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# Long-term Effect of the Complement Inhibitor Eculizumab on Kidney Function in Patients with Paroxysmal Nocturnal Hemoglobinuria

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**1 Abstract**

2 Paroxysmal nocturnal hemoglobinuria (PNH) is a debilitating and life-threatening disease  
3 in which lysis of PNH red blood cells frequently manifests with chronic hemolysis,  
4 anemia and thrombosis. Renal damage in PNH is associated with chronic hemosiderosis  
5 and/or microvascular thrombosis. We determined the incidence of renal dysfunction or  
6 damage, defined by stages of chronic kidney disease (CKD), in a large cohort of PNH  
7 patients, and evaluated the safety and efficacy of the complement inhibitor eculizumab in  
8 altering its progression. Renal dysfunction or damage was observed in 65% of the study  
9 population at baseline with 21% of patients with later stage CKD or kidney failure (GFR  
10  $\leq 60$  mL/min/1.73 m<sup>2</sup>; stage 3, 4 or 5). Eculizumab treatment was safe and well-tolerated  
11 in patients with renal dysfunction or damage and resulted in the likelihood of  
12 improvement as defined as categorical reduction in CKD stage ( $P < 0.001$ ) compared to  
13 baseline and to placebo ( $P = 0.04$ ). Improvement in renal function was more commonly  
14 seen in patients with baseline CKD stage 1-2 (67.1% improvement,  $P < 0.001$ ) although  
15 improvement was also observed in patients with CKD stage 3-4 ( $p = 0.05$ ). Improvements  
16 occurred quickly and were sustained for at least 18 months of treatment. Patients  
17 categorized at CKD stage 3-5 did not worsen during treatment with eculizumab. Overall,  
18 40 (21%) of 195 patients that demonstrated renal dysfunction or damage at baseline were  
19 no longer classified as such after 18 months of treatment. Administration of eculizumab  
20 to patients with renal dysfunction or damage was well tolerated and was usually  
21 associated with clinical improvement.

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## 1 Introduction

2 Intravascular hemolysis and the presence of cell-free plasma hemoglobin cause many of  
3 the serious clinical sequelae associated with various hemolytic disorders.<sup>1</sup> Paroxysmal  
4 nocturnal hemoglobinuria (PNH) is a rare and life-threatening blood disease that is  
5 clinically defined by significant intravascular hemolysis.<sup>2;3</sup> PNH arises from an acquired  
6 clonal genetic deficiency of glycosylphosphatidylinositol (GPI)-linked proteins on the  
7 surface of blood cells.<sup>3;4</sup> In particular, PNH red blood cells lack complement regulatory  
8 proteins, which render them susceptible to hemolysis mediated by the terminal  
9 components of complement. The release of excessive red cell hemoglobin during  
10 intravascular hemolysis usually exceeds the capacity of the hemoglobin scavenging  
11 molecule, haptoglobin, leading to high levels of cell-free hemoglobin and subsequently  
12 clinical manifestations of PNH.<sup>1</sup>

13  
14 Five-year mortality in patients with PNH is approximately 35% and median survival is  
15 approximately 10-15 years.<sup>2;5</sup> Kidney failure contributes to 8% – 18% of PNH related  
16 deaths.<sup>6</sup> Virtually all patients with PNH have evidence of kidney damage by biopsy or  
17 imaging techniques or at post-mortem examination.<sup>7-16</sup> Previous reports showed that  
18 repetitive exposure to cell-free hemoglobin causes renal hemosiderin accumulation,  
19 tubulointerstitial inflammation, and kidney damage.<sup>17</sup>

20  
21 Cell-free plasma hemoglobin in patients with PNH has also been shown to deplete nitric  
22 oxide (NO) leading to smooth muscle dystonias and pulmonary hypertension.<sup>18;19</sup> Nitric  
23 oxide is also a critical molecule in the regulation of vascular tone with the greatest effect  
24 on the afferent renal arterials.<sup>20</sup> Alterations in renal blood flow due to the sequestration  
25 of NO can have a direct effect on the glomerular filtration rate (GFR) and renal plasma  
26 flow.<sup>20-23</sup>

27  
28 Eculizumab (Soliris®) is a humanized monoclonal antibody that targets complement  
29 protein C5, thereby preventing production of the potent pro-inflammatory mediator C5a  
30 and the assembly of the terminal complement complex (also called the membrane attack  
31 complex or MAC) during complement activation.<sup>24</sup> In clinical studies, treatment of PNH

1 patients with eculizumab resulted in a reduction in hemolysis, cell-free plasma  
2 hemoglobin and NO depletion.<sup>19;25</sup> Data from treated patients also demonstrated an  
3 improvement in anemia, fatigue and quality of life, and a reduction in thrombotic  
4 events.<sup>25-28</sup> Herewith, we report the incidence of renal dysfunction or damage and  
5 evaluate the effect of long-term eculizumab treatment on the progression of this serious  
6 and life-threatening morbidity in a large, multinational cohort of patients with PNH  
7 enrolled in a phase 3b open-label extension study.

## 10 **Methods**

### 11 **Study design and patients**

12 Kidney function and the incidence of clinical kidney damage event rates were assessed in  
13 patients from three independent parent clinical studies (the phase 2 pilot study<sup>26;29</sup> and its  
14 extensions, the phase 3 TRIUMPH study,<sup>25</sup> and the phase 3 SHEPHERD study<sup>28</sup>) and the  
15 subsequent common phase 3 extension study.<sup>30</sup> All protocols were approved by the  
16 institutional review board at each center, and all patients gave written informed consent.

