The Role of Complement Inhibition in PNH

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Abstract

PNH has fascinated hematologists for many years. PNH is peculiar in that it is an acquired hemolytic disorder resulting from an abnormality that is intrinsic to the red cell. Almost all other acquired hemolytic conditions are due to an extrinsic attack on the red cells, for example, antibody-mediated or mechanical hemolysis. On the other hand, almost all congenital hemolytic conditions are due to intrinsic red cell abnormalities, such as glucose-6-phosphate dehydrogenase (G6PD) deficiency or sickle cell anemia. It was first suggested in the 1930s that complement may have a role to play in PNH and in the 1960s and 1970s Rosse and demonstrated that PNH cells were exquisitely sensitive to complement. Such an approach would be expected to treat the intravascular hemolysis, which is the hallmark of the disease, but also to address many of the complications of PNH, such as how to avoid complications of therapy, how to overcome some of the problems associated with treatment and who to select for treatment, as only a proportion of patients with a PNH clone will benefit. This article will review the evidence behind the use of eculizumab in PNH, the effect it will have on the complications of the disease, the most appropriate selection of patients for therapy, the optimal management and the potential complications of the therapy.

Introduction

PNH has fascinated hematologists for many years. PNH is a rare, chronic, debilitating, acquired disorder that most frequently presents in early adulthood and usually continues throughout the life of a patient. PNH results in the death of approximately half of affected individuals, mainly through thrombotic complications, and until recently had no specific therapy. In 2007 eculizumab, an anti-complement antibody targeting the C5 complement component was approved for PNH by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). Eculizumab is very effective in the treatment of intravascular hemolysis and all its sequelae, which include most of the symptoms and complications of PNH. Eculizumab has revolutionized our approach to hemolytic PNH and as it markedly reduces the principal complications of PNH, namely thrombosis and renal failure, should have a significant impact on survival. However, the development of eculizumab presents new challenges in PNH, such as how to avoid complications of therapy, how to overcome some of the problems associated with treatment and who to select for treatment, as only a proportion of patients with a PNH clone will benefit. This article will review the evidence behind the use of eculizumab in PNH, the effect it will have on the complications of the disease, the most appropriate selection of patients for therapy, the optimal management and the potential complications of the therapy.

Complement Inhibition

The concept of the inhibition of complement in PNH is not new. This was first considered in the 1950s when the relationship between PNH and the unopposed activity of terminal complement was being unravelled. The initial attempts to inhibit complement involved the use of corticosteroids. There is some evidence that at extremely large doses corticosteroids reduce the activity of complement. This was historically by many physicians looking after patients with PNH. However, there is very little evidence to support their use as very large doses are required to have any significant impact on complement activation and the toxicity of chronic steroid use is unacceptable.

Since most of the problems observed in PNH are due to the unopposed effect of complement against PNH cells then the obvious target for therapy in PNH is to inactivate complement. Such an approach would be expected to treat the intravascular hemolysis, which is the hallmark of the disease, but also to address many of the complications of PNH, such as venous thrombosis, renal failure and the relatively newly recognized entity of nitric oxide consumption by free hemoglobin. However, the potential consequences of complement inhibition are of concern since the complement system has evolved as a key part of our innate immune system. Deficiency of one of the early components of complement (before C5) results in an increased risk of both pyogenic infections and autoimmune phenomena, such as systemic lupus erythematosus and glomerulonephritis. In contrast, and somewhat surprisingly, deficiencies of the terminal complement pathway have relatively few consequences. The only apparently important adverse consequence of terminal complement deficiencies is an increased risk of infection from Neisseria meningitides and Neisseria gonorrhoeae. Therefore, the logical part of the complement cascade to target is the fifth component (C5), which would leave the early part of the complement pathway intact but...
prevent the assembly of the membrane attack complex and the release of the potent anaphylatoxin C5a (see Figure 1).

Eculizumab is a humanized monoclonal antibody that specifically binds to the human C5 complement protein, prevents its cleavage to C5a and C5b, and therefore prevents formation of the membrane attack complex.\(^5\) Eculizumab is an IgG kappa immunoglobulin with an engineered Fc portion that is a hybrid of IgG2 and IgG4 designed to have no downstream activity—thus eculizumab is a purely “blocking” antibody that prevents the cleavage and thus activation of C5. Eculizumab remains bound to the target until the complex is removed from the circulation. It is the first approved drug that specifically targets complement activation and was initially developed with a view to treating autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus and glomerulonephritis. Although the early studies in these disorders demonstrated some activity, the doses of eculizumab used were generally not high enough to completely block complement and the activity seen was not sufficient to encourage further development for these indications. However, these studies did demonstrate that a concentration of 35 μg/mL is the plasma level required to maintain complement inhibition. The studies demonstrated the safety of eculizumab in more than 700 hundred patients receiving therapy, with only a single case of meningococcal infection seen. In addition, there was no formation of human anti-human antibodies (HAHA) observed in these studies. These initial studies led to the development of eculizumab in PNH.

