Phase II trials of zilucoplan in paroxysmal nocturnal hemoglobinuria

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Phase II trials of zilucoplan in paroxysmal nocturnal hemoglobinuria

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AGK, PM, and HS contributed to the concept and design of the research, were study investigators, and contributed to the analysis of data. A-EL, CF, SG, MG, SK, GM, UO, CJP, HP, Y-MS, and JS were study investigators. RS was a study investigator and contributed to analysis of data. SR performed the mechanistic studies and contributed to the interpretation of
data. GDLB, PWD, and RF-F contributed to the interpretation of data. CES and DDV contributed to the design of mechanistic studies and interpretation of data. All authors reviewed and approved the manuscript for submission.

Data sharing statement:
Underlying data from this manuscript may be requested by qualified researchers 6 months after product approval in the US and/or Europe, or global development is discontinued, and 18 months after study completion. Investigators may request access to anonymized individual patient-level data and redacted study documents, which may include: analysis-ready datasets, study protocols, annotated case report forms, statistical analysis plans, dataset specifications, and clinical study reports. Prior to use of the data, proposals need to be approved by an independent review panel at www.Vivli.org and a signed data sharing agreement will need to be executed. All documents are available in English only, for a prespecified time, typically 12 months, on a password protected portal.

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and participated in a Medscape educational program funded by an educational grant from Apellis Pharmaceuticals. SK has received speaking and advisory board honoraria from Alexion Pharmaceuticals. GM has received research grant funding from AbbVie and consulting fees, speaking honoraria, and/or travel support from Amgen, Bristol Myers Squibb/Celgene, Janssen-Cilag, Novartis, Sanofi, and Takeda. PM has received lecture fees and travel support from Sobi and participated in an advisory board for Novartis. UO has received speaking and/or advisory board honoraria from Alexion Pharmaceuticals and Sobi. CJP has received speaking and/or advisory board honoraria from Alexion Pharmaceuticals, Apellis Pharmaceuticals, BioCryst Pharmaceuticals, and Sanofi. HP, Y-MS, and RS have no conflicts of interest to disclose. JS has received speaking and/or advisory board honoraria from Alexion Pharmaceuticals, Apellis Pharmaceuticals, BioCryst Pharmaceuticals, Novartis, Pfizer, Prevail Therapeutics, and Sanofi-Genzyme. GDLB, PWD, and SR, are employees of UCB and own stock and/or hold stock options in the company. CES and DDV are former employees of UCB and Ra Pharmaceuticals. RF-F is a former employee of Ra Pharmaceuticals. HS has received research funding and honoraria as a speaker in symposia or for service on advisory boards from Alexion Pharmaceuticals, Apellis Pharmaceuticals, Novartis, Ra Pharmaceuticals, Roche, and Sanofi (all to the institution of HS).

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Studies 201, 202, and 203 were funded by Ra Pharmaceuticals (Cambridge, MA, USA; now a part of UCB Pharma [Brussels, Belgium]). Medical writing and editorial assistance were provided by Jessica Deckman, PhD, CMPP, of The Lockwood Group (Stamford, CT, USA), and funded by UCB Pharma, in accordance with Good Publications Practice (GPP) 2022 guidelines (https://www.ismpp.org/gpp-2022).
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, chronic, clonal hematopoietic stem cell disorder. Uncontrolled complement activation is central in the pathogenesis of PNH.\textsuperscript{1-3} Approved first-line treatments of PNH are eculizumab and ravulizumab, which inhibit the activity of complement component 5 (C5).\textsuperscript{1,4-6}

Zilucoplan, a novel C5 inhibitor, is a small (3.5 kDa), 15-amino acid macrocyclic peptide that binds to C5 with high affinity and specificity.\textsuperscript{7} Zilucoplan inhibits complement via a dual mechanism that prevents cleavage of C5 into C5a and C5b and binds to the domain of C5 corresponding to C5b, thereby blocking the binding of C5b to C6.\textsuperscript{7} Zilucoplan prevents activation of the terminal complement pathway and assembly of the membrane attack complex that results in lysis of glycosylphosphatidylinositol (GPI)-anchored protein-deficient red blood cells (RBC) in PNH.\textsuperscript{7,8}

