

ORIGINAL ARTICLE

# Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria

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## ABSTRACT

### BACKGROUND

Paroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the *PIG-A* gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect the cells against attack by the complement system. We tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH.

### METHODS

Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12. Clinical and biochemical indicators of hemolysis were measured throughout the trial.

### RESULTS

Mean lactate dehydrogenase levels decreased from 3111 IU per liter before treatment to 594 IU per liter during treatment ( $P=0.002$ ). The mean percentage of PNH type III erythrocytes increased from 36.7 percent of the total erythrocyte population to 59.2 percent ( $P=0.005$ ). The mean and median transfusion rates decreased from 2.1 and 1.8 units per patient per month to 0.6 and 0.0 units per patient per month, respectively ( $P=0.003$  for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96 percent ( $P<0.001$ ), and measurements of the quality of life improved significantly.

### CONCLUSIONS

Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

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N Engl J Med 2004;350:552-9.

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THE MAJOR CLINICAL SIGNS OF PAROXYSMAL nocturnal hemoglobinuria (PNH) are intravascular hemolysis, venous thrombosis, and hemoglobinuria.<sup>1</sup> The disease arises from a somatic mutation of the PIG-A gene in a pluripotent hematopoietic stem cell. PIG-A encodes a protein that is essential for the synthesis of glycosylphosphatidylinositol (GPI), a lipid moiety that is embedded in the plasma membrane, where it serves to anchor a wide variety of proteins to the cell surface. The mutant stem cell subsequently expands to form a hematopoietic clone with a deficiency in proteins that are normally attached to the cell surface by the GPI anchor.<sup>2,3</sup> The mature blood cells derived from the hematopoietic clone can have a complete deficiency (type III) or a partial deficiency (type II) of GPI-linked proteins and almost always coexist with residual cells with a normal expression of these proteins (previously identified as type I).

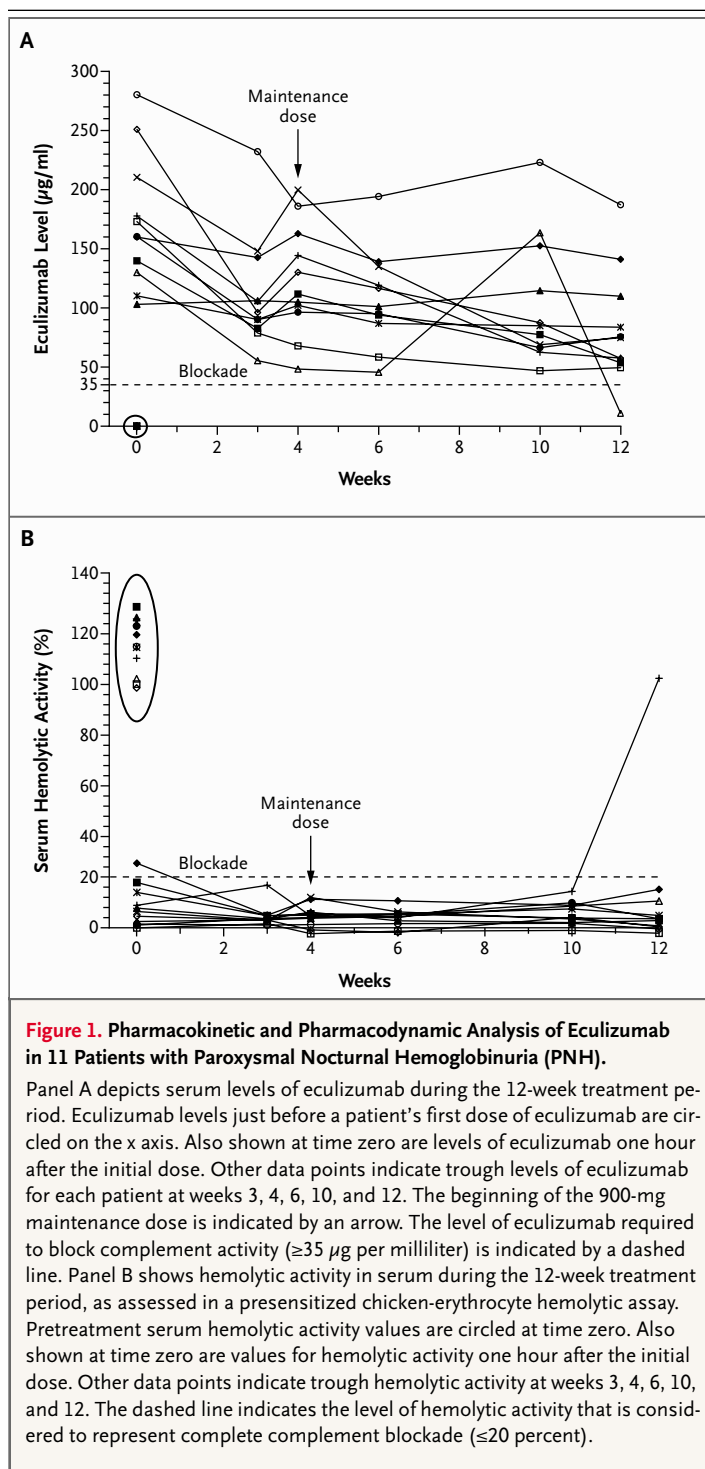
The clinical features of PNH result from the lack of one or more GPI-linked proteins that protect cells from complement-mediated attack. Two such proteins — CD55 and CD59 — are absent from PNH type III erythrocytes, platelets, and other blood cells.<sup>4-6</sup> CD55 regulates early complement activation by inhibiting C3 convertases,<sup>7</sup> whereas CD59 inhibits the assembly of the membrane-attack complex C5b-C9 by interacting with C8 and C9.<sup>4,5</sup> The lack of CD59 is probably responsible for the increased sensitivity of PNH erythrocytes and platelets to complement.<sup>4,8-13</sup>

