ORIGINAL ARTICLE

Nationwide study of eculizumab in paroxysmal nocturnal hemoglobinuria: Evaluation of treatment indications and outcomes


1Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands
2Department of Hematology, Internal Medicine, Maastricht University Medical Centre +, Maastricht, The Netherlands
3Department of Clinical Hematology, Amsterdam University Medical Centers, Academic Medical Center, Amsterdam, The Netherlands
4Department of Clinical Hematology, Amsterdam University Medical Centers, Academic Medical Center, Amsterdam, The Netherlands
5Department of Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, The Netherlands
6Department of Laboratory Medicine—Laboratory for Hematology, Radboud University Medical Center, Nijmegen, The Netherlands

Correspondence
Charlotte C. M. Schaap, Department of Hematology, Radboud University Medical Centre, Huispost 476, PO Box 9101, 6500 HB Nijmegen, The Netherlands.
Email: charlotte.schaap@radboudumc.nl

Funding Information
Alexion Pharmaceuticals

Abstract
Eculizumab is an effective treatment for paroxysmal nocturnal hemoglobinuria (PNH). However, considering the risk of life-threatening meningococcal disease, life-long duration and costs, there are strict criteria for initiation of therapy. To evaluate the application and real-world effectiveness of eculizumab in the Netherlands, a multicenter retrospective cohort study was conducted: indications and treatment outcomes were collected for 105 Dutch PNH patients. In all patients, eculizumab was initiated conforming to indications as formulated in the Dutch PNH guideline. According to recently published response criteria, 23.4% of the patients had reached a complete hematological response, 53.2% a good or partial response, and 23.4% a minor response after 12 months of therapy. In the majority of patients the response remained stable during long-term follow-up. The degree and relevance of extravascular hemolysis significantly differed between response groups (p = 0.002). Improvements of EORTC-QLQc30 and FACIT-fatigue scores were observed, however patients reported lower scores than the general population. A detailed evaluation of 18 pregnancies during eculizumab showed no maternal or fetal deaths, and no thromboembolic events during pregnancy. This study demonstrates that the majority of patients benefit from eculizumab when adhering to the indications as formulated in the Dutch PNH guideline. However, novel therapies are needed to further improve real-world outcomes, such as hematological responses and quality of life.

KEYWORDS
eculizumab, hematological response, paroxysmal nocturnal hemoglobinuria, pregnancy outcomes, treatment outcomes
1 | INTRODUCTION

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare disease caused by acquired somatic mutations in the x-linked phosphatidylinositol glycan complementation class A (PIG-A) gene in hematopoietic stem cells (HSCs). Affected hematopoietic cells lack expression of glycosylphosphatidylinositol (GPI) anchors, responsible for anchoring complement regulators DAF, recognized by CD55, and MIRL, recognized by CD59, at these cells. Due to the deficiency of these proteins, red blood cells are vulnerable to complement-mediated lysis. PNH is strongly associated with bone marrow failure syndromes in particular aplastic anemia (AA) and to a lesser extent myelodysplastic syndromes (MDS). The prevalence of clinical PNH in the Netherlands is estimated at 200–250 patients.

The clinical presentation of PNH is remarkably heterogeneous, with anemia due to intravascular hemolysis and its related symptoms being the hallmark of the disease. The most prominent symptom is fatigue which is often more intense than would be expected based on the degree of anemia. Furthermore, due to a severe nitric oxide (NO) scavenging by free hemoglobin, a variety of other symptoms may occur including abdominal pain, dysphagia and erectile dysfunction. Moreover, renal function is commonly affected by the activation of C5 into C5a and C5b, subsequent formation of the terminal complement complex C5b-9 and lysis of erythrocytes by the membrane attack complex. Clinical studies have shown that treatment with eculizumab leads to an impressive resolution of intravascular hemolysis, reduction in transfusion requirements, reduction in thrombosis rate, improvement or stabilization of renal function, successful pregnancy outcomes and improved survival.

