantine period to return for treatments will be at least 21 days, with days 19, 20, and 21 being symptom-free. After that time, if the patient remains symptom-free, crizanlizumab treatments may resume.

5.2 Dose Modifications

If a subject does not tolerate the protocol-specified dosing schedule, dose interruptions will be strongly considered by the PI based on the clinical situation. Dose reductions are not allowed.

If a subject experiences drug-induced toxicity, the subject will be closely monitored and a decision to continue or discontinue the subject from the study will be made at the next dose scheduled. Missing two consecutive doses for suspected drug-related AEs (drug toxicity) will be considered criteria for permanently discontinuing the patient from treatment.

Dose interruptions and discontinuation protocol are summarized in the below Table.

Dose interruption and re-initiation of investigational treatment	
Worst toxicity **CTCAE[a] Grade (CTCAE version 5) during a cycle of therapy**	
Investigations (Hematologic)	
Neutropenia (ANC)	
Grade 1 (ANC < LLN - 1.5 x 10^9/L) and Grade 2 (ANC < 1.5 x 10^9/L – 1.0 x 10^9/L)	**Maintain dose level.**
Grade 3 (ANC < 1.0 x 10^9/L – 0.5 x 10^9/L)	**Mandatory: Interrupt dose until resolved to ≤ Grade 2 or next dose scheduled. If abnormality persists, permanently discontinue the subject from the study.**
Grade 4 (ANC < 0.5 x 10^9/L)	**Mandatory: Permanently discontinue the subject from the study.**
Febrile neutropenia (ANC < 1.0 x 10^9/L, fever ≥ 38.3°C)	**Mandatory: Interrupt dose until resolved or next dose schedule. If abnormality persists, permanently discontinue the subject from the study.**
Thrombocytopenia	
Grade 1 (LPT < LLN - 75,000/mm^3) and Grade 2 (LPT < 75,000 - 50,000/mm^3)	**Maintain dose level.**
Grade 3 (LPT < 50,000 - 25,000/mm^3)	**Interrupt dose until resolved to ≤ Grade 2 or next dose scheduled. If abnormality persists, permanently discontinue the subject from the study.**
Grade 4 (LPT < 25,000/mm^3)	**Mandatory: Permanently discontinue the subject from the study.**
Investigations (Hepatic)	
Isolated Direct Bilirubin	
Grade 1 **>ULN - 1.5 x ULN if baseline was normal; > 1.0 - 1.5 x baseline if baseline was abnormal**	**Continue study treatment.**

Grade 2 and 3 **(>1.5 - 10.0 x ULN if baseline was normal; >1.5 - 10.0 x baseline if baseline was abnormal)**	**Interrupt study treatment. Monitor LFTs[b] weekly, or more frequently if clinically indicated, until resolved to ≤ 1.5 x ULN or baseline. Monitor for hemolysis. If resolved to ≤ Grade 1 or baseline, then continue study treatment.**
Grade 4 (>10.0 x ULN if baseline was normal; >10.0 x baseline if baseline was abnormal)	**Mandatory: Permanently discontinue study treatment.**
Isolated ALT elevation	
Grade 1 (> ULN - 3.0 x ULN)	**Maintain dose level.**
Grade 2 (> 3.0 - 5.0 x ULN)	**Maintain dose level. Repeat LFTs[b] as soon as possible, preferably within 48-72 hours from awareness of the abnormal results; if abnormal lab values are confirmed upon the repeat test, then monitor LFTs[b] weekly, or more frequently if clinically indicated, until resolved to ≤ 3.0 x ULN. If resolved, then continue with next dose scheduled.**
Grade 3 (> 5.0 - 20.0 x ULN)	**Interrupt dose. Repeat LFTs[b] as soon as possible, preferably within 48-72 hours from awareness of the abnormal results; monitor LFTs[b] weekly, or more frequently if clinically indicated, until resolved to ≤ 3.0 x ULN. If resolved, then continue with next dose scheduled.**
Grade 4 (> 20.0 x ULN)	**Mandatory: Permanently discontinue the subject from the study.**
Combined elevations of ALT and bilirubin (direct (conjugated))	
• **For subjects with normal baseline ALT and direct bilirubin value: ALT >3.0 x ULN combined with direct bilirubin >2.0 x ULN without evidence of cholestasis[c] OR** • For subjects with elevated baseline ALT or direct bilirubin value: ALT>2x baseline AND > 2.0 x baseline direct bilirubin	**Mandatory: in the absence of cholestasis (ALP< ULN), subject will be immediately discontinued from study treatment. Repeat LFTs as soon as possible, preferably within 48 hours from awareness of the abnormal results, then with weekly monitoring of LFTs[b]), or more frequently if clinically indicated, until ALT, or bilirubin have resolved to baseline or stabilization over 4 weeks.**
Infections	
Grade 1 and 2	**Maintain dose level.**
Grade 3	**Mandatory: Interrupt dose until resolved. If resolved, then continue with next dose scheduled.**

