SUPPLEMENTARY APPENDIX

Characterization of breakthrough hemolysis events observed in the phase III randomized studies of ravulizumab *versus* eculizumab in adults with paroxysmal nocturnal hemoglobinuria

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SUPPLEMENTAL MATERIAL

Details of Literature Review for Breakthrough Hemolysis Definition

A literature review was conducted (PubMed search terms: breakthrough hemolysis paroxysmal, breakthrough hemolysis nocturnal, breakthrough paroxysmal) to identify relevant reports to contribute to an operational definition. In addition, in May 2016 eight study investigators from the United States, Europe, and Asia were requested to describe three of the most recent cases from their respective clinical practices and provide clinical and laboratory measures used to define BtH.

Methodology of Post Hoc Analyses of Breakthrough Hemolysis Data

Analysis 1: This analysis was conducted to assess the contribution of lactate dehydrogenase (LDH) to treatment effect in patients with BtH, defined as LDH ≥2 times the upper limit of normal (ULN) after prior LDH reduction to <1.5× ULN on therapy, excluding other signs/symptoms of intravascular hemolysis. Proportions of patients with events were estimated by treatment and 95% CI for the difference between treatment groups was calculated using a stratified Newcombe method. The stratification factors are observed stratification groups of packed red blood cell (pRBC) units transfused in the 1 year prior to first dose of study drug (in 301 and 302) and screening LDH levels (in 301).

Analysis 2: This analysis was conducted to estimate BtH rates based on number of events, expressed in patient-years. Exposure adjusted incidence rates per 100 patient-years were estimated and compared between treatment groups using a Poisson

regression method with treatment, history of transfusion, and baseline LDH (study 301 only) level in the model.

Analysis 3: A Cox proportional hazard model was utilized to analyze and compare the treatment groups regarding time to first BtH due to any cause, time to first BtH due to pharmacodynamics/pharmacokinetics with adjustment for competing risk of complement-amplifying conditions and undetermined causality, and time to first BtH due to pharmacodynamics/pharmacokinetics and undetermined causality with adjustment for competing risk of complement-amplifying conditions.

Analysis 4: This analysis was conducted to assess correlation between serum free C5 concentrations <0.5 or ≥0.5 µg/mL and BtH events. Serum free C5 levels were assessed as previously described.[1] Percentages of patients who had all free C5 values <0.5 µg/mL and also experienced BtH were documented, as were percentages of patients with any free C5 value ≥0.5 µg/mL who also experienced BtH. Relative risk for BtH was calculated as the ratio of the percentage of patients with any free C5 level ≥0.5 µg/mL experiencing BtH over the ratio of the percentage of patients with any free C5 level <0.5 µg/mL experiencing BtH. Patients with biologically implausible free C5 data at end of infusion on day 1 were excluded from the analysis.

Results of Post Hoc Analyses of Breakthrough Hemolysis Data

Results for analyses 1 and 2 are reported in the main publication Results section.

Results for analysis 3: Results from a Cox proportional hazard analysis of time to the first BtH events due to any causality are shown in **Supplemental Figure 1**. Results from the analysis of time to first BtH events due to suboptimal C5 inhibition, with adjustment for competing risk of complement-amplifying conditions or undetermined causality, are shown in **Supplemental Figure 2**. Results from the analysis of time to first BtH events due to suboptimal C5 inhibition or undetermined causality, with adjustment for competing risk of complement-amplifying conditions are shown in **Supplemental Figure 3**.

Results for analysis 4 are shown in **Supplemental Table 3**.

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Supplemental Table 1. Overview of biomarkers used to define breakthrough hemolysis in patients with PNH reported in the medical literature

Biomarker
• LDH level ≥1500 U/L[2] or ≥1.5× ULN[3]
• LDH levels ≥1000 U/L with AST levels ≥90 IU/L[4]
• Elevated LDH levels with subtherapeutic levels of eculizumab[5, 6]
• Subtherapeutic levels of eculizumab (ie, <35 µg/mL)[7]
• CH50 hemolytic complement activity assay[8]
• Serum hemolytic activity >20% alone[9] or with subtherapeutic eculizumab levels (ie, <35 µg/mL) and elevated LDH[10]
• Persistent reticulocytosis with raised unconjugated bilirubin (ie, extravascular hemolysis)[11]

AST: aspartate aminotransferase; CH50: 50% hemolytic complement; LDH: lactate dehydrogenase; PNH: paroxysmal nocturnal hemoglobinuria; ULN: upper limit of normal.

