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RESEARCH ARTICLE

C3 inhibition with pegcetacoplan in subjects with paroxysmal nocturnal hemoglobinuria treated with eculizumab

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Abstract

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired, life-threatening hematologic disease characterized by chronic complement-mediated hemolysis and thrombosis. Despite treatment with eculizumab, a C5 inhibitor, 72% of individuals remain anemic. Pegcetacoplan (APL-2), a PEGylated C3 inhibitor, has the potential to provide more complete hemolysis control in patients with PNH. This open-label, phase Ib study was designed to assess the safety, tolerability, and pharmacokinetics of pegcetacoplan in subjects with PNH who remained anemic during treatment with eculizumab. Pharmacodynamic endpoints were also assessed as an exploratory objective of this study. Data are presented for six subjects in cohort 4 who received treatment for up to 2 years. In total, 427 treatment-emergent adverse events (TEAEs) were reported, 68 of which were possibly related to the study drug. Eight serious TEAEs occurred in two subjects; three of these events were considered possibly related to the study drug. Pegcetacoplan pharmacokinetic concentrations accumulated with repeated dosing, and steady state was reached at approximately 6-8 weeks. Lactate dehydrogenase levels were well controlled by eculizumab at baseline. Pegcetacoplan increased hemoglobin levels and decreased both reticulocyte count and total bilirubin in all six subjects. Improvements were observed in Functional Assessment of Chronic Illness Therapy Fatigue scores. Two subjects discontinued for reasons unrelated to pegcetacoplan. All four subjects who completed the study transitioned to pegcetacoplan monotherapy following eculizumab discontinuation and avoided transfusions. In this small study, pegcetacoplan therapy was generally well-tolerated, and resulted in an improved hematological response by achieving broad hemolysis control, enabling eculizumab discontinuation.

1 | INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired, life-threatening hematologic disease characterized by chronic complement-mediated hemolysis and thrombosis caused by the clonal expansion of hematopoietic stem cells that have acquired somatic mutations in the phosphatidylinositol glycan class A gene (*PIG-A*).[1,2] This gene is required for the biosynthesis of

glycosylphosphatidylinositol (GPI) anchors, which bind proteins to the cell surface.[1,2] Cells derived from hematopoietic stem cells with *PIG-A* gene mutations are consequently either partially (type II) or completely (type III) deficient of GPI-anchored proteins, including the complement regulatory proteins CD55 and CD59.[1-3]

Complement activation can be initiated by the lectin, classical, or alternative pathways. Central to all three pathways is C3, which is cleaved by C3 convertases into active fragments, including C3a, a proinflammatory anaphylatoxin, and C3b (Figure S1).[4] The alternative complement pathway is constantly activated at low levels, and C3b promotes the amplification loop within this pathway.[4] C3b is also involved in the formation of C5 convertase, which cleaves C5 into active C5 fragments, including C5a, a proinflammatory and prothrombotic anaphylatoxin, and C5b, a subunit of the membrane attack complex (MAC; also known as C5b-9).[4] CD55 accelerates the decay of convertases in the complement cascade, and CD59 inhibits the formation of the MAC; consequently, the partial or complete loss of CD55 and CD59 on the surface of red blood cells (RBCs) in patients with PNH renders these cells susceptible to complement-mediated attack and hemolysis.[5,6]

Intravascular hemolysis is mediated by the formation of the MAC and is associated with increased levels of lactate dehydrogenase (LDH) and reticulocytosis.[7] Extravascular hemolysis is initiated by the deposition of C3 fragments on the surface of type II and type III RBCs, which facilitates phagocytosis in the liver and spleen.[8] Intravascular or extravascular hemolysis can cause anemia in patients with PNH, which may result in a variety of symptoms, including fatigue, asthenia, and dyspnea. Patients experiencing severe anemia may require packed RBC transfusions.[9,10] Variations in phenotype between individuals with PNH appear to be affected by clone size. Research has shown that there is an association between clone size and LDH levels, and individuals with larger clone sizes are more likely to have a history of thrombosis.[11]

Eculizumab (Alexion Pharmaceuticals, Inc, Boston, MA) is an anti-C5 humanized monoclonal antibody, and was the first complement inhibitor approved by the US Food and Drug Administration (FDA) for the treatment of PNH.[12] Eculizumab binds to C5 and prevents its cleavage by C5 convertase, thus inhibiting MAC formation.[12] Although eculizumab has been shown to reduce intravascular hemolysis and the risk of thrombosis in patients with PNH, some patients receiving treatment continue to experience anemia and some remain transfusion-dependent.[12-15] In a retrospective analysis of 141 patients with PNH who had received treatment with eculizumab for ≥13 months, 72% of patients remained anemic and 36% required ≥1 transfusion per year.[13] Extravascular hemolysis can result in anemia in patients receiving treatment with eculizumab.[14] Episodes of breakthrough hemolysis have also been observed in patients receiving treatment with eculizumab.[8]

Ravulizumab (Alexion Pharmaceuticals, Inc, Boston, MA) is another anti-C5 humanized monoclonal antibody approved for the treatment of adults with PNH by the US FDA and the European Medicines Agency. Ravulizumab differs structurally from eculizumab by the substitution of four amino acids that extend its circulating half-life.[16,17] It has been shown to be non-inferior to eculizumab for transfusion avoidance and hemoglobin stabilization.[16] Currently, there are no therapeutic options that address extravascular hemolysis in patients with PNH.