Notwithstanding its effectiveness, treatment with eculizumab can cause major complications, the most important being the risk of life-threatening infections with encapsulated bacteria, including meningococcal disease. Moreover, eculizumab is one of world’s most expensive drugs and costs approximately €325 000 to €433 000 per patient per year in the Netherlands, depending on the dosage. It needs intravenous administration at a biweekly frequency, for a lifelong duration, without the possibility to temporarily interrupt due to the associated risk of severe breakthrough hemolysis.

Given the above, eculizumab is designated as an orphan drug for the treatment of PNH. As such, strict indications for its initiation have been defined, which in the Dutch guideline for diagnosis and treatment of PNH have been formulated as red blood cell (RBC) transfusion dependency, thromboembolic events related to PNH, hemolysis related severe impairment of quality of life (QoL), renal failure, pulmonary hypertension, pregnancy and exceptional cases after agreement of the Dutch PNH working group. These indications are in line with indications as formulated in other national guidelines, such as the National PNH Service guideline (UK) on eculizumab administration and the German/Austrian/Swiss onkopenidea guideline PNH.

In this study, we evaluated our real-world application and clinical- and patient reported outcomes of eculizumab therapy across the Netherlands, aiming to further improve our strategy for proper patient selection and optimizing eculizumab effectiveness for the Dutch patient population.

2 | MATERIALS AND METHODS

2.1 | Study design

We conducted a retrospective observational multicenter cohort study across the Netherlands, including all PNH patients treated with eculizumab during a period of at least 1 month until 23 September 2021. Patients who objected to the use of their medical data for research purposes were excluded. Across this study cohort, we evaluated the

Novelty statement

What is the new aspect of your work?

This is the first study of its kind describing both real-world application and five categories of clinical- and patient-reported outcomes (among which hematological responses according to recently published response criteria) of eculizumab therapy in a nation-wide cohort of PNH patients.

What is the central finding of your work?

Although the majority of PNH patients benefit from eculizumab therapy when adhering to the strict indications as formulated in the Dutch PNH guideline, novel therapies are needed to further improve real-world outcomes.

What is (or could be) the specific clinical relevance of your work?

Data reported in this study can be used to compare real-world outcomes of eculizumab with real-world outcomes of novel complement inhibition agents.
indications for initiation of treatment and five categories of outcomes, that is, hematological responses, effects on intravascular hemolysis and its related complications and symptoms, survival, effects on extravascular hemolysis, and pregnancy-related outcomes.

2.2 | Data collection and analysis

General patient- and treatment-related data was extracted from the electronic medical patient charts and collected using Castor EDC (Castor Electronic Data Capture, Ciwit BV, Amsterdam, The Netherlands). Recorded patient characteristics included gender, age at diagnosis, age at initiation of eculizumab, indication for start of treatment, duration of PNH until initiation of eculizumab, last preeculizumab granulocyte PNH clone size determined by flow cytometry, history of aplastic anemia, history of thromboembolism, and transfusion dependency. Transfusion dependency was defined as at least one red blood cell (RBC) transfusion during the 12 months prior to initiation of eculizumab.

Hematological responses were defined based on hemoglobin (Hb) responses and reductions in RBC transfusion exposure according to criteria proposed by Debureaux et al.23 (Table S1). For all patients who received at least 1 year of eculizumab, responses were evaluated at 6 months, at 12 months, and during the last 6 months of eculizumab therapy before September 2021.

To evaluate the effect of eculizumab on the degree of intravascular hemolysis, for all patients who received at least 1 year of therapy lactate dehydrogenase (LDH) levels were collected at baseline (last preeculizumab value), at 6 months, at 12 months, and at a single time point during the last 6 months of eculizumab therapy before September 2021. Moreover, the number of breakthrough hemolytic events which occurred between October 2013 (implementation of electronic medical record system) and September 2021 were collected for all patients who were treated at the Radboudumc during this period. Breakthrough hemolysis (BTH) was defined as LDH level >1.5× the upper limit of normal (ULN) and increased as compared to the steady-state, in combination with a Hb drop ≥2 g/dL and/or symptoms related to intravascular hemolysis (such as gross hemoglobinuria, painful crisis, dyspnea, or any other significant clinical finding).24

To study the effect of eculizumab on renal function, estimated glomerular filtration rates (eGFR) calculated using the Modification of Diet in Renal Disease (MDRD) equation25 were collected at baseline, at 6 months and at 12 months after initiation of eculizumab for all patients who received at least 1 year of therapy. To examine the effect of eculizumab therapy on thrombosis risk, number of thromboembolic events prior to- and during eculizumab therapy were collected. Furthermore, data on patient survival were gathered.