Supplemental Table 2. Description of breakthrough hemolysis cases by PNH experts

Expert	Symptomatic Manifestation of Breakthrough Hemolysis	Laboratory Indicators of Breakthrough Hemolysis	Comments
1	• Hemoglobinuria	• Decreased Hb • Increased LDH	• Check CH50
2	• PNH symptoms	• Elevated LDH	• Individualize LDH cutoff • Distinguish between low drug levels and CAC
3	• PNH symptoms (with or without cause)	• Increased LDH plus increased CH50	• 2× ULN in definition was too high; consider 1.5× ULN
4		• Decreased Hb • LDH increase for 2 consecutive troughs • LDH consistently high	• Focus on laboratory evidence • LDH at end of dosing interval • BtH event due to CAC • Recommend LDH cutoff of 2× ULN AND >2-fold greater than previous LDH
5	• Increased transfusion dependence	• Rising LDH at end of dosing interval • Decreased Hb	• Consider proportional change from stable LDH level, then individualize
6/7	• Continued transfusion dependence, not	• Increased CH50; low eculizumab level at end of dosing interval	• Distinguish between PK and CAC • Proposed definition (need all 3):

	explainable by other pathology • Symptoms after 8 days with abdominal pain and hemoglobinuria • PNH symptoms	• Increased LDH, increased bilirubin, decreased Hb	– PNH symptoms – 1.5 g/dL drop in Hb – LDH >2× ULN

BtH: breakthrough hemolysis; CAC: complement-amplifying condition; CH50: 50% hemolytic complement; Hb: hemoglobin; LDH: lactate dehydrogenase; PK: pharmacokinetics; PNH: paroxysmal nocturnal hemoglobinuria; ULN: upper limit of normal.

Supplemental Table 3: Correlation between serum free C5 concentration and patients with breakthrough hemolysis events[a].

Study	Treatment	Number of Patients With Breakthrough Hemolysis	Percentage (n/N) of Patients With All Free C5 Values <0.5 µg/mL Experiencing Breakthrough Hemolysis Events	Percentage (n/N) of Patients With Any Free C5 Value ≥0.5 µg/mL Experiencing Breakthrough Hemolysis Events	Relative Risk[b]
301	Ravulizumab (n=125)	5	4.0 (5/125)	NA	
	Eculizumab (n=121)	13	6.6 (7/106)	40.0 (6[b]/15[b])	
	Combined (N=246)	18	5.2 (12/231)	40.0 (6/15)	7.7
302	Ravulizumab (n=97)	0	0 (0/97)	NA	
	Eculizumab (n=98)	5	3.3 (3/91)	28.6 (2/7)	
	Combined (N=195)	5	1.6 (3/188)	28.6 (2/7)	17.9

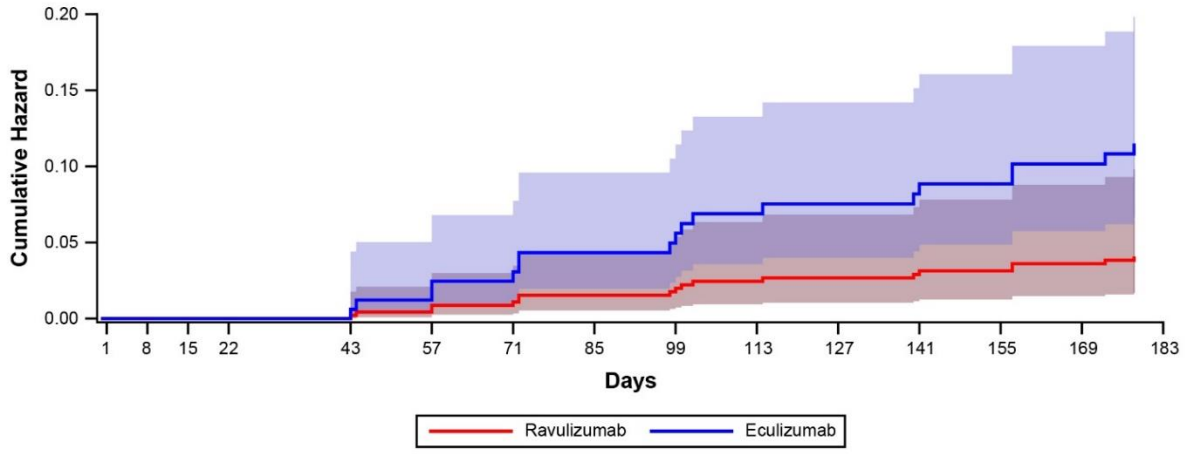
C5: complement component C5; NA: not applicable because no ravulizumab patients experienced free C5 ≥0.5 µg/mL.

