CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125166

PHARMACOLOGY REVIEW

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	BLA #125166
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	09/15/06
PRODUCT:	Eculizumab (Soliris™)
INTENDED CLINICAL POPULATION:	Patients with paroxysmal nocturnal hemoglubinuria
SPONSOR:	Alexion Pharmaceuticals Inc.
DOCUMENTS REVIEWED:	Electronic submission (eCTD)
REVIEW DIVISION:	Division of Medical Imaging and Hematology Products
PHARM/TOX REVIEWER:	Siham Biade, Ph.D.
PHARM/TOX SUPERVISOR:	Adebayo Laniyonu, Ph.D.
DIVISION DIRECTOR:	Rafel Rieves, M.D.
PROJECT MANAGER:	Florence Moore, M.S.

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I.   Recommendations

A. Recommendation on approvability: From the perspective of nonclinical pharmacology and toxicology, Soliris® is recommended for approval

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling:

Pregnancy (Category C)

8.1   Pregnancy
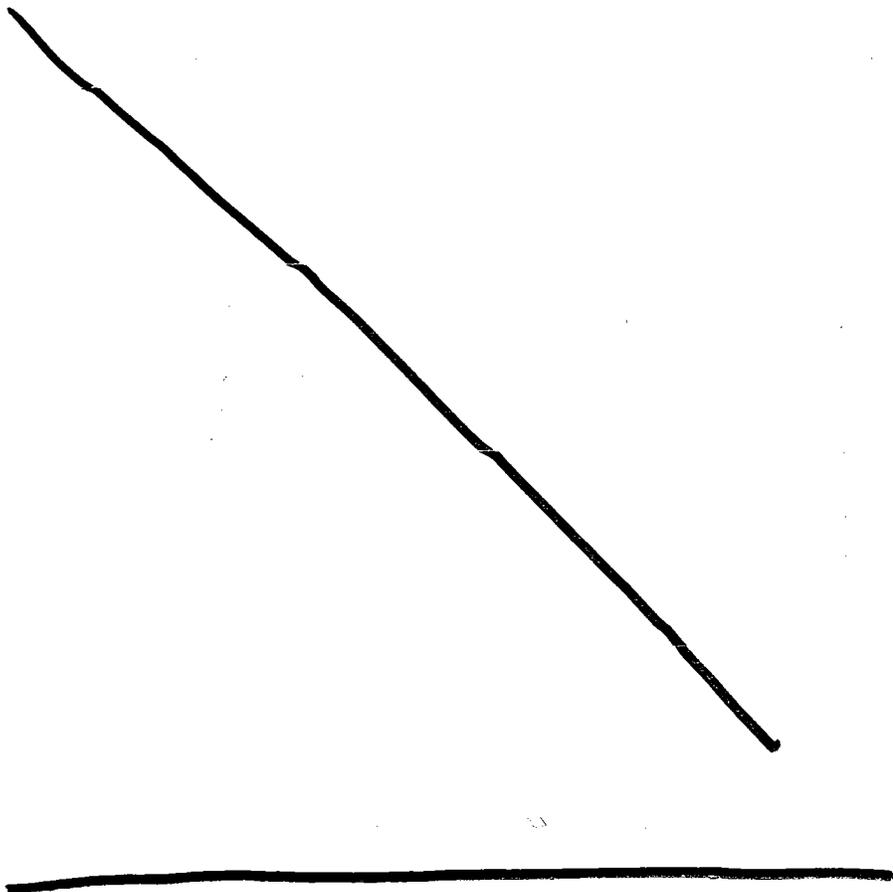
8.3   Nursing Mothers
It is not known whether Soliris is secreted into human milk.   IgG is excreted in human milk, so it is expected that Soliris will be present in human milk.

13.1   Carcinogenesis, Mutagenesis, Impairment of Fertility
Long-term animal studies have not been conducted to evaluate the carcinogenic
and genotoxic potential of Soliris. Effects of Soliris upon fertility have not been
studied in animals. Intravenous injections of male and female mice with a murine
anti-C5 antibody at up to 4-8 times the equivalent of the clinical dose of Soliris
had no adverse effects on mating or fertility.

II.   Summary of nonclinical findings

A. Brief overview of nonclinical findings

A.1. Pharmacodynamics/Pharmacokinetics

Kinetics of eculizumab interaction with purified human complement protein C5,
were determined using ▬▬▬▬▬ surface plasmon resonance (SPR). The association
and dissociation rates of C5 to eculizumab were faster at the higher temperature with a
K_D of 120 pM at 37°C and 46 pM at 25°C. (mean of three experiments). The binding
affinity of the parental murine m5G1.1-mAb (BB5.1) for human C5 (hC5), used in the
animal toxicology studies was obtained providing an estimated K_D of 29.5 pM.
No animal pharmacokinetics or toxicokinetics studies were conducted using the
surrogate murine antibody BB5.1. Intravenous administration of h5G1.1 G4 mAb (an
IgG4 isotype of eculizumab) to a C5-deficient mouse model reconstituted with human
C5, resulted in a rapid, dose-dependent and sustained inhibition of hC5-dependent serum
hemolytic activity with a $T_{1/2}\alpha$ of several hours, followed by a slow decline in serum
concentrations over the next 48 hours.
A species cross-reactivity study was conducted to assess the functional
complement C5 inhibition activity of eculizumab in human serum samples compared to
primate and non-primate serum samples including baboon, rhesus monkey, cynomolgus
monkey, chimpanzee, rat, pig, guinea pig, and rabbit using a hemolytic assay measuring
the hemoglobin release from chicken red blood cells. Eculizumab blocked human serum-
mediated hemolysis at 42nM (6.25 µg/mL), but did not inhibit hemolysis in the non-
primate and primate sera, indicating that eculizumab was only specific to human
complement C5.
An in vitro study was conducted to evaluate the potential cross-reactivity of
eculizumab with cryosections of a panel of 38 normal human tissues. The distribution
pattern of eculizumab staining of smooth muscle, skeletal muscle, and renal proximal
tubular epithelium appeared consistent with the expected localization of complement C5
in the tissues, with the exception of kidney in which significant cytoplasmic staining was
noted in both the tubular and the glomerular epithelium. In view of the absence of effects

of eculizumab on the kidney functions in clinical trials, this finding was not considered toxicologically relevant.

A.2. Toxicology

Two repeat dose toxicity studies and three reproductive studies (Seg I, II, and III) were conducted in mice, administered a surrogate mAb, the murine C5 specific BB5.1. For these studies, bolus intravenous injection was used whereas the intended clinical route is 45 minute infusion. Toxicokinetics and antibody measurement were not conducted in any of the toxicology studies. The hemolytic activity, used as surrogate of drug systemic exposure, did not provide consistent results in the reproductive toxicity studies. Pregnant females appeared to exhibit lower % hemolysis than males and non-pregnant females.

Repeat dose toxicity:

In a 4 week repeat dose toxicity study, no toxic signs, based on mortality, clinical observations and body weights, were observed in mice treated for 4 weeks with 30, 60, or 90 mg/kg/week (2-4, 4-8, and 6-12 times the human dose). Similar hemolytic inhibition was obtained at 60 and 90 mg/kg/week. Therefore, 60 mg/kg/week was selected as the maximum dose for subsequent studies.

In a 26 week toxicity study, mice were treated with 30 or 60 mg/kg/week (2-4, 4-8 times the human dose). Nine unscheduled deaths occurred (4/50 in control group and 5/50 in the 60 mg/kg/week group). No unscheduled death was recorded in the 30 mg/kg/week group. One high dose group male died during injection and exhibited lung thrombosis whereas one high dose female died after dosing. Death was considered accidental in a control male found with esophagus perforation, and in one control female death was attributed to bronchiolar-alveolar carcinoma. The cause of death for the others could not be determined, although macroscopic and microscopic findings differed between control and treated animals (e.g. pigmented foamy macrophages, lung congestion and thrombosis, subendocardial chronic active inflammation/hemorrhage observed in treated group).

Dose-dependent clinical signs included missing ears, sores/scabs in the ear or cranial area, rough hair coat, bent tail, and thin and/or hunched appearance. Macroscopic findings included skin sores, and sporadic findings in the treated groups. Ovary and uterus cysts, distended or with thickened wall, with lumen fluid were observed at higher incidence in treated females. AST and ALT mean values increased (~42 and 76% respectively) on week 31 for males. A statistically significant increase was observed in liver weights in high dose females, and an increase in heart weight (up to 12%) in high dose males and females were observed. At terminal sacrifice, the most remarkable findings included lung congestion, thrombosis, and/or hemorrhage, skin lesions (chronic active inflammation, necrotic epidermal cellular debris, ulcer, and acanthosis), and eyes retrobulbar inflammation and hemorrhage. Lens degeneration and bulbi phtysis was noted in one low dose female.

Lung neoplasms were observed in 2 unscheduled control deaths, pituitary B-adenoma and lung peribronchial lymphoid infiltration in 1/33 high dose female, multiple hematopietic neoplasms (lymphoma/lung/salivary glands/thymus) in 1/33 high dose

female. Lymphoid or lymphoreticular hyperplasia were observed respectively in mandibular lymph node (1 high dose female) and in thymus (1 high dose female). A NOAEL could not be established in this study.

Reproductive toxicology:

BB5.1 administered by intravenous injection to male and female mice prior to mating and until termination (for 28 days prior to mating until 2 days prior to necropsy for males) or through early gestation (from over 14 days prior to mating), did not affect female reproductive performance, female and litter reproductive parameters, and sperm count and motility. Sperm morphology was not performed. The NOEL for female toxicity and reproductive performance, for male and female fertility, and fetal effects was established at ≥ 60 mg/kg/week. The NOAEL was established at 30 mg/kg/week BB5.1 for male toxicity in view of two male deaths in the 60 mg/kg/week.

BB5.1 administered by IV injection to pregnant mice during the period of organogenesis did not affect maternal body weights, food consumption, and Cesarean section parameters. No toxicokinetic analysis was conducted to confirm fetuses exposure during pregnancy. Fetal malformations were sporadic with no dose-response pattern and there was an increased incidence of 14th rib and accessory bones in skull of treated fetuses. Two cases of retinal dysplasia and one case of umbilical hernia were observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. The NOAEL was established at 60 mg/kg/week for maternal toxicity and reproductive performance. The NOAEL for embryo/fetal development toxicity was established at 30 mg/kg/week.

In a prenatal and postnatal development study, the effects of BB5.1 were evaluated when administered by intravenous injection from implantation through weaning, on pregnant and lactating female mice, and on the development of the offspring. No toxicokinetics analysis, no maternal milk or placenta analysis for presence of murine antibodies were performed in any of the studies. Therefore, the assumption is that the neonates were exposed either during pregnancy or during lactation or both, since there were no data to support either one of the hypotheses. The results showed that one 60 mg/kg/week Fo female died on LD 14 with necropsy revealing a pale liver. In view of the number of dead (1/25 in low dose, 2/25 in high dose), and moribund male animals (1/25 in controls, 1/25 in low-dose, and 3/25 in high dose), and presence of clinical signs (skin sores, localized swelling and distended abdomen) in the F1 male generation, a NOAEL could not be established for these parameters. The NOAEL was established at ≥ 60 mg/kg/week for F1 pup development and reproductive performance.

B. Pharmacologic activity

Soliris, eculizumab antibody (h5G1.1-mAb), is a humanized IgG2/4 kappa antibody, consisting of two 448 amino acid heavy chains and two 214 amino acid light chains. The heavy chains are comprised of human IgG2 sequences in constant region 1, the hinge, and the adjacent portion of constant region 2, and human IgG4 sequences in the remaining part of constant region 2 and constant region 3. 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.

Eculizumab binds to the human C5 complement protein and inhibits terminal complement-mediated cell lysis and activation. The intravascular hemolysis in PNH results from the deficiency of the terminal complement regulatory protein CD59 on the surface of PNH erythrocytes. Normally, CD59 blocks the formation of the terminal complement complex (also called the membrane attack complex) on the erythrocyte surface, thereby preventing hemolysis. Restoration of terminal complement inhibition, therefore, should effectively stop intravascular hemolysis.

C. Nonclinical safety issues relevant to clinical use

None. There are no outstanding nonclinical safety issues.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number:	125166
Review number:	1
Sequence number/date/type of submission:	
Information to sponsor:	Yes ( ) No (x)
Sponsor and/or agent:	Alexion Pharmaceuticals, Inc., 352 Knotter Drive, Cheshire, Connecticut 06410
Manufacturer for drug substance:	Lonza Biologics Inc., 101 International Drive, Pease International Tradeport, Portsmouth, New Hampshire 03801, USA FDA Establishment Identifier: 3001451441
Reviewer name:	Siham Biade, Ph.D.
Division name:	Medical Imaging & Hematology Products
Review completion date:	February 1st, 2007

Drug:

Trade name: Soliris™

Generic name: Eculizumab (h5G1.1-mAb)

Code names: h5G1.1-mAb, LEX98, HAL1, 5G1.1 antibody, anti-C5 antibody, h5G1.1, h5G1.1VHC+h5G1.1VLC, h5G1.1HuG2/G4

Chemical name: immunoglobulin, anti-(human complement C5 a-chain) (human-mouse monoclonal 5G1.1 heavy chain), disulfide with human-mouse monoclonal 5G1.1 light chain, dimer

CAS registry number: 219685-50-4

Molecular formula/molecular weight: 148.523 kDA

Structure: [from sponsor's submission] The eculizumab antibody (h5G1.1-mAb) is a humanized IgG2/4 kappa antibody, consisting of two 448 amino acid heavy chains and two 214 amino acid light chains. The heavy chains are comprised of human IgG2 sequences in constant region 1, the hinge, and the adjacent portion of constant region 2, and human IgG4 sequences in the remaining part of constant region 2 and constant region 3. 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. Below is a depiction of the basic structure of the eculizumab antibody.
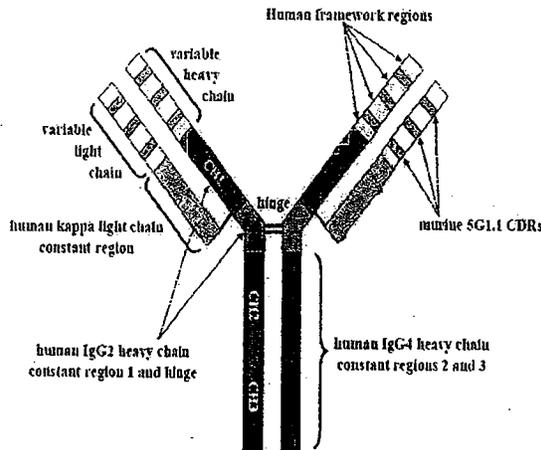
Relevant INDs/NDAs/DMFs: INDs# ████████████████████ 11075.

Drug class:
      Humanized complement 5 monoclonal antibody IgG2/IgG4 immunoglobulin

Intended clinical population:
      Patients with paroxysmal nocturnal hemoglobinuria (PNH)

Clinical formulation:
      Eculizumab is supplied as a 10 mg/mL sterile, preservative-free solution for intravenous infusion in 30 mL single use vials and contains sodium phosphate mono-and dibasic ██████████ , sodium chloride ██████████ , and polysorbate 80 ██ ██ . Prior to administration, it is diluted to a final concentration of 5mg/ml in either 0.9% Sodium Chloride, USP, 0.45% Sodium Chloride, USP, or 5% Dextrose in Water, USP.

Composition of Eculizumab Drug Product

Ingredients	Formulation Concentration	Quantity Per 30 mL Vial	Function	Quality Standard
Eculizumab		300 mg		In-house Reference Standard
Sodium phosphate monobasic		13.8 mg		USP, Ph. Eur.
Sodium phosphate dibasic		53.4 mg		USP, Ph. Eur.
Sodium chloride		263.1 mg		USP, Ph. Eur.
Polysorbate 80 (vegetable origin)		6.6 mg		NF, Ph. Eur.
Water for Injection		Q.S.		USP, Ph. Eur.

