SCIENTIFIC DISCUSSION

1. Introduction

Paroxysmal nocturnal haemoglobinuria

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare blood disorder with high morbidity and mortality. PNH is clinically defined by the deficiency of the endogenous glycosyl phosphatidylinositol (GPI)-anchored complement inhibitory protein CD59 on the surface of blood cells. CD59 normally blocks the formation of the terminal complement complex (also called the membrane attack complex) on the erythrocyte surface, thereby preventing haemolysis. The pathophysiology of PNH is directly linked to the complement-mediated destruction of the susceptible PNH red blood cells, which results in intravascular haemolysis, the primary clinical manifestation in all PNH patients. PNH is a clonal acquired genetic disease arising from a somatic mutation in the gene pig-A, located in the X-chromosome. Inactivating mutations appear only in a proportion of cells (PNH cells) and this proportion can vary among patients and over time in a single patient.

The estimated prevalence of PNH is 13 cases per million. Patients have an approximately 15 year median survival from its initial diagnosis. PNH is associated with multiple serious morbidities, several of which are potentially life threatening. The common clinical manifestations of PNH are haemolytic anaemia, venous thrombosis and deficient haematopoiesis. Excessive levels of cell-free plasma haemoglobin during intravascular haemolysis contribute to platelet activation, procoagulant activity and thromboembolism (TE), the leading cause of mortality in these patients (45%). Anaemia is highly variable with haematocrit values ranging from ≤20% to normal. Red blood counts (RBC) are normochromic and normocytic unless iron deficiency has occurred from chronic iron loss in the urine. Granulocytopenia and thrombocytopenia are common and reflect deficient haematopoiesis. Clinical haemoglobinuria is intermittent in most patients and never occurs in some, but haemosidenuria is usually present.

There are no therapies specifically approved for the treatment of PNH and no generally applicable therapy adequately treats the serious conditions associated with PNH. The only curative treatment available to patients is bone marrow transplantation, which allows the replacement of the defective cells, however, this treatment is available for only a small proportion of patients since a suitable donor is required. Furthermore, transplantation may be associated with substantial risks. Current treatments for PNH are palliative and do not address the underlying disease process. Transfusion therapy is useful for raising the haemoglobin level and also for suppressing the marrow production of RBC during episodes of sustained haemoglobonuria. Iron replacement may be used, however it usually exacerbates haemolysis because of the formation of many new RBC, which may be sensitive to treatment. This may be minimized by giving prednisone (60 mg/d) or by suppressing the bone marrow with transfusions. Thrombolytic agents are used for acute thrombosis and antithymocite globulin is often used for treating the marrow hypoplasia.

<u>About the product</u>

Eculizumab is a humanized monoclonal antibody that binds to the human C5 complement protein. The antibody is an $IgG_{2/4}$ kappa immunoglobulin comprised of human constant regions and murine complementarity-determining regions (CDRs) grafted onto human framework light- and heavy-chain variable regions. Eculizumab is composed of two 448 amino acid heavy chains and two 214 amino acid light chains and has a molecular weight of approximately 148 kDa.

Eculizumab recombinant antibody inhibits C5 cleavage to C5a and C5b, preventing the generation of the terminal complement complex C5b-9 and thus blocking complement-mediated cell lysis and activation.

2. Quality aspects

Introduction

The active substance of Soliris, eculizumab, is a recombinant humanised monoclonal antibody ($IgG_{2/4}$ kappa immunoglobulin) to human complement protein C5. Binding to this protein blocks its cleavage into C5a and C5b, thereby inhibiting terminal complement-mediated intravascular haemolysis.
Eculizumab is expressed in a NS0 murine myeloma cell line, produced in a 5,000 L-scale bioreactor and purified using a series of chromatography, concentration and diafiltration, and viral inactivation and filtration steps. The active substance is formulated with a sodium phosphate buffer, sodium chloride, polysorbate 80 and water for injections.
The medicinal product is manufactured by sterile filtration and aseptic filling into vials.
Soliris is presented as a concentrate for solution for infusion (300 mg/vial) and is diluted in sodium chloride or in a dextrose solution prior to administration.

Active Substance

- Nomenclature

INN Name:	eculizumab
Compendial Name:	not applicable
Chemical Name:	immunoglobulin, anti (human complement C5 α chain) (human mouse monoclonal 5G1.1 heavy chain), disulfide with human mouse monoclonal 5G1.1 light chain, dimer
Laboratory Code Names:	h5G1.1 mAb, LEX98, HAL1, 5G1.1 antibody, anti C5 antibody, h5G1.1, h5G1.1VHC+h5G1.1VLC, h5G1.1HuG2/G4
USAN/BAN/JAN Name:	eculizumab
CAS Registry Number:	219685504

- Description of the active substance

The eculizumab antibody (h5G1.1 mAb) is a humanised $IgG_{2/4}$ kappa antibody, consisting of two 448 amino acid heavy chains and two 214 amino acid light chains. The heavy chains are comprised of human IgG_2 sequences in constant region 1 (CH1), the hinge and the adjacent portion of constant region 2 (CH2), and human IgG_4 sequences in the remaining part of CH2 and constant region 3 (CH3). The light chains are comprised of human kappa sequences. The variable chains consist of human framework regions with grafted murine complementarity determining regions which form the antigen binding site.
There is one N-linked glycosylation site at Asn298 of the heavy chain that is occupied by a range of biantennary oligosaccharides.
Inter-chain disulfide bonds are expected between Cys136 of the heavy chain and Cys214 of the light chain and between Cys228 and Cys231 of the heavy chains.

The expected molecular mass of the protein is approximately 148 kDa based on the predicted amino-acid sequence and the expected glycosylation profiles.

- Manufacture

The active substance is manufactured at Lonza Biologics, Portsmouth, New Hampshire, USA and released by Alexion Pharmaceuticals Inc., Cheshire, Connecticut USA. These facilities are operated in accordance to EU current Good Manufacturing Practices (EU cGMP), with standard operating procedures in place to describe all procedures and controls.

Development genetics:
The expression plasmid containing the DNA encoding the genes for the heavy chain and light chain was transfected into NS0 cells obtained from a host cell bank. The cells were adapted to suspension culture and following cloning and subcloning steps, one clone was selected as the lead cell line.

Cell bank system:

A two-tiered cell banking system of MCB and Working Cell Bank (WCB) has been developed and maintained in accordance to cGMP and ICH guidelines.

One single ampoule of the pre-seed stock of the lead cell line was thawed and the MCB was obtained following culture and sub-culture in a selective medium, re-suspension of the cells and storage in the vapour phase of liquid nitrogen.
The WCB was prepared from one single ampoule of MCB, using the same procedures and media as described for the MCB.

Procedures followed in the preparation of MCB and WCB have been appropriately described. An extensive range of tests has been performed for their characterisation, in accordance with ICH guidelines, including identity, viability, stability, presence of adventitious agents.

Fermentation process
Eculizumab active substance is manufactured in a 5000-L scale production bioreactor. The cells used to inoculate the production bioreactor originate from a single ampoule taken from the WCB. Cell culture continues until pre-determined harvest criteria are reached.
Only one bioreactor train is used during the manufacture of a single batch of eculizumab.
There are no reprocessing steps for the cell culture and harvest process.

Purification process:
Eculizumab is purified using a series of chromatography, concentration and diafiltration, and viral inactivation and filtration steps.

Each step of the purification process has been adequately described, including description of the elution buffers, exchange buffers, column regeneration and storage conditions of both columns and product after each step. Suitable in-process controls (IPC) are in place, with acceptable limits.
Two reprocessing steps have been identified, consisting in the re-filtration following integrity test failure of the virus reduction filter or of the 0.22 µm final filter

Manufacturing process development and process validation:
A number of manufacturing changes have been introduced during development, resulting in five main processes for the active substance (A, B, C, D and the commercial process referred to as process E). These changes include:

- A change in cell line at an early stage of development: cell line LEX98 demonstrated higher expression than the original HAL1 cell line used in Process A and was therefore selected. The main clinical studies were conducted with material derived from LEX98 cell line;
- Changes to cell culture medium;
- Scale up of the fermentation process from 200 L to 2000 L and 5000 L;
- Various changes to the purification process;
- Change of manufacturing site.

The comparability exercise to support the different changes is considered adequate and the overall data provided were considered acceptable.

The validation program covers the validation of the upstream and downstream processes of five consecutively-manufactured batches of active substance using the proposed commercial process (process E) and scale (5000 L).

For the fermentation process, individual results for each lot were provided for all IPCs. The validation program included a complete analysis of several growth parameters, as well as other process parameters.

The validation of the purification process included analysis of intermediates obtained at each step, and purified after several chromatography cycles, with respect to product purity, identity, integrity and yield. Reduction of process-related impurities, bioburden and endotoxin were also covered by this program. The validation of the purification process was further supported by the evaluation of the re-

use of filtration membranes, column resins, and small-scale evaluation of DNA elimination. A validation protocol was also provided for the two reprocessing steps.
The overall results obtained generally support the consistency of the active substance manufacturing process.

Characterisation:
a) Elucidation of structure and other characteristics:

The structure of eculizumab antibody was mainly characterised using Process C material. Part of these studies was also performed on batches produced with the commercial Process E.
The primary structure was analysed by several methods and no variability has been detected, with the exception of the N- and C-terminal residues on the heavy chain.

The presence of intra-chain disulfide bonds was confirmed and found consistent with those expected for an $IgG_{2/4}$ antibody.
The amount of alpha-helix and beta-sheet was analysed by circular dichroism.

The oligosaccharide analysis predominantly showed bi-antennary core fucosylated glycans with varying amounts of terminal galactose.

The biological properties of eculizumab have been properly characterised and the assays that were chosen and the data provided are considered adequate.

b) Impurities

The potential product-related impurities identified are aggregates, fragments and other product variants. Potential process-related impurities include cell substrate derived impurities (host cell proteins, DNA), cell culture derived impurities.

Overall, impurities have been properly identified and characterised and are considered well controlled.

- Specifications

The proposed commercial active substance specifications have been established in accordance with ICH Q6B Guideline. Tests and acceptance criteria are based upon data obtained from batch analysis of clinical supplies and manufacturing process validation batches, stability studies, validation of analytical procedures and development activities. Justification for the specifications was provided.

- Stability

The design of the stability program, including the testing intervals and storage conditions, are in accordance with ICH guidelines. The tests chosen are a subset of tests from the release specifications selected for stability-indicating properties.
Long term stability data (at 2-8°C) were provided for commercial process E for up to 18 months. Materials from these batches were stored in a container representative of the active substance storage. All these batches were also monitored in accelerated stability studies at 20-25°C for up to 6 months.
Supportive stability data obtained from process D materials, stored in a non-representative container were also provided for up to 30 months at 2-8°C, and 12 months at 22-28°C.
All results were within the initially proposed specifications for up to 18 months at 2-8°C and up to 3 months at +20-25°C.
As discussed below, several specification limits initially proposed by the applicant were not considered acceptable. On the basis on the additional information provided during the evaluation procedure and the commitment that was made to provide additional stability data considering the new revised specifications, the claimed shelf life of 18 months at 2-8°C was acceptable.

Medicinal Product

- Pharmaceutical Development

The formulation for eculizumab was based on excipients which are commonly used in monoclonal antibody products intended for administration by intravenous infusion. The formulation did not change throughout the clinical development program and is the same as that proposed for the commercial product. Both long-term and accelerated stability data showed that this formulation is suitable for product stability. Parameters that can influence protein stability in solution, such as ionic strength, surfactant type and concentration, were evaluated in seven different formulations based on the use of sodium phosphate as a buffering agent.
The proposed container for the medicinal product is a single-use vial (type I glass) with a siliconised butyl stopper, an aluminium seal with a polypropylene flip-off cap. One vial contains 30 ml of concentrate to be diluted to a final concentration of 5 mg/ml by addition into the infusion bag of 0.45% or 0.9% sodium chloride, or 5% dextrose in water as diluent.

- Manufacture of the product

The medicinal product is manufactured at Ben Venue Laboratories, Ohio, USA and Almac Pharma Services, Craigavon, UK is responsible for batch release in the EU. These facilities are operated in accordance to EU cGMP, with standard operating procedures in place to describe all procedures and controls.

The medicinal product manufacturing process mainly consists in a double 0.22 µm filtration followed by an aseptic filling into vials. Filled vials are stoppered and sealed. Medicinal product vials are stored at 2-8°C prior to shipping for secondary packaging.
Sufficient information was provided on all process and operating parameters. Process validation data have shown that the sterile filtration and aseptic filling process are robust and well controlled and that the medicinal product can be consistently manufactured.

- Specifications

The control of medicinal product relies to a large extent on the same analytical methods as those used for the control of the active substance. The issues that were raised during the evaluation procedure for the active substance generally apply to the medicinal product.

- Stability of the Product

Real-time and accelerated stability studies were initiated in accordance with ICH guidelines and per protocol to monitor the time-temperature stability of cGMP lots of medicinal product. Based on the data provided, the approvable shelf life for the medicinal product is 24 months (2 years) at 2-8□C.