**Eculizumab in PNH**

*The Pilot study*

The Pilot study was the initial trial of eculizumab in PNH and commenced in two institutions in the United Kingdom in May 2002.\(^6\) In this study a total of 11 patients with hemolytic PNH who were transfusion-dependent (defined by 4 or more transfusions in the 12 months prior to inclusion in the study) were treated for a period of 3 months. The dose given was derived from the previous non-PNH trials in that it was expected to result in a rapid and complete blockade of complement. The dosing schedule used was a loading period of 600 mg every week for 4 doses followed by 900 mg the following week and then 900 mg every 14 days. The drug is given by intravenous infusion over a period of 30 minutes. The results of this study were dramatic. Immediately after the first dose of therapy patients experienced a huge improvement in measures of quality of life, cessation of hemoglobinuria and a significant fall or complete resolution of transfusion requirements. The Pilot study clearly confirmed that terminal complement was the principle culprit causing the intravascular hemolysis in PNH. All 11 patients continued on eculizumab at a dose of 900 mg every 14 days in an extension study.\(^7\) Now, over 6 years from the start of therapy, 10 patients remain on therapy and all except one of these patients remains on the 900 mg dose with one requiring a higher dose of 1200 mg every 14 days (see “Breakthrough from complement control,” below). The Pilot study led to two further studies, the TRIUMPH and SHEPHERD studies, which led to the approval of eculizumab by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA).

**TRIUMPH study**

The TRIUMPH study was a randomized placebo-controlled trial in which a similar dosing schedule as the Pilot study (see above) was compared to infusions of saline in a double-blind randomized controlled trial.\(^8\) The duration of therapy in the study was 6 months during which patients received either eculizumab or placebo. Following this 6-month pe-
period all patients were eligible for an Extension study in which the eculizumab continued at 900 mg every 2 weeks; the patients given placebo were induced with the standard regimen followed by 900 mg every 2 weeks. The key inclusion criteria were patients who were transfusion dependent (4 or more transfusions in the preceding 12 months) who had hemolytic PNH and no overt bone marrow failure (platelet count greater than $100 \times 10^9$). Patients were enrolled and then followed until they had a “qualifying” transfusion—this was defined as requiring a transfusion when their hemoglobin was within 1.5 g/dL of the level that they were previously transfused and under 9.0 g/dL. A total of 115 patients were screened in the TRIUMPH study and 87 fulfilled the criteria for a qualifying transfusion and were randomized (44 patients to eculizumab and 43 to placebo). The TRIUMPH Study convincingly met co-primary endpoints of the maintenance of hemoglobin levels above their previous set-point and a highly significant reduction in transfusion requirements. In addition, the secondary endpoints within the TRIUMPH study demonstrated the clinical benefit of eculizumab including a highly clinically meaningful improvement in fatigue and quality of life, reduction on parameters of intravascular hemolysis (see Figure 2), and that the drug was safe.

**SHEPHERD study**

The SHEPHERD study was an open-label non-randomized trial for patients with hemolytic PNH with much wider entry criteria than the TRIUMPH study. In the SHEPHERD study patients could have fewer prior transfusions (at least 1 in the previous 2 years) or had a religious belief that precluded transfusion. Also, patients with a lower platelet count (above $30 \times 10^9/L$) were entered into the study. A total of 97 patients were treated in the SHEPHERD study and the results were equally impressive as both the Pilot and the TRIUMPH studies with a marked reduction in transfusions compared to starting therapy and an improvement in quality of life. When the effect of eculizumab was compared across the TRIUMPH and SHEPHERD studies the efficacy in reducing hemolysis, stopping or lowering transfusion requirements and improving anemia and fatigue was seen across all severities of patients affected regardless of pretreatment transfusion requirements (see Figure 3).

Therefore a total of 195 patients were included in the 3 trials and all demonstrated consistent and very impressive responses in intravascular hemolysis.

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**Figure 2. 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 1-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. Reprinted with permission from Hillmen et al. Blood. 2007;110:4123-4128. © the American Society of Hematology.