In 2020, a flow cytometric test to measure the percentage of C3d opsonized PNH erythrocytes, a marker for extravascular hemolysis,26 was designed and validated in the Radboudumc (Supplementary methods). To compare the degree of extravascular hemolysis across the four hematological response groups, absolute reticulocyte count, percentage of C3d opsonized PNH erythrocytes, and hematological responses were determined at the time the flow cytometric test became available for patients receiving eculizumab treatment at the Radboudumc, independent of duration of eculizumab therapy.

To assess the effect of eculizumab on QoL and fatigue, patient reported outcomes on QoL and fatigue were extracted from the International PNH registry (NCT01374360) for a subset of patients who enrolled in the registry and who completed one Patient Assessment Questionnaire (PAQ) during the 12 months before initiation of eculizumab and one PAQ between 6 and 12 months after initiation of eculizumab. Patient reported outcomes on QoL were measured using the EORTC QLQ-C30 score (version 3); a continuous scale ranging from 0 to 100 with higher scores indicating better functioning. This score includes the domains physical-, role-, emotional-, social- and cognitive functioning and patients ratings of overall QoL and health.27 Fatigue was measured using the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue score: a continuous scale ranging from 0 to 52, with higher scores indicating less fatigue.28 Clinically meaningful improvements are considered as changes of 3 and 10 points in FACIT-Fatigue and EORTC QLQ-C30 scores, respectively.29–31 Additionally, physician-reported Karnofsky scores were extracted from the registry for a subset of patients from whom this score was available during the 12 months before initiation of eculizumab and between 6 and 12 months after initiation of eculizumab.

To study pregnancy outcomes during eculizumab therapy, obstetrical data from all patients who used eculizumab during pregnancy were accumulated. These data included number of pregnancies, maternal age at delivery, pregnancy outcome, term and mode of delivery, birth weight, possible PNH related complications during pregnancy and/or delivery, the need for blood and platelet transfusions during pregnancy and/or delivery, and data on the dosages of eculizumab during pregnancy.

2.3 | Statistical analysis

Categorical and continuous data are described by proportions and medians (range), while comparison analyses were performed using the Wilcoxon signed rank test, Mann–Whitney U test and Kruskal–Wallis H test. The incidence of thromboembolic events was calculated as number of events per 100 patient-years (PY) of PNH, and as number of events per 100 PY of eculizumab exposure. The incidence of breakthrough hemolytic events was calculated as number of events per 100 PY of eculizumab exposure. To assess overall survival rate, Kaplan–Meier analysis was used. p-values <0.05 were considered significant. Data were analyzed using IBM SPSS statistics 25.0.

3 | RESULTS

3.1 | Study population and patient characteristics at baseline

Baseline characteristics of 105 patients included in this study are depicted in Table 1. Fifty-two percent of the patients were female, and median age at initiation of eculizumab was 43 years (range 13–85 years). Median duration of PNH until start of eculizumab was

---

**Table S1**. For all patients, clinically meaningful changes were determined at the time the flow cytometric test became available for patients receiving eculizumab treatment at the Radboudumc, independent of duration of eculizumab therapy.
Since eculizumab was approved for the treatment of PNH in 2007, patients diagnosed following this date (n = 76) had a significantly shorter interval between diagnosis and initiation of eculizumab (median 2.1 years, range 0.0–9.4 years) as compared to patients diagnosed in the era before (median 13.5 years, range 1.7–44.1 years; p < 0.001). Cumulative exposure to eculizumab was 533.5 patient-years (PY) with a median duration of eculizumab therapy of 4.4 years (range 0.2–13.9 years). Ninety-five (90%) patients had received eculizumab for at least 1 year at the time of analysis.