However, a commitment was made to provide additional stability data considering the specifications that the applicant was asked to revise during the evaluation procedure (see below).

Studies were performed to demonstrate compatibility of the diluted medicinal product with the infusion diluents (0.9% sodium chloride, 0.45% sodium chloride, or 5% Dextrose in water) and infusion bag and support a 24 hour expiry of the diluted solution. However, after dilution, it is recommended to use the medicinal product immediately.

- Adventitious agents

The raw materials of animal origin used in the manufacturing process of eculizumab are:

- FBS used as a cryopreservative agent in the preparation of the MCB and WCB. FBS is sourced from New Zealand and TSE certificates were provided. Since non-inactivated FBS is used, the Applicant committed to screen cells derived from the WCB for bovine viruses. The Applicant also committed to use inactivated FBS for future cell banks.
- Bovine serum albumin (BSA) used as a medium component for cell banking and cell culture. This raw material is obtained from bovine blood and sourced from Australia and the USA. TSE certificates were provided.
- Cholesterol used as a medium and feed component for cell banking and cell culture. This raw

material is obtained from sheep wool grease sourced from Australia and New Zealand.

It was confirmed that polysorbate 80 is manufacture from substances of vegetable and chemical origin.

The low-pH treatment has been validated at pH 3.73 and 3.74 for X-MuLV and 3.78 for HSV. The inactivation kinetic of HSV has been performed in a single run. The Applicant committed to perform additional investigational studies on the efficacy of pH 3.7 treatment on HSV and X-MuLV viruses

Nanofiltration with Planova filters was validated using X-MuLV, HSV, BAV, and MVM as model viruses. The data were generally satisfactory but the applicant committed to perform an additional study to provide duplicate validation data for Planova filters on MVM clearance.

The viral safety of Soliris is considered acceptable due to i) the satisfactory level of cell bank and unprocessed bulk testing for adventitious viruses, and ii) the clearance efficiency of the production process. The applicant satisfactorily clarified some points relating to viral inactivation studies.

Discussion on chemical, pharmaceutical and biological aspects

In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines.
No major objection on Quality aspects was identified during the initial evaluation.

The generation of the original cell line, from the expression construct to the selection process was well described. Cell banks have been established and adequately characterised. The Applicant was asked to clarify several aspects of the cell culture procedure and the genetic stability of the cells. These points have been solved.

Other aspects of the active substance manufacturing process for which clarification and/or further documentation was requested have been either solved or will be addressed as follow-up measures. The same conclusion applies to the comparability exercise to support the changes introduced in the manufacturing process.

The medicinal product manufacturing process was described in sufficient detail and is considered adequately controlled. Further information was requested regarding process validation and the issues raised have been satisfactorily addressed.

The description and validation of analytical procedures for the active substance and medicinal product were generally satisfactory except for certain methods for which additional information was requested. These issues have been solved except for some minor points that will be addressed as follow-up measures.

Viral safety and safety concerning other adventitious agents including TSE were appropriately demonstrated although several minor points needed to be clarified. These points are now clear and no further concerns have been raised.

The main concerns after the initial assessment referred to the suitability of the methods and specifications in place to detect the major degradation species potentially expected in this type of product. Although in principle these methods could provide information on oxidised, deamidated and clipped forms, their limits were considered too wide to reflect variation of these variants. Consequently, the applicant was requested to:

i) Reconsider the proposed specifications for active substance and medicinal product, and discuss their suitability with regard to monitoring these variants.
ii) Provide the release data using tightened specifications for at least batches derived from processes D and E.
iii) Commit to develop appropriate methods to specifically monitor these variants, and to implement these methods with appropriate acceptance criteria into the specifications, if appropriate.

As a consequence, stability analysis would have to be revised taking into account the considerations expressed in the previous paragraph concerning oxidised, deamidated and clipped forms.

The Applicant revised the specifications for both batch release and stability studies, the acceptance criteria for several tests have been tightened as requested and the available data re-analysed accordingly. In addition, the suitability of the current tests to detect the major variants has been demonstrated by forced degradation studies.

Except for a number of quality points, which will be addressed as part of post-approval follow-up measures, the quality of Soliris has been adequately demonstrated.

3. Non-clinical aspects

Introduction

All non-clinical safety studies including the tissue cross reactivity study were GLP compliant.

Pharmacology

Eculizumab has minimal cross-reactivity with non-primate and primate species, therefore only *in vitro* pharmacodynamic studies were performed.

- Primary pharmacodynamics

To generate and select an anti human C5 antibody, a panel of mouse hybridomas specific for hC5 were generated and the antibodies produced by these hybridomas were evaluated for their ability to block complement-mediated haemolysis and C5a generation. One hybridoma, m5G1.1, was selected. The produced antibody m5G1.1 mAb blocked 100% of complement-mediated C5a release and haemolysis at a concentration of 10µg/mL. The affinity of m5G1.1 mAb for hC5 under equilibrium conditions in solution was assessed by an enzyme-linked immunosorbent assay (ELISA) method. The calculated Kd for m5G1.1 was 30 pM.

A humanized version of the molecule, h5G1.1 G2/G4 (eculizumab), was generated to minimize immunogenicity. The humanization strategy consisted on the graft of complementarity determining regions (CDRs) of the antibody of interest into a human antibody framework. Data presented on the haemolytic assay indicated that the humanization process had no effect on the functional activity of the antibody. The dissociation constant (KD) of eculizumab calculated following on Biacore analysis was 46 pM and 120 pM (at 25°C and 37°C, respectively).

The m5G1.1 mAb epitope on hC5 mapped to the alpha chain. Identical recognition of the hC5 epitope was demonstrated with the single-chain variant of eculizumab, h5G1.1-scFv, which has the variable regions responsible for antigen (hC5) specificity that are identical to those in eculizumab.

Species cross-reactivity was assessed using eculizumab and sera from 8 different species including human, baboon, Rhesus, Cynomolgus, chimpanzee, rat, pig, guinea pig and rabbit. Eculizumab effectively blocked haemolytic activity of human serum with complete inhibition demonstrated at approximately 100 nM of antibody. Eculizumab did not effectively block haemolytic activity of sera from any primate or non-primate species tested, even at extremely high concentrations of antibody.

Due to the species specificity of eculizumab, potential pharmacological activities *in vivo* have been explored with murine antibodies targeting rodent C5. Since there are no murine models of PNH, studies targeting other therapeutic areas with potential involvement of C5 activation (arthritis, lupus, airway inflammation and cardiac allograft models) have been presented.

- Secondary pharmacodynamics

Eculizumab was tested for potential cross-reactivity with normal human tissues. The antibody was

applied to cryosections (3 donors per tissue) at two concentrations, 5µg/mL and 30 µg/mL. C5-specific staining was observed in smooth and striated (skeletal) muscle in various tissues and expressed in multiple cell types including myoepithelium, myofibroblasts, renal tubular epithelium, and reticulum cells in the human tissues examined. These results are consistent with published reports of C5 expression.

- Safety pharmacology programme

No specific nonclinical safety pharmacology studies have been conducted.

- Pharmacodynamic drug interactions

No specific studies were performed.

Pharmacokinetics

The potential plasma disposition of eculizumab was evaluated measuring the PKs of an eculizumab variant, h5G1.1 G4 mAb, in a C5-deficient mouse model. The pharmacokinetic study was followed measuring hC5 and h5G1.1G4 mAb concentration using a haemolytic assay and an ELISA assay. Methods used in the plasma clearance study were not validated. The plasma clearance study was performed as a non-GLP research study.

- Absorption-Bioavailability

Intravenous (i.v.) or subcutaneous (s.c.) doses of 50, 100 or 150 µg of h5G1.1 G4 mAb were administered to C5-deficient male mice (B10.D2 oSn) that had been fortified with hC5. Serum samples were collected up to 48 hours after dosing and assayed for the presence of the mAb and hC5 as well as for haemolytic activity. A dose of 50 µg of h5G1.1 G4 mAb administered i.v. resulted in a rapid and potent dose-dependent inhibition of hC5-dependent serum haemolytic activity that was maintained for at least 48 hours. s.c. administration also provided protection from hC5-dependent serum haemolytic activity for at least 48 hours, although there was an initial 12-24 hour delay in this effect. Serum hC5 levels and antibody concentrations were also followed. After i.v. injection, a relatively rapid t 1/2 α of four hours was followed by a slow decline in serum concentrations over the next 48 hours. In contrast, s.c. administration was followed by a progressive rise in serum concentration. Serum levels of the antibody at 24 hours and thereafter were comparable in both s.c. and i.v. injections over the next 48 hours.

The molar ratio of antibody to hC5 required to inhibit *in vitro* serum complement haemolytic activity was approximately 0.5 to 1.

No pharmacokinetics studies of absorption after repeated doses were performed.

- Distribution

No specific studies were performed. No protein interaction studies were performed.

- Metabolism

No metabolism studies have been conducted.

- Excretion

No specific studies were performed.

- Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies have been performed.

Toxicology

- Single dose toxicity

No single-dose toxicity studies have been conducted with BB5.1 mAb in mice.

- Repeat dose toxicity (with Pharmacodynamic analysis)

In a four week range finding study BB5.1 mAb was administered to Crl:CD-1®(ICR) BR female mice by IV route, once, twice, or three times weekly resulting in weekly dose levels of 30, 60 or 90 mg/kg. Controls received vehicle three times weekly. Neither treatment-related clinical signs nor effects on body weight or body weight change were observed. Serum analysis indicated similar extents of serum complement inhibition (mean haemolysis <20%, on days 8, 15, 23 and 29) for mice treated with BB5.1 mAb at 60 versus 90 mg/kg/week. Since the extent of haemolytic prevention was so similar at both 60 and 90 mg/kg, and greater at these doses than at 30 mg/kg/day (34±12% haemolysis day 8, 20%±13% day 15, <20% days 22 and 29), the recommended high dose of BB5.1 mAb in subsequent future toxicity studies in mice was 60 mg/kg/week.

A twenty-six week study evaluated the toxicity of BB5.1 mAb. Crl:CD-1®(ICR) BR mice received by IV route BB5.1 mAb at 0 mg/kg twice weekly, 30 mg/kg once weekly, or 30 mg/kg twice weekly (resulting in dose levels of 0, 30 and 60 mg/kg/week respectively). No unscheduled deaths occurred in the 30 mg/kg/wk dose group. Nine unscheduled deaths occurred during the study (4 in controls, and 5 in high dose group); none were related to treatment. Treatment did not affect any of the toxicity parameters examined. Serum analysis indicated that the extent of mean % haemolysis decreased from pre-treatment levels of 70-80 % to below 20 % in treated mice at weeks 12 and 25. Although results were similar for both treated mice groups, the mean % haemolysis was slightly less for the 60 mg/kg/week mice in most instances. Following a four-week recovery period, the mean % haemolysis approximated pre-study values for both treatment groups.

- Genotoxicity

Genotoxicity studies have not been performed with eculizumab or any other surrogate anti-C5 antibodies.

- Carcinogenicity

Carcinogenicity studies have not been performed with eculizumab or any surrogate anti-C5 antibodies. The 26-week repeat dose toxicity study with a murine anti-mouse C5 antibody (BB5.1mAb) showed no cytotoxic or proliferative activities suggestive of carcinogenic risk at dose levels up to 60 mg/kg/week, i.e. a dose level showing significant inhibition of C5 activation.

- Reproduction Toxicity

All studies were claimed to be performed according to GLP.

Fertility and early embryonic development

BB5.1 mAb was administered by IV injection to male and female Crl:CD-1®(ICR) BR mice at doses of 0, 30 mg/kg/dose once weekly or twice weekly (resulting in 0, 30 or 60 mg/kg/week, respectively) prior to mating and until termination (males) or through early gestation (females).

BB5.1 mAb showed no effect on the reproductive performance. Sperm count and motility were unaffected and there were no changes observed upon caesarean section, indicative of effects on implantation or embryo-foetal viability.

Analysis of haemolytic activity showed systemic exposure. The mean haemolytic activity at week 10 from low and high dose males was 27.2% and 16.2% respectively. In females, where samples were taken on GD 12, both low and high dose groups had a mean haemolysis of 50.6 %, but the within group variability was significant. The NOAEL for male toxicity was determined to be 60 mg/kg/week, while the NOEL for female toxicity, male and female fertility and embryo-foetal viability was determined to be ≥60 mg/kg/week

Embryo-foetal development

Premated female Crl:CD-1®(ICR) BR mice were given BB5.1 mAb by IV injection either 0 or

30 mg/kg/dose on GD 6, 9, 12 and 15 (resulting in 0 or 60 mg/kg/week, respectively) or 30 mg/kg/dose on GD 6 and 12 (resulting in 30 mg/kg/week). There was no maternal toxicity and no effect on embryo/foetal viability and growth. No treatment-related foetal malformations were observed in mice treated with 30 mg/kg/week. A single incidence of umbilical hernia and two foetal incidences of retinal dysplasia (one foetus from two separate litters) were observed in offspring born from mothers exposed to the higher antibody dose.
The percentage of haemolysis was highly variable in the treated groups although mean percentage haemolysis was ≤ 62.4% indicating systemic exposure.