Ecuzumab is a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components.<sup>14,15</sup> It binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the cytolytic pore C5b-C9. Blockade of the complement cascade at C5 preserves the early components of complement that are essential for the opsonization of microorganisms and clearance of immune complexes.<sup>16</sup> In this trial, we investigated whether ecuzumab could reduce the incidence of intravascular hemolysis, hemoglobinuria, and transfusion requirements in patients with PNH.

## METHODS

### PATIENTS

The study was conducted from May through December 2002. Men and women (18 years of age and old-



er) who had received a diagnosis of PNH at least six months earlier, had a detectable GPI-deficient hematopoietic clone, and had received at least four red-cell transfusions in the preceding 12 months were

eligible. Patients were required to have a negative throat culture for *Neisseria meningitidis* and *N. gonorrhoeae*. All patients were vaccinated against *N. meningitidis* (Mengivac (A+C), Aventis Pasteur) before treatment. One patient had a stroke after consent but never received eculizumab and was excluded. Patients who were taking stable doses of immunosuppressive drugs (e.g., cyclosporine), warfarin, and iron supplements were permitted to continue them.

The trial was approved by the local research ethics committee and was performed according to the International Conference on Harmonisation and Good Clinical Practice Standards. Eleven patients gave written informed consent and were treated with eculizumab.

#### TREATMENT SCHEDULE

Patients received infusions of 600 mg of eculizumab weekly for four weeks, followed one week later by a 900-mg dose and then by a dose of 900 mg every other week through week 12.

#### INVESTIGATIONS

In this open-label pilot study, we obtained data on the pharmacokinetics, pharmacodynamics, and

immunogenicity of eculizumab and observed its clinical effects by measuring the following: lactate dehydrogenase, haptoglobin, bilirubin, and hemoglobin levels; reticulocyte counts; the proportion of GPI-deficient cells, as assessed by flow cytometry<sup>17</sup>; the rate of transfusion with packed red cells; the rate of occurrence of hemoglobinuria (assessed by daily comparison of the first morning urine sample with a standardized color chart before and during treatment); and the quality of life, as reflected by the scores on the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 instrument. The trigger for transfusion during the study period remained unchanged for each patient, as compared with their care before entry into the study: patients received blood transfusions when they had symptoms resulting from anemia.