### 3.2 | Indications for eculizumab therapy

In all patients, eculizumab was initiated according to Dutch guideline criteria (Table S2). Hemolysis related symptoms leading to severe impairment of quality of life (n = 50), transfusion dependency (n = 38), and PNH related thrombosis (n = 31) were the main reported indications for eculizumab. Other indications included pregnancy (n = 10), renal insufficiency (n = 8), and prevention of complications during allogenic stem cell transplantation (n = 1). Twenty-eight patients had more than one indication to start eculizumab.

### 3.3 | Efficacy of eculizumab therapy

#### 3.3.1 | Hematological response

Data on hematological responses to eculizumab after 6 months, 12 months and during the last 6 months of therapy were available from 94 out of 95 patients who received at least 1 year of treatment.
Comparison of markers for extravascular hemolysis and intravascular markers with a minor hematological response (response after 12 months of therapy. Response, 45.4% a good or partial response and 48.5% a minor response, 45.4% a good or partial response and 48.5% a minor response, 45.4% a good or partial response and 48.5% a minor response before completion of this study (152 patients with a good (response category of 55 patients. Reticulocyte count did not significantly differ between the groups (p

\[\text{median LDH levels of patients (n = 95) significantly} \]
\[\text{decreased from 1374 IU/L (range 254–5934 IU/L) at baseline to} \]
\[\text{246 IU/L (range 148–514 IU/L) after 6 months (p < 0.001), 250 IU/L} \]
\[\text{(range 158–454 IU/L) after 12 months (p < 0.001), and 243 IU/L (range} \]
\[\text{152–474 IU/L) measured at a single time point during the last 6 months} \]
\[\text{of therapy before completion of this study (p < 0.001).} \]

3.4 | Intravascular hemolysis

The optimal target of LDH reduction with eculizumab is <1.5 x ULN (ULN = 250 IU/L). Median LDH levels of patients (n = 95) significantly decreased from 1374 IU/L (range 254–5934 IU/L) at baseline to 246 IU/L (range 148–514 IU/L) after 6 months (p < 0.001), 250 IU/L (range 158–454 IU/L) after 12 months (p < 0.001), and 243 IU/L (range 152–474 IU/L) measured at a single time point during the last 6 months of therapy before completion of this study (p < 0.001).

3.5 | Breakthrough hemolysis

Data on breakthrough hemolytic events between October 2013 and September 2021 were collected for 88 patients. A total of 71 events were reported in 35 patients (40%) during 422.34 PY of eculizumab therapy. The majority of breakthrough events were attributed to infections (n = 57). Moreover, BTH occurred due to labor (n = 3), COVID vaccination (n = 3), surgery (n = 2), symptomatic cholelithiasis (n = 2), hyperemesis gravidarum (n = 1) and gout (n = 1). In one case BTH occurred during thrombosis. It is unknown if BTH was due to thrombosis, or was a risk factor for thrombosis in this case. Furthermore, in one case the trigger was not identified.

3.6 | Extravascular hemolysis

In Figure 2, markers for extravascular hemolysis are compared between hematological response categories of 55 patients. Reticulocyte count did not significantly differ between the groups (p = 0.119; Figure 2A). The percentage of C3d opsonized PNH erythrocytes was significantly lower in patients with a complete hematological response (n = 12) as compared to patients with a good (n = 21) or partial (n = 15) response (p = 0.040 and p < 0.001, respectively), but did not significantly differ from patients with a minor hematological response (n = 7; p = 0.227; Figure 2B).

