Evaluation of Potential Predictors of Disease Progression in Patients with aHUS, Including Genetics, Biomarkers, and Treatment (EVIDENCE)

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EVIDENCE

EVALUATION OF POTENTIAL PREDICTORS OF DISEASE PROGRESSION IN PATIENTS WITH aHUS, INCLUDING GENETICS, BIOMARKERS, AND TREATMENT

IND #011075

EUDRACT NUMBER: 2015-003135-35

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Amendment 1.1 (UK-specific) Date of Protocol:	05 August 2016
Amendment 2 Date of Protocol:	17 November 2016

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: EVIDENCE – EValuation of Potential PredIctors of Disease ProgrEssion in Patients with aHUS, Including GeNetiCs, BiomarkErs, and Treatment

PROTOCOL NUMBER: ECU-aHUS-403

PPD

11/17/2016

Date

Alexion Pharmaceuticals, Inc.

INVESTIGATOR'S AGREEMENT

I have read the ECU-aHUS-403 study protocol Amendment 2 and agree to conduct the study as outlined. I agree to conduct the trial in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Council on Harmonisation (ICH)/Good Clinical Practice (GCP) and applicable regulatory requirements. I also agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date:

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Role in Study	Name	Address and Telephone number
Clinical Study Leader	PPD	PPD Alexion Pharma Spain Passeig de Gracia 85, 08008 Barcelona Mobile: PPD Fax: PPD Email: PPD
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2. SYNOPSIS

Name of Sponsor/Company: Alexion Pharmaceuticals, Inc.	
Name of Investigational Product: All treatment decisions will be left to the Treating Physician's discretion and therefore no investigational product will be supplied during the study.	
Title of Study: EVIDENCE – EValuation of Potential PredIctors of Disease ProgrEssion in Patients with aHUS, Including GeNetiCs, BiomarkErs, and Treatment.	
Study center(s): No limit	
Principal Investigator: **Investigators:** A list containing all Investigators will be provided when site selection is completed.	
Studied period (years): Up to a maximum of 4 years Estimated date first patient enrolled: Q4 2015 Estimated date last patient enrolled: Q4 2017	**Phase of development:** Phase IV

Study Rationale:

Atypical hemolytic uremic syndrome (aHUS) is a rare, progressive, and life-threatening disease that affects both children and adults (Zimmerhackl, 2006). Atypical hemolytic uremic syndrome is a disease of complement dysregulation, frequently resulting from mutations in complement and/or coagulation pathway genes or from autoantibodies against complement proteins. Specific genetic complement mutations or antibodies are only currently identifiable in approximately 50% to 70% of patients diagnosed with aHUS (Noris, 2010; Matis, 1995). Chronic uncontrolled terminal complement activation causes complement-mediated thrombotic microangiopathy (TMA), a pro-thrombotic and pro-inflammatory state (Matis, 1995; Karpman, 2006), characterized by endothelial cell activation, the release of von Willebrand factor, platelet activation and aggregation, leukocyte recruitment and a pro-coagulant state (Ballermann, 1998). The prognosis in aHUS is poor with more than half of all patients dying, requiring dialysis, or having permanent kidney damage within 1 year of diagnosis (Caprioli, 2006).

Eculizumab is a high affinity humanized monoclonal antibody (mAb) that binds to and blocks the cleavage of C5 into the inflammatory, pro-thrombotic end molecule C5a and lytic membrane attack complex, C5b-9, leaving the upstream components of complement, most notably C3b, intact. Uncontrolled complement activation observed in aHUS patients coupled with the established activity of eculizumab to selectively inhibit terminal complement activation provided a mechanistic rationale for the evaluation of eculizumab in the treatment of aHUS. Eculizumab is approved for the treatment of aHUS in 39 countries, including the European Union and the USA in 2011, and Australia in 2012.

There is growing but limited information on the long term clinical status of aHUS patients who have previously received or are continuing to receive treatment with eculizumab. An observational registry study (M11-001 aHUS Registry) designed to collect clinical data to provide insight into the long-term outcomes of patients with aHUS and fulfill post-marketing commitments is ongoing. This study (ECU-aHUS-403) aims to provide greater insight into

risk factors for manifestations of TMA in patients with aHUS disease into risk of disease progression in relationship to standard of care treatment (defined as eculizumab approved treatment).

Several recent consensus papers regarding aHUS treatment clearly mention eculizumab as a part of the standard of care, and recommend its use as first-line treatment in many situations (Zuber, 2012; Campistol, 2013; Loirat, 2016; Nester, 2015). In order to provide further guidance on the chronicity of treatment in patients with aHUS, this study aims to assess TMA manifestations in relationship to treatment. This study will also seek to identify predictors of TMA manifestations, including genetic profile, and to further elucidate mechanisms of disease and response to treatment through biomarker assessment. Finally, long-term effects on kidney function in relationship to treatment and other factors will also be assessed.

This is a real-world study and as such, all treatment decisions will be left to the Treating Physician's discretion.

Study Objectives:

The main purpose of this study is to assess TMA disease manifestations in patients with aHUS with or without ongoing eculizumab treatment in a real-world setting.

Additional objectives of this study are to evaluate potential clinical predictors of disease manifestation and progression, including clinical characteristics and genetic profiling to evaluate disease activity and relationship to treatment as determined by levels of biomarkers associated with complement activation, and the use of associated supportive interventions such as plasma exchange/plasma infusion (PE/PI), dialysis, blood transfusions, and renal transplantation.

Study Design and Methodology:

Study (ECU-aHUS-403) is a prospective, non-randomized, open-label cohort study of patients with aHUS enrolled in the M11-001 aHUS Registry and currently receiving eculizumab with no prior intentional eculizumab discontinuations at any time (ie, not occasional, unplanned, or temporary missed doses of eculizumab, but intended long-term discontinuation).

The study will assess disease manifestation in patients receiving ongoing eculizumab treatment compared to those who intentionally discontinue eculizumab treatment. This is an open-label study with no randomization performed for patients. Treatment for aHUS will remain observational and at the discretion of the Treating Physician.

Approximately, 330 eligible patients, not to exceed 340 patients, enrolled in the M11-001 aHUS Registry are expected to participate in this study. Patients will enroll via competitive enrollment (ie, as patients become available at the site). Treatment is provided at the Treating Physician's discretion, therefore intentional discontinuation of eculizumab and re-initiation of eculizumab may occur during the study. At the Treating Physician's discretion, some patients may receive a reduced/increased eculizumab dosing regimen (ie, reduced/increased dose and or reduced/increased frequency of eculizumab).

Patients receiving ongoing eculizumab treatment (whether dosed as recommended in product labeling or on a reduced/increased dosing regimen) without any intentional eculizumab

discontinuation will have assessments every 2 weeks (Q2W) through Week 10 and monthly assessments thereafter beginning at Month 3 to Month 24, a period of 2 years, at which time they will complete the study.

Patients intentionally discontinued from eculizumab treatment before reaching Month 24 of eculizumab treatment, will commence a new schedule of assessments, immediately following the intentional discontinuation through 2 years from the time of their first intentional eculizumab discontinuation at which time they will complete the study.

The 2 year-assessment period will commence within 2 weeks after the last eculizumab infusion and this 2 year-assessment period following intentional discontinuation will not change if eculizumab treatment is re-initiated, ie, patients who re-initiate eculizumab after an intentional discontinuation will complete the study 2 years from the time of their first intentional eculizumab discontinuation. Consequently, the maximum possible time on the study for any patient is approximately 47 months, comprising 23 months of continuous eculizumab treatment, followed by an intentional discontinuation and another 24 months of study assessments, which may include periods of eculizumab re-initiation and subsequent intentional discontinuation of eculizumab treatment.

Prior to initiating any study procedure, the informed consent form (ICF) will be signed by the patient or patient's guardian and inclusion/exclusion criteria will be obtained and evaluated. At Day 0 (Baseline)/Visit 1, the patient will be assessed for study eligibility through medical history review, demographic data, and safety assessments (vital signs, physical examination, laboratory tests, assessments of TMA manifestations, eculizumab treatment status, and associated supportive interventions, adverse events [AEs], concomitant medications/therapies). Patient-reported outcomes (PRO) using Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue instrument, European Organization for Research and Treatment of Cancer quality of life questionnaire (EORTC QLQ-C30), and specific patient-reported aHUS symptoms will be assessed.

On Day 0 (Baseline)/Visit 1, safety assessments will be performed prior to blood or urine sample collection for biomarker tests. Blood samples for cRBC hemolytic assay, free C5, and pharmacokinetics (PK) will be collected. Samples for genetic testing will be collected from patients during the study at this visit or any other visit thereafter. A patient may be allowed to opt-out from providing a sample for genetic testing (eg, for religious reasons) and still participate in the study. Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab. If the scheduled eculizumab dose falls within a study visit window, the study visit should be scheduled on the same day of the dose or within 48 hours prior to the dose, such that laboratory samples can be taken prior to the eculizumab dose. If the scheduled dose falls on the same day as the scheduled visit, blood samples are to be taken 5 to 90 minutes prior to administration. It is preferred that the dose is scheduled on the same day as the visit, if possible. Patient-reported outcomes using FACIT-Fatigue instrument, EORTC QLQ-C30, and specific patient-reported aHUS symptoms will be assessed.

Study Year 1 and 2 - Patients Receiving Ongoing Eculizumab Treatment

After completing the Day 0 (Baseline), patients will return to the site for Q2W study visits from Week 2 through Week 10, followed by monthly study visits until the end of the study.

Patients receiving ongoing eculizumab treatment will have monthly study visits beginning at Month 3 through Month 24. If a patient completes all the study visits, then the end-of-study (EOS) visit will be scheduled at which time the patient completes the study. Safety assessments will be performed at every visit prior to collection of samples for biomarker analyses and administration of eculizumab. Blood and urine samples for biomarkers will be collected at every visit. Samples for genetic testing will be collected from patients on Day 0 (Baseline) however, may alternatively be collected at any other visit during the study after Day 0 (Baseline). Patient-reported outcomes using FACIT-Fatigue instrument, EORTC QLQ-C30, and specific patient-reported aHUS symptoms will be assessed at pre-specified visits.

For patients receiving eculizumab, blood samples for free C5, PK, and cRBC hemolytic assay will be collected every 6 months beginning at Month 6 through Month 24. For patients receiving eculizumab at a reduced/increased eculizumab dosing regimen, blood samples for free C5, PK, and cRBC hemolytic assay will be collected at the time of dose reduction and monthly thereafter as long as the patient continues to be on that reduced/increased eculizumab dosing regimen.

If a patient enters the study on a reduced/increased eculizumab dosing regimen, blood samples for PK and cRBC hemolytic assay will be collected monthly as long as the patient remains on a reduced/increased eculizumab dosing regimen.

If a patient experiences a TMA manifestation, assessments and sample collection as specified in the TMA manifestation visit will be performed. Additional blood samples may be collected as needed, at scheduled or unscheduled visits at the discretion of the Investigator or the treating physician.

Study Year 1 and 2 – Patients Who Discontinue Eculizumab

First Intentional Eculizumab Discontinuation:

Following the first intentional eculizumab discontinuation, patients will have a study visit within 2 weeks (±2 days) after the last eculizumab infusion, followed by Q2W study visits from Week 2 through Week 10. Beginning at Month 3, patients will have monthly study visits for a total of 2 years. If a patient completes all the study visits, then an EOS visit will be scheduled, at which time the patient completes the study.

Safety assessments will be performed at every visit prior to collection of samples for biomarker analyses. Blood and urine samples for biomarkers will be collected at every visit. Blood samples for cRBC hemolytic assay, PK, and free C5 will be collected at pre specified time points. Patient-reported outcomes using FACIT-Fatigue instrument, EORTC QLQ-C30, and specific patient-reported aHUS symptoms will be assessed at pre-specified visits.

Subsequent Intentional Eculizumab Discontinuation(s):

For any subsequent intentional eculizumab discontinuation, patients will follow the same assessments as with the first intentional eculizumab discontinuation visit followed by Q2W study visits thereafter from Week 2 through Week 10. Patients will have monthly visits beginning at Month 3 after intentional eculizumab discontinuation for a total of 2 years from the time of their first intentional eculizumab discontinuation. The monthly visits will follow

the schedule as determined by the date of the first intentional eculizumab discontinuation.

Study Year 1 and 2 – Patients Who Re-initiate Eculizumab

Re-initiation After the First Intentional Eculizumab Discontinuation:

Patients who re-initiate eculizumab will have a study visit within 1 week prior to re-initiation of eculizumab. At this visit, safety assessments will be performed prior to collecting blood and urine samples for biomarker tests. Blood samples will be collected for cRBC hemolytic assay, free C5, and PK.

Following the visit just prior to re-initiation of eculizumab, patients will have Q2W study visits from Week 2 through Week 10. Patients will have monthly study visits beginning at Month 3 through up to 2 years after the time of their first intentional eculizumab discontinuation. These monthly visits will follow the schedule as determined by the date of the first intentional eculizumab discontinuation. At these monthly visits, safety assessments will be performed prior to collection of samples for biomarker analyses. Blood samples for cRBC hemolytic assay, free C5, and PK will be collected at pre-specified time points.

Re-initiation After the Subsequent Intentional Eculizumab Discontinuation(s):

For any subsequent eculizumab re-initiation, patients will have a re-initiation study visit within 1 week prior to re-initiation of eculizumab and follow the same assessments as with the re-initiation after their first intentional eculizumab discontinuation. Patients will have Q2W study visits from Week 2 through Week 10, followed by monthly study visits thereafter for a total of 2 years from the time of the first intentional eculizumab discontinuation. These monthly visits will follow the original schedule as determined by the data of first intentional eculizumab discontinuation.

Assessments for TMA Manifestations:

The TMA manifestation visit should be performed as soon as possible on recognition of signs and/or symptoms of TMA manifestation onset by the Investigator. Changes in specific laboratory parameters (platelet count, serum creatinine, serum lactate dehydrogenase [LDH], and haptoglobin) along with renal and extra-renal signs and symptoms will be assessed at these visits, and can be derived from local and/or central laboratories. Data will be collected regardless of relationship to TMA in the opinion of the Investigator.

If a patient experiences a TMA manifestation, 2 TMA manifestation visits will be performed; the 1st TMA visit will be conducted as described above, as soon as possible on recognition of signs and symptoms of TMA manifestation onset by the Investigator and the 2nd TMA visit will be performed within 2 weeks of the first TMA visit.

Scheduled visits, when overlapping with other visits (eg, TMA visit, re-initiation visits), can be combined as appropriate.

Assessments of Eculizumab Treatment and Supportive Interventions:

For assessment of eculizumab treatment, the following information will be collected at every visit in the eculizumab treatment log:

- eculizumab dose administered

- eculizumab dosing frequency
- any changes in eculizumab dosing regimen (dose and/or frequency)
  - reason for change in dosing regimen
- whether eculizumab was discontinued
  - reason for intentional eculizumab treatment discontinuation
- whether eculizumab was re-initiated
  - reason for re-initiation

For the assessment of associated supportive interventions, the use of treatments such as PE/PI, dialysis, blood transfusions, and renal transplantation will be collected at every visit during the study.

Study Endpoints:

Primary endpoint:

Rate of TMA manifestations, where TMA includes:

1. Hematologic or renal events due to aHUS
2. Extra-renal clinical signs and symptoms of aHUS
3. Tissue (eg, kidney transplant) biopsy demonstrating TMA due to aHUS

Secondary endpoints:

- Change in estimated Glomerular Filtration Rate (eGFR) over time using the chronic kidney disease-epidemiology (CKD-EPI) formula
- Rate of TMA manifestations
  - while patient is on a labeled eculizumab dosing regimen
  - while patient is on a reduced/increased eculizumab dosing regimen (reduced/increased dose and or reduced/increased frequency)
- Incidence of PE/PI

Exploratory endpoints:

- Changes in exploratory biomarker levels:
  - Biomarkers may include, but are not limited to: Ba, TNFR1, sVCAM-1, TM, D-dimer, markers of renal injury (eg, Cystatin C or other) and assessments of complement deposition on endothelial cells
- Changes in PK (serum eculizumab concentration), pharmacodynamic (PD) (ie, cRBC hemolytic assay), and free C5
- Identification of novel genetic variants associated with aHUS
- Development of progressive proteinuria

- Incidence of change in dosing of eculizumab:
  - Re-initiation of eculizumab after discontinuation
  - Start of labeled dosing after reduced/increased dosing
- Incidence of transfusion
- Patient-reported outcomes (FACIT-Fatigue [Appendix 2 and Appendix 3], EORTC QLQ-C30 [Appendix 4], and patient-reported aHUS symptoms [Appendix 5])

Criteria for Site Reporting of Potential TMA Manifestations

Data on all hematologic, renal, extra-renal clinical signs and symptoms, and tissue biopsy results will be collected whether the physician deems related or not to aHUS. Information on etiology will be collected on clinical signs and symptoms deemed not related to aHUS will also be collected. Only criteria considered related to aHUS will be reported as a potential TMA manifestation under the primary endpoint.

