Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria

Peter Hillmen, M.B., Ph.D.,1 Petra Muus, M.D., Ph.D.,2 Ulrich Dührsen, D.M.,3 Antonio M. Risitano, M.D., Ph.D.,4 Jörg Schubert, M.D.,5 Lucio Luzzatto, M.D.,6 Hubert Schrezenmeier, M.D.,7 Jeffrey Szer, M.B., B.S.,8 Robert A. Brodsky, M.D.,9 Anita Hill, M.B., M.D.,1 Gerard Socié, M.D., Ph.D.,10 Monica Bessler, M.D., Ph.D.,11 Scott A. Rollins, Ph.D.,12 Leonard Bell, M.D.,12 Russell P. Rother, Ph.D.12 and Neal S. Young, M.D.13

1Leeds General Infirmary, Leeds, United Kingdom; 2Radboud University Medical Center, Nijmegen, The Netherlands; 3University Essen, Essen, Germany; 4Federico II University, Naples, Italy; 5Saarland University Medical School, Homburg-Saarland, Germany; 6Istituto Toscano Tumori, Florence, Italy; 7Institute of Transfusion Medicine, University Hospital, Ulm, Germany; 8Royal Melbourne Hospital, Melbourne, Australia; 9Johns Hopkins School of Medicine, Baltimore, MD; 10Hôpital Saint-Louis and INSERM, Paris, France; 11Washington University, St. Louis, MO; 12Alexion Pharmaceuticals, Inc., Cheshire, CT; 13National Heart, Lung, and Blood Institute, Bethesda, MD

Running title: ECULIZUMAB AND THROMBOSIS IN PATIENTS WITH PNH

Address correspondence to:
Dr. Peter Hillmen
Department of Haematology
Leeds General Infirmary
Great George Street
Leeds, LS1 3EX
United Kingdom
Phone: 011-44-113-392-3974

Copyright © 2007 American Society of Hematology
Fax: 011-44-113-392-6349

peter.hillmen@nhs.net
Abstract

Hemolysis and hemoglobinemia contribute to serious clinical sequelae in hemolytic disorders. In paroxysmal nocturnal hemoglobinuria (PNH) patients, hemolysis can contribute to thromboembolism (TE), the most feared complication in PNH, and the leading cause of disease-related deaths. We evaluated whether long-term treatment with the complement inhibitor eculizumab reduces the rate of TE in patients with PNH. Clinical trial participants included all patients in the three eculizumab PNH clinical studies which enrolled between 2002 and 2005 (n=195), and their continued treatment in a common multinational open-label extension study. Thromboembolism rate with eculizumab treatment was compared to the pre-treatment rate in the same patients. The TE event rate with eculizumab treatment was 1.07 events/100 patient-years compared to 7.37 events/100 patient-years ($P<.001$) prior to eculizumab treatment (relative reduction of 85%, absolute reduction 6.3 TE events/100 patient-years). With equalization of the duration of exposure before and during treatment for each patient, TEs were reduced from 39 events pre-eculizumab to 3 events during eculizumab ($P<.001$). The TE event rate in antithrombotic-treated patients (n=103) was reduced from 10.61 to 0.62 events/100 patient-years with eculizumab treatment ($P<.001$). These results show that eculizumab treatment reduces the risk of clinical thromboembolism in patients with PNH. This study is registered at [http://clinicaltrials.gov](http://clinicaltrials.gov) (Study ID: #NCT00122317).
**Introduction**

Intravascular hemolysis and cell-free plasma hemoglobin have been implicated in the serious clinical sequelae of various hemolytic disorders.\(^1\) Hemolysis is the primary clinical manifestation of the uncommon disease paroxysmal nocturnal hemoglobinuria (PNH) and has been shown to result in chronic disabling morbidities including anemia, severe fatigue, difficulty in functioning, pain and thrombosis, all of which have a major effect on the patient’s quality of life.\(^1\)\(^-\)\(^5\) Paroxysmal nocturnal hemoglobinuria is defined by the acquired genetic deficiency of glycosylphosphatidylinositol (GPI)-linked proteins from the surface of blood cells. The absence of GPI-linked complement regulatory proteins on PNH erythrocytes renders them susceptible to terminal complement-mediated hemolysis.