[1]The sodium phosphate concentration is approximately 10 mM (9.97 mM), which is comprised of sodium phosphate monobasic and the sodium phosphate dibasic.

The dosage regimen consists of an induction and maintenance phase. In the induction phase, 600 mg of eculizumab is administered every week for the first 4 weeks and 900 mg of eculizumab is administered in week 5. The maintenance phase consists of a 900 mg dose of eculizumab every 14 ± 2 days thereafter.

Route of administration:
Eculizumab is administered as a 25-45 min intravenous infusion.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

[For (b)(2) applications:

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of [NDA number] are owned by [name of sponsor] or are data for which [name of sponsor] has obtained a written right of reference. Any information or data necessary for approval of [NDA number] that [name of sponsor] does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that [name of sponsor] does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of [NDA number].]

Studies reviewed within this submission:

Study	*Title*
GTR-0056.00-AD01	Determination of Dissociation Constants for N19/8 and m5G1.1
GTR-0109.00	Eculizumab Binding Kinetics using ▬▬▬▬
BP-0026.00FR	Final Report for BP-0026, Species Cross-Reactivity of Eculizumab (h5G1.1-mAb)
▬ No IM1184	Cross-Reactivity Study of Eculizumab with Normal Human Tissues
GTR-0104.00	Pharmacodynamics and pharmacokinetics of C5-deficient Mice Reconstituted with Human C5 and Treated with an h5G1.1-mAb
6709-109	Four Week Intravenous Injection Range-Finding Study in Mice with BB5.1
6709-108	26-Week Intravenous Injection Toxicity Study of BB5.1 in Mice with a 4-Week Recovery
6709-104	Study of fertility and early embryonic development to implantation in mice with BB5.1
6709-105	Mouse Developmental Toxicity Study with BB5.1
6709-107	Study for effects on pre- and post natal development, including maternal function in the mice with BB5.1

Studies _not_ reviewed within this submission:

_____

Note: For NDA reviews, all section headings should be included.

2.6.2   PHARMACOLOGY

2.6.2.1 Brief summary

Introduction

[Large portions of this introduction and discussion of the complement system are excerpts from the sponsor's summary]

Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon, severely morbid and potentially fatal hemolytic disease that occurs at almost any age with an equal sex distribution and an incidence of 2-6 PNH patients per million population. Current treatments for PNH are palliative and consist of vitamin and mineral supplementation, and erythropoietin stimulating agents, blood transfusions, bone marrow transplantation, or corticosteroid and androgen therapy.

The intravascular hemolysis in PNH results from the deficiency of the terminal complement regulatory protein CD59 on the surface of PNH erythrocytes. Normally, CD59 blocks the formation of the terminal complement complex (also called the membrane attack complex) on the erythrocyte surface, thereby preventing hemolysis. Restoration of terminal complement inhibition, therefore, should effectively stop intravascular hemolysis, thereby reducing the morbidities in PNH.

The complement system

The complement cascade consists of more than 20 serum proteins that interact in a precise series of enzymatic cleavage and membrane binding events leading to the generation of products with immunoprotective, immunoregulatory, proinflammatory, and cytolytic properties (see schema from sponsor below)

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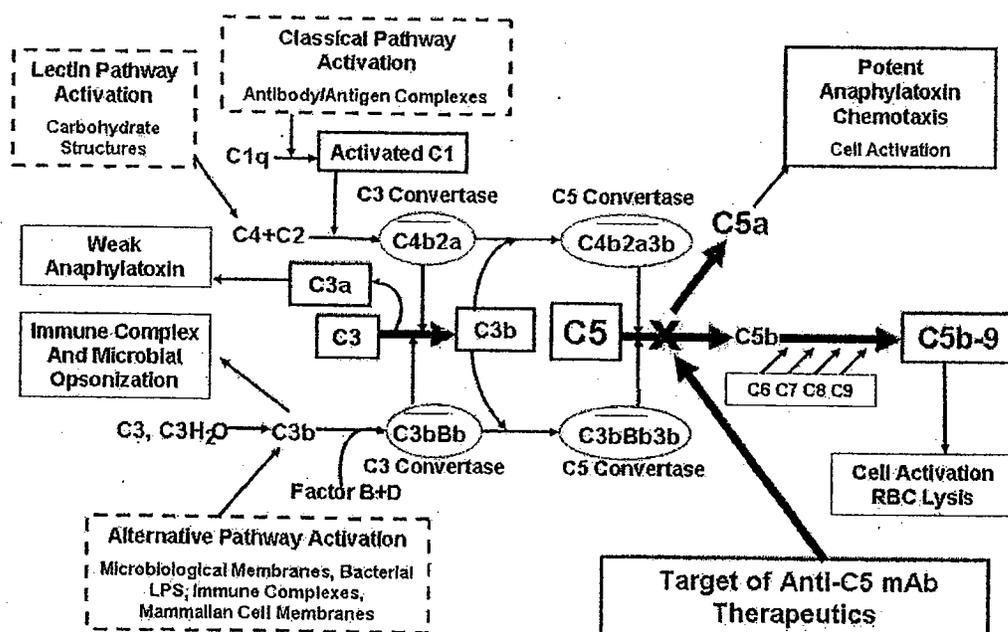
C5 is an attractive target in the rational design of a therapeutic complement inhibitor since C5 cleavage results in the generation of critical proinflammatory and cell lytic molecules (C5a and C5b-9) that can initiate inflammation, cell activation and, in the case of PNH, hemolysis. C5 is common to all three pathways of activation (see above diagram) enabling the blockade of terminal complement regardless of the mode of complement activation while preserving the ability to generate the critical immunoprotective and immunoregulatory functions of C3b-mediated opsonization and immune clearance.

Eculizumab is a humanized monoclonal antibody that binds to the human C5 complement protein and inhibits terminal complement-mediated cell lysis and activation. The antibody is an IgG2/4 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.

Summary of pharmacology studies

Kinetics of eculizumab interaction with purified human complement protein C5, were determined using ▬▬▬ surface plasmon resonance (SPR). The association and dissociation rates of C5 to eculizumab were faster at higher temperature with a K_D of 120 pM at 37°C and 46 pM at 25°C (mean of three experiments). The binding affinity of the parental murine m5G1.1-mAb (BB5.1) for human C5 (hC5) used in the animal toxicology studies was obtained providing an estimated K_D of 29.5 pM.

No animal pharmacokinetics or toxicokinetics studies were conducted using the surrogate murine antibody BB5.1. Intravenous administration of h5G1.1 G4 mAb (an IgG4 isotype of eculizumab) to a C5-deficient mouse model reconstituted with human C5, resulted in a rapid, dose-dependent and sustained inhibition of hC5-dependent serum hemolytic activity with a $T_{1/2}\alpha$ of several hours, followed by a slow decline in serum concentrations over the next 48 hours.

A species cross-reactivity study was conducted to assess the functional complement C5 inhibition activity of eculizumab in human serum samples compared to primate and non-primate serum samples including baboon, rhesus monkey, cynomolgus monkey, chimpanzee, rat, pig, guinea pig, and rabbit using a hemolytic assay measuring the hemoglobin release from chicken red blood cells. Eculizumab blocked human serum-mediated hemolysis at 42nM (6.25 μg/mL), but did not inhibit hemolysis in the non-primate and primate sera, indicating that eculizumab was only specific to human complement C5.

An in vitro study was conducted to evaluate the potential cross-reactivity of eculizumab with cryosections of a panel of 38 normal human tissues. The distribution pattern of eculizumab staining of smooth muscle, skeletal muscle, and renal proximal tubular epithelium appeared consistent with the expected localization of complement C5 in the tissues, with the exception of kidney in which significant cytoplasmic staining was noted in both the tubular and the glomerular epithelium. In view of the absence of effects of eculizumab on the kidney functions in clinical trials, this finding was not considered toxicologically relevant.

2.6.2.2 Primary pharmacodynamics

Study GTR-0056.00-AD01: Determination of Dissociation Constants for N19/8 and m5G1.1

Study objective:
The selection of the murine anti-human C5 antibody m5G1.1 was based on its superior potency over N19/8 as an inhibitor of both serum complement hemolytic activity and C5a generation. To further characterize the interaction between human C5 (hC5) and m5G1.1 or N19/8 that may explain this functional disparity, the dissociation constant (K_d) was determined for each antibody.

Study design
Immunoglobulins anti-human C5 mAbs N19/8 and m5G1.1 were purified from mouse ascites fluid. The affinity of either antibody for hC5 under equilibrium conditions in solution was evaluated by ELISA (*Friguet et al. 1985, Journal of Immunological Methods, 77:305-319*). This method uses unmodified antibody and antigen which eliminates potential alterations in their interaction that might be induced by chemical conjugations. The dissociation constant (K_d) of antibody-antigen was determined for each antibody.

The concentration of hC5 was titrated against a constant amount of antibody and allowed to reach equilibrium. The equilibrated solutions were then added to hC5 coated

plates, and unbound antibody was detected using a secondary antibody using protein concentration and average spectrophotometric absorbance values, the fraction of free antibody and the concentration of free antigen at equilibrium were calculated and plotted to generate binding curves (Figure 1). Linear regression analysis was then used to obtain the best fit. The reciprocal of the slope of this line represents the K_d.

Study results

Figure 1 shows the plots of the binding of the anti human C5 monoclonal antibodies m5G1.1 and N19/8, respectively, to human C5, and are representative of experiments carried out using different batches of antibodies. In each graph the x axis represents the fraction of free antibody and the y axis represents the concentration of free antigen at equilibrium.

Figure 1: K_d Determination for m5G1.1 (Left panel) and N19/8 (Right panel)
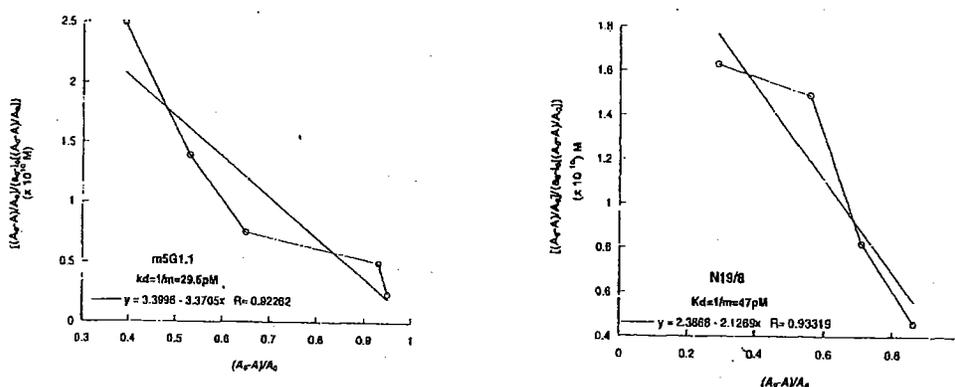
The calculated K_d for m5G1.1 is 29 pM and for N19/8 is 47 pM. Given the similar K_d values of the two antibodies, differences in affinity can not explain the superior complement inhibitory function of m5G1.1 over N19/8.

Study GTR-0109.00: Eculizumab Binding Kinetics using ▬▬▬ ⁄(Eculizumab Lot # 20182 QC-191; conducted by Alexion Pharmaceuticals Inc.)

Study objective

The objective of this study was to determine the kinetics of Eculizumab interaction with purified human complement protein C5.

Study design:

The ▬▬▬ uses a real-time biomolecular interaction technique which measures the association and dissociation of complexes. ▬▬▬▬▬

▬▬▬▬▬▬▬▬▬▬▬

▬▬▬▬▬▬ of human C5 were subsequently bound to the Anti-Fc/Eculizumab surface at both 25°C and 37°C. This technique allows the binding

ligand (eculizumab) to always be in the correct orientation and hence maximize the number of binding sites available for the analyte human C5. Eculizumab density is maintained at low level to avoid or minimize mass transport limitations, steric hindrance or aggregates. SPR measures a change in refractive index which is directly related to the mass concentration of molecules at the ligand analyte surface. If binding occurs as analyte sample of C5 passes over the prepared sensor surface, the response in the sensorgram increases. If equilibrium is reached a constant signal will be seen. Replacing sample with buffer (20 mM HCl, 0.01% P20) causes the bound molecules to dissociate and the response decreases. ▬▬▬ evaluation software generates the values of k_a and k_d by fitting the data to interaction models. The 25°C kinetics experiment was processed with a longer dissociation time due to slow dissociation. HBS-EP buffer was used as blanks and two concentration series of C5 were run in each experiment.

Results:

Non specific interactions were not observed. The values for ka, kd, K_D and Chi^2 were averaged for the triplicate experiment runs and shown in the tables below.

Table 1: Kinetics data for human C5 on an Anti-Fc/Eculizumab sensor chip surface at 25°C and 37°C

	Average value At 25°C	Average value at 37°C
Association rate constant ka (1/Ms)	$9.9 \times 10^5 \pm 8.48 \times 10^3$	$1.68 \times 10^6 \pm 4.16 \times 10^4$
Dissociation rate constant kd (1/s)	$4.57 \times 10^{-5} \pm 1.36 \times 10^{-6}$	$2.01 \times 10^{-4} \pm 1.28 \times 10^{-5}$
K_D (pM)	4.60 ± 1.56	$1.20 \times 10^{-10} \pm 5.51 \times 10^{-12}$
Chi^2	$5.91 \times 10^{-2} \pm 0.003$	0.203 ± 0.025

Figure 1: K_D (M) of human C5 to eculizumab kinetics at 25°C and 37°C
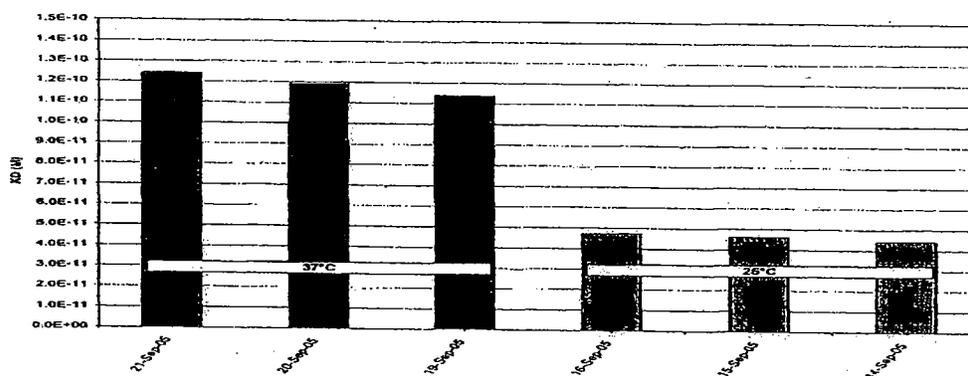
Conclusion

Both the association and dissociation rates of C5 to eculizumab were faster at 37°C compared to 25°C with K_D approximately twice as large (120 pM compared to 46 pM respectively).

Study BP-0026.00FR: Final Report for BP-0026, Species Cross-Reactivity of Eculizumab (h5G1.1-mAb) (Alexion Pharmaceuticals Inc., 352 Knotter Drive, Cheshire, CT 06410, Aug 04)

Study objective:

The purpose of this study was to assess the functional complement C5 inhibition activity of eculizumab in human serum samples compared to primate and non-primate serum samples including baboon, rhesus monkey, cynomolgus monkey, chimpanzee, rat, pig, guinea pig, and rabbit.

Study design:

The hemolytic assay measured the hemoglobin release from chicken red blood cells by determining the ability of eculizumab to block serum complement lysis of chicken red blood cells.

