Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Pittock, S.J., Barnett, M., Bennett, J.L., et al. Ravulizumab in Aquaporin-4–Positive Neuromyelitis Optica Spectrum Disorder

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# **Protocol Amendments Occurring After the Trial Started**

The protocol was finalized on August 12, 2019. Significant amendments to the protocol are detailed below.

### **June 30, 2020 (Amendments for All Countries)**

* **Study design**: updated the end of the primary treatment period: “to be triggered when 1) two patients have had an adjudicated on-trial relapse; or 2) all patients have completed, or discontinued prior to, 26 weeks on study, whichever comes later. However, if two patients have not had an adjudicated on-trial relapse by the time all patients have completed, or discontinued prior to, 50 weeks on study, the end of the primary treatment period will be triggered at that time. Patients who complete the week 26 or week 50 visit will remain on the trial in the primary treatment period until the primary treatment period ends”; updated that the total duration of study participation will be up to approximately 4.75 years, that the primary treatment period will be between 26 weeks and 2.5 years, and that the total treatment duration will be up to approximately 4.5 years; added that approximate enrollment for each geographic region will be between 28% and 40% of the total population.
* **Objectives and endpoints**: specified that endpoints of Expanded Disability Status Scale (EDSS), Hauser Ambulation Index (HAI), visual acuity (VA), color vision, and confrontational visual fields will be changes/worsening relative to baseline.
* **Schedule of activities**: added phone visits every two weeks to monitor patients’ safety and to evaluate potential relapse symptoms between dosing visits; removed pharmacokinetic (PK) cerebrospinal fluid collection at end of primary treatment period to reduce patient burden; changed days for week 170 visit to D1190 to correct a typo in the original protocol; removed week 6 biomarker and PK/cerebrospinal fluid collection to reduce patient burden; added monitoring of concomitant nondrug therapies/procedures and pregnancy tests.
* **Risk assessment**: added infusion reaction as one of the potential risks of study drug administration; provided guidance on management of infusion reaction.
* **Study population**: specified that anti-aquaporin-4 antibody–positive status should be obtained at screening; defined the study period as from screening visit until end of study; revised the requirement for pregnancy tests before the first dose of study drug to “negative serum pregnancy test at screening and a negative urine pregnancy test before the first dose of study drug”; specified the criterion excluding patients who are currently being treated with a biologic medication that may affect the functioning of the immune system, or those who have stopped treatment with a biologic medication that may affect the functioning of the immune system if five half-lives of the medication have not elapsed by the time of the screening visit; added a criterion excluding patients who previously participated in the PREVENT trial.
* **Study drug**: removed the sentence “the timing of Study ECU-NMO-301 (PREVENT) is contemporaneous”; removed the sentence “the dose of study drug and study patient identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study drug”; added intravenous (IV) immunoglobulin (Ig) or subcutaneous Ig used chronically as maintenance therapy and Bruton tyrosine kinase inhibitors to the list of disallowed medications.
* **Discontinuation of study drug and patient discontinuation/withdrawal**: added that an 8-week safety follow-up visit should be performed if a patient discontinues study drug definitively.
* **Study assessments and procedures**: specified the collection of medical history and collection time frame for history of neuromyelitis optica spectrum disorder (NMOSD); added additional instruction on ravulizumab supplemental dosing in the setting of IV Ig use for acute therapy; provided specific instructions for conducting off-site visits; added “and/or the new onset of neurologic symptoms or worsening of existing neurologic symptoms that required treatment. Treatment is defined as the use of high-dose IV steroids, PE/PP or IV Ig” to the definition of historical relapse; added definition and criteria of a case of interest; added the EQ-5D script for face-to-face administration; added a citation for HAI score, and updated the ordinal scale used to correct a minor error; added that “color vision will be assessed in this study using the first 21 Ishihara Plates”; changed “oral temperature” to “temperature” in monitoring of vital signs; specified that experimental drugs taken within the protocol-defined period should also be recorded in the electronic case report form (eCRF); changed the time window for PK and pharmacodynamic (PD) sample collection following end of study drug infusion from “at least 120 minutes” to “within 60 minutes”; added that samples collected for genetic biomarker research and future genetic biomarker research will be used for research and test/assay development of ravulizumab for NMOSD and related diseases; changed the terminology from “ocular coherence tomography” to “optical coherence tomography”.
* **Statistical considerations**: defined null and alternative hypotheses for testing adjudicated on-trial annualized relapse rate (ARR); added the modified full analysis set (mFAS) with the following definition: “the mFAS is a subset of the FAS, excluding patients who, for COVID-19–related reasons as determined by the investigator and documented in the eCRF (eg, infected with or exposure to COVID-19, quarantine, travel restrictions), received a dose of ravulizumab >35 days late or missed a dose altogether”; changed the PK set to the PK/PD analysis set, and provided the definition; updated that the statistical analysis plan (SAP) to support the primary analysis was developed and finalized shortly after the original protocol was final; updated that missing data will not be imputed unless indicated in the SAP; specified that, to account for potential differences resulting in missed or delayed dosing due to the coronavirus disease 2019 (COVID-19) pandemic that would have not occurred in the external placebo control, primary efficacy analyses will be performed using the mFAS; updated the time points provided as examples for which 95% confidence intervals (CIs) for the estimated proportion of patients who are relapse-free will be presented from (eg, week 26, week 50) and to (eg, week 24, week 48); added a section on medical resource utilization and health economics.
* **Supporting documentation and operational considerations**: removed a paragraph stating that the informed consent form will have a separate section regarding remaining samples; updated the instruction on reporting serious adverse events via electronic data collection tool and via paper case report form; updated the instruction on evaluating postmenopausal status to “before receiving study drug, female patients who consider themselves to be postmenopausal must provide evidence of postmenopause based on amenorrhea for at least 1 year prior to day 1 visit. Confirmatory serum follicle-stimulating hormone (FSH) level (>30 IU/L) may be obtained by the investigator at screening. In the absence of 1 year of amenorrhea, multiple elevated FSH levels will be required. The reason for not obtaining an FSH should be documented by the investigator at the time of screening”; specified that the Pregnancy Outcome and Breastfeeding Form should be used for initial information recording, and changed the duration of follow-up required after the estimated delivery date from 6 to 8 weeks to 3 months; updated VA #4 to VA 20/101–20/200, and changed VA #5 to VA 20/201–20/800.