Assessment of the safety of eculizumab included ascertainment of treatment-related adverse events, electrocardiography, and routine laboratory tests (e.g., serum chemical analyses and complete blood counts).

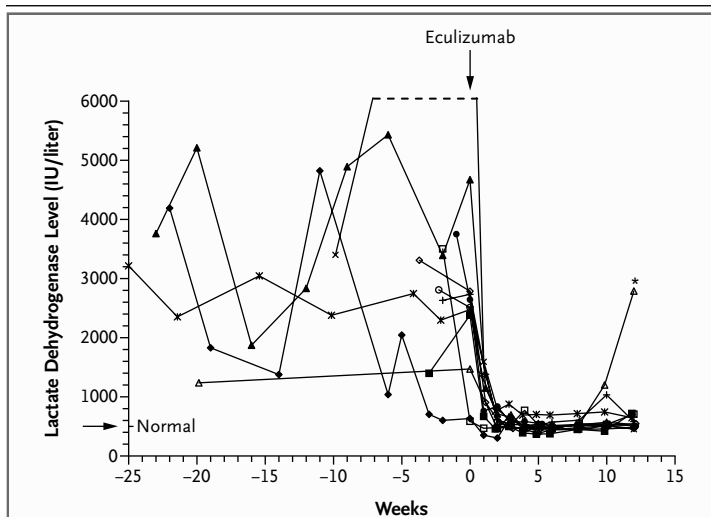
#### ASSAY METHODS

The pharmacokinetics of eculizumab were determined with an enzyme-linked immunosorbent assay that detects both free and C5-bound eculizumab.<sup>15</sup> The pharmacodynamics of eculizumab were determined by measuring the capacity of the patient's serum to lyse chicken erythrocytes in a standard total human serum-complement hemolytic assay.<sup>18</sup> The presence or absence of antibodies against eculizumab was assessed by an enzyme-linked immunosorbent assay.<sup>19</sup>

#### STATISTICAL ANALYSIS

Biochemical values were compared with the use of a paired Student's t-test, quality-of-life measurements with the use of a mixed-effect analysis of covariance, the median rate of transfusions with the use of a Wilcoxon signed-rank test, and the comparison of the number of days with paroxysms with the use of Fisher's exact test.

The corresponding author and the sponsor were jointly responsible for the design of this trial and the development of the protocol. Data were collected and analyzed by a clinical research organization, Kendle International, which maintained the trial data base and provided statistical support. The manuscript was prepared by the corresponding author, with substantial review and comments by the other authors and the sponsor. Final decisions on



**Figure 2.** Analysis of Lactate Dehydrogenase Levels, a Biochemical Indicator of Hemolysis, in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria up to 25 Weeks before and during 12 Weeks of Eculizumab Treatment.

The first dose of eculizumab is indicated by an arrow, as is the upper limit of the normal range of lactate dehydrogenase at the Leeds Teaching Hospital. The data point identified at week 12 by the asterisk represents a reading that was obtained from a duplicate serum sample since the original sample was lost. The dashed line represents off-scale points from one patient with a peak value of 12,100 IU per liter.

the content of the manuscript rested with the corresponding author in consultation with the other authors. All authors had access to the primary data.

## RESULTS

### DEMOGRAPHIC CHARACTERISTICS

Six men and five women (median age, 48 years; range, 21 to 67) with a median duration of PNH of 8.6 years (range, 1.7 to 37.4) participated in the trial. Five of the patients had platelet counts at base line of less than 150,000 per cubic millimeter. Eight patients had previously received a diagnosis of aplastic anemia, two were concomitantly receiving cyclosporine for aplastic anemia, and six were receiving warfarin.

