Eculizumab for paroxysmal nocturnal haemoglobinuria

Charles Parker

The complement system plays a central part in both innate and acquired immunity, but the contribution of complement activation to pathobiology is largely ancillary. An exception to the non-dominant role of complement in disease is the haemolytic anaemia of paroxysmal nocturnal haemoglobinuria (PNH). The intravascular haemolysis that is the clinical hallmark of PNH is a consequence of deficiency of the complement inhibitory proteins decay accelerating factor (DAF, CD55) and membrane inhibitor of reactive lysis (MIRL, CD59). Eculizumab is a humanised monoclonal antibody that binds and prevents activation of complement C5 and the subsequent formation of the cytolytic membrane attack complex of complement. Eculizumab inhibits the intravascular haemolysis of PNH, reduces transfusion requirements, stabilises haemoglobin concentration, and improves quality of life. Although chronic treatment with eculizumab increases the risk of infections with *Neisseria meningitides*, the drug is generally safe and well tolerated. But as is the case with other drugs developed for treatment of ultra-orphan diseases, eculizumab is expensive, and treatment must continue indefinitely because C5 inhibition does not affect the process (ie, clonal proliferation of haemopoietic stem cells with a mutant phosphatidylinositol glycan complementation class A [*PIGA*] gene) that underlies PNH. Moreover, due to the heterogeneous nature of the disease, treatment with eculizumab is not appropriate for all patients with PNH.

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Introduction

In March and June, 2007, the US Food and Drug Administration and the EU Commission, respectively, approved the use of the eculizumab (Soliris, Alexion, Cheshire, CT, USA) for treatment of patients with paroxysmal nocturnal haemoglobinuria (PNH) to reduce haemolysis. Eculizumab is a humanised monoclonal antibody that binds and thereby prevents activation of complement C5 by amplification convertases of the complement cascade. As a consequence, eculizumab blocks both generation of the C5a anaphylatoxin and formation of the cytolytic membrane attack complex (MAC) of complement (figure 1).[1–4]

Complement and PNH

The alternative pathway of complement (APC) is a component of innate immunity.[5] This ancient system evolved to protect the host against invasion by pathogenic microorganisms. Unlike the classical pathway of complement that is part of the system of acquired immunity and requires antibody for initiation of activation, the APC is in a state of continuous activation, armed at all times to protect the host.

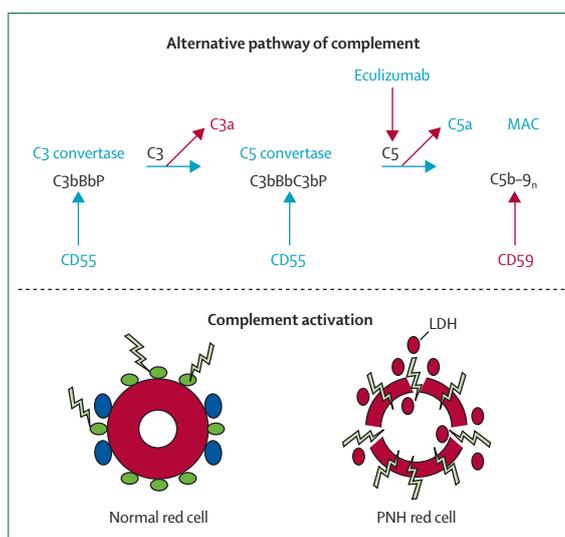The APC cascade can be divided into two functional parts. The first consists of the C3 and C5 convertases

(figure 1). These are enzymatic complexes that initiate and amplify the activity of the APC, by cleaving several substrate molecules, and ultimately generate MAC, the cytolytic subunit of the complement system. C3 convertase consists of activated C3 (C3b), activated factor B (Bb, enzymatic subunit of the complex), and factor P (a protein that stabilises the complex, previously called properdin). C5 convertase has the same components as C3 convertase except that two activated C3 molecules are needed to bind and position C5 for cleavage by activated factor B. C3a and C5a are bioactive peptides that are generated by cleavage of C3 and C5,

Figure 1: **Complement-mediated lysis in paroxysmal nocturnal haemoglobinuria (PNH)**
Red circles are haemoglobin. Bb=activated factor B. C3b=activated C3. CD55=decay accelerating factor (blue circles). CD59=membrane inhibitor of reactive lysis (green circles). C5b=activated C5. GPI=glycosyl phosphatidylinositol. MAC=membrane attack complex (consisting of C5b, C6, C7, C8, and several molecules of C9 [9n]. RBC=red blood cell. Modified from reference 6 with permission.