17  
18 The open-label, 12-week, phase 2 pilot study was conducted in 11 patients at two study  
19 centers in the United Kingdom.<sup>26</sup> Patients who completed the pilot study were eligible to  
20 enroll in a one-year extension study<sup>29</sup> followed by a two-year extension study. Patients  
21 18 years of age or older who had received at least 4 red cell transfusions in the previous 12  
22 months and had a PNH type III erythrocyte population  $\geq 10\%$  were eligible to enroll in  
23 the pilot study. Patients who were taking stable doses of immunosuppressive drugs (e.g.,  
24 cyclosporin), warfarin, and iron supplements were permitted to continue them during the  
25 study. Eculizumab was dosed at 600 mg via intravenous infusion every 7 days for 4  
26 doses, 900 mg 7 days later, and 900 mg every 14 days as a maintenance dose.

27  
28 The double-blind, placebo controlled, 26-week, phase 3 efficacy and safety study  
29 (TRIUMPH) was conducted in 87 patients in the U.S., Europe, Australia and Canada.<sup>25</sup>  
30 Patients 18 years of age or older, with at least 4 red cell transfusions in the previous 12  
31 months, a PNH type III erythrocyte population  $\geq 10\%$ , platelets  $\geq 100 \times 10^9/L$ , and lactate

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1 dehydrogenase (LDH)  $\geq 1.5$  times the upper limit of normal were eligible to enroll.  
2 Exclusion criteria were previously described patients who were taking stable doses of  
3 immunosuppressive drugs, anticoagulants, and iron supplements were permitted to  
4 continue during the study. Patients were centrally randomized (1:1) and stratified  
5 (according to red cell units transfused in the past year) to receive either placebo or  
6 eculizumab. Study medication was dosed as follows: eculizumab (600 mg) or placebo via  
7 intravenous infusion every 7 days for 4 doses; eculizumab (900 mg) or placebo via  
8 intravenous infusion 7 days later; followed by a maintenance dose of eculizumab (900  
9 mg) or placebo via intravenous infusion every  $14 \pm 2$  days for a total of 26 weeks of  
10 treatment.

Comment [GK1]: Added reference as requested by Reviewer #1


11  
12 The open-label, 52-week, phase 3 study (SHEPHERD) was conducted in 97 patients in  
13 the U.S., Europe, Australia and Canada.<sup>28</sup> Inclusion criteria for patients were broader than  
14 those for patients enrolled in TRIUMPH, as the pretreatment transfusion eligibility was  
15 adjusted to one or more red cell transfusions in the previous 24 months and the minimum  
16 platelet count requirement was lowered to  $30 \times 10^9/L$ ; all patients received the identical  
17 eculizumab dose as in the TRIUMPH study.

18  
19 Following completion of the parent clinical studies, 187 patients enrolled into the  
20 common open-label, 102-week, phase 3 extension study (195 patients were enrolled in  
21 parent studies).<sup>30</sup> Placebo-treated TRIUMPH patients entering the phase 3 extension  
22 study received the same eculizumab regimen outlined for the TRIUMPH and  
23 SHEPHERD studies. Eculizumab-treated patients from the pilot, TRIUMPH or  
24 SHEPHERD studies who entered the phase 3 extension study continued to receive the  
25 900 mg maintenance dose of eculizumab. Doses of immunosuppressive drugs,  
26 anticoagulants, and iron supplements could be altered at the discretion of the treating  
27 physician.

28


## 29 **Outcome measures**

30 For each of the 195 PNH patients entered into the eculizumab clinical trials, evidence of  
31 renal dysfunction or damage, as measured by CKD stage, was evaluated at baseline, 6

1 months, 12 months and 18 months.  All patients were required to have a urinalysis and  
 2 serum creatinine measures at baseline and during the trial. Patients with a positive  
 3 dipstick urine protein test were considered to have evidence of proteinuria. The  
 4 glomerular filtration rate (GFR) was estimated using the Modification by Diet of Renal  
 5 Disease study formula (MDRD) equation, as previously defined.<sup>31,32</sup> CKD stages were  
 6 defined using the Kidney Disease Outcomes Quality Initiative (KDOQI) as: Stage 5  
 7 CKD, GFR less than 15 mL/min/1.73 m<sup>2</sup>; Stage 4 CKD, GFR 15 – 30 mL/min/1.73 m<sup>2</sup>;  
 8 Stage 3 CKD, GFR 30 – 60 mL/min/1.73 m<sup>2</sup>; Stage 2 CKD, GFR 60 – 90 mL/min/1.73  
 9 m<sup>2</sup> and evidence of kidney damage which may include spot urinalysis with proteinuria or  
 10 by abnormal imaging findings; and Stage 1 CKD, GFR greater than 90 mL/min/1.73 m<sup>2</sup>  
 11 and evidence of kidney damage which may include spot urinalysis with proteinuria or by  
 12 abnormal imaging findings. Patients were classified as not having renal dysfunction or  
 13 damage if they failed to meet the criteria for Stages 1 - 5 CKD.<sup>33</sup> Initial measurements  
 14 were performed at screening prior to study enrollment. An improvement in renal function  
 15 was defined as a categorical documented reduction in CKD stage level (e.g., a change  
 16 from Stage 4 to Stage 3 or Stage 3 to Stage 1) or fulfilling the criteria of no CKD. A  
 17 worsening in renal function was defined as a categorical documented increase in CKD  
 18 stage level (e.g., a change from Stage 3 to Stage 4, or Stage 1 to Stage 2). No change in  
 19 renal status was defined as the patient remaining in the same CKD stage or the continued  
 20 absence of CKD.