### SAFETY

All patients completed the 12-week study. There were no deaths or thrombotic events, and all patients subsequently entered a 12-month extension study. Each patient reported one or more adverse events during the trial. Events reported by three patients included headache and upper respiratory tract infection. Events reported by two patients included influenza-like symptoms, rigors, dizziness, nausea, nasal congestion, and joint aches. None of these events were attributed to the study medication. Serious adverse events occurred in two patients. The first was hospitalized with a viral chest infection. The second reported nausea, vomiting, and headache after the first infusion, with dizziness and shivering the following day. The patient was hospitalized overnight, and subsequent infusions were well tolerated.

### PHARMACOKINETICS, PHARMACODYNAMICS, AND IMMUNOGENICITY OF ECULIZUMAB

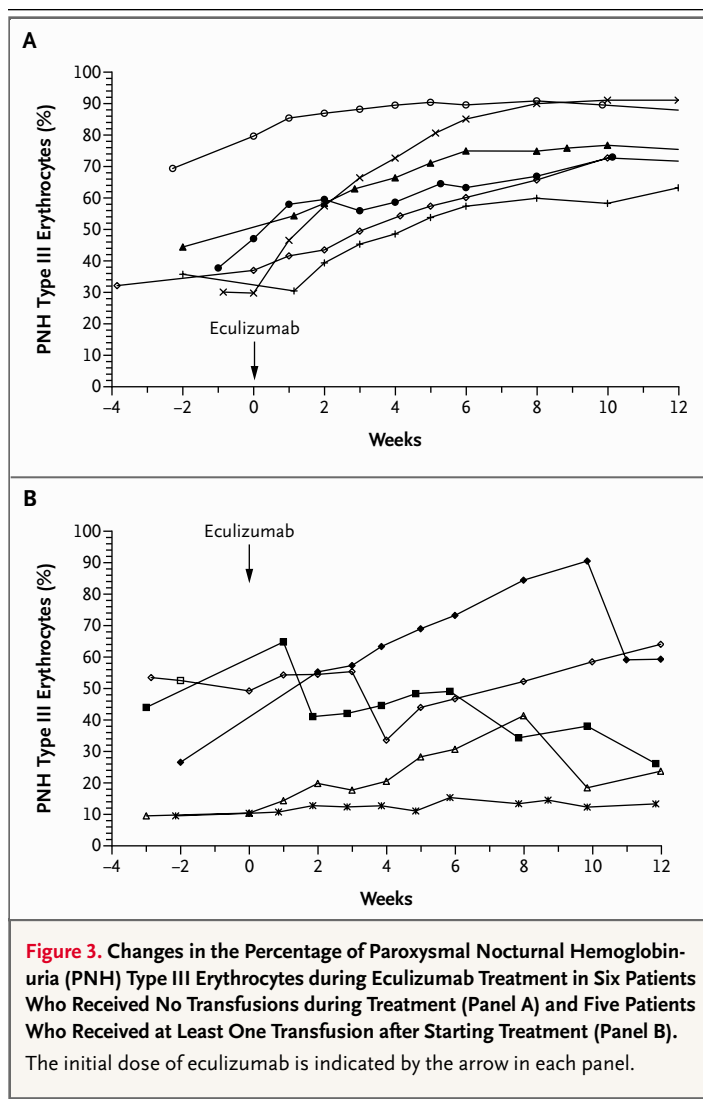
Peak and trough levels of eculizumab were well above 35  $\mu\text{g}$  per milliliter from one hour after the first dose through the completion of the 600-mg weekly dose period (Fig. 1A). In 10 patients, serum trough levels of eculizumab remained above 35  $\mu\text{g}$  per milliliter for the entire study.

The hemolytic activity of serum from these 10 patients was completely blocked (less than 20 percent in the chicken-red-cell assay) for essentially the entire treatment period (Fig. 1B). In 1 of the 11 patients, the trough level of eculizumab fell below 35  $\mu\text{g}$  per milliliter at week 12 and serum hemolytic activity returned. In no case were antibodies against eculizumab detected.