The species cross-reactivity was assessed and compared in two lots of eculizumab bulk samples (Lots # 20940 and # 20505). The complement activity of both primate and non-primate serum in comparison to human serum was also evaluated. Sera that showed very low complement activity were reordered. Eculizumab Lot# AP-3606 was used as the reference standard to prepare the quality control (QC) samples.

Method:

Eculizumab QC samples diluted to concentrations of 2 to 60 µg/mL were added to human serum (2.5 to 20% final concentration) to establish acceptable % hemolysis criteria. Cross reactivity was evaluated on serially diluted human, primate and non-primate sera using the chicken erythrocytes assay. Sensitized chicken erythrocytes were added at 2.5 x 10^6 cells/30µL to the plate containing serum and eculizumab, and incubated at 37°C for 30 min. Each plate contained wells of chicken erythrocytes incubated with 20% serum containing 2mM EDTA as blank, and wells containing buffer alone as negative control for spontaneous hemolysis. The plate was then centrifuged and the hemoglobin release was determined in the supernatant at 415nm.

Results:

1. Eculizumab QC samples at concentrations of 60µg/mL, 30µg/mL, 10µg/mL, and 2µg/mL produced % hemolysis of $\leq$ 10%, $\leq$ 20%, $\leq$ 35%, and $\geq$ 70% respectively in human serum, set as % hemolysis acceptance criteria.

2. The serum complement activity of baboon, rhesus monkey, cynomolgus monkey and chimpanzee serum was comparable to human serum at concentrations of 20%, 10%, and 5%. At 2.5% serum concentration, the average % hemolysis of baboon was similar to that of humans, whereas it was two-fold higher for the other primate sera.

3. The serum complement activity (% hemolysis) of non-primate sera is presented in the following table

Serum concentration	Human	Rabbit	Pig	Guinea pig	Rat
20%	88	47	51	63	87
10%	74	20	15	39	93
5%	37	4	3	15	85
2.5%	11	0	6	3	66

The average % hemolysis for human serum was calculated from 6 plates and that of non-primate serum was calculated from triplicate wells. Standard errors are shown for human sera only.

4. The lowest concentration of both of the eculizumab bulk lots which blocked human serum-mediated hemolysis was 42nM (6.25 µg/mL). At this concentration, eculizumab did not inhibit hemolysis in the non-primate and primate sera (Fig 1).

Figure 1: Analysis of 2 lots of eculizumab Cross-reactivity: human serum vs. primate and non primate serum. Data were plotted using average % hemolysis from triplicate wells for primate and non-primate animal sera, and from 6 plates for human serum.

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Cross-Reactivity Assay: Human Serum Vs. Primate Serum using Eculizumab Lot# 20840

Cross-Reactivity Assay: Human Serum Vs. Non-Primate Serum using Eculizumab Lot # 20840

Conclusion:

The sponsor concludes that there is no cross-reactivity of the eculizumab with non-primate species primate species and that the results suggest that eculizumab effectively inhibits C5 only in human serum.

Reviewer's comments:

Mouse serum was not included in this study and may have been used as an additional reference since it was the species selected for the toxicological studies. The sponsor did not provide individual data so the standard errors were not available in the figures for non human sera. It is of note that inhibition of hemolysis was variable in the toxicology studies using a surrogate murine antibody in mice.

Mechanism of action:

Eculizumab binds to the human C5 complement protein and inhibits terminal complement-mediated cell lysis and activation.

Drug activity related to proposed indication:

The intravascular hemolysis in PNH results from the deficiency of the terminal complement regulatory protein CD59 on the surface of PNH erythrocytes. Normally, CD59 blocks the formation of the terminal complement complex (also called the membrane attack complex) on the erythrocyte surface, thereby preventing hemolysis. Restoration of terminal complement inhibition, therefore, should effectively stop intravascular hemolysis.

2.6.2.3 Secondary pharmacodynamics

━ **Study No IM1184: Cross-Reactivity Study of Eculizumab with Normal Human Tissues (GLP study, ▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬**
▬▬▬▬▬▬▬▬▬▬▬▬ , July-September 2005)**

Study objective:

This study was conducted to evaluate the potential cross-reactivity of eculizumab with cryosections of normal human tissues.

Study design:

The unconjugated form of eculizumab was applied to cryosections of a panel of 38 normal human tissues (3 donors per tissue, where available) at two concentrations (30 µg/mL and 5 µg/mL). One sample from thymus was juvenile. Blood vessels were examined on all tissues. Immunoperoxidase staining method was used in this study.

The positive control consisted of human C5 and the negative control consisted of human hypercalcemia of malignancy peptide, amino acid residues 1-34 (designated PTHrP). Other controls were produced by omission of the test antibody (assay control) or substitution of a human myeloma-derived antibody with a different antigenic specificity than the test article (negative control antibody, HuIgG4).

For tissue immunohistochemical staining control, separate cryosections from each human tissue (except human blood cells) were stained in parallel for the expression of a human β2 microglobulin (a relatively ubiquitous epitope) using a rabbit antibody directed against human β2-microglobulin, to demonstrate overall tissue suitability for inclusion in the cross-reactivity study.

Results:

Eculizumab stained the positive control material consisting of human C5 spotted onto UV-activated resin slides, at both antibody concentrations. Eculizumab did not specifically react with the negative control material (human PTHrP). The negative control antibody HuIgG4 did not specifically react with either the positive or negative control material. There also was no staining of the assay control slides. β2-microglobulin localization was demonstrated in vascular endothelia and a variety of cells in all tissues, and in platelets and occasional intravascular lymphoreticular cells.

There was intracellular hC5-specific staining in multiple tissue elements in most of the following tissues in all the donors (except when specified otherwise):

Smooth muscle (intrinsic/vascular): Generally weak to intense staining of smooth muscle characterized as cytoplasmic filaments present in most tissues: adrenal capsule or capsule/trabeculae, bone marrow (1/3 donors), cerebrum, cerebellum, mammary gland (1/3 donors), eye, colon (2/3 donors), esophagus, small intestine, stomach, heart, kidney, liver, lung, lymph node capsule or trabeculae/medullary cords (2/3 donors), ovary, Fallopian tube, pancreas, parathyroid, peripheral nerve, pituitary, placenta (2/3 donors), prostate, salivary gland, skin intrinsic arrector pilus, spinal cord, spleen, skeletal muscle (2/3 donors), testis, thymus, thyroid, tonsil, ureter, urinary bladder, uterus, and cervix.

Striated (skeletal) muscle, myocytes: Weak to intense intracellular hC5-specific staining of skeletal muscle present in occasional tissues and characterized as cytoplasm or cytoplasmic striations: esophagus (2/3 donors), striated muscle, thyroid (1/3 donors), and tonsil (2/3 donors).

Myoepithelium: Strong to intense intracellular hC5-specific staining of myoepithelium characterized as cytoplasmic filaments present in occasional tissues: mammary gland, esophagus (1/3 donors), pancreas, and salivary gland.

Myofibroblasts: Weak to strong intracellular staining of smooth muscle present in rare tissues and characterized generally as cytoplasmic filaments: stroma of placenta (2/3 donors) and testis.

Renal tubular and glomerular epithelium: Moderate to strong intracellular staining of tubular and glomerular epithelium characterized as cytoplasmic filaments.

Reticulum cells: Weak to strong cytoplasmic staining of reticulum cells present in occasional lymphoid tissues. The dose dependent staining was seen in the red pulp reticulum cells in spleen (2/3 donors), in the medulla reticulum cells of the thymus (one juvenile sample out of 3 donors), and in the interfollicular reticulum cells in tonsil (3/3 donors).

Platelets: Moderate to strong intracellular staining of platelets seen at the high concentration in the blood. Residual endogenous peroxidase was noted in eosinophils.

Some non specific staining was considered due to endogenous/exogenous pigments such as melanin in eye and skin, fecal pigment in colon, lipofuscin in heart, endogenous pigment (hemosiderin) in lymph node, and exogenous pigment (carbon) in lung and lymph node.

Sponsor's conclusions:
C5-specific staining was observed in smooth and skeletal muscle in various tissues and was expressed in multiple cell types including myoepithelium, myofibroblasts, renal tubular epithelium, and reticulum cells in the human tissues examined. Moderate to strong staining of platelets was detected at the high concentration in all samples.

C5 expression in the human tissue panel examined in this study is consistent with published reports of C5 expression, as C5 has been reported in smooth muscle (Li *et al.*, 1999; Lin *et al.*, 1998), striated muscle (Yasojima et al., 1998), and renal proximal tubular epithelium (Fayyazi *et al.*, 2000, Immunology, 99(1):38-45). 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. These methods expose intracellular sites not normally present on the cell surface. No other reactivities or cross-reactivities were noted in this study.

Reviewer's comments:
The panel of adult human tissues examined was adequate. The concentrations tested were the clinical equivalent concentration and 6 times lower.
The distribution pattern and intensity of eculizumab staining of smooth muscle, skeletal muscle, and renal proximal tubular epithelium appeared consistent with the expected localization of complement C5 in the tissues, in which the presence of the target

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has been reported in the articles provided by the sponsor with the exception of the kidney. In Fayyazi et al.'s article, C5a receptor (C5aR) was reported to be expressed in the renal proximal tubular epithelium. However, in the present cross-reactivity study, eculizumab binds specifically to C5, and both the tubular and the glomerular epithelium were significantly stained. The staining was characterized as cytoplasmic. This observation was communicated to the clinical reviewer. In view of the absence of effects of eculizumab on the kidney functions in clinical trials, this finding was not considered toxicologically relevant.

2.6.2.4 Safety pharmacology

No animal studies were conducted to evaluate the safety pharmacology of eculizumab

2.6.2.5 Pharmacodynamic drug interactions

Not performed

2.6.3   PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.4   PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No animal pharmacokinetics or toxicokinetics studies were conducted using the surrogate murine antibody BB5.1. A study was conducted to analyze the pharmacodynamic and pharmacokinetic properties of an IgG4 isotype of eculizumab (h5G1.1 G4 mAb) in a C5-deficient mouse model reconstituted with physiologically relevant serum levels of human C5.

2.6.4.2 Methods of Analysis
[see under individual study reviews]

2.6.4.3 Absorption

Not applicable

2.6.4.4 Distribution

Not performed

2.6.4.5 Metabolism

Not performed

2.6.4.6 Excretion

Not performed

2.6.4.7 Pharmacokinetic drug interactions

Not performed

2.6.4.8 Other Pharmacokinetic Studies

Study GTR-0104.00: Pharmacodynamics and pharmacokinetics of C5-deficient Mice Reconstituted with Human C5 and Treated with an h5G1.1-mAb ━━━━ ━━━━ , approved 4 May 2005)

Study objective:

The purpose of this study was to analyze the pharmacodynamic and pharmacokinetic properties of the humanized anti-human C5 monoclonal antibody (mAb) h5G1.1. In view that h5G1.1 does not recognize C5 from other species, a C5-deficient mouse model reconstituted with physiologically relevant serum levels of human C5 was used. The analysis was performed using an IgG4 isotype of h5G1.1 (h5G1.1 G4 mAb), which is identical to h5G1.1-mAb (i.e. eculizumab) except that it contains the human IgG4 heavy constant region instead of a hybrid human IgG2/IgG4 heavy constant region. In study GTR-0084, the constant regions were shown to not affect C5 binding and complement inhibition in vitro.

Study design:

C5-deficient male B10.D2/oSn mice were reconstituted with 250 μg human C5, in PBS, then intravenously or subcutaneously injected 0.2 mL PBS, or a single dose of h5G1.1 G4 mAb at 5, 17, 50, 100, or 150 μg, or 190 μg of 2A2 HuG4 16 to 18 hours later, an unrelated antibody with a human IgG4 constant region used as a control antibody. Serum samples were collected at 2 min, and 2, 4, 8, 24, or 48 hours and in some cases at 72 hours following antibody injection. The hC5 serum levels and h5G1.1 G4 mAb serum levels were determined by ELISA assay. The pharmacodynamic or hemolytic activity was assessed with a hemolytic assay employing chicken erythrocytes and performed with 5% mouse serum samples plus 10% C5-depleted human serum.

Results:

1. Pharmacodynamics:

The serum hC5 levels averaged 25 to 45 μg/mL, as determined by ELISA, and levels were sustained up to at least 48 hours following hC5 injection. In PBS control mice, these levels of hC5 were able to reconstitute hemolytic activity. In contrast, rapid and dose-dependent inhibition of hC5-dependent hemolytic activity was obtained in mice IV injected with 50 μg or more of the h5G1.1 antibody (Fig.1). Hemolytic activity inhibition was maintained for at least 48 hours following the single IV administration of

50 μg antibody. The kinetics of systemic inhibition was delayed by 12-24 hours following subcutaneous injection (Fig.2). No inhibition was detected for the control antibody that has the same heavy constant region.

Figure 1: Inhibition of hC5-dependent serum hemolytic activity after IV administration of h5G1.1 G4 mAb

Hemolytic Activity (%)
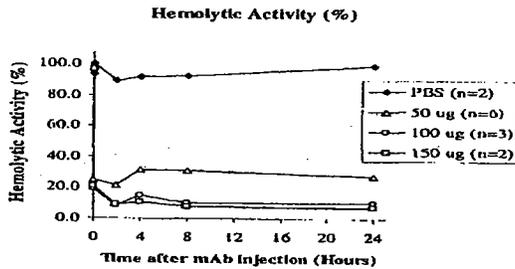
Figure 2: Pharmacodynamics of a 50 μg dose of h5G1.1 G4 mAb following IV or SC administration in hC5 reconstituted C5-deficient mice
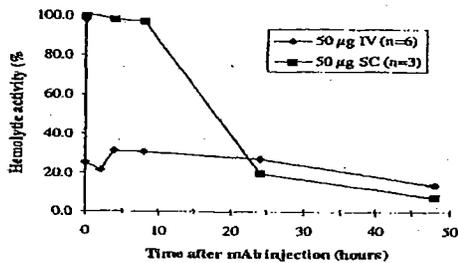
2. Pharmacokinetics:

Following IV injection, a relatively rapid $T_{1/2}\alpha$ of several hours was followed by a slow decline in serum concentrations over the next 48 hours. In contrast, SC administration was followed by a progressive rise in serum concentration such that serum levels of the antibody at 24 hours and thereafter were comparable to those measured following IV injection (Fig.3).

Figure 3: Pharmacokinetics of h5G1.1 G4 mAb after IV or SC administration in hC5 reconstituted C5-deficient mice
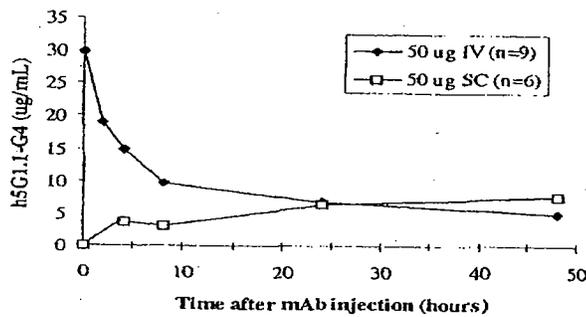
The concentrations of h5G1.1 G4 and hC5 calculated from each serum sample were used to determine the molar ratios of antibody to hC5 at the various time points (Fig.4). Taken together with the hemolytic activity determinations, it was determined that the h5G1.1 antibody completely blocks hC5-dependent hemolysis at a molar ratio of mAb to hC5 of approximately 0.5 to 1.

