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Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria

Russell P Rother[1], Scott A Rollins[1], Christopher F Mojcik[1], Robert A Brodsky[2] & Leonard Bell[1]

The complement system provides critical immunoprotective and immunoregulatory functions but uncontrolled complement activation can lead to severe pathology. In the rare hemolytic disease paroxysmal nocturnal hemoglobinuria (PNH), somatic mutations result in a deficiency of glycosylphosphatidylinositol-linked surface proteins, including the terminal complement inhibitor CD59, on hematopoietic stem cells. In a dysfunctional bone marrow background, these mutated progenitor blood cells expand and populate the periphery. Deficiency of CD59 on PNH red blood cells results in chronic complement-mediated intravascular hemolysis, a process central to the morbidity and mortality of PNH. A recently developed, humanized monoclonal antibody directed against complement component C5, eculizumab (Soliris; Alexion Pharmaceuticals Inc., Cheshire, CT, USA), blocks the proinflammatory and cytolytic effects of terminal complement activation. The recent approval of eculizumab as a first-in-class complement inhibitor for the treatment of PNH validates the concept of complement inhibition as an effective therapy and provides rationale for investigation of other indications in which complement plays a role.

The complement system: a potential target for therapeutics

The complement system consists of more than 20 serum proteins that interact in a precise series of enzymatic cleavage and membrane binding events leading to the generation of products with immunoprotective, immunoregulatory, proinflammatory and cytolytic properties[1,2].

Proximal complement. The cascade proceeds through three distinct pathways—alternative, classical and lectin—which all result in the generation of C3 convertase complexes that mediate the cleavage of C3 to C3a and C3b[1,3] (**Fig. 1**). Recent studies have delineated the structure of C3 and C3 fragments and provided important insights into their function[4–7]. The classical pathway is generally initiated by the interaction of C1q with antigen-antibody complexes. The lectin pathway is activated by the interaction of mannose-binding lectin (MBL) with specific mannose-containing carbohydrate structures. The alternative pathway is initiated by deposition of preformed C3b on a variety of substrates such as bacteria and cell membranes, including erythrocytes. This C3b is continuously available due to the interaction of C3 with water, a process that represents an important part of the innate immune system called complement 'tick-over'. In addition to its role in the initiation of alternative pathway activation, C3b is also necessary for the amplification and progression of the complement cascade through all pathways of activation and serves as a key immunoprotective and immunoregulatory molecule.

The importance of proximal complement components in immunity is illustrated by the clinical effects observed in people with genetic complement deficiencies. For example, individuals with C3 deficiency are at particular risk of infection from polysaccharide-coated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*, and frequently die at a young age from overwhelming sepsis[8]. The absence of C3 is generally thought to prevent the progression of complement through all pathways of activation, thereby reducing the body's ability to opsonize pathogens and mount an inflammatory response, although some studies suggest that C5 cleavage can occur in the absence of C3 by thrombin[3,9]. Individuals deficient in C1, C2 or C4 develop severe autoimmune symptoms such as a lupus-like syndrome and are also susceptible to recurrent overwhelming sepsis. Thus, deficiencies in early components of the complement cascade lead to impairment in the ability to generate C3b, which is critical for immune complex clearance and is the primary opsonin for many pathogenic microorganisms.

Terminal complement. All pathways of complement activation converge at the cleavage of C5 into C5a and C5b by the C5 convertase enzyme complexes. This cleavage event initiates the terminal complement cascade (**Fig. 1**). C5a is a potent anaphylatoxin that mediates leukocyte chemotaxis, increases vascular permeability, alters smooth muscle tone and induces secondary inflammatory mediators such as hydrolytic enzymes, reactive oxygen species, arachidonic acid metabolites and cytokines[10]. C5a receptors are present on a variety of cell types that directly contribute to inflammation, including monocytes, macrophages and neutrophils. The C5a receptor CD88 is a member of the N-formyl peptide receptor family, which transduces signals through a G protein–dependent pathway resulting in biochemical proinflammatory

[1]Alexion Pharmaceuticals, Inc., 352 Knotter Drive, Cheshire, Connecticut 06410, USA. [2]Johns Hopkins University School of Medicine, Division of Hematology, 720 Rutland Avenue, Ross Building Room 1025, Baltimore, Maryland 21205, USA. Correspondence should be addressed to R.P.R. (rotherr@alxn.com).

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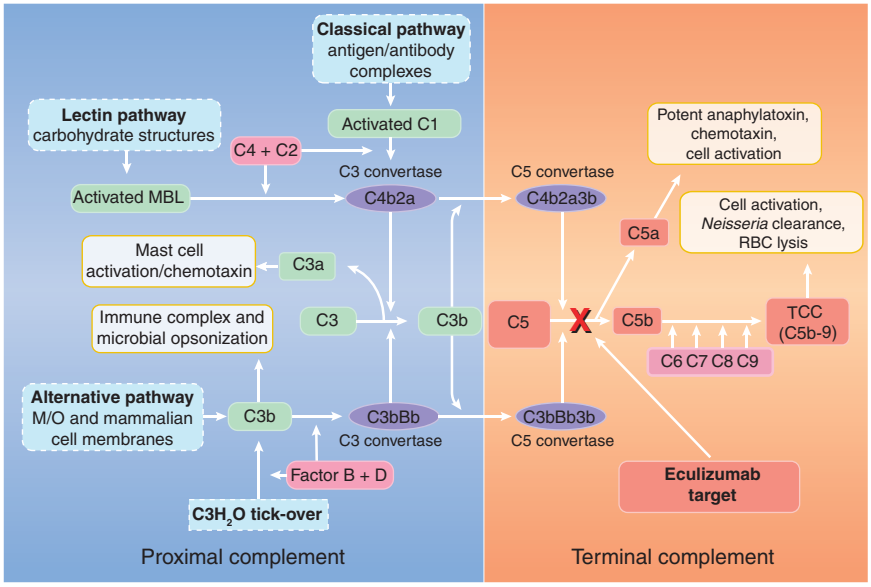