Grade 4	**Mandatory: Permanently discontinue the subject from the study.**
Infusion-related reactions	
Grade 1 **Mild transient reaction; infusion interruption not indicated; intervention not indicated**	**Continue study treatment and increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.** • **Consider slowing infusion rate.**
Grade 2 Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for <=24 hrs.	• **Interrupt infusion and increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.** • **Administer appropriate medical therapy (e.g. analgesics such as paracetamol /acetaminophen or NSAIDs and anti-histamines), as per local institutional guidelines and clinical presentation.** o **Steroids will be used with caution unless clinically indicated (e.g. management of hypersensitivity/anaphylaxis).** • **If symptoms resolve, restart infusion per investigator discretion at a slower rate under (e.g. 50%) continuous observation. Ensure a minimum of 1-hour observation period prior to restarting the infusion.** o **Before restarting, administer premedication (e.g. analgesics such as paracetamol/acetaminophen or NSAIDs and anti-histamines within 1 hour prior to dosing) as per local institutional guidelines for prophylaxis of infusion related reactions, including subsequent infusions.** • **In case of recurring infusion reactions despite premedication and prolonged infusion, consider discontinuation of study treatment.**
Grade 3 and 4 **Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement;**	**Mandatory: Permanently discontinue study treatment.** **If considered helpful, collect blood sample immediately after onset of the AE for further characterization of the IRR according with the**

hospitalization indicated for clinical sequelae **Life-threatening consequences; urgent intervention indicated**	**local institutional guidelines, clinical presentation and the responsible physician assessment**

General Note: Decision for dosing is made on prior lab results, not those from labs performed on the day of infusion. If lab results found to be abnormal, repeat (unscheduled) labs will be performed at least 1 week prior to scheduled dose in order to have results to show resolution of the abnormality before the scheduled dose is given

[a] Common Toxicity Criteria for Adverse Events (CTCAE Version 5)

[b] Core LFTs consist of ALT, AST, GGT, total bilirubin (fractionated [direct and indirect], LDH, albumin, creatinine kinase and ALP.

[c] "Cholestasis" defined as ALP elevation (> 2.0 x ULN and R value < 2). **Note**: The R value is calculated by dividing the ALT by the ALP, using multiples of the ULN for both values. It denotes whether the relative pattern of ALT and/or ALP elevation is due to cholestasis(R ≤ 2), hepatocellular (R ≥ 5), or mixed (R >2 and < 5) liver injury

5.2.1 Follow-up for toxicities

Subjects whose investigational treatment is interrupted or permanently discontinued due to an adverse event must be followed up at least once a week (or more frequently if required by institutional practices, or if clinically indicated) for 4 weeks, and subsequently at approximately 4-week intervals, until resolution or stabilization of the event, whichever comes first. Appropriate clinical experts (e.g. ophthalmologist, endocrinologist, dermatologist, psychiatrists) will be consulted as deemed necessary. All subjects must be followed up for adverse events and serious adverse events for 105 days following the last doses of investigational treatment.

Follow up on potential drug-induced liver injury (DILI) cases

Subjects with an elevation of transaminases in combination with an increase of total bilirubin (TBIL) and a normal ALP may be indicative of potential DILI, and will be considered as clinically important events.

