Coversin in paroxysmal nocturnal haemoglobinuria (PNH) in patients with resistance to eculizumab due to complement C5 polymorphisms

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List of Abbreviations

Abbreviation	Definition/Term
ACh	Acetylcholine
AChR	Acetylcholine Receptor
ADR	Adverse Drug Reaction
AE	Adverse Event
ANOVA	Analysis of Variance
BP	Blood Pressure
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computerised Tomography
DLT	Dose Limiting Toxicity
DSMB	Drug Safety Monitoring Board
EAMG	Experimental Autoimmune Myasthenia Gravis
EC	Ethics Committee
ECG	Electrocardiogram
ED	Effective Dose
EMA	European Medicines Agency
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HAHA	Human Antihuman Antibodies
HED	Human Equivalent Dose
ICH	International Committee on Harmonisation
IRB	Institutional Review Board (or equivalent, e.g. Ethics Committee)
ITT	Intention to Treat
i.v.	Intravenous
MAC	Membrane Attack Complex
MedDRA	Medical Dictionary of Regulatory Activities
MG	Myasthenia Gravis
MGFA	Myasthenia Gravis Foundation of America
MHRA	Medcines and Healthcare Regulatory Agency
MTD	Maximum Tolerated Dose
MW	Molecular Weight
NOAEL	No Observable Adverse Event Level
OmCI	*Ornithodoros moubata* Complement Inhibitor
PAD	Pharmacologically Active Dose
PBS	Phosphate Buffered Saline
PI	Principal Investigator
PNH	Paroxysmal Nocturnal Haemoglobinuria
POM	Prescription Only Medicine

Abbreviation	Definition/Term
PP	Per Protocol
p.r.n	*pro re nata* (as necessary)
QA	Quality Assurance
QC	Quality Control
QMG	Quantitative Myasthenia Gravis (score)
SAD	Single Ascending Dose
SAE	Serious Adverse Event
s.c.	Subcutaneous
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee
ULN	Upper Limit of Normal

Protocol Signature Page

Protocol Title:	Coversin in patients with paroxysmal nocturnal haemoglobinuria (PNH) and resistance to eculizumab due to complement C5 polymorphisms.
Protocol Number:	AK Study 578

Authorized Sponsor Representative Signature:

Signature: _____ Date: 11 NOV 2015

Dr Wynne H Weston-Davies MB FRCS
Rosebank, Salperton, Cheltenham,
GL54 4EF, UK

Principal PIs Signatures:

By my signature, I confirm that my staff and I have carefully read and understand this protocol and agree to comply with the conduct and terms of the study specified herein.

Signature: _____ Date: 12-11-2015

Dr Saskia Langemeijer
Radboud University Medical Centre
Nijmegen
Netherlands

Signature: _____ Date: 11 November 2015.

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Protocol synopsis

Protocol Title:	Coversin in paroxysmal nocturnal haemoglobinuria (PNH) in patients with resistance to eculizumab due to complement C5 polymorphisms.
Protocol Number:	AK Study 578
Sponsor:	Akari Therapeutics Plc 76 Wimpole Street London W1G 9RT UK
Investigational Product(s):	Coversin (rVA576) sterile solution for subcutaneous injection 7.2 mg/mL
Phase of Development:	II
Indication:	Paroxysmal nocturnal haemoglobinuria
PI(s):	Dr Saskia Langemeijer Dr Petra Muus Department of Haematology Radboud University Medical Centre Nijmegen Netherlands
Study Center(s):	Radboud University Medical Centre Nijmegen Netherlands
Objectives:	**Primary Objective:** Safety of Coversin (rVA576). **Secondary Objectives:** Efficacy of Coversin in the treatment of patients with PNH resistant to eculizumab.
Study Design:	Open label, non-comparative
Planned Number of Subjects:	Minimally 1 and maximally 6
Subject Population:	Patients with PNH, male or female, 18 years or older with proven resistance to eculizumab due to complement C5 polymorphisms
Diagnosis and Main Criteria for Inclusion and Exclusion:	**Inclusion Criteria:** • Patients with known paroxysmal nocturnal haemoglobinuria (PNH) • LDH ≥1.5 ULN • Resistance to eculizumab proven by both a recognised C5 polymorphism on genetic screening and complement inhibition on CH50 ELISA of <100% at concentrations of eculizumab in excess of 50µg/mL • Willing to give informed consent to treatment with Coversin • Willing to self-inject Coversin daily or to receive daily subcutaneous injections by a home nurse or in a doctor's office or hospital clinic • Willing to receive appropriate prophylaxis against *Neisseria* infection by both immunisation and continuous or intermittent antibiotics

	Exclusion Criteria: • Pregnancy (females) • Known allergy to ticks or severe reaction to arthropod venom (e.g. bee or wasp venom) • Failure to satisfy the PI of fitness to participate for any other reason
Treatment Regimen:	Single ablating dose of 0.57mg/kg per subject followed by daily maintenance doses. The initial maintenance dose will be 25% of the ablating dose. If this is insufficient to maintain complement activity at ≤10% of baseline (pre-treatment) level after 5 days of treatment the daily maintenance dose will be increased by doubling until that level of inhibition is achieved. In the event of 100% inhibition being achieved the dose may be titrated downwards at the PI's discretion until a satisfactory clinical result is obtained. If at any point in treatment complement activity (CH50) is 50% of baseline or more a further ablating dose of 0.57mg/kg should be given. An increased daily maintenance dose may be required in the event of intercurrent infection or illness. CH50 level should be checked in the event of deteriorating clinical response manifested by rising serum LDH.
Duration of Treatment:	Patients will undergo an initial period of 6 months treatment according to this protocol. At the expiry of this period, and on the recommendation of the PI, patients will have the option of remaining on long-term Coversin therapy and will enter the long term follow-up study.
Endpoints:	**Efficacy Endpoints:** Primary: Reduction in serum LDH from day 0 – Day 28 Secondary: Hb, haptoglobin, Fatigue (FACIT), Quality of Life (EORTC QL-C30), PNH RBC clone size, Transfusion requirement **Safety Endpoints:** Adverse events, ECG, Laboratory results, Vital signs.
Statistical Methods:	Descriptive statistics

List of prohibited medications:

1. Tizanidine (if on ciprofloxacin)

2. Eculizumab (*Soliris*®) should be discontinued before Coversin therapy is commenced. Ideally this should be 2 or more weeks before commencing Coversin unless, in the opinion of the investigator, it would not be in the best interests of the patient to do so.