Search strategy and selection criteria

I searched Medline for all articles, regardless of date of publication, with "eculizumab" (date of last search was August, 2008). I selected relevant articles and comprehensive overviews from the reference list. I also searched the reference lists of identified articles, and selected those I judged relevant, with emphasis on papers reporting basic and clinical research and dealing directly with eculizumab rather than those focusing on the pathophysiology or natural history of paroxysmal nocturnal haemoglobinuria.
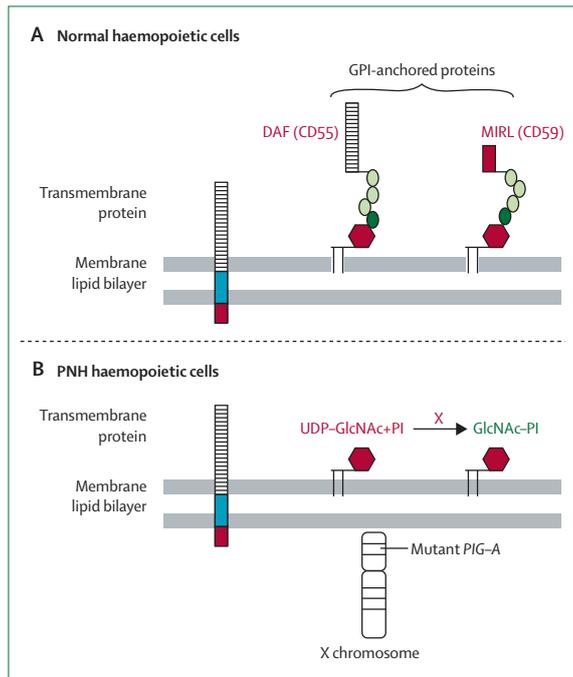
A Normal haemopoietic cells

GPI-anchored proteins

DAF (CD55)   MIRL (CD59)

Transmembrane protein

Membrane lipid bilayer

B PNH haemopoietic cells

Transmembrane protein

UDP–GlcNAc+PI   X   GlcNAc–PI

Membrane lipid bilayer

Mutant *PIG–A*

X chromosome

***Figure 2:* Molecular and genetic basis of paroxysmal nocturnal haemoglobinuria (PNH)**

(A) Normal haemopoietic cells express both transmembrane and glycosyl phosphatidylinositol (GPI)-anchored proteins (including two complement regulatory proteins, decay accelerating factor [DAF or CD55] and membrane inhibitor of reactive lysis [MIRL or CD59]). (B) PNH cells express transmembrane proteins normally, but GPI-anchored proteins are not expressed because of mutant phosphatidylinositol glycan complementation class A (*PIGA*). *PIGA* gene product is a glycosyl transferase that is essential for first step in synthesis of GPI-anchor moiety (transfer of nucleotide sugar [UDP-N-acetylglucosamine (UDP-GlcNAc)]) to phosphatidylinositol (PI). Modified from reference 6 with permission.

The molecular pathogenesis and genetic basis of PNH are known (figure 2). The disease is a consequence of clonal expansion of one or more haemopoietic stem cells with mutant phosphatidylinositol glycan complementation class A (*PIGA*) gene (located on Xp22.1; figure 2).[6] The protein product of *PIGA* is a glycosyl transferase[7] that is an obligate constituent of a complex biosynthetic pathway required for synthesis of the glycosyl phosphatidylinositol (GPI) moiety that anchors individual proteins belonging to diverse functional groups to the cell surface. As a result of mutant *PIGA*, progeny of the affected stem cells are deficient in all GPI-anchored proteins (GPI-APs). Although more than 20 GPI-APs are expressed by haemopoietic cells, the deficiency on red blood cells of the two GPI-anchored complement regulatory proteins—ie, CD55 and CD59, underlies the haemolytic anaemia of PNH.[6] Deficiency of CD55 and CD59 activates the complement cascade on the cell surface. Consequently, MACs form pores in the erythrocyte membrane, resulting in colloid osmotic lysis and release of haemoglobin and other contents of the red blood cell (including lactate dehydrogenase) into the intravascular space (figure 1).

Hypothetically, the PNH phenotype would result from inactivation of any of the more than 25 genes involved in synthesis of the GPI anchor, but somatic mutation of no gene involved in GPI-AP synthesis other than *PIGA* has been reported in patients with PNH. This finding is accounted for by the fact that, of the genes involved in the GPI-anchor synthesis pathway, only *PIGA* is located on the X-chromosome (figure 2). Therefore somatic mutation of only one allele is required for expression of the phenotype as men have one X-chromosome and, as a consequence of X-inactivation during embryogenesis, women have only one functional X-chromosome in somatic tissues. However, mutation of two alleles would be needed for inactivation of any of the autosomal genes involved in the GPI-anchor synthesis pathway.

The peripheral blood of patients with PNH is a mosaic of normal and abnormal cells. Although PNH is a clonal disease, it is not a malignant disease, and (for reasons that are unclear) the extent to which the *PIGA*-mutant clone expands varies widely among patients.[1,6,8] As an example, in some cases, more than 90% of the peripheral blood cells can be derived from the *PIGA*-mutant clone, whereas in others, less than 10% of the circulating cells might be GPI-AP deficient. This unique feature (variability in extent of mosaicism) is clinically relevant because patients with a small number of PNH clonal cells have minimum or no symptoms and do not need PNH-specific treatment, whereas those with a large number of PNH clonal cells are often debilitated by the consequences of chronic complement-mediated intravascular haemolysis and respond dramatically to treatment that inhibits complement.

Another remarkable feature of PNH is phenotypic mosaicism on the basis of *PIGA* genotype that determines

respectively, by their specific activation convertases. MAC is the common cytolytic subunit of the classical and lectin pathways of complement as well as the APC; it consists of activated C5, C6, C7, C8, and several molecules of C9.