### BIOCHEMICAL INDICATORS OF HEMOLYSIS

Levels of lactate dehydrogenase in serum were markedly elevated in all patients before eculizumab treatment. Mean ( $\pm$ SD) lactate dehydrogenase levels fell from  $3111\pm 598$  IU per liter during the 12 months before enrollment to  $594\pm 32$  IU per liter (normal range, 150 to 480) during treatment ( $P=0.002$ ) (Fig. 2).

The decrease in lactate dehydrogenase began after a single dose of eculizumab in all patients. Lactate dehydrogenase levels remained within or just above the normal range for the duration of the study (Fig. 2). In the one patient in whom eculizumab levels fell below 35  $\mu\text{g}$  per milliliter at week 12 (Fig. 1A), hemolytic activity returned (Fig. 1B) and lactate dehydrogenase levels increased transiently (Fig. 2).



**Figure 3.** Changes in the Percentage of Paroxysmal Nocturnal Hemoglobinuria (PNH) Type III Erythrocytes during Eculizumab Treatment in Six Patients Who Received No Transfusions during Treatment (Panel A) and Five Patients Who Received at Least One Transfusion after Starting Treatment (Panel B).

The initial dose of eculizumab is indicated by the arrow in each panel.

The dosing frequency was increased from 900 mg every 14 days to 900 mg every 12 days, reestablishing complete complement blockade during the ongoing 12-month extension study (data not shown).

Haptoglobin became detectable in the serum of 5 of the 11 patients after two weeks of eculizumab treatment but returned to undetectable levels soon thereafter (data not shown). Bilirubin levels were also elevated in most patients at base line and did not change significantly during treatment (data not shown).

#### EFFECT ON PNH CLONES

Type III erythrocytes are highly sensitive to lysis by complement and as a result have a short life span. In our study, the percentage of type III erythrocytes increased significantly from a mean of  $36.7 \pm 5.9$  percent before treatment to  $59.2 \pm 8.0$  percent at the end of 12 weeks of treatment ( $P=0.005$ ) (Fig. 3). The increase was particularly consistent in six patients who remained transfusion-independent during the study (Fig. 3A). In patients who received transfusions during the study, sudden drops in the proportion of type III cells were seen as the transfused red cells diluted the type III population (Fig. 3B). There were no significant changes in the per-

centages of PNH type III neutrophils, monocytes, or platelets during treatment with eculizumab; in most cases, these percentages were 90 to 100 percent before the study (data not shown).

#### TRANSFUSION REQUIREMENTS, HEMOGLOBIN LEVELS, AND RETICULOCYTE COUNTS

During the year preceding enrollment, the range of red-cell transfusions received by the 11 patients was 12 to 55 units, whereas during the three months of the study, the range was 0 to 8 units. Before eculizumab treatment, the mean and median transfusion rates were 2.1 and 1.8 units per patient per month, respectively (Table 1). These transfusion rates decreased to 0.6 and 0.0 unit per patient per month, respectively, during the three months of treatment with eculizumab ( $P=0.003$ ). Hemoglobin levels did not increase significantly during the treatment period, although hemoglobin values in six patients stabilized without transfusions (Table 1). Similarly, the numbers of reticulocytes remained relatively constant during eculizumab treatment.

#### HEMOGLOBINURIA

Our patients recorded the color of their urine each morning using a color chart designed to assess the

**Table 1. Transfusion Rates, Hemoglobin Levels, and Reticulocyte Counts 12 Months before and after 3 Months of Eculizumab Treatment.**