1.0 INTRODUCTION

Coversin is a small protein complement C5 inhibitor which prevents the cleavage of C5 by C5 convertase into C5a and C5b. It is effective in inhibiting terminal complement activity irrespective of the activating pathway. In *in vitro* experiments it has been found to be as effective as eculizumab (Soliris®) at a molar equivalent dose in preventing the haemolysis of affected clones of red blood cells taken from patients with paroxysmal nocturnal haemoglobinuria (PNH). Following an initial successful Phase Ia clinical trial in healthy human volunteers it is now being developed for the treatment of patients with PNH and other complement mediated diseases. Its small molecular size relative to the monoclonal antibody Soliris® makes it suitable for small volume subcutaneous injection with the advantage that patients can self-administer the drug and are not tied to bi-monthly intravenous infusions which necessitate either attendance at a hospital clinic or a home visit by a suitably qualified nurse.

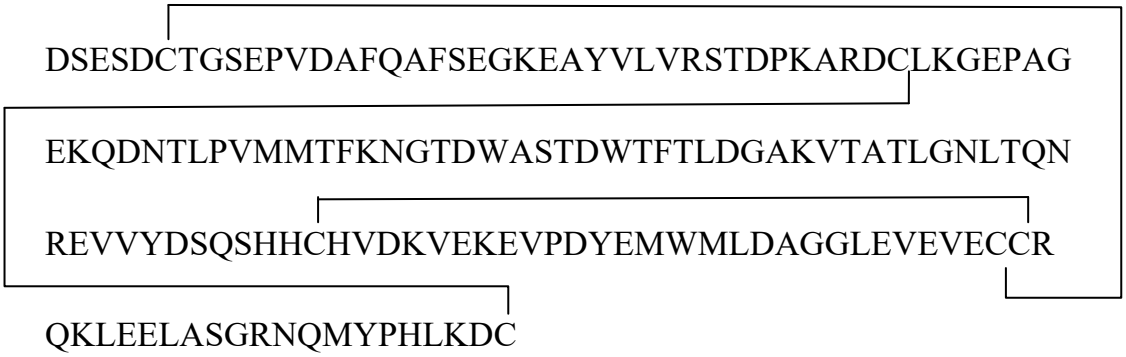
Under this protocol named patients with PNH and proven resistance to eculizumab will be treated with Coversin for 6 months in order to determine the safety and efficacy of the drug in these circumstances. If satisfactory control of the PNH is achieved, and at the discretion of the PI, patients will have the option of remaining on Coversin and being entered into the long term follow-up study.

1.1 Investigational Product

Coversin sterile solution for subcutaneous injection 7.2 mg/ml [*NB This is the concentration of the drug product (DP) to be used for the initial treatment of the first patient treated under this protocol. Coversin is still in development and this concentration may change. It is intended that fixed dosing for all patients between 50 and 100kg in weight will be introduced following Phase Ib clinical trials*]

Coversin is a compact small protein molecule with a lipocalin-like structure consisting of alpha helices and a beta barrel. There is a surface active site which binds to the complement C5 molecule with a high affinity (K_D 1.85 x 10-8) and an internalised active site which appears capable of binding small eicosinoid molecules such as leukotriene B4 [1]. The importance or otherwise of the latter site in the pharmacological and presumed therapeutic activity of Coversin has not been fully determined.

The molecular mass of Coversin as predicted by molecular modelling and confirmed by mass spectrometry is 16.7855 kDa.
The amino acid sequence of Coversin showing disulphide bridges is shown below:



Coversin drug substance is presented as a colourless aqueous solution stored frozen at -20^{o}C. Coversin drug product is presented as a colourless aqueous sterile-filtered solution for injection in 2ml vials filled to 0.5ml at a concentration of 8.9mg/ml. It will be prepared for s.c. administration by drawing up the requisite volume to deliver the desired dose in one or more syringes. No more than 1.0ml will be administered to a single injection site because of potential discomfort. Doses will be adjusted to the subject's weight.

1.2 Summary of Nonclinical and Clinical Studies Relevant to the Clinical Trial

Coversin is a recombinant small protein (MW 16.8kDa) which is derived from a native protein discovered in the saliva of the *Ornithodoros moubata* tick [2]. Its function in tick saliva is to assist the parasite in feeding by suppressing the host immune reactions that would otherwise alert the host to the presence of the parasite which could then be removed by scratching or grooming. It has been known for some time that all species of ticks, which can feed undisturbed on their hosts including rodents, cattle, dogs and man, for periods of 14 days or more, secrete an array of immunomodulatory peptides and proteins in their saliva in order to take control of their hosts' local and systemic immune and inflammatory responses [3].

The complement system is an important part of the innate immune system in many animal species including all mammals. There are three known pathways in the cascade: the classical, the alternative and the lectin but all converge on a final common pathway. At this point complement C5, a product of the classical pathway is acted upon by the enzyme C5 convertase, a product of the alternative pathway, to form C5a and a cluster of proteins (C5b, C6, C7, C8, and C9) which are collectively known as the membrane attack complex (MAC). It is known that many human autoimmune diseases are associated with inappropriate reactions to products of the final common pathway of the complement system. In particular myasthenia gravis, in which individuals form antibodies to their own acetyl choline receptors (approximately 70% of all MG patients) is associated with either inappropriate reaction to or over-production of MAC proteins [4].