For the purposes of reporting, a potential TMA manifestation is defined by at least one of the following criteria, although multiple criteria may be identified:

1. Hematologic or renal events due to aHUS

   A. The occurrence of two or more of the following 3 hematologic or renal events, where the changes from baseline* (see baseline definition below) in laboratory values are confirmed by a 2nd consecutive value derived from local and/or central laboratories; if local laboratory values are to be used, they must be reported to the sponsor):

- A decrease in platelet count by ≥25% below baseline and below the lower limit of normal (LLN)
- Evidence of hemolysis, defined by at least one of the following:
  - An increase in serum LDH by ≥25% above baseline and above the upper limit of normal (ULN)
  - A decrease in haptoglobin below the LLN
- A decline in kidney function that includes at least one of the following:
  - An increase in serum creatinine by ≥25% above baseline and above the ULN
  - An increase in proteinuria characterized by at least one of the following:
    - New onset of proteinuria: ≥2+ on urine dipstick or spot urine albumin to creatinine ratio ≥0.3 mg albumin/mg creatinine;
    - Worsening of existing proteinuria: ≥1.5 x increase in spot urine protein-to-creatinine ratio from baseline
    - New onset of nephrotic-range proteinuria: spot urine protein-to-creatinine ratio from baseline of >3.5 mg protein/mg creatinine in adults and >3.0 mg protein/mg creatinine in children
  - Requirement for renal replacement therapy, including dialysis or kidney

transplant

B. The occurrence of two or more of the following 3 hematologic or renal events, that do not necessarily meet all the criteria listed under #1A above, but are due to aHUS in the opinion of the Investigator:

- A clinically important decrease in platelet count
- Evidence of hemolysis, defined by at least one of the following:
  - A clinically important increase in serum LDH
  - A decrease in haptoglobin below the LLN
- A clinically important decline in kidney function that includes at least one of the following:
  - A clinically important increase in serum creatinine
  - A clinically important increase in proteinuria, for example: new onset proteinuria, worsening of existing proteinuria, or new onset of nephrotic range-proteinuria
  - Requirement for renal replacement therapy, including dialysis or kidney transplant related to a TMA event

*For reporting purposes, baseline is defined as follows:

For a patient on ongoing eculizumab treatment without any treatment discontinuation, the baseline is the value at Day 0. For a patient re-initiated on eculizumab treatment with at least one prior intentional discontinuation, the baseline for each on treatment period is the last laboratory value during the preceding intentional discontinuation period. For a patient intentionally discontinued from eculizumab treatment, the baseline for each off treatment period is the last value during the preceding ongoing eculizumab treatment period.

2. Extra-renal clinical signs and symptoms of aHUS

The occurrence of extra-renal clinical signs and symptoms that in the opinion of the Investigator are due to aHUS AND at least one other test result that is consistent with the diagnosis of TMA related to aHUS OR excludes alternative etiologies of extra-renal signs and symptoms:

- A single occurrence of any component of criterion #1A or #1B (hematologic or renal events due to aHUS) defined above (second/confirmatory value for laboratories not required)

  AND/OR

- Relevant imaging study/studies demonstrating either tissue damage or excluding etiologies other than aHUS (eg, abdominal ultrasound that shows no gallstone in a setting of pancreatitis)

  AND/OR

- Relevant laboratory data ruling out etiologies other than aHUS (eg, negative

hepatitis serologies)

3. Tissue biopsy demonstrating TMA due to aHUS

Adjudication of Potential TMA Manifestations

Events reported as meeting criteria (#1A, #1B, 2, or 3) listed above as well as potential events not indicated as meeting criteria will be adjudicated by an independent Endpoint Adjudication Committee as detailed in the Adjudication Committee Charter.

Diagnosis and main criteria for inclusion:

Inclusion Criteria:

Patients of any age, including minors, enrolled in the M11-001 aHUS Registry should meet all of the inclusion criteria below to be eligible for enrollment in the study:

1. Currently receiving eculizumab treatment in the M11-001 aHUS Registry
2. Two normal platelet counts (eg, >150,000/μL) at least 4 weeks apart while on eculizumab within the past 12 months
3. Two normal LDH levels (eg, <1.5 x ULN) at least 4 weeks apart while on eculizumab within the past 12 months
4. Willing, committed, and able to return for ALL clinic visits and complete all study-related procedures
5. Patient or patient's parent/legal guardian must be willing and able to give written informed consent. Patient (if minor) must be willing to give written informed assent (if applicable as determined by the central Institutional Review Boards/Independent Ethics Committees [IRB/IEC]).

Exclusion Criteria:

Patients enrolled in the M11-001 aHUS Registry Study who meet any 1 of the following criteria will be excluded from the study:

1. Any prior intentional discontinuation of eculizumab treatment
2. On chronic dialysis (defined as ≥3 months on dialysis)
3. Currently participating in another complement inhibitor trial
4. With life expectancy of <6 months
5. Patient or patient's parent/legal guardian unable to give written informed consent. Patient (if minor) unable to give written informed assent (if applicable as determined by the central Institutional Review Boards/Independent Ethics Committees [IRB/IEC]).

Duration of treatment:
Treatment is provided at the Treating Physician's discretion, therefore discontinuation of eculizumab and re-initiation of eculizumab may occur during the study. At the Treating Physician's discretion, some patients may receive a reduced/increased eculizumab dosing regimen (ie, reduced/increased dose and/or reduced/increased frequency of eculizumab).

Statistical Analysis:

This study (ECU-aHUS-403) aims to assess TMA manifestations in patients with aHUS with or without ongoing eculizumab treatment. Treatment for aHUS is non-randomized and is at the discretion of the Treating Physician. Hypothesis testing will be done using a two-sided α=0.05 Type 1 error rate, unless otherwise stated. For continuous variables, descriptive statistics (number of patients with non-missing values [N], mean, standard deviation [SD], median, minimum, and maximum) will be provided. For categorical variables, patient counts and percentages will be presented. The percentages will be calculated based on the total number of patients in the full analysis set (FAS) or the safety set.

Eculizumab Treatment Status:

All patients at their entry into the study will be receiving eculizumab treatment, dosed either on-label or at a reduced/increased dosing regimen, with no prior intentional discontinuation(s) of eculizumab treatment. Once enrolled in the study, patients are allowed to discontinue eculizumab treatment or re-initiate eculizumab treatment at the discretion of the Treating Physician.

Eculizumab treatment status will be classified for analytical purposes as follows:

- ***On eculizumab treatment:*** from the date of enrollment through three weeks after last infusion of eculizumab or until the patient withdraws from the study, or until the patient completes the study, whichever occurs first.
- ***Off eculizumab treatment:*** from three weeks after the last infusion of eculizumab and continue until the patient re-initiates eculizumab treatment or until the patient withdraws from the study, or until the patient completes the study, whichever occurs first.

For both the above categories, the time will accrue as specified until the patient changes treatment status category, or until the patient is withdrawn from the study, or until the patient completes the study.

Primary Endpoint Analyses:

Primary analyses will focus on comparison of rates (ie, adjusted appropriately for exposure periods) of new TMA disease manifestations between time on eculizumab treatment and time off eculizumab treatment.

In this context in which treatment exposure is a time varying confounder in that it affects the occurrence of a TMA disease manifestation and can subsequently be affected by a TMA disease manifestation, marginal structural models will be used. Marginal structural models estimation employs inverse-probability of treatment weighting (IPTW) and robust estimation methods. The goal of IPTW estimation is to obtain coefficients to create weights that will redistribute the population so that, in this case, treatment exposure is not temporally confounded (ie, creating a pseudo-population in which treatment exposure is no longer temporally confounded). The factors that may confound the association are considered in a treatment model (or weight model), which is used as part of the IPTW estimation methodology to obtain estimates of, again in this context, difference in incidence rates between time on and off treatment.

Differences between incidence rates of TMA disease manifestations will be estimated using negative binomial regression.

Secondary Endpoint Analyses:

Secondary endpoints include change over time for eGFR. Change over time will be summarized using repeated measures models starting from baseline. Mean changes from baseline will be analyzed using a restricted maximum likelihood based repeated measure approach. Summary statistics will be presented at baseline and follow-up time points and mean change from baseline will be estimated by eculizumab treatment status.

The primary analyses will be repeated with a distinction for the time on eculizumab treatment as on-label or as on reduced/increased dosing regimen. The focus will be on comparison of rates (ie, adjusted appropriately for exposure periods) of new TMA disease manifestations between:

- time off eculizumab treatment and time on on-label eculizumab treatment, and
- time on off-label eculizumab treatment and time on on-label eculizumab treatment

The number of occurrences of PE/PI per patient-years will be calculated and summarized by treatment status.

Safety Analyses:

AEs and general medical/surgical histories will be coded by primary system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) (Version 13.0 or the most current). All events from time of enrollment in the study will be included in the analyses. The number and percentage of patients with AEs will be summarized by preferred terms, SOC, severity (mild, moderate, severe), relationship to eculizumab treatment, and treatment status. If a patient has more than one occurrence of an AE, the most severe occurrence of the AE will be used in the severity summary table, and the strongest relationship to eculizumab treatment will be used in the relationship to eculizumab treatment table.

Similarly, the number and percentage of patients with SAEs will be summarized by preferred terms, SOC, severity (mild, moderate, severe), relationship to eculizumab treatment, and treatment status.

To account for differences in time spent on and off treatment, the number of AEs and SAEs per patient years will be calculated and summarized.

The number and percentage of patients with AEs resulting in death, study withdrawal, and intentional eculizumab treatment discontinuation will be summarized.

Physical Examinations and Vital Signs

Physical examinations will be collected at baseline for all patients and will be summarized. Vital signs (systolic and diastolic BP, temperature, and sitting or lying HR), height (only for pediatric patients and baseline only for adults), and weight and changes from baseline in vital signs (including height and weight) will be summarized by visit. Listings of physical examinations and vital signs will be produced.

Laboratory Assessments

Changes from baseline in laboratory assessments (chemistry and hematology,) will be summarized by visit. Likewise, shift tables (L [low], N [normal], H [high]) by visit and treatment status will be produced for clinical laboratory tests. Listings of laboratory data will be produced.

Exploratory Endpoint Analyses

Pharmacokinetic/Pharmacodynamic/Biomarker Analyses

Pharmacokinetic, PD, and biomarker analyses will be performed on all patients who have evaluable PK, PD, and biomarker data, respectively.

Graphs of plasma eculizumab concentration time profiles for individual patients and for means will be provided. Plasma eculizumab concentrations by collection day and time will be summarized by tabulations of mean, SD, median, minimum and maximum. Population PK analysis and PK-PD analyses will be conducted using eculizumab plasma concentrations, hemolytic activity and free C5 data, and selected biomarker data. PK and PK-PD model parameters and the variability will be estimated, and the potential covariates, such as demographics, disease severity, genetic markers, will be assessed. Graphic exploration of exposure versus the selected efficacy or safety endpoints will be made, and subsequent exposure response analyses may be performed if graphic exploration suggests that a relationship exists.

For PD and exploratory biomarker assessments, summary tabulations of mean, SD, median, minimum and maximum will be presented. The relationship between changes in PD biomarkers, exploratory biomarkers, and the effects of eculizumab treatment, as well as the effects of treatment discontinuation, will be evaluated.

For the exploratory biomarker analyses, the relationship between eculizumab concentration and the key and exploratory biomarkers or the correlation between clinical benefit and key exploratory biomarkers will be assessed by graphical display. Potential relationships between clinical outcomes, PK/PD, genetic profile and biomarker levels will be explored. Additional model-based exploratory analyses may be performed.

Repeated measures model analyses similar to the ones performed for the change in eGFR will be conducted for biomarkers (Ba, TNFR1, sVCAM-1, TM, D-dimer, markers of renal injury (eg, Cystatin C)) to be measured in the study. Summary statistics will be presented at baseline and follow-up time points and mean change from baseline will be estimated. The number of occurrences per patient-years of (1) re-initiation of eculizumab treatment after discontinuation, (2) change from reduced/increased to labeled dosing, and (3) blood transfusion, will be calculated and summarized. Summary statistics for proteinuria levels, FACIT-fatigue scores observed values and changes from baseline over time, and EORTC QLQ-C30 scale before and after discontinuation will be provided. Summary statistics (proportions) for patient reported aHUS symptoms, including severity, frequency, and impact will be reported before and after discontinuation.

Genetic Analyses

Genetic testing will include analysis of variants in genes known to be associated with aHUS.

Additional exploratory genetic analyses may be performed to identify novel genetic variants associated with aHUS or disease modifying genes. Such variants may be validated by a second sequencing method and validated variants will be summarized across the study population. Potential relationships between clinical outcomes, treatment status, biomarkers, and genetic variants will be explored.
Sample Size Rationale: The primary outcome of the study is TMA manifestation as a dichotomous variable. Preliminary data from the aHUS Registry shows about 20% of patients reporting discontinuation of eculizumab treatment. Additionally, longitudinal data on file at Alexion shows a difference of 15% in proportion of patients with TMA manifestations between patients who discontinue treatment compared to those who remain on treatment. The estimated sample size needed to detect a difference of 15% in TMA manifestations with power=80% in the proposed study is 300 patients (60 patients with eculizumab treatment discontinuation and 240 patients who continue ongoing treatment). Assuming a 10% loss to follow-up, the estimated sample size needed for the study is 330 patients.

3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2: List of Abbreviations and Definitions of Terms

Abbreviation or Specialist Term	Explanation
AE	Adverse event
aHUS	Atypical hemolytic uremic syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomic Therapeutic Chemical
BUN	Blood urea nitrogen
BP	Blood Pressure
CBC	Complete blood count
CFH	Complement factor H
CFI	Complement factor I
CKD-EPI	Chronic kidney disease-epidemiology
CI	Confidence Interval
CPK	Creatinine phosphokinase
cRBC	Chicken red blood cell
CRF	Case report form
EC	Ethics Committee
eCRF	Electronic case report form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
EIU	Exposure in-utero
EOI	Events of interest
EORTC QLQ-C30	European Organization for Research and Treatment or Cancer quality of life questionnaire
EOS	End-of-Study
ET	Early termination
FACIT	Functional Assessment of Chronic Illness Therapy
FAS	Full analysis set
GCP	Good Clinical Practice
HDL	High-density lipoprotein
HR	Heart Rate
ICH	International Council on Harmonisation
ICF	Informed Consent Form
ICU	Intensive care unit
IEC	Independent Ethics Committee
IPTW	Inverse-probability treatment weighting
IRB	Institutional Review Board
LDL	Low-density lipoprotein
LLN	Lower limit of normal
LDH	Lactate dehydrogenase
mAb	Monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
PD	Pharmacodynamics
PE	Plasma exchange
PI	Plasma infusion
PK	Pharmacokinetics
PRO	Patient related outcomes
Q2W	Every two-weeks
RBC	Red blood cell
RR	Respiration Rate
SD	Standard deviation
SOC	System organ class

Abbreviation or Specialist Term	Explanation
SAE	Serious adverse event
sVCAM1	Soluble vascular cell adhesion protein 1
TMA	Thrombotic microangiopathy
TNFR1	Tumor necrosis factor receptor 1
ULN	Upper limit of normal
UNS	Unscheduled
WBC	White blood cell

5. INTRODUCTION

5.1. Overview of Atypical Hemolytic Syndrome Disease

Atypical hemolytic uremic syndrome (aHUS) is a rare, progressive, and life-threatening disease that affects both children and adults (Zimmerhackl, 2006) . Atypical hemolytic uremic syndrome is a disease of complement dysregulation, frequently resulting from mutations in complement and/or coagulation pathway genes or auto-antibodies against complement proteins. Specific genetic complement mutations or antibodies are only currently identifiable in approximately 50% to 70% of patients diagnosed with aHUS (Noris, 2010; Matis, 1995) . Chronic uncontrolled terminal complement activation causes complement-mediated thrombotic microangiopathy (TMA), a pro-thrombotic and pro-inflammatory state (Matis, 1995; Karpman, 2006) , characterized by endothelial cell activation, the release of von Willebrand factor, platelet activation and aggregation, leukocyte recruitment and a pro-coagulant state (Ballermann, 1998) . The prognosis in aHUS is poor with more than half of all patients dying, requiring dialysis, or having permanent kidney damage within 1 year of diagnosis (Caprioli, 2006) .

Eculizumab is a high affinity humanized monoclonal antibody (mAb) that binds to and blocks the cleavage of C5 into the inflammatory, pro-thrombotic end molecule C5a and lytic membrane attack complex, C5b-9, leaving the upstream components of complement, most notably C3b, intact. Uncontrolled complement activation observed in aHUS patients coupled with the established activity of eculizumab to selectively inhibit terminal complement activation provided a mechanistic rationale for the evaluation of eculizumab in the treatment of aHUS. Eculizumab is approved for the treatment of aHUS in 39 countries, including the European Union and the USA in 2011, and Australia in 2012.

There is growing but limited information on the long-term clinical status of aHUS patients who have previously received or are continuing to receive treatment with eculizumab. An observational registry study (M11-001 aHUS Registry) designed to collect clinical data to provide insight into the long-term outcomes of patients with aHUS and fulfill post-marketing commitments is ongoing. This study (ECU-aHUS-403) aims to provide greater insight into risk factors for manifestations of TMA in patients with aHUS and risk of disease progression in relationship to standard of care treatment (defined as eculizumab approved treatment).

5.2. Rationale for the Study Design

Several recent consensus papers regarding aHUS treatment clearly mention eculizumab as a part of the standard of care, and recommend its use as first-line treatment in many situations (Zuber, 2012; Campistol, 2013; Loirat, 2016; Nester, 2015) . In order to provide further guidance on the chronicity of treatment in patients with aHUS, this study aims to assess TMA manifestations in in relationship to treatment. This study will also seek to identify predictors of TMA manifestations, including genetic profile, and to further elucidate mechanisms of disease and response to treatment through biomarker assessment. Finally, long-term effects on kidney function in relationship to treatment and other factors will also be assessed.

This is a real-world study and as such, all treatment decisions will be left to the Treating Physician’s discretion.

5.3. Rationale for Genetic and Biomarker Testing

It is widely recognized that aHUS is associated with genetic or acquired defects in genes of the complement pathway. Recent sequencing efforts have also identified additional pathogenic mutations in genes of the coagulation pathway (Bu, 2014). Mutational analysis of genes known to be associated with aHUS informs not only the disease process as likely complement-mediated, but also provides therapeutic and prognostic information in aHUS patients with regards to risk of progression, risk of relapse, and risk of recurrence. As the known pathogenic mutations can be identified in about one half of aHUS patients, additional pathogenic variants likely exist. Uncovering these novel variants will help advance our understanding of the disease pathogenesis. The goal of the genetic analysis is two-fold. First, known variants associated with aHUS will be analyzed in the patient population and the results will be communicated to the Investigators, which may be relayed also to the Treating Physicians, to inform patient management and treatment decisions. In addition, patients will also be evaluated for autoantibodies directed against complement factor I (CFI) and complement factor H (CFH); these results will also be provided to the Treating Physicians. Secondly, additional exploratory genetic analyses may be performed to identify novel variants associated with aHUS or disease modifying genes. This exploratory analysis may enable better, more rapid diagnosis of aHUS, a better understanding of the heterogeneity of the disease, and may help to identify those patients who are at highest risk of disease progression. The results of the exploratory analysis will not be provided to the Investigator.