The NOEL for maternal toxicity and embryo/foetal toxicity was determined to be ≥60 mg/kg/week, based on the lack of maternal and caesarean section findings at 60 mg/kg/week (i.e., the highest dose tested). Based on the observed foetal soft tissue malformations at 60 mg/kg/week, the NOEL for developmental toxicity was determined to be 30mg/kg/week.

Prenatal and postnatal development, including maternal function
Female Crl:CD-1®(ICR) BR mice received BB5.1 mAb at 30 mg/kg/dose once or twice weekly (groups 2 and 3, low and high dose, respectively) or vehicle. The control and high-dose groups were dosed on GD 6, 9, 12, 15, and 18 and on LD 2, 6, 9, 12, 15, and 18. The low-dose group was dosed on GD 6, 12, and 18 and LD 6, 12, and 18. There were no compound-related mortalities or clinical observations. Gestational and lactational body weights and gestational food consumption were unaffected by BB5.1 mAb. There were no compound-related necropsy findings. Natural delivery and litter data from the F_1 offspring were unremarkable. Survival indices and means for all maturation landmarks were similar across groups. Exploratory activity, learning and memory of the F_1 offspring were unaffected by BB5.1 mAb treatment. There were no compound-related mortalities or clinical observations during the maturation phase. BB5.1 mAb had no effect on body weight or body weight gain in F_1 males (maturation phase) or females (maturation and gestational phases), or overall reproductive performance in the F_1 generation. Analysis of haemolytic activity showed that mean levels were only slightly lower in treatment groups compared to control group

The NOEL for maternal toxicity and F1 pup development and reproductive performance through to parturition of the F2 generation was determined to be ≥60 mg/kg/week.

- Local tolerance

Local tolerance studies have not been performed with eculizumab or any other anti-C5 antibodies. There were no remarkable macroscopic observations or histomorphological findings at the injection site related to BB5.1 mAb treatment in the toxicity studies performed. Infusion reactions and injection site reactions have been monitored in over 700 patients exposed to eculizumab.

- Other toxicity studies

Immunotoxicity
There were no remarkable macroscopic observations or histomorphological findings in mandibular or mesenteric lymph nodes related to treatment with the surrogate antibody, BB5.1 mAb, in the twenty six week mouse study.

Ecotoxicity/environmental risk assessment

The main route of release will be in waste water, and the $PEC_{SURFACE/WATER}$ for eculizumab is below the threshold for Phase I assessment, according to the Guideline EMEA/CHMP/SWP/4447/00.

Discussion on the non-clinical aspects

Pharmacology
Results from *in vitro* pharmacology studies demonstrate that the humanized monoclonal antibody eculizumab inhibits the cleavage of C5 to C5a and C5b as well as blocking C5b-9-mediated serum haemolytic activity.

C5 expression has been reported in normal human tissues. Eculizumab binds to C5 on smooth muscle (intrinsic/vascular) and skeletal muscle, myoepithelium, myofibroblasts, renal tubular epithelium, and reticulum cells. Staining of platelets was also observed. The reticulum cell staining likely represents staining of C5 associated with intracellular filaments. Intracellular staining is due to the method of acetone fixation and cryotomy of the tissue/cell samples.

Pharmacodynamic studies have only been performed *in vitro*. Eculizumab is a highly specific monoclonal antibody binding only to human C5 and not to C5 from any other mammalian species tested. As stated in the CHMP scientific advice and in accordance with ICH S7, the approach to characterize the pharmacodynamic properties of eculizumab by *in vitro* methods is considered as acceptable in the light of the species specificity. Moreover, no specific safety concerns related to CNS, CV or RS functional effects are reported from clinical safety data. Therefore, the lack of safety pharmacology studies is considered justified.

Although no formal pharmacodynamic drug interaction studies have been performed, eculizumab has been administered to patients treated concomitantly with a broad range of medications commonly used in patients with PNH including erythropoietin, corticosteroids, anabolic steroids, anticoagulants, and immunosuppressants. Therefore, the absence of pharmacodynamic drug interaction studies is justified taking into account clinical data.

Pharmacokinetics
Pharmacokinetic studies show that the humanized mAb is functional in the murine vascular compartment for at least 48 hours after injection. Furthermore, based upon an average hC5 plasma concentration of 76 µg/mL [15], and a plasma volume of 3.0 L for a 70kg human, it is predicted that a single dose of 1.5-2.0 mg/kg of h5G1.1 G4 mAb should be sufficient to acutely mediate complete inhibition of complement-dependent serum haemolytic activity *in vivo* in humans.

No tissue distribution studies were performed, since eculizumab bind to a soluble protein, hC5, which is secreted into the blood. Eculizumab cannot bind to mC5 or function in a murine system. No placental transfer studies have been done due to the specificity of the antibody. However, IgG antibodies are known to cross the placental/foetal barrier from mother to offspring.

According to the Guideline CHMP/ICH/302/95, the lack of metabolism studies is acceptable.

No excretion studies have been performed. It is expected that since antibodies are transmitted in mother's milk, eculizumab will probably also be excreted in the milk.

Although no formal pharmacokinetic drug interaction studies have been performed, eculizumab has been administered to patients treated concomitantly with a broad range of medications commonly used in patients with PNH including erythropoietin, corticosteroids, anabolic steroids, anticoagulants, and immunosuppressants. Therefore, the absence of pharmacokinetic drug interaction studies is justified taking into account clinical data.

Toxicology
Toxicological studies were performed in mice using a surrogate murine anti-mouse C5 antibody (BB5.1 mAb). BB5.1 mAb specifically binds to mouse C5 but there was no information on whether the cross-reactivity profile of BB5.1 mAb with normal mouse tissues is similar to the cross-reactivity profile of eculizumab with normal human tissues.

Genotoxicity studies have not been performed with eculizumab or any other surrogate anti-C5 antibodies. Non-clinical genotoxicity studies are not generally considered relevant to biotechnology products (ICH S6 guideline). Antibodies, in general, do not interact directly with DNA, and therefore are unlikely to have any genotoxic potential.

Reproductive toxicity studies were conducted in 2002 and 2003 and reviewed by an independent consultant (Rochelle W Tyl, RTI international) that concluded that the observed foetal malformations were unlikely to be treatment related. Nevertheless, these findings could not be discarded and have been addressed in the SPC.

Local effects following therapeutic route of administration are well characterized following clinical monitoring and, in a lesser extend, repeat-dose toxicity studies performed with the surrogate antibody.

4. Clinical aspects

Introduction

Six clinical studies provide the basis for establishing the safety and efficacy of eculizumab therapy in the PNH patient population. These studies included 195 patients from 13 countries.

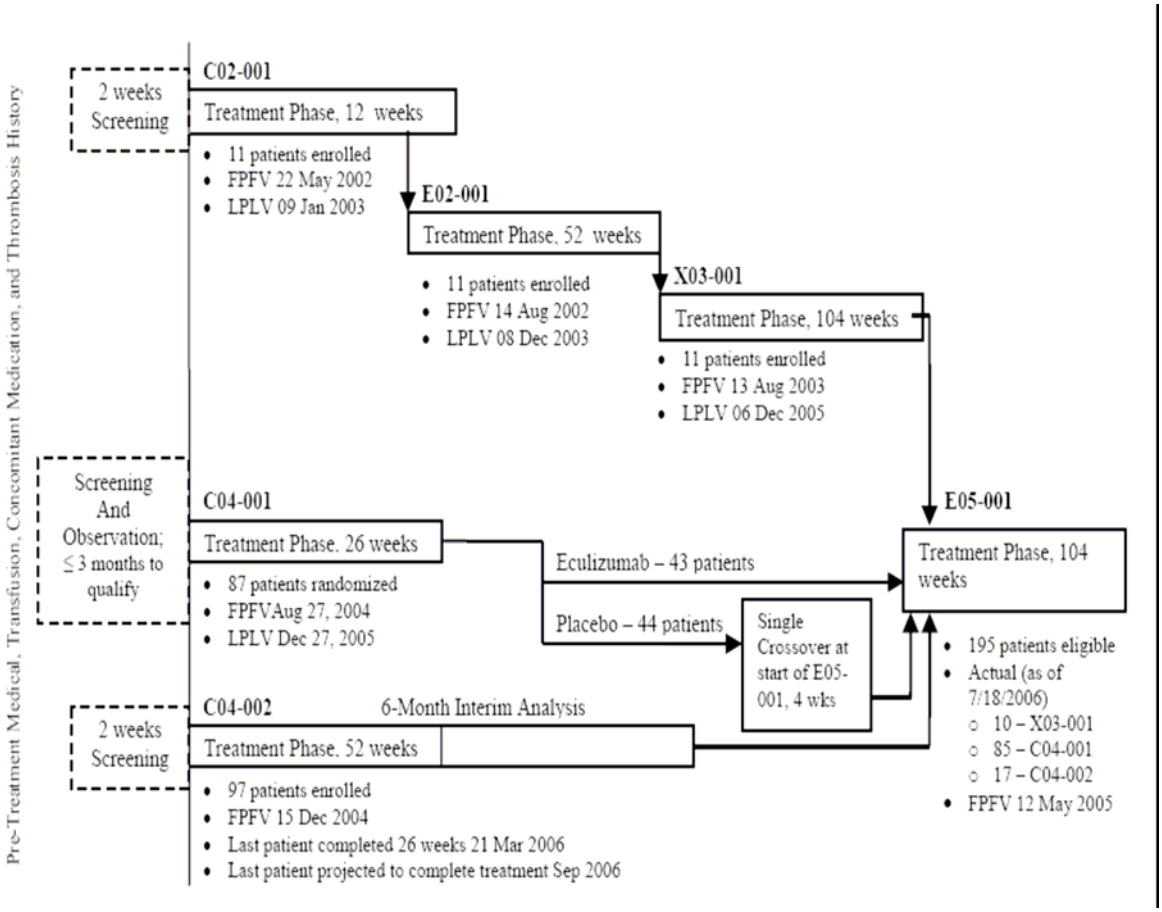
Listing of All PNH Studies

Study Number	Phase/Design[1]	Duration/Status	Total Patients Enrolled
C02-001	2[2] / OL	12 weeks/Complete	11
E02-001	2[2] / OL (C02-001 Extension)	52 weeks/Complete	11
X03-001	2[2] / OL (E02-001 Extension)	104 weeks/Complete	11
C04-001	3 / R, DB, PC	26 weeks/Complete	87
C04-002	3 / OL	52 weeks/Ongoing 26 week Interim Complete	97
E05-001	3b / OL (C04-001, C04-002, and X03-001 Extension)	104 weeks/Ongoing	187

[1]R = Randomized; [2]Also referred to as Phase I studies;
DB = Double Blind; PC = Placebo Controlled; OL = Open Label.

The following diagram illustrates the progression of each of the PNH studies conducted, which contributed to the application.

Schematic for Eculizumab PNH clinical studies



Additional supportive safety data was obtained in 11 studies in 716 patients in 6 non-PNH indications.

GCP

According to the applicant, the pivotal study was performed in full compliance with the principles of good clinical practices (GCP). Ethical principles according to the latest version of the Declaration of Helsinki were pursued in the major clinical studies.

No special reasons triggering a need for a GCP inspection has been identified.

Pharmacokinetics

The applicant has conducted 16 eculizumab clinical trials wherein pharmacodynamics (PD) and pharmacokinetics (PK) data were collected and analyzed.
These analyses were performed on patient serum samples derived from both single and multiple dose studies.

PK studies have not been conducted in healthy subjects.

The PK of six single doses of eculizumab was studied in fifty patients in two diseases in two studies, rheumatoid arthritis (RA) (C97-001-01) and systemic lupus erythematosus (SLE) (C97-002-01). These studies were conducted using a dense blood sampling schedule. In these phase I trials, serum eculizumab concentrations were recorded over doses ranging from 0.1 mg/kg to 8 mg/kg, after single IV bolus injections.
Phase II multiple-dose studies were conducted in patients with RA (C01-004), idiopathic membranous glomerulopathy (IMG) (C99-004), and PNH (C02-001). These multiple-dose studies measured only serum trough and peak eculizumab concentrations.

Pilot studies have been conducted with eculizumab in psoriasis (C99-007) and dermatomyositis (C99-006). These studies measured serum trough eculizumab concentrations.

PK simulations were used to predict eculizumab concentration variability in PNH patients and to assess the exposure and potential accumulation of eculizumab after repeated administration of fixed doses.

Methods

- Analytical methods

To measure the total concentration (bound + free) of eculizumab in human serum samples, an ELISA-based PK assay was developed.
The ruggedness of the assay was validated by evaluating the effects of day-to-day variability, instrument variability, and user variability on the reproducibility of assay results. No robustness variables were identified for the assay. Quality control samples containing eculizumab concentrations of 20, 120, and 240 µg/mL were evaluated by 2 separate analysts, using 2 different instruments, on 2 different days.
The stability of QC test samples at multiple concentrations of eculizumab (20, 120, and 240 µg/mL) was evaluated over time and at multiple storage conditions.