3.7 | Renal function

At baseline, 14.7% of the patients (n = 14) had an eGFR <60 mL/min, of whom five patients had acute kidney injury (median eGFR 30 mL/min, range 7–47 mL/min) and nine patients had chronic kidney disease (median eGFR 50 mL/min, range 35–56 mL/min). In all patients with acute kidney injury, renal impairment was the indication for initiation of eculizumab, and renal function significantly improved after 6 months of therapy (median eGFR 100 mL/min, range 77–128 mL/min), and after 12 months of therapy (median eGFR 101 mL/min, range 81–116 mL/min) as compared to baseline (p = 0.043 and p = 0.043, respectively). After initiation of eculizumab, in none of these patients renal failure recurred.

In patients with chronic kidney disease at baseline, renal function did not significantly improve after 6 months of therapy (median eGFR 48 mL/min, range 35–74 mL/min) and 12 months of therapy (median
3.8 | Thromboembolic events

Prior to initiation of eculizumab, 37 patients (35.2%) experienced 49 thromboembolic events during 639.84 PY of PNH. Seven patients on eculizumab therapy (6.7%) developed a nonlethal thromboembolic event (Table S3). Accordingly, the incidence rate decreased from 7.7 to 1.3 per 100 PY. Thrombosis developed in three eculizumab-treated patients despite therapeutic anticoagulant co-therapy at the moment of thrombosis. In two of seven patients, LDH levels were >1.5 × ULN at the time of thrombosis.

3.9 | Survival

Eleven patients (10.5%) died while on eculizumab. Five-years Kaplan–Meijer estimate of patient survival was 92.7% (95% confidence interval 84.3–96.7). Median age at death was 79 years (range 64–

FIGURE 3  Patient-reported outcomes on quality of life (QoL) (A) and fatigue (B), and physician-reported Karnofsky scores (C) before and during eculizumab therapy. Data were available for a subset of patients who enrolled in the international PNH registry and who completed one Patient Assessment Questionnaire (PAQ) in 12 months before initiation of eculizumab and one PAQ between 6 and 12 months after initiation of eculizumab. Boxes show median, and first and third quartiles. Whiskers extend to minimum and maximum values, p-values <0.05 are considered to be significant.
94 years). Causes of death included hematologic disorders other than PNH (n = 4), nonhematological malignancies (n = 3), pneumosepsis (n = 3) and unknown (n = 1). Mortality due to additional hematological diseases included therapy refractory aplastic anemia, MDS complicated by pulmonary invasive mould disease, chronic myelomonocytic leukemia and myeloproliferative neoplasm not otherwise specified. No patients died from meningococcal infections.

3.10 Patient-reported outcomes and physician-reported Karnofsky scores

Figure 3 shows patient-reported outcomes on QoL and fatigue, and physician-reported Karnofsky scores before and during eculizumab therapy. Median EORTC-QLQ-c30 scores on physical functioning, role functioning, emotional functioning, and cognitive functioning significantly increased during eculizumab therapy (median change of 10.00, 16.66, 8.33, and 16.66 points, respectively; Figure 3A). Moreover, there was a significant and clinically meaningful improvement of median FACIT-Fatigue score during eculizumab therapy (median change of 7.00 points; Figure 3B) as well as physician-reported Karnofsky scores (median change of 10.00 points; Figure 3C).

3.11 Pregnancy outcomes

Table 2 reports course and outcome of 18 pregnancies in 12 females during eculizumab exposure. In 10 pregnancies (55.6%) eculizumab was initiated during pregnancy while eight pregnancies (44.4%) were conceived under eculizumab therapy. All patients received therapeutic doses of low-molecular-weight heparin during pregnancy until conception. In 10 pregnancies (55.6%) eculizumab was initiated during pregnancy while eight pregnancies (44.4%) were conceived under eculizumab therapy. All patients received therapeutic doses of low-molecular-weight heparin during pregnancy until conception. All pregnant females had platelet counts >50 10^9/L. except for 1 patient who developed acute myeloid leukemia (AML) at 6 weeks of pregnancy. Sixteen pregnancies (88.9%) resulted in live, single births while 2 pregnancies (11.1%) terminated into a spontaneous abortion in the first trimester. No stillbirths occurred and no congenital abnormalities were observed in the newborns. No maternal deaths were reported and none of the pregnant women experienced thrombosis during pregnancy or in the postpartum period.