Coversin binds to the C5 molecule, preventing any C5 convertase from acting on it and cleaving it to form C5a and the MAC. It appears to do this by interfering with the interaction of C5 convertases rather than by blocking the actual cleavage site on the C5 molecule [5]. The binding to C5 is high affinity (K_D 1.85 x 10^{-8}) and appears to be irreversible in physiological conditions [6]. Other studies including X-ray crystallography have confirmed that Coversin complexes with human complement C5 [5].

Inhibition of the C5 complement system is a therapeutic target in a wide range of autoimmune and inflammatory diseases including rheumatoid arthritis, Crohn's disease, hypersensitivity pneumonitis, ischaemia reperfusion injury, sepsis, myasthenia gravis, paroxysmal nocturnal haemoglobinuria (PNH) and age related macular degeneration [7-14].

The initial proposed clinical indication is PNH in patients known to be resistant to eculizumab because of polymorphisms occurring in the complement C5 molecule. The humanised monoclonal antibody eculizumab (Soliris®) was approved in Europe and the USA for the treatment of PNH in 2007 and is now the standard of care in this condition. Since Coversin acts in exactly the same way as Soliris® it is believed that it will be effective as a therapeutic agent in the treatment of PNH but may offer patient benefits in terms of convenience since it can be self-administered by sub cutaneous injection rather than having to be administered by a health-care professional by intravenous infusion at two weekly intervals and in addition can be used in patients resistant to eculizumab.

There are no satisfactory animal models of PNH and the presumed efficacy of Coversin in this condition relies on the fact that, since its mode of action is identical to that of Soliris® with the exception that it binds to a different epitope on the C5 molecule, the clinical effects on patients with PNH are likely to be the same. In addition Coversin has been tested in an *in vitro* flow cytometry model using blood from patients with PNH with a large clone of Type III PNH cells . In this model the blood was either complement inactivated through heat treatment (negative control) or not and complement activation was achieved by acidification and the addition of magnesium chloride (MgCl). Blood so treated was first spiked with either Coversin or Soliris® in ascending concentrations and the effect of complement activation on the relative populations of Clone III to Clone I RBCs was observed. These results are shown in Figs 1and 2 below. They show that Coversin 10µg/mL and Soliris® 50µg/mL (molar equivalent concentrations) are equally effective in preventing haemolysis in blood from a patient with Type III red blood cells These results are illustrated below in Figs 1 and 2.

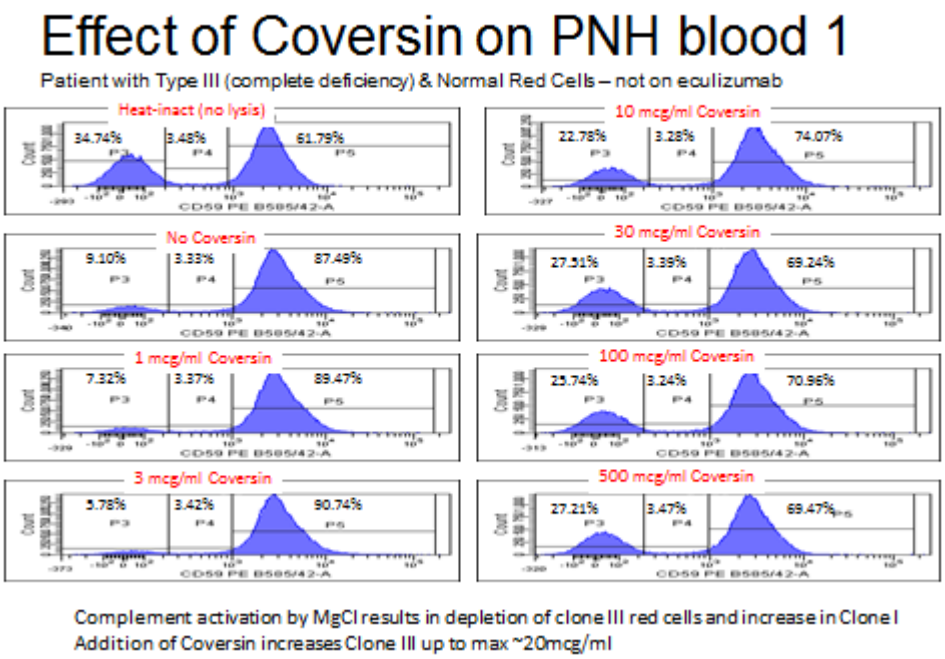


Fig 1 Effect of ascending concentrations of Coversin on complement activation in blood from a patient with PNH and type III PNH erythrocytes only.