Because the APC is primed for attack at all times, elaborate mechanisms for self-recognition and for protection of the host against APC-mediated injury have evolved. Both fluid-phase and membrane-bound proteins are involved in these processes. Normal human erythrocytes are protected against APC-mediated cytolysis primarily by decay accelerating factor (DAF, CD55) and membrane inhibitor of reactive lysis (MIRL, CD59). These proteins act at different steps in the complement cascade (figure 1). CD55 regulates the formation and stability of C3 and C5 convertases by destabilising the interaction between activated factor B and activated C3, whereas CD59 blocks the formation of MAC by inhibiting the binding of C9 to the nascent C5–8 complex. Deficiency of CD55 and CD59 on the erythrocytes of individuals with PNH is the pathophysiological basis of Coombs'-negative, intravascular haemolysis that is the clinical hallmark of the disease (figure 1). But why are PNH erythrocytes deficient in the two complement regulatory proteins?

the degree of GPI-AP deficiency.[9] PNH type III cells are completely deficient in GPI-APs, type II cells are partly (about 90%) deficient, and type I cells express GPI-APs at normal density (these cells are the progeny of residual normal stem cells). Phenotype varies among patients. Some patients have only cell types I and III (the most common phenotype), some have all three types (the second most common), and others have only cell types I and II (the least common). Further, the contribution of each phenotype to the composition of the peripheral blood varies. Phenotypic mosaicism is clinically relevant because PNH type II cells are relatively resistant to spontaneous haemolysis and patients with a high percentage of type II cells have a fairly benign clinical course.

The anaemia of PNH is multifactorial because an element of bone-marrow failure is present in all patients, although the degree of marrow dysfunction is variable.[9] In some patients, PNH arises in the setting of aplastic anaemia. In this case, marrow failure is the dominant cause of anaemia. In other patients, evidence of bone-marrow dysfunction might be subtle (eg, an inappropriately low reticulocyte count) with the degree of anaemia being determined mainly by the rate of haemolysis that is, in turn, determined by size of the PNH clone.

Clinical manifestations and diagnosis of PNH

The main clinical manifestations of PNH are haemolysis, thrombosis, and bone-marrow failure.[9] Constitutional symptoms (fatigue, lethargy, malaise, asthenia) dominate the history, but nocturnal haemoglobinuria is a presenting symptom in only about 25% of patients.[10] Directed questioning often elicits a history of episodic dysphagia and odynaphagia, abdominal pain, and male impotence. PNH should be suspected in patients with non-spherocytic, Coombs'-negative intravascular haemolysis. Reticulocytosis indicates the response to haemolysis, although the reticulocyte count might be lower than expected for the low haemoglobin concentration because of underlying bone-marrow failure. Serum concentration of lactate dehydrogenase is always abnormally high in patients with clinically significant haemolysis and serves as a valuable surrogate for measuring and following up the rate of intravascular haemolysis. Venous thrombosis, often in unusual sites (hepatic [Budd-Chiari syndrome], mesenteric, dermal, or cerebral veins), can complicate PNH. Arterial thrombosis is less common. Varying degrees of leucopenia, thrombocytopenia, and reticulocytopenia indicate the extent of bone-marrow insufficiency.

Once suspected, diagnosis of PNH is straightforward because deficiency of GPI-APs on peripheral blood cells is readily demonstrated by flow cytometry.[11] Analysis of both erythrocytes and polymorphonuclear neutrophils is warranted, since clone size will be underestimated if only erythrocytes are analysed because these are rapidly destroyed by complement. Recent transfusion will also affect the estimate of the clone size if only red cells are analysed.

Eculizumab as treatment for PNH

Background

PNH is an ultra-orphan disease. Its molecular basis has been known in considerable detail since the early 1990s (figure 1; figure 2), and a review of the pathobiology would suggest, even to the casual observer, that complement inhibition would be an effective way to treat the haemolytic anaemia of PNH.[12] Why then did more than a decade separate the development of a safe, effective complement inhibitor (eculizumab) and its use as treatment for PNH? The main reason for the delay is the rarity of the disease. The prevalence of PNH is not known with certainty. Prevalence estimates are mainly anecdotal and differ considerably, largely because of the heterogeneous nature of the disease. As noted above, the peripheral blood of patients with PNH is a mosaic of normal and abnormal cells, and the extent of the mosaicism varies widely among patients. Those with small clones have few or no symptoms related to haemolysis. Therefore an argument can be made that asymptomatic patients with small clones do not have clinically significant PNH and should be excluded from prevalence estimates. Others, however, might argue that any patient with flow-cytometric evidence of a population of GPI-AP-deficient cells, regardless of clone size, has PNH and should be included. The International PNH Interest Group has addressed the issue of disease heterogeneity by dividing PNH into three categories on the basis of flow-cytometric quantification of clone size and bone-marrow analysis (table).[9] Studies of prevalence that address disease heterogeneity are needed, but, by any definition, PNH is rare.