- Pharmacokinetic data analysis

Single-Dose Non-Compartmental PK Parameter Summary by Dose

	4mg/kg AUC(0-t) (h*mcg/mL)	**8mg/kg AUC(0-t)** (h*mcg/mL)	**4mg/kg AUC inf** (h*mcg/mL)	**8mg/kg AUC inf*** (h*mcg/mL)	**4mg/kg Cmax** (mcg/mL)	**8mg/kg Cmax** (mcg/mL)	**4mg/kg CL** (mL/hr/kg)	**8mg/kg CL*** (mL/hr/kg)
Average	17277.2	34060.6	19127.4	36654.2	139.63	174.68	0.2531	0.2303
SD	9149	7275.6	10248	7912.08	23.62	19.259	0.10628	0.06715
Median	13177	33736.00	16101	38595	144.7	175.3	0.248	0.207
%SD	53.0	21.4	53.6	21.6	16.9	11.0	42.0	29.2
N	7	9	7	7	7	9	7	7

Summary of the Single-Dose and Multiple-Dose Pharmacokinetic Parameters of Serum Eculizumab Estimated from the 2CLVFCLI Model

(a two compartment linear volume function model which was used to calculate PK parameters from single (C97-001) and multiple dose (C01-004) studies of RA patient)

Mean Pharmacokinetic Parameter	Single – Dose C97-001 n=10	Multiple – Dose C01-004 n=111
CL (mL/hr/kg)	0.26157	0.2998
V_2 (mL/kg)	20.039	44.3108
V_1 (mL/kg)	15.04	12.5061
V_{app} (mL/kg)	35.079	56.8169
$T_{1/2}$ (hr)	92.94	131.33
AUC(0-inf) (µg*hr/mL)	24467.6	25350.3
R_{acc}	n/a	1.065
$R_{e\ area}$	n/a	1.036
$T_{0.99}$ (hr)	n/a	1744.03

For multiple-dose patients the mean body-weight normalized clearance (CL) and volume of distribution (V_x) parameters were used to derive values which approximate the time to reach 99% steady-state (i.e. $T_{0.99}$) and the accumulation ratio (R_{acc}). Both of these derivations require calculation of the apparent volume of distribution (V_{app}) at steady-state and the effective half-life ($T_{1/2}$). The apparent volume (V_{app}) was calculated as the sum of the central (V_1 or V_c) and peripheral (V_2 or V_p) compartment volumes. The effective half-life ($T_{1/2}$) was calculated as 0.693*Vapp/CL. The AUC was calculated from the mean dose (µg/kg) divided by the mean clearance (mL/hr/kg). The time to reach 99% steady-state ($T_{0.99}$) and the accumulation ratio (R_{acc}) were then calculated using the formulas [$T_{0.99} = -3.32*T_{1/2}\text{Log}(1-0.9999)$], and [$R_{acc} = (1-2^{-\varepsilon})^{-1}$ where ε equals the ratio tau/$T_{1/2}$ and where Tau was estimated using the average repeated dosing interval of 504 hours. Re area was calculated as the ratio of the single and multiple dose areas under the curve (AUCss,inf/AUCsd,inf).

Kinetics appear linear regarding clearance. Elimination half-life and distribution volume are both dose-dependent.

- Absorption

Eculizumab is administered intravenously with 100% bioavailability.

- Distribution

Eculizumab‘s distribution seems to be primarily limited to the vascular space.
The amount of eculizumab associated with non-C5 plasma proteins and the effects of plasma protein binding on eculizumab distribution have not been determined.

- Elimination

Eculizumab has a mean elimination half-life of approximately 131 hours (from the multiple dose study in RA patients). Using sparse PK/PD data from 40 PNH patients in C04-001, a standard one-compartmental model analysis yielded higher estimates for $T_{1/2}$ (271 hours)

- Excretion

No specific clinical studies have been conducted to evaluate specific pathways of eculizumab excretion. According to the applicant, due to its molecular size (148 kD) eculizumab like other immunoglobulins are expected to be excluded from filtration in normal kidneys.

- Metabolism

Eculizumab contains only naturally occurring amino acids and has no known active metabolites. Human antibodies are predominately catabolised by lysosomal enzymes to small peptides and amino acids.

- Dose proportionality and time dependency

Eculizumab appears to demonstrate dose-dependent elimination and/or distribution in the therapeutic dosing range. While the exact mechanisms of its elimination are not completely understood, eculizumab clearance is thought to be mediated both by specific and non-specific pathways. Saturable specific pathways may be associated with the soluble antigenic target C5, and with the FcRn receptor.

- Intra- and inter-individual variability

No clinical studies have been specifically conducted to discern the effects of age, race, gender, or metabolic status (renal or hepatic impairment) on the PK of eculizumab.

- Pharmacokinetics in target population

PK parameters were determined from two PNH patient studies: C02-001 (pilot study) and C04-001 (pivotal study).

PK Parameter Summary using One-Compartment Model

N = 40	**CL (mL/hr/kg)**	**V_d (mL/kg)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
Average	0.311183	110.3	0.002776	271.7
SD	0.125097	17.9	0.000817	81.6
% SD	40.20	16.2	29.45	30.0
Min	0.150944	79.1	0.001376	134.1
Max	0.745052	144.1	0.005169	503.8
Median	0.289969	108.3	0.002793	248.2

- Special populations

No formal special patient population clinical studies have been performed

Pharmacodynamics

- Mechanism of action

Eculizumab binds with high affinity to human serum complement protein C5 (hC5) and inhibits the production of C5b-9 by blocking C5 cleavage in a dose-dependent manner such that a 1:1 molar ratio of eculizumab to C5 is sufficient for C5 inhibition.

A PD assay was developed to measure the haemoglobin release from antibody-sensitized chicken red blood cells (RBC) due to lysis by the terminal complement complex (C5b-9) deposited on the surface of these cells after activation of the classical pathway. C5 cleavage being an obligatory step, the ability of patient serum to lyse sensitized chicken RBCs in this assay is directly proportional to the amount of residual circulating C5 (not neutralised by eculizumab).

- Primary and Secondary pharmacology

Eculizumab inhibits complement-mediated haemolysis of sensitized red blood cells *in vitro* and similarly blocks the lysis of PNH red blood cells *in vivo* in a dose-dependent manner, thus demonstrating effective inhibition of the terminal complement pathway.

The affinity of eculizumab for C5 has been measured in non-clinical studies at 30 picomolar by ELISA and 120 picomolar by surface plasma resonance. Eculizumab demonstrates no appreciable species cross-reactivity. It is highly species specific. with no appreciable activity against chimpanzee, baboon, rhesus or cynomologous monkey, pig, rabbit, guinea pig, or rat complement.

There are no known secondary pharmacological effects of eculizumab because of the highly specific interaction of eculizumab with its target as indicated by the lack of cross-reactivity with C5 from other species.

- Relationship between plasma concentration and effect

Relationship between eculizumab serum concentration and inhibition of complement activity across multiple disease states suggests a specific minimum serum concentration that is sufficient to block complement activity in most patients. This relationship exists at any time during the PK profile, at peaks and troughs, whether in steady-state or not, and at any time during the dosing interval. Taken together, these data suggest that a target trough serum eculizumab concentration of approximately 35µg/mL will be required to maintain complete serum complement inhibition in patients regardless of their underlying disease. For the intended PNH population, maintaining terminal complement inhibition is necessary to mitigate intravascular haemolysis.

Thus, the dosing schedule of 600 mg weekly for four weeks followed one week later by 900 mg and then a maintenance schedule of 900 mg every 14 (± 2) days has been chosen to maintain the serum concentration of approximately 35 µg/mL in nearly all treated PNH patients.

No clinically relevant PD interactions between eculizumab and other medications or substances have been observed in any of the clinical subjects.

Clinical Efficacy

- Dose response study

A formal dose response study was not performed in PNH patients.
Prior to the commencement of the pilot PNH study, 722 unique patients had been exposed to eculizumab in dosing durations ranging from 1 day to almost 3 years. Pharmacokinetic and studies in all relevant patient populations demonstrated that an eculizumab serum concentration of 35 µg/mL or greater effectively inhibited complement-related haemolysis using in vitro assays on serum samples from these patients. Extensive PK/PD modelling from human studies indicated that fixed dosing was not obviously different (from dosing by weight. A review of safety data, including adverse events (AEs) and clinical laboratory results, and the potential for the development of human antihuman antibody (HAHA) revealed that the chosen dose would be expected to be safe and well tolerated in the PNH population. Data from the C02-001 open-label pilot PNH study showed that treatment with eculizumab at induction doses of 600 mg per week and maintenance doses of 900 mg every 2 weeks in PNH patients demonstrated statistically significant improvements in efficacy endpoints of serum lactate dehydrogenase (LDH) and units of PRBCs.

- Main study

Study C04-001 (TRIUMPH)

Study C04-001 (TRIUMPH): A Haemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi Centre, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Haemoglobinuria Patients

METHODS

Study Participants

This was a phase III, randomized, multicentre, double blind, placebo-controlled clinical trial using eculizumab in PNH patients.

The main inclusion criteria were age ≥18 years, patients must have required at least 4 episodes of transfusions in the 12 months prior to Visit 1 for anaemia or anaemia-related symptoms, patients must have a glycosylphosphatidylinositol-deficient red blood cell clone (type III cells) by flow cytometry of ≥10%, patients who were taking erythropoietin had to have been on a stable dose for 26 weeks prior to the screening visit (Visit 1) and the dose remained stable during the observation period and the treatment phase and patients must have had a platelet count of at least 100,000/mm3 either at Visit 1 or during the observation period.

The main inclusion criteria were patients whose mean haemoglobin level prior to transfusion over the previous 12 months was greater than 10.5 g/dL and patients whose absolute neutrophil count of less than or equal to 500/μL.

Treatments

The treatment was placebo or eculizumab. Placebo patients received placebo IV once a week (within 5-9 days) for 5 doses, then once every 2 weeks (within 12-16 days).

Eculizumab patients received 600 mg of eculizumab IV once a week (within 5-9 days) for 4 doses, followed by 900 mg eculizumab IV 1 week later for 1 dose, then 900 mg eculizumab IV every 2 weeks (within 12-16 days). There was a total of 26 weeks of treatment.

Objectives

The primary objective was to evaluate the safety and efficacy of eculizumab in the study population. The co-primary endpoints were haemoglobin stabilization and number of packed red blood cell (PRBC) units transfused.

Secondary objectives were transfusion avoidance, haemolysis as measured by lactate dehydrogenase (LDH) area under the curve (AUC) during the treatment period from baseline to Visit 18, and Functional Assessment of Chronic Illness Therapy fatigue (FACIT-Fatigue) scale changes from Baseline to Visit 18. Exploratory endpoints included LDH changes from baseline to Visit 18, European Organisation for Research and Treatment of Cancer quality-of-life (QoL) questionnaire (EORTC QLQ-C30) changes from baseline to Visit 18, thrombosis, platelet activity, and measures of nitric oxide (NO) and free haemoglobin from baseline to Visit 18.

Outcomes/endpoints

The co-primary endpoints were haemoglobin stabilization and units of PRBCs transfused during the treatment phase. For the haemoglobin stabilization endpoint, patients that reached or dropped below their predetermined haemoglobin set point did not achieve haemoglobin stabilization.

In order to assess the robustness of results for the co-primary endpoints, a sensitivity analysis was performed.

Secondary endpoints included transfusion avoidance, haemolysis as measured by the AUC of LDH, and QoL as measured by the FACIT-Fatigue scale.

Exploratory endpoints included changes of LDH from Baseline to Visit 18, QoL changes as measured by the EORTC QLQ-C30 instrument, thrombosis rate, platelet activity, and free haemoglobin and NO measures.

Sample size

The sample size was selected to show that incidence rates of haemoglobin stabilization during the treatment phase are 20% and 55% for the placebo and eculizumab groups, respectively. The median units of transfusion during the treatment phase were assumed to be 6 and 2 for the placebo and eculizumab groups, respectively. Based on these assumptions, with 35 patients per group and a 5% Type I error rate for each of the co primary endpoints, the study sample size of 75 patients was designed for approximately 82% power, using the 2-sided Fisher exact test for haemoglobin stabilization and the Wilcoxon rank sum test for units of PRBCs transfused.