Figure 1 Targeted blockade of complement protein C5. The complement cascade can be activated via the classical, lectin and alternative pathways. The proximal components of complement (proteins upstream of C5) are essential for microbial opsonization and immune complex clearance. All pathways of complement activation converge at the cleavage of the terminal complement protein C5 leading to the generation of molecules with proinflammatory and cell lytic properties. Targeted blockade at C5 with eculizumab therefore prevents the deleterious properties of terminal complement activation while preserving the immunoprotective and immunoregulatory functions of proximal complement. M/O, microorganisms. TCC, terminal complement complex.

responses[11]. A second C5a receptor has been identified (C5L2) that also facilitates C5a-mediated signaling in a variety of cell types[12]. C5b recruits the terminal complement components C6, C7, C8 and C9 to form C5b-9 or the terminal complement complex (TCC) on the surface of cells. The generation of the TCC at sublytic concentrations stimulates the release of many of the same proinflammatory molecules described for C5a. Most importantly, unimpeded assembly of the TCC on the cell surface results in cell lysis. Deposition of TCC on erythrocytes results in the destruction of these cells in hemolytic diseases such as PNH[13,14].

The importance of terminal complement components in immunity is demonstrated by individuals with genetic deficiencies of C5, C6, C7, C8 or C9, who show an increased incidence of infections with *N. meningitidis*, a bacterium that can cause meningitis[8]. Vaccines against *N. meningitidis* are recommended for terminal complement–deficient individuals, although breakthrough infections have been reported[15]. Interestingly, *N. meningitidis* infections in terminal complement–deficient people are typically less severe than in normal individuals, possibly due to the lack of TCC-mediated endotoxin release from the bacteria[15].

Targeted blockade of the complement cascade at C5. In the rational design of a therapeutic complement inhibitor, C5 is an attractive target[1]. Because C5 is common to all pathways of complement activation, blockade at this point stops the progression of the cascade regardless of the stimuli. In addition, prevention of C5 cleavage effectively blocks the generation of the potent proinflammatory molecule C5a and the cell lytic TCC. Importantly, C5 blockade preserves the critical immunoprotective and immunoregulatory functions of upstream components that culminate in C3b-mediated opsonization and immune complex clearance.

Development of eculizumab

To generate potent inhibitors of complement component C5, panels of murine anti-human C5 monoclonal antibodies were generated and initially screened for the ability to inhibit TCC-mediated lysis of antibody-sensitized chicken erythrocytes by human complement in a standard hemolytic assay[16]. The most potent candidates were cloned and purified, and functional activity was further characterized to identify anti-C5 antibodies that could also effectively block the generation of C5a. Of ~30,000 hybridomas screened, one monoclonal antibody (mAb), designated murine 5G1.1 (m5G1.1 mAb), was identified that effectively blocked both TCC-mediated hemolysis and the generation of C5a, at a 0.5:1 molar ratio of antibody to C5. Murine 5G1.1 mAb binding to human C5 mapped to the N-terminal region of the alpha chain.

Engineering. To reduce the potential for immunogenicity, we cloned and grafted the complementarity-determining regions of m5G1.1 mAb into human heavy and light chain antibody frameworks (**Fig. 2**)[16,17]. The human heavy-chain framework was derived from the H20C3H antibody variable region[18], which contained no amino acid changes relative to the original germline sequence. The human light-chain framework was derived from the I.23 antibody variable region[19], which contained only one amino acid change from the original germline sequence; the germline amino acid residue was restored at this position. The use of purely germline framework acceptor sequences should further minimize potential immunogenicity of the humanized antibody, because the immune system of every individual should be tolerant to these sequences.

The use of antibodies as therapeutics in some clinical settings is complicated by effector functions of such antibodies that serve to activate complement and/or to bind to antibody receptors (Fc receptors) on inflammatory cells after they have engaged their target. To reduce the potential for eculizumab to elicit proinflammatory responses, we replaced the heavy-chain constant region of the parental antibody with components of both human IgG2 and IgG4 constant regions (**Fig. 2**)[20]. The human IgG2 antibody isotype does not bind Fc receptors[21,22], whereas the human IgG4 isotype does not activate the complement cascade[23,24]. The IgG2/IgG4 hybrid constant region of eculizumab includes the CH1 and hinge regions of human IgG2 fused to the CH2 and CH3 regions of human IgG4, and lacks the ability to bind Fc receptor and to activate complement[20]. To avoid the generation of an antigenic site during the fusion, we used a restriction endonuclease cleavage site common to both IgG2 and IgG4 to join the two constant regions. Indeed, a total of 31 amino acids flanking the fusion site are identical between IgG2 and IgG4.

Binding characteristics. The humanized monoclonal antibody eculizumab maintained its ability to block the cleavage of C5 during complement activation as the antibody potently inhibited both the generation of C5a and C5b-9–mediated human serum hemolytic activity[16]. Eculizumab is highly species-restricted, with minimal activity against any other primate or mammalian C5. No nonspecific or unexpected

binding of eculizumab to any human tissue examined in a tissue cross-reactivity study was observed. In addition, the affinity of eculizumab for human C5 (K_d = 120 pM) is similar to that of the parental m5G1.1-mAb, indicating that the binding properties of the antibody were not compromised during the humanization process.

The eculizumab pharmacokinetic profile was analyzed using a one-compartmental model. When administered by intravenous infusion, eculizumab has a half-life of 272 ± 82 h, and its distribution appears to be primarily limited to the vascular space. Eculizumab serum concentrations appear to reach steady state after ~57 days. At steady state, the eculizumab accumulation ratio (R_{acc}) was calculated to be 1.075, which is indicative of minimal accumulation over time. Pharmacodynamic activity correlates directly with eculizumab serum concentrations and maintenance of trough levels above 35 μg/ml results in essentially complete blockade of hemolytic activity *in vivo*[25].