Although there are no international criteria for classification of a disease as ultra-orphan, in the UK, the term describes conditions with a prevalence of less than

	Rate of intravascular haemolysis*	Bone marrow	Flow cytometry	Benefit from eculizumab
Classic	Florid (macroscopic haemoglobinuria is frequent or persistent)	Cellular marrow with erythroid hyperplasia and normal or near-normal morphology†	Large proportion (>50%) of GPI-AP deficient PMNs§	Yes
PNH in setting of another bone-marrow failure syndrome‡	Mild to moderate (macroscopic haemoglobinuria is intermittent or absent)	Evidence of concomitant bone-marrow failure syndrome‡	Although variable, proportion of GPI-AP-deficient PMNs§ is usually small (<30%)	Dependent on size of PNH clone
Subclinical	No clinical or biochemical evidence of intravascular haemolysis	Evidence of concomitant bone-marrow failure syndrome‡	Small (<1%) proportion of GPI-AP-deficient PMNs detected by high-resolution flow cytometry	No

GPI-AP=glycosyl phosphatidylinositol-anchored protein. PMNs=polymorphonuclear neutrophils. Classification based on recommendations of International PNH Interest Group.[9] *Based on macroscopic haemoglobinuria, serum lactate dehydrogenase concentration, and reticulocyte count. †Karyotypic abnormalities are uncommon. ‡Aplastic anaemia and refractory anaemia or myelodysplastic syndrome are most commonly associated bone-marrow failure syndromes. §Analysis of PMNs is more informative than analysis of red cells due to selective destruction of GPI-AP-deficient red cells.

Table: **Classification of paroxysmal nocturnal haemoglobinuria (PNH)**

one case per 50 000 population.[13] The prevalence of clinically significant PNH (ie, classic disease plus some patients with fairly large clones that arise in the setting of another bone-marrow failure syndrome; table) is probably about less than one case per 200 000 population. Because of the rarity, PNH was not an attractive therapeutic target for companies, and, notably, eculizumab was not developed specifically as an inhibitor of the complement-mediated haemolysis of PNH (panel).

Development of eculizumab

In the early 1990s, investigators interested in developing complement inhibitors for therapeutic purposes chose C5 as a target because of its central position within the cascade (figure 1).[15] Inhibition of C5 not only prevents formation of MAC but also inhibits generation of C5a, the potent proinflammatory peptide that is released when C5 is cleaved by a C5 convertase. Another hypothetical advantage of targeting C5 is that immunoprotective (eg, C3b-mediated opsonisation) and immunomodulatory (eg, immune-complex solubilisation and clearance) processes that are effected by upstream constituents of the complement system remain unperturbed.

Murine monoclonal antibodies against human C5 were generated with hybridoma technology and tested for the capacity to block both complement-mediated haemolysis and C5a generation.[4,16] An IgG antibody m5G1.1 with these properties that was effective at a 0·5:1 molar ratio of IgG:C5 was chosen for further development. To reduce the potential immunogenicity of the murine immuno-globulin, m5G1.1 was humanised by cloning the variable region into human heavy-chain and light-chain IgG germline frameworks.[16] On binding to their target antigen, human IgG antibodies have the potential both to activate the classical pathway of complement and to bind to Fcγ receptors on immune effector cells. To remove these potential proinflammatory properties, humanised m5G1.1 was further modified by replacement of the heavy chain constant region of the parental antibody with components of both human IgG2 and IgG4 constant regions (figure 3).[16] The human IgG2 antibody isotype does not bind to Fcγ receptors, and the human IgG4 isotype does not activate the classical pathway of complement.

The binding affinity at equilibrium (Kd=120 pmol/L) of the humanised anti-C5 antibody (now called eculizumab) for human C5 was similar to that of the parental murine antibody m5G1.1, and the antibody retained the capacity both to block C5a generation and inhibit complement-mediated haemolysis by preventing MAC formation.[16]

Pharmacokinetics and pharmacodynamics

In a one-compartmental model, the half-life of eculizumab after intravenous infusion was 272 h (SD 82) with drug distribution being confined mainly to the vascular space.[4] Tissue accumulation was minimum and serum concentration reached a steady state after about 150 days.[4] Pharmacodynamic activity was concentration-dependent with a plasma concentration of 35 μg/mL or greater resulting in complete inhibition of in-vivo haemolytic activity.[17]

Preclinical and early clinical development

Because eculizumab is species-specific, a surrogate mouse anti-C5 (BB5.1) was used to study the effects of inhibition of MAC formation in murine models of both collagen-induced arthritis[18] and lupus-like autoimmune disease in NZB/WF1 mice.[19] The results provided safety and efficacy data that supported the feasibility of using an antibody therapeutically for sustained blockade of C5 activation and MAC formation.