[a]Measurement of free C5 methodology previously described. [b]Relative risk was calculated as the ratio of the percentage of patients with any free C5 ≥0.5 µg/mL experiencing BtH over the ratio of the percentage

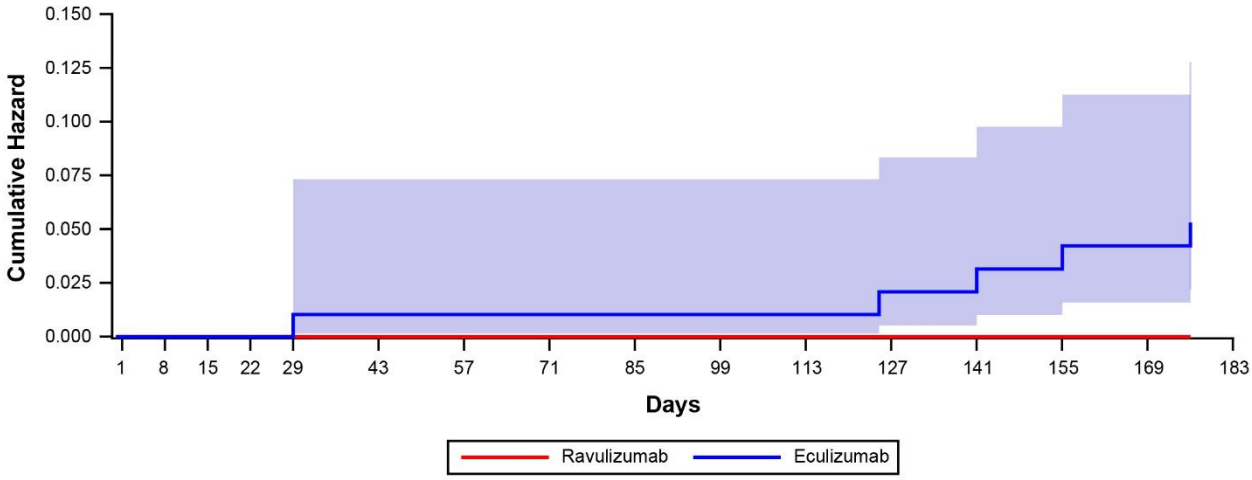
of patients with any free C5 <0.5 µg/mL experiencing BtH. [b]Excluded biologically implausible day 1 end of infusion data: 3 patients from the ravulizumab group, 4 patients from the eculizumab group.

Supplemental Figure 1. Time to first event of breakthrough hemolysis due to any causality. A) Study 301. B) Study 302. Shaded areas represent 95% confidence intervals.

A)

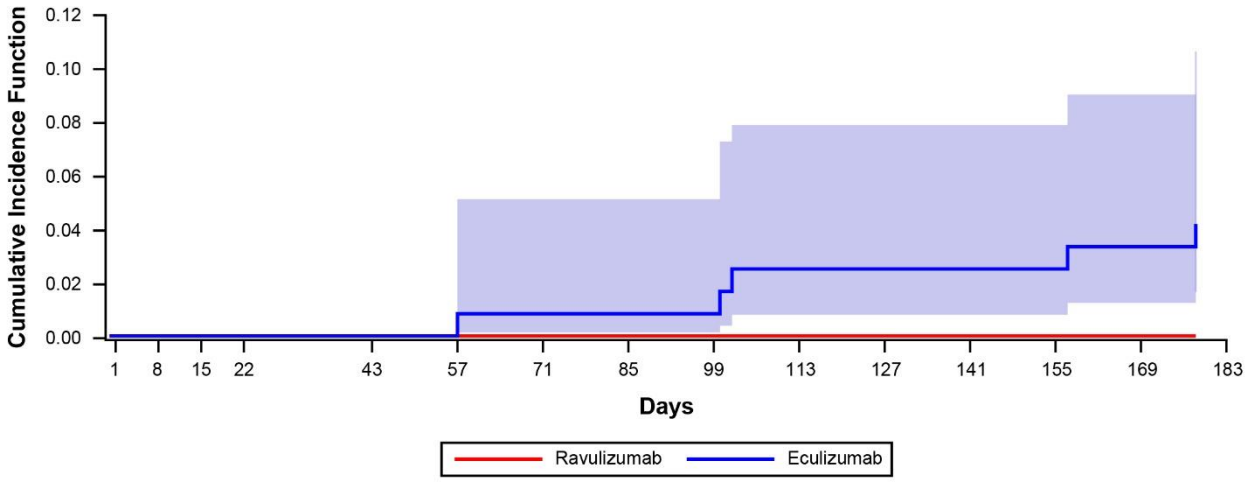


B)



Supplemental Figure 2. Time to first event of breakthrough hemolysis due to suboptimal C5 inhibition with adjustment for competing risk of complement-amplifying conditions or undetermined causality. A) Study 301. B) Study 302. Shaded areas represent 95% confidence intervals.