Given the clinical impact of C3-mediated extravascular hemolysis in patients with PNH, research into C3 as a possible therapeutic target is warranted.[8,18-20] We hypothesized that in subjects with PNH who remained anemic during treatment with eculizumab, complement blockade at the level of C3 with pegcetacoplan (APL-2; Apellis Pharmaceuticals, Waltham, MA) would improve hemoglobin levels and provide more complete hemolysis control.

Pegcetacoplan is an investigational, targeted C3 inhibitor consisting of two 15-amino acid cyclic peptides conjugated to a linear polyethylene glycol (PEG) molecule to increase its half-life. Pegcetacoplan binds to C3 and inhibits its activation.[21] It also binds to and prevents the activity of C3b, inhibiting the activity of convertases containing a C3b subunit, including C3 and C5 convertases associated with the alternative pathway, and C5 convertase associated with the classical pathway.[21] We report the results from an open-label, phase Ib study designed to assess the safety, tolerability, and pharmacokinetics (PK) of single and multiple subcutaneous (SC) doses of pegcetacoplan in subjects with PNH who remained anemic during treatment with eculizumab. The assessment of pharmacodynamic (PD) endpoints was an exploratory objective of this study. Here, we focus on results from subjects in cohort 4.

2 | METHODS

2.1 | Study design

This was an open-label, phase Ib, prospective, non-randomized, single and multiple ascending dose study conducted across seven clinical sites in the United States (PHAROAH, NCT02264639). The study was conducted in accordance with the guidelines for Good Clinical Practice and the Declaration of Helsinki. The study protocol was reviewed and approved by institutional review boards for each study site. Written informed consent was obtained from each participant.

2.2 | Inclusion/exclusion criteria

At screening, males and females aged ≥18 years, diagnosed with PNH, and weighing >55 kg were eligible to enter the study if they had been receiving treatment with eculizumab for at least 12 weeks. Subjects were required either to have received at least one transfusion within the previous 12 months or have hemoglobin levels <10 g/dL. Platelet count >30 000/mm^3 and absolute neutrophil count >500/mm^3 were also required. Prior to entry, subjects were vaccinated against *Neisseria meningitidis* types A, C, W, Y, and B; *Streptococcus pneumoniae*; and *Haemophilus influenzae* type B. Subjects were required to adhere to protocol-defined methods of contraception for the study duration, and women of childbearing potential were required to have a negative pregnancy test. Subjects with an active bacterial infection or known

infection with hepatitis B or C or HIV were excluded. Subjects with hereditary complement deficiency, a history of bone marrow transplantation or meningococcal disease, QTcF prolongation >450 ms for males and >470 ms for females, or creatinine clearance <50 mL/min were also excluded.

2.3 | Pegcetacoplan and eculizumab treatment schedules

This study consisted of four cohorts. Screening assessments were performed within 30 days prior to pegcetacoplan dosing initiation on day 1, and subjects could participate in multiple cohorts. Subjects in cohorts 1 and 2 initially entered a single-dose phase and received a pegcetacoplan dose of 25 and 50 mg, respectively. Following a 28-day waiting period, subjects then entered a multiple-dose phase (Part 1), in which cohort 1 subjects received pegcetacoplan 5 mg/d and cohort 2 subjects received 30 mg/d for a further 28 days. Subjects in cohort 3 entered directly into a multiple-dose phase and received pegcetacoplan 180 mg/d for 28 days. Subjects in cohort 4 also entered a multiple-dose phase and received 270 mg/d for 28 days (Part 1).

Subsequent to the review of available safety, PK, and PD data, subjects who demonstrated a clinical benefit from treatment continued to receive daily doses of pegcetacoplan until day 84 (Part 2A), day 364 (Part 2B), and day 729 (Part 2C). Subjects in cohorts 1-3 who completed the study entered an 8-week follow-up period (Part 3). Any subject that withdrew from the study also entered the follow-up period. Upon completion of Part 2C, subjects in cohort 4 could either enter Part 3 or enroll in an open-label extension study (APL2-307, NCT03531255) evaluating the long-term safety and efficacy of pegcetacoplan. Subject disposition is shown in Figure S2.