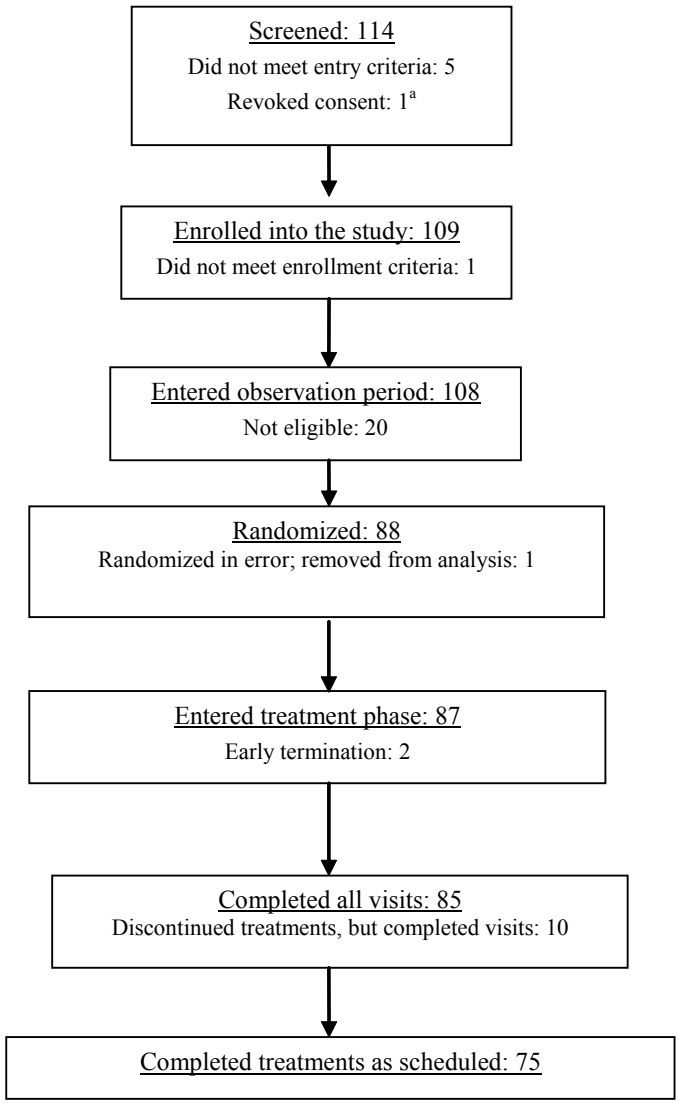
Randomisation
All the patients were randomly assigned in a 1:1 ratio to placebo or eculizumab by a centralized allocation method. Random assignment took place within 10 days of the qualifying transfusion in the observation period. Randomization was stratified according to the number of PRBC units transfused within 1 year prior to screening. The 3 randomization strata used were:
• between 4 and 14 units, inclusive
• between 15 and 25 units, inclusive, and
• greater than 25 units.

Blinding
The double-blind was maintained by using identical study drug kits and labels for eculizumab and placebo.

Statistical methods
The primary analysis method for the co-primary endpoints was the Wilcoxon rank sum test.
The analysis of the secondary endpoint of transfusion avoidance was carried out using the 2-sided Fisher exact test. As a sensitivity analysis, those patients who dropped out of the study during the treatment phase prior to having a transfusion are classified as not requiring a transfusion.
The AUC of LDH from Baseline to Visit 18 is presented for each patient and was analyzed using the Wilcoxon rank sum test. For the co-primary endpoints and all secondary endpoints, the primary analysis was based on the ITT population.
The FACIT-Fatigue scale collected secondary endpoint QoL data and was scored according to the scoring guideline for this instrument. The change of total FACIT-Fatigue scale score from Baseline was analyzed using a mixed-effects model with Baseline as a covariate, treatment and time as fixed effects, and patient as a random effect.
The exploratory endpoint of the changes of LDH from Baseline up to Visit 18 was analyzed using a mixed-effects model, with treatment and time as a fixed effect and patient as a random effect. Also, LDH change from Baseline to Visit 18 was analyzed using the Wilcoxon rank sum test.

RESULTS

Participant flow



[a] This patient was screened, but revoked consent. By local law, no part of this patient's data was permitted to be entered into the study database.

Recruitment

The patients were recruited at 34 sites located in North America, Europe, and Australia: the first patient/first visit in August 2004 and the last patient/last visit in December 2005.
The study period was 1.33 years.

Conduct of the study

Patients who discontinued study drug during the treatment phase but remained in the study in order to be eligible for a separate extension study underwent all the assessments that would normally have been done at the Visits 4, 5, 6, 7, 9, 11, 13, 15, 17, and 18, as appropriate, excluding PK/PD assessments.
Patients who discontinued the study drug and chose to leave the study before completing all visits were asked to complete all the assessments that would have been performed at Visit 18, the last scheduled study visit.

Baseline data

Demographic and disease baseline characteristics are shown below.

Baseline Demographics and Other Baseline Characteristics Population: ITT

	C04-001	
	Placebo N = 44	Soliris N = 43
Mean Age (SD)	38.4 (13.4)	42.1 (15.5)
Gender - Female (%)	29 (65.9)	23 (53.5)
History of Aplastic Anaemia or MDS (%)	12 (27.3)	8 (18.7)
Concomitant Anticoagulants (%)	20 (45.5)	24 (55.8)
Concomitant Steroids/Immunosuppressant Treatments (%)	16 (36.4)	14 (32.6)
Discontinued treatment	10[1]	2[2]
PRBC in previous 12 months (median (Q1,Q3))	17.0 (13.5, 25.0)	18.0 (12.0, 24.0)
Mean Hgb level (g/dL) at setpoint (SD)	7.7 (0.75)	7.8 (0.79)
Pre-treatment LDH levels (median, U/L)	2,234.5	2,032.0
Free Haemoglobin at baseline (median, mg/dL)	46.2	40.5

[1]Completed study visits. [2]One patient discontinued due to an AE unrelated to Soliris; one patient discontinued due to logistics.

Outcomes and estimation

Overview of Efficacy Endpoint Results

	C04-001		
	Placebo N = 44	**Soliris** N = 43	**P – Value**
Coprimary endpoints			
Percentage of patients with stabilised haemoglobin levels at end of study	0	49	< 0.001
PRBC transfused during treatment (median)	10	0	< 0.001
Secondary endpoints			
Transfusion Avoidance during treatment (%)	0	51	< 0.001
LDH levels at end of study (median, U/L)	2,167	239	< 0.001
LDH AUC at end of study (median, U/L x Day)	411,822	58,587	< 0.001
Free haemoglobin at end of study (median, mg/dL)	62	5	< 0.001
FACIT-Fatigue (effect size)		1.12	< 0.001

Primary efficacy results

Haemoglobin stabilization was achieved in 48.8% of eculizumab patients indicating that these patients did not require any transfusions during the 26-week study duration because their haemoglobin levels remained above their individual set points. Haemoglobin stabilization did not occur among any of the placebo patients and the difference between the treatment groups is statistically significant (P<0.001). The sensitivity analysis performed on the haemoglobin stabilization endpoint confirms this efficacy

outcome. Those Soliris treated patients who did not achieve haemoglobin stabilisation still experienced improvements in anaemia with statistically significant reductions in transfusion requirements (p<0.001).
After study treatment, statistically significant differences in haemoglobin stabilization were observed in the low and mid transfusion strata but not the high stratum (*P*=0.09).

Haemoglobin Stabilization (ITT)

Randomization strata	Haemoglobin stabilization?	Eculizumab N = 43 n/N (%)	Placebo N = 44 n/N (%)	*P* Value[a]
Overall (N=87)	Yes	21/43 (48.8)	0/44 (0.0)	0.000000014
	No	22/43 (51.2)	44/44 (100)	
4 to 14 units (n=30)	Yes	12/15 (80.0)	0/15 (0.0)	0.000010521
	No	3/15 (20.0)	15/15 (100)	
15 to 25 units (n=35)	Yes	5/17 (29.4)	0/18 (0.0)	0.019061584
	No	12/17 (70.6)	18/18 (100)	
>25 units (n=22)	Yes	4/11 (36.4)	0/11 (0.0)	0.090225564
	No	7/11 (63.6)	11/11 (100)	

Note: Stabilization was calculated between Baseline and 26 weeks after first dose.
P values were calculated using Fisher's exact test.

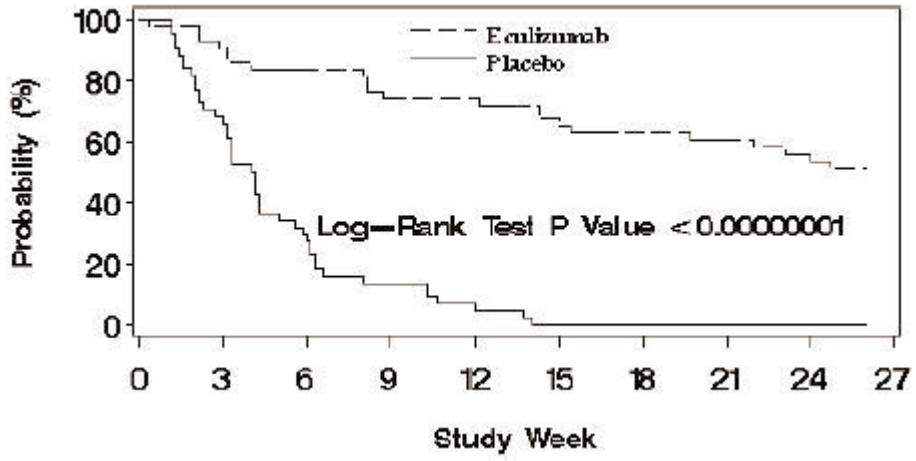
Summary of Units Transfused from Baseline to 26 Weeks (ITT)

Randomization strata	Eculizumab	Placebo	*P* value[a]
Overall (N)	43	44	<0.000000001
Mean (standard error)	3.0 (0.67)	11.0 (0.83)	
Median	0.0	10.0	
Range	(0.0, 16.0)	(2.0, 21.0)	
4 - 14 units (n)	15	15	0.000002311
Mean (standard error)	0.4 (0.29)	6.7 (0.72)	
Median	0.0	6.0	
Range	(0.0, 4.0)	(2.0, 12.0)	
15 - 25 units (n)	17	18	0.000665129
Mean (standard error)	4.2 (1.14)	10.8 (1.17)	
Median	2.0	10.0	
Range	(0.0, 15.0)	(2.0, 21.0)	
> 25 units (n)	11	11	0.000301977
Mean (standard error)	4.5 (1.59)	17.0 (1.04)	
Median	3.0	18.0	
Range	(0.0, 16.0)	(10.0, 20.0)	

P values were calculated using Wilcoxon's rank sum test.

Secondary efficacy results
Transfusion avoidance was achieved in half of the patients treated with eculizumab while in none of those treated with placebo. This difference was observed independently of the stratum considered.

Kaplan-Meier Plot of Time to First Transfusion During the Study



Treatment with eculizumab mitigates intravascular haemolysis, as measured by LDH AUC, with median LDH AUC of 411,822 U/L x Day in placebo-treated patients and 58,587 U/L x Day in eculizumab-treated patients (P<0.001).

Randomization strata	Eculizumab	Placebo	*P* value[a]
Overall (N)	43	44	<0.000000001
Mean (standard error)	3.0 (0.67)	11.0 (0.83)	
Median	0.0	10.0	
Range	(0.0, 16.0)	(2.0, 21.0)	
4 - 14 units (n)	15	15	0.000002311
Mean (standard error)	0.4 (0.29)	6.7 (0.72)	
Median	0.0	6.0	
Range	(0.0, 4.0)	(2.0, 12.0)	
15 - 25 units (n)	17	18	0.000665129
Mean (standard error)	4.2 (1.14)	10.8 (1.17)	
Median	2.0	10.0	
Range	(0.0, 15.0)	(2.0, 21.0)	
> 25 units (n)	11	11	0.000301977
Mean (standard error)	4.5 (1.59)	17.0 (1.04)	
Median	3.0	18.0	
Range	(0.0, 16.0)	(10.0, 20.0)	

P values were calculated using Wilcoxon's rank sum test.

Assessments of functional status as the Fatigue Scale Scoring also shown relevant differences between groups.

Minimally Important Difference Change in FACIT-Fatigue Score at Week 26 (ITT)

Improved by at least 4 points?	Eculizumab n (%)	Placebo n (%)	*P* Value[a]
Yes	22 (53.66)	8 (20.51)	0.0028
No	19 (46.34)	31 (79.49)	

The *P* value was calculated using Fisher's exact test.

A reduction in intravascular haemolysis as measured by serum LDH levels was sustained for the treatment period and resulted in increased transfusion avoidance, a reduced need for RBC transfusion and less fatigue.

- Clinical studies in special populations

No studies have been performed in patients with renal or hepatic impairment.