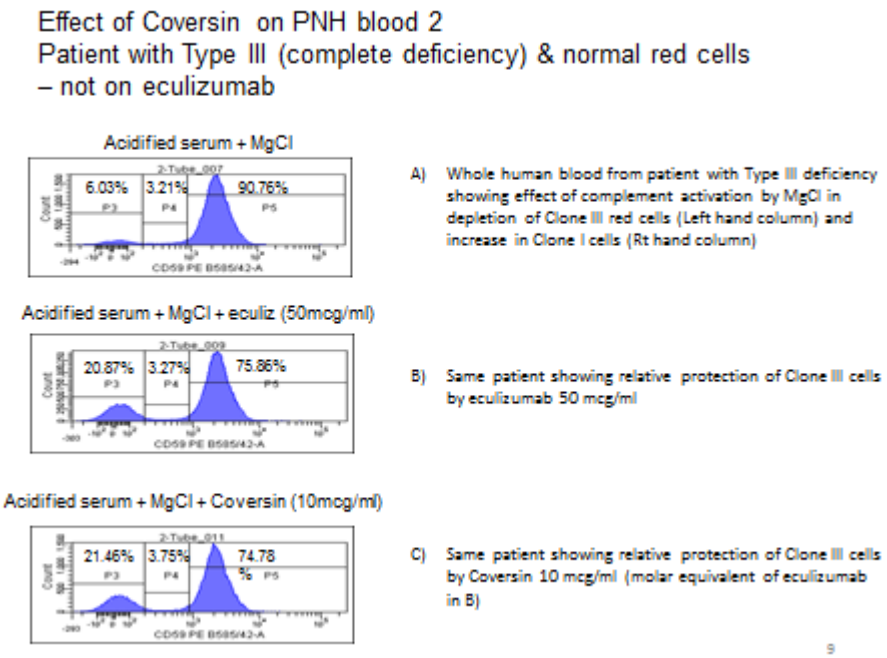


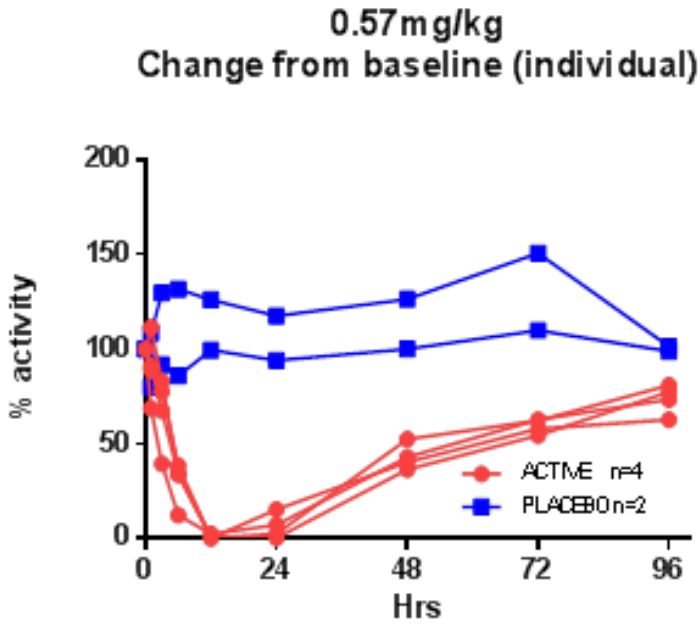
Fig 2 Comparison of Coversin 10µg/mL and Soliris® 50µg/mL on Type III PNH erythrocytes.

A single ascending dose (SAD) clinical trial in healthy volunteers, of Coversin administered by subcutaneous injection has been carried out in order to validate this route of administration and to confirm the dose needed to totally inhibit all C5 in the

vascular compartment. The starting dose was one eighth of the dose found to induce total C5 blockade in rodent and non-human primate (NHP) PK studies (0.57mg/kg) and doubled in each succeeding cohort of subjects until the target dose was reached. This dose was found to produce complete terminal complement blockade as determined by CH50 Equivalent assay in all four subjects and the result is shown in Figure 3 below. In this study there were no serious or dose related adverse events and the drug was well tolerated. There were three mild adverse reaction reported in two subjects who received active drug (intolerance to light, pain in the armunrelated to the injection site and light headedness). These adverse events were transient and self-limiting and were not dose related.

An immunogenicity study in BALBc mice given Coversin by daily subcutaneous injection showed that approximately 70% formed anti-Coversin antibodies by Day 28 but that these IgG antibodies were non-neutralising. There were no clinical or injection site reactions indicative of immuno- or allergenicity.

Figure 3 Phase I SAD clinical trial in normal volunteers. CH50 activity (% of baseline) in 4 active and 2 placebo treated subjects



A single human patient, a 4 year old child with a thrombotic microangiopathy (TMA) secondary to stem cell transplantation for chronic granulomatous disease has been treated with Coversin on a named patient basis for 58 days. The patient had previously been treated with eculizumab for 3 months with no apparent clinical benefit and was then found to have a polymorphism of C5 which prevented proper binding of the antibody to the complement protein. This polymorphism had previously only been reported in patients of Japanese origin although this patient was

Caucasian.[REF] Akari Therapeutics Plc has shown *in vitro* that when complement is activated in serum from this patient and from another non-Japanese PNH patient with resistance to eculizumab due to the same polymorphism it can be blocked with Coversin but not eculizumab and it was decided on that basis to attempt to treat the patient with Coversin.

The patient initially responded well to Coversin but the drug supply was limited and the patient died 10 days after the drug was withdrawn. During treatment there were no adverse events attributable to Coversin, no injection site reactions and no evidence of drug neutralisation. This patient represents the longest human exposure to date of Coversin.

1.3 Summary of Known and Potential Risks and Benefits to Human Subjects

Although there has been limited clinical experience with Coversin to date the clinical implications of complement C5 blockade can be deduced from a combination of genetic and epidemiological studies, animal studies and experience with eculizumab (*Soliris*®), an anti-complement C5 monoclonal antibody. Complement C5 deficiency in humans is a rare, familial condition caused by a variety of genetic defects including mutations in the C5 exons. Those affected have a predisposition to gram negative infections, particularly meningococcal meningitis, herpetic infections and seborrhoeic dermatitis. [17]

In clinical trials of eculizumab in paroxysmal nocturnal haemoglobinuria (PNH) 2 of 96 patients developed meningococcal meningitis and currently all subjects taking the drug are advised to maintain *Neisseria meningitidis* immunization with booster shots at recommended intervals. [18] In limited duration toxicological studies with both eculizumab and Coversin no adverse events or findings attributable to C5 blockade have been reported but PIs are advised to be alert for possible infectious events even in subjects who have received meningococcal immunisation. All subjects screened for entry into this trial will have pre-trial throat and nasal swabs taken within 5 days of entry and any positive *Neisseria sp.* results will be grounds for excluding that subject from the trial irrespective of immunisation status until the bacteria have been eliminated.