Hemolysis most likely contributes to thromboembolism (TE) in PNH, as patients with larger PNH clones have a higher incidence of TE and events have been temporally associated with increased hemolysis.\(^6\)\(^-\)\(^10\) Although the mechanism is not fully understood, hemolysis has been implicated in the initiation of platelet activation and aggregation.\(^1\) Additional *in vitro* studies have suggested that complement may directly activate platelets from PNH patients.\(^11\)\(^,\)\(^12\)

Thromboembolism is the leading cause of mortality in patients with PNH,\(^2\)\(^,\)\(^3\)\(^,\)\(^13\)\(^-\)\(^17\) and an initial thrombotic event increases the relative risk of death in PNH 5 to 10 fold.\(^15\)\(^,\)\(^17\) Retrospective studies suggest that, in non-Asian patients, TE accounts for approximately 40 – 67% of deaths with known causes.\(^13\)\(^-\)\(^17\) Further, 29 – 44% of patients with PNH have been reported to suffer from at least one TE event in the course of their disease.\(^13\)\(^-\)\(^16\)
In a retrospective study of 67 high risk patients with PNH not taking prophylactic anticoagulants, the TE rate was estimated at 3.7 events/100 patient-years (19 TE in 511.5 patient-years), while 0 TE in 117.8 patient-years were observed in a heterogeneous group of 39 patients with PNH treated with anticoagulants as primary prophylaxis. However, in other reports, new TE as well as progression of existing TE have been observed in patients with PNH, despite the use of anticoagulants and/or antiplatelet agents. The risk for fatal hemorrhage in patients with PNH is significant, in part due to the frequent occurrence of thrombocytopenia.

Eculizumab (Soliris™) is a humanized monoclonal antibody that targets complement protein C5, thereby preventing assembly of the terminal complement complex (also called the membrane attack complex or MAC) during complement activation. In clinical studies to date, eculizumab treatment reduced hemolysis and improved anemia, fatigue and quality of life in patients with PNH. In this report, we evaluate the effect of long-term treatment with eculizumab on the pre-specified clinical outcome of TE on an intention-to-treat basis in a multinational phase 3 open-label extension study that enrolled patients from three independent eculizumab PNH clinical studies (n=195). We explored the robustness of the effect of eculizumab treatment on TE in subgroup analyses.

Methods

Study design and patients

The number of TE events and the incidence rates were determined in patients from three independent parent clinical studies (the phase 2 pilot study and its extensions, the phase
3 TRIUMPH study,18 and the phase 3 SHEPHERD study20) and the common phase 3 extension study. All of the 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.19 Patients who completed the pilot study were eligible to enroll in a one year extension study 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.18 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 10⁹/L, and lactate dehydrogenase (LDH) ≥1.5 times the upper limit of normal were eligible to enroll. Exclusion criteria were previously described.18 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±2 days for 4 doses; eculizumab (900 mg) or placebo via
intravenous infusion 7±2 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.

The open-label 52-week phase 3 study (SHEPHERD) was conducted in 97 patients in the U.S., Europe, Australia and Canada. Patient characteristics were similar to those of patients treated in TRIUMPH except that the pre-treatment 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.

The common open-label 102-week phase 3 extension study is an ongoing study in which a total of 187 patients have enrolled following completion of the parent clinical studies (195 patients were enrolled in parent studies). 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 that 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 patients enrolling in the TRIUMPH, SHEPHERD, and common phase 3 extension studies, each of the respective protocols specified that all adverse events and past medical histories were to be evaluated by the principal investigator to identify clinical TE events
as defined by the same major adverse vascular event (MAVE) criteria in all studies. For each study, the protocol specified that the principal investigator record the description, location, method of diagnosis, date of diagnosis, and date resolved for each thrombosis/MAVE event in pre-identified case report form fields for events both prior to and during eculizumab treatment. Because of the diverse anatomical locations of thromboses in patients with PNH, multiple diagnostic tests were employed. At least 73% (91/124) of pretreatment MAVEs were objectively identified, and the most frequent tests were ultrasound, CT scan MRI and angiogram. Events classified as MAVE included thrombophlebitis/deep vein thrombosis, pulmonary embolus, cerebrovascular accident, amputation, myocardial infarction, transient ischemic attack, unstable angina, renal vein thrombosis, mesenteric vein thrombosis, portal vein thrombosis, gangrene, acute peripheral vascular occlusion, sudden death, and a category for other events. All adverse and past medical history events for patients enrolling in the pilot study were also evaluated in the same manner and the MAVE criteria were retrospectively applied. All MAVEs were independently source verified. The intention-to-treat analysis was based on all 195 patients who enrolled in all three parent clinical studies. The pre-treatment patient-years included the period extending from the earlier of the date of the diagnosis of PNH or the first thrombotic event to first eculizumab treatment. Pre-eculizumab treatment TE events included all events in all patients prior to enrollment in each of the studies (and during placebo treatment in TRIUMPH). The eculizumab treatment TE events included all events during the period commencing from the first eculizumab dose in the parent studies until the earlier of the follow-up after the last dose of eculizumab in the parent or extension studies or the data base lock in November 2006. For the
determination of TE events in patients receiving chronic antithrombotic agents, the Anatomical Therapeutic Chemical Classification System was used to identify relevant chronic therapies including anticoagulant and antiplatelet agents.21