**Figure 3. Mean (SE) units packed red blood cells transfused by pre-treatment transfusion strata during the TRIUMPH and SHEPHERD studies.**

$P$ values for data from the TRIUMPH study (A) were calculated using Wilcoxon’s rank sum test. $P$ values for data from the SHEPHERD study (B) were calculated using a sign rank test. Adapted from Schubert et al. Br J Haematol. 2008.
Impact of Eculizumab on PNH Complications

Thrombosis
Thrombosis is the major complication of PNH, and the pathophysiology of thrombosis is not clearly understood. The majority of the potential mechanisms, including platelet activation by complement due to nitric oxide consumption as a result of intravascular hemolysis and endothelial damage by the intravascular hemolysis, would be complement related. Therefore anti-complement therapy, such as with eculizumab, should prevent the occurrence of thrombosis. All patients from the three Pivotal studies were permitted to continue on eculizumab therapy in an Extension study. The responses in the three studies have been durable for over 6 years since the first patient was treated. In addition, the occurrence of thrombotic complications was assessed in all of the trials combined including the pivotal studies and the extension studies. The case notes of all patients were examined to assess the number and sites of thrombotic events prior to commencing eculizumab, and then any thrombotic events occurring on eculizumab were documented. Overall there were 124 thrombotic events in the 195 patients prior to starting eculizumab equating to 7.37 thromboses per 100 patient-years. In contrast, there were only 3 thromboses observed after commencing eculizumab which equates to 1.07 thromboses per 100 patient years. Thus there was a significant reduction in the thrombotic rate on eculizumab compared to the same patients prior to eculizumab ($P < .0001$). Further analysis revealed that even patients on anticoagulation with warfarin or heparin had a high rate of thrombosis prior to starting eculizumab and this was virtually absent when eculizumab was added to the anticoagulation (see Figure 4). Therefore, eculizumab has a profound effect on the occurrence of thrombosis in PNH even in cases where routine anticoagulation alone fails to prevent further thrombosis.

Renal failure
Renal failure has been noted as a complication of PNH for many years. In fact, between 8% and 18% of deaths in PNH have been reported to have renal failure as at least a contributing factor. In the eculizumab studies the rate of renal dysfunction increased with time from the diagnosis of PNH so that the actuarial risk of having renal dysfunction was approximately 75% at 30 years. This probably reflects the continuous intravascular hemolysis and thus the loading of the proximal renal tubules with iron. Early data from the eculizumab trials indicates that complement inhibition reduces the rate at which renal function deteriorates and in many patients results in an improvement in renal function.

Nitric oxide consumption
Many of the symptoms characteristic of PNH, such as the recurrent abdominal pain, dysphagia, erectile dysfunction and severe lethargy, appear to be due to nitric oxide (NO) consumption by free hemoglobin in the plasma. This syndrome of NO consumption with lethargy and pulmonary hypertension has been well described in sickle cell anemia but has only recently been recognized in PNH. In addition to the symptoms associated with NO consumption, this consequence of intravascular hemolysis can also result in pulmonary hypertension as has been observed in PNH. Eculizumab has a profound effect on intravascular hemolysis and as a consequence appears to reduce NO consumption with a marked improvement in associated symptoms.

Nitric oxide consumption

Figure 4. Analysis of thrombosis before and during eculizumab therapy. Comparison of the number and frequency of thromboses in patients in all of the eculizumab studies. (A) The analysis has been restricted to compare the same time period for each patient before they started eculizumab to the time they have spent on therapy. The aim is to remove a bias introduced due to the prolonged time for many patients between diagnosis and commencing therapy. (B) Demonstrates that thromboses are often seen even when patients are anticoagulated with warfarin or heparin and that these are reduced by eculizumab.
Specific Issues with Complement Inhibition

Breakthrough from complement control
Eculizumab must remain above 35 μg/mL to maintain complete blockade of complement.8 The dosing schedule for eculizumab of an induction period of 600 mg weekly for 4 doses and then 900 mg every 2 weeks is adequate to maintain complete complement blockade for the vast majority of patients with PNH. However, there are a small minority of patients, probably in the region of 10%, in whom this dosing schedule is inadequate. The trough drug levels in these patients fall below 35 μg/mL and the patient’s intravascular hemolysis breaks through, often with a sudden onset of hemoglobinuria in the day or two before the next dose of eculizumab.21 The onset of hemoglobinuria in this context suddenly returns to the level seen before treatment with eculizumab. There can be a drop in hemoglobin as the proportion of PNH red cells has usually increased during therapy in view of the lengthened survival of PNH red cells and the lack of transfused red cells. The symptoms and hemoglobinuria then resolve immediately after the next dose as the level of eculizumab is above 35 μg/mL. In most patients this type of breakthrough from complement control can be prevented by slightly increasing the dose of eculizumab either to 900 mg every 12 days or 1200 mg every 2 weeks.