In long term studies and post marketing surveillance of eculizumab patients for periods in excess of three years, apart from the increased risk of meningococcal infection referred to above, the most commonly reported side effects were headache, runny nose (nasopharyngitis), back pain, nausea and tiredness (fatigue). However these are all known to be associated with the use of antibodies rather than any known association with complement inhibition and, in any case, in trials the incidence was not significantly different from symptoms experienced by placebo treated patients.

There have been no adverse events in single or repeat dose animal toxicology studies of Coversin in doses up to 100 times the anticipated human dose. It is not anticipated that there will be any difference in the risk or adverse event profile of Coversin compared to eculizumab.

Because Coversin is a xenologous protein (derived from a tick salivary protein) there is always a possibility that its chronic use may be associated with the formation of antibodies which could neutralise the effect of the drug or cause untoward adverse reactions. The immunogenicity study mentioned above has gone some way to mitigating this possibility and experience with other parasite derived therapeutic molecules such as the leech-derived anticoagulant lepirudan suggest that parasite derived therapeutic molecules are not usulally associated with neutralising activity.

Potential benefits to patients with autoimmune disease remain to be evaluated but it is anticipated on the basis of results in animal models considered to be predictive of human disease that patients with autoimmune neurological conditions in which one or more specific antibodies to target tissues have been identified and which are known to be associated with complement activation will benefit from C5 inhibition. Such conditions include myasthenia gravis, Guillain Barré syndrome and varieties of multiple sclerosis associated with the MOG16 antibody.

1.4 Description of/and Justification for the Route of Administration, Dosage Regimen and Treatment Period(s)

The human complement C5 standing pool is about 200mg in a 70kg individual. Given the molecular weight of C5 (190kDa), the molecular weight of Coversin (16.8kDa) and the fact that it is a 1:1 binding, it would be expected that 1mg of Coversin would bind 11.3mg of C5. However, it has been shown that a 2.26:1 excess of Coversin is necessary to produce optimal binding conditions to C5 [19]. This means that it would take 40.0mg of Coversin to completely neutralise the standing pool of C5 in a 70kg human.

Pharmacokinetic studies in rats have shown that unbound Coversin is rapidly excreted by the renal route and that the serum half-life is ~30minutes whereas Coversin complexed with C5 has a circulating half-life of ~30 hours [6]. The rate of production of C5 by humans is not known precisely. Sissons *et al*. using the Fick Principle and ^{125}I labelled C5 injected intravenously calculated a daily turnover in normal humans of between 65 and 196µg/kg/24hrs (mean 90µg/kg/24hrs) [20]. It is generally believed that turnover in autoimmune disease is not substantially different to that in normal subjects. On this basis the mean amount of C5 produced daily is 151.2mg and, using the calculation explained above, it would take 30.0mg of Coversin to completely ablate it. These figures indicate that a loading ablation dose for a human is 0.57mg/kg and a maintenance dose is 0.018mg/kg/hr.

In preclinical models of myasthenia gravis in rats doses of 0.5, 5.0 and 50mg/kg were all found to be significantly more effective than control (vehicle) in mitigating the signs of experimental autoimmune myasthenia gravis (EAMG) in a dose dependent fashion [16]. The Pharmacologically Active Dose (PAD) in laboratory animals (rodents) is therefore estimated at ≤0.5mg/kg. In a human *in vitro* model using whole blood from patients with paroxysmal nocturnal haemoglobinuria (PNH), an autoimmune complement dependent condition, it was found that the maximal effect in preventing red cell haemolysis was achieved by a dose equivalent to between 0.3 and 0.7 mg/kg. Above 0.7 mg/kg no additional benefit was seen.

In pharmacokinetic studies in mice it was found that when a single loading dose was administered by the subcutaneous injection route serum complement activity as determined by the CH50 Eq ELISA was totally suppressed and did not return to normal levels until 24 hours later. At 12 hours there was still 45% or more inhibition of terminal complement activity compared to baseline. This level of inhibition was significantly longer than when the drug was administered intravenously and is believed to be because of a reservoir effect combined with relatively slower renal clearance. These observations have yet to be fully confirmed by more extensive metabolic studies but are considered to be a reasonable basis for selecting administration by subcutaneous rather than intravenous injection.

Toxicokinetic samples were taken at intervals following first dose in the cynomolgus monkey repeat dose toxicology study. As the only dose used in this study was 50x the expected human therapeutic dose interpretation must be guarded. Serum complement activity at 15 minutes, 1, 3, 6, 9 and 12 hours was measured using the CH50 ELISA. The results showed that complement activity fell to about 50% of baseline after 15 minutes and to zero at one hour and it remained at that level for the whole of the 12 hour period. Since it is known that any circulating unbound Coversin would have been excreted by the renal route within 2 – 3 hours it may be assumed that the prolonged effect was due to some depot effect associated with the subcutaneous route of administration.

Single and repeat dose toxicology studies in rat and cynomolgus monkey have been performed using doses of up to 200 times (single dose) and 50 times (repeat dose) the expected human dose. In these studies no signs of toxicity were observed, no haematological or biochemical observations fell outside the range of normal and no gross or microscopic signs of pathology were found in any organ or tissue. It has therefore not been possible to set a No Observable Adverse Effect Level (NOAEL) and so a Human Equivalent Dose (HED) has not been established.

In pharmacology studies no toxicity has been observed to date in any species at any dose by any route of administration. These have included up to 50mg/kg intraperitoneally and up to 20mg/kg intravenously and by inhalation. The current

toxicology studies include single and repeat doses of up to 100 fold the likely HED (i.e. 57.0mg/kg in rats and primates).