Sixteen pregnancies (88.9%) resulted in live, single births while two pregnancies (11.1%) terminated into a spontaneous abortion in the first trimester. No stillbirths occurred and no congenital abnormalities were observed in the newborns. No maternal deaths were reported and none of the pregnant women experienced thrombosis during pregnancy or in the postpartum period.

Preterm birth was reported in five pregnancies (31.3%): in three cases labor started spontaneously at 30, 35 and 36 weeks, respectively; in one patient a cesarean section was performed at 36 weeks because of severe maternal thrombocytopenia; and in one patient an emergency cesarean section was performed at 31 weeks because of severe placental insufficiency. This latter patient was diagnosed with PNH in the third trimester of pregnancy when she presented with hemolytic anemia, preeclampsia and intrauterine growth restriction. Eculizumab was started immediately, but 15 days after initiation of therapy an emergency cesarean section had to be performed because of fetal distress.

Four deliveries were complicated by maternal hemorrhage of >1000 mL, mainly due to retained placental products (n = 3). In all cases, therapeutic low-molecular-weight heparin was interrupted >24 h prior to delivery, and platelet counts were (either spontaneously or via transfusion) above 50 10^9/L. Six patients received RBC or platelet transfusions during pregnancy and/or delivery. Except for one patient who developed acute myeloid leukemia (AML), all were transfusion independent at 6 weeks postpartum. Three pregnancies were complicated by clinical and

| Patients, n | 12 |
| Total pregnancies, n | 18 |
| Age at delivery, median in years (range) | 34 (30–41) |
| PNH granulocyte clone size at first pregnancy visit, median in percentage (range) | 74 (31–99) |
| Pregnancy outcome, n (%) |  |
| Live birth | 16 (88.9) |
| Miscarriage | 2 (11.1) |
| Gestational age at delivery (live births), n (%) |  |
| ≥37 weeks | 11 (68.8) |
| 35–36 weeks | 3 (18.7) |
| 32–34 weeks | 0 (0.0) |
| <32 weeks | 2 (12.5) |
| Birth weight (live births), median in grams (range) |  |
| Newborns born ≥37 weeks | 3110 (2185–3810) |
| Newborns born 35–36 weeks | 2812 (2770–2944) |
| Newborns born <32 weeks | 1263 |
| Mode of delivery (live births), n (%) |  |
| Vaginal delivery | 11 (68.8) |
| Spontaneous | 4 (36.4) |
| Induced | 7 (63.6) |
| Cesarean section | 5 (31.2) |
| Emergency section | 2 (40.0) |
| Elective section | 3 (60.0) |
| Complications during pregnancy and/or delivery (live births), n (%) |  |
| Thrombosis | 0 (0.0) |
| Blood loss >1000 cc within 24 h after delivery | 4 (25.0) |
| Retained placenta and/or placental products | 5 (31.3) |
| Placental insufficiency* | 3 (18.8) |
| Development of acute myeloid leukemia | 1 (6.3) |
| Temporary dose adjustment of eculizumab during pregnancy (live births), n (%) | 3 (18.8) |
| Transfusion requirement (live births), n (%) |  |
| RBC transfusions during pregnancy | 6 (37.5) |
| RBC transfusions during delivery | 6 (37.5) |
| Platelet transfusions during pregnancy | 4 (25.0) |
| Platelet transfusions during delivery | 9 (25.0) |

Abbreviation: PNH, paroxysmal nocturnal hemoglobinuria.

*Gestational hypertension, preeclampsia, intrauterine growth restriction.

aBirth weight of one preterm was not recorded.
laboratory signs of breakthrough hemolysis, managed by temporary dosage increase of eculizumab. Except for one patient, all patients continued eculizumab after pregnancy. In one patient eculizumab was interrupted after the postpartum period because of a spontaneous decrease of the PNH clone during pregnancy.