A)



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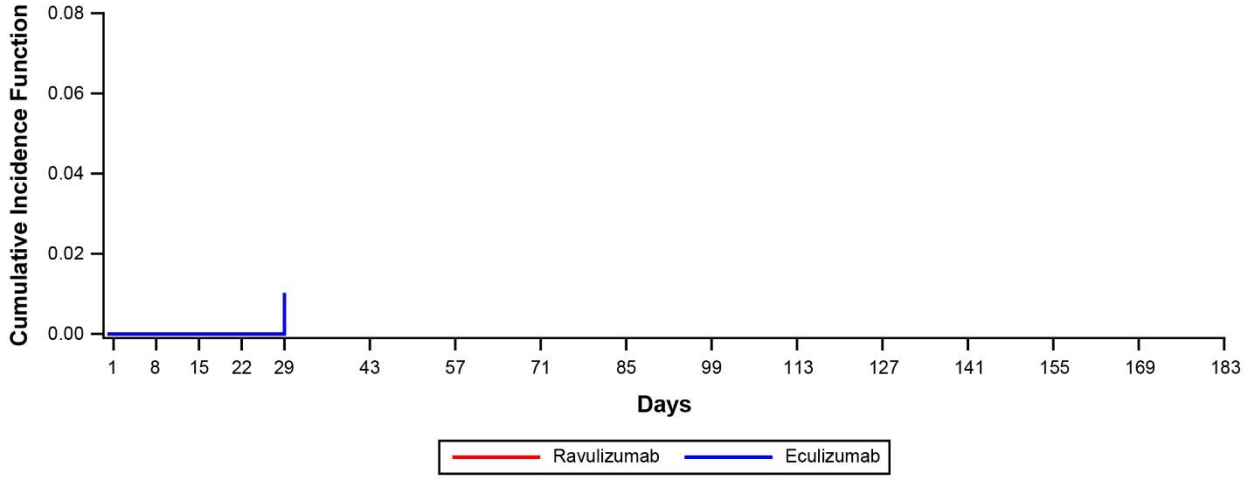
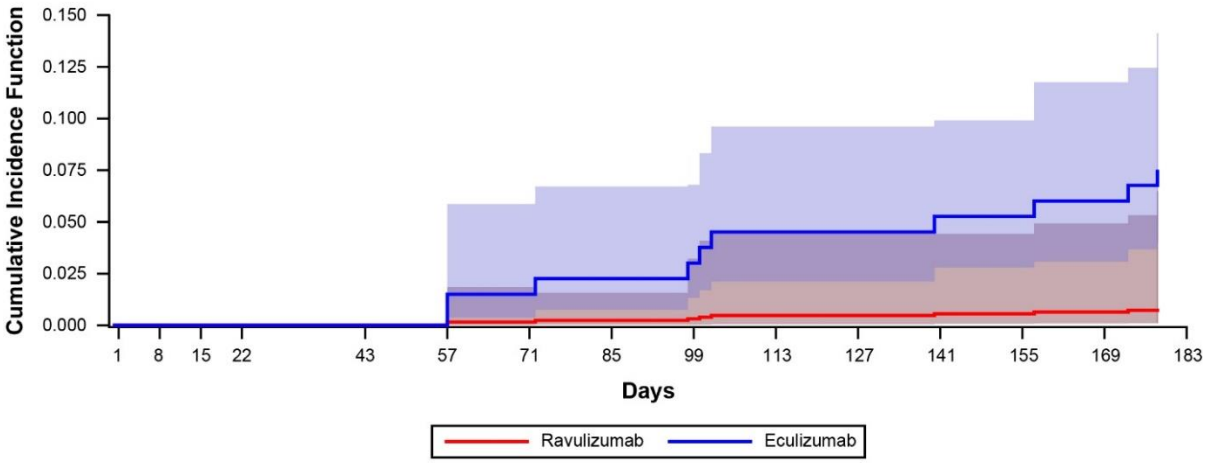


Supplemental Figure 3. Time to first event of breakthrough hemolysis due to suboptimal C5 inhibition or undetermined causality with adjustment for competing risk of complement-amplifying conditions. A) Study 301. B) Study 302. Shaded areas represent 95% confidence intervals.

A)



B)