Subjects meeting the following criteria will require further follow-up as outlined below:

- **For subjects with normal ALT and Direct BIL value at baseline:** ALT > 3.0 x ULN combined with Direct BIL > 2.0 x ULN without evidence of cholestasis
- **For subjects with elevated ALT or Direct BIL value at baseline:** ALT > 2.0 x baseline AND Direct BIL > 2.0 x baseline
- **For subjects with normal ALT at baseline:** ALT > 5.0 x ULN for more than 2 weeks
- **For subjects with elevated ALT at baseline:** ALT >3.0 x baseline for more than 2 weeks

For these patients, repeat LFTs as soon as possible, preferably within 48-72 hours. Patients will be closely monitored and workup for competing etiologies initiated, including hemolysis or cholestasis, defined as ALP elevation > 2.0 x ULN with R value < 2.

Note: (The R value is calculated by dividing the ALT by the ALP (alkaline phosphatase), using multiples of the ULN for both values. It denotes whether the relative pattern of ALT and/or ALP elevation is due to cholestatic (R ≤ 2), hepatocellular (R ≥ 5), or mixed (R >2 and < 5) liver injury).

In the absence of cholestasis or an alternative explanation, these subjects will be immediately discontinued from study treatment. The evaluation will include laboratory tests, detailed history, physical assessment and the possibility of liver metastasis or new liver lesions, obstructions/compressions as described below:

1. Laboratory tests will include ALT, AST, albumin, creatine kinase, total bilirubin, direct and indirect bilirubin, GGT, LDH, prothrombin time (PT)/INR and alkaline phosphatase.
2. A detailed history, including relevant information, such as review of ethanol, concomitant medications, herbal remedies, supplement consumption, and history of any pre-existing liver conditions or risk factors, will be collected.
3. Further local testing for acute hepatitis A, B, C or E infection and liver imaging (e.g. biliary tract) may be warranted.
4. Obtain PK sample, as close as possible to last dose of study drug.
5. Additional local testing for other hepatotropic viral infection (CMV, EBV or HSV), autoimmune hepatitis or liver biopsy may be considered as clinically indicated or after consultation with specialist/hepatologist.

All cases confirmed on repeat testing meeting the laboratory criteria defined above, with no other alternative cause for LFT abnormalities identified will be considered as “medically significant”, and thus, meet the definition of SAE and will be reported as SAE using the term “potential drug-induced liver injury." All events will be followed up with the outcome clearly documented.

5.3 Concomitant therapy

All medications (other than crizanlizumab) and significant non-drug therapies (including physical therapy, and herbal/natural medications) administered during treatment will be noted in the patient’s medical record.

Please refer to the below sections for information on permitted and prohibited medications and non-drug therapies.

5.3.1 Permitted concomitant therapy

Please refer to the IB.

Aspirin, NSAIDs and prophylactic doses of anticoagulants are permitted, while other anti-platelets agents or anticoagulants at doses targeting therapeutic levels are prohibited.

Other approved medications for supportive care (antiemetics, anxiolytics, hypnotics, antihistamines) are permitted, including marinol.

Infusion-related reactions have been reported in patients treated with crizanlizumab. Prophylactic pre-medication is permitted, and sites will follow local practice and guidelines for administration of monoclonal antibodies; pre-medications may be adjusted based on clinical presentation as deemed appropriate (e.g. for pain management).

There is no existing clinical data on concomitant use of crizanlizumab and corticosteroids. For patients presenting for acute pain related to sickle cell disease, the 2020 guideline from American Society of Hematology suggests against corticosteroids for acute pain management.1

If a participant experiences a grade 3 or grade 4 infusion related reaction, study drug will be discontinued.

Vitamin and mineral supplements (e.g. fish oil, folic acid, L-arginine, L-citrulline, magnesium, riboflavin, vitamin C, vitamin D, vitamin E, and Zinc) are also permitted, though caution is advised when taking amounts exceeding 100% of the recommended daily allowance.