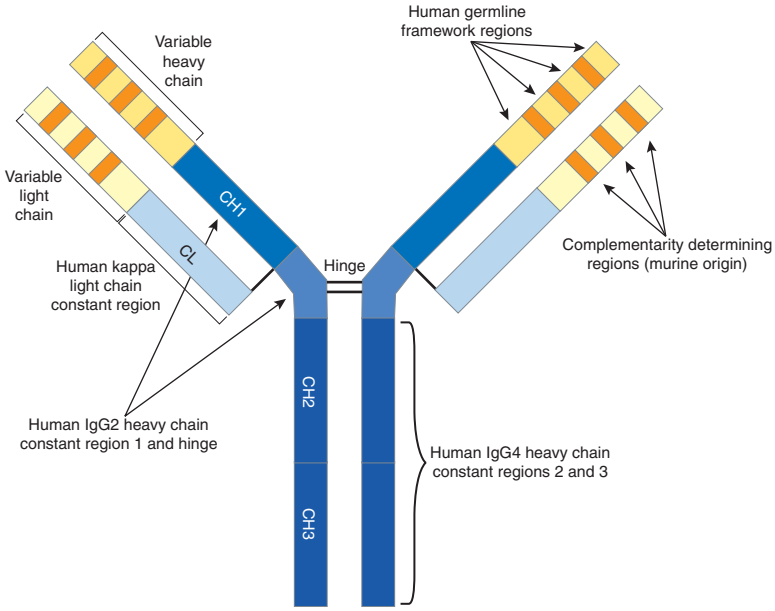

Figure 2 Engineering of eculizumab to reduce immunogenicity and eliminate effector functions. To minimize immunogenicity, we grafted murine complementarity-determining regions into human heavy and light chain germline antibody framework sequences. Additionally, human IgG2 and IgG4 heavy chain sequences were combined to form a hybrid constant region that is unable to bind Fc receptors or to activate the complement cascade. Eculizumab exhibits high affinity for human C5, effectively blocking its cleavage and downstream proinflammatory and cell lytic properties.

Preclinical development. Because of the species-restricted nature of eculizumab, pharmacological activities *in vivo* were explored with a surrogate anti-mouse C5 antibody (BB5.1) that demonstrated terminal complement–inhibitory activity comparable to that of eculizumab. As no adequate mouse models of PNH exist, *in vivo* studies targeting other therapeutic indications with potential involvement of terminal complement activation were used. In a collagen-induced model of arthritis, BB5.1 was shown to prevent the onset of disease and to improve the course of previously established disease[26]. Treatment with BB5.1 also resulted in an improvement in renal disease and in dramatic prolongation of survival in a lupus-like autoimmune model[27]. These models provided substantial evidence that a functionally blocking anti-C5 antibody could effectively and consistently block terminal complement activation leading to a reduction in terminal complement-mediated proinflammatory sequelae. The surrogate BB5.1 antibody was also used in mouse toxicity studies in which no compound-related mortalities or significant abnormalities were observed.

Early clinical development. Initial human clinical studies of eculizumab, and a single-chain antibody variant of eculizumab called pexelizumab, were performed in individuals with rheumatoid arthritis and systemic lupus erythematosus[28,29] and in individuals with coronary artery bypass graft surgery and myocardial infarction, respectively[30,31]. The initial eculizumab studies served two purposes, to determine a dosing schedule for future trials and to establish initial safety of terminal complement inhibition in humans. Participants received a single dose of eculizumab ranging from 0.1 mg/kg to 8 mg/kg; all doses were observed to be safe and well-tolerated. The 8 mg/kg dose (~600 mg total) provided complete complement blockade for 7–14 days. Studies exploring multiple chronic dose regimens with eculizumab were also performed in individuals with membranous nephritis and rheumatoid arthritis[32,33].

PNH—a disease in search of a complement inhibitor

Etiology of PNH. PNH is a rare form of hemolytic anemia with an estimated incidence of 1.3 cases per million per year and a prevalence of 15.9 cases per million[34]. The disease is manifest by an acquired genetic deficiency of endogenous complement inhibitors on the surface of blood cells; these cells are referred to as the PNH clone(s). PNH clones are deficient in proteins that are normally linked to the cell surface by a glycosylphosphatidylinositol (GPI)-anchor. The inability of cells to attach proteins through this lipid moiety is caused by somatic mutations occurring in the X-linked gene, phosphatidylinositol glycan–complementation class A (*PIGA*)[35–37], a critical molecule in the GPI-anchoring pathway.

In individuals with PNH, cells deficient in GPI-anchored proteins exist in all blood lineages including erythroid, myeloid and lymphoid cells. These PNH cells are clonal and harbor matching *PIGA* mutations indicating that the mutations occur in multipotent hematopoietic stem cells[38,39]. Granulocytes from the blood of healthy controls also possess *PIGA* mutations at an exceedingly low frequency (~1 in 50,000), suggesting that random mutations in this gene are important in the pathogenesis of PNH[40]. Thus, it has been hypothesized that the development of PNH requires two independent events to occur; hematopoietic stem cells must acquire a *PIGA* mutation and an immune attack that preferentially targets normal stem cells must be present[41,42]. This proposal presumes that the bone marrow insult targets a GPI-anchored protein on the hematopoietic stem cell, thereby providing a survival advantage to GPI-anchoring–deficient stem cells (that is, cells possessing a *PIGA* mutation). It should be noted however that *PIGA* mutations characterized in healthy controls appear to occur in more differentiated colony-forming cells rather than multipotent hematopoietic stem cells[39]. These colony-forming cells would not have self-renewal capacity and therefore would not be propagated beyond their terminally differentiated progeny.

There is clinical evidence to support a survival advantage of *PIGA* mutated hematopoietic stem cell clones over normal stem cells in damaged bone marrow. The mAb alemtuzumab (Campath) is used to treat