- Supportive studies

Study C04-002 (SHEPHERD): Safety in Haemolytic PNH Patients Treated with Eculizumab: A Multi-centre Open-label Research Design Study

This was a phase III, open-label, multicentre study of eculizumab administered as an intravenous (IV) infusion to 97 PNH patients with haemolysis for a total of 52 weeks, with a pre-specified 26-week interim analysis.
The primary objective was to evaluate the safety of eculizumab in patients with at least one blood transfusion in the previous 24 months.
Patients ≥ 18 years who had received at least 1 blood transfusion in the previous 24 months were eligible. A PNH type III erythrocyte population ≥ 10%, platelets ≥ 30,000/mL, and lactate dehydrogenase (LDH) ≥ 1.5 times the upper limit of normal were also required as inclusion criteria. Patients who had received another investigational drug within 30 days of the first visit or had an absolute neutrophil count < 500/μL were excluded. Patients with complement deficiency, active bacterial infection, prior meningococcal disease, or prior bone marrow transplant were also excluded.
During the induction period, patients received 600 mg of eculizumab administered by IV infusion once a week for 4 doses, followed by 900 mg eculizumab IV 1 week later for 1 dose. During the maintenance period, patients received 900 mg eculizumab IV every 2 weeks for a total of 52 weeks treatment. Each dose was administered by IV infusion over 25 to 45 minutes.
The primary surrogate of efficacy was haemolysis as measured by LDH area under the concentration curve (AUC). The secondary endpoints were haemolysis as measured by LDH change from baseline and quality of life (QoL) as measured by the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue). Exploratory endpoints were Quality of life as measured by the European Organisation for Research and Treatment of Cancer Quality-of-Life Questionnaire Core 30 (EORTC QLQ-C30), thrombosis, platelet activity, nitric oxide (NO), and free haemoglobin measures.

RESULTS

Overview of Efficacy Results

	Soliris N = 97	**P – Value**
PRBC transfused during treatment (median)	0.0	< 0.001
Transfusion Avoidance during treatment (%)	51	< 0.001
LDH levels at end of study (median, U/L)	269	< 0.001
LDH AUC at end of study (median, U/L x Day)	-632,264	< 0.001
Free Haemoglobin at end of study (median, mg/dL)	5	< 0.001
FACIT-Fatigue (effect size)	1.14	< 0.001

* Results from study C04-002 refer to pre- versus post-treatment comparisons.

Treatment with eculizumab mitigates intravascular haemolysis, as measured by LDH AUC change from baseline (primary endpoint), with a median reduction in LDH AUC at 52 weeks of 632,264 U/L x Day (P<0.001).

Treatment with eculizumab mitigates intravascular haemolysis, as measured by median LDH change

from baseline, with median LDH decreasing from 2,051 U/L pre-treatment to 269 U/L at 52 weeks (P<0.001). Assessments of functional status as the Fatigue Scale Scoring also showed relevant differences from baseline.

Study E05-001 (An extension study of C04-001, C04-002, and C02-001): A Phase III, Open-Label, Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic Paroxysmal Nocturnal Haemoglobinuria (PNH) Who Have Participated in the TRIUMPH (C04-001), SHEPHERD (C04-002) or X03-001 Studies.

E05-001 is a phase III, open-label, extension study of eculizumab in patients with transfusion dependent, haemolytic PNH who have participated in the TRIUMPH (C04- 001), SHEPHERD (C04-002), or X03-001 Studies. Thus virtually all patients enrolled in eculizumab trials in PNH were entered in this extension protocol. TE events is the leading cause of morbid-mortality in patients with PNH.

The E05-001 statistical analysis plan prospectively identified that TE event rates would be analyzed as a cumulative event rate in the combined eculizumab-treated study population including data from all PNH trials (C02-001, E02-001, X03-001, C04-001, and C04-002). As was specified for all E05-001 planned comparisons, TE event rates during eculizumab treatment were compared to pre-eculizumab event rates in the same patients. However, it should be noted that findings from these studies were shown in non-controlled clinical trials.

Eculizumab treatment substantially reduced TE events in the combined eculizumab-treated PNH patient population. The reduction in TE event rate during eculizumab treatment as compared to individual matched patients pre-treatment was statistically significant (P<0.001).

Updated study E05-001 analysis of November 2006 data base lock of all eculizumab-treated patients which includes an additional 120 patient years of exposure shows that the reduction in thromboembolism risk has been maintained. When compared to the rate of thromboembolism events in all enrolled patients before treatment, eculizumab treatment results in a reduction in the thromboembolism event rate in the same patients in each of the individual clinical studies and a significant 7-fold reduction in the thromboembolism event rate overall from 7.37 events per 100 patient years pre-eculizumab treatment to 1.07 events per 100 patient years during eculizumab treatment (P<0.001) (see table below).

E05-001; Comparison of Thrombosis/MAVE rates Across all Eculizumab PNH Studies: Total Pre-Eculizumab Treatment Rate vs. Eculizumab Treatment Rate

	C04-001	C04-002	C02-001/ E02-001/ X03-001	E05-001 All studies combined
Pre-treatment				
Patients (n)	43	97	11	195
Thrombosis events (n)	16	91	5	124
Patient years (n)	309.0	718.3	161.7	1683.4
Rate per 100 years	5.18	12.67	3.09	7.37
Eculizumab treatment				
Patients (n)	43	97	11	195
Thrombosis events (n)	0	2	0	3
Patient years (n)	21.8	96.9	34.2	281.0
Rate per 100 years	0.00	2.06	0.00	1.07[1]

[1]p = 9 x 10-14

- Discussion on clinical efficacy

The demonstration of efficacy of Soliris in PNH patients with haemolysis was assessed in a randomized, double-blind, placebo-controlled 26 week study (C04-001). PNH patients were also treated with Soliris in a single arm 52 week study (C04-002) and in a long term extension study (E05-001)

In TRIUMPH, study patients treated with Soliris had significantly reduced ($p < 0.001$) haemolysis resulting in improvements in anaemia as indicated by increased haemoglobin stabilization and reduced need for RBC transfusions compared to placebo treated patients. These effects were seen among patients within each of the three pre-study RBC transfusion strata (4 - 14 units; 15 - 25 units; > 25 units). After 3 weeks of Soliris treatment, patients reported less fatigue and improved health-related quality of life. In SHEPHERD, a reduction in intravascular haemolysis as measured by serum LDH levels was sustained for the treatment period and resulted in increased transfusion avoidance, a reduced need for RBC transfusion and less fatigue. From the 195 patients that originated in C04-001, C04-002 and C02-001, Soliris-treated PNH patients were enrolled in a long term extension study (E05-001). All patients sustained a reduction in intravascular haemolysis over a total Soliris exposure time ranging from 10 to 54 months. There were fewer thrombotic events with Soliris treatment than during the same period of time prior to treatment. However, this finding was shown in non-controlled clinical trials.

Clinical safety

Actually, the safety and efficacy of eculizumab were assessed in three separate studies including

- Study C02-001: an 11 patient open-label 12 week phase 2 study; this study had two study-specific extension studies (E02-001 and X03-001) totalling an additional 156 weeks).
- Study C04-001 (TRIUMPH): a 87 patient randomized, double-blind, placebo-controlled 26-week phase 3 study,
- Study C04-002 (SHEPHERD) : an ongoing 97 patient open-label 52 week phase 3 study,

Since eculizumab is a humanised monoclonal antibody, expected class effects included immune responses such as post-infusion reactions and human-antibodies (HAHA), though the frequency of these across PNH and non-PNH patients has been similar between Soliris and placebo treated patients. In addition, due to the inhibition of the distal complement components, an increased incidence of infections by encapsulated bacteria was expected. Literature describes an increased susceptibility to *Neisseria* in patients with terminal complement deficiencies, particularly *N. meningitides*. Pneumococcal or *Haemophilus influenzae* infections are not described as increased in the literature as opposed to in patients with early complement components (C1-C4). Nevertheless, although with a less empirical basis, general infections were considered as a relevant potential risk. The potential for severe haemolysis following discontinuation of eculizumab due to the possible increase of the PNH RBC clone during treatment also emerged as a potential risk haemolysis.

- Patient exposure

All patients successfully completing studies previously mentioned were eligible to enrol in an ongoing open-label 104 week phase 3 extension study (Study E05-001) which is anticipated to enrol approximately 190 patients.

The PNH safety database reflects a cumulative 264 patient-years during review of the dossier. Supportive safety data were obtained in 11 clinical studies that included 716 patients (492.20 patient years) exposed to eculizumab in six other indications.

- Adverse events

Adverse events reported at a very common (≥10.0%) or common (5.0 – 10.0%) frequency with eculizumab in a total of 140 patients in C04-001 and C04-002 are listed by system organ class and preferred term in the following table. Adverse events were mostly mild to moderate in severity.

Adverse Events Reported in Studies C04-001 and C04-002

Adverse Event	**Preferred Term**	
System Organ Class	Very Common (>1/10)	Common (5/100 – 1/10)
Infections and infestations	Urinary tract infection Nasopharyngitis Upper Respiratory Tract Infection	Respiratory Tract Infection Herpes Simplex Sinusitis Viral Infection Gastroenteritis
Psychiatric disorders		Insomnia
Nervous System Disorders	Dizziness Headache	
Respiratory, Thoracic and Mediastinal Disorders		Epistaxis Pharyngolaryngeal pain Cough
Gastrointestinal Disorders	Diarrhoea Nausea	Vomiting Abdominal Pain Abdominal Pain Upper Constipation
Skin and Subcutaneous Tissue Disorders		Rash Pruritis
Musculoskeletal and Connective Tissue Disorders	Back Pain Arthralgia	Myalgia Pain in extremity Muscle cramp
General Disorders and Administration Site Conditions	Pyrexia	Influenza-Like Illness Fatigue
Injury, Poisoning and Procedural Complications	Contusion	

In the combined Phase III PNH studies C04-001 and C04-002, headache was the most common adverse event observed with eculizumab. Headaches were observed in 49.3% (69/140 patients) of patients treated with eculizumab and 27.3% (12/44 patients) in placebo-treated patients and were mild/moderate in all but 5 eculizumab treated and 1 placebo-treated patients. Most headaches were mild and did not persist after the initial administration phase of Soliris. In addition, the following adverse events were increased in frequency by 5% or more with Soliris as compared to placebo: headache (49.3%), nasopharyngitis (25.0%), nausea (17.1%), pyrexia (14.3%), myalgia (7.9%), fatigue (7.9%), and herpes simplex (5.7%). 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. In C04-001 and C04-002, Soliris treatment was discontinued following adverse events in one patient (pregnancy). This event was unrelated to Soliris.

- Serious adverse events and deaths

In study C04-001, 4 eculizumab patients reported 5 severe TEAEs including haemolysis, abscess limb (left arm), headache, pregnancy, and exacerbation of PNH that required hospitalization. Eight placebo patients reported 14 severe TEAEs including anaemia, neutropenia (2 events), constipation, cellulitis, folliculitis, viral infection, dehydration, headache, abdominal pain, upper abdominal pain, and exacerbation of PNH that required hospitalization (3 events). Serious adverse reactions occurred among 4 (9%) patients receiving Soliris and 9 (21%) patients receiving placebo. The serious reactions included infections and progression of PNH. No deaths occurred in the study.

In C04-001 and C04-002 combined, SAE occurred in more than one patient and at a frequency greater than placebo for two SAEs, namely: intervertebral disc protrusion (1.4%, vs 0%) and headache (1.4% vs 0%).

The SAE incidence rates for patients who were enrolled in C04-001 and have accumulated 6 months of exposure in E05-001 were generally similar compared to those observed in the C04-001 eculizumab and placebo treatment groups.

There were three deaths in PNH patients treated with eculizumab and two deaths in non-PNH patients treated with eculizumab. No death related to eculizumab was reported by the applicant. The first patient with PNH died due to cerebral herniation with a previous history of multiple TE events. He had experience a TE event following subtherapeutic anticoagulation, after the withdrawal of eculizumab. The investigator considered not to be related to eculizumab treatment. The second died due to chronic myelomonocytic leukaemia in a patient with a previous history of myelodisplastic syndrome (considered not related to eculizumab treatment by the investigator). The third died due to a cerebrovascular accident in a patient treated under compassionate use with life-threatening anaemia and presumed intermittent sepsis (considered also not related to eculizumab treatment by the investigator). The two cases occurred in non-PNH patients were: an accidental electrocution (eculizumab unrelated, according to the investigator) and a sepsis due to Candida species following gallbladder surgery complicated by abscess formation and bowel resection for a perforation of the diverticulum.

- Laboratory findings

In 151 patients included in the eculizumab population versus 44 with placebo, increased RBC indices MCH, MCHC and MVC, as well as a positive shift in the reticulocyte differential count were observed in eculizumab-treated patients but not in the placebo arm (shift table analysis). LDH, AST, CK and free haemoglobin levels shifted from high to normal. There was also a shift to high ferritin in eculizumab-treated PNH-patients.
The shifts from normal to low WBC (24 patients treated with eculizumab vs 0 with placebo) and absolute neutrophil counts were slight and without apparent clinical significance. There was also a shift from normal to high in alkaline phosphatase (normal to high in 19 patients treated with eculizumab versus 0 in placebo), direct bilirubin levels (42 patients treated with eculizumab vs 8 with placebo) and APTT (43 patients treated with eculizumab versus 2 treated with placebo). No shifts were detected for INR or ATT ratio. 28/43 of these patients were on concomitant anticoagulant medication. Three patients for eculizumab and 0 for placebo shifted from normal to high for creatinine. Serum total protein and uric acid showed shifts towards normal.