Efficacy, pharmacodynamics, safety, and tolerability of zilucoplan were evaluated in adult patients with PNH in 2 phase 2 12-week studies (study 201, NCT03078582; study 203, NCT03030183) and a long-term extension study (NCT03225287; Figure 1A and 1B). Eligibility criteria are summarized in Supplementary Table S1. The primary endpoint of the 12-week studies was change from baseline in serum lactate dehydrogenase (LDH) levels. This analysis included 10 eculizumab-naïve patients and 19 with prior eculizumab treatment (eculizumab-switch cohort; Figure 1B). All 10 eculizumab-naïve patients entered the extension study, 2 (20.0%) of whom discontinued. In the switch cohort, 8 of 19 (42.1%) patients discontinued and 11 (57.9%) entered the extension study; 2 (10.5%) patients discontinued and 9 (47.4%) were still receiving zilucoplan treatment at data cutoff (November 2020).
Patient demographics and baseline characteristics are provided in Supplementary Table S2. As expected, the naïve cohort had higher baseline LDH and median-free hemoglobin than the switch cohort.

In the naïve cohort, treatment with zilucoplan resulted in consistent, complete, and sustained inhibition of both the classical and alternative complement pathways (Figure 2A), leading to rapid, substantial, and sustained LDH decreases from baseline (median LDH, 378.0 U/L [1.6× upper limit of normal (ULN) of 234 U/L]) (Figure 2B). Of the 5 patients who required ≥1 transfusion (irrespective of number of units) in the 6 months before the study start, 2 (40.0%) became transfusion-independent after zilucoplan treatment initiation (Figure 2C). Zilucoplan treatment led to a consistent decrease of median free hemoglobin (baseline 7.10 mg/dL) at all post-baseline time points (median change [range], −3.90 to −5.95 mg/dL). Mean changes from baseline to each post-baseline time point in all other secondary endpoints, including total bilirubin, total hemoglobin, haptoglobin, reticulocytes, and hemoglobinuria were generally small or variable, displaying no clear trend for the naïve cohort (data not shown).

Zilucoplan treatment in the switch cohort led to complete and sustained inhibition of both the classical and alternative complement pathways (Figure 3A). Patients treated in the switch cohort resulted in a 230.3 U/L median LDH increase from baseline during the primary evaluation period (Figure 3B). In the 7 (36.8%) patients who were transfusion-independent, the mean (standard deviation) baseline LDH value was 232.6 (22.6) U/L. After an initial increase in mean LDH that peaked at Week 6, values remained consistent at approximately 1.5× ULN in transfusion-independent patients in the switch cohort (Figure 3B).

In transfusion-dependent patients in the switch cohort, mean baseline LDH value was significantly higher than in the transfusion-independent group. Despite zilucoplan treatment,
transfusion-dependent patients in the switch cohort experienced increased mean LDH values that reached their highest levels at Week 20 (924.7 U/L; Figure 3B). Based on the investigators’ medical evaluation, patients with evidence of increased hemolysis discontinued zilucoplan and resumed eculizumab treatment, resulting in stabilization of LDH. Among the 12 transfusion-dependent patients in the switch cohort, including some who received treatment for <6 months, 4 were transfusion-independent after initiation of zilucoplan (Figure 3C).

Within the switch cohort, patients who discontinued and were considered to have had switch failure had higher reticulocyte counts at baseline than those who were considered switch successes (Figure 3D). At baseline, the switch cohort had a median free hemoglobin of 1.80 mg/dL; variable changes with a median range of 0.0-1.70 mg/dL were observed across all post-baseline time points. Mean changes from baseline to each post-baseline time point in all other secondary endpoints were generally small or variable with no clear trends.

Zilucoplan, a self-administered at-home subcutaneous small-volume (<1 mL) injection with a thin (29G) needle, was well tolerated, with >18.6 patient-years of exposure and mean duration of exposure of 36.4 weeks. In the initial 12-week study period (N=29), all patients experienced adverse events (AEs), of which 11 (37.9%) patients had treatment-related AEs (most common [occurring in >1 patient]: headache [n=4], hemolysis [n=4], dizziness [n=2], fatigue [n=2], injection site bruising [n=2]). No events of thrombosis were observed. During the 12-week study period, treatment-related AEs occurred in fewer patients in the naïve cohort (20.0% [n=2/10]) versus the switch cohort (47.4% [n=9/19]). Four (13.8%) patients experienced serious AEs (pyrexia and febrile nonhemolytic transfusion reaction [ naïve cohort; n=1], urinary tract infection, gastroenteritis, and pyrexia [switch cohort; n=1 each]); none were considered treatment-related.
In the long-term extension study (n=19), all patients experienced AEs, of which 4 (21.1%) patients had treatment-related AEs (most common [occurring in >1 patient]: headache [n=2], injection site bruising [n=2]). Treatment-related AEs occurred at similar frequencies in the naïve (20.0% [n=2/10]) and switch (22.2% [n=2/9]) cohorts. Six (31.6%) patients experienced serious AEs (anemia [n=2]; deep vein thrombosis [n=1]; headache, nausea, osteoarthritis, and rotator cuff syndrome [n=1]; infectious enterocolitis and tongue hematoma [n=1]; encephalopathy, pneumococcal pneumonia, and suicide attempt [n=1]), of which headache and nausea experienced by 1 (5.3%) patient were deemed treatment-related.