Doses in cohort 4 could be increased to 360 mg/d if a subject had a sub-optimal clinical response but acceptable tolerability to treatment. Increases were subject to approval by the Sponsor and implemented on an individual subject basis. Pegcetacoplan doses investigated in the study were selected based on data from standard toxicology studies and initial clinical testing in healthy volunteers. Final data from healthy volunteer trials were not available at the time of study initiation; consequently, conservative doses based on PK modeling predictions were selected for cohorts 1 and 2. Following review of data from healthy volunteer studies, the doses for cohorts 3 and 4 were amended to 180 and 270 mg/d, respectively.

Pegcetacoplan was initially administered as SC injections by trained research personnel. Subjects transitioned to self-administered SC infusions following the introduction of an ambulatory syringe pump. Administered volumes of pegcetacoplan ranged from 0.25 mL to 9 mL (Table S1). If the dose volume was ≤3 mL, doses were administered as 1 or 2 bolus SC injections. If the volume was >3 mL, doses were administered as SC infusions lasting approximately 20 minutes.

Treatment with eculizumab was a prerequisite for entry into the study. The product labeling approved by the FDA for eculizumab is 900 mg every 2 weeks. During the study, investigators adjusted eculizumab dosage as required, based on the subject's clinical status. Adjustments to eculizumab included dose reduction and discontinuation of therapy. Any subject who discontinued eculizumab therapy continued to receive pegcetacoplan monotherapy. Prophylactic antibiotic therapy was prescribed to all subjects and was initiated on day 1 and continued until 2 weeks after the final dose of pegcetacoplan.

2.4 | Outcome measures

Primary endpoints were the number and severity of treatment-emergent adverse events (TEAEs) and pegcetacoplan PK parameters. Exploratory PD endpoints included levels of hemoglobin, LDH, and total bilirubin; reticulocyte count; serum C3 levels; and an assessment of the hemolytic activity of the classical and alternative pathways using CH50 and AH50 assays. The clonal distribution of PNH RBCs and degree of C3 fragment deposition on these cells was assessed. The number of packed RBC transfusions administered during the study was also recorded. To assess quality of life, the Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) questionnaire was self-administered by subjects during clinic visits. Blood samples were collected for PK and PD assessments. Additional details are provided in the Methods section of the Appendix.

2.5 | Statistical analysis

Due to the small sample size, treatment responses are shown by plotting individual subject data over time.

3 | RESULTS

3.1 | Subject demographics and characteristics

In total, 10 subjects were screened and nine (eight female and one male) were enrolled into the study. Two subjects entered multiple cohorts. Baseline demographics and characteristics of the subjects in cohort 4 are shown in Table 1. Five of the six subjects enrolled in this cohort were receiving higher or more frequent doses of eculizumab at screening than the FDA-approved label dose.

Four subjects completed the study and enrolled into the extension study. Subject 9 withdrew on day 353 due to comorbidities unrelated to pegcetacoplan, and subject 11 withdrew on day 253 due to pregnancy. All four subjects who completed the study discontinued eculizumab treatment between days 457 and 626 (Table S2), and continued to receive pegcetacoplan monotherapy.

Pegcetacoplan dose was increased above 360 mg/d in subject 12. Following approval by the Sponsor and institutional review board, this subject received pegcetacoplan 360 mg/d with a 720 mg dose administered every fourth day from day 616 until the end of the study. This was equivalent to a dose of 440 mg/d.

TABLE 1 Baseline demographics and characteristics

Parameter	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11[a]	Subject 12[b]
Age, years	50	52	35	57	38	48
Sex	Female	Female	Female	Female	Female	Female
BMI, kg/m^2	35.2	22.8	59.2	27.8	25.6	45.9
Time since diagnosis, years	22	11	1	9	21	9
Number of packed RBC transfusions in the year prior to first enrolled cohort[c]	1	9	1	3	10	8
Pre-dose C3 complement,[d] mg/dL	117	91	133	82	89	126
Pre-dose CH50 complement,[e] CAE units	6	0	0	0	23	0
Pre-dose AH50 complement,[f] % normal	<35	<35	<35	<35	<35	<35
Pre-dose clonal distribution of PNH RBCs, %						
CD59 type I	7.5	52.5	21.6	66.6	80.9	0.4
CD59 type II	51.6	1.0	53.6	13.1	0.2	48.7
CD59 type III	40.9	46.5	24.8	20.7	18.9	50.9
Pre-dose hemoglobin,[g] g/dL	8.4	9.6	7.4	10.5	9.8	7.0
Pre-dose LDH,[h] U/L	199	225	535	211	307	204
Pre-dose total bilirubin,[i] mg/dL	1.8	1.9	1.8	2.0	1.3	2.7
Pre-dose reticulocyte count,[j] $\times 10^9$/L	362.4	295.7	159.8	272.0	515.2	398.5
Pre-dose FACIT-F total score	49	28	33	28	17	40
Pre-dose PNH granulocytes, % FLAER	81.0	98.6	90.5	95.7	55.2	99.2
Pre-dose PNH monocytes, % FLAER	91.8	96.9	85.0	91.3	50.4	97.1
Eculizumab treatment at screening	1200 mg approx. every 2 wk	900 mg approx. every 2 wk	1200 mg approx. every 2 wk	900 mg once weekly	1200 mg approx. every 2 wk	900 mg once weekly