666 patients treated with eculizumab were included and 221 patients with placebo. In the non-PNH patients treated with eculizumab, haematological parameters (monocytes and RBCs) tended to shift towards normal. ALT (15 for eculizumab, 4 for placebo), AST (12 for eculizumab, 2 for placebo), BUN (40 for eculizumab, 10 for placebo) and uric acid (27 for eculizumab, 8 for placebo) shifted to high in treated populations and creatinine levels (27 for eculizumab, 8 for placebo) in both treated and untreated patients. There were no shifts in creatinine levels. Serum albumin and total protein count rose to normal.

- Safety in special populations

There have been no studies of eculizumab in patients with any disease and less than 18 years of age.

- Immunological events

Adverse events related to the specific humanized monoclonal antibody drug class may include infusion reactions and immunogenicity. For all HAHA (Human Anti-Human Antibody) analyses, positive and negative both IgG and IgM responses were provided at each study visit where a sample was collected and analysed.

In the C04-001 study, a larger proportion of patients reported a 24 hour post-infusion AE with eculizumab than with placebo (79.1% versus 65.9%), and a slightly larger proportion of eculizumab patients reported a 48 hour post-infusion AE (86.0% versus 70.5%).

1 of 43 eculizumab-treated patients and 1 of 44 placebo-treated patients demonstrated a detectable HAHA response during the C04-001 study. The single response in the eculizumab cohort was of low titer, and had no apparent effect on the PK/PD of eculizumab. There was no evidence of reduced biological or clinical activity of eculizumab in PNH patients irrespective of HAHA. Infusion reactions, including allergic reactions, were not associated with any HAHA responses.

In general, no neutralizing HAHA responses have been observed with eculizumab treatment in 911 patients, some of whom have had chronic exposure for up to 4 years.

	PNH		**Non-PNH**		**All Eculizumab Studies**	
	Placebo	Eculizumab	Placebo	Eculizumab	Placebo	Eculizumab
Total Patients with HAHA Measurement, n	44	151	206	677	250	828
Patients with a Positive HAHA Response, n (%)	1 (2.3)	2 (1.3)	11 (5.3)	26 (3.8)	12 (4.8)	28 (3.4)
Patients with an IgM Response, n (%)		1 (0.7)	5 (2.4)	7 (1.0)	5 (2.0)	8 (1.0)
Patients with an IgG Response, n (%)	1 (2.3)	1 (0.7)	7 (3.4)	20 (3.0)	8 (3.2)	21 (2.0)
Patients with and IgG and IgM Response, n (%)	-	-	1 (0.5)	1 (0.1)	1 (0.4)	1 (0.1)
Patients with Persistent Response	-	1 (0.7)[2]	1 (0.5)[3]	1 (0.1)[3]	1 (0.4)[3]	2 (0.2)[4]

[1]Source: Table 2.7.4.7-94
[2]One, IgM
[3]One, IgG.
[4]One, IgG and one IgM

- Safety related to drug-drug interactions and other interactions

Soliris was investigated in PNH studies in which patients received conventional treatments as background treatment. Although no formal drug interaction studies have been performed, Soliris has been administered to patients treated concomitantly with a broad range of medications commonly used in patients with PNH including erythropoietin, corticosteroids, anabolic steroids, anticoagulants, and immunosuppressants. Specific drug interaction studies have not been conducted with Soliris.

- Discontinuation due to AES
  - The single DCAE [explain] with eculizumab in C04-001 was due to pregnancy. The patient's pregnancy continued through to the full term with no foetal abnormalities reported.
  - Subsequent to the database lock, a single E05-001 patient withdrew from the study due to an SAE, progression of his pre-existing MDS to CMML, which was determined to be unrelated to study medication. The patient subsequently died due to this pre-existing condition.
  - A second patient discontinued due to newly recognized MDS. The patient was withdrawn to prepare for possible bone marrow transplantation. The cause of the MDS was identified to be the patient's underlying indication (PNH) and possibly related to eculizumab.
  - One patient discontinued C04-002 due to intervertebral disc protrusion AE. This patient subsequently died. Both events were unrelated to eculizumab treatment.

Discontinuation of or non-compliance with eculizumab treatment regimen was not associated with subsequent serious haemolysis in PNH studies C02-001, C04-001, C04-002, and E05-001. Serious haemolysis did not ensue in each of five patients who discontinued eculizumab treatment after receiving eculizumab for a range of 29-715 days prior to withdrawal. Updated safety database shows that in PNH clinical studies, 16 patients discontinued the Soliris treatment regimen. Serious haemolysis was not observed.

Furthermore, in an additional 9 patients with 12 episodes of non-compliance with the eculizumab

dosing regimen, there were no episodes observed in which patients demonstrated increased haemolysis over pre-treatment levels with signs and/or symptoms of serious haemolysis.
Patients that discontinued eculizumab treatment were effectively managed by standard of care and none reported serious haemolysis subsequent to discontinuation of eculizumab therapy.

- Post marketing experience

N/A

- Discussion on clinical safety

Safety data for eculizumab as a treatment for PNH was collected in six clinical studies that included 195 patients (147.44 patient-years) treated with eculizumab with approximately 70 % (138) treated for at least 26 weeks and approximately 20 % treated for at least 52 weeks. Updated safety information in PNH reflects 264 patient-years of Soliris exposure. Supportive data were obtained in 11 clinical studies that included 716 patients (492.20 patient years) in other indications.
The most commonly reported adverse reactions in eculizumab treated patients are headache, nasopharyngitis, nausea, pyrexia, myalgia, fatigue, and herpes simplex, each occurring in 5 or more out of 100 patients. Eculizumab treatment did not seem to be associated with an increase in severity of AEs in treated patients. No cumulative, irreversible toxicities or treatment related deaths were reported.

Identified risks are general infections, especially meningococcal infections, haemolysis after drug discontinuation and haematologic abnormalities.

Three cases of meningococcal infections were reported, one in an unvaccinated non-PHN (idiopathic membranous glomerulonephritis –IMG-) patient and one in each of two vaccinated PNH patients. The first case was considered as possibly related to eculizumab whilst the second two were considered to be probably related to eculizumab by investigators. Prior to initiating Soliris therapy, it is recommended that PNH patients should receive immunizations according to current immunization guidelines. Additionally, all patients must be vaccinated against meningococcus at least 2 weeks prior to receiving Soliris. If available, tetravalent, conjugated vaccines are recommended.

Infrequent, low titre antibody responses have been detected in Soliris treated patients across all PNH and non-PNH studies with a frequency (3.4%) similar to that of placebo (4.8%). No patients have been reported to develop neutralizing antibodies following therapy with Soliris, and there has been no observed correlation of antibody development to clinical response or adverse events. However, the detection of anti-eculizumab antibodies is highly dependent on the specificity and sensitivity of the technique and the applicant has committed to develop a specific HAHA technique to be used in future trials as a follow-up measure (FUM).
A number of laboratory parameters shifted from normal to high in ¨PNH and non-PNH patients. The applicant states that available information on haematological findings for PNH tends to reflect changes related to the mechanism of action of eculizumab, although this statement might be arguable. Most laboratory parameter alterations in PNH were not confirmed in non-PNH patients. Of particular relevance are the increases in APTT, direct bilirubin and alkaline phosphatase. The applicant notes that a number of PNH patients with a APTT increase were on anticoagulant treatment, which is in fact quite frequent in PNH patients, although the type of treatment is not specified (e.g. heparin treatment). The presence of antibodies against anticoagulation factors was not detected. The rest of the findings were not confirmed in the non-PNH patients.

With the provided information, however, no specific laboratory parameter was consistently found for PNH or non-PNH population and not for placebo. To date there are no reasons to suspect any clinically relevant laboratory adverse event.

No second tumours, other than transformation in CMML in a patient with MDS, were detected. Due to eculizumab's proposed mechanism of action, tumoural immunity is not expected to be affected in a major manner. There were no preclinical findings regarding this issue either. However, since limited long-term safety data are available and long term treatment is expected to be the rule, it seems sensible to keep carcinogenic potential in focus, particularly regarding haematological abnormalities.

Due to its mechanism of action, Soliris therapy should be administered with caution to patients with active systemic infections. The overall severity and frequency of infections in Soliris treated patients was similar to placebo treated patients in clinical studies, although an increase in the number and severity of infections, particularly due to encapsulated bacteria, cannot be excluded. Patients should be provided information from the Patient Information Leaflet to increase their awareness of potential serious infections and the signs and symptoms of them.

Further limitations of the safety database include renal or hepatic impairment, absence of data in children or in pregnant and lactating women and the lack of information regarding drug-drug interactions.

Treatment with Soliris should not alter anticoagulant management. If serious haemolysis occurs after Soliris discontinuation the procedures and treatments are described in the SPC.

Contraindications for the use of Soliris are hypersensitivity to eculizumab, murine proteins or to any of the excipients. Soliris therapy shall not be initiated in patients:

- with unresolved *Neisseria meningitides* infection.
- who are not currently vaccinated against *Neisseria meningitides.*
- who have known or suspected hereditary complement deficiencies.

Given the need to further collection of information of most important identified risks, and the need to complement global and long-term safety data, the safety registry has been included as a FUM with clearly predefined timelines for the provision of the revised registry protocol and its implementation. Periodic information from such registry will be provided with the PSURs.

The CHMP, having considered the data submitted in the application is of the opinion that the risk minimisation activities listed under 3.5 are necessary for the safe and effective use of the medicinal product

5. Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Risk Management Plan

Safety specifications have considered most important risks. There are some limitations regarding the size of the safety database and the duration of a treatment. Further experience will be gained from post-marketing experience.

Regarding specific risks, the immunogenicity of the drug in clinical trial appeared to be similar to the immunogenicity profile of placebo in both PNH and non-PNH studies. No signal regarding autoimmune-like AE related to eculizumab's administration have been detected. However, the detection of anti-eculizumab antibodies is highly dependent on the specificity and sensitivity of the technique and the applicant has committed to develop a specific HAHA technique to be used in future trials (FUM).

No second tumours, other than transformation in CMML in a patient with MDS, were detected. Due to eculizumab's proposed mechanism of action, tumoural immunity is not expected to be affected in a major manner. There were no preclinical findings regarding this issue either. However, since limited

long term safety data are available and long term treatment is expected to be the rule, it seems sensible to keep carcinogenic potential in focus, particularly regarding haematological abnormalities.

Even though, in general, interactions with antibodies are difficult to predict, no formal interaction studies have been performed. The lack of this information is clearly stated in the SPC.

Anticipated use in populations in which data are missing has been discussed (e.g. use during pregnancy). However, the absence of a paediatric clinical development program has not been fully justified. The MAH will address this issue as a part of the registry that will be set up with patients treated in clinical practice.

A global safety registry has been proposed as an additional pharmacovigilance activity, which is considered of the highest interest. The proposed activities seem adequate and appear to be feasible taking into account the incidence of the disease and the need of follow up usually required for patients with PNH.
Following review, the current safety protocol and CRF should be revised and an agreement with the CHMP should be reached prior to the actual launch of the product (FUM). The applicant should also assess the immunogenicity of eculizumab using samples collected in a cohort of patients through the Soliris Safety Registry (FUM). The risk of haematological abnormalities, of *Neisseria gonorrhoea*, pneumococcal infection and *Haemophilus influenzae* as well as the risk of off label use and paediatric off label use should be followed through the Soliris Registry.