In addition to genetic markers of disease, analysis of biomarkers of complement pathway proteins and biomarkers of disease progression will further advance our understanding of both the mechanisms of disease and the ability of eculizumab to act on these mechanisms. Levels of free C5 and functional assays of terminal complement activity are expected to provide a better understanding of the relationship between eculizumab levels and complement-mediated injury. Exploratory biomarkers of complement alternative pathway activation (eg, Ba), coagulation (eg, D-dimer), inflammation (eg, TNFR1), endothelial activation (eg, sVCAM-1), endothelial damage (eg, thrombomodulin), renal injury (eg, cystatin C), and other markers of complement activation or disease progression may be performed to help elucidate the level of ongoing alternative pathway activation and the effect of eculizumab on complement, inflammation, and coagulation signaling networks and resultant endothelial injury and tissue damage. The series of exploratory tests to be done may differ based on the study time point, eg the biomarkers performed during a TMA may differ in order to explore the specific changes caused by disease manifestation. This exploratory analysis will also evaluate whether the underlying mechanisms of disease are affected when eculizumab treatment is discontinued. The biomarker analyses are exploratory and these results will not be provided to the Investigator.

6. STUDY OBJECTIVES AND PURPOSE

The main purpose of this study is to assess TMA disease manifestations in patients with aHUS with or without ongoing eculizumab treatment in a real-world setting. The study hypothesis is that the rate of TMA manifestation will be highest during periods of eculizumab treatment discontinuation.

Additional objectives of this study are to evaluate potential clinical predictors of disease manifestation and progression, including clinical characteristics and genetic profiling to evaluate disease activity and relationship to treatment as determined by levels of biomarkers associated with complement activation, and the use of associated supportive interventions such as plasma exchange/plasma infusion (PE/PI), dialysis, blood transfusions, and renal transplantation.

7. INVESTIGATIONAL PLAN

Study (ECU-aHUS-403) is a prospective, non-randomized, open-label cohort study of patients with aHUS enrolled in the M11-001 aHUS Registry and currently receiving eculizumab with no prior intentional eculizumab discontinuations at any time (ie, not occasional, unplanned, or temporary missed doses of eculizumab, but intended long-term discontinuation). The study will assess disease manifestation in patients receiving ongoing eculizumab treatment compared to those who intentionally discontinue eculizumab treatment. This is an open-label study with no randomization performed for patients. Treatment for aHUS will remain observational and at the discretion of the Treating Physician.

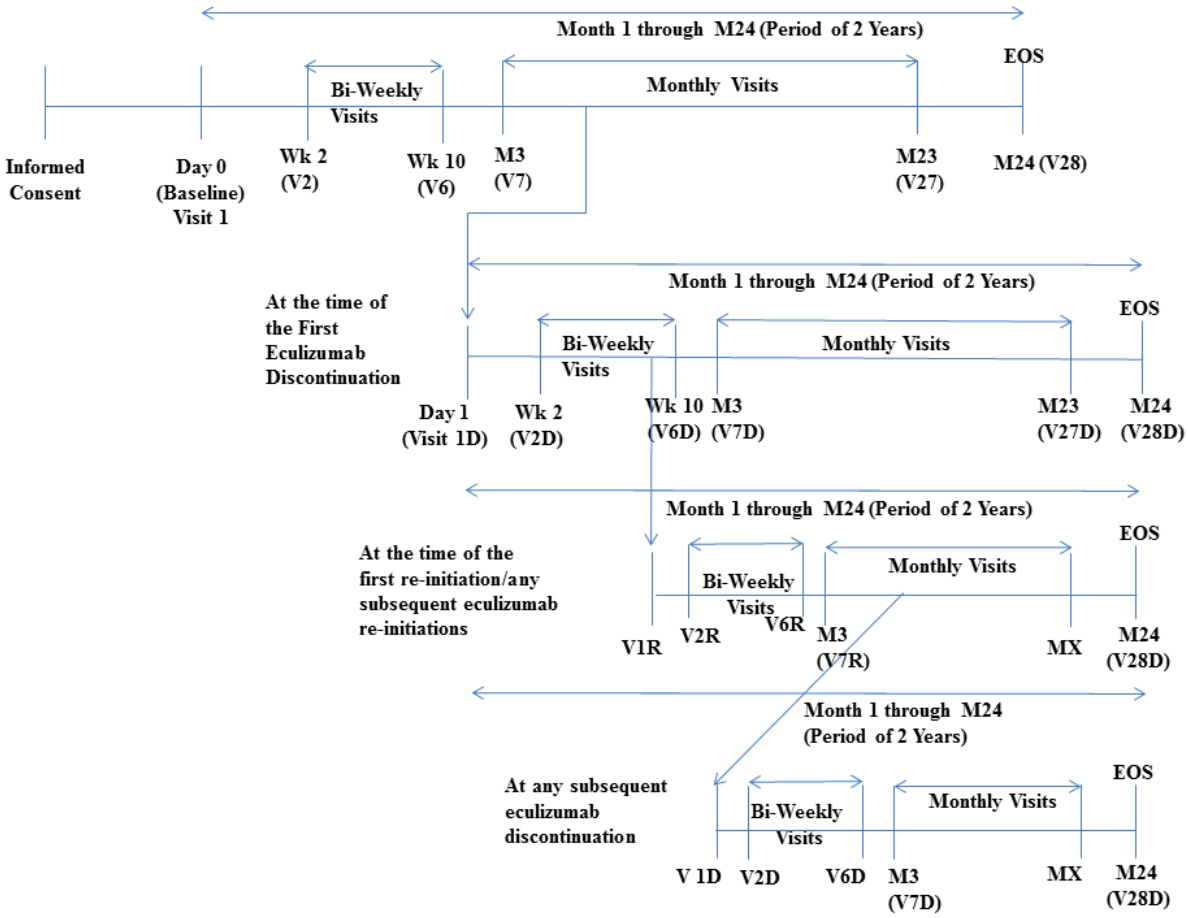
7.1. Overall Study Design and Plan Description

Approximately, 330 eligible patients, not to exceed 340 patients, currently enrolled or to enroll in the M11-001 aHUS Registry are expected to participate in this study. Patients will enroll via competitive enrollment (ie, as patients become available at the site). Treatment is provided at the Treating Physician's discretion, therefore intentional discontinuation of eculizumab and re-initiation of eculizumab may occur during the study. At the Treating Physician's discretion, some patients may receive a reduced/increased eculizumab dosing regimen (ie, reduced/increased dose and/or reduced/increased frequency of eculizumab).

Patients receiving ongoing eculizumab treatment (whether dosed as recommended in product labeling or on a reduced/increased dosing regimen) without any intentional eculizumab discontinuation, will have assessments every 2 weeks (Q2W) through Week 10 and monthly assessments thereafter beginning at Month 3 to Month 24, a period of 2 years, at which time they will complete the study (Figure 1).

Patients intentionally discontinued from eculizumab treatment before reaching Month 24 of eculizumab treatment, will commence a new schedule of assessments, immediately following the intentional discontinuation through 2 years from the time of their first intentional eculizumab discontinuation at which time they will complete the study (Figure 1). The 2-year assessment period will commence 2 weeks after the last eculizumab infusion and this 2-year assessment period following intentional discontinuation will not change if eculizumab treatment is re-initiated, ie, patients who re-initiate eculizumab after an intentional discontinuation will complete the study 2 years from the time of their first intentional eculizumab discontinuation (Figure 1). Consequently, the maximum possible time on the study for any patient is approximately 47 months, comprising 23 months of continuous eculizumab treatment, followed by an intentional discontinuation and another 24 months of study assessments, which may include periods of eculizumab re-initiation and subsequent intentional discontinuation of eculizumab treatment.

Figure 1: Study Flow Diagram



Prior to initiating any study procedure, the informed consent form (ICF) will be signed by the patient or patient's guardian and inclusion/exclusion criteria are obtained and evaluated. At Day 0 (Baseline)/Visit 1, the patient will be assessed for study eligibility through medical history review, demographic data, and safety assessments (vital signs, physical examination, laboratory tests, assessments of TMA manifestations, eculizumab treatment status, and associated supportive interventions, adverse events [AEs], concomitant medications/therapies). Patient-reported outcomes (PRO) using Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue instrument, European Organization for Research and Treatment of Cancer quality of life questionnaire (EORTC QLQ-C30), and specific patient-reported aHUS symptoms will be assessed.

On Day 0 (Baseline)/Visit 1, safety assessments will be performed prior to blood or urine sample collection for biomarker tests. Blood samples for cRBC hemolytic assay, free C5, and pharmacokinetics (PK) will be collected. Samples for genetic testing will be collected from patients during the study at this visit or any other visit thereafter. A patient may be allowed to opt-out from providing a sample for genetic testing (eg, for religious reasons) and still participate in the study. Patient-reported outcomes using FACIT-Fatigue instrument, EORTC QLQ-C30,

and specific patient-reported aHUS symptoms will be assessed. Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab.

7.1.1. Study Year 1 and 2 - Patients Receiving Ongoing Eculizumab Treatment

After completing the Day 0 (Baseline)/Visit 1, patients will return to the site for Q2W study visits from Week 2 through Week 10 (Visit 2 through Visit 6), followed by monthly study visits until the end of the study (Figure 1).

Patients receiving ongoing eculizumab treatment will have monthly study visits beginning at Month 3 (Visit 7) through Month 24 (Visit 28), see Table 3. If a patient completes all the Study Visits (Visits 1 through Visit 27), then Visit 28, will be the end-of-study (EOS) visit, at which time the patient completes the study. Safety assessments will be performed at every visit prior to collection of samples for biomarker analyses and administration of eculizumab. Blood and urine samples for biomarkers will be collected at every visit (Table 3). Samples for genetic testing will be collected from patients on Day 0 (Baseline)/Visit 1 however, may alternatively be collected at any other visit during the study after Day 0 (Baseline). Patient-reported outcomes using FACIT-Fatigue instrument, EORTC QLQ-C30, and specific patient-reported aHUS symptoms will be assessed on Day 0 (Baseline)/Visit 1, Month 3 (Visit 7), and Month 6 (Visit 10) (Table 3). Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab. If the scheduled eculizumab dose falls within a study visit window, the study visit should be scheduled on the same day of the dose or within 48 hours prior to the dose, such that laboratory samples can be taken prior to the eculizumab dose. If the scheduled dose falls on the same day as the scheduled visit, blood samples are to be taken 5 to 90 minutes prior to administration. It is preferred that the dose is scheduled on the same day as the visit, if possible.

For all patients receiving eculizumab, blood samples for free C5, PK, and cRBC hemolytic assay will be collected every 6 months beginning at Month 6 (Visit 10) through Month 24 (Visit 28) (Table 3). For patients receiving eculizumab at a reduced/increased eculizumab dosing regimen, additional blood samples for free C5, PK, and cRBC hemolytic assay will be collected at the time of dose reduction and monthly thereafter as long as the patient continues to be on that reduced/increased eculizumab dosing regimen. If a patient enters the study on a reduced/increased eculizumab dosing regimen, blood samples for free C5, PK, and cRBC hemolytic assay will be collected monthly as long as the patient remains on a reduced/increased eculizumab dosing regimen (Table 6).

If a patient experiences a TMA manifestation, 2 TMA visits will be performed; the 1st TMA visit (Visit 1T) will be conducted as soon as possible on recognition of signs and symptoms of TMA manifestation onset by the Investigator. A 2nd TMA visit (Visit 2T) should be performed within 2 weeks of the first visit. Assessments and sample collection as specified in TMA Visits (Visit 1T and Visit 2T) in Table 3 will be performed.

Additional blood and/or urine samples may be collected as needed, at scheduled or unscheduled visits at the discretion of the Investigator.

7.1.2. Study Year 1 and 2 – Patients Who Discontinue Eculizumab

First Intentional Eculizumab Discontinuation:

Following the first intentional eculizumab discontinuation, patients will have a study visit (Visit 1D) within 2 weeks (±2 days) after the last eculizumab infusion followed by Q2W study visits from Week 2 through Week 10 (Visit 2D through Visit 6D) (Table 4). Beginning at Month 3 (Visit 7D), patients will have monthly study visits for a total of 2 years (Visit 7D-28D). If a patient completes all the Study Visits (Visits 1D through Visit 27D), then Visit 28D will be the EOS visit, at which time the patient completes the study (Table 4).

At the study visit (Visit 1D), on the date of first intentional eculizumab discontinuation, safety assessments will be performed prior to collecting samples for biomarker tests. At the Q2W study visits (Visit 2D through Visit 6D), safety assessments will be performed prior to collecting blood and urine samples for biomarker tests. During these Q2W study visits, blood samples for cRBC hemolytic assay, free C5, and PK will be collected at time points specified in (Table 4).

At monthly visits beginning at Month 3 (Visit 7D) through Month 24 (Visit 28D/EOS), monthly safety assessments will be performed prior to collecting blood and urine samples for biomarker tests. Blood samples for cRBC hemolytic assay, free C5, and PK will be collected at Month 3 and Month 24/EOS after intentional discontinuation of eculizumab as specified in Table 4. Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab.

Patient-reported outcomes using FACIT-Fatigue instrument, EORTC QLQ-C30, and specific patient-reported aHUS symptoms will be assessed at the study visit (Visit 1D), Month 3 (Visit 7D), and Month 6 (Visit 10D).

If a patient experiences a TMA manifestation, 2 TMA visits will be performed; the 1st TMA visit (Visit 1TD) will be conducted as soon as possible on recognition of signs and symptoms of TMA manifestation onset by the Investigator. A 2nd TMA visit (Visit 2TD) should be performed within 2 weeks of the first visit. Assessments and sample collection as specified in TMA Visits (Visit 1TD and Visit 2TD) in Table 4 will be performed.

Additional blood and/or urine samples may be collected as needed, at scheduled or unscheduled visits at the discretion of the Investigator or Treating Physician.

Subsequent Intentional Eculizumab Discontinuation(s):

For any subsequent intentional eculizumab discontinuation, patients will follow the assessments as specified in Visit 1D as with the first intentional eculizumab discontinuation followed by Q2W study visits thereafter as specified in Visit 2D through 6D (Table 4). Patients will have monthly visits beginning at Month 3 after intentional eculizumab discontinuation for a total of 2 years (24 months) from the time of the first intentional eculizumab discontinuation (Table 4). The monthly visits will follow the schedule as determined by the date of their first intentional eculizumab discontinuation.

7.1.3. Study Year 1 and 2 –Patients Who Re-initiate Eculizumab

Re-initiation After the First Intentional Eculizumab Discontinuation:

Patients who re-initiate eculizumab will have a study visit within 1 week prior to re-initiation of eculizumab (Visit 1R). At Visit 1R, safety assessments will be performed prior to collecting blood and urine samples for biomarker tests. Blood samples will be collected for cRBC hemolytic assay, free C5, and PK (Table 5).

After Visit 1R, patients will have Q2W study visits from Week 2 through Week 10 (Visit 2R through Visit 6R) as specified in Table 5. Patients will have monthly study visits beginning at Month 3 through up to 2 years after the time of the first intentional eculizumab discontinuation. These monthly visits will follow the schedule as determined by the date of the first intentional eculizumab discontinuation. At these monthly visits, safety assessments will be performed prior to collection of samples for biomarker analyses. Blood samples for cRBC hemolytic assay, free C5, PK will be collected at time points specified in Table 5.

If a patient experiences a TMA manifestation, 2 TMA visits will be performed; the first TMA visit (Visit 1TR) will be conducted as soon as possible on recognition of signs and symptoms of TMA manifestation onset by the Investigator. A 2nd TMA visit (Visit 2TR) should be performed within 2 weeks of the first visit. Assessments and sample collection as specified in TMA Visits (Visit 1TR and Visit 2TR) in Table 5 will be performed.

Additional blood and/or urine samples may be collected as needed, at scheduled or unscheduled visits at the discretion of the Investigator or Treating Physician. Scheduled visits, when overlapping with other visits (eg, TMA visit, re-initiation visits), can be combined as appropriate.

Re-initiation After the Subsequent Intentional Eculizumab Discontinuation(s):

Similarly, for any subsequent eculizumab re-initiation, patients will have a re-initiation study visit within 1 week prior to re-initiation of eculizumab and follow the assessments as specified in Visit 1R (Table 5), Q2W study visits as specified in Visit 2R through Visit 6R, and monthly study visits thereafter for a total of 2 years from the time of the first intentional eculizumab discontinuation (ie, 2 years from Visit 1D). These monthly visits will follow the original schedule as determined by the data of the first intentional eculizumab discontinuation.