Abbreviations: BMI, body mass index; CAE, complement activity enzyme; FACIT-F, Functional Assessment of Chronic Illness Therapy Fatigue scale; FLAER, fluorescein-labeled proaerolysin; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell.

[a]Subject 11 was also enrolled in cohort 2 and cohort 3.

[b]Subject 12 was also enrolled in cohort 3.

[c]Number of transfusions in the last 12 months prior to randomization.

[d]C3 complement reference range at baseline 82-160 mg/dL.

[e]CH50 complement reference range at baseline 60-144 CAE units.

[f]AH50 complement reference range at baseline >59% normal.

[g]Hemoglobin reference range at baseline 12-15 g/dL.

[h]LDH reference range at baseline 110-209 U/L.

[i]Total bilirubin reference range at baseline 0.2-1.2 mg/dL.

[j]Reticulocyte count reference range at baseline 39-123 $\times$ 10^9/L.

3.2 | Safety

In cohort 4, 427 TEAEs were reported, of which 68 were considered possibly related to the study drug (Table 2). In total, 57 injection site reactions (ISRs) were recorded for >3300 SC injections/infusions. The majority of ISRs were mild and quickly resolved during the treatment period. Two ISRs (an injection site erythema and an injection site induration) were reported as moderate in severity, though neither resulted in any interruption to pegcetacoplan dosing. Of the 68 TEAEs considered possibly related to the study drug, 48 were related to the injection site. TEAEs summarized by System Organ Class are shown in Table S3.

Twelve serious adverse events (SAEs) were reported, of which eight were TEAEs. The three urinary tract infections and single pyrexia episode occurred >30 days after the final dose of pegcetacoplan (Table 3). The 12 SAEs were reported in subjects 9 and 11.

Events of increased ALT and AST, all of which occurred in subject 11, were considered possibly related to pegcetacoplan. The first instance of increased ALT was recorded on day 28. Pegcetacoplan dosing was temporarily suspended between days 29 and 56 to investigate this event. Pegcetacoplan treatment was reinitiated on day 57 at a dose of 180 mg/d, and increased to 270 mg/d on day 71. During this period of dosing suspension, an SAE of anemia necessitating a blood transfusion was reported on day 48. Hemoglobin levels for this subject improved following the resumption of pegcetacoplan dosing. A recurrence of increased ALT coincided with an increase in AST on day 101 in this subject. Following endoscopic retrograde cholangiopancreatography and sphincterotomy, transaminase levels in this subject normalized, though an SAE of pancreatitis was reported on day 103.

A lower gastrointestinal hemorrhage was recorded on day 39 in subject 9. This event was due to hemorrhoids and necessitated

TABLE 2 Summary of AEs

Event	Number of subjects (N = 6)	Number of events
Any TEAE	6	427
TEAE at least possibly related to study drug	4	68
Serious TEAE	2	8
TEAE of special interest	5	269
TEAE of special interest at least possibly related to study drug	4	55
TEAE leading to study drug discontinuation	0	0
TEAE leading to death[a]	0	0
Injection site reactions	4	57

Note: A subject could be counted more than once per category in total events; but when counting total unique AEs, a subject was only counted once for each AE Preferred Term.
Abbreviations: AE, adverse event; TEAE, treatment-emergent adverse event.
[a]No subjects in cohort 4 died due to an AE. One subject in cohort 1 died from an intracranial hemorrhage on day 35 prior to entering the multiple-dose phase; this event was a serious AE and not a TEAE, as it occurred >30 days after the final dose of pegcetacoplan. This event was considered unlikely to be related to the study drug and possibly related to the subject's history of thrombocytopenia. The platelet count for this subject was 45×10^9/L at screening and 44×10^9/L on day 34 (reference range 150-450 $\times 10^9$/L).