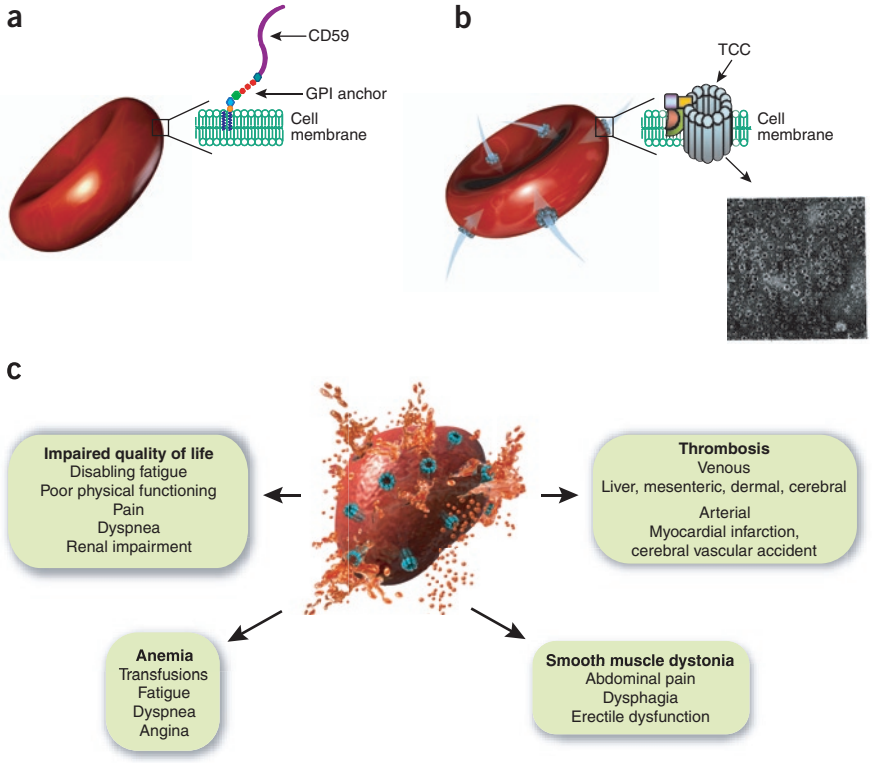

Figure 3 Terminal complement deposition on the surface of PNH RBCs results in chronic hemolysis and serious clinical morbidities. (**a**) Normal RBCs express complement regulatory proteins including the terminal complement inhibitor CD59. CD59 is tethered to the RBC lipid bilayer of the cell membrane by a GPI anchor. The presence of CD59 protects these cells from autologous complement-mediated destruction. (**b**) PNH RBCs lack GPI-anchored proteins including CD59. Continuous complement activation leads to formation of the terminal complement complex (TCC) that transverses the lipid bilayer. These complexes form numerous pores in the RBC membrane, as shown by the electron micrograph (inset). As pores form in the membrane, water enters the RBC (arrows), resulting from osmotic pressure. (**c**) As the concentration of TCCs increases, the PNH RBC swells and ultimately ruptures (hemolysis). Intravascular hemolysis causes the release of the RBC contents such as hemoglobin into the plasma. Hemolysis in PNH is chronic and leads to multiple clinical sequelae, including severe anemia requiring transfusions, disabling fatigue, dyspnea, impaired quality of life, recurrent pain associated with smooth muscle dystonia, and thrombosis. Artwork by Edmond Alexander, ©2007 Edmond Alexander, printed with permission. Micrograph image is from E.R. Podack. Molecular mechanisms of cytolysis by complement and cytolytic lymphocytes. *J. Cell. Biochem.* **30**, 133–170 (1986). Copyright © 1986 Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

chronic lymphocytic leukemia by targeting and destroying malignant T- and B-cells that express the GPI-anchored protein CD52. In many individuals, a PNH T-cell clone expands after alemtuzumab treatment. In this case alemtuzumab supplies the bone marrow insult and the GPI-anchoring deficient progenitor T-cells that lack CD52 escape immune destruction and expand[43].

Pathophysiology of PNH. Although PNH blood cells of all lineages are missing the wide array of GPI-anchored proteins from their surface, they are remarkably normal with regard to their physiological functions (e.g., fighting infection, clotting, carrying oxygen). However, the PNH red blood cell (RBC) is exquisitely sensitive to destruction because of the inability to regulate autologous terminal complement assembly (**Fig. 3**). This defect is primarily triggered by the absence of terminal complement inhibitor function[13,14,44,45]. CD59 normally blocks formation of the TCC on the RBC surface, thereby preventing intravascular hemolysis[46–48]. Hemolysis in PNH is chronic because of a continuous state of complement activation (tick-over; **Fig. 1**), but brisk episodes of hemolysis (paroxysms) coincident with increases in complement activation triggered by infections, surgery, strenuous exercise, excessive alcohol intake and blood transfusions frequently occur[49].

Chronic hemolysis is central to the morbidities in PNH and contributes to mortality in those with the disease (**Fig. 3**). It may result in severe anemia requiring transfusion, and refractory transfusion-dependent hemolytic anemia has been considered a major complication of PNH warranting consideration of bone marrow transplantation[35]. Fatigue in PNH patients is tied directly to hemolysis, strikingly disproportionate to the degree of anemia, frequently debilitating and similar to the severity observed in anemic cancer patients[25,49–52]. Hemolysis is also directly linked to diminished health status and functioning[25,49]. Other disabling morbidities evidenced by individuals with PNH that are likely a direct result of hemolysis include dysphagia, abdominal pain, erectile dysfunction, pulmonary hypertension and renal failure[49,53,54]. Thromboembolism, the most feared complication in PNH, is also directly associated with hemolysis and may be induced by the release of free hemoglobin, consumption of nitric oxide, and subsequent clotting[53,55–63].

Thrombosis is clinically evident in ~40% of individuals with PNH and is the leading cause of premature mortality[64–68]. Although venous thrombosis is more typical in PNH, arterial thrombosis, particularly in the cerebral circulation, is not uncommon[56,64]. Thrombosis is evident in individuals with small and large PNH clone sizes and varying degrees of hemolysis, although it appears to be more common in those with larger clones[56,57,66,68]. Individuals with hemolysis but without a history of transfusion also experience thrombosis, including fatal first thrombosis[57]. Subclinical thrombosis has been reported in 60% of a cohort of PNH patients, despite use of anticoagulation, and unrelated to transfusion requirements[69].