**Comment [GK2]:** As requested by reviewer #3 comment 1.

**Comment [GK3]:** Addressing Reviewer #2 comment 8

21  
 22 In addition, major clinical kidney (MCK) events were determined by querying all  
 23 medical history events prior to use of eculizumab and all adverse events during treatment  
 24 with eculizumab. Medical history events prior to diagnosis of PNH were excluded. By  
 25 definition, all medical history events obtained in these analyses were non-fatal. MCK  
 26 events were defined as event terms  severe reversible or irreversible renal damage  
 27 including acute or chronic renal failure, renal insufficiency, renal impairment, dialysis  
 28 and procedures (i.e. hemodialysis and/or placement of catheters for dialysis; renal artery  
 29 angioplasty, ureteral catheterization), nephrotoxicity, scarred or necrotic kidney,  
 30 pyelonephritis, glomerulopathy, or Stage 3 – 5 CKD (complete list described in Table 5).  
 31 MCK events during placebo treatment in the TRIUMPH study were considered with the

**Comment [GK4]:** Addressing reviewer #3 comment 2

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**Comment [GK5]:** As requested by reviewer #3 comment 5





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2 1 group of events prior to eculizumab treatment. These analyses included all events during  
3  
4 2 the period commencing from the first eculizumab dose in the parent studies until the last  
5  
6 3 follow-up of either the last dose of eculizumab or the data base lock in July 2007.  
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8 4

## 9 5 **Statistical Analysis**

10 6 The changes for all patients in each CKD stage were compared within each stage and  
11  
12 7 across the overall treatment population to the observations at screening using chi-square  
13  
14 8 analyses and the null hypothesis that the probability of worsening CKD stage was equal  
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16 9 to the probability of improving CKD stage. The changes in CKD stage for patients  
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18 10 treated with eculizumab in the TRIUMPH study and for patients treated with placebo in  
19  
20 11 the TRIUMPH study were also each compared to the respective observations at screening  
21  
22 12 using chi-square analyses and the null hypothesis that the probability of worsening CKD  
23  
24 13 stage was equal to the probability of improving CKD stage for each treatment group. The  
25  
26 14 changes in CKD stage for patients treated with eculizumab in the TRIUMPH study were  
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28 15 compared to the changes in CKD stage for patients treated with placebo in the TRIUMPH  
29  
30 16 study using chi-square analyses and the null hypothesis that the probability of worsening  
31  
32 17 and improving with eculizumab treatment was equal to the probabilities with placebo  
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34 18 treatment.  
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37 20 The incidence rate of MCK events was tabulated and the MCK event rate per patient year  
38  
39 21 was determined for the pre-eculizumab and the eculizumab treatment periods for the  
40  
41 22 overall patient population. A time-to-event analysis was performed for MCK events prior  
42  
43 23 to trial enrollment. The MCK event rate was determined as events per 100 patient-years  
44  
45 24 before and during eculizumab treatment. Patients with a MCK event prior to treatment  
46  
47 25 were included in the patients evaluated to have a potential MCK event during eculizumab  
48  
49 26 treatment. Event rates were compared on an intention-to-treat basis with the Wilcoxon  
50  
51 27 signed-rank test.  
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53 28

## 54 29 **Results**

### 55 30 Patient characteristics

1 Baseline characteristics of patients in each of the parent clinical studies are shown in  
 2 Table 1. Ninety-six percent of all eligible patients chose to enroll in the common  
 3 extension study.

4  
 5 Renal dysfunction or damage was a common finding in patients with PNH as 65% of the  
 6 study population exhibited Stage 1 – Stage 5 CKD (Table 2). Approximately 21% of  
 7 patients had renal insufficiency comprising CKD Stages 3, 4 or kidney failure (Stage 5).

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8 Age can play a contributing role into the development of CKD. The National Kidney  
 9 Foundation estimates the prevalence of CKD stage 3-5 in people  $\geq 65$  years of age is  
 10 17%. Of the 15 patients  $\geq 65$  years of age that entered the trials, we found that 10 (66%)

11 patients had CKD stage 3 to 5. Of the 180 patients < 65 years of age, we observed 114

Comment [GK6]: As requested by  
Reviewer #2 comment 10

12 (63%) had CKD and 30 (16.7%) had CKD 3-5 (4 times higher than the general  
 13 population with CKD 3-5).<sup>32</sup> Taken together, these data suggest that patients with PNH

14 are equally likely to have CKD at different ages, although CKD at an older age may be  
 15 more likely to be more severe. Additionally, there were 14 patients with a history of acute

16 renal failure, 5 of which had required dialysis, which showed at least partial recovery and

17 21% (3/14) of these patients subsequently developed chronic renal insufficiency (chronic

18 GFR < 60 ml/min/1.73 m<sup>2</sup>).

Comment [GK7]: As requested by  
Reviewer #2 comment 11

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 20 MCK events were common in the PNH patient population, as 27% of patients (52 of 195)  
 21 were diagnosed with an event, 67% of which occurred within 10 years of diagnosis.

22 Further, the Kaplan-Meier probability of patients progressing to an MCK event was 15  
 23 out of 103 (15%) at five years, 16 out of 60 (27%) at 10 years, 9 out of 21 (44%) at 20

24 years and 3 out of 4 (75%) at 30 years as indicated in Figure 1.

#### 25 26 Effect of eculizumab on renal function

27 Eculizumab administration was associated with a reduction in median LDH from 1,832 to  
 28 292 U/L (P<0.0001) at 6 months in patients with more severe renal damage (CKD stages

29 3-5) and sustained out to 18 months (274 U/L; P<0.001). In similar fashion, eculizumab

30 reduced LDH levels from 2,722 U/L to 372 U/L at 6 months in patients with CKD stages

31 1-2 (P<0.001) and was sustained out to 18 months (318 U/L; P<0.001).