Statistical analysis

For each type of TE event and for the total group of events collected in the thrombosis record case report form, the incidence rate was tabulated. Rate of thrombosis per patient year for the pre-eculizumab and the eculizumab treatment periods was analyzed for the overall patient population and for each parent clinical study.

TE event rates for pre-eculizumab and eculizumab treatment periods were calculated as the total number of TE events divided by the time in years on a per patient basis. Wilcoxon signed-rank test was used for the prespecified analysis on an intention-to-treat basis and for all subanalyses of the differences between the pre-eculizumab and eculizumab treatment TE events and incidence rates.

Results

Study population and baseline characteristics

The pre-specified TE clinical outcome was determined on an intention-to-treat basis in the multinational phase 3 open-label extension study that enrolled patients from 3 independent eculizumab PNH clinical studies (n=195). 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 into the common extension study.
**Clinical outcomes**

Hemolysis is the primary clinical manifestation of PNH and is associated with serious morbidities in PNH including TE.\textsuperscript{1,6-9} Inhibition of terminal complement with eculizumab rapidly and consistently reduced hemolysis to near normal levels in patients from all parent clinical studies while placebo treatment in the TRIUMPH study showed no effect (Figure 1). Subsequent eculizumab treatment of placebo-treated patients also resulted in a rapid and sustained reduction in hemolysis.

Prior to receiving eculizumab, application of the specified MAVE criteria to the evaluation of medical history events identified 124 TE events in the 195 study patients. Pre-treatment TE events in the study group occurred in both venous (85\%) and arterial (15\%) sites (Table 2). The most common sites of venous thrombosis were lower extremity deep veins (18.5\%), mesenteric/splenic veins (18.5\%) and hepatic/portal veins (16.9\%) while the most common site of arterial thrombosis was cerebrovascular accident/transient ischemic attack (13.7\%).

**Main Outcome Measure.** To determine the effect of treatment with eculizumab on the incidence of TE events in PNH, the TE event rate in patients with and without eculizumab treatment were compared. Eculizumab treatment resulted in a reduction in the TE event rate in each of the individual clinical studies (Table 3). The pre-specified overall TE event rate was reduced from 7.37 events/100 patient-years (124 total events) pre-eculizumab treatment to 1.07 events/100 patient-years (3 total events) with eculizumab treatment in the same patients (\( P < .001 \)). This represented a relative reduction of 85\% and an absolute reduction of 6.3 TE events/100 patient-years. The TE event rate in placebo-treated patients from the double-blind placebo-controlled
TRIUMPH study increased from 2.34 events per 100 patient-years before entering the study to 4.38 events per 100 patient-years during the 6 month period of placebo treatment.

In order to eliminate a potential bias arising from an unexpected change in the number of TE events immediately preceding trial entry, and in order to adjust for the difference between the number of patient-years accumulated in the pre-eculizumab versus the eculizumab treatment periods, the duration of exposure before and during treatment was equalized for each patient. Compared to the number of TE events pre-treatment, eculizumab treatment resulted in a significant reduction in the overall number of TE from 39 events to 3 events ($P < .001$; Table 4). This represented a relative reduction of 92%.

To determine the pre-eculizumab TE event rate immediately preceding eculizumab treatment, the period 12 months before treatment was examined; the TE event rate during this period in the overall patient population was 17.21 TE events per 100 patient-years (191.8 total patient-years) indicating an increase in rate nearer to initiation of the eculizumab studies.