7.2. Schedule of Assessments

Table 3: Schedule of Assessments for Patients Receiving Ongoing Eculizumab Treatment (Recommended and Reduced/Increased Dosing Regimen)

	Baseline	Q2W (±2 days) Visits (Wk 2 through Wk 10)			Monthly (± 1 week) Visits for Patients Receiving Ongoing Eculizumab Treatment[a] Month 3 through Month 24 (Year 2)[b]										
Study Visits	**V1**	**V2**	**V3**	**V4 – V6**	**V7 – V9**	**V10**	**V11 - V15**	**V16**	**V17 – V21**	**V22**	**V23- V27**	**V28/ EOS*/ ET****	**TMA Visit**		**+UNS Visit**
Days/Months	**Day 0**	**Wk 2**	**Wk 4**	**Wk 6- Wk 10**	**M3 - M5**	**M6**	**M7 - M11**	**M12**	**M13- M17**	**M18**	**M19- M23**	**M24**	**^1T**	**^^2T**	
Study Assessments/Procedures															
Informed Consent[c]	X														
Inclusion/ Exclusion	X														
Medical history[d]	X														
Demographics	X														
Safety Assessments/Blood and Urine Samples for Laboratory Tests[e]															
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X		
Height[f] and Weight	X	X	X	X	X	X	X	X	X	X	X	X	X		
Complete Physical Examination[g]	X														
AEs	X	X	X	X	X	X	X	X	X	X	X	X	X		
Concomitant Medication/Therapy	X	X	X	X	X	X	X	X	X	X	X	X	X		
Laboratory Tests[h]															
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood Chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X		
Urinalysis	X	X	X	X	X	X	X	X	X	X	X	X	X		
Assessment of TMA manifestation[i]															
Changes in Specific Laboratory Parameters	X	X	X	X	X	X	X	X	X	X	X	X	X		
Renal Signs and Symptoms	X	X	X	X	X	X	X	X	X	X	X	X	X		
Extra-renal Signs and Symptoms	X	X	X	X	X	X	X	X	X	X	X	X	X		

Table 3: Schedule of Assessments for Patients Receiving Ongoing Eculizumab Treatment (Recommended and Reduced/Increased Dosing Regimen (Continued)

	Baseline	Q2W (±2 days) Visits (Wk 2 through Wk 10)			Monthly (± 1 week) Visits for Patients Receiving Ongoing Eculizumab Treatment[a] Month 3 through Month 24 (Year 2) [b]										
Study Visits	**V1**	**V2**	**V3**	**V4 – V6**	**V7 – V9**	**V10**	**V11 - V15**	**V16**	**V17 – V21**	**V22**	**V23- V27**	**V28/ EOS*/ ET****	**TMA Visit**		**+UNS Visit**
Days/Months	**Day 0**	**Wk 2**	**Wk 4**	**Wk 6- Wk 10**	**M3 - M5**	**M6**	**M7 - M11**	**M12**	**M13- M17**	**M18**	**M19- M23**	**M24**	**^1T**	**^^2T**	
Assessment of Eculizumab Treatment and Associated Supportive Interventions[j]															
Eculizumab Treatment Log	X	X	X	X	X	X	X	X	X	X	X	X	X		
PE/PI	X	X	X	X	X	X	X	X	X	X	X	X	X		
Dialysis	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood Transfusion	X	X	X	X	X	X	X	X	X	X	X	X	X		
Renal Transplantation	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood/Urine Samples for Genetic and Biomarker Analyses															
Genetic Tests[k]	X														
Biomarker Tests[l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood samples for the following															
cRBC Hemolytic assay[m]	X					X		X		X		X	X	X	
Free C5[m]	X					X		X		X		X	X	X	
PK[n]	X					X		X		X		X	X	X	
PRO Assessments[o]															
FACIT-Fatigue	X				M3 only	X									
EORTC QLQ-C30	X				M3 only	X									
Specific patient reported aHUS symptoms	X				M3 only	X									
Additional samples[p]	**At the Discretion of the Investigator, additional/more frequent samples may be collected at Scheduled Study Visits/Unscheduled Visits**														

Abbreviations: AE = adverse event; cRBC = chicken red blood cell; EIU = exposure in-utero; EORTC QLQ-C30 = European Organization for Research and Treatment of Cancer quality of life questionnaire; EOS = end-of-study; ET = early termination; FACIT-Fatigue = Functional Assessment of Chronic Illness Therapy Fatigue scale; PE/PI = plasma exchange/plasma infusion; T = trough sample; TMA = thrombotic microangiopathy; PK = pharmacokinetics; UNS = unscheduled visit

* If a patient completes all the Study Visits (Visits 1-28), Visit 28, the last Study Visit will be the end-of-study (EOS) visit, at which time the patient completes the study.

** If a patient withdraws early from the study during the Study Visits (Visits 1-28), an Early Termination (ET) Visit will be performed.

^ If a patient experiences a TMA manifestation as defined in Section 7.3, the TMA visit (Visit 1T) must be performed as soon as possible of symptom onset. Additional evaluation visits can be scheduled at the discretion of the investigator.

^^ The TMA visit (Visit 2T) must be performed within 2 weeks (±2 days) of Visit 1T.

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

[a] Treatment in this study is provided at the Treating Physician's discretion. Some patients may receive a reduced/increased eculizumab dosing regimen. All assessments and procedures will be performed prior to administration of eculizumab. If a patient's dose is reduced/increased after enrolling in the study, study assessments as specified in Section 7.4.2.2 and Table 6 will be performed at a Study Visit (UNS) at least 2 days prior to the visit when the reduced/increased eculizumab dosing regimen will be administered.

[b] Patients receiving ongoing eculizumab treatment (recommended or reduced/increased dosing regimen) without any intentional eculizumab discontinuation will have monthly assessments performed beginning at Month 3 to Month 24, a period of 2 years, at which time they will complete the study. Monthly visits are defined as every 28 days ie, every 4 weeks.

[c] On Day 0 (Baseline), patient or patient's guardian sign the informed consent. Patient or patient's parent/legal guardian must be willing and able to give written informed consent and the patient (if minor) must be willing to give written informed assent.

[d] Medical history data should be reviewed in order to determine eligibility, and this review should be documented; however, medical history data are only to be reported in the M11-001 Registry database and not through this study's database.

[e] Safety assessments (ie, vital signs, physical examination, laboratory tests, assessments of TMA manifestations and associated supportive interventions, AEs, and concomitant medications/therapies) will be collected at the specified clinic visits prior to sample collection for biomarker tests. For a summary of clinical laboratory tests see Appendix 1. In addition, pregnancy-, lactation-, and follow-up-data on neonates for 3 months after delivery, especially when the neonate has experienced eculizumab EIU, will also be collected.

[f] Height will be collected for adult patients only at Day 0 (Baseline). Height will be collected for pediatric patients at every visit.

[g] A complete physical examination will be performed only at Day 0 (Baseline)..

[h] Laboratory tests will include platelet count, serum albumin, serum LDH, serum creatinine, hemoglobin, haptoglobin, total bilirubin, full CBC, and urinalysis. Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab. For patients on eculizumab, if the scheduled dose falls within a study visit window, the study visit should be scheduled on the same day of the dose or within 48 hours prior to the dose, such that laboratory samples can be taken prior to the eculizumab dose. If the dose falls on the same day as the scheduled visit, blood samples are to be taken 5 to 90 minutes prior to administration. It is preferred that the dose is scheduled on the same day as the visit, if possible. Among patients receiving PE/PI or dialysis, blood and urine samples for laboratory tests will be collected prior to initiation of PE/PI or dialysis.

[i] TMA manifestation assessments of aHUS including renal and extra-renal manifestations.

[j] Associated supportive interventions including PE/PI, dialysis, blood transfusion, and renal transplantation. Among patients receiving PE/PI or dialysis, samples for analyses and assessments will be collected prior to initiation of PE/PI or dialysis.

[k] Sample for genetic testing will be collected on Day 0 (Baseline) of the study however, may alternatively be collected at any other visit after Day 0 (Baseline) during the study. A patient may be allowed to opt-out from providing a sample for genetic testing (eg, for religious reasons) and still continue to participate in the study.

[l] At Day 0 (Baseline), blood and urine samples collected for genetic and biomarker analysis should be collected after safety assessments have been performed and prior to administration of eculizumab. For all other time points, samples for biomarker analyses should be collected after safety assessments have been performed and prior to administration of eculizumab. Among patients receiving PE/PI or dialysis, blood and urine samples will be collected prior to initiation of PE/PI or dialysis. Unused samples will be stored for future analysis (see Section 14.1.3).

[m] For all patients receiving eculizumab, blood samples for cRBC hemolytic assay and free C5 will be collected every 6 months beginning at Month 6 (Visit 10) through Month 24 (Visit 28). For patients receiving eculizumab at a reduced/increased eculizumab dosing regimen, additional trough blood samples for cRBC

hemolytic assay and free C5 will be collected at the time of dose reduction and monthly thereafter until the patient continues to be on that reduced/increased eculizumab dosing regimen (see Schedule of Assessments provided in Table 6).

[n] Trough blood samples for PK tests are to be taken 5 to 90 minutes prior to administration of eculizumab. For patients on eculizumab, if the scheduled dose falls within a study visit window, the study visit should be scheduled on the same day of the dose or within 48 hours prior to the dose, such that laboratory samples can be taken prior to the eculizumab dose. If the dose falls on the same day as the scheduled visit, blood samples are to be taken 5 to 90 minutes prior to administration. It is preferred that the dose is scheduled on the same day as the visit, if possible. For all patients receiving eculizumab, blood samples for PK will be collected every 6 months beginning at Month 6 (Visit 10) through Month 24 (Visit 28). For patients receiving eculizumab at a reduced/increased eculizumab dosing regimen, additional blood samples for PK will be collected at the time of dose reduction and monthly thereafter until the patient continues to be on that reduced/increased eculizumab dosing regimen (see Schedule of Assessments provided in Table 6).

[o] Using FACIT-Fatigue (Appendix 2 and Appendix 3), EORTC QLQ-C30 (Appendix 4), and patient-reported aHUS symptoms (Appendix 5), PRO assessments will be performed.

[p] If a patient is experiencing a TMA manifestation additional blood samples may be collected at scheduled clinical visits/unscheduled visits, at the discretion of the Investigator.

Table 4: Schedule of Assessments for Patients Who Discontinue Eculizumab

	Patients Who Discontinue Eculizumab Treatment[a,b]											
	At Discontinuation (Within 2 Wks [±2 days] after last eculizumab dose)[c]	**Q2W (±2 days) after DC (Wk 2 through Wk 10)**			**Monthly (± 1 week) Visits Month 3 through Month 24 (Year 2)**							
Study Visits	**V1D**	**V2D**	**V3D**	**V4D – V6D**	**V7D**	**V8D –V9D**	**V10D**	**Visit 11D through Visit 27D**	**28D/ EOS* /ET****	**TMA**		**+UNS Visit**
Study Month/Week	**Day 0**	**Wk 2**	**Wk 4**	**Wk 6- Wk 10**	**M3**	**M4 M5**	**M6**	**Month 7 through Month 23**	**Month 24**	**^1TD**	**^^2TD**	
Safety Assessments[d]												
Vital Signs	X	X	X	X	X	X	X	X	X	X		
Height[e] and Weight	X	X	X	X	X	X	X	X	X	X		
AEs	X	X	X	X	X	X	X	X	X	X		
Concomitant Medication/Therapy	X	X	X	X	X	X	X	X	X	X		
Laboratory Tests[f]												
Hematology	X	X	X	X	X	X	X	X	X	X		
Blood Chemistry	X	X	X	X	X	X	X	X	X	X		
Urinalysis	X	X	X	X	X	X	X	X	X	X		
Assessments of TMA Manifestations[g]												
Changes in Specific Laboratory Parameters	X	X	X	X	X	X	X	X	X	X		
Renal Signs and Symptoms	X	X	X	X	X	X	X	X	X	X		
Extra-renal Signs and Symptoms	X	X	X	X	X	X	X	X	X	X		
Assessment of Eculizumab Treatment and Associated supportive interventions[h]												
Eculizumab Treatment Log	X	X	X	X	X	X	X	X	X	X		
PE/PI	X	X	X	X	X	X	X	X	X	X		
Dialysis	X	X	X	X	X	X	X	X	X	X		
Blood Transfusion	X	X	X	X	X	X	X	X	X	X		

Table 4: Schedule of Assessments for Patients Who Discontinue Eculizumab (Continued)

	Patients Who Discontinue Eculizumab Treatment[a,b]											
	At Dc (Within 2 Wks [±2 days] from last eculizumab dose)[c]	Q2W (±2 days) after DC (Wk 2 through Wk 10)			Monthly (± 1 week) Visits Month 3 through Month 24 (Year 2)							
Study Visits	**V1D**	**V2D**	**V3D**	**V4D – V6D**	**V7D**	**V8D –V9D**	**V10D**	**Visit 11D through Visit 27D**	**28D/ EOS* /ET****	**TMA**		**+UNS Visit**
Study Month/Week	**Day 0**	**Wk 2**	**Wk 4**	**Wk 6- Wk 10**	**M3**	**M4 M5**	**M6**	**Month 7 through Month 23**	**Month 24**	**^1TD**	**^^2TD**	
Renal Transplantation	X	X	X	X	X	X	X	X	X	X		
Blood/Urine Samples for Genetic and Biomarker Analyses												
Genetic Tests[i]												
Biomarker Tests[j]	X	X	X	X	X	X	X	X	X	X	X	
Blood samples for the following												
cRBC Hemolytic assay[k]	X	X	X		X				X	X	X	
Free C5[k]	X	X	X		X				X	X	X	
PK[l]	X	X	X		X				X	X	X	
PRO Assessments[m]												
FACIT-Fatigue	X				X		X					
EORTC QLQ-C30	X				X		X					
Specific patient reported aHUS symptoms	X				X		X					
Additional samples[n]	**At the Discretion of the Investigator, additional/more frequent samples may be collected at Scheduled Study Visits/Unscheduled Visits**											

Abbreviations: AE = adverse event; cRBC = chicken red blood cell; EIU = exposure in-utero; EORTC QLQ-C30 = European Organization for Research and Treatment of Cancer quality of life questionnaire; EOS = end-of-study; ET = early termination; FACIT-Fatigue = Functional Assessment of Chronic Illness Therapy Fatigue scale; PE/PI = plasma exchange/plasma infusion; T = trough sample; TMA = thrombotic microangiopathy; PK = pharmacokinetics; UNS = unscheduled visit

* If a patient completes all the Study Visits (Visits 1D-28D), Visit 28D, the last Study Visit will be the end-of-study (EOS) visit, at which time the patient completes the study.

** If a patient withdraws early from the study during the Study Visits (Visits 1D-28D), an Early Termination (ET) Visit will be performed.

^ If a patient experiences a TMA manifestation as defined in Section 7.3, the TMA visit (Visit 1TD) must be performed as soon as possible of symptom onset. Additional evaluation visits can be scheduled at the discretion of the investigator.

^^ The TMA visit (Visit 2TD) must be performed within 2 weeks (±2 days) of Visit 1TD.

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

[a] Patients who discontinue eculizumab will have monthly study visits for 2 years after the time of the first intentional eculizumab discontinuation. Q2W visits are defined as every 2 weeks ie, every 14 days. Monthly visits are defined as every 28 days ie, every 4 weeks.
[b] Reasons for intentional eculizumab treatment discontinuation will be systematically collected.
[c] Following the first intentional eculizumab discontinuation, patients will have a study visit within 2 weeks (±2 days) after the last eculizumab infusion.
[d] Safety assessments (ie, vital signs, physical examination, laboratory tests, assessments of TMA manifestations and associated supportive interventions, AEs, and concomitant medications/therapies) will be collected at the specified clinic visits prior to sample collection for biomarker tests. For a summary of clinical laboratory tests see Appendix 1. In addition, pregnancy-, lactation-, and follow-up-data on neonates for 3 months after delivery, especially when the neonate has experienced eculizumab EIU, will also be collected.
[e] Height will be collected ONLY for pediatric patients.
[f] Laboratory tests will include platelet count, serum albumin, serum LDH, serum creatinine, hemoglobin, haptoglobin, total bilirubin, full CBC, and urinalysis. Among patients receiving PE/PI or dialysis, blood and urine samples for laboratory tests will be collected prior to initiation of PE/PI or dialysis.
[g] TMA manifestation assessments of aHUS including renal and extra-renal manifestations.
[h] Associated supportive interventions including PE/PI, dialysis, blood transfusion, and renal transplantation. Among patients receiving PE/PI or dialysis, samples for analyses and assessments will be collected prior to initiation of PE/PI or dialysis.
[i] Sample for genetic testing will be collected on Day 0 (Baseline) of the study however, may alternatively be collected at any other visit after Day 0 (Baseline) during the study. A patient may be allowed to opt-out from providing a sample for genetic testing (eg, for religious reasons) and still continue to participate in the study.
[j] Samples for biomarker analyses should be collected after safety assessments have been performed. Among patients receiving PE/PI or dialysis, samples for analyses and assessments will be collected prior to initiation of PE/PI or dialysis. Unused samples will be stored for future analysis (see Section 14.1.3).
[k] Blood samples for cRBC hemolytic assay and free C5 will be collected as specified until 2 years after the first intentional eculizumab discontinuation.
[l] Blood samples for PK will be collected as specified until 2 years after the first intentional eculizumab discontinuation. Samples will not be evaluated for PK once levels of eculizumab are no longer detectable. Unused samples will be stored for future analysis (see Section 14.1.3).
[m] Using FACIT-Fatigue (Appendix 2 and Appendix 3), EORTC QLQ-C30 (Appendix 4), and patient-reported aHUS symptoms (Appendix 5), PRO assessments will be performed.
[n] If a patient is experiencing a TMA manifestation additional blood samples may be collected at scheduled clinical visits/unscheduled visits, at the discretion of the Investigator.

