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

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Abstract

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

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Introduction

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

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

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

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

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

Methods

Study design and patients

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

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

The double-blind, placebo controlled, 26-week, phase 3 efficacy and safety study (TRIUMPH) was conducted in 87 patients in the U.S., Europe, Australia and Canada.[25] Patients 18 years of age or older, with at least 4 red cell transfusions in the previous 12 months, a PNH type III erythrocyte population ≥ 10%, platelets ≥ 100 x 10^9/L, and lactate

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

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

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

Outcome measures

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

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

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

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

Statistical Analysis

The changes for all patients in each CKD stage were compared within each stage and across the overall treatment population to the observations at screening using chi-square analyses and the null hypothesis that the probability of worsening CKD stage was equal to the probability of improving CKD stage. The changes in CKD stage for patients treated with eculizumab in the TRIUMPH study and for patients treated with placebo in the TRIUMPH study were also each compared to the respective observations at screening using chi-square analyses and the null hypothesis that the probability of worsening CKD stage was equal to the probability of improving CKD stage for each treatment group. The changes in CKD stage for patients treated with eculizumab in the TRIUMPH study were compared to the changes in CKD stage for patients treated with placebo in the TRIUMPH study using chi-square analyses and the null hypothesis that the probability of worsening and improving with eculizumab treatment was equal to the probabilities with placebo treatment.

The incidence rate of MCK events was tabulated and the MCK event rate per patient year was determined for the pre-eculizumab and the eculizumab treatment periods for the overall patient population. A time-to-event analysis was performed for MCK events prior to trial enrollment. The MCK event rate was determined as events per 100 patient-years before and during eculizumab treatment. Patients with a MCK event prior to treatment were included in the patients evaluated to have a potential MCK event during eculizumab treatment. Event rates were compared on an intention-to-treat basis with the Wilcoxon signed-rank test.

Results

Patient characteristics

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

Renal dysfunction or damage was a common finding in patients with PNH as 65% of the study population exhibited Stage 1 – Stage 5 CKD (Table 2). Approximately 21% of patients had renal insufficiency comprising CKD Stages 3, 4 or kidney failure (Stage 5). Age can play a contributing role into the development of CKD. The National Kidney Foundation estimates the prevalence of CKD stage 3-5 in people ≥ 65 years of age is 17%. Of the 15 patients ≥ 65 years of age that entered the trials, we found that 10 (66%) patients had CKD stage 3 to 5. Of the 180 patients < 65 years of age, we observed 114 (63%) had CKD and 30 (16.7%) had CKD 3-5 (4 times higher then the general population with CKD 3-5).[32] Taken together, these data suggest that patients with PNH are equally likely to have CKD at different ages, although CKD at an older age may be more likely to be more severe. Additionally, there were 14 patients with a history of acute renal failure, 5 of which had required dialysis, which showed at least partial recovery and 21% (3/14) of these patients subsequently developed chronic renal insufficiency (chronic GFR < 60 ml/min/1.73 m^2).

MCK events were common in the PNH patient population, as 27% of patients (52 of 195) were diagnosed with an event, 67% of which occurred within 10 years of diagnosis. Further, the Kaplan-Meier probability of patients progressing to an MCK event was 15 out of 103 (15%) at five years, 16 out of 60 (27%) at 10 years, 9 out of 21 (44%) at 20 years and 3 out of 4 (75%) at 30 years as indicated in Figure 1.

Effect of eculizumab on renal function

Eculizumab administration was associated with a reduction in median LDH from 1,832 to 292 U/L (P<0.0001) at 6 months in patients with more severe renal damage (CKD stages 3-5) and sustained out to 18 months (274 U/L; P<0.001). In similar fashion, eculizumab reduced LDH levels from 2,722 U/L to 372 U/L at 6 months in patients with CKD stages 1-2 (P<0.001) and was sustained out to 18 months (318 U/L: P<0.001).

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

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

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

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

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

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

Eculizumab treatment improved CKD irrespective of patient age. Improvement with eculizumab treatment was similar in patient groups younger and older than 65 years of age at 18 months (no significant difference in eculizumab benefit between ≥ 65 years and

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

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

Safety

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

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

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Comment [GK21]: We removed the safety results on GFR <60 as requested by Reviewer #2 comment7.

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Discussion

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

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

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

The observation that a substantial proportion of patients treated with eculizumab stabilized renal function during 18 months of treatment is clinically important. In the general population, patients without PNH with CKD stages 1-5 followed for an average of 4.2 years, the rate of CKD stage progression has been reported as 12 patients per 100 patient-years.[34] During 1.5 years of treatment with eculizumab, the rate of CKD stage progression is 2.5 patients per 100 patient-years. Further, the rate of improvement of CKD in the general population was reported as 0.9 patients per 100 patient-years over 4.2 years.[34] In contrast, treatment with eculizumab is associated with an improvement rate of 35.2 patients per 100 patient-years. In PNH patients with more severe CKD stages 3-5 at baseline, the rates of CKD improvement during treatment with eculizumab is 33 patients per 100 patient-years. The observed improvements with eculizumab treatment demonstrate the significant contribution of chronic terminal complement-mediated hemolysis to the progression of CKD in patients with PNH.