TABLE 3 Summary of serious AEs

Preferred Term	AE maximum severity	Number of subjects (N = 6)	Number of events
Subjects with ≥1 serious AE		2	12
Pyrexia	Severe	1	1
Anemia	Severe	1	1
Lower gastrointestinal hemorrhage	Moderate	1	1
Pancreatitis	Severe	1	1
Portal vein thrombosis	Severe	1	1
Sepsis	Severe	1	1
Urinary tract infection	Severe	1	3
ALT increased	Severe	1	2
AST increased	Severe	1	1

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

hospitalization. Sepsis was recorded on day 199 in subject 9 and was considered to be caused by a procedure for the placement of a kidney stent and nephrostomy tube. Pegcetacoplan dosing was withheld on day 200 in this subject and resumed on day 201. On day 207, an SAE of portal vein thrombosis that lasted for 23 days was reported in this subject. Pegcetacoplan dosing was withheld from day 207 and was not resumed prior to this subject's withdrawal from the study.

3.3 | PK properties of pegcetacoplan

PK analyses revealed a dose-response relationship. In the single-dose phase, pegcetacoplan serum concentration increased with increasing dose. Peak serum concentration was generally observed a week after dosing, reflecting a slow absorption phase by SC administration. Concentrations decreased in a monoexponential manner after reaching maximum serum concentration. Elimination half-life could not be calculated due to limited data, but data from healthy volunteer studies suggested approximately 9-10 days.

In the multiple-dose phase, pegcetacoplan serum concentration accumulated with repeated dosing and increased with increasing dose. Steady state for the majority of subjects was reached approximately 6-8 weeks after dosing initiation, although individual subjects may have reached steady state between 4-6 weeks. After reaching steady state, the change in pegcetacoplan serum concentration appeared to be minimal during treatment. Figure 1 shows semi-logarithmic concentrations of pegcetacoplan in cohort 4. The temporary suspension of pegcetacoplan between days 29 and 56 in subject 11 resulted in a drop in study drug concentration.

3.4 | PD endpoints

Blood samples were analyzed at a central laboratory, and the reference ranges for several endpoints changed during the course of this 2-year study. Data for each of these endpoints were evaluated in the context of the reference ranges provided by the central laboratory at each time point.

3.4.1 | Hemolytic markers and transfusions

Individual hemoglobin, reticulocyte count, total bilirubin, and LDH data for all six subjects in cohort 4 are shown in Figure S3. Individual trellis plots for the four subjects in cohort 4 who completed the study are shown in Figure S4. The trellis plots present hemoglobin values divided by the lower limit of normal (LLN) and reticulocyte count, total bilirubin, and LDH values divided by the upper limit of normal (ULN).

Hemoglobin

The hemoglobin reference ranges provided by the central laboratory during the course of this study were 12-15 g/dL and 11.1-15.9 g/dL. Hemoglobin levels were below the LLN in all subjects at baseline (range 7.0-10.5 g/dL) using either reference range (Figure S3A). Hemoglobin levels increased in all subjects by day 29 and remained steady throughout the study, though some fluctuations occurred during the course of treatment. Following this increase, two subjects were able to undergo regular phlebotomies as a prescribed procedure

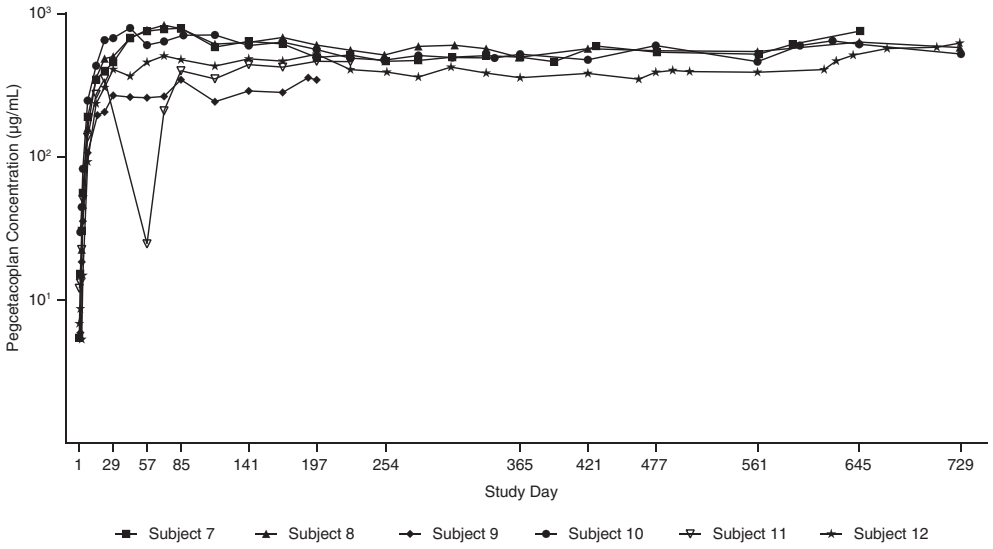

FIGURE 1 Individual semi-logarithmic concentrations of pegcetacoplan in cohort 4

to address severe transfusion-related iron overload. The reference range at the end of the study was 11.1-15.9 g/dL, and three of the four remaining subjects had hemoglobin levels within this range after 2 years of treatment (Figures S3A and S4).