5.3.2 Prohibited Medications

Participants should not take tocilizumab (Actemra®), etanercept (Enbrel®), adalimumab (Humira®), infliximab (Remicade®), rituximab (Rituxan®), abatacept (Orencia®), anakinra (Kineret®), certolizumab pegol (Cimzia®), golimumab (Simponi®), cyclosporine, tofacitinib (Xeljanz®), baricitinib (Olumiant®) or other immunosuppressive drugs while taking crizanlizumab. Taking crizanlizumab with these medicines may increase risk of infection.

Please refer to the IB.

As far as possible avoid co-administering drugs with a “Known”, “Possible”, or “Conditional” risk of Torsades de Pointes as per www.qtdrugs.org during the course of treatment:

• If concomitant administration of drugs with a “Known risk of Torsades de Pointes” is required and cannot be avoided, crizanlizumab will be interrupted. Treatment may only be resumed after 5 half-lives (of the QT-prolonging drug) from last dose of the QT prolonging drug, and close ECG monitoring is advised.

• If during the course of treatment, concomitant administration of a drug with ¨Possible risk¨ or ¨Conditional risk of Torsades de Pointes¨ is required, based on the Treating Physician assessment and clinical need, treatment may be continued under close ECG monitoring to ensure patient safety.

A list of drugs associated with QT prolongation and/or TdP is available online at www.qtdrugs.org

The use of other investigational agents is prohibited during treatment of crizanlizumab. In addition, the administration of monoclonal antibodies other than crizanlizumab is prohibited, due to the theoretical potential for cross-reactivity and/or overlapping toxicities with other monoclonal antibodies. If investigational agents or other monoclonal antibodies have been used in the past, they must have been discontinued at least 30 days (or 5 half-lives of that agent, whichever is greater) prior to starting crizanlizumab treatment.

Infusion-type reactions were rarely reported in prior studies with crizanlizumab, so prophylaxis for such reactions is not recommended. If a patient experiences severe infusion- related reaction, crizanlizumab will be discontinued and appropriate treatment provided.

5.4 Dose Forms, Strengths and Storage

Crizanlizumab 10 mg/ml vials (100 mg per vial). Drug will be stored in the appropriate conditions, similar to other biologic drugs administered to our patients.

5.5 Availability

Crizanlizumab will be provided by Novartis.

5.6 Warnings and Precautions

Crizanlizumab is not recommended for use during pregnancy or lactation and will not be given to pregnant or breastfeeding mothers, neonates, or children. Crizanlizumab administration will be avoided, where possible, to patients with acute infection

5.6.1 Pregnancy Risks – Precautions and Restrictions

It is not known conclusively what effects crizanlizumab has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Non-sterilized female patients of a reproductive age group should use effective methods of contraception through defined periods during and after study treatment as specified below:

- Female patients must meet one of the following: postmenopausal or surgically sterile for at least 1 year before the screening visit, agree to practice two effective methods of contraception from the time of signing of the informed consent form through

90 days after the last dose of the study drug, or agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)

- If a patient is suspected to be pregnant, the study drug will be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

6.0 VISIT SCHEDULE AND ASSESSMENTS

	Baseline[1]	Week 1	Week 3	Week 5	Week 7 and every 4 weeks for up to 2 years (7, 11, 15, etc.)	Study Discontinuation	Follow-up[2]
Informed Consent	X						
Medical History	X						
Physical Exam[3]	X				X	X	X
Retinal Exam[4]	X				X[10]	X	X
SF-36 survey	X				X[10]	X	X
Brain Magnetic Resonance Imaging (MRI)[5]	X				X[10]	X	X
Cognitive Testing[6]	X				X[10]	X	X
Complete Blood Count (CBC)[7]	X			X	X[11]	X	
Comprehensive Metabolic Panel (CMP)[8]	X			X	X[11]	X	
Infection Screening[9]	X						
Crizanlizumab Administration		X	X	-	X		

Note: Crizanlizumab administration and all assessments allowed +/- 1-week window for scheduling issues.