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

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

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

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

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

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reduced the load of cell-free plasma hemoglobin delivered to the kidneys in patients with PNH, as indicated by the 96% (P<0.001) reduction in the frequency of hemoglobinuria .[26]

Biochemical analyses of patients with PNH have demonstrated high levels of cell-free plasma hemoglobin and the resultant depletion of NO.[1;19] Interestingly, reduction of available NO in animal models results in an increase in renal arteriole resistance, a reduction in renal plasma flow and glomerular filtration rate (GFR), and subsequently poor renal function.[20-23] In addition, pooled data from 310 human subjects with normal renal function demonstrated that administration of the NO synthase inhibitor L-NGmonomethyl arginine (L-NMMA) significantly reduced renal plasma flow, and that GFR was dependent on NO synthase activity, suggesting a direct role of NO in glomerular hemodynamics.[20] In clinical trials, eculizumab rapidly reduced hemolysis and cell-free plasma hemoglobin resulting in a repletion of NO in patients with PNH.[19] While eculizumab treatment improved renal function at all stages of CKD, it was particularly noteworthy in patients classified with stage 1 or 2 CKD at baseline. In a higher proportion of patients with Stage 1 or Stage 2, eculizumab treatment resulted in improvement in renal function (progression to less severe CKD stage or transition to no CKD) in 67% of treated patients. In addition, the improvements in renal function in all stages of CKD occurred rapidly (within 6 months). Taken together, these data suggest the possibility that the availability of NO and subsequent changes in renal vascular tone after eculizumab treatment may play a role in the improvement of renal dysfunction or damage demonstrated in this study. Nonetheless, whether renal dysfunction is due to NO-mediated vasomotor responses in the renal arteries or renal disease due to the direct effects of cell-free plasma hemoglobin and hemosiderin, mitigation of hemolysis in these patients with eculizumab improves renal function. The impact of eculizumab on retarding progression and/or improving function may be more substantial with intervention early in the course of the disease.

It has been suggested that large vessel thrombosis or microvascular thrombosis may contribute to renal damage in patients with PNH.[12] However, clinical observations have been only infrequently supportive of large vessel thrombosis as a cause of renal

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dysfunction or damage in PNH with many studies demonstrating renal damage in the absence of large vessel occlusions.[9;13-15] Additionally, no incidence of renal vein or renal arterial thrombosis was reported amongst 195 patients in the current study population.[30] Nonetheless, subclinical microvascular venous or arterial thrombosis could conceivably contribute to renal insufficiency in PNH.[12]

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

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

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

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

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

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

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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%)
	median (range)
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, $x10^{12}$/L	0.164 (0.036 – 0.757)
Lactate dehydrogenase level, U/L	2139 (499 – 10,300)
Platelet count, $x10^{9}$/L*	149 (23 - 547)

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

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Table 2. Renal characteristics in patients		
CKD Stage	**Baseline n (%)**	**18 Months treatment n (%)**
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.

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

	Change in Stage of CKD with Treatment			
Baseline Stage	**Improvement**	**No Change**	**Worsening**	**P value[1]**
Eculizumab				
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
Placebo				
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[2]

[1]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.

[2]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.

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

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

[1]Improvement, no change, and worsening as defined in the methods section.

[2]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.

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Table 5. Major Clinical Kidney Events Before and During Eculizumab Treatment		
All patients, N = 195	**Pre-Treatment**	**Eculizumab Treatment**
MCK event rate (Events per 100 patient-years)	**4.22**	**2.10**
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
Eculizumab vs Placebo		**P<0.001**

* 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.

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

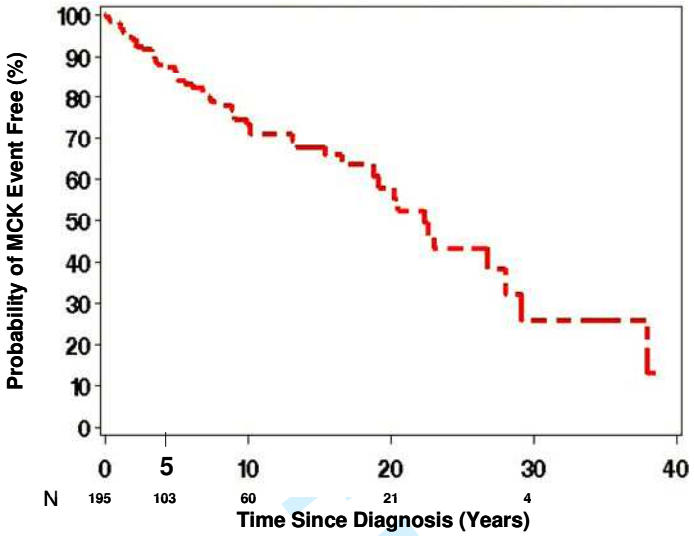

Figure 1.The Kaplan-Meier probability of patients progressing to an MCK event. Event free; 85% (95% CI; 77.9% - 89.6%) at five years , 73% (95% CI: 63% - 80%) at 10 years, 56% (95% CI 42% - 67%) at 20 years and 25% (95% CI: 10% - 44%) at 30 years.

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