Extravascular hemolysis
The major symptoms of PNH are due to the intravascular hemolysis resulting from the unopposed activity of terminal complement due to the deficiency of the principal inhibitor of the formation of the membrane attack complex, CD59. However, PNH cells are deficient in a large number of glycosyl phosphatidyl inositol (GPI)–anchored proteins including another complement regulatory molecule, decay accelerating factor (DAF or CD55). DAF acts earlier in the complement cascade to prevent the formation of the C3-convertase and C3b generation. Since eculizumab blocks beyond C3 it might be expected that C3 generation accelerates and, due to the deficiency in DAF, this may lead to an accumulation of C3b on the cell surface. The consequence of this is extravascular hemolysis as the PNH red cells are loaded with C3b and C3d and therefore are cleared more rapidly by the reticulo-endothelial system.22 This probably explains the observation that, despite the resolution of intravascular hemolysis with eculizumab the hemoglobin levels do not always return to normal. The majority of patients on eculizumab therapy have a usually well-compensated extravascular hemolysis, which is manifest by a slight to moderate anemia, reticulocytosis, raised bilirubin, low haptoglobin and a positive direct antiglobulin (Coombs’) test (but for complement only; there is no immunoglobulin on the red cell surface). In most patients this is of little clinical consequence, but in some the degree of extravascular hemolysis may result in a continued requirement for transfusions, although usually at a greatly reduced frequency. In addition, the hemoglobin level may fall during times when there is increased complement activation, for example, during infections, and this appears most likely due to an increase in extravascular hemolysis. Some patients with extravascular hemolysis may respond to either erythropoietin or even a small dose of steroids.

Withdrawal from eculizumab
Most patients with PNH who are treated with eculizumab will cease to require transfusions. This leads to an increase in the proportion of PNH red cells as they are not diluted by transfusions and also have a more normal life span.21 In some patients the proportion of PNH red cells may exceed 90%. Therefore, there is a theoretical risk that when eculizumab is stopped for any reason there may be a sudden fall in hemoglobin and brisk hemoglobinuria. It is wise to observe patients very closely if eculizumab has to be stopped and consider giving a red cell transfusion if any symptoms develop. Needless to say, patients need educating that they cannot simply miss a scheduled dose of therapy.

Meningococcal infection
It seems bizarre that the complement cascade can be blocked at the C5 level with abolition of terminal complement activation and that this results in relatively few problems. In fact both inherited deficiencies of terminal complement in humans and knock-out animals have been described and, in contrast to when early complement components are affected, the deficiency of terminal complement results in surprisingly few problems. The only major concern of individuals with inherited terminal complement deficiency is an increased risk of developing infections with encapsulated organisms, particularly Neisseria species (meningitides or gonorrhoeae).7 But while the frequency of developing meningococcal infection is higher, the risk of mortality from the infection is lower. Therefore, it was entirely predictable that patients treated with eculizumab would have an increased risk of meningococcal infection as it mimics inherited terminal complement deficiency. To date a small number of patients, who have been vaccinated against N meningitides, have developed meningococcal infection (thus far all septicemia and not meningitis) and this occurs in approximately 0.5 cases per 100-patient years on eculizumab.21 The two mainstays to reduce the risk of meningococcal infection are vaccination and early therapy. Patients should be vaccinated with the tetra-valent vaccine (meningococcal ACWY vaccine), if available, as this covers all except the strain B, for which there is no vaccine. In fact, all of the cases of meningococcal disease reported to date in vaccinated patients on eculizumab have been due to strain B. Ideally, patients should be vaccinated 2 weeks before commencing eculizumab (the only excep-
tion being those patients who are critically ill from their PNH when starting eculizumab immediately may be vital) and re-vaccinated every 2 to 3 years. Early treatment is also vital. Patients need to be instructed that if they develop any symptoms suggesting meningococcal infection, such as very high temperatures, a hemorrhagic rash or neck stiffness, that they contact their doctor immediately. Meningococcal septicemia is life-threatening but almost always responds to either penicillin or cephalosporins and should be treated immediately even before a positive culture is established. The most important message is to be VIGILANT for meningococcal disease at all times.