1. Within 30 days prior to initiating crizanlizumab
2. Every 6 months following end of study visit for up to 2 years
3. Including vital signs (BP, HR and temperature)
4. Performed by Drs. M. Gilbert Grand, Rajendra Apte or P. Kumar Rao (See Appendix B)
5. Performed by Dr. Andria Ford on HRPO 201509147 via Fluid-attenuated inversion recovery (FLAIR) MRI. MRI scans not dependent on participation in Dr. Ford's study
6. See Appendix A for list of assessments
7. Including differential
8. Sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, AST [SGOT], and ALT [SGPT]
9. All patients will receive pre-screening to rule out infections (e.g., hepatitis B / C, HIV, tuberculosis, etc.) according to package insert for crizanlizumab
10. Weeks 27, 51, ~~and~~ 75, and 99~~9~~ only. Assessments may be +/- 2 weeks.
11. Weeks 15, 27, 39. 51, 6~~3~~7, 7~~5~~9, 87, and 9~~9~~5 only. Assessments may be +/- 2 weeks.

7.0 SAFETY MONITORING AND REPORTING

In compliance with the University Institutional Data and Safety Monitoring Plan, the Principal Investigator will complete a Data and Safety Monitoring (DSM) report semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and complete a semi-annual report. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study Principal Investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the Principal Investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO according to institutional guidelines.

7.1 Adverse Events (AEs) and Safety Reporting

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website: *http://evs.nci.nih.gov/ftp1/CTCAE/About.html*

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:

http://www.hhs.gov/ohrp/policy/advevntguid.html

7.2 Reporting to Novartis

The following safety information must be reported to Novartis:

7.2.1 Serious Adverse Events

Any serious Adverse Events (SAEs), including the transmission of an infectious agent via a medicinal product, or reports of drug exposure during pregnancy or lactation (including initial and follow up reports) in patients exposed to the “Product” within 24 hours of becoming aware of it, including those which may have been the reason for the patient to discontinue treatment,

Serious adverse event (SAE) is defined as one of the following:

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Note that hospitalizations for the following reasons will NOT be reported as serious adverse events:
- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- Social reasons and respite care in the absence of any deterioration in the patient’s general condition

Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event

To ensure patient safety, every SAE, regardless of causality assessment, occurring after the patient has provided informed consent and until 105 days after the patient has stopped participation to the MAP must be reported to Novartis within 24 hours of learning of its occurrence.

Any SAEs experienced after this 105-day period will only be reported to Novartis if the Treating Physician or other involved health care professional suspects a causal relationship to the treatment with Crizanlizumab.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. This report must be submitted within 24 hours of the Treating Physician or other involved health care professional receiving the follow-up information. An SAE that is considered completely unrelated to a previously reported one will be reported separately as a new event.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form. The Treating Physician or other involved health care professional must assess the relationship to the treatment with crizanlizumab, complete the SAE Report Form and send the completed, signed form by fax within 24 hours.

The telephone and telefax number of the contact persons in the local Novartis Patient Safety, specific to the site, are listed in the Treating Physician or other involved health care health care professional folder provided to the site. The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the case report form documentation at the site participating in this MAP.

Follow-up information is sent to the same person to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. The follow-up information will describe whether the event has resolved or continues, if and how it was treated, whether the patient continued or withdrew from participation to this MAP.

If the SAE is not previously documented in the Investigator's Brochure a local Patient Safety Department associate or representative may urgently require further information from the Treating Physician or other involved health care professional for Health Authority reporting.

7.2.2 Pregnancies

To ensure patient safety, any occurrence of a pregnancy in a patient on treatment with Crizanlizumab must be reported to Novartis within 24 hours of learning of its occurrence. Any SAE experienced during pregnancy must be reported on the SAE Report Form. The pregnancy will be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy will be recorded on a Pregnancy Form and reported by the Treating Physician or other involved health care professional to the local Novartis Patient Safety Department. In case of any congenital abnormality, birth defect or maternal and newborn complications, the possible relationship to Crizanlizumab will be reported.