Complement inhibitors in PNH. As PNH is defined by the acquired genetic deficiency of the endogenous terminal complement inhibitor CD59 on the surface of blood cells, restoration of terminal complement inhibition should effectively reduce hemolysis in PNH and abrogate the serious clinical morbidities associated with this life-threatening disease. Initial attempts to circumvent the functional defect in PNH cells focused on replacing CD59 on the surface of PNH cells through gene therapy. A recombinant, transmembrane form of CD59 (CD59-TM) was generated and analyzed for the ability to regulate complement activity[70]. A GPI-anchoring deficient complement-sensitive B-cell line derived from an individual with PNH was virally transduced with CD59-TM, resulting in protection against classical complement-mediated membrane damage. These data established that a functional transmembrane form of CD59 can be expressed on the surface of PNH cells

through a gene therapy approach. Retroviral transduction of PNH erythroid cells with a functional *PIGA* gene has also been shown to restore GPI-anchor protein expression on the surface of these cells, thereby protecting them from complement-mediated destruction[71]. However, to provide a renewable source of complement-protected erythrocytes in individuals with PNH through a gene therapy approach, efficient targeting of early erythroid progenitor cells would be required, which has not been achieved to date. This led to the development of the complement inhibitor eculizumab, which binds to and blocks the cleavage of C5 in plasma, thereby reestablishing terminal complement regulation in the plasma of individuals with PNH. The potential effect of terminal complement blockade on hemolysis in PNH was observed in a person with coexistent C9 deficiency and PNH who had no signs of hemolysis[72]. PNH was diagnosed in this individual only after the administration of a whole blood transfusion during a routine surgery, which served to reconstitute C9 and trigger hemolysis.

Table 1 Efficacy results of the open-label phase 2 pilot study of eculizumab in individuals with PNH

	Before treatment	During eculizumab	*P*-value
Units of packed RBCs transfused/patient/month			
Median	1.8	0	0.003
Mean ± s.e.m.	2.1 ± 0.83	0.6 ± 0.67	
LDH (mean ± s.e.m.; U/L)[a]	3111 ± 598	594 ± 32	0.002
PNH RBCs (proportions)	36.7 ± 5.9	59.2 ± 8.0	0.005
Days with hemoglobinuria/patient/month	2.9	0.12	<0.001

[a]The normal range for LDH in this study was 150–480 U/L.

Eculizumab in PNH: clinical trials

Eculizumab was evaluated in PNH in three separate parent studies—a phase 2 pilot study and two phase 3 studies. All participants in these trials were allowed to continue eculizumab in follow-on extension studies.

Pilot study. The first eculizumab PNH study (beginning in May 2002) evaluated terminal complement inhibition in a 3-month open-label phase 2 pilot study in 11 participants with hemolytic PNH at two sites in the United Kingdom[73]. In this study, participants with at least four transfusions in the previous 12 months received 600-mg infusions of eculizumab every week for 4 weeks, followed 1 week later by a 900-mg dose and then by 900 mg every other week through week 12. In this study and all subsequent trials, participants were vaccinated against *N. meningitidis* at least 2 weeks before the first dose of eculizumab. Clinical and biochemical indicators of hemolysis were measured throughout the trial. Mean lactate dehydrogenase (LDH) levels decreased from 3111 U per liter before treatment to 594 U per liter during treatment ($P = 0.002$; **Table 1**). The mean percentage of PNH erythrocytes increased from 36.7% of the total erythrocyte population to 59.2% ($P = 0.005$). The mean and median transfusion rates decreased from 2.1 and 1.8 units per individual per month to 0.6 and 0.0 units per individual per month, respectively ($P = 0.003$, for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96% ($P < 0.001$), and measurements of the quality of life substantially improved. From this study, it appeared that eculizumab was safe and well-tolerated in individuals with PNH. This terminal complement inhibitor reduced hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in quality of life.

After completion of the initial 12-week study, all 11 participants from the initial PNH study chose to continue treatment in a 52-week extension study; all participants completed the study[25]. The dramatic reductions in hemolysis and the need for transfusion that were observed in the pilot study were maintained throughout the extension study, and significant improvements in quality-of-life measures also continued. In this study, long-term eculizumab administration continued to be safe and well-tolerated. Clinical breakthrough occurred in two individuals with insufficient eculizumab levels; shortening the dosing interval (increasing the total dose) resulted in rapid restoration of complement suppression, abrogation of hemolysis, and resolution of disease signs and symptoms. These data demonstrate the close relationship between sustained terminal complement inhibition, hemolysis, and symptoms in individuals with PNH. Further, dramatic clinical improvements in signs and symptoms attributed specifically to hemolysis including hemoglobinuria, and the smooth muscle dystonias—abdominal pain, dysphagia and erectile dysfunction—were observed in these participants[54]. Ten of the original 11 participants have remained on eculizumab for more than 5 years of continuous treatment.

Phase 3 pivotal program. The results from the initial open-label phase 2 pilot study were sufficiently compelling that discussions were initiated with the FDA and the EMEA in order to plan phase 3 studies for registration in the United States and Europe, respectively. In 2003,

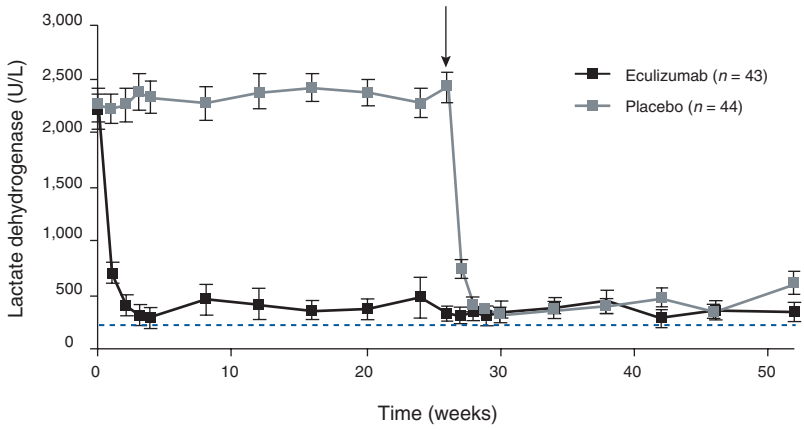

Figure 4 Reduction in hemolysis during treatment with eculizumab. Mean levels of lactate dehydrogenase reflect the degree of hemolysis from baseline to week 52. The dashed line indicates the upper limit of the normal range for lactate dehydrogenase (normal range, 103 to 223 U per liter). In eculizumab-treated patients, the mean level of lactate dehydrogenase was rapidly reduced to just above the upper limit of the normal range. In the placebo group, the mean level of lactate dehydrogenase remained highly elevated. The arrow depicts the transition of placebo-treated patients in TRIUMPH to eculizumab treatment in the phase 3 extension study at which time levels of lactate dehydrogenase rapidly reduced to near normal values.