Transfusions

In the 12 months prior to screening, all six subjects had received at least one packed RBC transfusion (Table 1). During the course of the study, two subjects required transfusions. In total, subject 9 received nine packed RBC transfusions, seven of which were administered either before pegcetacoplan serum concentration had reached steady state or after treatment with pegcetacoplan had been suspended. Subject 11 received a 3-unit packed RBC transfusion on day 48 (hemoglobin level 7.1 g/dL), which coincided with a suspension of pegcetacoplan dosing between days 29 and 56. Transfusion avoidance was achieved in all four subjects who completed the study.

Reticulocyte count

The reticulocyte count reference ranges provided by the central laboratory were 39-123 $\times$ 10^9/L and 35-101 $\times$ 10^9/L. Reticulocyte counts were above the ULN in all subjects at baseline using either reference range, and reductions were observed in all subjects during the study (Figures S3B and S4). Peaks in reticulocyte count were observed in subject 11 on days 57, 113, and 225, and in subject 12 on day 616. The reference range at the end of the study was 35-101 $\times$ 10^9/L. Three of the four subjects who completed the study had reticulocyte counts recorded at this time point, and all were ≤ 1.5 $\times$ ULN.

Total bilirubin

The total bilirubin reference ranges provided by the central laboratory were 0.2-1.2 mg/dL and 0.0-1.2 mg/dL. Total bilirubin levels were above the ULN in all subjects at baseline using either reference range (Figure S3C). Decreases in total bilirubin levels were observed in all subjects during the course of the study. A total bilirubin level of 2.5 mg/dL was recorded in subject 10 on day 169 (Figure S4C). The test was subsequently repeated and gave a reading of 0.4 mg/dL, suggesting that the initial value was an anomalous result. A total bilirubin level of 2.3 mg/dL was recorded in subject 12 on day 421 (Figure S4D). This increase occurred after an initial attempt to discontinue eculizumab therapy with no increase in pegcetacoplan dosing. After 2 years of treatment, all four subjects who completed the study had total bilirubin levels within the 0.0-1.2 mg/dL reference range provided by the central laboratory.

LDH

The LDH reference ranges provided by the central laboratory were 110-209 U/L and 119-226 U/L. At baseline, five subjects had LDH levels ≤1.5 $\times$ ULN using both reference ranges (Figure S3D). LDH levels remained stable, with some fluctuations occurring during the course of treatment. An LDH level of 1571 U/L was recorded in subject 12 on day 421 (Figure S4D). This increase occurred after an initial attempt to discontinue eculizumab therapy with no increase in pegcetacoplan dosing. After 2 years of treatment, three of the four subjects who completed the study had LDH levels ≤1.5 $\times$ ULN and one subject had an LDH level above the ULN (Figure S4).

3.4.2 | Effect on PNH RBC clone size and C3 deposition

An increase in type II and III cells as a proportion of the clonal distribution of total RBCs was observed in all 6 subjects, with values >90% observed in five subjects by day 85, at which point values had generally plateaued (Figure S5A). A decrease was observed in subject 11 on day 57 following a temporary suspension of pegcetacoplan dosing between days 29 and 56. The proportion of type II and III RBCs recorded in this subject increased following resumption of pegcetacoplan. A decrease in the deposition of C3 fragments on the surface of type II and III RBCs was observed in all subjects in cohort 4 (Figure S5B). It took 90-120 days for values to decrease to a

minimum close to zero. This time lag is similar to the lifespan of RBCs, suggesting that newly generated RBCs did not accumulate C3 fragments and that existing cells with prior C3 fragment deposition might continue to circulate with a lifespan close to normal.

3.4.3 | Complement markers

The serum C3 reference ranges provided by the central laboratory during the course of this study were 82-160 mg/dL and 82-167 mg/dL. An increase in serum C3 levels was observed in all subjects in cohort 4 (Figure S6). A decrease in serum C3 levels from 415 mg/dL to 107 mg/dL was observed between day 29 and day 57 in subject 11, which coincided with a suspension of pegcetacoplan dosing.

The central laboratory used two different units to quantify CH50 during the course of the study: complement activity enzyme (CAE) units and U/mL. The three CH50 reference ranges provided by the central laboratory during the course of the study were 60-144 CAE units, 41-60 U/mL, and >41 U/mL. No significant effect on CH50 was observed among the six subjects during the first year of treatment (Figure S7A). The reference range provided by the central laboratory at the end of the study was >41 U/mL, and CH50 levels were within this range for all four remaining subjects (Figure S7B).