Figure 4: Molar ratio of h5G1.1 G4 mAb to hC5 in hC5 reconstituted C5-deficient mice dosed with 50 μg of h5G1.1 G4 mAb.
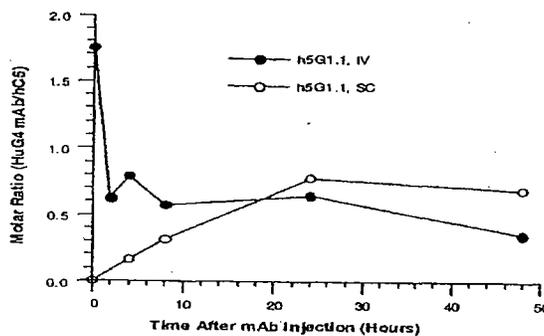
Conclusion:

It was determined that the h5G1.1 antibody completely blocks hC5-dependent hemolysis at a molar ratio of mAb to hC5 of approximately 0.5 to 1. Based on an average hC5 plasma concentration of 76 μg/mL (Kohler and Mueller-Eberhard 1967, J Immunol., 99(6):1211-6), and a plasma volume of 3.0 L, the sponsor predicted that a single dose of 1.5-2.0 mg/kg of h5G1.1 mAb should be sufficient to acutely mediate complete inhibition of complement-dependent serum hemolytic activity in vivo in humans.

Reviewer's comments:

In comparison, the proposed clinical dose ranges from 450 to 900 mg/week, equivalent to 7.5 to 15 mg/kg/week or 1.1 to 2.1 mg/kg/day of eculizumab for a 60 kg adult, indicating that the clinical dose should yield complete inhibition of hemolysis. Additional studies using the murine antibody and C5-sufficient mice would have been of

value to help understand the variable results obtained in the hemolysis activity in the toxicology studies.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary
Two repeat dose toxicity studies and three reproductive studies (Seg I, II, and III) were conducted in mice, administered a surrogate mAb, the murine C5 specific BB5.1. Bolus intravenous injection was used whereas the intended clinical route is 45 minute infusion. Toxicokinetics and antibody measurement were not conducted in any of the toxicology studies. The hemolyitic activity, used as surrogate of drug systemic exposure, did not provide consistent results in the reproductive toxicity studies. Pregnant females appeared to exhibit lower % hemolysis than males and non pregnant females.

Repeat dose toxicity:
In a 4 week repeat dose toxicity study, no toxic signs, based on mortality, clinical observations and body weights, were observed in mice treated for 4 weeks with 30, 60, or 90 mg/kg/week (2-4, 4-8, and 6-12 times the human dose). Similar hemolytic inhibition was obtained at 60 and 90 mg/kg/week. Therefore, 60 mg/kg/week was selected as the maximum dose for subsequent studies.

In a 26 week toxicity study, mice were treated with 30 or 60 mg/kg/week (2-4, 4-8 times the human dose). Nine unscheduled deaths occurred (4/50 in control group and 5/50 in the 60 mg/kg/week group). No unscheduled death was recorded in the 30 mg/kg/week group. One high dose group male died during injection and exhibited lung thrombosis whereas one high dose female died after dosing. Death was considered accidental in a control male found with esophagus perforation, and in one control female death was attributed to bronchiolar-alveolar carcinoma. The cause of death for the others could not be determined, although macroscopic and microscopic findings differed between control and treated animals (e.g. pigmented foamy macrophages, lung congestion and thrombosis, subendocardial chronic active inflammation/hemorrhage observed in treated groups).

Dose-dependent clinical signs included missing ears, sores/scabs in the ear or cranial area, rough hair coat, bent tail, and thin and/or hunched appearance. Macroscopic findings included skin sores, and sporadic findings in the treated groups. Ovary and uterus cysts, distended or with thickened wall, with lumen fluid were observed at higher incidence in treated females. AST and ALT mean values increased (~42 and 76% respectively) on week 31 for males. A statistically significant increase was observed in liver weights in high dose females, and an increase in heart weight (up to 12%) in high dose males and females were observed. At terminal sacrifice, the most remarkable findings included lung congestion, thrombosis, and/or hemorrhage, skin lesions (chronic active inflammation, necrotic epidermal cellular debris, ulcer, and acanthosis), and eyes retrobulbar inflammation and hemorrhage. Lens degeneration and bulbi phtysis was noted in one low dose female.

Lung neoplasms were observed in 2 unscheduled control deaths, pituitary B-adenoma and lung peribronchial lymphoid infiltration in 1/33 high dose female, multiple

hematopietic neoplasms (lymphoma/lung/salivary glands/thymus) in 1/33 high dose female. Lymphoid and lymphoreticular hyperplasia were observed respectively in mandibular lymph node (1 high dose female) and in thymus (1 high dose female). A NOAEL could not be established in this study.

Reproductive toxicology:

BB5.1 administered by intravenous injection to male and female mice prior to mating and until termination (for 28 days prior to mating until 2 days prior to necropsy for males) or through early gestation (from over 14 days prior to mating), did not affect female reproductive performance, female and litter reproductive parameters, and sperm count and motility. Sperm morphology was not performed. The NOEL for female toxicity and reproductive performance, for male and female fertility, and fetal effects was established at ≥ 60 mg/kg/week. The NOAEL was established at 30 mg/kg/week BB5.1 for male toxicity in view of two male deaths in the 60 mg/kg/week.

BB5.1 administered by IV injection to pregnant mice during the period of organogenesis did not affect maternal body weights, food consumption, and Cesarean section parameters. No toxicokinetic analysis was conducted to confirm fetuses exposure during pregnancy. Fetal malformations were sporadic with no dose-response pattern and there was an increased incidence of 14th rib and accessory bones in skull of treated fetuses. Two cases of retinal dysplasia and one case of umbilical hernia were observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. The NOAEL was established at 60 mg/kg/week for maternal toxicity and reproductive performance. The NOAEL for embryo/fetal development toxicity was established at 30 mg/kg/week.

In a prenatal and postnatal development study, the effects of BB5.1 were evaluated when administered by intravenous injection from implantation through weaning, on pregnant and lactating female mice, and on the development of the offspring. No toxicokinetics analysis, no maternal milk or placenta analysis for presence of murine antibodies were performed in any of the studies. Therefore, the assumption is that the neonates were exposed either during pregnancy or during lactation or both, since there were no data to support either one of the hypothesis. The results showed that one 60 mg/kg/week Fo female was found dead on LD 14 with necropsy revealing a pale liver. In view of the number of dead (1/25 in low dose, 2/25 in high dose), and moribund male animals (1/25 in controls, 1/25 in low-dose, and 3/25 in high dose), and presence of clinical signs (skin sores, localized swelling and distended abdomen) in the F1 male generation, a NOAEL could not be established for these parameters. The NOAEL was established at ≥ 60 mg/kg/week for F1 pup development and reproductive performance.

2.6.6.2 Single-dose toxicity

None submitted

2.6.6.3 Repeat-dose toxicity

Study title: Four Week Intravenous Injection Range-Finding Study in Mice with BB5.1

Study objective:

This study was designed to select a dose (based on hemolytic assay) for repeat dose toxicity studies.

Key study findings: Based on mortality, clinical observations and body weights, no toxic signs were observed in mice treated for 4 weeks with 30, 60, or 90 mg/kg/week. The sponsor selected the dose of 60 mg/kg/week for future toxicology studies, based on the fact that the hemolytic activity was similar between 60 and 90 mg/kg/week treatment groups.

Study no.:   6709-1 09
Volume #, and page #:   N/A (e-submission)
Conducting laboratory and location: ▬▬▬▬▬▬▬▬
▬▬▬▬▬

Date of study initiation: 28 June 2001
GLP compliance: Yes
QA report: Yes (x) no ( )
Drug, lot #, and % purity: Referred to as BB5.1 IgG and/or BB5.1 (syn. Mouse anti-C5), Lot No. 2640001 FBP, SDS PAGE 174 KDa, 3.0 mg/mL
Vehicle 0.2 M Tris-buffered saline Lot 416576/1 20701

Methods

Doses: Once, twice, or three times/week to obtain 30, 60, or 90 mg/kg/week for 4 weeks. Vehicle diluted to 20 mM was administered 3 times/week.
Species/strain: mice/▬ :CD-IB(1CR)BR
Number/sex/group or time point (main study): 7 females/dose.
Route, formulation, volume, and infusion rate: IV, 3mg/mL solution, 10 mL/kg/dose, bolus.
Satellite groups used for toxicokinetics or recovery: none; hemolytic activity was used as an indicator of systemic exposure.
Age: ~8-week-old
Weight: 18.5 to 30.7 g
Sampling times: Blood samples (200 μL) were collected from all mice under anesthesia on Days 8, 15, 22, and 29 for determination of hemolytic activity.
Unique study design or methodology (if any): none
The study was conducted according to the following design:

Group	Females #	Dose level mg/kg/dose	Dose level doses/week	Dose level mg/kg/week	Concentration mg/Ml	HDM*
1(control)	7	0	3	0	0	0
2 (low)	7	30	1	30	3	2-4 XHD
3 (mid)	7	30	2	60	3	4-8 XHD
4 (high)	7	30	3	90	3	6-12 XHD

* HDM: Human Dose Multiple based on weight. The clinical dosage regimen consists of an induction phase of 600 mg/week for the first 4 weeks followed by 900 mg in week 5 and a maintenance phase of 900 mg every ~2weeks thereafter. With a dose range of 450mg-900mg/week, the clinical dose could range from 7.5 to 15 mg/kg/week for a 60kg adult.

.Observations and times:

Mortality/moribundity: Twice daily
Clinical signs: Cageside observations were made once daily and detailed
                           observations once prior to treatment and once weekly
                           thereafter.
Body weights: Prior to treatment (at time of randomization), on the first day
                           of treatment (prior to dosing), and once weekly thereafter.

All mice were weighed, euthanized with carbon dioxide, exsanguinated, and discarded without necropsy on Day 30.

Results

Mortality: One mouse in control Group 1 died and was replaced by another mouse on treatment day 3. However, the sponsor states that all mice survived until scheduled sacrifice.

Clinical signs: There were no treatment-related clinical observations.

Body weights: There were no treatment-related effects on body weight or body weight change.

Hemolytic activity: Results are summarized in the following table

Avg. % Hemolysis (mean ± SD)				
Animal groups	Day 8	Day 15	Day 22	Day 29
Group 1 (Vehicle, n=6)	94 ± 11	92 ± 15	93 ± 16	92 ± 20
Group 2 (30 mg/kg/week, n=7)	34 ± 12	20 ± 13	9 ± 6	3 ± 9
Group 3 (60 mg/kg/week, n=7)	13 ± 14	10 ± 16	6 ± 9	1 ± 2
Group 4 (90 mg/kg/week, n=7)	15 ± 7	9 ± 15	1 ± 2	0 ± 0

Serum analysis results indicated that the extent of hemolysis decreased over the duration of the study (from Day 8 to Day 29) at all dose levels. In contrast, the mean percent hemolysis in the control mice (92 to 94%) did not change over the duration of the study. Similar extents of hemolytic prevention were noted for serum obtained from mice treated with BB5.1 at 60 versus 90 mg/kg/week: 13, 10, 6, and 1% at 60 mg/kg/day and 15, 9, 1, and 0% at 90 mg/kg/day, for Days 8, 15, 22, and 29 respectively. At 30 mg/kg/day, the extent of hemolytic prevention was less than noted in the 60 and 90 mg/kg/week mice with 34, 20, 9, and 3% for Days 8, 15, 22, and 29, respectively.

Conclusion

No indications of toxicity as measured by mortality/morbidity, clinical observations, and body weights, were noted in CD-1 female mice intravenously administered BB5.1 once, twice or three times weekly for 4 consecutive weeks to provide dose levels of 30, 60, and 90 mg/kg/week, respectively. Serum analysis indicated similar hemolytic activity for mice treated with BB5.1 at 60 and 90 mg/kg/week, and a lower activity for mice treated with 30 mg/kg/week. The sponsor concluded that the recommended high dose of BB5.1 in future toxicology studies is 60 mg/kg/week.

Reviewer's comments:

The highest dose selected for future studies will be 4 to 8 times the human clinical dose [600mg/week for the first 4, 900mg on week 5, and 900 mg/kg every 2 weeks] for a 60 kg adult.

The same batch was used in all the toxicology studies. One sample of the bulk purified product tested positive for fungus (1 colony at Day7). Analysis of another sample was negative, and the product was filtered again. Although the sterility of the product was not re-confirmed in a repeat test, the biological activity of the drug was conserved as shown in the hemolytic assay, and this finding is unlikely to affect the study results.

Study title: 26-Week Intravenous Injection Toxicity Study of BB5.1 in Mice with a 4-Week Recovery

Key study findings: There were 9 unscheduled deaths in this study (4 control and 5 high dose animals). Because of the higher incidence in clinical signs and histopathological findings (lungs, ears, eyes) in the low and high dose treatment groups compared to the control group, a NOAEL could not be established in this study.

Study no.:	6709-108
Volume #, and page #:	N/A (e-submission)
Conducting laboratory and location:	▬▬▬▬▬▬▬▬▬▬
	▬▬▬▬▬▬
Date of study initiation:	9 August 2001
GLP compliance:	Yes (with an important deviation on Day –4, serum samples collected for serum hemolytic activity were not centrifuged within 60 minutes of collection).
QA report:	yes (X) no ( )
Drug, lot #, and % purity:	BB5.1 IgG and/or BB5.1 (syn. Mouse anti-C5), Lot No. 2640001 FBP, Protein concentration: 3mg/mL, SDS PAGE 174 KDa.

Methods

Doses: 0, 30 or 60 mg/kg/week for 26 weeks
Species/strain: mice/ ➡:CD-1®(ICR)BR

Number/sex/group or time point (main study): 65/sex/dose

Route, formulation, volume, and infusion rate: Intravenous, 3 mg/mL in Tris-Buffered saline, 10 mL/dose, bolus.

Stability and Routine Analysis: Following thawing and prior to use, a 1-mL aliquot of each test article and vehicle/control solutions was collected at Weeks 1, 4, 7, 13, 20, 24, and 26 for analysis.

Hemolytic Activity: A hemolytic assay employing chicken erythrocytes was used to assess the pharmacodynamic activity of BB5.1 in mouse serum samples collected at various intervals during the study: once prior to initiation of treatment on Day 1, once during the latter part of Weeks 12 and 25, and once during Week 30.

Satellite groups used for toxicokinetics or recovery: none

Age: ~8-week-old at dosing

Weight: 26.9-37.1 g for males and 21.3-28.5 g for females

Unique study design or methodology (if any): none

The treatment was intravenously administered to mice according to the following design

	Number of mice*		Dose levels				
Group	Male	Female	mg/kg/dose	Dose/week	mg/kg/week	Concentration Mg/mL	HDM[#] Based on weight
1 (control)	25	25	0	2	0	0	0
2 (low)	15	15	30	1	30	3	2-4 XHD
3 (high)	25	25	30	2	60	3	4-8 XHD

*The first 15 mice/sex/group were designated for terminal sacrifice following at least 26 weeks of treatment. The last 10 mice/sex in Groups 1 and 3 were designated to undergo at least 4 weeks of recovery following at least 26 weeks of treatment.

[#]HDM: Human Dose Multiple based on weight. The clinical dosage regimen consists of an induction phase of 600 mg/week for the first 4 weeks followed by 900 mg in week 5 and a maintenance phase of 900 mg every ~2weeks thereafter. With a dose range of 450mg-900mg/week, the clinical dose could range from 7.5 to 15 mg/kg/week for a 60kg adult.

Observations and times:

Mortality/Morbidity: Twice daily

Clinical signs: Cageside observations made once daily, detailed observations once prior to treatment and once weekly thereafter.

Body weights: Recorded once prior to treatment, on the first day of treatment and once weekly thereafter.