4 | DISCUSSION

Eculizumab has proven to be an effective treatment for patients with PNH. However, in light of the necessity for life-long treatment, potential side effects and costs, there are strict criteria for initiation of therapy. In this study we show that, in real-world practice, the adherence to the indications as formulated in the Dutch guideline for diagnosis and treatment of PNH is good, resulting in selection of patients most likely to benefit from treatment. Indeed, in accordance with experiences from other international cohorts, in the majority of our Dutch patients important improvements were seen in both objective and subjective parameters, that is, significant increases in hemoglobin level and organ functioning, important reductions in thrombosis risk, and impressive improvements in patient-reported outcomes. Furthermore, the reported 5-year survival estimates of 92.7% in a mainly elderly patient cohort imply a major survival benefit.

Despite these impressive benefits, only a minority of patients responded to a complete hematological response and half of the patients who started eculizumab because of transfusion dependency had a minor response after 12 months of treatment. It is important to zoom into these patients. An inadequate erythropoietic response due to an underlying bone marrow disorder such as aplastic anemia can account for an incomplete hematological response.

In addition, in almost all patients on eculizumab, a fraction of C3d opsonized PNH erythrocytes arise which become target of phagocytosis due to complement receptor binding by macrophages in liver and spleen. This results in a variable degree of extravascular hemolysis. We here confirm previous reports on a strong association between hematological response failure with underlying bone marrow failure and/or ongoing extravascular hemolysis. Patients with suboptimal hematological responses demonstrated low reticulocyte counts and/or high C3d opsonization, resulting into a net limited increase in hematological parameters.

To improve the treatment of PNH, it is important to discriminate between the different causes of residual anemia during eculizumab therapy. Patients with bone-marrow failure may require other treatments than complement inhibitors, while patients with prominent extravascular hemolysis will likely benefit more from agents affecting the complement pathway proximal of C5, such as C3, factor B and factor D. Indeed, the novel complement C3-inhibitor pegcetacoplan was recently shown to be superior to eculizumab in terms of hemoglobin change and transfusion independency in PNH patients on eculizumab with hemoglobin levels <10.5 g/dL, and has recently been approved by the FDA and EMA.

Our study shows that despite suboptimal hematological responses patients still benefit from eculizumab therapy. We show that across all hematological response groups, treatment with eculizumab led to a marked reduction of intravascular hemolysis, as evidenced by a significant decrease of LDH levels after initiation of therapy. One of the major complications of PNH is an increased risk of thrombomelobosis, which is the main cause of mortality in untreated PNH. Although the underlying mechanism of thrombosis has not been fully clarified yet, thrombomelobosis appears to be related to ongoing complement activation that makes the management of thrombomelobosis difficult. We show in our nationwide cohort that the incidence of thrombomelobosis was reduced by 83.1% with eculizumab therapy. These results are in accordance with results of previous studies. We reported a significant increase in euglobulin clot lysis time after up to 12 months of therapy in PNH patients who presented with acute kidney injury, and showed that these patients did not experience new episodes of renal failure once eculizumab had been initiated. Although we cannot exclude that renal function would have improved spontaneously once the hemolytic crisis had passed, these results suggest that controlling intravascular hemolysis with eculizumab improves kidney function in PNH patients with acute kidney injury, and prevents recurrent episodes of renal dysfunction.

We reported a significant and clinically meaningful improvement in all almost domains of QoL after 12 months of eculizumab therapy as measured by EORTC-QLQ-c30 and FACT-fatigue scores. These results are in line with the results of clinical trials investigating eculizumab. Unfortunately we did not analyze patient reported outcomes on QoL and fatigue during long-term eculizumab therapy because only few patients who completed PAQs before initiation of eculizumab, completed PAQs during long-term therapy. Although initiation of eculizumab improved QoL in PNH, patients on eculizumab still reported lower scores in all six domains of EORTC-QLQ-c30 and FACT-fatigue scores as compared to a representative general (Dutch) population. Fatigue is one of the most frequently reported symptoms in PNH and has a major impact on overall QoL.