Initial clinical development

As noted above, eculizumab was not developed to treat the haemolysis of PNH because initial studies in human beings were done on patients with systemic lupus erythematosis,[20] rheumatoid arthritis, and membranous nephritis.[4] These phase I investigations provided safety and dose-finding information. Doses ranging from 0·1 mg/kg to 8 mg/kg were safe and well tolerated, and the 8 mg/kg dose completely inhibited MAC formation for 7–14 days.[15,20]

Clinical trials

Phase II pilot study

No clinically informative animal models of PNH exist. The initial clinical study of eculizumab in the treatment of PNH began in May, 2002, and enrolled six men and five women at two sites in the UK.[21] Adult patients with a history of PNH for at least 6 months who had received at least four red-cell transfusions in the preceding 12 months were eligible. Before starting treatment, patients had to have a negative throat culture for *Neisseria meningitides* and *N gonorrhoeae*, and all patients were vaccinated against *N meningitides* with Mengivac (A+C) (Aventis Pasteur, Lyons, France).[22–24] Patients were infused weekly for 4 weeks with eculizumab 600 mg. The dose was increased to 900 mg at week 5, and, subsequently, patients were infused with 900 mg every other week until week 12.

Pharmacokinetic and pharmacodynamic analyses showed that ten of 11 patients maintained a serum concentration of eculizumab (>35 μg/mL) sufficient to sustain near complete inhibition of MAC formation (measured with a haemolytic assay) through the 12 weeks of observation. In one patient, near the end of the treatment, the serum eculizumab concentration fell below the effective threshold, and concomitantly, serum haemolytic activity became detectable.

All patients completed the study with no thromboembolic complications (six of the patients were on warfarin). Each patient reported one or more adverse events, with headache and upper respiratory-tract infection reported by three patients, and influenza-like symptoms, rigors, dizziness, nausea, nasal congestion, and joint aches reported by two patients; however, none of the symptoms was attributed to treatment with eculizumab. Two patients had serious adverse events (a viral chest infection requiring hospitalisation, and nausea, vomiting, and headache after the first infusion, with dizziness and shivering the following day requiring overnight hospitalisation).

During the year before enrolment, the range of red-cell transfusions received by the 11 patients ranged from 12 units to 55 units, whereas during the 3 months of the study, the range was 0–8 units. Before eculizumab treatment, the median transfusion rate was 1·8 units per month, and during the 12 weeks of treatment, the median was 0 units (p=0·003). Patients remained anaemic (mean haemoglobin concentration at the end of the 12 week study was 100·3 g/L) and the reticulocyte count after treatment with eculizumab (191·2×10³ per L) was similar to that (161·4×10³ per L) during the 12 months before enrolment. As shown by a reduction in serum concentration of lactate dehydrogenase, eculizumab inhibited the intravascular haemolysis of PNH. In the 12 months before treatment with eculizumab, mean serum lactate dehydrogenase concentration for the study patients was 3111 IU/L (SD 598; normal range 150–480 IU/L) and fell to 594 IU/L (32) during treatment. Serum lactate dehydrogenase concentration fell after administration of the first dose of eculizumab and stayed low in
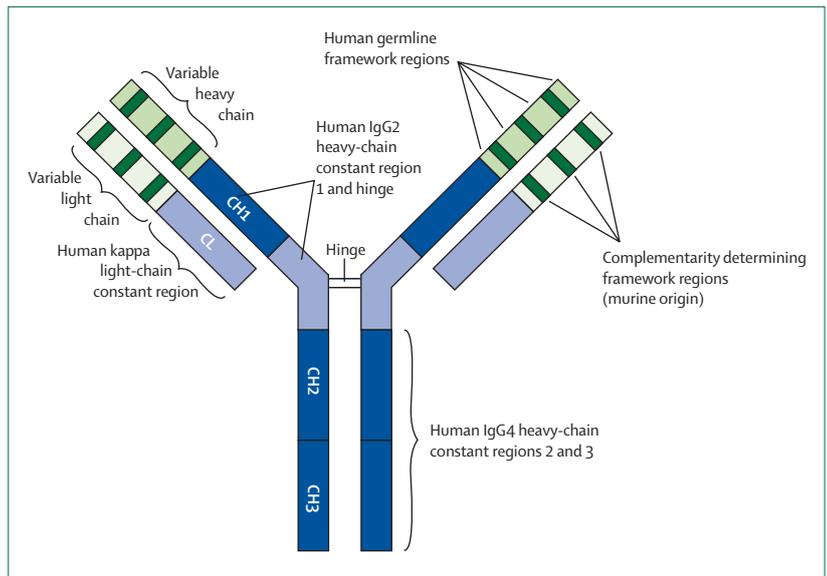

Figure 3: Schematic representation of eculizumab
Reproduced from reference 4 with permission.

all but one patient whose serum concentration of eculizumab fell to a subtherapeutic concentration during week 12 of treatment. This patient required a change in dose frequency from 900 mg every 14 days to 900 mg every 12 days. With this modification in dose, the serum concentration of eculizumab remained therapeutic and haemolysis resolved. The percentage of complement-sensitive PNH cells (called PNH type III cells) increased from a mean of 36·7% (5·9) before treatment to 59·2% (8·0) at the end of the study (p=0·005). Episodes of macroscopic haemoglobinuria were reduced during treatment from 2·9 days per patient per month during the 2–4 week screening period before starting treatment, to 0·12 days per patient per month during the 12 weeks of treatment (p<0·001).

Assessment of quality of life with QLQ-30 showed substantial improvement during treatment compared with the pretreatment baseline in global health, physical functioning, emotional functioning, cognitive functioning, fatigue, dyspnoea, and insomnia.