Table 5: Schedule of Assessments for Patients who Re-initiate Eculizumab

Patients Who Re-initiate Eculizumab Treatment[a,b]							
	Prior to Re-initiation of Eculizumab (within 1 week prior)	**Q2W (±2 days) after Re-initiation Wk 2 through Wk 10**	**Monthly (± 1 week) Visits, following schedule in Table 2 Determined by the Date of First Intentional Eculizumab Discontinuation**	**Patients Who Completed Monthly Study Visits For 2 Years After the First Intentional eculizumab discontinuation**			
Study Visits	**Visit 1R**	**V2R – V6R (Wk 2 through Wk 10 [±2 days] after Re-initiation)**	**Monthly from Visit 7R Through Up to 2 Years After the First Intentional eculizumab discontinuation Visit**	**EOS* /ET****	**TMA**		**+UNS Visit**
					^1TR	**^^2TR**	
Safety Assessments[c]							
Vital Signs	X	X	X	X	X		
Height[d] and Weight	X	X	X	X	X		
AEs	X	X	X	X	X		
Concomitant Medication/Therapy	X	X	X	X	X		
Laboratory Tests[e]							
Hematology	X	X	X	X	X		
Blood Chemistry	X	X	X	X	X		
Urinalysis	X	X	X	X	X		
Assessment of TMA manifestations[f]							
Changes in Specific Laboratory Parameters	X	X	X	X	X		
Renal Signs and Symptoms	X	X	X	X	X		
Extra-renal Signs and Symptoms	X	X	X	X	X		
Associated supportive interventions[g]							
Eculizumab Treatment Log	X	X	X	X	X		
PE/PI	X	X	X	X	X		

Table 5: Schedule of Assessments for Patients who Re-initiate Eculizumab (Continued)

Patients Who Re-initiate Eculizumab Treatment							
	Prior to Re-initiation of Eculizumab (within 1 week prior)	**Q2W (±2 days) after Re-initiation Wk 2 through Wk 10**	**Monthly (± 1 week) Visits, following schedule in Table 2 Determined by the Date of First Intentional Eculizumab Discontinuation**	**Patients Who Completed Monthly Study Visits For 2 Years After the First Intentional eculizumab discontinuation**			
Study Visits	**Visit 1R**	**V2R – V6R (Wk 2 through Wk 10 [±2 days] after Re-initiation)**	**Monthly from Visit 7R Through Up to 2 Years After the First Intentional eculizumab discontinuation Visit**	**EOS* /ET****	**TMA**		**+UNS Visit**
					^1TR	**^^2TR**	
Dialysis	X	X	X	X	X		
Blood Transfusion	X	X	X	X	X		
Renal Transplantation	X	X	X	X	X		
Blood/Urine Samples for Genetic and Biomarker Analyses							
Genetic Tests[h]							
Biomarker Tests[i]	X	X	X	X	X	X	
Blood samples for the following							
cRBC Hemolytic assay[j]	X		Only Every 6 Months Until 2 Years After the First Intentional Eculizumab Discontinuation (V10R, V16R, V22R)	X	X	X	
Free C5[j]	X			X	X	X	
PK[k]	X			X	X	X	
Additional samples[l]	**At the Discretion of the Investigator, additional/more frequent samples may be collected at Scheduled Study Visits/Unscheduled Visits**						

Abbreviations: AE = adverse event; aHUS = atypical hemolytic uremic syndrome; cRBC = chicken red blood cell; ET = early termination; PK = pharmacokinetics; EOS = end-of-study; TMA = thrombotic microangiopathy; UNS = unscheduled visit

* If a patient has completed all required study visits (for a total of 2 years from the time of the first intentional eculizumab discontinuation [Visit 1D]), the last Study Visit will be the end-of-study (EOS) visit, at which time the patient completes the study.

** If a patient withdraws early from the study prior to completing all required monthly visits, an ET Visit will be performed.

^ If a patient experiences a TMA manifestation as defined in Section 7.3, the TMA visit (Visit 1TR) must be performed as soon as possible of symptom onset. Additional evaluation visits can be scheduled at the discretion of the investigator.

^^ The TMA visit (Visit 2TR) must be performed within 2 weeks (±2 days) of Visit 1TR.

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

[a] Patients who re-initiate eculizumab treatment will have monthly study visits for 2 years after the time of the first intentional eculizumab discontinuation. Q2W visits are defined as every 2 weeks ie, every 14 days. Monthly visits are defined as every 28 days ie, every 4 weeks.

[b] Reasons for re-initiation will be systematically collected.

[c] Safety assessments (ie, vital signs, laboratory tests, assessments of TMA manifestations and associated supportive interventions, AEs, and concomitant medications/therapies) will be collected at the specified clinic visits prior to sample collection for biomarker tests. For a summary of clinical laboratory tests see Appendix 1. In addition, pregnancy-, lactation-, and follow-up-data on neonates for 3 months after delivery, especially when the neonate has experienced eculizumab EIU, will also be collected.

[d] Height will be collected ONLY for pediatric patients.

[e] Laboratory tests will include platelet count, serum albumin, serum LDH, serum creatinine, hemoglobin, haptoglobin, total bilirubin, full CBC, and urinalysis. Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab. Among patients receiving PE/PI or dialysis, blood and urine samples for laboratory tests will be collected prior to initiation of PE/PI or dialysis.

[f] TMA manifestation assessments of aHUS including renal and extra-renal manifestations.

[g] Associated supportive interventions including PE/PI, dialysis, blood transfusion, and renal transplantation.

[h] Samples for genetic testing will be collected on Day 0 (Baseline) of the study however, may alternatively be collected at any other visit after Day 0 (Baseline) during the study. A patient may be allowed to opt-out from providing a sample for genetic testing (eg, for religious reasons) and still continue to participate in the study.

[i] Samples for biomarker analyses should be collected after safety assessments have been performed and prior to administration of eculizumab. Among patients receiving PE/PI or dialysis, blood and urine samples will be collected 5 to 90 minutes prior to initiation of PE/PI or dialysis. Unused samples will be stored for future analysis (see Section 14.1.3).

[j] Blood samples for cRBC hemolytic assay and free C5 will be collected as specified until 2 years after the first intentional eculizumab discontinuation.

[k] Blood samples for PK will be collected as specified until 2 years after the first intentional eculizumab discontinuation.

[l] If a patient is experiencing a TMA manifestation additional blood samples may be collected at scheduled clinical visits/unscheduled visits, at the discretion of the Investigator.

Table 6: Schedule of Additional Assessments for Patients Receiving Eculizumab on a Reduced/Increased Dosing Regimen

Assessments	Patients Receiving Eculizumab on a Reduced/Increased Dosing Regimen	
	At time of dose reduction/increase (- 2 days)[a]	Monthly[a,b] (± 1Wk)
Biomarker Tests	X	
PK	X	X
cRBC Hemolytic Assay	X	X
Free C5	X	X

Abbreviations: cRBC = chicken red blood cell; PK = pharmacokinetics

[a] These assessments may occur during pre-scheduled monthly study visits or at unscheduled visits. This visit should be scheduled prior to the dose when possible; the visit window is within 2 days prior (-2D) to the reduction/increase. As with all other study visits, samples for assessments should be collected 5 to 90 minutes prior to the administration of eculizumab.

[b] For patients receiving eculizumab on a reduced/increased dosing regimen, monthly PK, cRBC, and free C5 will be drawn as long as the patient remains on a reduced/increased eculizumab dosing regimen.

7.3. Study Endpoints

7.3.1. Primary endpoint:

Rate of TMA manifestations, where TMA includes:

1. Hematologic or renal events due to aHUS
2. Extra-renal clinical signs and symptoms of aHUS
3. Tissue biopsy demonstrating TMA due to aHUS

7.3.2. Secondary endpoints:

- Change in estimated Glomerular Filtration Rate (eGFR) over time using the chronic kidney disease-epidemiology (CKD-EPI) formula
- Rate of TMA manifestations
  - while patient is on a labeled eculizumab dosing regimen
  - while patient is on a reduced/increased eculizumab dosing regimen (reduced/increased dose and or reduced/increased frequency)
- Incidence of PE/PI

7.3.3. Exploratory endpoints:

- Changes in exploratory biomarker levels:
  - Biomarkers may include, but are not limited to: Ba, TNFR1, sVCAM-1, TM, D-dimer, markers of renal injury (eg, Cystatin C or other) and assessments of complement deposition on endothelial cells
- Changes in PK (serum eculizumab concentration), pharmacodynamic (PD) (ie, cRBC hemolytic assay), free C5
- Identification of novel genetic variants associated with aHUS
- Development of progressive proteinuria
- Incidence of change in dosing of eculizumab:
  - Re-initiation of eculizumab after intentional discontinuation
  - Start of labeled dosing after reduced/increased dosing
- Incidence of transfusion
- Patient-reported outcomes (FACIT-Fatigue [Appendix 2 and Appendix 3], EORTC QLQ-C30 [Appendix 4], and patient-reported aHUS symptoms [Appendix 5])

7.3.4. Safety endpoints:

- Nature, severity, and relatedness to treatment of AEs (including serious adverse events [SAEs])

- laboratory tests (blood chemistry, hematology, and urinalysis)
- other assessments (vital signs)

7.3.5. Criteria for Site Reporting of Potential TMA Manifestations

Data on all hematologic, renal, extra-renal clinical signs and symptoms, and tissue biopsy results will be collected whether the physician deems related or not to aHUS. Information on etiology will be collected on clinical signs and symptoms deemed not related to aHUS will also be collected. Only criteria considered related to aHUS will be reported as a potential TMA manifestation under the primary endpoint.

For the purposes of reporting, a potential TMA manifestation is defined by at least one of the following criteria, although multiple criteria may be identified:

1. Hematologic or renal events due to aHUS

   A. The occurrence of two or more of the following 3 hematologic or renal events, where the changes from baseline* (see baseline definition below) in laboratory values are confirmed by a 2nd consecutive value derived from local and/or central laboratories; if local laboratory values are to be used, they must be reported to the sponsor):

- A decrease in platelet count by ≥25% below baseline and below the lower limit of normal (LLN)
- Evidence of hemolysis, defined by at least one of the following:
  - An increase in serum LDH by ≥25% above baseline and above the upper limit of normal (ULN)
  - A decrease in haptoglobin below the LLN
- A decline in kidney function that includes at least one of the following:
  - An increase in serum creatinine by ≥25% above baseline and above the ULN
  - An increase in proteinuria characterized by at least one of the following:
    - New onset of proteinuria: ≥2+ on urine dipstick or spot urine albumin to creatinine ratio ≥0.3 mg albumin/mg creatinine;
    - Worsening of existing proteinuria: ≥1.5 x increase in spot urine protein-to-creatinine ratio from baseline
    - New onset of nephrotic-range proteinuria: spot urine protein-to-creatinine ratio from baseline of >3.5 mg protein/mg creatinine in adults and >3.0 mg protein/mg creatinine in children
  - Requirement for renal replacement therapy, including dialysis or kidney transplant

   B. The occurrence of two or more of the following 3 hematologic or renal events, that do not necessarily meet all the criteria listed under #1A above, but are due to aHUS in the opinion of the Investigator:

- A clinically important decrease in platelet count

- Evidence of hemolysis, defined by at least one of the following:
  - A clinically important increase in serum LDH
  - A decrease in haptoglobin below the LLN
- A clinically important decline in kidney function that includes at least one of the following:
  - A clinically important increase in serum creatinine
  - A clinically important increase in proteinuria, for example: new onset proteinuria, worsening of existing proteinuria, or new onset of nephrotic range-proteinuria
  - Requirement for renal replacement therapy, including dialysis or kidney transplant related to a TMA event

*For reporting purposes, baseline is defined as follows:

For a patient on ongoing eculizumab treatment without any treatment discontinuation, the baseline is the value at Day 0. For a patient re-initiated on eculizumab treatment with at least one prior intentional discontinuation, the baseline for each on treatment period is the last laboratory value during the preceding intentional discontinuation period. For a patient intentionally discontinued from eculizumab treatment, the baseline for each off treatment period is the last value during the preceding ongoing eculizumab treatment period.

2. Extra-renal clinical signs and symptoms of aHUS

   The occurrence of extra-renal clinical signs and symptoms that in the opinion of the Investigator are due to aHUS AND at least one other test result that is consistent with the diagnosis of TMA related to aHUS OR excludes alternative etiologies of extra-renal signs and symptoms:

   - A single occurrence of any component of criterion #1A or #1B (hematologic or renal events due to aHUS) defined above (second/confirmatory value for laboratories not required)

     AND/OR

   - Relevant imaging study/studies demonstrating either tissue damage or excluding etiologies other than aHUS (eg, abdominal ultrasound that shows no gallstone in a setting of pancreatitis)

     AND/OR

   - Relevant laboratory data ruling out etiologies other than aHUS (eg, negative hepatitis serologies)

3. Tissue biopsy demonstrating TMA due to aHUS

7.3.6. Adjudication of Potential TMA Manifestations

Events reported as meeting criteria (#1A, #1B, 2, or 3) listed in Section 7.3.5 above as well as potential events not indicated as meeting criteria will be adjudicated by an independent Endpoint Adjudication Committee as detailed in the Adjudication Committee Charter (Section 9.2.1).

7.4. Study Visit Procedures

Treatment for aHUS will remain observational and at the discretion of the Treating Physician. At a given time, patients may be receiving ongoing eculizumab treatment, intentionally discontinued from eculizumab treatment, or re-initiated on eculizumab treatment. Based on the eculizumab treatment status of the patient, assessments and procedures as specified in the following sections (Section 7.4.1 through Section 7.4.6) will be performed, as applicable.

At visits where blood and urine samples are collected for biomarker analyses, safety assessments will be performed prior to sample collection for biomarker analyses.

At visits where administration of eculizumab is scheduled, all sample collection should be performed 5 to 90 minutes prior to eculizumab infusion. If the scheduled eculizumab dose falls within a study visit window, the study visit should be scheduled on the same day of the dose or within 48 hours prior to the dose, such that laboratory samples can be taken prior to the eculizumab dose. If the scheduled dose falls on the same day as the scheduled visit, blood samples are to be taken 5 to 90 minutes prior to administration. It is preferred that the dose is scheduled on the same day as the visit, if possible.

Due to blood volume limitations for lower body weight patients or due to other clinical factors per the Investigator's discretion, blood samples will be collected based on the prioritization scheme described in the Laboratory Manual for this study. In addition, detailed instructions for sample collection, preparation, and handling are provided in the Laboratory Manual.

7.4.1. Core Procedures Performed At <u>All</u> Regularly Scheduled Study Visits and the 1st TMA Visits

The following will be performed at <u>all</u> study visits for patients on ongoing treatment (V1 through V28/EOS/ET), with intentional discontinuation (V1D through V28D/EOS/ET), or re-initiation of eculizumab, (V1R through V28R/EOS/ET) as well as the 1st TMA visit (TMA 1T, 1TD, and 1TR): (*note: these procedures are <u>not</u> required for the **TMA 2T, 2TD, or 2TR visits***)

- Core Procedure Safety assessments (Note: All safety assessments and sample collection should be performed prior to eculizumab infusion)
  - Measure vital signs, including assessments of systolic and diastolic blood pressure (BP), temperature, respiration rate (RR), and heart rate (HR)
  - Height (to be done at baseline for all patients; at subsequent visits, only required for pediatric patients) and weight
  - Evaluate and record any new AEs or changes in AEs since the previous visit; at baseline, an initial assessment of AEs should occur
  - Record any new medications or changes to concomitant medications/therapies
  - Obtain blood and urine samples for the laboratory tests: Hematology, Blood Chemistry, and Urinalysis; see Appendix 1
  - Assessments of TMA manifestations including changes in specific laboratory parameters, renal and/or extra-renal signs and symptoms (see Section 7.3)

  - For assessments of eculizumab treatment and associated supportive interventions, record the following:
    - eculizumab treatment regimen in treatment log
    - PE/PI
    - blood transfusion
    - dialysis
    - renal transplantation

- Core Procedure Study-specific tests: After completing the safety assessments, the following study-specific tests will be performed:
  - Obtain blood and urine samples for biomarker analyses

7.4.2. Visits for TMA Manifestation for all patients regardless of eculizumab treatment status: Visit 1T/1TD/1TR and Visit 2T/2TD/2TR

The procedures at Visit 1T are the same as for Visit 1TD and Visit 1TR; however, the appropriate visit label and visit label abbreviations should be utilized to allow for clear data collection regarding the patient's eculizumab treatment status (ie, "D" denotes intentional discontinuation, "R" denotes re-initiation following an intentional discontinuation, and for patients on ongoing treatment the additional letter is omitted). Similarly, the procedures at Visit 2T are the same as for Visits 2TD and 2TR; however, the appropriate visit label/abbreviation should be used according to the patient's eculizumab treatment status.

In summary, 2 TMA manifestation visits will be performed. The 1st TMA manifestation visit (Visit 1T or 1TD or 1TR) should be performed as soon as possible on recognition of signs and/or symptoms of TMA manifestation onset by the Investigator. A 2nd TMA manifestation visit (Visit 2T or 2TD or 2TR) should be performed within 2 weeks of Visit 1T/1TD/1TR. These visits may be combined with already scheduled study visits, if overlapping with the scheduled study visit window.

Additional evaluation visits can be scheduled at the discretion of the Investigator.

7.4.2.1. First (1st) TMA Visit - Upon Recognition of TMA Manifestation

The 1st TMA manifestation visit (Visit 1T/1TD/1TR) should be performed as soon as possible on recognition of signs and/or symptoms of TMA manifestation onset by the Investigator. Additional evaluation visits can be scheduled at the discretion of the Investigator.

In addition to the core procedures listed in Section 7.4.1, the following tests and procedures will be performed for Visit 1T/1TD/1TR (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing

7.4.2.2. Second (2nd) TMA Visit - Within 2 weeks [±2 days] of the 1st TMA Visit

Note that the core procedures listed in Section 7.4.1 are not required at this visit.

Only the following tests and procedures will be performed at the 2nd TMA visit (Visit 2T/2TD/2TR):

- Obtain blood and urine sample, as applicable, for the following:
  - Biomarker analyses
  - cRBC hemolytic assay
  - PK testing
  - Free C5

7.4.3. Data Collection (During Year 1 and 2) for Patients Receiving Ongoing Eculizumab

Following the Day 0 / Baseline Visit 1, patients will have study visits every two weeks (Q2W) until Week 10 (Visit 2 through Visit 6). Beginning at Month 3 (Visit 7), patients will have monthly study visits for a total of 2 years (Visit 7-28), unless there is an intentional discontinuation in eculizumab treatment. If there is an intentional discontinuation of eculizumab, the procedures in Section 7.4.5 should be followed.

All Q2W and monthly visits (V1 through V28) for patients receiving ongoing Eculizumab Treatment will contain the **core procedures** listed in Section 7.4.1.