The Phase I single ascending dose SAD study showed a maximum onset of action between 6 and 12 hours after injection and a slow recovery due to replenishment of C5 by the liver and other tissues so that at 96 hours post-dose CH50 had only returned to about 75% of baseline. This result was unexpected since both onset and duration of action in animals including NHP was substantially faster and it is considered likely that it is due to both a depot effect since humans generally have more subcutaneous adipose tissue than laboratory animals and because the production of C5 by the liver appears to be slower in humans.

The result suggests that, following a single ablating dose of Coversin, it should be possible to dose once a day using a maintenance dose between 25 and 50% of the ablating dose and keep terminal complement activity totally inhibited which is considered necessary in diseases such as PNH. If a fixed dose of 60mg, 50% higher than the 40mg needed to completely ablate C5 in a 70kg individual, is used it should provide adequate initial ablation in all subjects up to 100kg in weight. Similarly a daily maintenance dose of between 25 and 50% of this should be sufficient to provide 24 hour complement inhibition. Paediatric patients and patients of 50kg or less could be treated using a half dose.

In the first patient in the current study however, because the drug supply will be limited for the first 3 – 6 months it is proposed that a dose tailored the patient's actual weight will be used. When lyophilised drug product becomes available and when the supply situation is improved this and other patients may be switched to a fixed dosing regimen.

1.5 Compliance Statement

This Phase II clinical trial will adhere to the trial protocol and will be conducted in compliance with current ICH Good Clinical Practice (GCP) guidelines and all applicable regulatory requirements.

1.6 Description of the Trial Population

The study population will be minimally 1 patient and maximally 6 patients above the age of 18with PNH and known resistance to eculizumab. Patients may be male or non-pregnant females using adequate methods of contraception if of childbearing potential.

2.0 TRIAL OBJECTIVE AND PURPOSE

The trial objectives of this study are to demonstrate the safety and efficacy of Coversin when given to patients with PNH and known resistance to eculizumab.

1) To assess the safety and tolerability of Coversin at a single ablating dose followed by repeat daily maintenance doses calculated by body weight.

2) To assess whether this dosage regimen is sufficient to control the signs and symptoms of PNH (see Endpoints below).

3.0 TRIAL DESIGN

Treatment with Coversin comprises an ablating dose on day 1 and daily maintenance treatment from day 2 onwards. Calculated according to body weight, the ablating dose will be 0.57mg/kg. Thereafter the daily repeat maintenance dose will be titrated according to clinical response and complement inhibition as determined by CH50 ELISA. The initial daily maintenance dose will be 25% of the ablating dose (0.14 mg/kg) and this will be adjusted up or down if necessary once steady state is reached (5 days). This is further explained in chapter 6, TRIAL PROCEDURE .

In order to mitigate the risk of infection by *Neisseria sp* all subjects or patients taking part in the trial will be carefully instructed about precautions to be taken in case of actual or suspected infection. Patients may either be given permanent antibiotic prophylaxis (oral penicillin daily) or be provided with high dose ciprofloxacin and instructed to immediately commence taking it in the event of a suspected infection. In the latter case they will also be told to make immediate contact with the PI or her deputy. In addition all patients will receive immunisation with quadrivalent meningoccal vaccine Anti meningococcal B vaccine has recently been developed and will be given once approved in the country where the patient is treated. Vaccination against meningococci typ ACW135Y is required at the start of the study. Vaccination against meningococci typ B is not mandatory for inclusion in the study. Should the first vaccination take place $\leq$ 14 days of commencement of Coversin, then the patient must take high dose antibiotic for the first 2 weeks. This will generally be ciprofloxacin daily (2x 750mg orally, until 2 weeks have elapsed since first vaccination) or alternative antibiotic treatment directed against meningococci.

3.1 Endpoints

Safety:

- Frequency, type and relationship to treatment of AEs and SAEs
- Out of range laboratory parameters (haematology and chemistry)
- Vital signs
- ECG abnormalities Day 0 – Day 90

Primary efficacy endpoint:

- Serum lactic dehydrogenase (LDH) AUC from Day 0 to Day 28 compared with 28 days pre-treatment

Secondary efficacy endpoints:

- Haemoglobin at Day 28 and day 90 and Day 180, absolute and change from baseline
- LDH at days 90 and 180
- Haptoglobin at day 28, day 90 and day 180, absolute and change from baseline
- Dependency on blood transfusion
- Quality of Life at days assessed by the FACIT and EORTC QL-C30 instruments compared to Day 0, day 28, 90 and 180

4.0 CONTRACEPTION

At this point in time the reproductive safety of Coversin is unknown. Female patients entered into this study should not be pregnant at the time of commencing Coversin therapy and should observe suitable contraceptive methods until such time as reproductive toxicity data become available. Should inadvertent pregnancy occur this should be discussed with the PI in order to decide how to proceed. It is anticipated that reproductive toxicity data will become available by early 2017.

4.1 Exposure to Partners During the Study

There is a unknown risk of drug exposure through the ejaculate (which also applies to vasectomised males) that might be harmful to the sexual partners, including pregnant partners, of male subjects. Although the risks of this are at present unknown this should be carefully explained to all male patients taking Coversin and they should be advised to use barrier contraception until reproductive toxicity data become available.

4.2 Sperm Donation

Male subjects should not donate sperm for the duration of the study and for at least 90 days after the last day of Coversin administration.

5.0 SUBJECT WITHDRAWAL CRITERIA

Subjects may be withdrawn from the trial in the following circumstances:

- An adverse event graded serious or severe which in the opinion of the PI is possibly, probably or definitely related to administration of the trial drug.

- Any other reason for which, in the opinion of the PI it would not be in the best interests of the subject to remain in the trial.

- At the request of the subject for any reason.

- Non-compliance or behaviour not compatible with the proper conduct of the trial.

Appropriate clinical and serological data will be entered in the CRFs. In addition the Subject Withdrawal page of the CRFs will be completed. All data that would normally have been entered on the final day of dosing will be collected and entered together with such additional data as the PI considers clinically necessary to ensure the subject's safety or to elucidate the nature of the AE (e.g. special tests, X Rays, scans, photographs etc.)