Comment [GK8]: As requested by  
Reviewer #2 comment 9 and reviewer #3  
comment 3

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4 In the randomized, placebo-controlled, double-blind TRIUMPH study, eculizumab  
5 treatment was associated with a significant improvement in renal function, demonstrated  
6 by improvement in CKD stages, compared to placebo treatment (P=0.04; Table 3). Left  
7 untreated, on balance patients were not likely to improve (16.7% improve / 14.3%  
8 worsen; P=0.78). In contrast, PNH patients treated with eculizumab were 6.0 times more  
9 likely to improve than worsen in renal function, showing a significant improvement at 6  
10 months compared to baseline (29.3% of patients improved, 4.9% of patients worsened;  
11 P=0.005). Patients with CKD stage 1-2 at baseline were 6 times more likely to improve  
12 than worsen (Table 4, P=0.006) with eculizumab treatment, while kidney function did not  
13 change with placebo (P=0.25). The improvement in kidney function with eculizumab  
14 treatment in patients with CKD stage 3-5 at baseline in the TRIUMPH study was not  
15 significant (limited patient number, n=9).

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Comment [GK9]: Addressing reviewer #2 comment 4.

16 As indicated in Table 4, long-term eculizumab treatment significantly increased the  
17 likelihood of improved renal function in all CKD stages (P<0.001). The improvements  
18 occurred early and were sustained to at least 18 months of treatment. When combining  
19 data from all three clinical trials, eculizumab-treated patients were 4 times more likely to  
20 improve than worsen at 6 months (31.7% improve / 7.9% worsen) and this outcome  
21 improved to 6 times more likely to improve over 18 months of treatment (Table 4).  
22 Patients with CKD stage 3-5 prior to eculizumab treatment were 4 times more likely to  
23 improve than worsen (22.9% improve / 5.7% worsen; P=0.05) with subsequent  
24 eculizumab treatment at 18 months and patients with milder renal dysfunction (stage 1 or  
25 2) at baseline were 25 times (67.1% improve / 2.7% worsen, P<0.001) more likely to  
26 improve than worsen with eculizumab treatment.

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28 CKD stage 3-5 is characterized by eGFR (<60 ml/min/1.73 m<sup>2</sup>). The mean eGFR across  
29 the CKD 3-5 cohort demonstrated a trend in improvement at 18 months of eculizumab  
30 treatment but did not reach statistical significance. However, the mean change in eGFR  
31 in patients treated with eculizumab who improved with CKD stage 3-5 at baseline was

Comment [GK10]: As requested by reviewer #2 comment 1

1 clinically significant: 10.3 ml/min/1.73 m<sup>2</sup> at 6 months, 12.8 ml/min/1.73 m<sup>2</sup> at 12  
 2 months, and 11.5 ml/min/1.73 m<sup>2</sup> at 18 months of treatment (Table 4). Of the 46 patients  
 3 with CKD stage 2 at baseline (defined as GFR between 60-90 ml/min/1.73 m<sup>2</sup> and  
 4 evidence of proteinuria), 63% improved at 6 months of treatment (vs 13.0% deteriorated)  
 5 and this improvement was sustained for 18 months (68% improved vs 2.3% worsened).  
 6 Of the 36 patients with CKD stage 1 at baseline, 65%, 71%, and 66% showed  
 7 improvement in CKD after 6, 12, and 18 months of treatment, respectively. Ninety four  
 8 percent of patients with CKD stage 1 at baseline maintained eGFR ( $\geq 90$  ml/min/1.73 m<sup>2</sup>)  
 9 and with complete elimination of proteinuria after 18 months of treatment.

Comment [GK11]: As requested by  
Reviewer #2 comment 2

Comment [GK12]: As requested by  
Reviewer #2 comment 2

10  
 11 Overall, during the course of 18 months of eculizumab treatment, 40/195 (21%) patients  
 12 classified with renal dysfunction or disease were no longer classified as having CKD.  
 13 This included 5 patients initially with pre-dialysis Stage 3, 20 patients with Stage 2, and  
 14 15 patients with Stage 1. Of the 5 patients with CKD stage 3 at baseline the median  
 15 change in eGFR with eculizumab treatment was 14.6 ml/min/1.73 m<sup>2</sup> and all patients  
 16 with proteinuria had a resolution of the proteinuria. Twenty patients with baseline CKD  
 17 stage 2 no longer had CKD (CKD stage 0) after 18 months of eculizumab treatment.  
 18 Objective evidence of renal damage, as indicated by proteinuria, was eliminated with  
 19 eculizumab treatment in these patients and 15%, 17% and 25% of patients achieved  
 20 normal eGFR ( $\geq 90$  ml/min/1.73 m<sup>2</sup>) at 6, 12 and 18 months of treatment, respectively.

Comment [GK13]: Addressing  
Reviewer #3 comment 1

Comment [GK14]: As requested by  
Reviewer #2 comment #3

21  
 22 In patients with either one or no transfusions in the 12 months prior to study entry (n=22),  
 23 the distribution of renal dysfunction or disease by stage of CKD at baseline was similar to  
 24 the overall population (Stage 4, 4.5%; Stage 3, 13.6%; Stage 2, 22.7%; Stage 1, 18.2%;  
 25 No CKD, 40.9%). After 6 months of treatment with eculizumab, patients with minimal or  
 26 no transfusion support exhibited either an improvement in renal dysfunction stage (27%)  
 27 or stabilized renal function (73%), and no patients worsened (P=0.008).