Food consumption: Weekly

Ophthalmoscopy: Prior to treatment and once during Week 25

EKG: Not performed

Hematology: Sampling was conducted approximately 1 week prior to terminal and recovery sacrifices

Clinical chemistry: Sampling was conducted at terminal and recovery sacrifices

Urinalysis: Not performed

Gross pathology: Performed on all mice that died or were sacrificed in a moribund condition, or on surviving mice after 26 weeks of treatment or, after 26 weeks of treatment and 4 weeks of recovery.

Organ weights: Adrenal (2), brain, heart, kidney (2), liver (with gallbladder), ovary (2), spleen, testis with epididymis (2), thyroid with parathyroid.

Histopathology: Adequate Battery: yes (X); Peer review: no (X)
Tissues (as appropriate) from each terminal-sacrifice mouse in the control and high-dose groups and from each mouse that died or was sacrificed at an unscheduled interval were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Macroscopic lesions were examined microscopically from each mouse in the low-dose group. No histopathology was performed after the recovery period.

Results

Dose analysis results using the hemolytic assay:

Analysis of hemolytic activity for the dose formulations demonstrated hemolysis inhibition. The mean hemolysis values of a 50 and 1 µg/mL dilution of the dose formulations were approximately 4% and 91% respectively throughout the study. Mean values for the Tris Buffer dilutions were ≥ 95%.

Mortality:

Nine unscheduled deaths occurred during the study: 3 males (on weeks 12, 14, and 23) and 1 female (week 17) in the control group and 3 males (weeks 6, 12, and 22) and 2 females (weeks 21 and 23) in the 60 mg/kg/week group. One control male death was accidental as determined by the esophagus perforation and the death in a control female was attributed by the sponsor to bronchiolar alveolar carcinoma. Two animals of the high dose group died during or following dose injection. The cause of unscheduled deaths in the remaining animals was not determined.

There were no unscheduled deaths reported in the 30 mg/kg/week group

Clinical signs:

The most remarkable clinical signs likely to be treatment-related, started around the 8th week of treatment, and consisted of missing left, right or both ears (2/50 control, 3/30 low dose, 8/50 high dose), often associated with sores/scabs in the ear or cranial area (5/50 control, 6/30 low dose, and 10/50 high dose). Clinical findings also included rough hair coat (4 controls, 5 low dose, and 11 high dose), bent tail (1 low dose, 3 high dose) and thin and hunched appearance (1 low and high dose).

Body weights:

Although statistically significant changes were occasionally noted, these changes did not occur consistently, and overall mean body weight changes were not affected by treatment.

Food consumption:

Although statistically significant changes were occasionally noted, these changes did not occur consistently, and overall mean food consumption was not affected by treatment.

Ophthalmoscopy:

There were no treatment-related ophthalmic observations.

Hematology:

        There were no treatment-related changes.

Hemolytic activity evaluation:

The results of hemolytic activity are shown in the following tables:

	Mean % Hemolysis			
	Week 1	Week 12	Week 25	Week 30
Group 1 ♂	72.5	81.4	85.6	86.6
Group 2 ♂	79.2	15.7	18.8	NS
Group 3 ♂	69.1	11.0	21	67.5
Group 1 ♀	68.3	74.2	85.3	85.1
Group 2 ♀	70.8	13.3	14.5	NS*
Group 3 ♀	50.9	3.9	4.5	41.6

    *NS: no sample

Clinical chemistry:

        There were no treatment-related clinical pathology findings with the following exceptions: AST and ALT mean values increased (~42 and 76% respectively) on week 31 for males and there was a statistically significant decrease (~35%) in mean ALK P in high dose females on week 31.

Gross pathology:

Unscheduled deaths

        Remarkable findings included mottled lungs, grey mass (carcinoma) in the lung, perforated esophagus, enlarged kidneys in control animals and soft brain, mottled lungs, dark stomach, ovary cyst, fluid in the thoracic cavity, and hunched and thin appearance in the treated group.

Terminal sacrifice

        Remarkable findings consisted of skin sores mostly in males (1 control, 3 low and 4 high dose) and alopecia (one low dose), raised area of pituitary (1 high-dose female), enlarged spleen (1 low-dose), enlarged mandibular lymph node (1 low-dose, 1 high-dose), and distended urinary bladder with lumen fluid (1 high dose). Ovary cysts (3 control, 6 low-dose, 2 high dose), and uterus cysts (1 low, 2 high dose), with thickened wall (2 low-dose), or distended with lumen fluid (1 control, 2 high dose) were observed.

Recovery sacrifice

        Macroscopic effects were somewhat less marked, except for the distended uterus and fluid lumen found in 2 controls and 4 high-dose mice, and the uterus cysts noted in 2 controls and 5 high dose females.

Organ weights:

Significant organ weight changes are shown in the table below. Changes are expressed as % of corresponding control groups.

% Weight Change		Terminal sacrifice			Recovery		
		Absolute	Relative to body	Relative to brain	Absolute	Relative to body	Relative to brain
Adrenals	G2♂	↑163#	↑203#	↑162#	-	-	-
	G3♂	-	-	-	↓14	↓22	↓22
Ovary	G2♀	↓23	↓25	↓22	-	-	-
Spleen	G2♂	↑80	↑82	↑67	-	-	-
	G3♂	↑20	↑9	↑5	↓13	↓14	↓12
Thyroid/Parathyroid	G3♂	↑27	↑20	↑20	↑14	↑8	↑10
	G3♀	-	-	-	↓22	↓21	↓16
Heart	G3♂	-	-	-	↑12	↑9	↑9
	G3♀	-	-	-	↑11	↑5	↑9
Liver	G3♀	-	-	-	↑14*	↑8*	↑12*

*Statistically significant; #Due to large standard deviation
G2: 30 mg/kg/week group; G3: 60 mg/kg/week

The most remarkable changes were a statistically significant increase in liver weights in the high dose females, and an increase in heart weight (up to 12%) in high dose males and females.

Histopathology:

No histopathology was performed for the recovery groups.

Unscheduled deaths

In the control animals, unscheduled death was considered accidental in the mouse with perforated esophagus, and may have been caused by bronchiolar-alveolar neoplasm (1M with mottled red dark lungs), and bronchiolar-alveolar carcinoma (1F). The 4th control animal had both kidneys enlarged with no histopathology correlate.

In the treated animals, findings consisted of pigmented foamy macrophages in adrenal cortex, spleen, or mesenteric lymph nodes, lung thrombosis (1M) and congestion (3), and one case of subendocardial chronic active inflammation/hemorrhage.

Terminal sacrifice

The most remarkable microscopic findings were observed in lungs, skin and eyes. Findings included lung congestion (3 control, 7 treated females) and hemorrhage (1 control, 3 treated), lung thrombosis (one high dose), and lung lymphoid peribronchial infiltration (1 high-dose female). Eye retrobulbar inflammation (1 low dose, 2 high dose), eye hemorrhage (2 high dose), lens degeneration and bulbi phtysis (one low dose female) were observed mostly in female mice. Sores/scabs were associated with chronic active inflammation and necrotic cellular debris of epidermal surface (1 control, 3 low dose, 4 high dose), ulcer and acanthosis (2 low dose, 4 high dose).

Lung neoplasms were observed in 2 control unscheduled deaths, pituitary B-adenoma and lung peribronchial lymphoid infiltration in 1/33 high dose female, multiple hematopietic neoplasms (lymphoma/lung/salivary glands/thymus) in 1/33 high dose female. Mandibular lymph node lymphoid hyperplasia and thymus lymphoreticular hyperplasia were observed in one high dose female each.

Other remarkable microscopic findings in the high dosed groups included: kidney tubule dilatation (1 control, 3 high dose), liver pigment sinusoidal cell (1), pancreas chronic inflammation (1), salivary gland chronic inflammation (1), jejeunum or ileum amyloidosis (1 control, 1 high dose)

Toxicokinetics: Hemolytic activity was measured as an indicator of systemic exposure. (See results of hemolytic activity)

The sponsor established the NOAEL at 60 mg/kg/week.

Reviewer's comments and conclusions:

Toxicokinetics analysis was not performed. Moreover, there was no measurement of both total and neutralizing antibodies against the biologic agent. At a minimum, samples should have been collected prior to study initiation, at scheduled necropsy, following the final dose, and at the completion of the recovery period. However, the hemolytic activity assay results were indicative of systemic exposure, which was dose-dependent in females but not in males. Some blood samples were not centrifuged within the protocol-required 60 minutes of collection. The biological activity was sustained through the duration of the study, and decreased at the end of the treatment-free recovery period.

Because of the higher incidence in clinical signs and histolopathological findings (lungs, ears, eyes) in the low and high dose treatment groups compared to the control animals, a NOAEL could not be established in this study.

Histopathology inventory (optional)

Study	6709-108
Species	Mice
Adrenals	X*
Aorta	
Bone Marrow smear	
Bone (femur) with bone marrow	X
Brain	X*
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*

Lachrymal gland	
Larynx	
Liver	X*
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X
Thyroid	X*
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology
Not performed

2.6.6.5 Carcinogenicity
Not performed

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Study of fertility and early embryonic development to implantation in mice with BB5.1

Key study findings: 30 mg/kg/week BB5.1 is the NOAEL for male toxicity in view of the deaths in the 60 mg/kg/week group. The NOEL for female toxicity, for male and female fertility, reproductive performance, and fetal effects is ≥ 60 mg/kg/week.

Study no.:	6709-104
Volume #, and page #:	N/A, electronic submission (e-CTD)
Conducting laboratory and location:	▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬
	▬▬▬▬▬▬▬▬▬
Date of study initiation:	9 August 2001
GLP compliance:	Yes
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	BB5.1 IgG and/or BB5.1 (syn. Mouse anti-C5), Lot No. 2640001 FBP, Protein concentration: 3mg/mL, SDS PAGE 174 KDa.

Objective: This study was designed to assess the effects of BB5.1 when administered by intravenous injection to male and female mice prior to mating and until termination (approx. week 10 for males) or through early gestation (approx. week 6 for females). This study included assessment of potential effects on general toxicity, gonadal function, mating behavior, implantation, and general fertility according to the following design:

Group	Number of animals		Dose levels			
	Male	Female	mg/kg/dose	Dose/week	Mg/kg/week	Human Dose Multiple based on weight
1 (control)	25	25	0	2	0	0
2 (low)	25	25	30	1	30	2-4 XHD
3 (high)	25	25	30	2	60	4-8 XHD

Methods

Study design:

One hundred and fifty Crl:CD-1®(ICR)BR mice, 9 weeks old, weighing 23.3 to 36.0 g and 24.1 to 29.9 g for males and females respectively, were dosed by IV injection via tail vein. The control and high dose groups were dosed twice weekly and the low dose group was dosed once weekly. Males were dosed over at least a 28-day period prior to mating and until one or two days prior to necropsy (~10 week treatment). Females were dosed over at least a 14-day period prior to mating, throughout the mating period, and during early gestation, with the last dose administered no later than GD 7 (~6 week treatment).

Parameters and endpoints evaluated:

Survival and moribundity: recorded twice daily.

Clinical signs: evaluated daily post dose during the first 2 weeks of dosing.

Estrus cycle was determined daily during the mating period until confirmation of mating occurred or until the mating period ended.

Body weights: recorded at randomization, twice weekly during treatment, and at termination for males. Females were weighed twice weekly during the premating treatment phase and during mating. Females' body weights were also recorded on GDs 0, 3, 7, 10, and 13.

Food consumption: recorded weekly during the premating treatment period.

Serum chemistry for the determination of hemolytic activity:
Blood was collected from the orbital sinus under anesthesia, from the first five males per group during weeks 4 and 10, and from the first five confirmed-mated females per group on week 7 or GD 12 (~6 days after the last dosing). The biological activity of BB5.1 was assessed with a hemolytic assay using chicken erythrocytes. Assays with BB5.1 dosing formulations, placebo, and mouse serum samples were performed with a 5% mouse samples plus 10% C5-depleted human serum. The males were bled on weeks 4 and 10 and the females on week 7, allowing sufficient time for significant clearance by the normal routes.

Disposition of animals:
On GD 13, necropsy was performed on all confirmed females and on females that did not confirm to have mated for abnormalities of the cervical, thoracic, or abdominal viscera. The number and distribution of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were recorded.
Necropsy was performed on surviving males at termination and on dead males. Epididymes, testes, seminal vesicles (with coagulating gland), and prostate were weighed and preserved in 10% neutral-buffered formalin, except for the right epididymis and testis used for assessment of reproductive capacity on the first 10 surviving males/group. The left epididymis was used for motility assessment. The right testis was stored in 10% neutral buffered formalin for possible future spermatogenic staging.

Results

Mortality:
There were two unscheduled deaths in the 60 mg/kg/week male group. There were no deaths in female groups. One mouse was found dead on Day 43. Prior to death, there were no remarkable clinical observations. Necropsy revealed a severely distended bladder and dark bright orange lungs. The other mouse was found dead on Day 70. Prior to death, the animal was reported as hypoactive, cold to the touch, with tremors. These clinical observations were not observed in this animal on other days prior to Day 70 or in any other mice. There were no remarkable findings at necropsy. No histopathology was performed.

Clinical signs:

There were no remarkable drug- related effects on clinical signs of the surviving animals. The only clinical signs noted prior to the death in the 60 mg/kg/week male on Day 70, which was hypoactivity, cold to the touch, with tremors. The estrus cycle was unaffected by BB5.1.

Body weight:
There were no remarkable drug-related effects on body weight and body weight changes for any animals of any group including pregnant females.

Food consumption:
There were no remarkable drug-related effects on food intake for any animals of any group.

Hemolytic activity:

The hemolytic assay results are summarized in the following table

Average % hemolysis for mouse sera

	Week 4	Week 7	Week 10
Group 1 males	90.2		100
Group 2 males	24.6		27.2
Group 3 males	7.0		16.2
Group 1 females		98.4	
Group 2 females		50.6	
Group 3 females		50.6	

Sera from Groups 1 (control article) did not protect erythrocytes from hemolysis as evidenced by hemolysis percentages $\geq$ 90%. In contrast, mice treated with BB5.1 sera display lower serum hemolysis indicating the presence of biologically active drug with hemolytic activity generally $\leq$ 50% in the treated groups. In some treated female mice, individual hemolytic activity values were similar to those of controls.

Necropsy:
There were no remarkable drug related effects seen at gross necropsy for males or females of any of the treated groups when compared to the control group of animals.

Organ weights:
No remarkable treatment-related findings

Summary of overall reproductive performance data:

	Group 1 0 mg/kg/week	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
No. of male/female pairs	25	25	25
No. (%) of animals mated	24	25	25
(Male/Female Copulation Index)	96%	100%	100%
No. (%) of animals successfully mated	24	24	22

(Male/Female Fertility Index)	100%	96%	88%

BB5.1 had no effect on reproductive performance; most of the females mated within the first 4 days of the mating period.

Summary of female and litter reproductive parameters:

Parameters	Group 1 Control	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
Females Assigned	25	25	25
Female Mated	24	25	25
Dam Deaths	0	0	0
No. Pregnant	24	24	22
Aborted	0	0	0
Delivered Early	0	0	0
Pregnant at C-Section	22	24	21
Dams with Viable Fetuses	22	24	21
Dams with no Viable Fetuses	0	0	0
Total Live Fetuses	268	278	250
Total Dead Fetuses	0	0	0
Mean live Fetuses (#/%)	12.2/92.7	11.6/88.9	11.9/89.3
Total Corpora Lutea	303	338	296
Mean Corpora Lutea	13.8	14.1	14.1
Implantation Sites	289	312	276
Mean Implantation Sites	13.1	13.0	13.1
Pre-Implantation Loss (%)	4.7	7.5	6.2
Post-Implantation Loss (%)	7.3	11.1	10.7
Total Resorptions (Total/Mean/%)	21/1.0/7.3	34/1.4/11.1	26/1.2/10.7
Early Resorptions (Total/Mean/%)	15/0.7/4.8	31/1.3/10.0	20/1.0/8.0
Late Resorptions (Total/Mean/%)	6/0.3/2.5	3/0.1/1.1	6/0.3/2.7

The number and distribution of corpora lutea, implantation sites, early and late resorptions, live and dead fetuses, and indices for pre-implantation loss and post-implantation loss were unaffected by treatment.