**Patients Receiving Antithrombotics.** In order to evaluate the potential impact of antithrombotic therapy on TE event rates with and without eculizumab treatment, the TE event rate in patients treated with antithrombotic agents was examined. Compared to the pre-eculizumab TE event rate in antithrombotic treated patients, eculizumab treatment resulted in a reduction in the TE event rate in each of the individual clinical studies and a significant reduction in the overall TE event rate (Table 5). The TE event rate in patients receiving antithrombotics was 10.61 events/100 patient-years pre-eculizumab and was reduced to 0.62 events/100 patient-years in the same patients during eculizumab
treatment \( (P < .001) \). This represented a relative reduction of 94\%, and an absolute reduction of 9.99 TE events per 100 patient-years. When the analysis of thromboses in patients receiving antithrombotics was restricted to patients on anticoagulants \( (n=91) \); excludes patients on antiplatelet agents), the TE event rate was reduced from 11.54 events/100 patient-years pre-eculizumab to 0.72 events/100 patient-years during eculizumab treatment \( (P < .001) \).
Discussion

Intravascular hemolysis and the release of red blood cell hemoglobin have been linked to the occurrence of thrombosis and other serious morbidities in various hemolytic disorders. Thromboembolism is a serious and life-threatening complication in PNH and the single most frequent cause of death in patients. Prospective analysis of patient data pooled from three clinical studies and a common extension study enabled us to carry out a comparison of the TE event rate in non-treated and in eculizumab-treated patients with PNH. This analysis demonstrates that eculizumab treatment resulted in a dramatic reduction in the TE event rate from 7.37 to 1.07 TE events/100 patient-years. The reduction in the number of TE events and the TE incidence rates with eculizumab were robust and were not diminished in several clinically relevant sensitivity analyses.

Interestingly, TE events in this study occurred in both venous and arterial systems as has been previously described, confirming that TE is not restricted to the venous system in PNH.

The TE event rate without eculizumab of 7.37 events per 100 patient-years in our patient population, which comprised 195 patients, is more than twice that of the TE event rate published previously for 67 high risk patients in a single-center descriptive study. The current TE event assessment was systematic, protocol specified, and performed on a multicenter, multinational, and controlled basis. While most patients in the current study had large populations of PNH granulocytes, TE was also observed pre-eculizumab in patients who would be expected to have less severe disease with smaller PNH cell populations (<50%), as has been previously reported. The observed variability in the pre-eculizumab TE event rates among the individual studies may be related in part to the
different inclusion criteria. For example, the higher pre-treatment TE event rate in the SHEPHERD study could reflect inclusion of patients with lower platelet counts which may be due to defective hematopoiesis or platelet consumption as a result of hypersplenism associated with previous thrombosis in the portal system. Despite heterogeneity in the individual pre-study TE event rates, eculizumab treatment consistently resulted in a marked reduction in this rate in each individual study. It is important to note that the TE event rate was higher in the 12-month period immediately preceding eculizumab treatment. The increasing TE event rate of 17.21 events per 100 patient-years observed in the study population immediately prior to trial enrollment may indicate that the overall event rate of 7.37 TE events per 100 patient-years may have been diminished due to the loss of patients who had suffered fatal TE events and did not survive to enroll in the clinical trials or progression of underlying disease.

The observed reduction of TE events during eculizumab treatment is unlikely to be due to improvements in patient care or more aggressive anti-thrombotic therapy leading up to and during the clinical studies since the TE event rate did not decrease in the period of time immediately preceding study enrollment nor during the study in placebo-treated patients. The pre-eculizumab TE event rate during the 12-month period just prior to study initiation was markedly elevated, reaching a rate of 17.21 events per 100 patient-years. In addition, the TE event rate in placebo-treated patients from the double-blind placebo-controlled TRIUMPH study increased from 2.34 events per 100 patient-years before entering the study to 4.38 events per 100 patient-years during the 6 month evaluation period. Further, initiation of anti-thrombotic therapy during eculizumab
treatment did not contribute to the observed reduction in TE events; only 3 out of 195 patients commenced anti-thrombotic treatment after eculizumab initiation.

Three TE events occurred in patients during eculizumab treatment: a deep vein thrombosis occurred after 1 year of eculizumab therapy in an anticoagulated patient with no history of TE; a pulmonary embolism occurred within 2 months of starting eculizumab therapy in a non-anticoagulated patient with a history of two previous TE events (pulmonary embolism and deep vein thrombosis); and a deep vein thrombosis occurred within 6 months of starting eculizumab therapy in a non-anticoagulated patient with a history of one TE event (deep vein thrombosis). Hemolysis was effectively controlled in all three patients during eculizumab treatment.