Pregnancy outcomes must be collected for the female partners of any males who were treated with Crizanlizumab. Consent to report information regarding these pregnancy outcomes will be obtained from the mother.

7.2.3 Non-Serious Adverse Events

Any non-serious Adverse Events (AEs) (including initial and follow up reports) in patients exposed to the "Product" twice annually through AE reconciliations, including those which may have been the reason for the patient to discontinue treatment.

7.2.4 Other Relevant Safety Information

Any other relevant safety information on the list below, in patients exposed to the "Product", including initial and follow up reports twice annually:

- lack of efficacy (with or without clinical symptoms),
- overdose (with or without clinical symptoms),
- withdrawal reactions and rebound effects (with or without clinical symptoms),
- intentional drug misuse/ abuse (with or without clinical symptoms),
- drug dependence/addiction (with or without clinical symptoms),
- medication errors (including maladministration, dispensing or prescribing errors) (with or without clinical symptoms),
- drug-drug or drug-food interaction (with or without clinical symptoms),

• disease progression and aggravation (with or without clinical symptoms),

• unexpected beneficial effects and

• treatment non-compliance with clinical symptoms.

7.3 Unanticipated Problems

Definition:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.4 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.5 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.6 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

7.7 Reporting to the Human Research Protection Office (HRPO)

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at any WU, BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event will be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

7.8 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements.

PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO. It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes:
    - Death
    - A life-threatening adverse drug experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
    - Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

  An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration

Center for Drug Evaluation and Research

Division of Pulmonary, Allergy and Rheumatology Products

5901-B Ammendale Rd.

Beltsville, MD 20705-1266

FAX: 301-796-9728

7.9 Timeframe for Reporting Required Events

Reportable adverse events will be collected from first dose of study treatment through 28 days following the last study treatment.

8.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

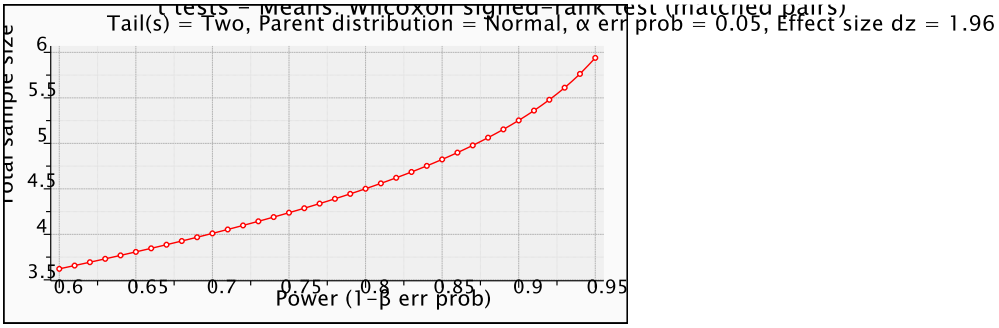
Case Report Form	Submission Schedule
Original Consent Form	Prior to Registration
Registration Form Eligibility Form On-Study Form Medical History Form	Baseline
Safety Labs Form (CBC & CMP)	Baseline Within 30 days of initiating crizanlizumab Every 3 months, with labs reviewed prior to infusions End of Study
SF-36 Survey	Baseline Every 6 months End of Study Each Follow-Up
Response Assessment Form (Imaging, Clinical, & Retinal)	Baseline Every 6 months End of Study Each Follow-Up
Adverse Events Form	Ongoing

10.0 STATISTICAL METHODS AND DATA ANALYSIS

This is a Phase II trial evaluating the safety, tolerability, and efficacy of crizanlizumab for the treatment of RVCL. A maximum of 20 patients will be enrolled.

Primary Endpoint Statistical analysis: The primary endpoint is rate of progression in cerebral lesions as assessed by FLAIR MRI. The rate of progression (calculated in percentage) can be variable across patients but we estimate it to be ~15% per year on average. The analysis will be a pre-post intervention comparison of the rate (number of new or worsened lesions) measured the year after initiation of crizanlizumab compared to the year (+/- 3 months) prior to initiation. A Wilcoxon signed rank test will test the hypothesis that rate of progression is decreased post crizanlizumab intervention. For those who the pre- evaluation is not within +/- 4 weeks of the one-year mark, the actual rate in the pre-treatment period will be used to estimate the one-year rate assuming that progression occurs in a linear fashion. Only patients with pre- and post-evaluations available will be considered evaluable for the primary endpoint. Those not evaluable for the primary analysis will still be enrolled and followed on protocol for all other analyses.