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28  
 29 Eculizumab treatment improved CKD irrespective of patient age. Improvement with  
 30 eculizumab treatment was similar in patient groups younger and older than 65 years of  
 31 age at 18 months (no significant difference in eculizumab benefit between  $\geq 65$  years and

Comment [GK15]: Addressing  
Reviewer #1 comment 8

1 < 65 years; p=0.10). The likelihood of an improvement in CKD with eculizumab  
 2 treatment at 18 months stratified by age quartile was statistically significant in all age  
 3 quartiles (Q1 <29 years, P<0.001; Q2, 29- 39 years, P=0.009; Q3, 39 -51 years, P=0.004;  
 4 Q4, >51 years, P=0.009). Patients with CKD stage 1-2 at baseline showed a significant  
 5 improvement in CKD with eculizumab treatment at all age quartiles. While there was  
 6 significant improvement in CKD in the overall study population with baseline CKD stage  
 7 3-5, when analyzed by the smaller cohorts of age quartiles there was a demonstrated  
 8 trend in improvement but it did not reach statistical significance in the individual age  
 9 quartiles (n=0, n= 6, n=7 and n=22 patients with stage 3-5 CKD at baseline in each of the  
 10 age quartiles, respectively).

11  
 12 The effect of eculizumab treatment on clinically identified MCK events is shown in  
 13 Table 5. The MCK event rate was reduced 50% from 4.22 events per 100 patient-years  
 14 prior to eculizumab treatment to 2.10 events per 100 patient-years with eculizumab  
 15 treatment (P <0.001).

### 16 Safety

17 Eculizumab was safe and well tolerated. Safety observations are consistent in all 3 parent  
 18 stud and the open-label extension study. Most treatment-emergent AEs (95.0%) were  
 19 mild or moderate in severity and 90.8% of AEs were determined to be unrelated to study  
 20 drug. The most frequently occurring treatment-emergent AEs were: nasopharyngitis,  
 21 (39.6%); headache (36.9%) and upper respiratory tract infection, (31.0%). The most  
 22 frequently reported AE related to study drug by preferred term was headache (15.5%).  
 23 Notably, headaches were generally mild and treated with over-the counter analgesics. As  
 24 was reported previously, the frequency of headaches declined after the first 2 treatment  
 25 doses and were similar in frequency to that observed with placebo. The most common  
 26 SAEs reported were associated with infections and infestations (12.8% of patients),  
 27 nervous system disorders (4.3%), and blood and lymphatic disorders (8.0%). The  
 28 majority of SAEs were moderate in intensity and unrelated to the study drug. Two  
 29 patients experienced meningococcal infections during the study. Both patients fully  
 30 recovered. One patient discontinued treatment and one patient continued treatment with

Comment [GK16]: As requested by Reviewer #1

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Comment [GK17]: As requested by Reviewer #1 comment 6

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Comment [GK18]: Hillmen, TRIUMPH, NEJM 2006

Comment [GK19]: Reviewer #1 comment 5

1 study drug. Positive tests for HAMA were rare, recorded in only 5 patients, transient, not  
 2 sustained in any patient, and not associated with any pharmacokinetic or clinical effect.  
 3 Three deaths were reported during this study. Two deaths were determined to be  
 4 unrelated to study drug, and in one patient drug discontinuation could not be excluded as  
 5 a contributing factor.

**Comment [GK20]:** As requested by reviewer #1 comment 7

**Comment [GK21]:** We removed the safety results on GFR <60 as requested by Reviewer #2 comment 7.

**Deleted:** In patients with pre-dialysis or kidney failure stages (CKD Stage 3 – Stage 5), eculizumab treatment was generally well tolerated. The most common reported adverse events for patients with GFR less than 60 mL/min/1.73 m<sup>2</sup> treated with eculizumab were upper respiratory tract infection, nasopharyngitis, and arthralgia which were similar in frequency to patients with GFR less than 60 mL/min/1.73 m<sup>2</sup> treated with placebo in the TRIUMPH study. ¶

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## 7 Discussion

8 Due to the recognized high incidence (65%) of renal dysfunction or damage at baseline in  
 9 patients from the TRIUMPH study<sup>27</sup> and the serious and life-threatening nature of poor  
 10 renal function in patients with PNH,<sup>12</sup> analyses were performed to assess the overall  
 11 effect of eculizumab treatment on renal function. Eculizumab treatment was significantly  
 12 more likely to improve CKD stage than placebo treatment through six months. Long-term  
 13 eculizumab treatment resulted in a significant improvement and prevention of worsening  
 14 of renal dysfunction or damage at all stages of baseline disease. Patients were 6 times  
 15 more likely to improve renal function at 18 months of eculizumab treatment, rather than  
 16 deteriorate, with 34% of patients showing improvement in renal function.

17  
 18 CKD stage was selected as the primary measure of renal outcome, as opposed to solely  
 19 eGFR, in order to align our results with clinically validated assessments of renal function  
 20 in other populations, as recommended by the National Kidney Foundation (NKF).<sup>33</sup> [The  
 21 assessment of CKD stages 1-2 identifies the importance of early damage, as measured by  
 22 proteinuria, since eGFR can remain at a relatively high functional level (>60  
 23 mL/min/1.73 m<sup>2</sup>), but the presence of proteinuria indicates further objective evidence of  
 24 clinically significant glomerular and/or tubular damage. Further, NKF guidelines  
 25 recommend treatment of the comorbid conditions in patients with CKD stages 1-2 that  
 26 may contribute to the increase in renal damage or proteinuria in order to ultimately slow  
 27 progression of renal dysfunction.<sup>33</sup> In alignment with these NKF guidelines, the results  
 28 of the current clinical study indicate that blocking the on-going complement-mediated  
 29 hemolysis with eculizumab treatment was associated with a significant improvement in  
 30 CKD and also resulted in a reduction in the percent of patients who progressed (from  
 31 11.1% at 6 months to 2.7% at 18 months of eculizumab treatment). [The staging of CKD

**Comment [GK22]:** Addressing reviewer #2 comment 2

**Comment [GK23]:** Addressing Reviewer #2 comment 1.

1 stages 3, 4 and 5 is defined by eGFR less than 60 mL/min/1.73 m<sup>2</sup>, a measure of poor  
 2 renal filtration, and without the concomitant requirement for proteinuria.<sup>33</sup> Therefore, for  
 3 patients with baseline stages 3-5 CKD, in alignment with NKF guidelines, the current  
 4 analysis focused on categorical changes in eGFR, with particular focus on maintenance  
 5 of renal function and delay of deterioration. In the current study, results demonstrate that  
 6 22.9% of patients with CKD 3-5 improved, (vs 5.7% worsened) and 71.4% maintain  
 7 stable renal function. There was a mean increase of 11.5 mL/min/1.73 m<sup>2</sup> eGFR in the  
 8 patients with baseline CKD stages 3-5 who improved with eculizumab treatment.