Table 2 Primary and secondary endpoints of the phase 3 pivotal efficacy study TRIUMPH

	Treatment period		
	Placebo	Eculizumab	*P*-value
Stabilization of hemoglobin levels (percent of patients)[a]	0	49	<0.000001
Units transfused/patient[a]			
Median	10	0	<0.000001
Mean ± s.e.m.	11.0 ± 0.83	3.0 ± 0.67	
Transfusion Avoidance (percent of patients)	0	51	<0.000001
FACIT-Fatigue score (change from baseline)	– 4.0 ± 1.7	6.4 ± 1.2	0.000006
LDH (area under the curve; U/L × day)	411,822	58,587	<0.000001

[a]Co-primary endpoints.

eculizumab received orphan designation for PNH in both the United States and Europe. The phase 3 eculizumab in PNH program consisted of: TRIUMPH, the pivotal trial that examined eculizumab safety and efficacy in a hemolytic PNH population with at least 4 transfusions in 12 months; SHEPHERD, which examined safety and efficacy of eculizumab in a broader and more heterogeneous hemolytic PNH population in individuals with a history of transfusion; and a phase 3b extension study to evaluate long-term safety and efficacy, including the potential anti-thrombotic effect of eculizumab.

TRIUMPH was a double-blind, randomized, placebo-controlled, multicenter, phase 3 trial[74]. Individuals were first enrolled and treated in 2004 and received either placebo or eculizumab intravenously; eculizumab was given at a dose of 600 mg weekly for 4 weeks, followed 1 week later by a 900-mg dose and then 900 mg every 14 ± 2 days through week 26. Eighty-seven participants (43 eculizumab, 44 placebo) at 34 international sites were enrolled. The two primary endpoints were the stabilization of hemoglobin levels and the number of units of packed red cells transfused. Biochemical indicators of hemolysis and the participant's fatigue and quality of life were also assessed. After enrollment, participants were observed for up to 13 weeks. Those who did not require a transfusion during the observation period were considered ineligible for randomization. Eighty-seven individuals underwent randomization. Every participant treated with eculizumab experienced an objective response. Across all treated participants, a significant reduction in hemolysis (as measured by LDH) was observed after 1 week of treatment and maintained throughout the 26 week study in the eculizumab group but not in placebo (**Fig. 4**). There was an 85.8% lower median area under the curve for LDH plotted against time (in days) in the eculizumab group, as compared with the placebo group (58,587 versus 411,822 U per liter, respectively; $P < 1 \times 10^{-6}$; **Table 2**). After completion of the study, a similar reduction in hemolysis was demonstrated with placebo participants transitioning to eculizumab. Stabilization of hemoglobin levels in the absence of transfusions was achieved in 49% (21 of 43) of the individuals assigned to eculizumab and none (0 of 44) of those assigned to placebo ($P < 1 \times 10^{-6}$). During the study, a median of 0 units of packed red cells was administered in the eculizumab group, as compared with 10 units in the placebo group ($P < 1 \times 10^{-6}$). A total of 51% of the eculizumab group remained transfusion independent during the entire 26-week study, whereas no one in the placebo group achieved this status. Even among participants receiving eculizumab in whom transfusion independence was not achieved, transfusion requirements were reduced by 44% as these individuals also demonstrated a clinically and statistically significant reduction in hemolysis. The reduced but continued requirement for transfusion support in some individuals may reflect more severe bone marrow aplasia and/or residual low-level hemolysis through the extravascular compartment[74]. Clinically and statistically significant improvements were also found in fatigue, as measured by scores on the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) instrument ($P = 6 \times 10^{-6}$) and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30; $P < 1 \times 10^{-6}$). Participants that did not achieve complete transfusion independence during eculizumab treatment also experienced significant improvement in fatigue and other measures of quality of life. Of the 87 participants, 4 in the eculizumab group and 9 in the placebo group had serious adverse events, none of which were considered to be treatment-related; all these patients recovered without sequelae. The most common adverse events reported in the eculizumab group were headache, nasopharyngitis, back pain and nausea. Headache and back pain occurred more frequently in the eculizumab group than in the placebo group. Anti-eculizumab antibody responses were of low titer, transient, and occurred at a frequency similar to that of placebo. The TRIUMPH study demonstrated that eculizumab is a well-tolerated and effective therapy for PNH.

After initiation of the TRIUMPH study, eculizumab was evaluated in SHEPHERD, an open-label, non-placebo controlled, phase 3 clinical safety and efficacy study in a more diverse PNH population including patients with marked thrombocytopenia and minimal transfusion requirements. Participants were first enrolled and treated in 2005, and eculizumab was administered for a total of 52 weeks using a dosing schedule identical to that of TRIUMPH. Ninety-seven individuals at 33 international sites were enrolled. Adverse events were similar to those with placebo in the TRIUMPH study. Hemolysis was significantly reduced leading to improved anemia with a reduction in or elimination of transfusion requirement, lessened fatigue and improved overall health-related quality of life[75]. Compared to the overall population, efficacy with eculizumab was similar in individuals who would be expected to have less severe disease, including patient subgroups with lower baseline hemolysis, mild anemia, and minimal or no pretreatment transfusion requirement[76].

Of the 195 individuals entering the pilot, TRIUMPH or SHEPHERD studies, 187 participants completed these studies and elected to receive eculizumab in a common 104-week phase 3b open-label extension study. Overall, there was no evidence of an increased incidence of infection across PNH studies with eculizumab as compared to placebo, including serious infections, severe infections or multiple infections. Two individuals vaccinated against *N. meningitidis* experienced meningococcal sepsis; they were treated and recovered without clinical sequelae. Serious hemolysis was not observed in any of the initial 16 participants who discontinued treatment. Long-term eculizumab treatment was associated with a reduction in hemolysis up to more than 54 months of continuous treatment. Importantly, the design of the phase 3b extension study specified that the thrombosis rate for each participant would be compared before and during eculizumab treatment. Early evaluation shows that the 7.37 thrombotic events per 100 patient-years experienced before eculizumab was reduced to 1.07 events per 100 patient-years with eculizumab treatment ($P < 1 \times 10^{-6}$)[77].