All 11 patients in the initial phase II pilot study enrolled in a 52 week extension study with the same maintenance dose schedule (900 mg of eculizumab every 14 days).[17] For two patients whose serum concentration of eculizumab fell below the therapeutic threshold (35 μg/mL) during days 13 and 14 of treatment and who concordantly developed signs and symptoms of haemolysis, the interval between infusions was reduced to 12 days. This modification in the schedule resulted in sustained concentrations of eculizumab that were sufficient to block complement activity and prevent haemolysis. In the 52 week extension period, the efficacy of eculizumab, as measured by reduction in intravascular haemolysis (on the basis of serum lactate dehydrogenase and days

with macroscopic haemoglobinuria), reduction in red-cell transfusion requirement, and improvement in quality of life, were sustained. No patient had a thrombotic episode (six of 11 patients were on chronic warfarin before and during the study). Only one serious adverse event was reported (transient neutropenia) and was judged by the principal investigator to be unrelated to the study medication.

TRIUMPH phase III randomised study

Transfusion Reduction Efficacy and Safety Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria (TRIUMPH)[25] was a randomised, double-blind, placebo-controlled multinational study of eculizumab for the treatment of PNH. The trial started in October, 2004, and ended in June, 2005. Because there was no standard treatment for PNH, the effects of eculizumab were compared with placebo. Patients with PNH aged 18 years or older and needing four or more transfusions during the preceding 12 months were eligible. Other inclusion criteria were a PNH type III erythrocyte proportion of 10% or more, platelet count of at least 100 000 per μL and serum lactate dehydrogenase concentration of at least 1·5 times the upper limit of normal. The trial consisted of a 2 week screening period, an observation period of up to 3 months and a 26 week treatment period. Patients who did not need a red-cell transfusion during the 3 month observation were not eligible for randomisation. The two primary endpoints of the study were haemoglobin stabilisation and the number of units of packed red cells transfused. Biochemical indicators of intravascular haemolysis and quality of life were also assessed.

87 patients (35 men and 52 women) underwent randomisation (44 in placebo group and 43 in eculizumab group). The treatment schedule was the same as that in the phase II pilot study. Haemoglobin stabilisation in the absence of transfusion was noted in 21 (49%) of 43 patients in the treatment group compared with none in the placebo group (p<0·001). The median number of units of packed red cells administered to patients assigned to eculizumab treatment was 0 compared with 10 for the controls (p<0·001). Eculizumab inhibited intravascular haemolysis as shown by a reduction in serum lactate dehydrogenase concentration. Patients assigned to eculizumab had an 85·8% lower median area under the curve for this enzyme plotted against time compared with the placebo group (p<0·001). Of 41 patients in the treatment group who completed the study, 15 had a normal serum lactate dehydrogenase, whereas all patients in the placebo group had a concentration of at least five-fold greater than the upper limit of normal at the end of the study. Quality-of-life analysis showed significant improvements (most notably in symptoms of fatigue) for the group assigned to eculizumab compared with the placebo group. Serious adverse effects were reported for four patients in the eculizumab group and nine in the placebo group, none were considered to be treatment

related. No thrombotic episodes were seen in the eculizumab group (21 of 43 patients in this group were on anticoagulant drugs), whereas one thrombotic episode was reported in the placebo group (11 of 44 in this group were on anticoagulant drugs).

SHEPHERD non-randomised safety and efficacy study

The eligibility criteria for Safety in Hemolytic PNH Patients Treated with Eculizumab: A Multi-centre Open-label Research Design Study (SHEPHERD),[26] a multi-national, single-arm, safety and efficacy study,—differed from those of TRIUMPH.[25] The criteria included a platelet count of 30 000 per μL or greater (vs 100 000 per μL or more for TRIUMPH) and a need to have had at least one red-cell transfusion within the 2 years before enrolment (vs at least four transfusions within the preceding year for TRIUMPH). Although entry criteria for SHEPHERD were different from those of TRIUMPH, no detailed statistical comparison between the demographic and baseline characteristics of the populations of the two studies was presented. Therefore, the extent of the difference could not be discerned, but data in a subsequent report showed considerable overlap in the two populations, including platelet count (162 000 per μL for TRIUMPH vs 136 000 per μL for SHEPHERD) and size of PNH granulocyte clone (95% for TRIUMPH vs 96% for SHEPHERD).[14] The eculizumab schedule in SHEPHERD was identical to that in TRIUMPH with the duration of treatment being 52 weeks (vs 26 weeks for TRIUMPH).

Of 97 patients who were enrolled, 96 completed the study. Efficacy outcomes for SHEPHERD were similar to those reported in both the phase II pilot study and TRIUMPH with transfusion-independent stabilisation of haemoglobin concentration, reduction in red-cell trans-fusion requirement, reduction in intravascular haemo-lysis (on the basis of a reduction in serum lactate dehydrogenase concentration), and improvement in quality of life, particularly fatigue, seen in all studies.