Anticoagulation is commonly used in patients with PNH who have a history of thrombosis and has been proposed as a prophylaxis for higher risk patients. However, physicians have reported new TE events and advancing TE in patients with PNH while on antithrombotics. In addition, hemorrhagic adverse events are a significant risk of chronic anticoagulant therapy particularly as some patients are thrombocytopenic, and therefore primary prophylaxis may be contraindicated in this patient population. Chronic anti-coagulation was shown to be associated with an overall risk of 7.6 bleeding complications per 100 patient-years with the risk increasing to 11.0 bleeding complications per 100 patient-years during the first 90 days of treatment. The risk for hemorrhage with anticoagulant therapy in patients with PNH has been reported to be approximately 5% or higher, including fatal hemorrhage. In the present study, the pre-eculizumab TE event rate remained elevated in patients treated either therapeutically or prophylactically with antithrombotics, such as warfarin, suggesting that such therapies
were not adequate to prevent TE in this patient population. By contrast, chronic administration of eculizumab, a therapy that targets the underlying hemolysis in PNH, significantly reduced the overall TE event rate. Whether anticoagulants can be reduced or eliminated in patients with PNH receiving eculizumab is the subject of future investigation. Further, while eculizumab discontinuation in 16 study patients has not been associated with hemolysis above pretreatment levels and concomitant thrombosis, patients should be monitored following eculizumab discontinuation.24

The pretreatment TE event rate was elevated, and substantial improvement with eculizumab was observed, even in patients who would be expected to have less severe disease. The TE event rate was reduced from 10.76 events per 100 patient-years before treatment to 2.88 events per 100 patient-years during eculizumab in patients with lower levels of baseline hemolysis (lowest pretreatment LDH quartile). Similarly, The TE event rate was reduced from 4.87 events per 100 patient-years before treatment to 0.00 events per 100 patient-years during eculizumab in patients with minimal pretreatment anemia (0 or 1 transfusions in the year prior to eculizumab treatment). Taken together, these data demonstrate that thrombotic risk is elevated in patients who would be expected to have less severe disease and the benefit of eculizumab in this patient population is maintained.

Hemolysis is characteristic of various hemolytic diseases and has been reported to contribute to serious clinical consequences. In patients with PNH, thromboembolic events have been linked to hemolysis, potentially through the build-up of cell-free plasma hemoglobin.1,6-9 The effect of free hemoglobin on platelet function and hypercoagulability and the recognized increase in thrombotic tendencies in PNH may be largely due to its ability to scavenge nitric oxide.25-32 Other potential mechanisms of
thrombosis in PNH include the generation of pro-coagulant platelet microvesicles due to the absence of the terminal complement inhibitor CD59, and the interaction of red cell microvesicles and soluble urokinase plasminogen activator receptor.\textsuperscript{11,12,33,34} Terminal complement inhibition with eculizumab would be expected to have a beneficial effect on TE occurring through any of these mechanisms.

The current findings show that long-term administration of eculizumab, in a diverse population of patients, including patients who would be expected to have less severe disease, substantially reduces the risk of thrombosis. In clinical studies to date, eculizumab therapy has controlled hemolysis, improved anemia and quality of life, and appeared to be safe and well tolerated.\textsuperscript{18,19,35} Considering that thrombosis has been demonstrated to cause the majority of deaths in PNH, it is reasonable to expect that eculizumab treatment, by decreasing the risk of thrombosis, may increase the life-expectancy of these patients.
Acknowledgements

Author Contributions

P.H. designed the study, performed the research and collected the data, analyzed and interpreted the data and drafted the manuscript; P.M. performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript; U.D. performed the research and collected the data and reviewed the manuscript; A.M.R. performed the research and collected the data and reviewed the manuscript; J.S. designed the study, performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript; L.L. performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript; H.S. performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript; J.S. performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript; R.A.B. performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript; A.H. performed the research and collected the data and reviewed the manuscript; G.S. analyzed and interpreted the data, drafted the manuscript and reviewed the manuscript; M.B. designed the study, performed the research and collected the data and reviewed the manuscript; S.A.R. designed the study, analyzed and interpreted the data and reviewed the manuscript; L.B. designed the study, analyzed and interpreted the data, drafted the manuscript and reviewed the manuscript; R.P.R. designed the study, analyzed and interpreted the data, drafted the manuscript and reviewed the manuscript; N.S.Y. designed the study, performed the research and collected the data, analyzed and interpreted the data and reviewed the manuscript.
Conflict of Interest Disclosure