Summary of sperm analysis parameters:

Sperm parameters		Group 1 0 mg/kg/week	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
Motility (%)	Mean	85	91	87
	Number examined	10	10	10
Epididymal count (Million sperm/count)	Mean	1549.7	1542.3	1450.4
	Number examined	10	9	10

Sperm count and motility were examined in the first surviving 10 males per group and were not affected by BB5.1. Sperm morphology was not performed.

Report conclusion:

Based on the results of this study, the NOAEL for male toxicity is 60 mg/kg/week, while the NOEL for female toxicity, male and female fertility, and embryo-fetal viability, is ≥ 60 mg/kg/week.

Reviewer's Comments:

Hemolytic activity analyses show a significant difference between male and female groups suggesting less efficient inhibitory activity or other unidentified mechanistic effects of BB5.1 in pregnant females   Although the potential for immunogenic reaction in mice is low in view that BB5.1 is a murine antibody, both total and neutralizing antibodies against the biologic agent should have been measured and the possible effect of pregnancy on their production evaluated. In addition toxicokinetics was not performed. In some female mice, the individual hemolytic activity was similar to that of controls.

There were two unscheduled deaths in the 60 mg/kg/week male group. Necropsy revealed a severely distended bladder and dark bright orange lungs in one of the mice. (Lung appeared to be a target organ in the 26 week repeat dose study). Although no death was ascribed to BB5.1 by the sponsor in any of the toxicology studies, the relationship between drug and death was not completely ruled out because the incidence of death was often higher in treated animals compared to control animals across the studies: There were two unscheduled deaths in the 60 mg/kg/week male group in the present study and dead/moribund male animals (1/25 in controls, 2/25 in low-dose, and 5/25 in high dose) in the Seg III study (see review) in the F_1 generation males.

In view of the deaths in the 60 mg/kg/week and the slightly lower mean absolute and relative prostate weights in the 60 mg/kg/week group, 30 mg/kg/week BB5.1 is the NOAEL for male toxicity. The NOAEL for female reproductive toxicity is 30 mg/kg/week, and the NOAEL for female toxicity, male and female fertility, and fetal effects is ≥ 60 mg/kg/week.

Embryofetal development

Study title: Mouse Developmental Toxicity Study with BB5.1

Key study findings: The NOAEL for BB5.1 administered by IV injection to pregnant mice during organogenesis is 60 mg/kg/week for maternal toxicity and reproductive performance (based on C-section parameters). The NOAEL for embryo/fetal development toxicity was established at 30 mg/kg/week in view of the fetal malformations consisting of two retinal dysplasia and one hernia observed in the 60 mg/kg/week.

Study no.:   6709-105
Volume #, and page #:   N/A Electronic submission (e-CTD)

Conducting laboratory and location: ▬▬▬▬▬▬▬▬▬▬▬▬

Date of study initiation: 9 August 2001
GLP compliance: Yes (ICH & OECD)
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Referred to as BB5.1 IgG and/or BB5.1 (syn. Mouse
                                 anti-C5), Lot No. 2640001 FBP, Protein concentration:
                                 3mg/mL
                                 Control: 20 mM Tris-Buffered saline, Lot 416576/1

Methods

      This study assessed the maternal and embryo/fetal effects of BB5.1 when administered by IV injection to pregnant mice during the period of organogenesis. Eighty-seven females premated at the supplier ▬▬▬▬▬▬▬▬▬▬▬ using males of the same strain, 12.5 week old, weighing 24.3 to 32.8 g, were received on GD 3 or 4. The study was conducted according to the following design:

	Number of mice	Dose levels				
Group	Female	mg/kg/dose	Dose/week	mg/kg/week	Concentration mg/mL	Human Dose Multiple
1 (control)	25	0	2	0	0	0
2 (low)	25	30	1	30	3	2-4
3 (high)	25	30	2	60	3	4-8

      Females were administered intravenous doses via a tail vein. The control and high dose groups were dosed on GD 6, 9, 12, and 15. The low dose group was dosed on GD 6 and 12. The dosing volume was 10 mL/kg/day. Dosages were selected based on results of a pilot mice study (▬▬▬ 6709-109) in which dose levels of 60 and 90 mg/kg/week administered IV to CD1-1 mice produced similar activity results for prevention of red blood cell hemolysis when the sera were assayed.

Parameters evaluated

<u>Mortality/moribundity</u>: All mice were observed twice daily for mortality and moribundity.

<u>Clinical signs:</u> Cage side observations were made on dosing days 1 hour post dose and abnormal findings were recorded. Detailed observations were made at each body weight recording, and abnormal observations or an indication of normal was recorded. The supplier provided GD 0 clinical observations.

<u>Body weight and food consumption</u>: Recorded on GD 0, 4, 6, 8, 10, 12, 14, 16, 18, with feed consumption recorded starting GD 4.

<u>Serum chemistry for determination of hemolytic activity:</u>
      Prior to termination on GD 18, 200 μL of blood was collected from the orbital sinus of the first five mice/group; the day of dosing was GD 15 for the control and the high dose groups and GD 12 for the low dose group. The pharmacodynamic or

biological activity of BB5.1 was assessed with a hemolytic assay using chicken erythrocytes. Assays with BB5.1 dosing formulation, placebo, and mouse serum samples were performed with a 5% mouse samples plus 10% C5-depleted human serum.

Disposition of animals

Dams were sacrificed by carbon dioxide inhalation and exsanguination on GD 18 and examined grossly for abnormalities of the cervical, thoracic, or abdominal viscera. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. The placenta or amniotic sac was examined for any abnormalities. Gross lesions were preserved in 10% neutral-buffered formalin.

All fetuses were weighed, evaluated for external abnormalities, sex recorded, and sacrificed via intra-peritoneal injection of sodium methohexital. Approximately half of the fetuses from each litter were randomly selected and processed for visceral examination by the Wilson technique for assessing soft tissue development. The other half were eviscerated and processed for skeletal evaluation using the Alizarin Red S staining methods. All fetuses were retained in Bouin's fixative or glycerin with thymol added as a preservative.

Results

Mortality (dams):
All mice survived until GD 18.

Clinical signs (dams):
No remarkable treatment-related effect

Body weight (dams):
Mean body weight and body weight gain were similar among the groups and were unaffected by BB5.1 treatment.

Food consumption (dams):
Mean consumption was similar across groups and was unaffected by BB5.1 treatment.

Hemolytic activity:

The results of the hemolytic activity assay are presented in the following table

Mean % hemolysis for mouse sera

	Group 1	Group 2	Group 3
	90±6	73±5	17±1
	100±1	90±6	100±4
	100±11	0±1	95±4
	0±0	0±1	71±15
	100±10	100±9	29±3
Mean	78.0	52.6	62.4

<u>Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):</u>

Maternal necropsy observations were unremarkable.

Mean gravid uterine weights and corrected terminal body weights were similar across groups and were unaffected by BB5.1 treatment.

Three premature deliveries occurred during the treatment period, one in the 30 mg/kg/week and two in the 60 mg/kg/week groups. The sponsor ascribed this effect to the time-mating procedure. All groups had dams with no viable fetuses and the incidence of this finding was not affected by BB5.1 treatment. There was an apparent decrease in post-implantation loss % in the 60 mg/kg/week group (10.2%), compared to the control group (22.9 %) and the 30 mg/kg/week group (24.6%). However, this finding is not toxicologically relevant.

Other mean Cesarean section parameters were not affected by the treatment and the following table summarizes the data.

Summary of cesarean section parameters

PARAMETER	Group 1 0 mg/kg/week	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
Dams mated	25	25	25
Dams pregnant (#/%)	20/80	20/80	20/80
Aborted	0	0	0
Delivered early	0	1	2
Dams with viable fetuses (%)	17 (85%)	16 (80)	19 (95%)
Dams with no viable fetuses (%)	3 (15%)	4 (20%)	1 (5%)
Mean Corpora Lutea	12.6	13.2	13.7
Mean Implantation Sites	12.0	12.2	12.6
Total Live Fetuses	202	196	230
Mean Live Fetuses	10.1	9.8	11.5
Mean Total Resorptions (%)	1.9 (22.9%)	2.5 (24.6%)	1.1 (10.2%)
Mean Early Resorptions (%)	1.9 (22.5%)	2.1 (21.9%)	0.8 (8.5%)
Mean Late Resorptions (%)	0.1 (0.4%)	0.3 (2.7%)	0.2 (1.7%)
Total Dead Fetuses	0	0	0
Pre-Implantation Loss (%)	6.4	7.0	8.3
Post-Implantation Loss (%)	22.9	24.6	10.2
Gravid Uterus Weights (g)	17.60	17.48	19.37
Corrected Gravid Uterus Weights (g)	33.25	33.60	33.85
Gravid Uterus Weights* (g)	20.66	22.05	20.50
Corrected Gravid Uterus Weights* (g)	33.70	35.08	34.30
Mean Fetal Weight (g) (M/F)	1.38/1.36	1.40/1.36	1.36/1.30
Sex Ratio (M/F)	1.0	1.08	1.13

*Means calculated excluding dams with no viable fetuses or with no pups delivered.

Offspring (malformations, variations) are summarized in the following table

	Variations & Malformations (Fetal incidence /litter incidence)	Group 1	Group 2	Group 3
Fetal external variations		None	None	None

Fetal external malformations	Open eye + Exencephaly*	1	-	-
	Malrotated hindlimbs	-	1	-
	Ombilical hernia	-	-	1
Fetal soft tissue variations	Distended urinary bladder	2	1	3
Fetal soft tissue malformations	High arched palate + Acrania*	1	-	-
	Cleft palate	-	1	-
	Retinal dysplasia#	-	-	2/2
Fetal skeletal variations	Accessory bones in skull	8/5	15/8	13/8
	Incomplete ossification of skull	-	1/1	1/1
	Less than four caudal vertebrae ossified	1/1	-	-
	Sternebrae asymmetrically ossified	7/5	6/5	6/5
	Sternebrae extra ossification sites	6/3	12/7	5/3
	$5^{th}/6^{th}$ sternebrae incomplete ossification	1/1	-	-
	Minor fusion of sternebrae	1/1	-	-
	$5^{th}/6^{th}$ sternebrae bipartite	1/1	1/1	1/1
	Other sternebrae incomplete ossification	1/1	-	-
	14th full rib	9/6	15/11	18/10
	14th rudimentary rib	17/10	17/9	26/13
	14th unilateral full rib	3/2	1/1	4/3
Fetal skeletal malformations	Sternebrae malformed	1/1	1/1	-
	Major fusion of sternebrae	-	1/1	-
	Forked/fused rib(s)	1/1	-	-

*Findings noted in the same fetus
#Finding noted in fetuses from two different litters

Conclusions

Three premature deliveries occurred during the treatment period, one in the 30 mg/kg/week and two in the 60 mg/kg/week groups. The sponsor ascribed this effect to the time-mating procedure. There was an apparent decrease in post-implantation loss % in the 60 mg/kg/week group compared to the control and low-dose groups. Other mean Cesarean section parameters were not affected by the treatment with BB5.1

Reviewer's comments:

Hemolytic inhibitory activity was lower than that described in the non-pregnant females in the 26 week toxicity study. Although the potential for immunogenic reaction in mice is low in view that BB5.1 is a murine antibody, both total and neutralizing antibodies against the biologic agent should have been measured and the possible effect of pregnancy on their production evaluated. In addition toxicokinetics was not performed. In some female mice, the individual hemolytic activity was similar to that of controls.

The umbilical hernia and retinal dysplasia, which were observed in the 60 mg/kg/week group only, constitute the most potentially relevant findings. Fetal malformations were sporadic with no dose-response pattern and there was an increased incidence of 14th rib and accessory bones in skull of treated fetuses. Therefore, the NOAEL for BB5.1 administered by IV injection to pregnant mice during organogenesis is 60 mg/kg/week for maternal toxicity and reproductive performance (based on C-section parameters). The NOAEL for embryo/fetal development toxicity could be established at 30 mg/kg/week.

Prenatal and postnatal development

Study title: Study for effects on pre- and post natal development, including maternal function in the mice with BB5.1

The purpose of this study was to evaluate the effects of BB5.1 administered by intravenous injection from implantation through weaning on pregnant and lactating female mice and the development of the offspring.

Key study findings: In view of the number of deaths and presence of clinical signs in the F_1 generation males in the low and high dose treated groups, the NOAEL was not established for these parameters. The NOAEL was established for F_1 pup development and reproductive performance at $\geq$ 60 mg/kg/week.

Study no.:	6709-107
Volume #, and page #:	N/A electronic submission (e-CTD)
Conducting laboratory and location:	▬▬▬▬▬▬▬▬▬▬
	▬▬▬▬▬▬
Date of study initiation:	10 August 2001
GLP compliance:	Yes (ICH & OECD)
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	Referred to as BB5.1 IgG and/or BB5.1 (syn. Mouse anti-C5), Lot No. 2640001 FBP, 3mg/mL, SDS PAGE 174 KDa.
	Control: 20 mM Tris-Buffered saline, Lot 416576/120701

Methods

   Doses: 0, 30 and 60 mg/kg/week.
   Species/strain: Mice/▬ CD-1®(ICR) BR
   Number/sex/group: 37 females/group
   Route, formulation, volume, and infusion rate: Intravenous, solution, 10 mL/kg/day, bolus
   Satellite groups used for toxicokinetics: none
   Study design: One hundred twenty-seven ▬ CD-1®(ICR) BR mice were pre-mated by the supplier ▬▬▬▬▬▬▬▬▬ using males of the same strain. Pre-mated female mice, approximately 12-week-old, weighing 19.8 to 35.2 g, were assigned to 3 groups (37/group). The control group (Group 1) received 20 mM Tris-buffered saline, while the low-dose (Group 2) and high-dose (Group 3) groups received respectively 30 mg/kg/week and 60 mg/kg/week BB5.1, according to the following protocol:

	Females (#)*	Doses levels				Human dose multiple	Dosing schedule[#] Gestation-Lactation
		mg/kg/dose	Dose/week	mg/kg/week	Mg/mL		
G1	37	0	2	0	0	0	GD 6, 9, 12, 15, & 18 LD 2, 6, 9, 12, 15, & 18
G2	37	30	1	30	3	2-4	GD 6, 12, & 18

							LD 6, 12, & 18
G3	37	30	2	60	3	4-8	GD 6, 9, 12, 15, & 18 LD 2, 6, 9, 12, 15, & 18

*GD 0: day of mating

Parameters and endpoints evaluated:

Hemolytic activity

Blood samples collected on LD 14 from the first 5 Fo female mice per group with litters for determination of hemolytic activity,

Fo generation – Observation of animals

Fo females were evaluated for mortality and moribundity (twice daily), cageside observations (1 hr post dosing for the first 2 weeks), and detailed clinical observations, body weights, and food consumption (GD 0, 4, 6, 9, 12, 15, and 18, and LD 0, 2, 4, 6, 9, 12, 15, 18, and 21). Fo females that delivered raised their young to Day 21 postpartum.

F1 pups were sexed. Dead pups were examined for cervical, thoracic, and abdominal viscera abnormalities and preserved in alcohol and live pups were weighted and examined for external abnormalities. On LD 4, 7, 14, and 21, the number of live pups, body weights, and clinical observations were recorded. Pups were evaluated for mortality, cannibalization, developmental landmarks, abnormal behavior or ill health throughout lactation.