Pregnancy
The risks of pregnancy in PNH are very significant. There is a very high risk of thrombotic complications for the expectant mother as well as a risk of developing hypoplastic anemia.23 It is difficult to give an accurate assessment of the risks, as most of the reports are single cases or very small series and it is much more likely that complicated pregnancies would be reported. However, the risk of maternal mortality is probably around 20% (5 of 24 patients in one reported series—3 deaths due to thrombosis and 2 due to infection) and of thrombotic complications in over 10% of patients. The literature also suggests an increased risk of fetal loss, but these cases are also subject to over-reporting. There is no obvious increased risk of fetal abnormalities in PNH. Therefore the management of pregnancy in PNH is a challenge. It is generally recommended that patients receive full anticoagulation with low-molecular-weight heparin. Alternatively, warfarin may be substituted after the first trimester. Given the benefit of eculizumab in reducing thrombosis in PNH, a key question is whether the drug can be given safely in pregnancy. The design of eculizumab with a hybrid Fc portion with IgG2 and IgG4 components is designed to abolish any effector mechanisms of the antibody. An added advantage may be that IgG2 isotypes do not cross the placenta, so there should be little impact on the fetus. However, this needs to be confirmed before firm guidelines can be proposed for the management of pregnancy in PNH.

Economic Aspects of Eculizumab
PNH is an extremely uncommon disorder with a prevalence of patients with a PNH clone in the region of 16 cases per million population and an incidence of 1.3 per million population.24 In addition, not all of these patients will require therapy with eculizumab, as the main feature of some patients’ disease may be hypoplastic anemia rather than hemolysis. Also some patients with hemolysis may have mild symptoms, which are insufficient to justify the use of eculizumab. Due to the rarity of PNH, eculizumab falls into the ultra-orphan drug category as defined by the National Institute of Clinical Excellence (NICE). Ultra-orphan drugs are those that are indicated for fewer than 1 patient per 50,000 population.25 For this reason, eculizumab is expensive compared to drugs for more frequent indications, but the cost for eculizumab is similar to therapies for other ultra-orphan diseases, such as enzyme replacement therapy for lysosomal storage diseases (for example, Gaucher’s disease) or inhibitors for hemophiliacs.26

Monitoring Eculizumab Therapy
The loss of iron in the urine through hemosiderinuria is a peculiar feature of PNH. This means that patients can sometimes receive very large numbers of transfusions, often several hundred, without suffering iron overload. One of the consequences of eculizumab in PNH is that, as a result of the lack of intravascular hemolysis, this protective mechanism is lost. Even if patients do not require transfusions, they may have a significant level of extravascular hemolysis and a slight anemia, resulting in the accumulation of iron. It is therefore very important to monitor ferritin levels in patients regularly during eculizumab therapy. If patients become iron overloaded then iron chelation therapy may become necessary.

The risk of meningococcal infection is always present although the current vaccinations are protective against most of the meningococcal strains. It is important that the patients maintain up-to-date vaccinations—for example, vaccinations will need to be repeated every few years according to the manufacturers guidelines. It is also important to continue to educate patients and where appropriate their family as to the risks of meningococcal disease and ensure that as soon as any signs suggestive of infection occur that they immediately present for medical evaluation.

Patient Selection for Eculizumab
Eculizumab is very effective at controlling intravascular hemolysis with a marked improvement in quality of life, fatigue, transfusion requirements and a reduction in the risk of thrombosis. The preliminary data indicate that other sequelae of intravascular hemolysis, such as the development of chronic renal failure, are improved. However, treatment with eculizumab requires a detailed knowledge of the potential consequences of therapy and experience in their management. Patients are likely to have to receive infusions every 2 weeks for the remainder of their lives or until their PNH spontaneously remits, which occurs in a minority of patients (12 out of 80 patients in one series reported before eculizumab27). If patients stop eculizumab they will immediately revert to hemolytic PNH with all the symptomatology and risks of complications. The frequency of infusions inevitably has an impact on the patients’ lives and is a clear sign to the patient that they have a chronic disorder. This, of course, must be balanced against the often extremely disruptive effect of PNH on a patient’s life, such as the regular transfusions and often severe as well as
unpredictable symptoms. In addition, there is the small but definite risk of meningococcal infections, which must be carefully considered as well the cost of eculizumab, which is important in some healthcare systems. Therefore the selection of patients for treatment with eculizumab is extremely important. We need to identify patients who either have moderate to severe symptoms of PNH (such as significant fatigue or poor quality of life) or those who have developed, or are at significant risk of developing, complications of their PNH, such as thrombosis or renal dysfunction. These are the patients for whom eculizumab is definitely indicated.

Conclusion
The development of anti-complement therapy with eculizumab has revolutionized our approach to and, to some extent, our understanding of PNH. Eculizumab is effective in the management of the most prominent feature of PNH, namely, the intravascular hemolysis and its consequences, such as severe fatigue, thrombosis and renal failure. The challenges that are presented are how to manage patients during eculizumab therapy and the selection of patients for treatment.

Disclosures
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References