The most common treatment-emergent adverse events were headache, nasopharyngitis, and upper respiratory-tract infections. Most (96%) of the adverse events were mild to moderate, and most (76%) were judged by the investigators to be unrelated to treatment with eculizumab. Fewer headaches were reported during the second 26 weeks of the study, and for patients who had headache, symptoms were usually noted within the first 48 h of drug infusion and resolved after the first 2 weeks of the induction phase. Two patients with a history of thrombosis had a thrombotic event during the study. Of 44 serious adverse events reported, seven were considered possibly related to eculizumab (pyrexia [n=2], headache [n=1], abdominal distension [n=1], viral infection [n=1], anxiety [n=1], and renal impairment [n=1]). Infections were common (89 of 97 patients reported at least one infection during the 52 week treatment), but 99% of infections were reported

as mild to moderate with 91% judged as being unrelated to eculizumab. Seven infection-related adverse events in six patients were judged to be serious (three episodes of pyrexia and one each of cholangitis, endometritis, pylonephritis, and viral infection).

Low-titre, non-inhibitory anti-eculizumab antibodies were found in two patients. Complete inhibition of serum complement haemolytic activity was seen in 89 of 97 study patients with eight showing a return of complement haemolytic activity during the last 1 or 2 days of the 14 day maintenance-dosing interval. In six patients, sustained complement blockade was accomplished by reduction of the dosing interval from 14 days to 12 days.

All patients who participated in the three clinical trials described above were eligible for the extension study in which patients continued to receive eculizumab at 900 mg every 14 days (±2 days). Of 195 eligible patients, 187 enrolled in the study. The study lasted 102 weeks, with the database being locked in November, 2006.[14] Continued inhibition of complement-mediated intravascular haemolysis as shown by a large reduction in serum lactate dehydrogenase (figure 4) was recorded throughout the observation period.[14] No evidence of tolerance or development of neutralising antibodies has been reported.[4] Three patients who had been vaccinated against *N meningitides* developed meningococcal sepsis (personal observation);[4] all three were treated and recovered. Two of three patients have remained on chronic treatment with eculizumab. The overall incidence of infection was not increased versus that in placebo-treated patients from TRIUMPH.[4]

During treatment, the percentage of PNH erythrocytes in peripheral blood increases[21] because eculizumab enhances survival of the abnormal cells by protecting them against complement-mediated lysis. The fact that treatment with eculizumab increases the proportion of these cells initially raised concerns that discontinuation of the drug might result in a haemolytic catastrophe. However, 16 patients have discontinued treatment with eculizumab without having exacerbation of haemolysis,[4] probably due to some protection of PNH erythrocytes by factor H, a plasma protein that regulates the APC by accelerating decay of the C3 and C5 convertases in a manner that is similar to the action of CD55[27]

Thromboembolic events are the major cause of morbidity and mortality in PNH.[9,28-33] To test whether eculizumab affects the incidence of thromboembolism, patients enrolled in the extension study were assessed with the major adverse vascular event (MAVE) criteria. Events classified as MAVE include thrombophlebitis or deep-vein thrombosis, pulmonary embolus, cerebrovascular accident, amputation, myocardial infarction, transient ischaemic attack, unstable angina, and renal-vein, mesenteric-vein, or portal-vein thrombosis, gangrene, acute peripheral vascular occlusion, sudden death, and a category for other events. The principal investigators were responsible for the description, location, method of diagnosis, date of
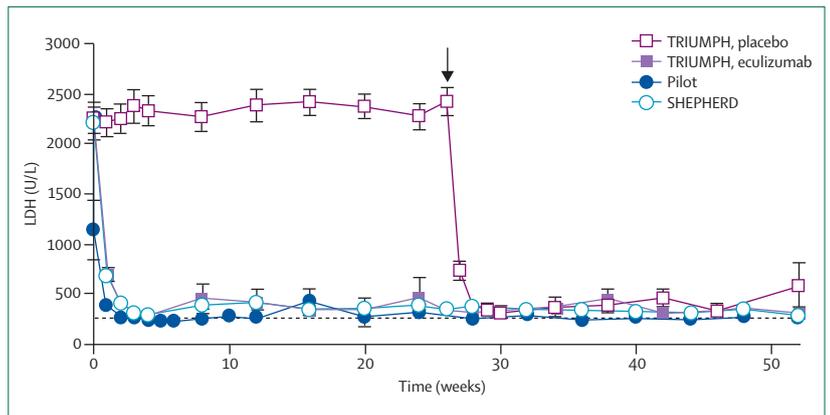

Figure 4: Serum concentration of lactate dehydrogenase (LDH) during treatment with eculizumab
Dashed line is the upper limit of normal (range 103–223 U/L). Values from pilot study were normalised to those of TRIUMPH and SHEPHERD studies and include data from 1 year extension study.[14] Arrow shows transition of placebo-treated patients in TRIUMPH to eculizumab. In eculizumab-treated patients, mean concentration of LDH fell to just above upper limit of normal, and effects were sustained throughout entire 1 year observation.[14] Error bars represent SD. Reproduced from reference 14 with permission.