Table Summary of the risk management plan

Safety Concern	**Risk**	**Proposed Pharmacovigilance activities**	**Proposed Risk Minimisation activities**
Meningococcal Infection	Identified	1. Routine Pharmacovigilance 2. Soliris Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including :Events of Interest as part of additional Pharmacovigilance	1- SPC : • Contraindication : patients with unresolved Neisseria meningitides infection, not vaccinated patients, patients with known or suspected hereditary complement deficiencies • Special warnings and precautions section 4.4: all patients must be vaccinated and re-vaccinated; consideration on appropriate use of antibacterial agents; monitoring, evaluation and treatment of infections mentioned as the most serious adverse event in section 4.8 2- PL • All patients must be vaccinated against meningococcal infection • Vigilance for risks of meningococcal infection • Early detection of symptoms of meningococcal infection and steps to manage 3-Patient Safety Card • Warning for early detection of symptoms and advice to contact medical facility • To be shown to consulted physician for acknowledgement of the risk 4- Vaccination reminders 5- Annual physician and patient surveys to assess their understanding of risks 6-Process to confirm patient Neisseria vaccination prior to treatment in each country 7-Physician's Guide 8-Educational Brochure

Safety Concern	Risk	Proposed Pharmacovigilance activities	Proposed Risk Minimisation activities
General infections	Potential	1. Routine Pharmacovigilance 2. Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including :Events of Interest as part of additional Pharmacovigilance	1- SPC : • Contraindication : patients with known or suspected hereditary complement deficiencies • Warning section 4.4 • Mentioned as adverse events in section 4.8 2-PL • Vigilance for risks of infections • Early detection of symptoms of serious infection and steps to manage 3--Patient Safety Card • Warning for early detection of symptoms and advice to contact medical facility • To be shown to consulted physician for acknowledgement of the risk 4--Annual physician and patient surveys to assess their understanding of risks 5-:Physician's Guide 6-Educational Brochure
Serious haemolysis after drug discontinuation	Potential	1. Routine Pharmacovigilance 2. Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including : Events of Interest as part of additional Pharmacovigilance	1-SPC • Warning: Treatment Discontinuation and Laboratory Monitoring sections in section 4.4 2-PL • Vigilance for risks of discontinuation • Need to carefully monitor for signs and symptoms of serious haemolysis following drug discontinuation 3-Annual physician and patient surveys to assess their understanding of risks 4-Physician's Guide 5-Educational Brochure

Safety Concern	Risk	Proposed Pharmacovigilance activities	Proposed Risk Minimisation activities
Headache	Identified	1.Routine Pharmacovigilance	1-SPC section 4.8 2-Medical Information for physicians as needed This risk does not require further mitigation activities 3-Physician's Guide 4-Educational Brochure
Infusion reactions	Potential	1. Routine Pharmacovigilance 2. Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including : Events of Interest as part of additional Pharmacovigilance	1-SPC: warning in section 4.4 2- PL: sections 2 and 3 3-Physician's Brochure 4-Educational Brochure
Immunogenicity	Potential	1. Routine Pharmacovigilance 2. Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including :Events of Interest as part of additional Pharmacovigilance	1-SPC: warning in section 4.4 2- PL : section 2 3-Physician's Guide

Malignancies Haematologic abnormalities	Potential	1. Routine Pharmacovigilance 2. Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including :Events of Interest as part of additional Pharmacovigilance	This risk does not require further mitigation activities
Pregnancy and lactation	Missing information	1. Routine Pharmacovigilance 2. Safety Registry • Annual survey • Maintained at least 5 years • Includes collecting information for specific events 3. Specific reporting including :Events of Interest as part of additional Pharmacovigilance	1-SPC : Section 4.6 • Reflects lack of information • "Soliris should be given only if clearly needed" • Recommendation of contraception for child bearing potential women • Lactation should be discontinued 2- PL : section 2 recommendation of contraception methods, Need of contraception methods use 3- Physician's Guide 4- Educational Brochure
Children	Missing information	1- Routine Pharmacovigilance 2- PNH Safety registry pre-specified checklist 3- PK sub-study within Soliris Safety Registry	1-SPC : lack of experience in children, mentioned in Sections 4.2 and 5.2 2-PL: section 2 *"Soliris has not been administered to patients less than 18 years of age"* 3-Physician's Guide 4-Educational Brochure
Renal impairment	Missing information	1- Routine Pharmacovigilance 2- PNH Safety registry pre-specified checklist	1-SPC: lack of information reflected in sections 4.2 and 5.2 2-Physician's Guide
Hepatic impairment	Missing information	1- Routine Pharmacovigilance 2- PNH Safety registry pre-specified checklist	1-SPC: lack of information reflected in sections 4.2 and 5.2 2-Physician's Guide

The CHMP, having considered the data submitted in the application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product:

Risk minimisation activities

1. The MAH shall agree the details of a distribution system with the National Competent Authorities and must implement such programme nationally to ensure that:

- drug distribution will only be possible after checking that the patient has effectively received a meningococcal vaccination with a written confirmation
- prior to distribution, all health care professionals are provided with information on the following key safety concerns:
  - Headache
  - Infusion reaction
  - Neisseria and general infection
  - Risk of serious haemolysis following eculizumab discontinuation and proposed management
  - Pregnancy and need of adequate contraception in women of childbearing potential
  - Immunogenicity
  - Renal and hepatic impairment.

2. Prior to launch, the MAH shall agree on the implementation of a patient card system in each Member Sate. This patient card will provide details of the signs and symptoms of infection as well as instruction for the patient to immediately seek medical care. The card will also provide information to health care professionals that the patient is receiving Soliris treatment.

3. The MAH shall propose a detailed methodology for the Health Care Professionals and Patient survey. In addition to the assessment of the satisfaction of eculizumab information provided, the proposed questionnaire will investigate other methods for assessing the effectiveness of the risk minimisation program (e.g. surveys to follow compliance to vaccination). The evaluation of the goals for distribution of educational materials should also be considered.

6. Overall conclusions, risk/benefit assessment and recommendation

Quality

In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The fermentation and purification of the active substance have been adequately described, controlled and validated. The active substance has been well characterised with regard to its physicochemical and biological characteristics, using state-of the-art methods, and the specifications that have been set are acceptable. The manufacturing process of the medicinal product has been satisfactorily described and validated. The quality of the medicinal product is controlled by adequate test methods and specifications. The viral safety and the safety concerning other adventitious agents including TSE have been sufficiently assured. Except for a number of quality points, which will be addressed as part of post-approval follow-up measures, the overall quality of Soliris is considered acceptable.

Non-clinical pharmacology and toxicology

Pharmacology
Eculizumab is a highly specific monoclonal antibody binding only to human C5 and not to C5 from any other mammalian species tested. As stated in the CHMP scientific advice and in accordance with ICH S7, the approach to characterize the pharmacodynamic properties of eculizumab by *in vitro* methods is considered as acceptable in the light of the species specificity. Moreover, no specific safety concerns related to CNS, CV or RS functional effects are reported from clinical safety data. Therefore, the lack of safety pharmacology studies is considered justified.
In vitro pharmacology studies demonstrate that the humanized monoclonal antibody eculizumab inhibits the cleavage of C5 to C5a and C5b as well as blocking C5b-9-mediated serum haemolytic activity.

C5 expression has been reported in normal human tissues. Eculizumab binds to C5 on smooth muscle (intrinsic/vascular) and skeletal muscle, myoepithelium, myofibroblasts, renal tubular epithelium, and reticulum cells. Staining of platelets was also observed. The reticulum cell staining likely represents staining of C5 associated with intracellular filaments. The tissue and cell staining likely represents staining of intracellular C5. Intracellular staining is due to the method of acetone fixation and cryotomy of the tissue/cell samples.
Although no formal pharmacodynamic drug interaction studies have been performed, eculizumab has been administered to patients treated concomitantly with a broad range of medications commonly used in patients with PNH including erythropoietin, corticosteroids, anabolic steroids, anticoagulants, and immunosuppressants. Therefore, the absence of pharmacodynamic drug interaction studies is justified taking into account clinical data.

Pharmacokinetics
Pharmacokinetic studies show that the humanized mAb is functional in the murine vascular compartment for at least 48 hours after injection. Furthermore, based upon an average hC5 plasma concentration of 76 µg/mL, and a plasma volume of 3.0 L for a 70kg human, it is predicted that a single dose of 1.5-2.0 mg/kg of h5G1.1 G4 mAb should be sufficient to acutely mediate complete inhibition of complement-dependent serum haemolytic activity *in vivo* in humans.
No tissue distribution studies were performed, since eculizumab bind to a soluble protein, hC5, which is secreted into the blood. Eculizumab cannot bind to mC5 or function in a murine system. No placental transfer studies have been done due to the specificity of the antibody.

According to the Guideline CHMP/ICH/302/95, the lack of metabolism studies is acceptable.
No excretion studies have been performed. It is expected that since antibodies are transmitted in mother's milk, eculizumab will probably also be excreted in the milk.
Although no formal pharmacokinetic drug interaction studies have been performed, eculizumab has been administered to patients treated concomitantly with a broad range of medications commonly used in patients with PNH including erythropoietin, corticosteroids, anabolic steroids, anticoagulants, and

immunosuppressants. Therefore, the absence of pharmacokinetic drug interaction studies is justified taking into account clinical data.

Toxicology
Toxicological studies were performed in mice using a surrogate murine anti-mouse C5 antibody (BB5.1 mAb). BB5.1 mAb specifically binds to mouse C5 but there was no information on whether the cross-reactivity profile of BB5.1 mAb with normal mouse tissues is similar to the cross-reactivity profile of eculizumab with normal human tissues.
Genotoxicity studies have not been performed with eculizumab or any other surrogate anti-C5 antibodies. Antibodies, in general, do not interact directly with DNA, and therefore are unlikely to have any genotoxic potential.
Reproductive toxicity studies revealed, that although the observed foetal malformations were unlikely to be treatment related, they can not be discarded.
Local effects following therapeutic route of administration are well characterized following clinical monitoring and, in a lesser extend, repeat-dose toxicity studies performed with the surrogate antibody.

Efficacy

The efficacy of Soliris in PNH patients with haemolysis were assessed in a randomized, double-blind, placebo-controlled 26 week study (C04-001). PNH patients were also treated with Soliris in a single arm 52 week study (C04-002) and in a long term extension study (E05-001). In all studies, the dose of Soliris was 600 mg study drug every 7 ± 2 days for 4 weeks, followed by 900 mg 7 ± 2 days later, then 900 mg every 14 ± 2 days for the study duration. Soliris was administered as an intravenous infusion over 25 - 45 minutes.

Patients treated with Soliris had significantly reduced ($p< 0.001$) haemolysis resulting in improvements in anaemia as indicated by increased haemoglobin stabilization and reduced need for RBC transfusions compared to placebo treated patients. These effects were seen among patients within each of the three pre-study RBC transfusion strata (4 - 14 units; 15 - 25 units; > 25 units). After 3 weeks of Soliris treatment, patients reported less fatigue and improved health-related quality of life. All patients sustained a reduction in intravascular haemolysis over a total Soliris exposure time ranging from 10 to 54 months. There were fewer thrombotic events with Soliris treatment than during the same period of time prior to treatment.

Safety

Safety data for eculizumab as a treatment for PNH was collected in six clinical studies that included 195 patients (147.44 patient-years) treated with eculizumab with approximately 70 % (138) treated for at least 26 weeks and approximately 20 % treated for at least 52 weeks. Supportive data were obtained in 11 clinical studies that included 716 patients (492.20 patient years) in other indications.
The most commonly reported adverse reactions in eculizumab treated patients are headache, nasopharyngitis, nausea, pyrexia, myalgia, fatigue, and herpes simplex, each occurring in 5 or more out of 100 patients. Eculizumab treatment did not seem to be associated with an increase in severity of AEs in treated patients. No cumulative, irreversible toxicities or treatment related deaths were reported.

Identified and potential risks are general infections, especially meningococcal infections, haemolysis after drug discontinuation and haematologic abnormalities.

Further limitations of the safety database include renal or hepatic impairment, absence of data in children or in pregnant and lactating women and the lack of information regarding drug-drug interactions.

Given the need to further collection of information of most important identified risks, and the need to complement global and long-term safety data, the safety registry has been included as a FUM with clearly predefined timelines for the provision of the revised registry protocol and its implementation. Periodic information from such registry will be provided with the PSURs.

The CHMP, having considered the data submitted in the application is of the opinion that the risk minimisation activities listed under 3.5 are necessary for the safe and effective use of the medicinal product

- User consultation

The Applicant performed a readability testing ("user consultation") and a satisfactory report has been provided.

Risk-benefit assessment

PNH is associated with increased morbidity and mortality. Available therapies have a limited effect and do not affect the course of the disease. Eculizumab reduces C5-mediated intravascular haemolysis in patients with PNH. This effect results in a relevantly reduced need of transfusions and improved functional and quality of life status. All patients sustained a reduction in intravascular haemolysis over a total Soliris exposure time ranging from 10 to 54 months. There were fewer thrombotic events with Soliris treatment than during the same period of time prior to treatment.
The identified safety risks have been adequately addressed by the applicant. The applicant agreed to collect further information on the most important identified and potential risks and to complement global and long-term safety data using a global safety registry with agreed timelines for the provision of the revised registry protocol and its implementation. Periodic information from such registry will be provided with the PSURs.

The CHMP, having considered the data submitted, was of the opinion that:

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.

- the following additional risk minimisation activities were required:

1. The MAH shall agree the details of a distribution system with the National Competent Authorities and must implement such programme nationally to ensure that:

    - drug distribution will only be possible after checking that the patient has effectively received a meningococcal vaccination with a written confirmation
    - prior to distribution, all health care professionals are provided with information on the following key safety concerns:
        - Headache
        - Infusion reaction
        - Neisseria and general infection
        - Risk of serious haemolysis following eculizumab discontinuation and proposed management
        - Pregnancy and need of adequate contraception in women of childbearing potential
        - Immunogenicity
        - Renal and hepatic impairment.

2. Prior to launch, the MAH shall agree on the implementation of a patient card system in each Member Sate. This patient card will provide details of the signs and symptoms of infection as well as instruction for the patient to immediately seek medical care. The card will also provide information to health care professionals that the patient is receiving Soliris treatment.

3. The MAH shall propose a detailed methodology for the Health Care Professionals and Patient survey. In addition to the assessment of the satisfaction of eculizumab information provided, the proposed questionnaire will investigate other methods for assessing the effectiveness of the risk minimisation program (e.g. surveys to follow compliance to

vaccination). The evaluation of the goals for distribution of educational materials should also be considered.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Soliris was favourable and therefore recommended the granting of the marketing authorisation in the following indication:
"Soliris (eculizumab) is indicated for the treatment of patients with paroxysmal nocturnal haemoglobinuria (PNH).
Evidence of clinical benefit of Soliris in the treatment of patients with PNH is limited to patients with history of transfusions."