7.4.3.1. Additional Data Collection on Day 0 (Baseline)/Visit 1 for Patients Receiving Ongoing Eculizumab Treatment

In addition to the **core procedures** listed in Section 7.4.1, at the Day 0 (Baseline)/Visit 1, after obtaining the signed ICF, the following will be performed:

- Review inclusion and exclusion criteria
- Review medical history including aHUS diagnosis history. Note: medical history data are only to be reported in the M11-001 aHUS Registry database and not through this study's database
- Record demographics including age and sex of the patient
- Complete physical examination will include assessments of the following organ/body systems: skin, head, ears, eyes, nose, throat, neck, lymph nodes, pulse, chest, heart, abdomen, extremities, musculoskeletal, and general neurologic examination

After completing the safety assessments, the following will be performed:

- Obtain blood sample for genetic testing (this sample may be collected at this visit or at any visit after Day 0 (Baseline)/Visit 1 during the study)
- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolysis assay

  - PK testing

- Patient-reported outcomes:
  - FACIT-Fatigue
  - EORTC QLQ-C30
  - aHUS symptoms

7.4.3.2. Additional Data Collection at the Month 3 and Month 6 Visits for Patients Receiving Ongoing Eculizumab Treatment

In addition to the **core procedures** listed in Section 7.4.1, the following will be performed at the Month 3 and Month 6 Visits.

- Patient-reported outcomes:
  - FACIT-Fatigue
  - EORTC QLQ-C30
  - aHUS symptoms

7.4.3.3. Additional data Collection at 6 Month Visits for Patients Receiving Ongoing Eculizumab Treatment

In addition to the **core procedures** listed in Section 7.4.1, the following will be performed at 6 Month Visits ie Visits V10/Month 6, V16/Month12, V22/Month 18, and V28/Month24/EOS/ET:

- Obtain blood sample for the following (note: protocol-specific tests should always be collected after the safety assessments):
  - Free C5
  - cRBC hemolysis assay
  - PK testing

7.4.3.4. Visit 28 (Month 24)/End-of-Study (EOS)/Early Termination (ET) Visit for Patients Receiving Ongoing Eculizumab Treatment:

An End-of-Study (EOS) visit will be performed when a patient has completed all Study Visits (Visits 1 through 27) before a patient leaves the study or the study is terminated by the Sponsor or by a Regulatory Authority. An Early Termination (ET) visit will be performed if a patient withdraws early during the study. The procedures indicated in Section 7.4.3.3 should be performed.

7.4.4. Additional Data Collection (During Year 1 and 2) for Patients Receiving Ongoing Eculizumab Treatment at a Reduced/Increased Dosing Regimen

Evaluations for patients receiving ongoing eculizumab treatment at a reduced/increased dosing regimen will be the same as for patients receiving ongoing eculizumab treatment at a label-recommended dosing regimen.

Tests, procedures, and assessments listed in Section 7.4.3, as appropriate, will be performed.

7.4.4.1. Additional Data Collection at the Time of Eculizumab Dose/Dosing Reduction/Increase

As specified earlier, at the discretion of the Treating Physician, patients may receive a reduced/increased eculizumab dose or reduced/increased dosing frequency (Section 7.1). If a patient's dose is reduced or increased after enrolling in the study, the following assessments and procedures will be performed on the visit day when the reduced/increased eculizumab dose regimen will be administered, or within 2 days prior, per the visit window. These visits may occur during pre-scheduled monthly study visits or at unscheduled visits.

The tests and procedures performed are:

- Obtain blood and urine samples for biomarker analyses
- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing

7.4.4.2. Additional Data Collection Every Month for Patients on a Reduced/Increased Dosing Regimen

In addition to the procedures for patients receiving a label-recommended dosing regimen, patients receiving eculizumab at a reduced/increased dosing regimen will require the following data to be collected on a monthly basis following the initial reduction/increase:

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing

These monthly procedures are recommended to occur during pre-scheduled monthly study visits or may occur at unscheduled visits.

7.4.5. Data Collection for Patients After Intentional Eculizumab Treatment Discontinuation and After any Subsequent Intentional Discontinuation(s)

Following the first intentional eculizumab discontinuation, patients will have a study visit (Visit 1D) within 2 weeks after the last eculizumab infusion. Patients will then have Q2W study visits until Week 10 (Visit 2D through Visit 6D). Beginning at Month 3 (Visit 7D), patients will have monthly study visits for a total of 2 years (Visit 7D-28D).

For any subsequent intentional eculizumab discontinuation, patients will follow the assessments as specified in Visit 1D, and Q2W study visits thereafter as specified in Visit 2D through 6D. Patients will have monthly visits beginning at Month 3 after the intentional eculizumab discontinuation for a total of 2 years from the time of the first intentional eculizumab

discontinuation. The monthly visits will follow the schedule as determined by the date of the first intentional eculizumab discontinuation visit (Visit 1D).

All Q2W and monthly visits for patients with a first or subsequent intentional eculizumab discontinuation will contain the **core procedures** listed in Section 7.4.1. The following visits listed in Section 7.4.5.1 through 7.4.5.3 will require additional procedures as listed below

7.4.5.1. Additional Data Collection at the Time of Intentional Discontinuation/Visit 1D (2 weeks [±2 days] After the Last Eculizumab Infusion)

In addition to the **core procedures** listed in Section 7.4.1, the following will be performed (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing
- Patient-reported outcomes:
  - FACIT-Fatigue
  - EORTC QLQ-C30
  - aHUS symptoms

7.4.5.2. Additional Data Collection at V2D/Week 2, 3D/Week 4, and V7D/Month 3 for Patients After Intentional Eculizumab Treatment Discontinuation

In addition to the **core procedures** listed in Section 7.4.1, the following will be performed (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing

7.4.5.3. Additional Data Collection at V7D/Month 3 and V10D/Month 6 for Patients After Intentional Eculizumab Treatment Discontinuation

In addition to the core procedures listed in Section 7.4.1, the following PRO will be collected.

- Patient-reported outcomes:
  - FACIT-Fatigue
  - EORTC QLQ-C30
  - aHUS symptoms

7.4.5.4. Visit 28D/Month 24/End-of-Study (EOS)/Early Termination (ET) Visit for Patients After Intentional Eculizumab Treatment Discontinuation

An EOS visit will be performed when a patient has completed all Study Visits (Visits 1D through 27D) before a patient leaves the study or the study is terminated by the Sponsor or by a Regulatory Authority. An ET visit will be performed if a patient withdraws early during the study.

In addition to the **core procedures** listed in Section 7.4.1, the following will be performed (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing

7.4.6. Data Collection for Patients who Re-initiate Eculizumab Treatment After an Intentional Discontinuation: Re-initiation After the First Intentional Eculizumab Discontinuation and Any Subsequent Re-initiation(s)

Following an intentional eculizumab discontinuation, patients who re-initiate eculizumab treatment will have a study visit (Visit 1R) at the time of re-initiation i.e. within 1 week prior to re-initiation. Patients will then have Q2W study visits until Week 10 (Visit 2R through Visit 6R). Beginning at Month 3 (Visit 7R), patients will have monthly study visits for a total of 2 years (Visit 7R-EOS/ET) from the first intentional eculizumab discontinuation.

All Q2W and monthly visits for patients who re-initiate eculizumab will contain the **core procedures** listed in Section 7.4.1. The following visits listed in Section 7.4.6.1 through 7.4.6.3 will require additional procedures as listed below.

7.4.6.1. Additional Procedures at the Time of Eculizumab Re-initiation/Visit 1R (1 Week Prior to Re-initiation [±2 days])

In addition to the core procedures listed in Section 7.4.1, the following will be performed (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolytic assay
  - PK testing

7.4.6.2. Additional data Collection at 6 Month Visits: Visits V10R/Month 6, V16R/Month 12, V22R/Month 18 for Patients who Re-initiate Eculizumab Treatment After an Intentional Discontinuation

In addition to the core procedures listed in Section 7.4.1, the following will be performed at 6 Month Visits ie, Visits V10R/Month 6, M16R/Month 12, V22R/Month 18 (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolysis assay
  - PK testing

7.4.6.3. End-of-Study (EOS)/Early Termination (ET) Visit for Patients who Re-initiate Eculizumab Treatment After an Intentional Discontinuation

An EOS visit will be performed when a patient has completed all required study visits (for a total of 2 years from the time of the first intentional eculizumab discontinuation visit [Visit 1D]) or the study is terminated by the Sponsor or by a Regulatory Authority. An ET visit will be performed if a patient withdraws early during the study.

In addition to the core procedures listed in Section 7.4.1, the following will be performed (note: protocol-specific tests should always be collected after the safety assessments):

- Obtain blood sample for the following:
  - Free C5
  - cRBC hemolysis assay
  - PK testing

7.4.7. Visits at the Discretion of the Investigator

If a patient is experiencing a TMA manifestation additional blood samples may be collected at scheduled clinical visits/unscheduled visits, at the discretion of the Investigator.

7.4.8. Unscheduled Visits

Additional (Unscheduled) visits outside the specified visits are permitted at the discretion of the Investigator. Procedures, tests, and assessments will be performed at the discretion of the Investigator. If an Unscheduled Visit is performed, any tests, procedures, or assessments performed at the Unscheduled Visits must be recorded on the eCRFs.

7.4.9. Missing Visits

Patients who fail to return for a scheduled visit must be contacted by the site study staff to determine the reason for missing the appointment. For patients who are being treated with eculizumab and who miss a dose, the Investigator may contact the Medical Monitor to discuss the status and future treatment of these patients. Patients should be strongly encouraged to return to the study site for evaluation if a TMA manifestation or an AE is suspected to have occurred.

As it is vital to obtain information on a patient's missing visit to assure the missing appointment was not due to a TMA manifestation or an AE, every effort must be made to follow-up with the patient. Follow-up due diligence documentation will consist of 3 phone calls followed by 1 registered letter to the patient's last known address, and documented in both the source documents and the eCRF.

In cases where a scheduled study visit at the site cannot occur, data may be collected via telephone, where possible, or remotely, as permissible by and in accordance with all applicable regulatory requirements.

8. SELECTION AND WITHDRAWAL OF PATIENTS

8.1. Patient Participation

Any M11-001 aHUS Registry patient currently on eculizumab treatment and meeting all the eligibility criteria will receive information from the Investigator regarding participation and will sign a consent form prior to their enrollment in this study. Patients must remain enrolled in the M11-001 aHUS Registry in order to remain eligible to participate in the current study. Any patient discontinuing from the M11-001 aHUS Registry must discontinue from the current study. Day 0 (Baseline) assessments of a patient will commence only after patient's consent is documented.

8.2. Inclusion Criteria

Patients of any age, including minors, enrolled in the M11-001 aHUS Registry should meet all of the inclusion criteria below to be eligible for enrollment in the study:

1. Currently receiving eculizumab treatment in the M11-001 aHUS Registry
2. Two normal platelet counts (eg, >150,000/μL) at least 4 weeks apart while on eculizumab within the past 12 months
3. Two normal LDH levels (eg, <1.5 x ULN) at least 4 weeks apart while on eculizumab within the past 12 months
4. Willing, committed, and able to return for ALL clinic visits and complete all study-related procedures
5. Patient or patient's parent/legal guardian must be willing and able to give written informed consent. Patient (if minor) must be willing to give written informed assent (if applicable as determined by the central Institutional Review Boards/Independent Ethics Committees [IRB/IEC]).

8.3. Exclusion Criteria

Patients enrolled in the M11-001 aHUS Registry Study who meet any 1 of the following criteria will be excluded from the study:

1. Any prior intentional discontinuation of eculizumab treatment
2. On chronic dialysis (defined as ≥3 months on dialysis)
3. Currently participating in another complement inhibitor trial
4. With life expectancy of <6 months
5. Patient or patient's parent/legal guardian unable to give written informed consent. Patient (if minor) unable to give written informed assent (if applicable as determined by the central Institutional Review Boards/Independent Ethics Committees [IRB/IEC]).

8.4. Patient Withdrawal Criteria

8.4.1. Withdrawal of Patients From the Study

Patients may decide to withdraw participation in the study (by notifying the Investigator verbally or in writing) at any time without penalty and without affecting future medical care. Every effort should be made to ensure patients are willing to comply with study participation prior to conducting the Day 0 (Baseline) procedures.

Patients may decide to withdraw participation in the study and can still continue to participate in the M11-001 aHUS Registry. However, any patient discontinuing from the M11-001 aHUS Registry must discontinue from the current study.

Investigators may choose to withdraw a patient because of an AE or because of conditions or illnesses that preclude compliance with the protocol from the standpoint of the patient's safety or well-being (safety, behavioral or administrative reasons).

8.4.2. Handling of Withdrawals

When a patient withdraws or is withdrawn from the study, the Investigator shall record the withdrawal reason(s). Whenever possible, all patients who prematurely withdraw from the study will undergo all assessments at the ET visit for safety as per the Schedule of Assessments (Table 3, Table 4, and Table 5).

If a patient is withdrawn due to an AE, the event will be followed until it is resolved or in the opinion of the Investigator the patient is determined to be medically stable. Every effort will be made to undertake protocol-specified safety follow-up procedures.

Patients who fail to return for final assessments will be contacted by the study site staff in an attempt to request them to comply with the protocol. As it is vital to obtain follow-up data on any patient withdrawn because of an AE or SAE, follow-up due diligence documentation will consist of 3 phone calls followed by 1 registered letter to the patient's last known address. Every effort must be made to undertake protocol-specified safety follow-up procedures.

8.4.3. Sponsor's Termination of Study

Alexion may terminate the study at any time for any reason including, for example, clinical or administrative reasons.

8.4.4. End of Study Definition

The end of study is defined as the last visit completed by the last patient.

9. STUDY ASSESSMENTS

9.1. Safety Parameters

9.1.1. Vital Signs

Vital signs will be measured at every visit and will include assessments of systolic and diastolic blood pressure (BP), temperature, respiration rate (RR) and heart rate (HR). Vital signs will be obtained after the patient has been supine or seated for at least 5 minutes. Ideally, each patient's BP should be measured using the same arm. Systolic and diastolic BPs will be documented in mmHg. Temperature will be obtained in degrees Celsius or Fahrenheit. HR will be documented in beats per minute.

9.1.2. Physical Examination

A complete physical examination will be performed only at the Baseline visit. The complete physical examination will include assessments of the following organ/body systems: skin, head, ears, eyes, nose, throat, neck, lymph nodes, pulse, chest, heart, abdomen, extremities, musculoskeletal, and general neurologic examination.

9.1.3. Laboratory Tests

Blood and urine for laboratory tests should be collected 5 to 90 minutes prior to administration of eculizumab. For patients on eculizumab, if the scheduled dose falls within a study visit window, the study visit should be scheduled on the same day of the dose or within 48 hours prior to the dose, such that laboratory samples can be taken prior to the eculizumab dose. If the scheduled dose falls on the same day as the scheduled visit, blood samples are to be taken 5 to 90 minutes prior to administration. It is preferred that the dose is scheduled on the same day as the visit, if possible.

Blood Chemistry

Sodium	Total protein, serum	Lactate dehydrogenase (LDH)
Potassium	Creatinine	Isoenzymes
Chloride	Blood urea nitrogen (BUN)	Total bilirubin
Carbon dioxide/bicarbonate	Aspartate aminotransferase (AST)	Total cholesterol*
Calcium	Alanine aminotransferase (ALT)	Triglycerides
Glucose	Alkaline phosphatase	Uric acid
Albumin	Creatine phosphokinase (CPK)	

*(low-density lipoprotein [LDL] and high-density lipoprotein [HDL])

Hematology

Hemoglobin	Red blood cells (RBCs)
Haptoglobin	White blood cells (WBCs)
Platelet count	Complete blood count

Urinalysis

Color	Glucose	RBC
Clarity	Blood	Hyaline and other casts
pH	Bilirubin	Bacteria
Specific gravity	Leukocyte esterase	Epithelial cells
Ketones	Nitrite	Crystals
Protein	WBC	Yeast
Protein/Creatinine ratio		

9.2. Assessments for TMA Manifestations

Assessments for TMA manifestations will be performed at every visit. Changes in specific laboratory parameters (platelet count, serum creatinine, serum LDH, haptoglobin, and urinalysis) along with renal and extra-renal signs and symptoms as defined in Section 7.3 will be assessed at these visits, and can be derived from local and/or central laboratories. Data will be collected regardless of relationship to TMA in the opinion of the Investigator.

9.2.1. Endpoint Adjudication Committee

Data supporting the primary endpoint of TMA manifestations, blinded to eculizumab treatment status, will be reviewed by the Endpoint Adjudication Committee on a regular basis for purposes of adjudicating the primary endpoint. A description of the data included in the review, composition of the committee, and other relevant information will be detailed in the Endpoint Adjudication Committee Charter.

9.3. Assessments of Eculizumab Treatment and Associated Supportive Interventions

For assessment of eculizumab treatment, the following information will be collected at every visit in the eculizumab treatment log:

- eculizumab dose administered
- eculizumab dosing frequency
- any changes in eculizumab dosing regimen (dose and/or frequency)

  - reason for change in dosing regimen
- whether eculizumab was discontinued
  - reason for intentional eculizumab treatment discontinuation
- whether eculizumab was re-initiated
  - reason for re-initiation

For the assessment of associated supportive interventions, the use of treatments such as PE/PI, dialysis, blood transfusions, and renal transplantation will be collected at every visit during the study.

9.4. Contraception

All patients entering into the study are treated with Soliris per recommendations of local marketing authorization approval. Although there have been no adequate and well-controlled studies of Soliris in pregnant women, Soliris is expected to cross the placenta. Local Soliris labeling may recommend contraception during treatment for women of childbearing potential. For example, the European SmPC recommends for women of childbearing potential to use effective contraception during treatment and up to 5 months after treatment with Soliris. Investigators must provide recommendations on contraception, if necessary, as per local label.

9.5. Adverse Events

9.5.1. Detection of Adverse Events

The Investigator is responsible for detecting, assessing, documenting and reporting of all Adverse Events (AEs).

AEs reported by the patient and/or parent or legal guardian and/or identified in response to an open-ended question from study personnel or revealed by observation, physical examination, or other study procedures must be collected and recorded.