P.H., P.M. and G.S. have received grant support from Alexion Pharmaceuticals, Inc.
P.H., J.S., H.S., R.A.B., G.S. L.L. and A.H. have received lecture fees from Alexion Pharmaceuticals, Inc. P.H., J.S and M.B. have served as consultants for Alexion Pharmaceuticals, Inc. P.M., A.H., J.S. and L.L. have served on Advisory Committees for Alexion Pharmaceuticals, Inc. S.A.R., L.B. and R.R. are employees of Alexion Pharmaceuticals, Inc. and have equity ownership in the company. They have assigned to Alexion Pharmaceuticals their inventions made as employees and have received no royalties from the company for these inventions.

Funding/Support

The studies described in these analyses were funded by Alexion Pharmaceuticals, Inc.

Individual Acknowledgments

Additional investigators and institutions who participated in the clinical studies can be found in the Supplemental Appendix.

We are indebted to Christopher Mojcik, M.D., Ph.D., Henk-Andre Kroon, M.D., MBA, Michael Bombara, B.S. and Jing Jing Wang, M.D., MS (Alexion Pharmaceuticals, Inc.) for trial oversight; to Jason Chan, Ph.D. (Alexion Pharmaceuticals, Inc.) for the statistical analysis; and to Kerry Quinn-Senger, Ph.D. (Alexion Pharmaceuticals, Inc.) for assistance with technical writing.
References


15. Socié G, Mary JY, de Gramont A et al. Paroxysmal nocturnal haemoglobinuria: 

16. Araten DJ, Thaler HT, Luzzatto L. High incidence of thrombosis in African-
American and Latin-American patients with Paroxysmal Nocturnal 

17. Nishimura J, Kanakura Y, Ware RE et al. Clinical course and flow cytometric 
analysis of paroxysmal nocturnal hemoglobinuria in the United States and Japan. 

18. Hillmen P, Young NS, Schubert J et al. The complement inhibitor eculizumab in 

19. Hillmen P, Hall C, Marsh JC et al. Effect of eculizumab on hemolysis and 
transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. 

20. Young NS, Antonioli E, Rotoli B et al. Safety and Efficacy of the Terminal 
Complement Inhibitor Eculizumab in Patients with Paroxysmal Nocturnal 
2006;108:971.

Center. Ref Type: Report


Figure 1. Levels of Lactate Dehydrogenase during Treatment with Eculizumab.

Mean levels of lactate dehydrogenase reflect the degree of hemolysis in each of the parent clinical studies from baseline to week 52. The dashed line indicates the upper limit of the normal range for lactate dehydrogenase (normal range, 103 to 223 U per liter). Values from the pilot study were normalized to that of the TRIUMPH and SHEPHERD studies and include data from the subsequent one year extension study. In eculizumab-treated patients, the mean level of lactate dehydrogenase was rapidly reduced to just above the upper limit of the normal range. In the placebo group, the mean level of lactate dehydrogenase remained highly elevated. The arrow depicts the transition of placebo-treated patients in TRIUMPH to eculizumab treatment in the phase 3 extension study at which time levels of lactate dehydrogenase rapidly reduced to near normal values.
Table 1. Baseline Characteristics of Patients with PNH from Parent Trials