Approval of eculizumab. After completion of TRIUMPH and after the pre-specified interim 26-week analysis in SHEPHERD, applications for marketing authorization of eculizumab in the treatment of PNH were submitted to the FDA and EMEA in September 2006. The FDA

accepted the application for priority review and in March 2007, less than 6 months after the completion of SHEPHERD, FDA approved eculizumab for the treatment of patients with PNH to reduce hemolysis. The EMEA granted review of the eculizumab application under the Accelerated Assessment procedure, the fastest evaluation time frame for full approval awarded by EMEA. In June 2007, eculizumab became the first medicinal product to receive approval by the European Commission within the Accelerated Assessment procedure and it is indicated for the treatment of patients with PNH.

Conclusions and perspectives

Clinical development of eculizumab spanned a full decade before its first approval in the rare, disabling and life-threatening, clonal blood disorder PNH (**Fig. 5**). The studies before initiation of the PNH clinical program provided an important assessment of the safety profile and immunomodulatory effects of eculizumab. Once initiated, the PNH program has been one of the most extensive pre-approval clinical programs for an 'ultra-orphan' disease; the design of the multinational trials enrolling 195 participants allowed sequential, controlled and long-term evaluation of both biochemical and clinically validated measurements in the PNH population. This paradigm of careful evaluation may be used in the consideration of eculizumab in other uncommon, debilitating and life-threatening diseases for which there is evidence suggesting that terminal complement may significantly contribute to the pathophysiology of the disease, including antibody-mediated transplant rejection[78], myasthenia gravis and other peripheral neuropathies[79–83], fulminant lupus[27,29], catastrophic anti-phospholipid syndrome[84,85] and pattern 2 multiple sclerosis[86]. The understanding of critical pharmacokinetic and pharmacodynamic properties in PNH patients and the accomplishment of large-scale commercial manufacturing of eculizumab may also permit the evaluation of new formulations of eculizumab in future development programs for still other diseases with evidence suggesting complement-mediated pathology, including asthma[87] and age-related macular degeneration[88].

Clinical trials have demonstrated that eculizumab dramatically and reproducibly abrogates intravascular hemolysis, the primary clinical manifestation of PNH. These trials have shown that the reduction in hemolysis is independently associated with improvements in major morbidities in individuals with PNH including anemia, fatigue and health-related quality of life, as well as fewer thrombotic events. Because of the extremely rare nature of the disease and the ethical dilemma posed by the efficacy evident with eculizumab in the treatment of PNH, a prospective randomized controlled clinical trial is not a feasible means to examine the long-term impact of eculizumab on survival in individuals with PNH. However, given the evident anti-hemolytic effect and attendant clinical benefit of eculizumab administration, it is possible that eculizumab could beneficially impact several of the leading causes of premature mortality in PNH including venous and arterial thrombosis, hemorrhage that may be worsened by platelet consumption, renal failure and liver failure. In a *post hoc* analysis of the survival of eculizumab-treated PNH patients compared to historical controls[64], ~15 deaths would have been expected during the 2-year period of eculizumab treatment; however, two deaths were observed during this period, or 87% fewer than expected ($P = 0.0005$). This preliminary analysis suggests that long-term eculizumab treatment may beneficially affect survival in individuals diagnosed with PNH.

The humanization strategy used to construct eculizumab has resulted in only very rare evidence of responses to eculizumab, with an incidence similar to that found with placebo treatment. There is no evidence of tolerance or a neutralizing antibody response in any treated individual to date and infusion-related immune effects are similar in frequency to placebo. It is interesting to speculate that the selection of purely germline framework acceptor regions, seen as native by all recipients, has allowed for this important safety benefit.

Rigorous evaluation of eculizumab in PNH has helped tease out the role of hemolysis in mediating critical patient-reported outcomes, including fatigue and patient functioning. The reproducible demonstration that patient improvement with eculizumab, due to the abrogation