On LD14, blood was collected for determination of hemolytic activity from the first 5 mice/group with litters.

Necropsy was performed on all Fo females.

F1 generation – Observation of animals

Mortality, moribundity, maturation, locomotor activity (open field tests), learning, memory and reverse learning evaluation, body weight recording, and physical examinations were performed.

Following the 7-week postweaning maturation phase, males and females within each treatment group were cohabited for up to 14 days for mating.

Post-mating Phase – F1 females

F1 females were observed for mortality/moribundity (twice/day), and clinical observations and body weight (GD 0, 7, 14, 17, and LD0).

F2 pups were sexed. Dead pups (Days 0 and 1) were examined for cervical, thoracic, and abdominal viscera abnormalities and preserved in alcohol and live pups were weighted and examined for external abnormalities.

Cannibalized pups were recorded and discarded without necropsy; F2 pups were killed on LD1 and preserved in 10% neutral-buffered formalin.

Necropsy was performed on all F1 females.

Results

Hemolytic activity:

Percent hemolysis (%) was measured in the first 5 mice/group with litters on LD 14, which is 2 days after the 5th dose and the 8th dose in the low and high dose respectively. The results of the hemolytic activity assay are shown in the following table

	Group 1 0	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
	84 ± 1	46 ± 0	92 ± 2
	100 ± 1	87 ± 3	56 ± 0
	100 ± 2	79 ± 1	85 ± 2
	94 ± 2	95 ± 0	85 ± 2
	100 ± 2	96 ± 1	100 ± 3
Mean	95.6	80.6	83.6

F_0 generation – Observation of animals

F_0 in-life:
One 60 mg/kg/week female was found dead on LD 14 with necropsy revealing a pale liver. Clinical signs, body weight gain, and food consumption were unremarkable in this mouse and the death was not attributed to BB5.1 by the sponsor. There were no unscheduled deaths during gestation. One female each in the 0 and 30 mg/kg/week groups was sacrificed after having total litter death (LD 3 and 8, respectively).

On LD18, opaque eye was observed in one female of each of the control and the 60 mg/kg/week groups. During lactation, one pup in each treatment group was cold to touch and had no visible milk in stomach.

Clinical observations, mean body weight data, and mean food consumption values were unremarkable and were generally similar among the groups through gestation and lactation.

Results of Natural delivery and litter data (Mating/Fertility) - F_0 generation:

		Group 1 0 mg/kg/week	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
Females mated		37	37	37
Females pregnant		23/37 (62%)	20/37 (54%)	16/37 (43%)
Females delivering		23 (100%)	18 (90%)	15 (94%)
Duration of gestation		18.8	19.0	18.7
Females with liveborn pups		23	18	15
Gestation index (Parturition index)		100%	90%	94%
Females with stillborn pups/F pregnant		2/23 (8.7%)	3/20 (15%)	1/16 (6.3%)
Pups delivered (#/Mean)		251/10.91	217/12.06	171/11.40
Pups liveborn/stillborn/uncertain		249/2/0	208/5/4	166/2/3
Implantation sites (#/mean)		277/12.04	234/13.00	187/12.46
Pup survival indices* (%)	Livebirth index	99	96	97
	Viability index	95	98	97
	Weaning index	96	92	95
Pup disposition	Culled day 4	72	61	42
	Killed	0	0	0
	Died	15	10	9
	Cannibalized	0	1	0

Missing	1	3	2
Pups surviving at 21 days	161	131	113
Mean male percent by litter at 21 days	50	51	53
Live pups/litter with live pups	Unremarkable		
Pup weight/litter (g)	Unremarkable		

*Livebirth index (number born alive/number born)
Viability index (number alive day 4 precull/ number liveborn)
Weaning index (number alive at weaning/number alive at day 4 postcull)
Gestation index = number of females delivering live pups/number of females pregnant

The pregnancy rates were 62, 54, and 43% in the control, 30 mg/kg/week, and 60 mg/kg/week groups respectively. The delivery rates of pregnant mice in the control and 30 and 60 mg/kg/week groups were 100, 90, and 94%, respectively. All females that delivered had viable pups. The duration of gestation and pup survival indices (i.e., livebirth, viability, and weaning) were similar across groups. The decreased lower absolute values in the 60 mg/kg/week group for pups delivered, liveborn pups, pups culled on Day 4, and pups surviving at 21 days are a reflection of the small number of pregnant dams in that group and are not attributed to BB5.1. Mean and covariate-adjusted pup weights were similar across groups.

Fo necropsy:

Fo dams' necropsy: Findings were limited to one ovary cyst and one renal cyst in the 30 mg/kg/week group, a pale liver in the 60 mg/kg/week mouse found dead on LD 14, two gravid uteri and two uterus hydrometra in each of the treated groups, and uterus hydrometra in a control mouse.

F1 pups necropsy: The percent of stillborn was 2.3, 6.3, and 3.8%, and post mortem autolysis was observed in 1 pup (from 1 litter), 1 pup (from 1 litter), and 5 pups (from 2 litters) in the control, 30 mg/kg/week and 60 mg/kg/week groups respectively. Empty stomach was observed in 7 pups from one litter of the 30 mg/kg/week group total litter death female, and in 1 pup from one litter in each the control and the 60 mg/kg/week groups.

F1 pups maturation:
No remarkable findings in development and behavior

F1 generation – Observation of animals

F1 in life:

Survival/Clinical observations/Body weights:
Mortality/moribundity: There were three unscheduled deaths and five moribund animals were removed from study, all in the F1 males group. Deaths included one male the 30 mg/kg/week group on Day 71 and two in the 60 mg/kg/week group on Days 90 and 119. Five moribund males included one control on day 87, one 30 mg/kg/week on Day 68, and three 60 mg/kg/week on Days 71 and 112.
No unscheduled deaths or moribundity were reported in the F1 female group.

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Clinical observations: Clinical signs often associated with the deaths and moribund state, observed only in males and starting around weeks 6-10, consisted of urine stains (similar across groups), skin sores (2 controls, 6 low dose, 6 high dose), localized swelling (1 control, 3 low dose, 3 high dose) and distended abdomen (2 high dose). Hunched appearance was noted in one 60 mg/kg/week male on Day 112, hypoactivity in one low dose and 2 high dose males whereas one male was cold to touch on Day 112. One female in the 60 mg/kg/week group had a total litter death and one female of the 30 mg/kg/week group was thin on LD 0.

Statistical analysis shows no significant changes in mean body weights in F_1 males and females, and during gestation or lactation of F_1 generation females.

Natural delivery and litter data/reproductive performance – F_1 generation:

	Group 1 0 mg/kg/week	Group 2 30 mg/kg/week	Group 3 60 mg/kg/week
Females mated	25	25	25
Females pregnant	24 (96%)	21 (84%)	24 (96%)
Females delivering	24 (100%)	20 (95%)	24 (100%)
Duration of gestation	19.1	18.9	19.2
Females with liveborn pups	24	20	24
Gestation index (Parturition index)	100%	95%	100%
Females with stillborn pups/F pregnant	4 (17%)	4 (20%)	1 (4.2%)
Pups delivered (#/Mean)	308/12.83	250/12.50	295/12.29
Pups liveborn/stillborn/uncertain	302/6/0	245/5/0	294/1/0
Implantation sites (#/mean)	335/13.96	267/13.35	323/13.46
Pup survival indices* (%) Livebirth index	98	98	100
Pup disposition Killed	8	31	23
Died	8	14	13
Cannibalized	0	1	0
Missing	5	7	5
Pups surviving at Day 0	300	245	292
Entire litter died	1	3	2
Live pups/litter with live pups	Unremarkable		
Pup weight/litter (g)	Unremarkable		

*Livebirth index (number born alive/number born)
Gestation index = number of females delivering live pups/number of females pregnant

Natural delivery and litter data: The pregnancy rates were 96, 84, and 96% in the control, 30 mg/kg/week, and 60 mg/kg/week groups respectively. The delivery rates of pregnant mice in the respective groups were 100, 95, and 100%. All females that delivered had viable pups. The duration of gestation and pup livebirth index were similar across groups. Data from other delivery and litter parameters were unremarkable. Mean and covariate-adjusted pup weights were similar across groups.

Reproductive performance: BB5.1 had no remarkable effect on reproductive performance of the F_1 generation.

F1 necropsy:

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F1 parental necropsy:

Necropsy of surviving males revealed moderately to severely distended or dilated renal pelvis and urinary bladder with white, yellow to dark fluid in 2 males of the control group, 2 males of the 30 mg/kg/week group, and 3 males of the 60 mg/kg/week group.

Necropsy of moribund animals revealed similar findings in 1 control and one low dose male, and in 3 high dose males. In addition, enlarged kidneys were observed in 1 male of each of the treatment group, with a raised area in a 60 mg/kg/week animal and pale spleen in another one.

Necropsy of dead males revealed similar findings in the kidney and the urinary bladder in 1 male of each treatment group, whereas the other 60 mg/kg/week male exhibited a dark brown nonglandular mucosa in the stomach.

In the females, necropsy findings were limited to ovarian cysts in one 30 mg/kg/week mouse, and two 60 mg/kg/week mice. One of these 60 mg/kg/week mouse had a severe dilated renal pelvis.

F2 pups necropsy: Findings were limited to postmortem autolysis in two pups from a 60 mg/kg/week/litter.

Post-mating Phase – F₁ females

There were no remarkable clinical observations or body weight changes in the F₁ females during the rest phase.

Sponsor's conclusion:

The no-observed-effect level (NOEL) for maternal toxicity and F₁ pup development and reproductive performance through to parturition of the F₂ generation is ≥ 60 mg/kg/week.

Reviewer's comments:

One 60 mg/kg/week F₀ female was found dead on LD 14 with necropsy revealing a pale liver. Pregnancy rates were decreased in the treated groups (54% and 43% in the low and high dose groups respectively) compared to the control groups (62%) in F₀ females. In contrast, pregnancy rates were only slightly decreased in the fertility study (respectively 100, 96, and 88%) and were identical in the developmental study (80% in all groups). No explanation was provided for this discrepancy. However, in the Seg II and Seg III studies, females were pre-mated at the supplier and later transported to the conducting facility, which may induce stress, and explain such low pregnancy rates. There were no remarkable effects on reproductive performance of the F₁ generation.

Three F₁ generation males died and four were found in moribund state in the treated groups compared to one moribund animal in the control group. Skin sores, localized swelling and distended abdomen incidence was significantly increased and dose-dependent in the treated groups compared to controls. The clinical signs and necropsy findings (distended or dilated renal pelvis and urinary bladder with fluid) in F₁ generation males were somewhat similar to those observed in the 26 week repeat dose toxicity study.

Analysis of hemolytic activity measured on LD 14 in the first 5 mice having litters, showed that the mean percent hemolysis was similar across groups (95.6, 80.6,

and 83.6 % for the control, the 30 mg/kg/week, and the 60 mg/kg/week groups respectively). The results of the hemolytic assay were not consistent across the studies conducted by the sponsor. Although pregnant females appeared to exhibit lower % hemolysis no clear correlation could be established between the % hemolytic activity inhibition and the duration and time of treatment, and time of blood collection. Again, both total and neutralizing antibodies against the biologic agent should have been measured and the possible effect of pregnancy on their production evaluated. In addition toxicokinetics was not performed.

Because the drug was administered intravenously, one can assume that its availability nears 100% in the plasma.

In conclusion, the pregnancy rates were too low and therefore should be considered non reliable. Because of the number of dead/moribund male animals (1/25 in controls, 2/25 in low-dose, and 5/25 in high dose) and the presence of clinical signs in the F_1 male generation, no NOAEL was established for these parameters. The NOAEL could be established at $\geq$ 60 mg/kg/week for F_1 pup development and reproductive performance.

2.6.6.7 Local tolerance

Not performed

2.6.6.8 Special toxicology studies

None performed

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Two repeat dose toxicity studies and three reproductive studies (Seg I, II, and III) were conducted in mice intravenously administered a surrogate mAb, the murine C5 specific BB5.1. No toxicokinetics or antibodies measurements were conducted in any of the toxicology studies and the hemolytic activity, used as surrogate of drug systemic exposure, did not provide consistent results. There was wide variation within the same groups

A NOAEL could not be established in the 26 week toxicity study. There were nine unscheduled deaths (4/50 in control group and 5/50 in the 60 mg/kg/week group) and no unscheduled deaths in the 30 mg/kg/week group. Dose-dependent clinical signs included missing ears, sores/scabs in the ear or cranial area, rough hair coat, bent tail, and thin and/or hunched appearance. Macroscopic findings included skin sores, and sporadic findings in the treated group. Statistically significant increase in liver weights in high dose females, and an increase in heart weight (up to 12%) in high dose males and females were observed. At terminal sacrifice, the most remarkable findings included lung congestion, thrombosis, and/or hemorrhage, skin lesions (chronic active inflammation,

necrotic epidermal cellular debris, ulcer, and acanthosis), and eye retrobulbar inflammation and hemorrhage.

BB5.1 administered by intravenous injection to male and female mice prior to mating and until termination (males) or through early gestation (females), did not affect reproductive performance, female and litter reproductive parameters, sperm count and motility. The NOEL for female toxicity, for male and female fertility, reproductive performance and fetal effects was established at ≥ 60 mg/kg/week. In view of two deaths in the 60 mg/kg/week, 30 mg/kg/week BB5.1 was the NOAEL for male toxicity.

BB5.1 administered by IV injection to pregnant mice during the period of organogenesis did not affect maternal body weight and food consumption, Cesarean section parameters. Fetal malformations were sporadic with no dose-response pattern and there was an increased incidence of 14th rib and accessory bones in skull of treated fetuses. There were two cases of retinal dysplasia and one case of umbilical hernia observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. The NOAEL was established at 60 mg/kg/week for maternal toxicity and reproductive performance and the NOAEL for embryo/fetal development toxicity was established at 30 mg/kg/week.

In a prenatal and postnatal development study, the effects of BB5.1 were evaluated when administered by intravenous injection from implantation through weaning, on pregnant and lactating female mice, and the development of the offspring. One 60 mg/kg/week Fo female was found dead on LD 14 with necropsy revealing a pale liver. In view of the number of dead/moribund male animals (1/25 in controls, 2/25 in low-dose, and 5/25 in high dose), and presence of clinical signs (skin sores, localized swelling and distended abdomen) in the F1 male generation, a NOAEL could not be established for these parameters. The NOAEL could be established at ≥ 60 mg/kg/week for F1 pup development and reproductive performance.

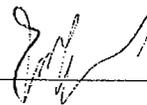Unresolved toxicology issues (if any): None

Recommendations:
From the perspective of nonclinical pharmacology and toxicology, Soliris® is recommended for approval. No additional nonclinical studies are required.

Suggested labeling:
Recommendations on labeling are included in the Executive Summary.