diagnosis, and date of resolution for each MAVE. Events that antedated treatment with eculizumab were identified retrospectively from the period starting from the earlier of either the date of diagnosis of PNH or the date of the first thrombotic event to the time of the first eculizumab treatment. With this method of comparing retrospective data with observational data, a substantial reduction in thromboembolic events was noted in patients treated with eculizumab. For example, the rate of thromboembolism was 7·37 events per 100 patient years before eculizumab treatment compared with 1·07 events per 100 patient years during treatment (p<0·001), and thromboembolic events were reduced from 39 before treatment to three during eculizumab treatment (p<0·001). Event rate was also reduced for patients on antithrombotic drugs (10·61 events per 100 patient years before eculizumab to 0·62 events per 100 patient years with eculizumab).[14]

These results suggest that eculizumab ameliorates the thrombophilia of PNH, but the study design makes assessment of the effect of treatment nebulous. The major concerns are the use of MAVE criteria that did not require uniform documentation to characterise the thromboembolic event and the use of retrospective data to estimate the rate of thrombosis before starting treatment. In the only part of the study that was randomised and included a placebo control (TRIUMPH), one thromboembolic event occurred in the placebo group (11 of 44 patients were on anticoagulant drugs) and no thromboembolic events occurred in the eculizumab-treated group (21 of 43 patients were anticoagulated). A large difference in the pretreatment thromboembolic rate was also seen among the treatment groups. For example, in the placebo group of TRIUMPH, the thromboembolic event rate was 2·34 per 100 patient years versus 12·67 per 100 patient years for SHEPHERD. This difference does not seem to be due to differences in baseline characteristics of the patients because the PNH

clone size on the basis of the percentage of GPI-AP deficient granulocytes was equivalent.[14] A high rate of pretreatment thromboembolic events (10·31 per 100 patient years) was reported in patients treated with antithrombotic drugs, whereas complete protection against thromboembolism in patients with PNH treated with warfarin (although that study also relied on retrospective data) was reported in a previous study.[28] The effects of eculizumab on the thrombophilia of PNH need to be measured in a dedicated randomised study. Such a study is unlikely, however, because a placebo control would be difficult to justify in view of the efficacy of eculizumab in controlling intravascular haemolysis and improving quality of life in patients with PNH.

Cost

Eculizumab is expensive (nearly $400 000 per year in the USA and about £250 000 per year in the UK), and it has no effect on the underlying stem-cell abnormality, which means that indefinite treatment will be needed. But the cost of the drug must be weighed against expenditures associated with supportive care (eg, transfusions for symptomatic anaemia, management of acute renal failure secondary to haemoglobinuria, and hospitalisation to control abdominal pain and treat dehydration in the event of haemolytic exacerbation), and the cost of eculizumab is within the range of treatments for other ultra-orphan diseases, such as enzyme-replacement therapy for lysosomal storage diseases (estimated at £150 000–180 000).[34] Support both for development and for reimbursement of drugs to treat orphan and ultra-orphan diseases are actively debated.[13,34]

Other treatment options

Other than eculizumab, no specific treatment for PNH exists, and for patients not being treated with eculizumab, management is largely supportive. Although haemolysis is ameliorated in some patients by treatment with glucocorticoids or androgens, the use of steroids in the management of patients with PNH is controversial.[9] PNH can be cured by allogeneic bone-marrow transplantation, but not all patients have suitable donors, and the potential benefits must be weighed against the morbidity and mortality associated with the procedure. Several unique features (eg, size of PNH clone, red-cell phenotype, degree of bone-marrow failure, and underlying bone-marrow disease) must be taken into account in managing patients with PNH.[9]

Conclusions

Eculizumab was not developed to treat the complement-mediated haemolysis of PNH. Is there a more rational approach to drug development for PNH? Conceivably, an inhibitor of complement C9 would effectively block formation of MAC and thereby control the haemolysis of PNH[35] while leaving intact the downstream functions of complement, including the capacity to generate the proinflammatory C5a peptide. Additionally, inhibition of C9 rather than C5 might reduce the risk of *Neisseria* spp infections[36] But eculizumab has proven to be effective, well tolerated, and fairly safe, and evidence of an improvement on the therapeutic index of eculizumab by another inhibitor would require human studies because good animal models of PNH do not exist. In view of the rarity of PNH and the established role of eculizumab, enthusiasm for a phase III clinical trial would probably be limited unless a new compound offered the possibility of a major advantage over eculizumab (eg, oral administration, substantially lower cost). The ultimate goal of elimination of the PNH clone and restoration of normal bone-marrow function will involve development of a totally different therapeutic approach. Although bone-marrow transplantation can accomplish both these goals, such treatment is restrained by availability of donors and treatment-related morbidity and mortality.

Eculizumab has the potential to ameliorate symptoms associated with other diseases in which C5 activation, MAC formation, or both underlie or contribute greatly to pathophysiology. Except for paroxysmal cold haemoglobinuria (the Donath-Landsteiner antibody is a potent complement activator) there are no obvious clinical situations in which aberrant complement regulation has the kind of dominant role that it does in PNH. But complement activation might contribute in an ancillary role to the pathophysiology of several other diseases.[4,37–39] In view of the safety and efficacy data generated as a result of the careful investigation of treatment of PNH, clinical studies of eculizumab efficacy in human diseases in which aberrant complement regulation might contribute to the pathophysiology could be done with impunity.

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