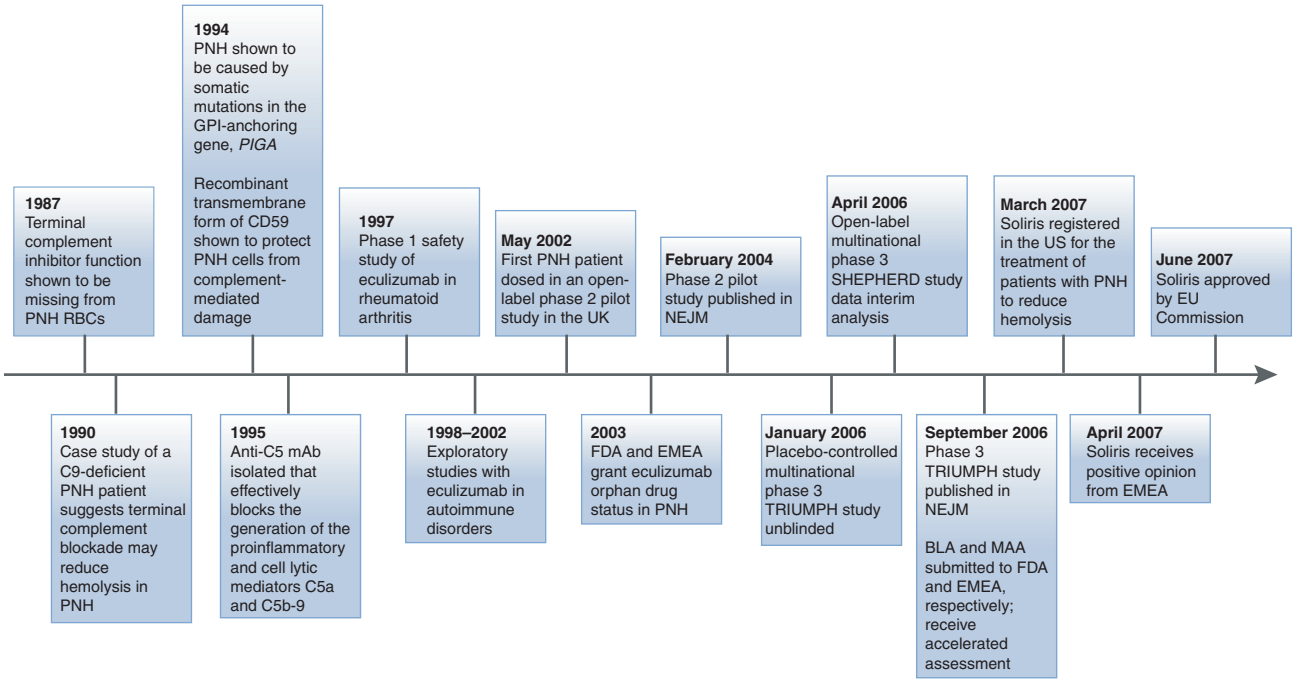

Figure 5 Eculizumab in PNH development timeline. BLA, biologic license application; MAA, marketing authorization application.

of the underlying hemolysis, is distinct from associated improvements in anemia may have applications to understanding the potential multifactorial etiology of fatigue in individuals with anemia in other disease settings, such as cancer. Similarly, evident improvement in smooth muscle dystonias and thrombosis with eculizumab treatment in PNH has helped to establish new and important clinical paradigms, particularly with regard to the role of cell-free plasma hemoglobin and nitric oxide scavenging in thrombosis, smooth muscle dystonia and vasomotor abnormalities[53]. In this case, focus on a rare disease may not only lead to benefit for afflicted individuals but may also contribute to the overall foundation of knowledge. Such knowledge may provide insight into more common diseases with disordered nitric oxide regulation, including systemic and pulmonary hypertension, diabetes, sickle cell disease, and other genetic or acquired hemolytic anemias.

In summary, data from six clinical studies, enrolling a total of 195 participants, demonstrate the robust efficacy of eculizumab for the treatment of individuals with PNH. Every individual treated with eculizumab to date has shown an objective improvement in hemolysis. Treatment with eculizumab modifies the clinical course of PNH and markedly reduces the serious morbidities associated with the disease. In particular, therapy with eculizumab reduces anemia, blood transfusion requirements, and the risk for life-threatening thrombosis. Eculizumab also improves other morbidities that are often serious, including fatigue, pain, dyspnea and impaired overall quality of life. Treated individuals will continue to be monitored in a global registry. Overall, the therapeutic benefit and safety of eculizumab in the treatment of patients with the rare blood disease PNH validates the utility of terminal complement inhibitor therapeutics for the treatment of medical conditions where complement activation is involved.

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COMPETING INTERESTS STATEMENT
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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Erratum: Funding crunch forces stem cell company to abandon therapies

Monya Baker
***Nat. Biotechnol.* 25, 951–952, 2007; published online 3 September 2007**

In the version of this article originally published, in the penultimate paragraph, the name of the Monash University scientist was misspelled; the correct spelling is Colin Pouton. In addition, the statement in paragraph 2 "the SSCC, which is being supported by the same group that funded ESI, the Agency for Science, Technology and Research (A*STAR)" is incorrect. ESI is not funded by A*STAR. Its major investor is Bio-One Capital, a venture capital fund controlled through the Singapore Government's Economic Development Board. The SSCC is funded by A*STAR.

Erratum: Mice with a human touch

Christopher Thomas Scott
***Nat. Biotechnol.* 25, 1075–1077, 2007; published online 6 October 2007**

In the version of this article initially published, it was incorrectly stated on p. 1076, paragraph 2, line four from bottom, that "Aya Jakobovits, originally at Cell Genesys and later at Abgenix, shepherded the XenoMouse through its entire development." Five of the six XenoMouse strains developed at Abgenix were initiated after Jakobovits left in 1997.

Erratum: Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria

Russell P Rother, Scott A Rollins, Christopher F Mojcik, Robert A Brodsky & Leonard Bell
***Nat. Biotechnol.* 25, 1256–1264 (2007); published online 7 November 2007; corrected after print 7 December 2007**

In the version of this article initially published, on p. 1258, paragraph 2, the incorrect serum concentrations to reach steady state and the incorrect accumulation ratio (R_{acc}) for eculizumab were given. The corrected sentence reads "Eculizumab serum concentrations appear to reach steady state after ~57 days. At steady state, the eculizumab accumulation ratio (R_{acc}) was calculated to be 1.075." In addition, in Figure 5, in the PDF version only, the word "assessment" was cut off the bottom of the box beginning "September 2006." The final sentence should read "BLA and MAA submitted to FDA and EMEA, respectively; receive accelerated assessment." And in Table 1, the heading "Units of packed RBCs transfused/patient/month" now appears in a separate row. The errors have been corrected in the HTML and PDF versions of the article.

Corrigendum: A diverse family of thermostable cytochrome P450s created by recombination of stabilizing fragments

Yougen Li, D Allan Drummond, Andrew M Sawayama, Christopher D Snow, Jesse D Bloom & Frances H Arnold
***Nat. Biotechnol.* 25, 1051–1056 (2007); published online 26 August 2007; corrected after print 7 December 2007**

In the version of this article initially published, the sentence starting with "The most thermostable 450 P450 chimera" contains an extra "450." The correct sentence should start as follows "The most thermostable P450 chimera". The error has been corrected in the HTML and PDF versions of the article.

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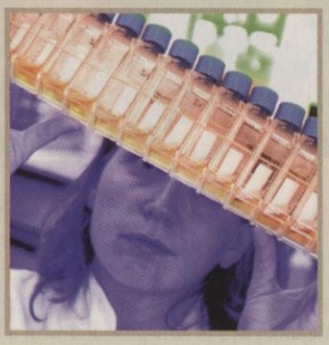
nature biotechnology

Insect pests such as the cotton bollworm *Helicoverpa armigera* consume a substantial portion of global agricultural outputs. Mao *et al.* (p 1307) and Baum *et al.* (p 1322) show that ingestion of transgenic plant material that expresses gene-specific double-stranded RNA can trigger potentially lethal RNA interference in several herbivorous insect pests. Credit: © Alessio Di Leo http://www.alessiodileo.com/



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