<table>
<thead>
<tr>
<th></th>
<th>Pilot (n=11)</th>
<th>TRIUMPH (n=87)*</th>
<th>SHEPHERD (n=97)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6 (55)</td>
<td>35 (40)</td>
<td>48 (49)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (45)</td>
<td>52 (60)</td>
<td>49 (51)</td>
</tr>
<tr>
<td><strong>Median age, years (range)</strong></td>
<td>48 (21 – 67)</td>
<td>38 (18 – 85)</td>
<td>41 (18 – 78)</td>
</tr>
<tr>
<td><strong>Median disease duration, years (range)</strong></td>
<td>8.7 (1.7 – 37.9)</td>
<td>6.5 (0.5 – 38.5)</td>
<td>4.9 (0.1 – 31.4)</td>
</tr>
<tr>
<td><strong>Reticulocyte count x 10^{12}/L (range)</strong></td>
<td>0.14 (0.07 – 0.37)</td>
<td>0.21 (0.04 – 0.57)</td>
<td>0.14 (0.04 – 0.76)</td>
</tr>
<tr>
<td><strong>Platelet count x 10^{9}/L (range)</strong></td>
<td>182 (36 – 466)</td>
<td>162 (59 – 547)</td>
<td>136 (23 – 355)</td>
</tr>
<tr>
<td><strong>Median PNH granulocyte population size (%) (range)</strong></td>
<td>97.0 (47.8 – 99.8)</td>
<td>95.3 (82.6 – 99.5)†</td>
<td>96.0 (1.1 – 99.9)‡</td>
</tr>
<tr>
<td><strong>Median PNH type III red cell population size (%) (range)</strong></td>
<td>37.0 (10.4 – 79.6)</td>
<td>29.5 (2.4 – 88.0)</td>
<td>33.5 (7.7 – 98.8)</td>
</tr>
<tr>
<td><strong>History of aplastic anemia or myelodysplastic syndromes, n (%)</strong></td>
<td>8 (73)</td>
<td>20 (23)</td>
<td>31 (32)</td>
</tr>
<tr>
<td><strong>History of TE events, n (%)</strong></td>
<td>3 (27)</td>
<td>17 (19)</td>
<td>42 (43)</td>
</tr>
<tr>
<td><strong>Use of antithrombotic agents, n (%)</strong></td>
<td>6 (55)</td>
<td>44 (51)</td>
<td>59 (61)</td>
</tr>
<tr>
<td><strong>Use of steroids, n (%)</strong></td>
<td>0 (0)</td>
<td>28 (32)</td>
<td>36 (37)</td>
</tr>
</tbody>
</table>

*Includes 43 eculizumab-treated patients and 44 placebo-treated patients

†Based on 31 patients with baseline values

‡Based on 94 patients with baseline values
Table 2. Sites of Pre-Treatment Thromboembolism Events*

<table>
<thead>
<tr>
<th>Type of Event</th>
<th>No. Events</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous Thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep Vein Thrombosis</td>
<td>41</td>
<td>33.1</td>
</tr>
<tr>
<td>-lower extremity</td>
<td>23</td>
<td>18.5</td>
</tr>
<tr>
<td>-other†</td>
<td>18</td>
<td>14.5</td>
</tr>
<tr>
<td>Mesenteric/Splenic Vein Thrombosis</td>
<td>23</td>
<td>18.5</td>
</tr>
<tr>
<td>Hepatic/Portal Vein Thrombosis</td>
<td>21</td>
<td>16.9</td>
</tr>
<tr>
<td>Pulmonary Embolus</td>
<td>8</td>
<td>6.5</td>
</tr>
<tr>
<td>Cerebral/Internal Jugular Thrombosis</td>
<td>7</td>
<td>5.6</td>
</tr>
<tr>
<td>Superficial Vein Thrombosis</td>
<td>5</td>
<td>4.0</td>
</tr>
<tr>
<td>Arterial Thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular Accident/</td>
<td>17</td>
<td>13.7</td>
</tr>
<tr>
<td>Transient Ischemic Attack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial Infarction/Unstable Angina</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>124‡</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

*Pre-treatment period includes placebo-treated patients in the TRIUMPH study

†Includes inferior vena cava, bilateral lower extremity, pelvic, ureter, axillary, subclavian and brachiocephalic veins

‡Occurred in 63 patients; 1 event in 35 patients, 2 events in 15 patients, 3 events in 7 patients and 5 or more events in 6 patients
### Table 3. Thromboembolism Events in Patients with and without Eculizumab

<table>
<thead>
<tr>
<th></th>
<th>Pilot*</th>
<th>TRIUMPH</th>
<th>SHEPHERD</th>
<th>Extension† (All studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n)</td>
<td>11</td>
<td>44</td>
<td>43</td>
<td>97</td>
</tr>
<tr>
<td>TE Events (n)</td>
<td>5</td>
<td>11</td>
<td>16</td>
<td>91</td>
</tr>
<tr>
<td>Patient-years (n)</td>
<td>161.7</td>
<td>470.4</td>
<td>309.0</td>
<td>718.3</td>
</tr>
<tr>
<td>TE Event Rate (n per 100 patient-years)</td>
<td>3.09</td>
<td>2.34</td>
<td>5.18</td>
<td>12.67</td>
</tr>
<tr>
<td><strong>Eculizumab Treatment‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n)</td>
<td>11</td>
<td>44</td>
<td>43</td>
<td>97</td>
</tr>
<tr>
<td>TE Events (n)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Patient-years (n)</td>
<td>34.2</td>
<td>22.9</td>
<td>21.8</td>
<td>96.9</td>
</tr>
<tr>
<td>TE Event Rate (n per 100 patient-years)</td>
<td>0.00</td>
<td>4.38</td>
<td>0.00</td>
<td>2.06</td>
</tr>
</tbody>
</table>