10 The observation that a substantial proportion of patients treated with eculizumab  
 11 stabilized renal function during 18 months of treatment is clinically important. In the  
 12 general population, patients without PNH with CKD stages 1-5 followed for an average  
 13 of 4.2 years, the rate of CKD stage progression has been reported as 12 patients per 100  
 14 patient-years.<sup>34</sup> During 1.5 years of treatment with eculizumab, the rate of CKD stage  
 15 progression is 2.5 patients per 100 patient-years. Further, the rate of improvement of  
 16 CKD in the general population was reported as 0.9 patients per 100 patient-years over 4.2  
 17 years.<sup>34</sup> In contrast, treatment with eculizumab is associated with an improvement rate of  
 18 35.2 patients per 100 patient-years. In PNH patients with more severe CKD stages 3-5 at  
 19 baseline, the rates of CKD improvement during treatment with eculizumab is 3  
 20 patients per 100 patient-years. The observed improvements with eculizumab treatment  
 21 demonstrate the significant contribution of chronic terminal complement-mediated  
 22 hemolysis to the progression of CKD in patients with PNH.

24 Clark and colleagues previously reported that 32% (6/19) of patients with PNH had  
 25 creatinine clearance of less than 60 mL/min/1.73 m<sup>2</sup> (CKD stages 3-5) indicating kidney  
 26 damage.<sup>12</sup> The exact mechanisms responsible for kidney damage in patients with PNH  
 27 are unclear, but a substantial number of investigations implicate chronic hemolysis  
 28 leading to hemosiderin accumulation, inflammation, and renal scarring. Magnetic  
 29 resonance imaging (MRI) studies reproducibly show that virtually all patients with PNH  
 30 are observed to have renal cortical areas of low signal intensity including patients with  
 31 PNH who have lower levels of hemolysis indicated by no hemoglobinuria for more than

Comment [GK24]: Addressing Reviewer #2 comment 1

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Comment [GK26]: Addressing Reviewer #1 comment 3.

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1 8 years, aplastic anemia, MDS/CMML, or with smaller PNH clones.<sup>7;10;16;35</sup> Cortical  
2 abnormalities detected by intravenous pyelogram have been noted in patients with lower  
3 levels of hemolysis as represented by the absence of overt hemoglobinuria.<sup>12</sup> Autopsy or  
4 biopsy findings typically show heavy hemosiderin accumulation in the proximal tubules,  
5 hemoglobin tubular casts, signs of chronic interstitial nephritis and fibrosis.<sup>12</sup> When  
6 examined, functional tubular defects are commonly found with impaired ability to  
7 concentrate urine, renal tubular acidosis, decreased reabsorption of phosphate, glucosuria  
8 with normal blood sugar and aminoaciduria.<sup>12;36</sup>

9  
10 The renal cortical damage in PNH is typical of intravascular hemolysis and not generally  
11 found in patients with extravascular hemolysis.<sup>11</sup> Cell-free plasma hemoglobin can lead  
12 to progressive tubulointerstitial inflammation with upregulation of monocyte  
13 chemoattractant protein-1 and Nuclear Factor-kappa B (NF-κB) and upregulation of both  
14 interstitial and basement membrane extracellular matrix components. Additionally,  
15 hemosiderosis in the kidney with direct generation of free radicals has also been  
16 suggested as a mechanism for both acute and chronic kidney inflammation and fibrosis in  
17 PNH.<sup>14;37</sup> Hemosiderosis was found in 9 of 10 patients in a recent MRI study of patients  
18 with PNH.<sup>16</sup> The fate of iron deposits in the kidneys of these patients following resolution  
19 of hemolysis with eculizumab treatment is currently under investigation. Terminal  
20 complement inhibition with eculizumab would be expected to inhibit the direct  
21 hemolysis-mediated renal damage as well as the damage secondarily due to inflammation  
22 associated with kidney injury.

**Comment [GK27]:** Paragraph to address Reviewer #3 comment 6.

23  
24 Eculizumab administration is associated with an immediate and sustained reduction in  
25 intravascular hemolysis in all treated patients.<sup>25;26;28</sup> Inhibition of terminal complement  
26 with eculizumab rapidly and consistently reduced intravascular hemolysis to near normal  
27 levels in patients from all PNH clinical studies while placebo treatment in the TRIUMPH  
28 study showed no effect.<sup>25</sup> Moreover, the reduction in intravascular hemolysis with  
29 eculizumab treatment resulted in similar reductions in cell-free plasma hemoglobin to  
30 near normal levels.<sup>19</sup> In a previous study, treatment with eculizumab significantly