Patient No.	12 Mo before Eculizumab Treatment				After 3 Mo of Eculizumab Treatment			
	Transfusions		Hemoglobin	Reticulocytes	Transfusions		Hemoglobin	Reticulocytes
	no. of units	rate*	g/dl	$\times 10^{-3}/\text{mm}^3$	no. of units	rate†	g/dl	$\times 10^{-3}/\text{mm}^3$
1	22	1.8	10.3	77.5	2	0.7	10.0	100.7
2	23	1.9	8.3	200.0	8	2.9	8.8	182.6
3	20	1.6	10.1	169.5	0	0.0	10.7	175.9
4	28	2.3	9.3	282.0	0	0.0	9.4	333.3
5	12	1.0	11.9	96.3	2	0.7	10.6	121.8
6	14	1.2	9.8	346.8	0	0.0	10.6	259.0
7	34	2.8	12.8	100.6	0	0.0	13.5	166.8
8	21	1.7	9.5	164.5	0	0.0	9.8	239.6
9	55	4.5	10.7	138.0	3	1.1	11.4	285.8
10	41	3.4	8.5	108.7	5	1.8	8.8	140.1
11	14	1.2	8.5	91.4	0	0.0	10.0	97.4
Median‡		1.8				0.0		
Mean		2.1	10.0	161.4		0.6	10.3	191.2

\* The rate (in units per month) was calculated as (number of units  $\div$  365 days)  $\times$  30.

† The rate (in units per month) was calculated as (number of units  $\div$  84 days)  $\times$  30.

‡  $P=0.003$  for the change in the median rate of transfusion by the Wilcoxon signed-rank test.

degree of hemoglobinuria (Fig. 4A) during both the 2-to-4-week screening period and the 12-week treatment period. Paroxysms of hemoglobinuria were prospectively defined as dark-colored urine with a colorimetric level of 6 or more. In nine patients for whom urine scores were assessed, the mean incidence of paroxysms was reduced from 2.9 days to 0.12 day per patient per month ( $P < 0.001$ ) (Fig. 4B).

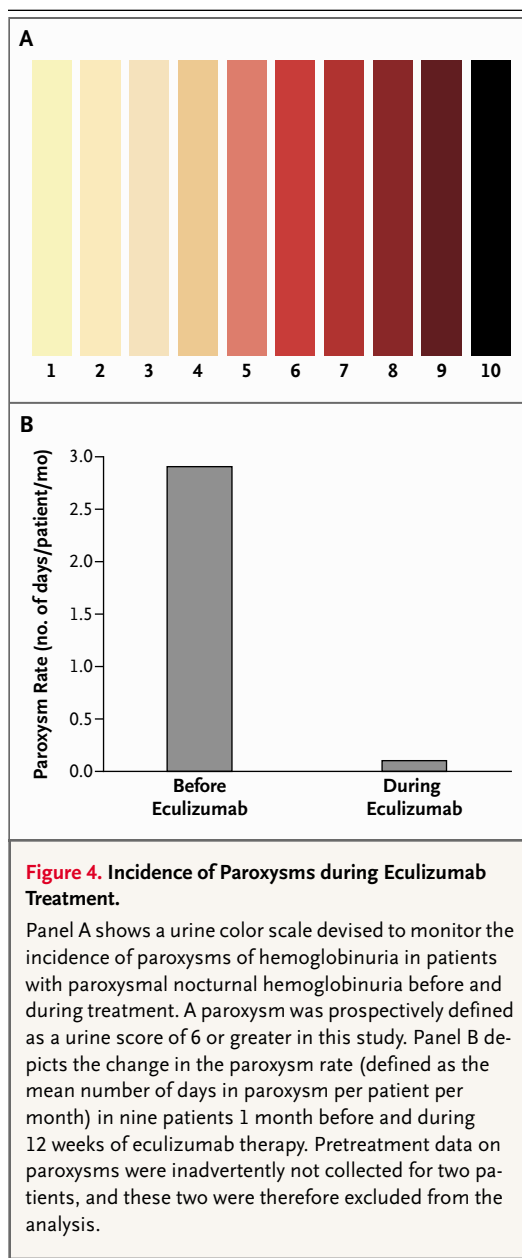
#### QUALITY OF LIFE

The quality of life was assessed with the use of the EORTC QLQ-C30 instrument. When responses at base line were compared with responses during 12 weeks of eculizumab treatment, there were significant improvements in the domains of global health status ( $P = 0.02$ ), physical functioning ( $P < 0.001$ ), emotional functioning ( $P < 0.001$ ), cognitive functioning ( $P = 0.002$ ), fatigue ( $P < 0.001$ ), dyspnea ( $P = 0.002$ ), and insomnia ( $P = 0.049$ ) (Table 2).