*Includes a one-year and a two-year extension study

†Includes TRIUMPH placebo-treated patients that transitioned to eculizumab treatment in the phase 3 extension study

‡Three of 195 patients began anticoagulant treatment during eculizumab; 2 of which were following the occurrence of a TE event

‖The 3 TE events occurring during eculizumab treatment included two events during SHEPHERD and 1 event during the Extension

∫*P < .001 for comparisons of eculizumab treatment versus pre-treatment; signed rank test
Table 4. Thromboembolism Events in Patients during the Eculizumab Treatment Period Compared to TE Events during the Same Period of Time Pre-Eculizumab Treatment

<table>
<thead>
<tr>
<th></th>
<th>Pilot*</th>
<th>TRIUMPH</th>
<th>SHEPHERD</th>
<th>Extension† (All studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n)</td>
<td>11</td>
<td>43</td>
<td>97</td>
<td>195</td>
</tr>
<tr>
<td>Patient-years (n)</td>
<td>33.0</td>
<td>21.8</td>
<td>93.6</td>
<td>272.1</td>
</tr>
<tr>
<td>TE Events</td>
<td>5</td>
<td>0</td>
<td>21</td>
<td><strong>39</strong></td>
</tr>
<tr>
<td><strong>Eculizumab Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n)</td>
<td>11</td>
<td>43</td>
<td>97</td>
<td>195</td>
</tr>
<tr>
<td>Patient-years (n)</td>
<td>34.2</td>
<td>21.8</td>
<td>96.9</td>
<td>281.0</td>
</tr>
<tr>
<td>TE Events</td>
<td>0.00</td>
<td>0.00</td>
<td>2</td>
<td><strong>3‡</strong></td>
</tr>
</tbody>
</table>

*Includes a one-year and a two-year extension study

†Includes TRIUMPH placebo-treated patients that transitioned to eculizumab treatment in the phase 3 extension study

‡P < .001 for comparisons of eculizumab treatment versus pre-treatment; signed rank test
Table 5. Thromboembolism Events in Patients Receiving Previous Antithrombotics

<table>
<thead>
<tr>
<th></th>
<th>Pilot*</th>
<th>TRIUMPH</th>
<th>SLEEPHERD</th>
<th>Extension† (All studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n)</td>
<td>9</td>
<td>23</td>
<td>51</td>
<td>103</td>
</tr>
<tr>
<td>TE Events (n)</td>
<td>4</td>
<td>9</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>Patient-years (n)</td>
<td>45.3</td>
<td>70.6</td>
<td>168.2</td>
<td>377.1</td>
</tr>
<tr>
<td>TE Event Rate (n per 100 patient-years)</td>
<td>8.83</td>
<td>12.74</td>
<td>15.46</td>
<td><strong>10.61</strong></td>
</tr>
<tr>
<td><strong>Eculizumab Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n)</td>
<td>9</td>
<td>23</td>
<td>51</td>
<td>103</td>
</tr>
<tr>
<td>TE Events (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient-years (n)</td>
<td>28.8</td>
<td>11.9</td>
<td>51.0</td>
<td>161.9</td>
</tr>
<tr>
<td>TE Event Rate (n per 100 patient-years)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td><strong>0.62‡</strong></td>
</tr>
</tbody>
</table>

†To qualify as a TE event on antithrombotic therapy, the event must have been verified to have occurred more than two weeks following initiation of antithrombotics

*Includes a one-year and a two-year extension study

†Includes TRIUMPH placebo-treated patients that transitioned to eculizumab treatment in the phase 3 extension study

‡P < .001 for comparisons of eculizumab treatment versus pre-treatment; signed rank test
Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria

Peter Hillmen, Petra Muus, Ulrich Duhrsen, Antonio M Risitano, Jorg Schubert, Lucio Luzzatto, Hubert Schrezenmeier, Jeffrey Szer, Robert A Brodsky, Anita Hill, Gerard Socie, Monica Bessler, Scott A Rollins, Leonard Bell, Russell P Rother and Neal S Young