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3 1 reduced the load of cell-free plasma hemoglobin delivered to the kidneys in patients with  
4 PNH, as indicated by the 96% ( $P<0.001$ ) reduction in the frequency of hemoglobinuria.<sup>26</sup>  
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7 4 Biochemical analyses of patients with PNH have demonstrated high levels of cell-free  
8 plasma hemoglobin and the resultant depletion of NO.<sup>1:19</sup> Interestingly, reduction of  
9 available NO in animal models results in an increase in renal arteriole resistance, a  
10 reduction in renal plasma flow and glomerular filtration rate (GFR), and subsequently  
11 poor renal function.<sup>20-23</sup> In addition, pooled data from 310 human subjects with normal  
12 renal function demonstrated that administration of the NO synthase inhibitor L-  
13 NGmonomethyl arginine (L-NMMA) significantly reduced renal plasma flow, and that  
14 GFR was dependent on NO synthase activity, suggesting a direct role of NO in  
15 glomerular hemodynamics.<sup>20</sup> In clinical trials, eculizumab rapidly reduced hemolysis and  
16 cell-free plasma hemoglobin resulting in a repletion of NO in patients with PNH.<sup>19</sup> While  
17 eculizumab treatment improved renal function at all stages of CKD, it was particularly  
18 noteworthy in patients classified with stage 1 or 2 CKD at baseline. In a higher  
19 proportion of patients with Stage 1 or Stage 2, eculizumab treatment resulted in  
20 improvement in renal function (progression to less severe CKD stage or transition to no  
21 CKD) in 67% of treated patients. In addition, the improvements in renal function in all  
22 stages of CKD occurred rapidly (within 6 months). Taken together, these data suggest the  
23 possibility that the availability of NO and subsequent changes in renal vascular tone after  
24 eculizumab treatment may play a role in the improvement of renal dysfunction or damage  
25 demonstrated in this study. Nonetheless, whether renal dysfunction is due to NO-  
26 mediated vasomotor responses in the renal arteries or renal disease due to the direct  
27 effects of cell-free plasma hemoglobin and hemosiderin, mitigation of hemolysis in these  
28 patients with eculizumab improves renal function. The impact of eculizumab on retarding  
29 progression and/or improving function may be more substantial with intervention early in  
30 the course of the disease.  
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45 29 It has been suggested that large vessel thrombosis or microvascular thrombosis may  
46 contribute to renal damage in patients with PNH.<sup>12</sup> However, clinical observations have  
47 been only infrequently supportive of large vessel thrombosis as a cause of renal  
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4 dysfunction or damage in PNH with many studies demonstrating renal damage in the  
5 absence of large vessel occlusions.<sup>9;13-15</sup> Additionally, no incidence of renal vein or renal  
6 arterial thrombosis was reported amongst 195 patients in the current study population.<sup>30</sup>  
7  
8 Nonetheless, subclinical microvascular venous or arterial thrombosis could conceivably  
9 contribute to renal insufficiency in PNH.<sup>12</sup>

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12 The study population presented here indicates that the overall prevalence of renal  
13 dysfunction or disease in PNH, as measured by CKD staging, is increased 6.6 fold over  
14 that observed in the general population in the United States.<sup>32</sup> It is possible that drug  
15 therapies occasionally used in PNH patients such as cyclosporine or NSAIDs may  
16 contribute to renal dysfunction or damage. However, in our study 6.6% of patients were  
17 taking cyclosporine and 8.7% were on NSAIDs, making it unlikely that these  
18 concomitant medications significantly impacted the analyses.

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21 Later-stage renal dysfunction or failure is a major cause of morbidity and mortality in  
22 PNH.<sup>6;12</sup> In the current study, of the 40 patients with later stage CKD (stages 3 to 5), 10  
23 patients had been identified as specifically having chronic renal impairment in a review  
24 of their medical histories. Eighteen of these 40 patients had been identified to have had  
25 one or more major clinical kidney event and 22 had not been diagnosed with any renal  
26 disorder. These data suggest that kidney damage has been underappreciated as a  
27 significant symptom or clinical consequence of PNH.

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30 Eculizumab was safe and well tolerated, consistent with the safety findings in all 3 parent  
31 clinical trials. In keeping with established precautions, all patients were vaccinated with  
32 a meningococcal vaccine at least 14 days before initial eculizumab treatment.

Comment [GK28]: As requested by  
reviewer #1 comment 5



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42 In summary, PNH is a life-threatening blood disorder in which patients are at risk for  
43 end-organ damage and organ failure due to chronic hemolysis. Renal dysfunction or  
44 damage is a common and progressive medical complication in patients with PNH that  
45 also contributes to mortality in these patients. The current findings show that long-term  
46 treatment with eculizumab in patients with hemolytic PNH requiring transfusions

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1 substantially improved kidney function or reduced progression of kidney disease as a  
2 consequence of controlling the intravascular hemolysis.

For Peer Review

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**Table 1. Baseline Demographics of Patients in All PNH Studies (N = 195)**

Description	n (%)
Gender, male	89 (45.6)
History of AA or MDS	59 (30.3)
History of previous thrombosis	62 (31.8)
Use of Cyclosporin	13 (6.6%)
Use of NSAID	17 (8.7%)
	<b>median (range)</b>
Age in years	39 (18 – 85)
Disease duration in years	5.5 (0.12 – 38.5)
PNH type III RBC proportions, %	32.3 (2.4 – 98.8)
Reticulocyte counts, $\times 10^{12}/L$	0.164 (0.036 – 0.757)
Lactate dehydrogenase level, U/L	2139 (499 – 10,300)
Platelet count, $\times 10^9/L^*$	149 (23 - 547)

AA, aplastic anemia; MDS, myelodysplastic syndrome; \*N = 194;

<b>CKD Stage</b>	<b>Baseline n (%)</b>	<b>18 Months treatment n (%)</b>
Stage 5	3 (1.5%)*	1 (0.6%)
Stage 4	7 (3.6%)	8 (4.7%)
Stage 3	30 (15.4%)	25 (14.8%)
Stage 2	48 (24.6%)	18 (10.7%)
Stage 1	36 (18.5%)	14 (8.3%)
No CKD	69 (35.4%)	103 (60.9%)

\* one of the three patients was receiving dialysis at treatment entry.