9.5.2. Definition of an Adverse Event

Adverse event means any untoward medical occurrence in a patient or clinical study patient administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

All observed or volunteered AEs regardless of causal relationship will be recorded and reported as described in the sections below.

For all AEs the Investigator must obtain adequate information for the following: (1) determine the outcome of the AE; (2) determine if the event meets criteria for a serious adverse event (SAE); (3) assess the severity of the AE, and (4) determine the causality of the AE. For AEs with a causal relationship to the treatment, the Investigator must follow-up on the outcome of the event until the event or sequelae either resolve or stabilize.

For each AE, the Investigator will be responsible for assessing seriousness, severity, and relationship of the AE to product.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition, and abnormal laboratory findings that are considered to be of clinical significance are all to be considered AEs.

The definition of AE covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital), and anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen are not AEs.

9.5.2.1. Abnormal Test Finding

Abnormal test findings may be considered AEs or SAEs; however, Investigators are strongly encouraged to report the diagnosis, sign or symptom instead of just the abnormal result. The criteria for an abnormal test finding being classified as an AE or SAE are as follows:

- Test result is associated with a sign or symptom;
- Test result requires additional diagnostic testing;
- Test result requires a medical or surgical intervention;
- Test result leads to a change in study dosing outside of the protocol defined dosing or discontinuation from the study;
- Test result requires significant additional treatment (ie, addition of new medication, significant increase in dose of current medication).

9.5.2.2. Serious Adverse Event and SAE Criteria

Any AE that fulfills any 1 of the criteria listed below must be recorded as an SAE. An SAE (experience) or reaction is described as any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Important medical event

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately

life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

9.5.2.3. Hospitalization

AEs that are associated with hospitalization or prolongation of hospitalization are considered SAEs. All admissions to a health care facility meet this criteria, even if less than 24 hours. Criteria for seriousness are also met if transfer within the hospital is done to receive more intense medical/surgical care (eg, medical floor to the intensive care unit [ICU]).

Hospitalization does not include the following:

- Rehabilitation facility
- Hospice facility
- Nursing facility
- Emergency Room
- Same day surgery

Hospitalization or prolongation of hospitalization not associated with an AE is not an SAE, examples include:

- Admission for a pre-existing condition not associated with either a new AE or with worsening of a pre-existing AE
- Protocol-specified admission
- Pre-planned admission

9.5.2.4. Procedures

Elective procedures that were pre-planned prior to the time that written ICF was obtained are not AEs. Any complication or worsening of a pre-existing condition leading to the procedure must be considered an AE. In addition, any AE that could occur as an outcome of the planned procedure should be considered as an AE.

Diagnostic and therapeutic procedures (invasive and non-invasive) such as surgery or angiography should not be reported as an AE or SAE. However, the medical condition or the diagnosis that was responsible for the procedure should be recorded. The procedure should be recorded in the narrative as treatment for the AE or SAE (eg, laparoscopic cholecystectomy is the procedure or treatment for an SAE of necrotic gall bladder).

9.5.2.5. Events of Interest

Events of Interest (EOI) will be identified by the Drug Safety Physician and if applicable also by the Clinical Trial Team Physician during the evaluation of safety data for the Clinical Study Report. Significant AEs of particular clinical importance, other than SAEs and those AEs

leading to discontinuation of the patient from the study will be classified as other AEs. For each EOI, a narrative may be written and included in the Clinical Study Report.

9.5.2.6. Severity Assessment

AE severity will be rated by the Investigator as mild, moderate, or severe using the following criteria:

- Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate: events result in a low level of inconvenience or concerns with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

If an AE changes in severity over the duration of the event, the maximum (ie, worst) severity should be reported.

Severity and seriousness must be differentiated. Severity describes the intensity of an AE, while the term seriousness refers to an AE that has met the criteria for an SAE.

9.5.2.7. Causality Assessment

An Investigator causality assessment (Unrelated, Unlikely, Possible, Probable, or Definite) must be provided for all AEs (both serious and non-serious). This assessment must be recorded in the eCRF and any additional SAE forms as appropriate. The definitions for the causality assessments appear below.

- Not related (unrelated): This relationship suggests that there is no association between eculizumab and the reported event.
- Unlikely related: This relationship suggests that the clinical picture is highly consistent with a cause other than eculizumab, but attribution cannot be made in absolute certainty and a relationship between eculizumab and AE cannot be excluded with complete confidence.
- Possibly related: This relationship suggests that treatment with eculizumab may have caused or contributed to the AE, i.e. the event follows a reasonable temporal sequence from the time of drug administration and/or follows a known response pattern to eculizumab, but could also have been produced by other factors.
- Probably related: This relationship suggests that a reasonable temporal sequence of the event with eculizumab administration exists and the likely association of the event with eculizumab. This will be based upon the known pharmacological action of eculizumab, known or previously reported adverse reactions to eculizumab or class of drugs, or judgment based on eculizumab's clinical experience.
- Definitely related: Temporal relationship to eculizumab, other conditions (concurrent illness, concurrent medication reaction, or progression/expression of disease state) do

not appear to explain event, corresponds with the known pharmaceutical profile, improvement on discontinuation, re-appearance on re-challenge.

9.5.2.8. Exposure during Pregnancy and Lactation

Pregnancy data will be collected during this study for all patients.

For all Alexion products, both in development or post approval, exposure during pregnancy must be recorded and followed. Exposure during pregnancy also called exposure in-utero (EIU) can be the result of either maternal exposure or transmission of drug product via semen following paternal exposure.

If a patient within this study or a patient's partner becomes or is found pregnant while treated or exposed to eculizumab, the Investigator must submit a pregnancy form to Alexion via the same method as SAE reporting. Pharmacovigilance will supply the Investigator with a copy of a "Pregnancy Reporting and Outcome Form / Breast Feeding". Pharmacovigilance must be notified.

Exposure of an infant to an Alexion product during breastfeeding would need to be reported in the "Pregnancy Reporting and Outcome Form / Breast Feeding", and any AEs an infant may experience following breastfeeding needs to be reported to Alexion Pharmacovigilance or its delegate.

The patient or a female partner should be followed until the outcome of the pregnancy is known (spontaneous miscarriage, elective termination, normal birth or congenital abnormality), even if the patient discontinued treatment with eculizumab or withdraws from the study. When the outcome of the pregnancy becomes known the form should be completed and returned to Alexion Pharmacovigilance or its delegate. If additional follow-up is required, the Investigator will be requested to provide the information.

Pregnancy in itself is not regarded as an AE unless there is a suspicion that eculizumab may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs and many may meet criteria for a SAE. Complications of pregnancy and abnormal outcomes of pregnancy such as ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly would meet criteria of a SAE and thus, should be reported as an SAE. Elective abortions without complications should not be handled as AEs. Pregnancy-, lactation-, and follow-up-data on neonates for 3 months after delivery, especially when the neonate has experienced eculizumab EIU, will be collected.

9.5.2.9. Withdrawal due to an AE

Withdrawal from the study due to an AE or SAE must be clearly differentiated from withdrawal due to other reasons.

9.5.2.10. Reporting Period

For SAEs, the reporting period to Alexion Pharmacovigilance or its delegate begins following the patient's signing of the ICF (providing consent to participate in the study) and continues through the patient's EOS/ET Visit.

For non-serious AEs, the reporting period begins following the patient's signing of the ICF (providing consent to participate in the study) and continues through the last visit (EOS/ET).

All medical events that occurred prior to patient's signing of the ICF and do not meet the SAE criteria are considered as medical history and do not need to be reported as AEs (pre-existing conditions).

9.5.2.11. Reporting Requirements for AEs

All AEs must be assessed by the Investigator to determine if they meet criteria for an SAE. If criteria are met for an SAE the event must be reported to Alexion Pharmacovigilance or its delegate as per requirements in Section 9.5.2.14.

9.5.2.12. Reporting Requirements for Non-serious AEs

All non-serious AEs must be recorded in the eCRF upon awareness.

9.5.2.13. Reporting Requirements for SAEs

All AEs must be assessed by the Investigator to determine if they meet criteria for an SAE. For SAEs, Alexion Pharmacovigilance or its delegate must be notified immediately or within 24 hours of the Investigator and/or study site staff becoming aware of the event, regardless of the presumed relationship to eculizumab or to study procedure. If the event meets criteria for a fatal or life threatening SAE criteria, the Investigator should notify Alexion Pharmacovigilance or its delegate immediately. These reporting timelines need to be followed for all initial SAE cases and follow-up versions to the initial case.

The Investigator must verify the accuracy of the information recorded on the SAE pages of the eCRF with the corresponding source documents, and submit the SAE electronically via the Safety Gateway. If the eCRF is not available, the Investigator must complete, sign, and date paper SAE pages and send a copy via Email or fax to contact information provided below:

Email: PPD

Fax: PPD

When further information becomes available, the SAE Form should be updated with the new information and reported immediately via the same contact information.

Additional follow-up information, if required or available, should be entered into the eCRF or sent to the Sponsor or designee within 24 hours of the Investigator or study site staff becoming aware of this additional information. These reporting timelines should be followed for all initial and follow-up SAEs.

For all SAEs, the Investigator must provide the following:

- Appropriate and requested follow-up information in the time frame detailed above
- Causality of the SAE(s)
- Outcome of the SAE(s)
- Medical records and laboratory/diagnostic information

If a patient experiences a SAE with an outcome of death:

- The SAE resulting in death should have an outcome documented as death/fatal with an end date being the date of death.
- If the patient had additional AE/SAEs that were ongoing at the time of death, these events would be documented as ongoing with no end date.
- Only one event should have an outcome of death/fatal unless an autopsy report or investigator states otherwise.

9.5.2.14. Sponsor Reporting Requirements

The Sponsor or legal representative is responsible for notifying the relevant regulatory authorities of SAEs meeting the reporting criteria as per regional and local regulations.

9.5.2.15. Investigator Reporting Requirements

The investigator must fulfill all local regulatory obligations required for study investigators. It is the PI's responsibility to notify the IRB or IEC of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical study. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

9.6. Pharmacokinetic/Pharmacodynamic Assessments

Blood samples for PK (plasma eculizumab concentrations)/PD (cRBC hemolysis assay) assessments will be collected as specified in Table 3 through Table 6 in Section 7.2. Samples for free C5 will also be collected as specified in the Schedule of Assessments tables (Table 3 through Table 6) in Section 7.2.

9.7. Genetic and Biomarker Assessments

Blood and urine samples for biomarker tests will be collected at specified time points (See Schedule of Assessments tables [Table 3 through Table 6] in Section 7.2). For genetic testing, blood samples collected on Day 0 (Baseline/Visit 1) or any visit thereafter will be used. The genetic testing kit will also contain samples to be collected for CFH and CFI antibody testing, to be analyzed by the designated genetic lab. Genetic mutations of known clinical relevance in aHUS may be communicated to the patient or patient's guardian by the Investigator together with appropriate genetic counseling. Genetic mutations of unknown significance (novel variants) identified through exploratory analysis will not be communicated to patients or their Investigator.

9.8. Patient Related Outcomes Assessments

Patient related outcomes including the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue instrument (Appendix 2 and Appendix 3), European Organization for Research and Treatment of Cancer quality of life questionnaire (EORTC QLQ-C30) (Appendix 4), and pre-specified patient-reported aHUS symptoms (Appendix 5) will be assessed as specified in Table 3 and Table 4.

10. SAMPLE SIZE AND STATISTICAL METHODS

10.1. Sample Size Estimation

The primary outcome of the study is TMA manifestation as a dichotomous variable. Preliminary data from the aHUS Registry shows about 20% of patients reporting discontinuation of eculizumab treatment. Additionally, longitudinal data on file at Alexion shows a difference of 15% in proportion of patients with TMA manifestations between patients who discontinue treatment compared to those who remain on treatment. The estimated sample size needed to detect a difference of 15% in TMA manifestations with power=80% in the proposed study is 300 patients (60 patients with eculizumab treatment discontinuation and 240 patients who continue ongoing treatment). Assuming a 10% loss to follow-up, the estimated sample size needed for the study is 330 patients.

10.2. Statistical Methods

10.2.1. Analysis Sets

The full analysis set (FAS) is defined as all aHUS patients who signed an informed consent for this study (ECU-aHUS-403). The per protocol set includes all enrolled patients who did not have any major protocol deviations deemed to potentially influence the primary analysis endpoint. This set will be determined prior to database lock. The safety set is defined the same way as the FAS and consists of all patients who signed an informed consent for this study.

10.2.2. General Considerations

Study ECU-aHUS-403 aims to assess TMA manifestations in patients with aHUS with or without ongoing eculizumab treatment. Treatment for aHUS is non-randomized and is at the discretion of the Treating Physician. Hypothesis testing will be done using a two-sided α=0.05 Type 1 error rate, unless otherwise stated. For continuous variables, descriptive statistics (number of patients with non-missing values (N), mean, standard deviation [SD], median, minimum, and maximum) will be provided. For categorical variables, patient counts and percentages will be presented. The percentages will be calculated based on the total number of patients in the FAS or the safety set. The statistical analysis plan will define specific analyses that will be repeated on the per protocol set as sensitivity analyses. Analyses will be conducted using SAS Version 9.2 or higher.

Eculizumab Treatment Status:

All patients at their entry into the study will be receiving eculizumab treatment, dosed either on-label or at a reduced/increased dosing regimen, with no prior intentional discontinuation(s) of eculizumab treatment. Once enrolled in the study, patients are allowed to discontinue eculizumab treatment or re-initiate eculizumab treatment at the discretion of the Treating Physician.

Eculizumab treatment status will be classified for analytical purposes as follows:

- ***On-eculizumab treatment***: from the date of enrollment through three weeks after last infusion of eculizumab or until the patient withdraws from the study, or until the patient completes the study, whichever occurs first.
- ***Off-eculizumab treatment:*** from three weeks after the last infusion of eculizumab and continue until the patient re-initiates eculizumab treatment or until the patient withdraws from the study, or until the patient completes the study, whichever occurs first.

For both the above categories, the time will accrue as specified until the patient changes treatment status category, or until the patient is discontinued from the study, or until the patient completes the study.

10.2.3. Patient Disposition

For patients who withdraw from the study, the reason for study discontinuation will be summarized. For patients who intentionally discontinued eculizumab treatment, reasons for intentional discontinuation will be summarized as recorded in the clinical database. A by-patient disposition status listing will be provided for all enrolled patients with the following information: patient ID, date of signed informed consent, date of the intentional eculizumab discontinuation, reason for intentional eculizumab treatment discontinuation, date of study withdrawal, and the reason for study withdrawal.

10.2.4. Patient Characteristics

All demographic and baseline characteristics information will be summarized using frequency counts and percentages for categorical variables, and summary statistic for continuous variables.

10.2.5. Concomitant Therapy

Concomitant medications will be summarized with counts and percentages for each medication and Anatomic Therapeutic Chemical (ATC) level 3 class. Patients will be reported just once for a particular drug or ATC class. Additionally, concomitant medications will be presented in a listing.

10.2.6. Treatment Compliance

As previously stated, all patients enrolled in this study will be patients on eculizumab treatment at the time of entry into the study. Patients are allowed to discontinue eculizumab treatment as well as re-initiate eculizumab treatment at the discretion of the Investigator. It is expected that some patients may also be receiving eculizumab following a reduced/increased dosing regimen (reduced/increased dose and or reduced/increased frequency). Analyses to evaluate how this affects the primary endpoint will be performed.

10.2.7. Primary Endpoint Analyses

Primary analyses will focus on comparison of rates (ie, adjusted appropriately for exposure periods) of new TMA disease manifestations between time on eculizumab treatment and time off eculizumab treatment.

In this context in which treatment exposure is a time varying confounder in that it affects the occurrence of a TMA disease manifestation and can subsequently be affected by a TMA disease manifestation, marginal structural models will be used. Marginal structural models estimation employs inverse-probability of treatment weighting (IPTW) and robust estimation methods. The goal of IPTW estimation is to obtain coefficients to create weights that will redistribute the population so that, in this case, treatment exposure is not temporally confounded (ie, creating a pseudo-population in which treatment exposure is no longer temporally confounded). The factors that may confound the association are considered in a treatment model (or weight model), which is used as part of the IPTW estimation methodology to obtain estimates of, again in this context, difference in incidence rates between time on and off treatment.

Differences between incidence rates of TMA disease manifestations will be estimated using negative binomial regression. A generalized linear negative-binomial model for rates consists of following:

t_j the length of observation for patient j (expressed in days, months or years)

Y_j the count for patient *j* during t_j

x_j the vector of baseline covariates including study treatment for patient j

μ_j the mean of the negative binomial distribution of Y_j

The conditional mean for the distribution of the rate μ_j/t_j for patient j given his/her baseline covariates x_j, is modeled by the following equation:

$\log(\mu_j/t_j)=x'_j\theta$, ie, $\log(\mu_j)=x'_j\theta+\log(t_j)$, where θ is the vector of unknown fixed model parameters for baseline covariates including study treatment.

Within the negative binomial regression model, the null hypothesis of no treatment effect ($\theta_{treatment}=0$) is tested against the alternative hypothesis ($\theta_{treatment}<0$) by a one-sided Wald-type level α test. A two-sided (1-2α) x 100% Wald-type CI is calculated for the relative reduction in mean rate for, in this context, on treatment versus off.

In the event that the data manifest excess zeros, zero-inflated (count) models provide a way of modeling the excess zeros by assuming there are two possible data generation processes (process 1 and process 2), and the result of a Bernoulli trial (eg, throwing a biased coin) determines which process is used for patient j: process 1 is chosen with probability φ_j and process 2 with probability 1- φ_j, and φ_j may depend on patient j's baseline covariates (including study treatment), denoted as $\mathbf{z}_j$, typically via a logistic regression. Process 1 generates only zero counts, whereas process 2 generates counts from either a Poisson or negative binomial distribution (which may depend on patient j's baseline covariates (including study treatment), denoted as $\mathbf{x}_j$). The set of baseline covariates **z** and **x** may be identical or may have some or no covariates in common.