Twenty-one injection site reactions occurred in 12 (41.4%) patients across the 12-week and extension study periods; all were mild except for 1 event of moderate severity. Headache was the most common AE across all study periods, occurring in 12 (41.4%) patients. No deaths or meningococcal infections occurred during the studies.

To understand the mechanism for switch failure, an experimental analysis was performed in which the impact of treatment on hemolytic protection of commercially sourced type III PNH RBCs after complement activation and C3b opsonization was studied by flow cytometry (Supplementary Figure S1). In the absence of complement activation (heat-inactivated sera condition), type III RBCs (absence of CD59 expression) accounted for 60% of the total RBC pool in the analyzed PNH donor, while the type II RBC population (partial/reduced CD59 levels) was small in this donor and consequently excluded from further analysis. Low levels of C3b were detected on type III but not type I (high CD59 expression) RBCs. Acidification of complement-competent serum resulted in alternative pathway activation and lysis of type III RBCs in the absence of C5 inhibition (data not shown). Blocking C5 activation with either eculizumab or zilucoplan resulted in partial protection of type III RBCs from lysis and deposition
of C3b on type III RBCs. In the presence of both eculizumab and zilucoplan, type III RBCs were protected from lysis and present at levels similar to controls (heat-inactivated serum; 

**Supplementary Figure S1A**), and a larger proportion of highly C3b-opsonized type III RBCs were generated compared with eculizumab treatment or zilucoplan alone (**Supplementary Figure S1B and C**). During the switch protocol, eculizumab and zilucoplan are both circulating in the blood at therapeutic concentrations for several days to over a week. The combination of eculizumab and zilucoplan enabled the accumulation of high densities of C3b on PNH type III RBCs, which was not observed in type I RBCs. High concentrations of C3b may enable a non-enzymatic cleavage of C5 on the surface of RBCs that cannot be inhibited by a C5 inhibitor, including zilucoplan. Prior studies have suggested that high density of membrane-bound C3b can directly activate C5, leading to membrane attack complex formation without proteolytic cleavage of C5 into C5a and C5b. These prior analyses demonstrated conformational activation of C5 in absence of convertases or other enzymes that cannot be inhibited by different individual C5 inhibitors alone. We hypothesize that after eculizumab washout, densely C3-opsonized RBCs bind C5, which then adopts a C5b-like conformation that cannot be efficiently inhibited by zilucoplan, resulting in intravascular hemolysis of this cell population (**Supplementary Figure S1D**).

Management of patients with PNH should seek to achieve complete and sustained inhibition of terminal complement. Residual free C5 was associated with increased risk of breakthrough intravascular hemolysis in patients on other C5 inhibitors. Free C5 as not assessed in our trial, but complete complement inhibition was seen using functional assays in the current studies (Figures 2A and 3A) and in other populations, patients with generalized myasthenia gravis.
In conclusion, in eculizumab-naïve patients with PNH treated with zilucoplan, LDH reductions were similar to those previously reported with eculizumab,\(^4\) which agrees with the pharmacodynamic effect of zilucoplan. Despite confirmed complete complement inhibition, transfusion-dependent patients in the switch cohort with high reticulocyte counts failed to respond sufficiently to zilucoplan.

We expand upon the findings of other research groups to provide a rationale for increased hemolysis in patients who switched from eculizumab to zilucoplan.\(^10,11\) This phenomenon is thought to be PNH-specific as a result of the disease-induced absence of complement inhibitors on RBCs. Overall, zilucoplan therapy was safe and well tolerated in patients with PNH.