The central laboratory used two different units to quantify AH50 during the course of the study: % normal units and U/mL. The two AH50 reference ranges provided by the central laboratory during the course of this study were >59% normal and 77-159 U/mL. At baseline, all six subjects had AH50 levels below the reference range of >59% normal (Figure S8A). AH50 levels increased following eculizumab discontinuation, and two of the four subjects who completed the study had AH50 levels within the 77-159 U/mL reference range at the last evaluation time point (Figure S8B).

3.4.4 | Impact on quality of life (fatigue): FACIT-F scores

At baseline, subject 7 had a high FACIT-F score of +49 (compared with the other five subjects, range 17-40), which remained steady throughout treatment. Increases in FACIT-F score were reported in all other subjects during the course of the study (Figure S9). Change from baseline in FACIT-F total score was >3 points in three of the four subjects who completed the study. Subjects 9 and 11, who withdrew from the study, had changes from baseline in FACIT-F total scores of +15 and +16, respectively, at the last evaluation time point.

3.4.5 | Treatment compliance

The majority of pegcetacoplan doses were administered according to the protocol. Pegcetacoplan dosing was withheld in subject 9 from day 207 and was not resumed prior to withdrawal from the study on day 353. Subject 11 did not receive pegcetacoplan between days 29 and 56 due to an SAE. Eight pump malfunctions were reported in three subjects. Five events were experienced by subject 12, and four resulted in the subject missing a dose of pegcetacoplan. Overall treatment compliance was 90.7% for subject 12 and >99% for the other five subjects.

4 | DISCUSSION

Eculizumab was the first complement inhibitor approved by the US FDA for the treatment of PNH. Although eculizumab has been shown to reduce intravascular hemolysis, transfusion dependence, and the risk of thrombosis in these patients, some patients remain anemic and transfusion-dependent.[12,14,15]

This residual anemia is partly due to extravascular hemolysis arising from C3 fragment deposition on RBCs.[14] In a study of biological samples from patients with PNH treated with eculizumab, the percentage of C3 fragment-positive PNH RBCs was highly variable among patients, with a median of 22.6% (range 0.5%-61.3%).[14] The percentage of C3 fragment-positive PNH RBCs was also shown to correlate with absolute reticulocyte count.[14]

We hypothesized that complement blockade upstream of C5 at the level of C3 with pegcetacoplan would improve hemoglobin levels and provide more complete hemolysis control in subjects with PNH who remained anemic during treatment with eculizumab.[18-20] The primary objectives of this study were to assess the safety, tolerability, and PK of single and multiple SC doses of pegcetacoplan in subjects with PNH who were still anemic during treatment with eculizumab. The assessment of PD endpoints was an exploratory objective of this study. Five of the six subjects in cohort 4 presented at baseline with LDH levels ≤1.5 × ULN, indicating that MAC formation was inhibited by eculizumab prior to enrollment. However, five subjects were receiving higher or more frequent doses of eculizumab than the FDA-approved dose and, despite treatment, all six subjects were anemic and had received at least one transfusion within the year prior to screening.

Pegcetacoplan was found to be generally well-tolerated in this small population. The majority of TEAEs were considered either unrelated or unlikely to be related to the study drug. Of the TEAEs possibly related to pegcetacoplan, the majority were related to the injection site. Pegcetacoplan PK concentrations accumulated with repeated dosing in cohort 4, and steady state was reached at approximately 6-8 weeks. The observed PK levels were similar to those predicted in clinical testing in healthy volunteers.

The changes observed in biochemical markers over the course of this study support the broad control of hemolysis by pegcetacoplan, which allowed for a dose reduction in eculizumab therapy and, ultimately, a switch to pegcetacoplan monotherapy in all four subjects who completed the study. Furthermore, transfusion avoidance was achieved in all four of these subjects. At baseline, hemoglobin levels were below the LLN in all subjects and increased over the course of the study. The temporary suspension of pegcetacoplan therapy between days 29 and 56 in subject 11 provided valuable insights as, despite continued eculizumab therapy, hemoglobin levels decreased

during this period. This decrease was attributed to extravascular hemolysis, as further evidenced by persistently-low LDH levels throughout this period and normalization of the subject's hemoglobin level following resumption of pegcetacoplan.

Reticulocyte counts were above the ULN in all subjects at baseline and were shown to decrease during the course of the study. On-treatment decreases were also observed in total bilirubin levels. LDH levels did not change substantially during the course of the study, which may be due to the fact that LDH levels were well controlled by eculizumab at baseline. Outcomes from the FACIT-F questionnaire showed that pegcetacoplan elicited a clinically-meaningful improvement, demonstrated by an increase of >3 points in three of the four subjects who completed the study.[22]

An increase in the proportion of PNH type II and III RBCs was observed, along with reduced C3 fragment deposition on these cells. Given that C3 fragment deposition is an indicator of opsonization and extravascular hemolysis, these findings suggest that pegcetacoplan protects type II and III RBCs from complement-mediated attack and extravascular hemolysis. An increase in serum C3 levels was observed in all subjects. The C3 level at week 4 was approximately 180% higher than at baseline. After completing 2 years of treatment, C3 level was 270% higher than at baseline, which demonstrated robust C3 inhibition through repeated pegcetacoplan dosing. Residual complement activity was also assessed.