**Table 3. Effect of 6-month eculizumab treatment on renal function in the placebo-controlled TRIUMPH study**

Baseline Stage	Change in Stage of CKD with Treatment			P value <sup>1</sup>
	Improvement	No Change	Worsening	
<b>Eculizumab</b>				
All patients (n=41)	29.3%	65.9%	4.9%	0.005
Stage 3 – 5 (n=9)	11.1%	88.9%	0.0%	0.30
Stage 1 – 2 (n=17)	64.7%	23.5%	11.8%	0.006
<b>Placebo</b>				
All patients (n=42)	16.7%	69.0%	14.3%	0.78
Stage 3 – 5 (n=9)	22.2%	66.7%	11.1%	0.56
Stage 1 – 2 (n=14)	35.7%	50.0%	14.3%	0.25
Eculizumab vs. Placebo				0.04 <sup>2</sup>

<sup>1</sup>The comparison (except for eculizumab vs. placebo) performed for each group or for all patients, tested the null hypothesis that patients are as likely to improve as they are to worsen.

<sup>2</sup>The comparison of eculizumab vs. placebo tests the null hypothesis that the probability of patients improving or worsening with eculizumab is equal to the same probabilities with placebo.

**Table 4. Effect of long-term eculizumab treatment on renal function**

Baseline Stage	Change in Stage of CKD with Treatment			P value <sup>2</sup>
	Improvement <sup>1</sup>	No Change <sup>1</sup>	Worsening <sup>1</sup>	
<b>6 months</b>				
All patients (n=189)	31.7%	60.3%	7.9%	<0.001
Stage 3 – 5 (n=40)	20.0%	75.0%	5.0%	0.05
(Mean change in GFR; ml/min/1.73 m <sup>2</sup> )	(+10.3)	(-0.3)	(-2.9)	
Stage 1 – 2 (n=81)	64.2%	24.7%	11.1%	<0.001
<b>12 months</b>				
All patients (n=179)	35.2%	58.1%	6.7%	<0.001
Stage 3 – 5 (n=39)	20.5%	76.9%	2.6%	0.02
(Mean change in GFR; ml/min/1.73 m <sup>2</sup> )	(12.8)	(-0.6)	(-4.2)	
Stage 1 – 2 (n=77)	71.4%	23.4%	5.2%	<0.001
<b>18 months</b>				
All patients (n=166)	34.3%	60.2%	5.4%	<0.001
Stage 3 – 5 (n=35)	22.9%	71.4%	5.7%	0.05
(Mean change in GFR; ml/min/1.73 m <sup>2</sup> )	(11.5)	(0.3)	(-6.3)	
Stage 1 – 2 (n=73)	67.1%	30.1%	2.7%	<0.001

<sup>1</sup>Improvement, no change, and worsening as defined in the methods section.

<sup>2</sup>The comparison is performed for each group or for all patients at each time point, testing the null hypothesis that patients are as likely to improve as they are to worsen.

<b>Table 5. Major Clinical Kidney Events Before and During Eculizumab Treatment</b>		
<b>All patients, N = 195</b>	<b>Pre-Treatment</b>	<b>Eculizumab Treatment</b>
<b>MCK event rate (Events per 100 patient-years)</b>	<b>4.22</b>	<b>2.10</b>
Patient years	1683.6	381.6
MCK events (n)	71	8
CKD 3-5 #	22	
Renal impairment	12	5
Acute renal failure	7	
Pyelonephritis	5	
Renal Failure	3	2
Hemodialysis	3	
Renal dialysis	2	
Kidney stone	2	
Other‡	15	1
<b>Eculizumab vs Placebo</b>		<b>P&lt;0.001</b>

\* P Value based on signed rank test , # patients with CKD but no documented history of renal events

‡ includes Bilateral atherosclerotic renal artery stenosis, calculus of kidney, hematuria, hemochromatosis, kidney abscess, nephrotic syndrome, mild secondary hydronephrosis, nephrotoxicity, proliferative glomerulonephritis, renal colic, scarred kidney, hypertension, insertion of left ureteric stent and potassium wasting nephropathy.

Figure 1. Time to Major Clinical Kidney Event in 195 Patients with PNH Prior to Eculizumab Treatment

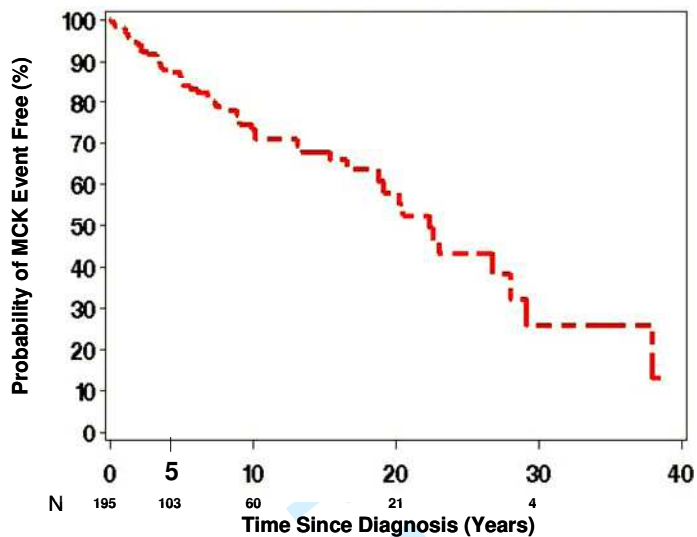


Figure 1. The Kaplan-Meier probability of patients progressing to an MCK event. Event free; 85% (95% CI; 77.9% - 89.6%) at five years , 73% (95% CI: 63% - 80%) at 10 years, 56% (95% CI 42% - 67%) at 20 years and 25% (95% CI: 10% - 44%) at 30 years.