6.0 TRIAL PROCEDURE

The trial will comprise an open label, non-comparative study of minimally 1 patient and maximally 6 patients with PNH and known resistance to Eculizumab, who meet the inclusion and exclusion criteria (see below). Patients agreeing to participate in the trial will be admitted to the hospital for a minimum of 2 days for initiation of Coversin treatment, for clinical observation and for bloodsampling (PK/PD). Treatment will be continued on an out-patient basis with daily visits until stabilization or adjustment of dose.

Patients deemed capable of self-administration will be taught how to calculate and draw up the appropriate volume of solution for injection and how to inject subcutaneously. They will also be taught how to store and thaw Coversin prior to injection..

For Patients deemed not capable of self administration, alternative facilities for administration at home will be sought (General Physician or suitably qualified home care nurse).

Only if, in the opinion of the PI, the patient is capable of self injecting the correct dose of Coversin or adequate alternative facilities existfor safety monitoring and dosing supervision, treatment will be transferred to admisitration at home.

If not already immunised against *Neisseria sp*. immunisation and prophylactic antibiotics will be administered as set out in Section 10 below. Nose and throat swabs will be taken although growth of *Neisseria* species will not be grounds for exclusion but will prompt particular vigilance on the part of the PI.

The ablating dose will be divided between as many injection sites as are necessary to avoid injection of >1.2mL of Coversin solution for injection. These sites may be chosen from the deltoid regions, the anterior abdominal region or the anterior femoral regions. The injection sites used will be recorded in the patient notes and will be inspected at 24 hour intervals for 5 days for injection site reactions.

Doses of Coversin will be given at the same time each day, preferably between 6 and 8am. The time of each dose will be recorded in the CRFs. Patients self-administering Coversin will use suitable sites on the anterior femoral and anterior abdominal regions and the site, date and time of injection will be entered on the appropriate CRF sheet (patient diary) and be initialled by the supervising nurse.

All patients will return to the hospital out patient clinic for follow-up at a minimum of monthly intervals according to the schedule in appendix 1.

Blood and urine samples will be taken from all patients according the schedule on appendix 1.

Patients who have satisfactorily mastered the technique of self-administration of Coversin may be discharged home when considered appropriate to do so by the PI providing that adequate arrangements for daily pre-dose blood sampling and sample handling for CH50 samples have been arranged (see below).

All patients will be followed up at a minimum of monthly intervals by the PI. If at the 6 month follow-up the patient wishes to continue Coversin treatment and if the PI is satisfied as to the suitability of Coversin treatment, the patient may be transferred to the long-term follow-up protocol.

It is expected that the majority of patients will respond to an ablating dose of 0.57mg/kg followed by a daily maintenance dose of 25% of the ablating dose.

On a per patient basis this will be adjusted as necessary on the basis of clinical response and CH50 assay. The protocol allows for a flexible, tailored dose adaptation on day 7 and any subsequent follow-up. in patients who are not adequately controlled as is outlined below ..

If patients are not adequately controlled at the first (7 day) or subsequent follow up either of three options may be followed:

Patient will be reinduced with an identical ablating dose (0.57 mg/kg) **and /or** the maintenance dose may be increased with one step at the time for a maximum of 2

times according to the below schedule of increments . Each incremental dose level must be given for a minimum of 4 days.

The definition of adequately controlled coversin treatment is:

LDH <1.5xULN
AND
CH50 ≤10% of baseline.

It is expected that this adequate control is achieved at day 5.

If a recent CH50 result is not available at the time of assessment then the PI will make a decision on clinical grounds alone on the adequacy or otherwise of disease control.

If adequate control is not achieved on day 5 of maintenance therapy at the standard dose level (and day 5 of increment dose levels if applicable) the following dose adaptation will take place:

- If CH50 ≤10% and LDH is reducing but still ≥1.5xULN, treatment at the same maintenance dose level is continued for another week.

- If LDH is decreasing and if CH50 is between 50 and 10% of baseline, the maintenance dose may be increased according to the schedule of increments of maintenance dose shown below.

- If LDH is not reducing and CH50 is >50% of baseline the patient must either be discontinued from the trial and switched to alternative therapy or must be readmitted to hospital for dose stabilisation following another ablating dose (0.57mg/kg) of Coversin. In either case the findings and course of action must be discussed by a Trial Steering Committee comprised of, at minimum, the PI, and the Medical Director of the Sponsor company.

The schedule of increments for the maintenance dose is as follows:

Ablating dose	**First maintenance dose level**	**Second maintenance dose level**	**Third maintenance dose level**
0.57 mg/kg	0.14 mg/kg	0.29 mg/kg	0.57 mg/kg

Within each patient the first incremeneted maintenance dose should be given at 24 hour intervals for at least 4 days until steady state is reached and at that point a CH50 assay should be performed. If the CH50 is not ≤10% of baseline (adjusted to 100%) then the next incremental dose (Second maintenance dose level should be given for at least another 4 days before switching to the Third maintenance dose level. If at any

stage the CH50 is >50% of baseline another ablating dose should be given before using the next incremental dose.