Despite the cessation of clinical development of zilucoplan in PNH, the efficacy and safety profile of this novel C5 inhibitor in generalized myasthenia gravis,\(^7\) along with the flexibility of once-daily at-home subcutaneous injections, has established zilucoplan as another potential option in the growing armamentarium of C5 inhibitors.\(^3\) It has been suggested that combined treatment targeting of different components of the complement cascade might overcome the residual hemolysis seen in a proportion of patients with PNH treated with a C5 inhibitor.\(^2,14\)
Meeting presentation: European Hematology Association; June 14-17, 2018; Stockholm, Sweden.
References


FIGURE LEGENDS

Figure 1. Study design and patient flow for Studies 201 and 203. (A) Study designs for Studies 201 and 203 and (B) CONSORT diagram.

The two 12-week, single-arm studies (Study 201; conducted April 2017 to January 2018, and Study 203; conducted September 2017 to February 2018) enrolled 26 and 3 patients, respectively. The analysis includes 10 patients from Study 201 who were eculizumab-naïve and 19 with prior eculizumab treatment (eculizumab switch cohort; 16 patients from Study 201 and 3 patients from Study 203). All 10 patients in the naïve cohort and 11/19 patients in the switch cohort entered the open-label extension study. *The naïve cohort included patients with no prior exposure to eculizumab. †The switch cohort included patients with prior exposure to eculizumab for ≥6 months before screening. On Day 1, a single loading dose of 0.3 mg/kg zilucoplan was administered subcutaneously. Thereafter, patients self-injected subcutaneous daily zilucoplan at home for the subsequent 12 weeks. Dose escalation to 0.3 mg/kg daily could be initiated at Week 2 if LDH <1.5× ULN was not achieved or an overt breakthrough hemolysis episode occurred (assessed via investigator judgement). Dose increase to 0.3 mg/kg in 10 patients in the naïve cohort and 16 patients in the switch cohort after a median time of 19 days (range, 15-669 days) and 19.5 days (range, 1-57 days), respectively. §Blood samples for pharmacodynamics were collected within 1 hour of the first dose administration and at 1, 3, and 6 hours post-dose on Day 1. ¶For patients who had a zilucoplan dose increase to 0.3 mg/kg, samples for pharmacodynamics were collected at pre-dose, on Day 1 of the new dose, and thereafter at scheduled visits. *No patients from either study were lost to follow-up. D, day; LDH, lactate dehydrogenase; ULN, upper limit of normal; W, week.
Figure 2. Effect of zilucoplan on patients with PNH in the eculizumab-naïve cohort. (A)
Mean complement activity as measured by sRBC assay (classical CP; left y-axis, orange
circles)\textsuperscript{15} and Wieslab ELISA (alternative CP; right y-axis, blue triangles),\textsuperscript{16} (B) mean LDH
levels, and (C) transfusion requirements before and after initiation of zilucoplan. Change in
serum LDH, sRBC lysis, and Wieslab ELISA at each time point were analyzed using the 2-sided
Wilcoxon signed-rank test in each cohort. Missing data were not imputed. *Baseline is the
average of the screening and Day 1 LDH values per patient. C5, complement component 5; CP,
complement pathway; ELISA, enzyme-linked immunosorbent assay; LDH, lactate
dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; SD, standard deviation; SEM,
standard error of the mean; sRBC, sheep red blood cell; ULN, upper limit of normal; W, week.

Figure 3. Effect of zilucoplan on patients with PNH in the eculizumab switch cohort. (A)
Mean complement activity as measured by sRBC assay (classical CP; left y-axis, orange
circles)\textsuperscript{15} and Wieslab ELISA (alternative CP; right y-axis, blue triangles),\textsuperscript{16} (B) mean LDH
reductions, (C) transfusion requirements before and after initiation of zilucoplan, and (D) mean
reticulocyte counts at baseline in the switch cohort, as stratified by switch success (n=8) versus
switch failure (n=11; 282×10\textsuperscript{9}/L vs 159×10\textsuperscript{9}/L; male ULN: 130×10\textsuperscript{9}/L; female ULN:
120×10\textsuperscript{9}/L). Switch failure was defined as zilucoplan discontinuation during the first 12 weeks;
patients could resume eculizumab treatment per individual investigator procedures. Change in
serum LDH, sRBC lysis, and Wieslab ELISA at each time point were analyzed using the 2-sided
Wilcoxon signed-rank test in each cohort. Missing data were not imputed. *Most recent non-
missing value obtained immediately before administration of first dose of zilucoplan; mean (SD)
baseline LDH values: transfusion-independent (n=7), 232.6 (22.6) U/L; transfusion-dependent (n=12), 320.8 (44.4) U/L ($P=0.0296$). C5, complement component 5; CP, complement pathway; ELISA, enzyme-linked immunosorbent assay; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; SD, standard deviation; SEM, standard error of the mean; sRBC, sheep red blood cell; ULN, upper limit of normal; W, week.
A