#### DISCUSSION

Patients with PNH have chronic, often disabling symptoms of fatigue and intermittent episodes of dysphagia, abdominal pain, and hemoglobinuria. These symptoms are thought to be related to the intravascular destruction of PNH type III erythrocytes, which are deficient in complement inhibitors, by autologous complement. The hemolytic anemia frequently renders the patients transfusion-dependent. In addition, patients have an extremely high risk of potentially life-threatening thrombosis, particularly thrombosis of the hepatic and cerebral veins. Approximately 50 percent of patients with PNH die of the disease; the median duration of survival after diagnosis is 10 years.<sup>1</sup>

We found that the defect in the membrane-bound inhibitor of terminal complement components in PNH was ameliorated by the administration of eculizumab. This antibody specifically prevents cleavage of C5, which is necessary for assembly of the membrane-attack complex. Blockade of terminal complement components presumably prolongs the survival of type III erythrocytes (since there was no simultaneous increase in reticulocytes), which are highly sensitive to lysis by complement, thereby increasing the proportion of these cells in the blood and reducing signs of hemolysis in most patients. In some patients, the percentage of type III erythrocytes increased to more than 80 percent of the total erythrocyte population. This interpretation of the mechanism of action of eculizumab is consistent



with the report of an asymptomatic patient with PNH who had more than 80 percent type III erythrocytes and a concomitant deficiency in the terminal complement protein C9.<sup>20</sup> Thus, inhibition of the assembly of C5b–C9 by an antibody or by a congenital deficiency of a terminal component of the complement system can protect type III erythrocytes from complement-mediated lysis.

The long-term effects of protecting PNH type III erythrocytes from complement are not known. For example, will removing the negative pressure

**Table 2. Change in the Quality of Life during Eculizumab Treatment.\***

Domain	Mean Base-Line Score†	Change from Base Line‡§	P Value¶
Global health status	56.1	13.7	0.02
Physical functioning	70.9	13.0	<0.001
Emotional functioning	70.5	12.7	<0.001
Cognitive functioning	77.3	11.8	0.002
Fatigue	47.5	-15.3	<0.001
Dyspnea	39.4	-12.4	0.002
Insomnia	30.3	-10.8	0.049

\* The quality of life was assessed with the European Organization for Research and Treatment of Cancer QLQ-C30 instrument.

† Numbers represent mean values of linearly transformed scores.

‡ Values for change from base line represent least-square means. A positive value indicates an improvement in the score for global health status, physical functioning, emotional functioning, and cognitive functioning, whereas a negative value indicates an improvement in the score for fatigue, dyspnea, and insomnia.

§ Values are from a mixed analysis-of-covariance model with visit as a fixed effect, patient as a random effect, and base line as a covariate.

on type III hematopoietic cells alter the rate of expansion of the PNH clone? What might occur in a patient with an increased population of PNH type III erythrocytes if treatment with eculizumab is stopped? Two of the patients who entered the eculizumab extension study had transient breakthroughs (lasting two to three days) in complement blockade until the dosing interval was adjusted. Both patients had hemoglobinuria with mild symptoms, but the episodes were not life threatening and were easily managed (data not shown). Definitive answers to these questions will require further study.

In this trial, lactate dehydrogenase levels declined rapidly and remained reduced as long as the serum level of eculizumab exceeded 35 µg per milliliter. The importance of maintaining this level of antibody was demonstrated in a single patient, in whom the eculizumab level transiently dropped below 35 µg per milliliter at week 12, resulting in a return of serum complement activity and an increase in lactate dehydrogenase levels. Subsequent administration of eculizumab reestablished complement blockade and rapidly reduced lactate dehydrogenase levels. Interestingly, lactate dehydrogenase levels were reduced in most patients to just above the upper limit of normal. The slightly elevated levels of this enzyme during treatment with eculizumab could reflect persistent, low-level C3b-mediated extravascular hemolysis or, possibly, undefined

mechanisms of hemolysis that are unrelated to complement.