Signatures (optional):

Reviewer Signature _____ 2/23/07

Supervisor Signature _____ Concurrence Yes ✓ No ___

2/24/07

Pediatric and Maternal Health Staff
Office of New Drugs
Center for Drug Evaluation and Research
Food and Drug Administration
Silver Spring, MD 20993
Tel 301-796-0700
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MEMORANDUM

Date: February 22, 2007   **Date Consulted:** January 26, 2007

From: Karen B. Feibus, M.D.
               Team Leader, Pediatric and Maternal Health Staff

Through: Lisa Mathis, MD
               Associate Director, Pediatric and Maternal Health Staff   for Dr. Mathis 02/28/2007

               Sandra Kweder, MD
               Deputy Director, Office of New Drugs   Sandra Kweder 2/28/07

To: Division of Medical Imaging and Hematology Products (DMIHP)

Drug: Soliris (eculizumab)

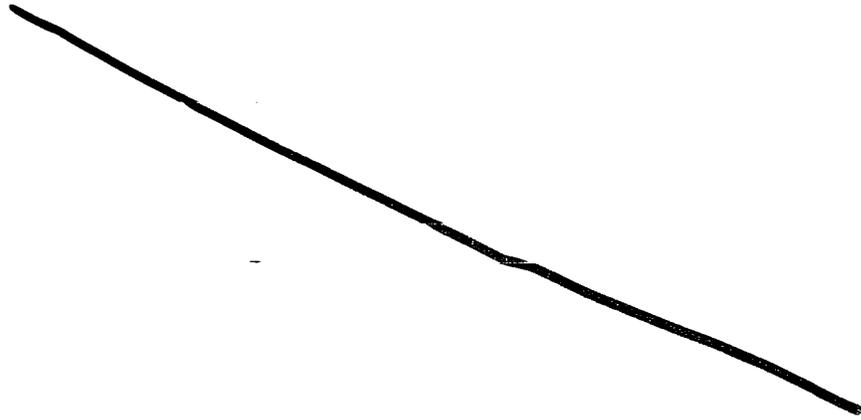**Indication:** Paroxysmal Nocturnal Hemaglobinuria (PNH)

Subject: Labeling for use during pregnancy and lactation

Materials
Reviewed: Proposed label
               Pharmacology/Toxicology Review

Consult Question:
Please review Package Insert and advice on the pregnancy, labor & delivery and Nursing
Mothers sections and advise us regarding appropriate text for the PI. The company has neither
clinical nor animal data pertaining to pregnancy, labor nor lactation. We provide the following
text as a possible option based upon other biologicals: is this reasonable? if not, please advise
appropriate text:

Pregnancy (text to be finalized by Pharm tox but probably category C): Category C

1

EXECUTIVE SUMMARY

Eculizumab is a humanized murine monoclonal antibody that prevents complement-mediated RBC hemolysis in individuals with paroxysmal nocturnal hemoglobinuria (PNH). PNH is a serious medical condition that significantly shortens life expectancy. In pregnancy, PNH is associated with a high risk of venous thrombosis and significant maternal and fetal morbidity and mortality. Current management options are limited and rely on transfusion of packed RBCs and platelets to manage anemia and frequently associated thrombocytopenia, corticosteroid therapy, and prophylactic anticoagulation.

Animal reproductive studies in one animal species (mice) using a murine surrogate antibody molecule suggest some developmental abnormalities and increased deaths (males only) among offspring exposed to the antibody during gestation. It is not clear how these findings relate to potential outcomes in humans if eculizumab is used by pregnant women with PNH.

Women with PNH are discouraged from becoming pregnant; however, for those who do, there are limited treatment options and a high risk of venous thrombosis, serious infection, or death. The overall benefits of eculizumab use in certain pregnant women with PNH may outweigh the potential fetal risks.

IgG is present in human breast milk, so it is likely that eculizumab will be present in human milk as well. However, neonatal and infant absorption of IgG from the intestine is very limited. Breastfeeding offers infants many short term and long term health benefits. In certain situations, the benefits of breastfeeding may outweigh the risks of limited exposure to eculizumab.[1]

Alexion, Inc. is establishing a registry for patients with PNH who use eculizumab. This registry should be used to prospectively follow pregnancies with eculizumab exposure and their maternal and neonatal outcomes.

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[1] American Academy of Pediatrics, Section on Breastfeeding. Breastfeeding and the Use of Human Milk. Pediatrics 2005; 115: 496-506.

The pregnancy and lactation labeling recommended by the Maternal Health Team (see recommendations) considers all of the potential benefits and poorly defined risks eculizumab use during pregnancy and lactation.

BACKGROUND

The sponsor, Alexion, Inc., submitted a NDA under priority review and Subpart H for eculizumab for the treatment of paroxysmal nocturnal hemaglobinuria (PNH). Eculizumab is a humanized murine complement 5 monoclonal antibody IgG 2/4 immunoglobulin administered by intravenous infusion once weekly for five doses and then every two weeks. Clinical trials demonstrate maintenance of significant decreases in hemolysis as documented by decreases in serum lactose dehydrogenase levels over 26 – 52 weeks of treatment.

PNH is caused by a somatic mutation of the PIG-A gene on chromosome X, which codes for the enzyme glycosyltransferase. This enzyme catalyzes the first step in the production of the complement regulatory proteins CD55 and CD59, which protect against complement activation. The red blood cells (RBCs) of PNH patients are susceptible to intravascular hemolysis due to decifiencies in these two regulatory proteins and their GP1 anchor. Most PNH patients make some normal RBCs and some deficient RBCs. Symptoms often don't fully manifest until early adulthood with the onset of pallor, sporadic dark urine (especially in the morning), severe fatigue, and difficulty concentrating. Laboratory assessment reveals severe anemia with inadequate reticulocytosis and low white blood cells (WBCs) and/or platelets. Fifty percent of patients present with pancytopenia and hemorrhagic or infectious symptoms. PNH patients may experience hyperhemolysis, increased infections, thrombosis, and bone marrow insufficiency. RBC transfusion is the primary treatment. Other treatments used include glucocorticoids and danacrine.[2] Following diagnosis, PNH patients have a median survival of 12 years.

Pregnant women with PNH are rare but have a substantial risk of maternal and fetal morbidity and mortality. Pregnancy and PNH are both hypercoagulable states. In 2006, Fieni et al published a case report and thorough review of the literature on pregnancies in women with PNH. They reviewed 26 published clinical reports describing pregnancy outcomes of 43 women with PNH. During pregnancy, 74% of these women experienced minor complications that did not require hospitalization, such as increased need for RBC and/or platelet transfusion, and 16% experienced a major maternal complication that required hospitalization or intensive care. Postpartum rates of minor and major complications were 35% and 30% respectively. Nearly 40% of the pregnancies ended in preterm delivery with a fetal and neonatal death rate of 7.2%.

Venous thrombosis was the most common major maternal complication both during pregnancy and postpartum. Two women developed Budd-Chiari syndrome due to venous thrombus formation during pregnancy. During the postpartum period, two women developed cerebral vein thrombosis, one developed isolated hepatic and splenic vein thrombosis, and two developed Budd-Chiari syndrome (one with hepatic, splenic, and femoral thrombosis). Three of these

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[2] Harrington WJ, Kolodny L, Horstman LL, Jy W, Aahn YS. Danazol for Paroxysmal Nocturnal Hemoglobinuria. Am J Hematol. 1997; 54: 149-154.

women died. Two other women died from infectious complications – one with amebic colitis and one with sepsis. The maternal mortality rate was 11.6%.[3]

The authors advise avoidance of pregnancy for women with PNH based on the high rates of maternal and fetal/neonatal mortality. Their recommendations for clinical management of pregnancy in women with PNH include: close monitoring to control maternal anemia and detect infection early; routine prophylactic anticoagulation during pregnancy and for six weeks postpartum; screening for thrombophilias like Factor V Leiden; and frequent ultrasound to assess fetal growth and cervical length.

REVIEW OF DATA
Pregnancy:
The sponsor did not conduct any studies of Soliris in pregnant women. Reproductive toxicology studies (Segments I, II, and III) were conducted in mice using doses of a murine anti-C 5 IgG antibody that approximated 2-4 times (low dose) and 4 - 6 times (high dose) the recommended human Soliris dose. Siham Biade, Ph.D., the pharmacotoxicology reviewer, reviewed these studies and discussed the following findings in her review:

> When animal exposure to the antibody occurred in the time period from before mating until early gestation, no decrease in fertility or reproductive performance was observed. When maternal exposure occurred during organogenesis, two cases of retinal dysplasia and one case of umbilical hernia observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. When maternal exposure to the antibody occurred in the time period from implantation through weaning, one pregnant female died (high dose group) and decreased reproductive performance was observed in surviving females (low and high doses). Antibody-treated mothers also had male offspring that experienced a higher rate of death, skin sores, localized swelling and distended abdomen. Surviving offspring had normal development and reproductive performance.

> Necropsy of surviving F_1 males revealed moderately to severely distended or dilated renal pelvis and urinary bladder with white, yellow to dark fluid in two males of the control group, two males of the 30 mg/kg/week group, and three males of the 60 mg/kg/week group. Necropsy of moribund animals revealed similar findings in one control male, one low dose male, and three high dose males. Enlarged kidneys were observed in one male from each treatment group, with a raised area in a 60 mg/kg/week animal and pale spleen in another one. Necropsy of dead males revealed similar findings in the kidney and the urinary bladder in one male of each treatment group, whereas the other 60 mg/kg/week male exhibited a dark brown nonglandular mucosa in the stomach. In the females, necropsy findings were limited to ovarian cysts in one 30 mg/kg/week mouse, and two 60 mg/kg/week mice. One of these 60 mg/kg/week mouse had a severe dilated renal pelvis.

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[3] Fieni S, Bonfanti L, Gramellini D, Benassi L, Delsignore R. Clinical Management of Paroxysmal Nocturnal Hemoglobinuria in Pregnancy: A Case Report and Updated Review. Obstet Gynecol Survey 2006 Sep; 61(9): 593-601.

Clinical safety and efficacy studies submitted to the Soliris BLA included a randomized, double-blind, placebo-controlled study that examined Soliris effects in RBC transfusion-dependent PNH patients over a 26 week period (Study 1). Single arm, uncontrolled studies that provided additional Soliris safety data included a 52 week study (Study 2) and a long term follow-up study. Patients received meningococcal vaccination prior to receipt of Soliris and were monitored for signs and symptoms of infection. In all studies, Soliris-treated patients were administered 600 mg study drug every 7 ± 2 days for 4 weeks, followed by 900 mg 7 ± 2 days later, then 900 mg dose every 14 ± 2 days for the study duration. Soliris was administered as an intravenous infusion over 25-45 minutes. Results from these studies demonstrated statistically and clinically significant reductions in hemolysis, anemia, fatigue, and thrombotic events. These effects persisted throughout treatment periods of 52 weeks and longer.

Among 140 subjects with PNH who participated in Studies 1 and 2, there was one pregnancy. The subject began Soliris therapy on ▬▬▬▬▬ and reported the pregnancy to investigators on ▬▬▬▬ She was immediately discontinued from the study. Pregnancy outcome was normal for the subject and her neonate.

Lactation:
There are no animal or human studies evaluating the levels of eculizumab in maternal milk or the serum levels in nursing offspring. A 2003 article by Philippe Vande Perre states that newborns of many mammalian species are hypogammoglobulinemic and milk immunoglobulins are their principal source of antibodies. In these animals, duodenal and jejunal Fc receptors on the enterocyte membrane recognize and bind IgG and deliver it to the submucosa and neonatal blood. This is not the case in humans. In humans, breast milk antibodies do not enter the neonatal/infant circulation in substantial amounts. The most important immunoglobulin in human milk is secretory IgA, which serves as a first line defense for the immature intestinal mucosa.[4]

Labels:
The Soliris® labeling proposed by the sponsor was reviewed. The sponsor, Alexion, is establishing a registry for PHN patients, which could be used to follow pregnancies that occur in women with PHN who are treated with eculizumab. In addition, the pregnancy and lactation sections of currently approved labeling for other humanized murine monoclonal antibody therapeutic biologic products were reviewed prior to making the recommendations below. For examples, please refer to the current labeling for Raptiva® and Tysabri®.

DISCUSSION/CONCLUSIONS
PNH is a serious medical condition that significantly shortens life expectancy. In pregnancy, PNH is associated with a high risk of venous thrombosis and significant maternal and fetal morbidity and mortality. Current management options are limited and rely on transfusion of packed RBCs and platelets to manage anemia and frequently associated thrombocytopenia, corticosteroid therapy, and prophylactic anticoagulation.

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[4] Van der Perre P. Transfer of antibody via mother's milk. Vaccine 2003; 21: 3374-3376.

Eculizumab is a humanized murine monoclonal antibody that prevents complement-mediated RBC hemolysis in individuals with PNH. Animal reproductive studies in one animal species (mice) using a murine surrogate antibody molecule suggest some developmental abnormalities in offspring exposed to the antibody during gestation. It is not clear how these findings relate to potential outcomes in humans if eculizumab is used by pregnant women with PNH. During clinical trials, one woman became pregnant while receiving eculizumab therapy. Therapy was immediately discontinued and her pregnancy outcome was normal.

Women with PNH are discouraged from becoming pregnant; however, for those who do, there are limited treatment options and a high risk of venous thrombosis, serious infection, or death. The overall benefits of eculizumab use in certain pregnant women with PNH may outweigh the potential fetal risks.

IgG is present in human breast milk, so it is likely that eculizumab will be present in human milk as well. However, neonatal and infant absorption of IgG from the intestine is very limited. Breastfeeding offers infants many benefits. Human milk feeding decreases the incidence and/or severity of a wide range of infectious diseases (including bacterial meningitis, respiratory tract infection, necrotizing enterocolitis, and otitis media) and reduces post-neonatal infant mortality by 21% in the United States. Some studies suggest decreased rates of sudden infant death syndrome in the first year of life and reduction in the incidence of the following condition in older children: insulin-dependent and non-insulin-dependent diabetes, lymphoma, leukemia, Hodgkin disease, overweight and obesity, hypercholesterolemia, and asthma. In certain situations, the benefits of breastfeeding may outweigh the risks of limited exposure to eculizumab.[5]

Alexion, Inc. is establishing a registry for patients with PHN who use eculizumab. This registry should be used to prospectively follow pregnancies with eculizumab exposure and their maternal and neonatal outcomes.

RECOMMENDATIONS
The Maternal Health Team recommends the following wording for the pregnancy and lactation sections of the Soliris® label:

8.1 Pregnancy

Pregnancy (Category C):

PNH is a serious illness. Pregnant women with PNH and their fetuses have high rates of morbidity and mortality during pregnancy and the postpartum period. There are no adequate and well-controlled studies of Soliris in pregnant women. Soliris, a recombinant IgG molecule (humanized murine anti-C5 antibody), is expected to cross the placenta. Animal studies using a mouse analog of the Soliris molecule (surrogate murine anti-C5 antibody) showed increased rates of developmental abnormalities and an increased rate of dead and moribund offspring at doses 2-8 times the human dose. Soliris should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

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[5] American Academy of Pediatrics, Section on Breastfeeding. Breastfeeding and the Use of Human Milk. Pediatrics 2005; 115: 496-506.

Animal reproduction studies were conducted in mice using doses of a murine anti-C 5 antibody that approximated 2-4 times (low dose) and 4-8 times (high dose) the recommended human Soliris dose. When animal exposure to the antibody occurred in the time period from before mating until early gestation, no decrease in fertility or reproductive performance was observed. When maternal exposure to the antibody occurred during organogenesis, two cases of retinal dysplasia and one case of umbilical hernia were observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. When maternal exposure to the antibody occurred in the time period from implantation through weaning, one pregnant female died (high dose group); antibody-treated mothers also had a higher number of male offspring that became moribund or died (1/25 controls, 2/25 low dose group, 5/25 high dose group) and surviving male offspring with skin sores, localized swelling, and distended abdomen. Surviving offspring had normal development and reproductive performance.

8.2 Labor and Delivery

No information is available on the effects of Soliris during labor and delivery.

8.3 Nursing Mothers

It is not known whether Soliris is secreted into human milk. IgG is excreted in human milk, so it is expected that Soliris will be present in human milk. However, published data suggest that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts. Caution should be exercised when Soliris is administered to a nursing woman. The unknown risks to the infant from gastrointestinal or limited systemic exposure to Soliris should be weighed against the known benefits of breastfeeding.

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