Study 201
- Eculizumab-naïve cohort (n=10)
- Eculizumab switch cohort (n=16)

Study 203
- Eculizumab switch cohort (n=3)

Pooled eculizumab switch cohort (n=19)

0.1 mg/kg zilucoplan

W1

0.3 mg/kg loading dose

W2

W3

W4

W6

W8

W10

W12

0.1 mg/kg zilucoplan or dose escalate to 0.3 mg/kg

Review of safety & efficacy

Evaluation period for primary efficacy endpoint (change in LDH from baseline to mean of W6–W12)

Blood draw for efficacy and pharmacodynamic assessments

B

Study 201
- Eculizumab-naïve
  - Assessed for eligibility (n=10)
    - Included (n=10)
  - Allocated to intervention (n=10)
    - Received allocated intervention (n=10)
    - Analyzed (n=10)
    - Withdrew (n=0)

Study 203
- Eculizumab switch
  - Assessed for eligibility (n=16)
    - Included (n=16)
  - Allocated to intervention (n=19)
    - Received allocated intervention (n=19)
    - Analyzed (n=19)
    - Withdrew (n=8)
    - Adverse event (n=8)
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- Number of transfusions during a 4W period
- Discontinued

**D**

P-value: 0.091

- Reticulocyte count (SEM; x 10⁹/L)

Switch success (n=11) vs Switch failure (n=8)
### Supplementary Table S1. Key inclusion and exclusion criteria for patients in Studies 201, 202, and 203.

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<th>Inclusion criteria</th>
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<td><strong>Study 201</strong></td>
<td>Platelet count $&lt;30,000/\mu L$ or ANC $&lt;500$ cells/\mu L at screening</td>
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<tr>
<td>$\geq$18 years of age</td>
<td>Glomerular filtration rate of $&lt;30$ mL/min/1.73 m$^2$</td>
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<tr>
<td>Diagnosis of PNH by flow cytometry</td>
<td>Alanine aminotransferase $&gt;2\times$ ULN</td>
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<td>Females of childbearing potential must have had a negative pregnancy test at screening and 24 hours before the first dose of study drug and using effective contraception during the study</td>
<td>or direct bilirubin and alkaline phosphatase $&gt;2\times$ ULN</td>
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<td>C5 inhibitor-naïve cohort: not received treatment with eculizumab before or during screening with LDH level $\geq2\times$ ULN during screening</td>
<td>History of meningococcal disease</td>
</tr>
<tr>
<td>C5 inhibitor switch cohort: treated with eculizumab for $\geq$6 months</td>
<td></td>
</tr>
<tr>
<td><strong>Study 203</strong></td>
<td></td>
</tr>
<tr>
<td>$\geq$18 years of age</td>
<td>Platelet count $&lt;30,000/\mu L$ or ANC $&lt;500$ cells/\mu L at screening</td>
</tr>
<tr>
<td>Diagnosis of PNH by flow cytometry</td>
<td>Glomerular filtration rate of $&lt;60$ mL/min/1.73 m$^2$</td>
</tr>
<tr>
<td>Females of childbearing potential must have been not pregnant at screening and 24 hours before the first dose of study drug and using effective contraception during the study</td>
<td>Direct bilirubin, alanine aminotransferase, or aspartate aminotransferase $&gt;1.5\times$ ULN</td>
</tr>
</tbody>
</table>
| Inadequate response to eculizumab defined as treatment with eculizumab for $\geq$6 months plus $\geq$1 of the following:  
  o Documented LDH level $\geq1.5\times$ ULN within 90 days of screening  
  o Presence of known C5 mutation conferring resistance to eculizumab | History of meningococcal disease |