Primary Endpoint Power analysis: Based on the uncertainty of the proportion of patients enrolled who will be evaluable, definitive power analyses are not feasible. Therefore, sample power calculations were performed a range of possibilities. Assuming the average rate of progression in the year prior to initiation of crizanlizumab is ~15%, we estimate that the effect size the corresponds with a 50% rate reduction (from 15% to 7.5%, for example) is ~0.5 - ~1 based on interpatient variation. A sample size of 20 patients evaluable for the primary endpoint will allow us to detect an effect size of >0.6 with 80% power at one-sided 0.05 alpha. A sample size of 10 evaluable patients will allow us to detect an effect size >0.9. This study is designed to generate preliminary data rather than confirm hypotheses and, thus, not definitively powering the study is acceptable.



Secondary endpoint analyses include:

1) Rate of progression in white matter atrophy two years after initiation of crizanlizumab compared to one year prior to initiation of crizanlizumab. Statistical analysis: a Wilcoxon signed rank test (matched pairs pre-post intervention) will test the hypothesis that rate of progression is decreased two years post crizanlizumab initiation compared to one-year pre crizanlizumab initiation.
2) Rate of progression in retinal lesions development two years after initiation of crizanlizumab compared to one year prior to initiation of crizanlizumab. Statistical analysis: a Wilcoxon signed rank test (matched pairs pre-post intervention) will test the hypothesis that rate of progression is decreased two years post crizanlizumab initiation compared to one-year pre crizanlizumab initiation.
3) Rate of progression in cognitive decline two years after initiation of crizanlizumab compared to one-year prior to initiation of crizanlizumab. Statistical analysis: a Wilcoxon signed rank test (matched pairs pre-post intervention) will test the hypothesis that rate of progression is decreased two years post crizanlizumab initiation compared to one-year pre crizanlizumab initiation.
4) Safety of crizanlizumab will be measured by serious and non-serious adverse events deemed by the study PI to be related to crizanlizumab treatment in any participants. Statistical analysis: Descriptive
5) Tolerability of crizanlizumab measured by dose-lowering and/or discontinuation of crizanlizumab for each participant throughout the duration of the study. Statistical analysis: Descriptive
6) Quality of life while receiving crizanlizumab will be assessed by SF-36 score. Statistical analysis: Descriptive

12.0 REFERENCES

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4. Wood KC, Hebbel RP, Granger DN. Endothelial cell P-selectin mediates a proinflammatory and prothrombogenic phenotype in cerebral venules of sickle cell transgenic mice. American journal of physiology Heart and circulatory physiology 2004; 286:H1608-14.
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Appendix A: Cognitive Testing

The following assessments will be performed:

- Montreal Cognitive Assessment Test (MoCA) score
- Free and Cued Selective Reminding (FCSRT) memory test
- Coding Symbol Test
- Verbal fluency
- Gait speed
- Neurological exam
- Barthel Index of Activities of Daily Living (ADLs)
- Geriatric depression scale
- State-Trait Anxiety scale

Appendix B: Retinal Exam

Specific retinal findings typical of RVCL include microaneurysms, cotton wool spots, capillary drop out, proliferative retinopathy and pre-retinal hemorrhage. The tests below will be done at baseline, and every sixth months of treatment to assess RCVL.

- Examination including: Snellen, acuity, slit lamp, fundus biomicroscopy, and indirect ophthalmoscopy of the fundus
- Standard Digital Retinal photographs of both eyes as per the ETDRS protocol or Wide-field color photographs with corresponding angiographic image protocol
- Fluorescein angiography of the retinal circulation including wide-field angiography
- High Resolution OCT scans of the macula