In a zero-inflated negative binomial model, the probability of $\{Y_j = y_j \mid \mathbf{x}_j\}$ is:

$$P(Y_j = y_j|\boldsymbol{x}_j, \boldsymbol{z}_j) = \begin{cases} \varphi(\boldsymbol{\gamma}'\boldsymbol{z}_j) + \left(1 - \varphi(\boldsymbol{\gamma}'\boldsymbol{z}_j)\right) P_{Negbin}(0|\boldsymbol{x}_j) & \text{if } y_j = 0 \\ \left(1 - \varphi(\boldsymbol{\gamma}'\boldsymbol{z}_j)\right) P_{Negbin}(y_j|\boldsymbol{x}_j) & \text{if } y_j > 0 \end{cases}$$

10.2.8. Secondary Endpoint Analyses

Secondary endpoints include change over time for eGFR. Change over time will be summarized using repeated measures models starting from baseline. Mean changes from baseline will be analyzed using a restricted maximum likelihood based repeated measure approach. Summary statistics will be presented at baseline and follow-up time points and mean change from baseline will be estimated by eculizumab treatment status.

The primary analyses will be repeated with a distinction for the time on eculizumab treatment as on-label or as on reduced/increased dosing regimen. The focus will be on comparison of rates (ie, adjusted appropriately for exposure periods) of new TMA disease manifestations between:

- time off eculizumab treatment and time on on-label eculizumab treatment, and
- time on reduced/increased dosing of eculizumab treatment and time on on-label eculizumab treatment

The number of occurrences of PE/PI per patient-years will be calculated and summarized by eculizumab treatment status.

10.2.9. Safety Analyses

Adverse events and general medical/surgical histories will be coded by primary system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) (Version 13.0 or the most current). All events from time of enrollment in the study will be included in the analyses.

The number and percentage of patients with AEs will be summarized by preferred terms, SOC, severity (mild, moderate, severe), relationship to eculizumab treatment, and treatment status. If a patient has more than one occurrence of an AE, the most severe occurrence of the AE will be used in the severity summary table, and the strongest relationship to eculizumab treatment will be used in the relationship to eculizumab treatment table.

Similarly, the number and percentage of patients with SAEs will be summarized by preferred terms, SOC, severity (mild, moderate, severe), relationship to eculizumab treatment, and treatment status.

To account for differences in time spent on and off treatment, the number of AEs and SAEs per patient years will be calculated and summarized.

The number and percentage of patients with AEs resulting in death, study withdrawal, and eculizumab treatment discontinuation will be summarized.

10.2.10. Physical Examinations and Vital Signs

Physical examinations will be collected at baseline for all patients and will be summarized. Vital signs (systolic and diastolic BP, temperature, and sitting or lying HR), height (only for pediatric patients), and weight and changes from baseline in vital signs (including height and weight) will be summarized by visit. Listings of physical examinations and vital signs will be produced.

10.2.11. Laboratory Assessments

Changes from baseline in laboratory assessments (chemistry and hematology) will be summarized by visit. Likewise, shift tables (L [low], N [normal], H [high]) by visit and treatment status will be produced for clinical laboratory tests. Listings of laboratory data will be produced.

10.2.12. Exploratory Endpoint Analyses

10.2.12.1. Pharmacokinetic/Pharmacodynamic/Biomarker Analyses

Pharmacokinetic, PD (cRBC hemolytic assay), and biomarker analyses will be performed on all patients who have evaluable PK, PD, and biomarker data, respectively.

Graphs of plasma eculizumab concentration time profiles for individual patients and for means will be provided. Plasma eculizumab concentrations by collection day and time will be summarized by tabulations of mean, SD, median, minimum and maximum. Population PK analysis and PK-PD analyses will be conducted using eculizumab plasma concentrations, hemolytic activity and free C5 data, and selected biomarker data. PK and PK-PD model parameters and the variability will be estimated, and the potential covariates, such as demographics, disease severity, genetic markers, will be assessed. Graphic exploration of exposure vs the selected efficacy or safety endpoints will be made, and subsequent exposure response analyses may be performed if graphic exploration suggests that a relationship exists.

For PD and exploratory biomarker assessments, summary tabulations of mean, SD, median, minimum and maximum will be presented. The relationship between changes in PD biomarkers, exploratory biomarkers, and the effects of eculizumab treatment, as well as the effects of treatment discontinuation, will be evaluated.

For the exploratory biomarker analyses, the relationship between eculizumab concentration and the key and exploratory biomarkers or the correlation between clinical benefit and key exploratory biomarkers will be assessed by graphical display. Potential relationships between clinical outcomes, PK/PD, genetic profile and biomarker levels will be explored. Additional model-based exploratory analyses may be performed.

Repeated measures model analyses similar to the ones performed for the change in eGFR will be conducted for biomarkers (Ba, TNFR1, sVCAM-1, TM, D-dimer, markers of renal injury (eg, Cystatin C)) to be measured in the study. Summary statistics will be presented at baseline and follow-up time points and mean change from baseline will be estimated. The number of occurrences per patient-years of (1) re-initiation of eculizumab treatment after discontinuation, (2) change from reduced/increased to labeled dosing, and (3) blood transfusion, will be calculated and summarized. Summary statistics for proteinuria levels, FACIT-fatigue scores observed values and changes from baseline over time, and EORTC QLQ-C30 scale before and after discontinuation will be provided. Summary statistics (proportions) for patient reported aHUS symptoms, including severity, frequency, and impact will be reported before and after discontinuation.

10.2.12.2. Genetic Analyses

Genetic testing will include analysis of variants in genes known to be associated with aHUS. Additional exploratory genetic analyses may be performed to identify novel genetic variants

associated with aHUS or disease modifying genes. Such variants may be validated by a second sequencing method and validated variants will be summarized across the study population. Potential relationships between clinical outcomes, treatment status, biomarkers, and genetic variants will be explored.

10.2.13. Other Statistical Considerations

10.2.13.1. Missing or Invalid Data

Missing post-baseline data will not be imputed unless indicated in the described analysis in the SAP.

10.2.13.2. Interim Analysis

An interim analysis will be conducted dependent upon enrollment and data availability when approximately 1 year of follow-up data is available for analysis for 50% of the target sample.

11. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

11.1. Study Monitoring

Before a study site can enter a patient into this study, a representative of Alexion Pharmaceuticals, Inc. or its designee will communicate with the study site to:

- Determine the adequacy of facilities and equipment
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and responsibilities of the Sponsor or its representatives. This will be documented in a Clinical Study Agreement between the Sponsor and the Investigator, as appropriate.

During the study, a Sponsor's representative or designee will have regular contact with the study site for the following:

- Provide information and support to the Investigator(s)
- Confirm that facilities and equipment remain acceptable
- Confirm that all patients have been properly consented
- Confirm that the study team is adhering to the protocol, that data are being accurately recorded in the CRFs/eCRFs
- Perform source data verification. This includes a comparison of data in the CRFs/eCRFs with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (eg, clinic charts).
- Record and report any protocol deviations not previously sent to the Sponsor.
- Confirm that AEs and SAEs have been properly documented on CRFs/eCRFs, and confirm that any SAEs have been forwarded to the Sponsor. Confirm that SAEs meeting criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the Investigator(s) or other staff needs information or advice.

11.2. Data Capture System

An electronic data capture (EDC) system will be used in this study. The site maintains a separate source for the data entered by the site into the sponsor-provided EDC system.

Data will be entered and stored in the EDC system, as applicable. Where possible, data from the M11-001 aHUS Registry may be used in combination with the data collected in this study in order to limit duplication of reporting or in program-level analysis of eculizumab patient data. Case report form data will be encoded and stored in a clinical trial database. Data managed by a central vendor, such as laboratory test data, will be stored electronically in the central vendor's database system. Data will subsequently be transferred from the central vendor to the Sponsor.

11.3. Access to the Study Data

All patients will be assigned a number upon enrollment in the study. Patient details will be de-identified as necessary. Physicians will then enter data as periodic updates as required. The Sponsor's designee and automated email messages will remind the Investigator to update information in the study. Investigators will have access to the secure database for entering patient data as well as accessing patient data already entered. Paper copies of the CRF will also be available to the Investigator if required.

11.4. Audits and Inspections

Authorized representatives of the Sponsor, a regulatory authority, an EC, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection by the Sponsor is to examine systematically and independently, all study-related activities and documents, to determine whether study-related activities were conducted, and whether data were recorded, analyzed, and accurately reported, according to the protocol, Good Clinical Practice (GCP) guidelines of the International Council on Harmonisation (ICH), and any applicable regulatory requirements. The Investigator should contact the Sponsor immediately if contacted by a regulatory agency about an inspection.

11.5. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

The PI must obtain IRB /IEC approval for the investigation. Initial IRB /IEC protocol approval, and all materials that have been submitted and approved by the IRB/EC for this study including the patient ICF and recruitment materials must be maintained by the Investigator and made available for inspection.

11.6. Data Monitoring

Due to the study's observational nature with respect to eculizumab treatment, an independent data monitoring committee will not be implemented.

12. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, the Sponsor or designee may conduct quality assurance audit(s) (Section 11.4).

13. ETHICS

This study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH GCP guidelines.

Investigators and other study personnel must comply with all instructions and regulations specified in this protocol, and applicable ICH GCP guidelines, and must conduct this study in accordance with all local, federal, and regulatory agency regulations.

In accordance with ICH guidelines, pediatric participation in these studies should occur in qualified pediatric centers, with personnel who are properly trained and experienced in studying the pediatric population, and in evaluating and managing potential pediatric AEs. Site personnel should be knowledgeable and skilled in dealing with the pediatric population and its age appropriate needs, and be encouraged to use measures that minimize discomfort from procedures (eg, topical anesthesia to place IV catheters, use of indwelling catheters rather than repeated venipunctures for blood sampling, and collection of some protocol-specified blood samples when routine clinical samples are obtained).

13.1. Ethics Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or EC, as appropriate. The Investigator must submit written approval to the Sponsor before he/she can enroll any patient into the study.

No modifications to the protocol should be made without the approval of both the Investigator and the Sponsor. Changes that significantly affect patient safety, the scope of investigation, or scientific validity of the study will require IRB/EC notification prior to implementation, except where the modification is necessary to eliminate an apparent immediate hazard to patients. Any deviations from protocol must be fully documented. The Investigator is responsible for informing the IRB or EC of any amendment to the protocol, in accordance with local requirements. In addition, the IRB or EC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or EC upon receipt of amendments and annually, as local regulations require.

The Investigator is also responsible for providing the IRB/IEC with any reportable serious adverse drug reactions from any other study conducted with the study drug. The Sponsor will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or EC, according to local regulations and guidelines.

13.2. Ethical Conduct of the Study

The Investigator will conduct all aspects of this study in accordance with all national, provincial, and local laws of the pertinent regulatory authorities.

13.3. Written Informed Consent

The Investigator at each center will ensure that the patient's parent or legal guardian is given full and adequate oral and written information about the nature, purpose, possible risk, and possible benefit of the study. The patient's parent or legal guardian also must be notified that he/she is free to discontinue his/her child from the study at any time. The parent or legal guardian should be given the opportunity to ask questions and time to consider the information provided.

The signed and dated ICF must be obtained before conducting any study procedures.

The Investigator(s) must maintain the original, signed and dated ICF. A copy of the signed ICF must be given to the patient's parent or legal guardian.

Should new information become available during the study that might affect the parent's or legal guardian's willingness to continue his/her child's study participation, the parent or legal guardian will be notified in a timely manner about the new information and asked to sign a new ICF.

14. DATA HANDLING AND RECORDKEEPING

14.1.1. Inspection of Records

The Sponsor or designee will be allowed to conduct site visits for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the patient charts, study source documents, and other records relative to study conduct.

14.1.2. Retention of Records

The Investigator must maintain all study-related documentation, in accordance with local retention requirements, for a period of 2 years after the last marketing application approval or, if not approved, 2 years following discontinuation of the test article for investigation. If it becomes necessary for the Sponsor or the regulatory authority to review any study-related documentation, the Investigator must permit access to such records. No records may be destroyed without Sponsor written approval.

14.1.3. Retention of Biological Samples

If a patient's parent or legal guardian has provided consent for storage of biological samples, and if permitted by local regulatory authorities, any remaining sample will be stored at a central location for a maximum of 15 years after the last patient's last study visit. These samples may be used for future exploratory biochemical or genetic tests to confirm aHUS diagnosis, to develop early diagnostic methods or to better understand the pathogenesis, course, and outcome of aHUS. If permitted according to local regulations and if patients or their guardian consent, blood samples may be used for additional exploratory aHUS-specific genetic analyses.

Blood or DNA samples may be used for analysis of candidate genes believed to be responsible for aHUS in order to develop improved diagnostic methods. Blood or DNA samples may also be stored for future testing to discover novel variants in genes not previously known to be mutated in aHUS patients, to discover disease modifying genes, or to identify gene mutations which affect the PK or PD of eculizumab. The stored samples will not be used for any clinical study database with the exception of this study; however, the results obtained may be used to inform a future clinical study. All samples will be stored as de-identified under the patient ID number assigned at study enrollment. This will constitute a single code. Any results produced from genetic analysis will be double coded for de-identification by using a second code in place of the patient ID number to further protect patient privacy. The link between the patient ID number and the genetic results will be maintained and stored in a secure environment, with restricted access. The link will be used to facilitate correlation of genetic results with clinical data and allow for regulatory audit. Genetic samples can be traced for destruction via the patient ID number in the case of withdrawal of consent when the patient or consenting guardian has requested disposal/destruction of collected samples not yet analyzed. All other biochemical analyses not containing genetic data that are produced from stored samples, such as PK analyses or biomarker analyses, will be stored as de-identified under the patient ID number.

14.1.4. Data Privacy

The Sponsor will make every effort to protect patient privacy. For all study data collection, patients will be identified by a unique patient ID number and date of birth in regions where permitted. All data will be securely stored with restricted access. Genetic results will be further protected as described in Section 14.1.3. Results from this study may be presented at meetings or in articles. However, patient name or initials that could identify a patient will not be used in any such meetings or articles.

15. PUBLICATIONS

The terms for publication are based on the permissions and responsibilities outlined in the study specific Clinical Trial Agreement.

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APPENDIX 1. SUMMARY OF LABORATORY PANELS AND TESTS

Blood Chemistry

Sodium	Total protein, serum	Lactate dehydrogenase (LDH)
Potassium	Creatinine	Isoenzymes
Chloride	Blood urea nitrogen (BUN)	Total bilirubin
Carbon dioxide/bicarbonate	Aspartate aminotransferase (AST)	Total cholesterol*
Calcium	Alanine aminotransferase (ALT)	Triglycerides
Glucose	Alkaline phosphatase	Uric acid
Albumin	Creatine phosphokinase (CPK)	

*(low-density lipoprotein [LDL] and high-density lipoprotein [HDL])

Hematology

Hemoglobin	Red blood cells (RBCs)
Haptoglobin	White blood cells (WBCs)
Platelet count	Complete blood count

Urinalysis

Color	Glucose	RBC
Clarity	Blood	Hyaline and other casts
pH	Bilirubin	Bacteria
Specific gravity	Leukocyte esterase	Epithelial cells
Ketones	Nitrite	Crystals
Protein	WBC	Yeast
Protein/Creatinine ratio		

APPENDIX 2. FACIT-FATIGUE ASSESSMENT FOR PEDIATRIC PATIENTS

Pediatric (Paediatric) Functional Assessment of Chronic Illness Therapy – Fatigue

Below is a list of statements that other people with your illness have said are important. **Please circle or mar one number per line to indicate your response as it applies to the past 7 days.**

		None of the time	A little bit of the time	Some of the time	Most of the time	All of the time
pF1	I feel tired	0	1	2	3	4
pF2	I have energy (or strength)	0	1	2	3	4
pF3	I could do my usual things at home	0	1	2	3	4
pF4	I had trouble starting things because I was too tired	0	1	2	3	4
pF5	I had trouble finishing things because I was too tired	0	1	2	3	4
pF6	I needed to sleep during the day	0	1	2	3	4
pF7	I got upset by being too tired to do things I wanted to do	0	1	2	3	4
pF8	Being tired made it hard for me to play or go out with my friends as much as I'd like	0	1	2	3	4
pF9	I needed help doing my usual things at home	0	1	2	3	4
pF10	I feel weak	0	1	2	3	4
pF11	I was too tired to eat	0	1	2	3	4
pF12	Being tired made me sad	0	1	2	3	4
pF13	Being tired made me mad (angry)	0	1	2	3	4

APPENDIX 3. FACIT-FATIGUE ASSESSMENT FOR ADULT PATIENTS

FACIT Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

		Not at all	A little bit	Some-what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

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APPENDIX 4. EUROPEAN ORGANIZATION FOR RESEARCH AND TREATMENT OF CANCER QUALITY OF LIFE QUESTIONNAIRE (EORTC QLQ-C30)

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: _ _ _ _

Your birthdate (Day, Month, Year): _ _ _ _ _ _ _ _

Today's date (Day, Month, Year): 31 _ _ _ _ _ _ _ _

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

APPENDIX 5. PATIENT-REPORTED aHUS SYMPTOMS

During the past 3 months, did you have any of the following symptoms?	Symptoms Present?	If YES, how often did you have it?				If YES, how severe was it usually?				If YES, how much did it distress or bother you?				
		Rarely	Occasionaly	Frequently	Almost Constantly	Slight	Moderate	Severe	Very Severe	Not at all	A little bit	Somewhat	Quite a bit	Very Much
Yellow discoloration of eyes and/or skin (jaundice)	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Chest pain	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Shortness of breath	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Headache	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Confusion	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Irritability	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Anxiety	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Weakness	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Fatigue	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Easy bruising / abdominal bleeding	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Abdominal Pain	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Nausea/Vomiting	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Diarrhea	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Swelling	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4
Other, specify: _____	☐ No ☐ Yes	1	2	3	4	1	2	3	4	0	1	2	3	4