We also found that eculizumab treatment significantly reduced transfusion requirements, even though the levels of hemoglobin did not change significantly. However, the hemoglobin level in an individual patient before study entry was artificially maintained as a result of the transfusion of normal red cells. The transfused red cells survive far longer than PNH cells. Therefore, the stabilization of hemoglobin levels with a reduced need or no need for transfusion is a result of the protection of PNH red cells from complement-mediated lysis by eculizumab.

The decrease in transfusion requirements was most apparent in the six patients without a clinically significant degree of bone marrow failure (as defined by a normal platelet count). All but one of these patients were transfusion-independent during the study and remained so during an extension study. The remaining patient received a single 3-unit transfusion during the study, as compared with the receipt of 55 units in the 12 months preceding enrollment.

There was a rapid improvement in the quality of life during eculizumab therapy, as measured by the EORTC QLQ-C30. These clinical observations support the hypothesis that many of the important co-existing clinical conditions in patients with PNH are directly related to chronic and acute episodes of hemolysis, possibly through the scavenging of nitric oxide by plasma free hemoglobin.<sup>21-25</sup>

Eculizumab was safe and well tolerated during this open-label pilot study. The adverse events reported by patients were similar in type and frequency to those reported with either eculizumab or placebo in other controlled trials. All patients are currently participating in a one-year extension study in which the drug continues to be well tolerated. Furthermore, the rates of intravascular hemolysis, as measured by lactate dehydrogenase levels and hemoglobinuria, remain reduced in all patients, with 5 of 11 patients having been transfusion-independent for at least one year since starting eculizumab treatment.

The PIG-A mutation in patients with PNH causes deficiencies in the membrane-bound complement inhibitors CD55 and CD59, resulting in intravascular hemolysis.<sup>2,3</sup> However, patients who have genetic deficiencies in the surface expression of CD55 (Inab phenotype) with normal levels of CD59 have no clinical signs of hemolysis.<sup>8,9</sup> Conversely, a pa-

tient with a genetic deficiency in the expression of CD59 but normal levels of CD55 had symptoms indistinguishable from those of PNH.<sup>10,11</sup> Therefore, the somatic mutation in the PIG-A gene that causes the deficiency of the membrane-bound terminal complement inhibitor CD59 is critical to the pathogenesis of PNH. We found that terminal complement inhibition with eculizumab ameliorated the untoward effects of this deficiency.

In summary, eculizumab appears to enhance the survival of type III PNH erythrocytes, improving the quality of life and reducing the extent of hemolysis, hemoglobinuria (the clinical hallmark of PNH), and the need for blood transfusions in patients with

PNH. This study confirms that terminal complement activation is the key mediator of erythrocyte destruction in PNH.

Funded by Alexion Pharmaceuticals, Cheshire, Conn.

Dr. Hillmen reports serving as a consultant to Alexion Pharmaceuticals and receiving grant support from the company; Drs. Rollins, Mojcik, and Rother, Mr. Bombara, and Ms. Petro report having equity ownership in Alexion Pharmaceuticals; and Drs. Rollins and Rother have assigned to Alexion Pharmaceuticals their inventions made as employees of the company and have received no royalties from Alexion for these inventions. Dr. Rollins receives royalties for inventions he made before becoming an employee of Alexion.

We are indebted to Drs. Leonard Bell, Stephen Squinto, and Paul Finnegan for critical review of the manuscript; to Karen Hannon for excellent technical support; and to Jan Farish, David Buchanon, Pilar Hernandez-Campo, and the nurses of Healthcare-at-Home for their assistance in carrying out the study.

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