### **April 28, 2021 (Germany Only)**

* **Supporting documentation and operational considerations**: removed the term “his/her legally authorized representative” and content related to this term because, based on enrollment criteria, patients participating in this study are capable of providing consent; added “withdrawal of the favorable opinion or the approval” and “inability to adjust the required maximum sum of insurance” to the list of reasons for early site closure or termination; added description of remote verification of source data during COVID-19 pandemic.

### **June 7, 2021 (UK Only)**

* **Supporting documentation and operational considerations**: included language for risk assessment of COVID-19 and of COVID-19 vaccines.

### **September 1, 2021 (Amendments for All Countries)**

* **Schedule of activities**: added study visits at weeks 210, 218, 226, and 234; added additional schedule-of-activities table to accommodate new study visits.
* **Study design**: added the following to the overall study design: “patients may be switched from the 10 mg/mL to the 100 mg/mL formulation of ravulizumab with no change to the weight-based dose regimen during the long-term extension period”; changed the term “relapse” to “on-trial relapse” when defining the end of the primary treatment period; changed the term “time to first relapse” to “time to first adjudicated on-trial relapse” when referencing the study endpoint; removed the mFAS and related text.
* **Study drug**:added 100 mg/mL dose strength; added text clarifying that treatment for relapse is ultimately at the discretion of the investigator.
* **Study assessments and procedures**: revised text clarifying the EDSS; clarified the Landolt C assessment procedure; clarified that body temperature assessment may be oral, temporal, or axillary.
* **Statistical considerations**: revised the hypothesis statement for the adjudicated on-trial ARR from emphasis on the upper 95% CI to being based on the mean adjudicated on-trial ARR; changed the term “time to first relapse” to “time to first adjudicated on-trial relapse”; added that immunogenicity study data will be included in the final analysis of the primary treatment period; removed the statement that the mFAS would be the primary analysis set to account for potential differences resulting from missed or delayed dosing due to the COVID-19 pandemic, and clarified that sensitivity analyses would account for baseline imbalances between treatment groups; added that Firth’s Penalized Likelihood will be used to estimate the hazard ratio, risk reduction, and profile likelihood 95% CIs if there is no observed event in a treatment arm; added language on censoring; added a statement regarding how the change from baseline to the analysis time point at 6 weeks after relapse/the end of the primary treatment period will be calculated for patients who missed a dose for reasons related to COVID-19; changed the language regarding what would be considered statistically significant from the 95% CI approach to the two-sided *p* value approach, and added language to describe how the ARR will be calculated in the event that a patient missed a dose for COVID-19–related reasons; replaced the approach using nonparametric analysis of covariance (ANCOVA) of the EQ-5D endpoints with an approach using ANCOVA of the ranks of the change from baseline (with treatment as a factor and the ranks of the baseline values as a covariate); removed reference to the upper 95% CI when describing statistical significance.
* **Supporting documentation and operational considerations**:removed the term “his/her legally authorized representative” and content related to this term when describing enrollment; added “withdrawal of the favorable opinion or the approval” and “inability to adjust the required maximum sum of insurance” to the list of reasons for early site closure or termination; added description of remote verification of source data during COVID-19 pandemic; added language for the risk assessment of COVID-19; updated history of protocol amendment.

# **Additional Statistical Considerations**

### **Sample-Size Calculation**

The sample size for this trial was based on the percentage of patients who were estimated to be relapse-free at 12 months (92% for ravulizumab, 63% for placebo). Forty-seven patients were included in the PREVENT placebo group, and there was a two-sided 5% level of significance and an estimated dropout rate of 2–10%, so a sample size of approximately 55 patients in the ravulizumab treatment group would provide a power of ≥90% to detect a treatment difference in time to first adjudicated on-trial relapse (primary endpoint).

### **Secondary Efficacy Endpoints**

The adjudicated on-trial ARR for patients treated with ravulizumab was calculated using a Poisson regression analysis, in which the log of time in the study period was used as the offset variable and the historical ARR was used as a covariate. This endpoint would be considered statistically significant if the adjudicated on-trial ARR was <0.25 (two-sided *p* ≤ 0.05).

The remaining variables were evaluated as the change from baseline (day of first dose of study drug) to the end of the study period time point. The end of the study period was the week 6 visit following a physician-determined relapse. This was consistent with the placebo patients’ trial experience, in which patients rolled over to eculizumab dosing after the week 6 visit of a physician-determined relapse. Patients with no physician-determined relapse were evaluated at the end of the primary treatment period visit (ravulizumab arm) or the end of study visit (placebo arm).

Clinically important worsening from baseline in HAI score was analyzed using a logistic regression model, with treatment group as a factor and baseline HAI score included as a covariate.

Change from baseline in EQ-5D index score and EQ-5D visual analog score was calculated using ANCOVA of the ranks of the change from baseline, with treatment group as a factor and the ranks of the baseline values as a covariate.

Clinically important worsening from baseline in EDSS score was analyzed using logistic regression, with treatment group as a factor and baseline EDSS as a covariate.

If statistical significance in one endpoint was not achieved (*p* > 0.05), then subsequent endpoints were not considered statistically significant.