No significant effect on CH50 was observed during the first year of treatment; however, as of day 640, all four remaining subjects had CH50 values within the reference range. AH50 values at baseline reflected inhibition of the alternative complement pathway by eculizumab. The effect of pegcetacoplan on AH50 levels could not be determined until after eculizumab discontinuation, after which AH50 levels generally increased. The majority of doses were administered according to protocol requirements and treatment compliance was very high in all subjects.

Previous research suggests that reticulocytosis in the absence of elevated LDH and bilirubin levels could be an indicator of extravascular hemolysis.[8,14] Hence, for patients receiving eculizumab with controlled LDH, reticulocytosis could indicate C3 opsonization of RBCs and may serve as an indicator for those patients who might benefit from the addition of a C3 inhibitor. Owing to its function as a convergence point for activation pathways within the complement cascade and its position upstream of C5, C3 is an attractive therapeutic target.[18-20] There are, however, concerns that chronic impairment of C3 activation may increase susceptibility to infections and immune-mediated diseases, especially with long-term inhibition.[23,24] Although further research is needed to investigate the long-term safety of pegcetacoplan, safety concerns relating to infections did not arise in the study.

Research has shown PEGylated molecules can elicit adaptive changes in cells and tissues; consequently, the safety of the long-term use of PEGylated molecules such as pegcetacoplan may be of concern.[25] However, a review of published literature on PEGylated therapeutic products currently approved for human use showed these products to be safe and well-tolerated.[26] Pegcetacoplan was shown to be well-tolerated in healthy volunteer trials[27] and, although it was a small study, four subjects in PHAROAH received pegcetacoplan for 2 years without any safety concerns arising.

In this small study, pegcetacoplan was generally well-tolerated and resulted in an improved hematological response by achieving broad hemolysis control through the inhibition of C3. This study demonstrated for the first time (to our knowledge) that systematic C3 inhibition using pegcetacoplan can be achieved safely and effectively for at least 2 years and enabled eculizumab discontinuation in four subjects. Research into the therapeutic potential of pegcetacoplan is ongoing with phase Ib and phase 3 studies in subjects with PNH who are treatment naïve (PADDOCK, NCT02588833; PRINCE, NCT04085601). A phase 3 study (PEGASUS, NCT03500549) investigating the efficacy and safety of pegcetacoplan compared with eculizumab is also ongoing. Data from PHAROAH and an improved PK model allowed for the modeling of less-frequent dosing regimens providing the same pegcetacoplan serum levels; consequently, subjects in PEGASUS received pegcetacoplan 1080 mg twice weekly. These studies will further define the efficacy and safety profile of pegcetacoplan as a treatment for patients with PNH.

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CONFLICT OF INTERESTS

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DATA AVAILABILITY STATEMENT

The data generated during the current study are proprietary and not publicly available, but data are available from the corresponding author on reasonable request and with permission of Apellis Pharmaceuticals.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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APPENDIX A.

Methods

TEAEs were defined as AEs that developed or worsened after the first dose of pegcetacoplan and up to 30 days after the final dose. AEs of special interest were also monitored and included local or systemic infection of any origin, ISRs, thromboembolic events, infusion pump-related events, and clinically significant decreases in

kidney function. Local ISRs were rated according to FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (Sep 2007).[28] Any reaction that did not fulfill these criteria was designated an AE.

The clonal distribution of PNH RBCs was assessed by flow cytometry using an FACS Canto II IVD flow cytometer (Becton Dickinson). A minimum of 90 000 events were required for the quantification of the percentage of type II and type III PNH RBCs. Fluorescein isothiocyanate (FITC)-labeled anti–human glycophorin A (clone 11E4B-7-6; Beckman Coulter) and phycoerythrin (PE)-labeled anti-human CD59 (clone MEM-43; Life Technologies) were used to detect RBCs and CD59, respectively. C3 deposition on PNH RBCs was detected using biotinylated monoclonal antibody to human C3d (A702; Quidel) and streptavidin-APC (BioLegend). Appropriate isotype control from Becton Dickinson was included (mouse immunoglobulin G [IgG]-FITC and IgG-PE, both clone X40; and mouse IgG-biotin, clone MOPC-21). Flow cytometry was also used to assess the expression of GPI-anchored proteins on the surface of white blood cells in peripheral whole blood. This assay utilized a panel of analytes including CD14, CD15, CD24, CD45, CD64, and fluorescein-labeled proaerolysin to identify specific cell populations and measure GPI-anchored protein expression.