ANC, absolute neutrophil count; C5, complement component 5; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; ULN, upper limit of normal.
Supplementary Table S2. Demographics and baseline characteristics.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Eculizumab-naïve patients (n=10)</th>
<th>Eculizumab switch patients (n=19)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>56.0 (32-81)</td>
<td>53.0 (21-72)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>6 (60.0)</td>
<td>8 (42.1)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>10 (100.0)</td>
<td>15 (78.9)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>0</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>Not reported</td>
<td>0</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Mean body mass index, kg/m² (SD)</td>
<td>27.3 (6.2)</td>
<td>28.3 (5.6)</td>
</tr>
<tr>
<td>Median disease duration, y (range)</td>
<td>0.8 (0.0-12.0)</td>
<td>4.2 (0.5-36.0)</td>
</tr>
<tr>
<td>Median reticulocyte count, ×10⁹/L (range)</td>
<td>120 (94-331)</td>
<td>196 (41-382)</td>
</tr>
<tr>
<td>Median platelet count, ×10⁹/L (range)</td>
<td>132.5 (19.0-293.0)</td>
<td>142.0 (53.0-224.0)</td>
</tr>
<tr>
<td>Median monocyte clone size, % (range)</td>
<td>82.7 (45.8-99.7)</td>
<td>97.0 (21.5-100.0)</td>
</tr>
<tr>
<td>Median RBC clone size, % (range)</td>
<td>40.2 (8.3-63.3)</td>
<td>60.2 (5.4-99.0)</td>
</tr>
<tr>
<td>Median free hemoglobin, mg/dL (range)</td>
<td>7.1 (1.5-31.2)</td>
<td>1.8 (0.4-148.7)</td>
</tr>
<tr>
<td>Mean LDH, U/L (ULN: 234 U/L) (range; SD)</td>
<td>1174.1 (462-2435; 601.4)§</td>
<td>288.3 (159-797; 132.4)§</td>
</tr>
<tr>
<td>Transfusion-dependent within prior 6 months, n (%)</td>
<td>5 (50.0)</td>
<td>12 (63.2)</td>
</tr>
<tr>
<td>Median duration of eculizumab treatment, y (range)</td>
<td>0</td>
<td>3.6 (0.4-12.0)</td>
</tr>
<tr>
<td>Eculizumab dose &gt;900 mg/Q2W, n (%)</td>
<td>0</td>
<td>7 (36.8)</td>
</tr>
</tbody>
</table>

C5, complement component 5; LDH, lactate dehydrogenase; RBC, red blood cell; SD, standard deviation; Q2W, every 2 weeks; ULN, upper limit of normal.
*Data are representative of subgroup analyses of 2 cohorts.
†Includes 16 patients from Study 201 and 3 patients from Study 203.
‡Average of screening and study Day 1 values.
§Most recent non-missing value obtained immediately before administration of first dose of zilucoplan.
Supplementary Figure S1. Effect of treatment with zilucoplan and eculizumab on C3b opsonization of PNH patient donor cells and mechanistic hypothesis for the role of C5 inhibition with zilucoplan and eculizumab in PNH. (A) Representative flow cytometric plots from a single commercially sourced PNH donor, (B) quantification of mean fluorescence intensity (MFI) from 3 commercially sourced PNH donors, (C) percentage of C3b-positive cells on Type III PNH RBCs from 3 commercially sourced PNH donors, and (D) mechanistic hypothesis for the role of C5 inhibition with zilucoplan and eculizumab in PNH. In healthy individuals, RBCs express complement regulators CD55 and CD59 on the cell surface that are protective against alternative complement pathway–mediated hemolysis via production of the MAC. In patients with PNH in the absence of treatment with zilucoplan or eculizumab, RBCs lacking CD55 and CD59 can be found at a significant proportion (type III clones) in circulation and these cells are susceptible to MAC-mediated intravascular hemolysis. In patients with PNH in the presence of either zilucoplan or eculizumab, MAC-mediated intravascular hemolysis of PNH RBCs is mostly inhibited; however, C3b opsonization of PNH RBCs can still occur, resulting in elimination of RBCs through extravascular hemolysis by macrophages in the liver. In patients with PNH in the presence of both zilucoplan and eculizumab, MAC-mediated intravascular hemolysis is effectively blocked, allowing the accumulation of highly C3b-opsonized RBCs. These highly opsonized RBCs support an elevated density of C5 convertases. Further, this high C3b loading promotes the recruitment of C5, and non-enzymatic rearrangement to a C5b-like conformation that can insert in the membrane and initiate MAC assembly. Upon eculizumab withdrawal, zilucoplan alone cannot overcome MAC-mediated intravascular hemolysis of highly C3b-opsonized RBCs. APC, allophycocyanin; C3b, complement component 3b; C5, complement component 5; CD55, cluster of differentiation 55;
CD59, cluster of differentiation 59; FITC, fluorescein isothiocyanate; HI, heat-inactivated; MAC, membrane attack complex; MFI, mean fluorescence intensity; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell; RES, reticuloendothelial system.