Reasons for persistent fatigue despite adequate inhibition of intravascular hemolysis can be multifactorial and may include remaining anemia due to extravascular hemolysis, lasting effects of thrombosis, and psychological complaints associated with chronic illness. Future research is needed to investigate if improvements in QoL and fatigue will sustain during long-term eculizumab therapy and if novel agents, such as the complement C5 inhibitor ravulizumab, which can be administered every eight instead of 2 weeks, and inhibitors targeting the proximal complement system further improve QoL. The EORTC-QLQ-c30 is originally developed for clinical studies of cancer patients. To gain better insight in the QoL of PNH patients on complement inhibitory therapies the implementation of PNH specific QoL questionnaires is important.

We evaluated pregnancy outcomes during eculizumab exposure. Previously, pregnancy was discouraged in women with PNH because of a great risk of fetal and maternal morbidity and mortality. The advent of eculizumab has importantly improved outcomes, and disabled previous recommendations against pregnancy. Despite these major improvements, it is important to emphasize that pregnancy itself necessitated start of eculizumab in the majority of our pregnant PNH females, consequently implying the need for a lifelong continuation of treatment in most patients. Second, although no pregnancy resulted in thrombotic events or lethal outcomes, our dataset underlines that the risk of (nonlethal) complications during the course of pregnancy and labor is not normalized compared to the healthy Dutch population. Ultimately, these results warrant the
importance of proper preconception patient counseling, and joined hematologic and obstetric treatment at an experienced PNH center.

An important issue with the chronic use of eculizumab is the occurrence of breakthrough hemolytic events due to suboptimal C5 inhibition or complement activating events such as infections, surgery or pregnancy. Ex vivo experiments showed that complement activation leads to accumulation of C3b on the cell surface, resulting in a conformation change in C5 that limits the ability of eculizumab to block terminal complement.65 According to current literature, approximately 11%–27% of PNH patients experience breakthrough hemolytic events during eculizumab therapy.55-57 We report a higher prevalence of BTH during longer-term follow-up, mainly due to infections. However, as there is no consensus yet regarding the definition of BTH, incidence rates may depend on which definition is used.

In conclusion, this study shows that the majority of PNH patients benefit from eculizumab therapy when adhering to the strict indications as formulated in the Dutch guideline on diagnosis and treatment of PNH. However, novel therapies (e.g. proximal complement inhibitors) are needed to further improve real-world outcomes, such as hematological responses and QoL. Data reported in this study can be used to compare real-world outcomes of eculizumab with real-world outcomes of novel complement inhibitory agents.

AUTHOR CONTRIBUTIONS
Charlotte C. M. Schaap and Saskia M. C. Langemeijer designed the study; Charlotte C. M. Schaap, Floor C. J. I. Heubel-Moenen, Erfan Nur and Marije Bartels acquired the data; Charlotte C. M. Schaap, Olivier W. H. van der Heijden, Emiel de Jonge, Frank W. M. B. Preijers and Saskia M. C. Langemeijer analyzed and/or interpreted the data; Charlotte C. M. Schaap and Saskia M. C. Langemeijer wrote the manuscript; all authors critically reviewed and approved the final manuscript; Nicole M. A. Blijlevens and Saskia M. C. Langemeijer supervised the study.

FUNDING INFORMATION
No external funding was received for this analysis. Patient-reported outcomes on quality of life and fatigue, and physician-reported Karnofsky scores were extracted from the international PNH registry (NCT01374360), which was funded by Alexion Pharmaceuticals, Inc.

CONFLICT OF INTEREST STATEMENT
The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
Charlotte C. M. Schaap https://orcid.org/0000-0001-6445-1079
Floor C. J. I. Heubel-Moennen https://orcid.org/0000-0002-3281-926X
Erfan Nur https://orcid.org/0000-0002-7069-930X
Marije Bartels https://orcid.org/0000-0001-9685-1755

REFERENCES


56. Nakayama H, Usuki K, Echizen H, Ogawa R, Orii T. Eculizumab dosing intervals longer than 17 days may be associated with greater risk of


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