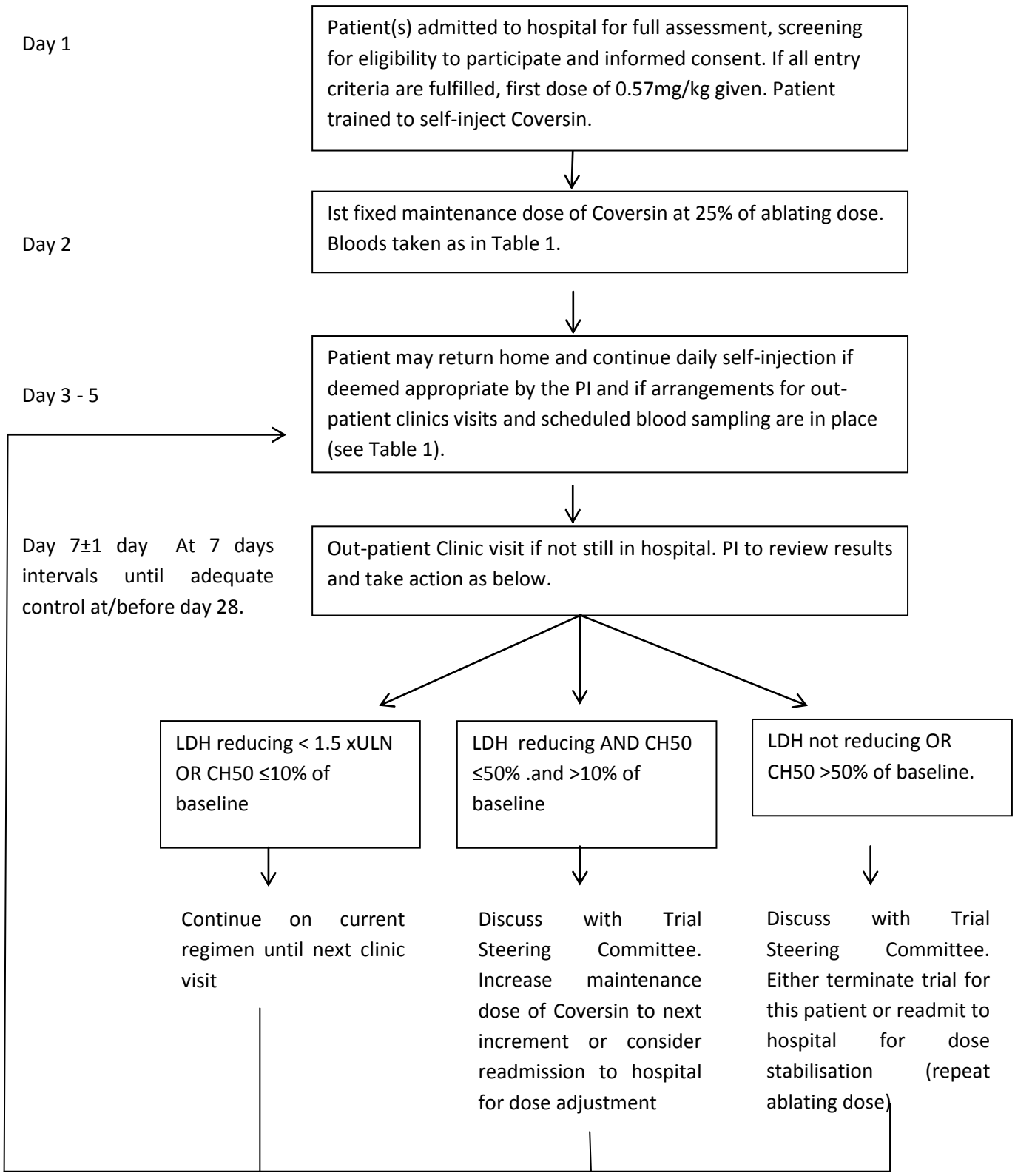
Blood samples and timing is shown in Appendix 1. Samples for CH50, antibodies and drug level should be processed as shown in Section 20 and sent to the Akari Therapeutics Plc complement laboratory for assaying. All other laboratory tests should be done locally in the PI's hospital laboratory.

If an ablating dose is repeated , PK/PD and other assessment will be performed as indicated in Appendix 1 at 1hr, day 2 and day 5.

At increments of the maintenance dose PK and PD and other assessments are repeated at day 5 as indicated in Appendix 1.

Extra samples for PK/PD may be taken at the discretion of the investigators during illness or infection.

Flow chart: Schedule of Procedures and Visits



The trial will continue for 6 months or until it has been decided by the PI in consultation with the Sponsor Company that further Coversin treatment is futile or not in the patient's best interests. If the patient is satisfactorily controlled and wishes to continue on Coversin he/she will be permitted to enter the long-term follow-up study. Drug will, if necessary, be supplied free of charge by the Sponsor until it is approved for this indication and reimbursed through the normal channels.

6.1 Endpoints

Primary Efficacy Endpoint:

- Significant reduction in mean serum LDH from Day 0 (pre-dose) to Day 28 (AUC)

Secondary Efficacy Endpoints:

- Change in LDH from Day 0 (pre-dose) at monthly intervals after Day 28
- Change in proportion of PNH clone Day 0 – Day 90
- Change in mean Hb Day 0 – Day 90
- Change in mean Hp Day 0 – Day 90
- Change in FACIT score Day 0 – day 28- Day 90 - Day 180
- Change in EORTC QLQ C30 score Day 0 – -Day 28-Day 90 – Day 180

Safety Endpoints:

- Frequency, type and relationship to treatment of AEs and SAEs
- Out of range laboratory parameters (haematology and chemistry)
- Vital signs
- ECG abnormalities Day 0 – Day 90

6.2 Description of Trial Treatments, Dosage and Dosage Regimens

Coversin for subcutaneous injection will be supplied initially as vials of frozen solution, each 2mL vial containing 1.2mL of 7.2mg/mL solution. Vials should be stored frozen at -20^{o}C until between 12 and 24 hours of use when they should be allowed to thaw at ambient or refrigerated (2 – 6^{o}C) temperature. Ideally they should be thawed overnight in refrigerated conditions.

Following the initial batch for use in the first patient treated under this protocol it is likely that all future batches will be supplied as lyophilised powder 15mg in 5mL glass vials. Until reconstituted it can be stored at ambient temperature for the duration allowed by the expiry date printed on the label. It will be reconstituted within 6 hours of administration with 0.5mL of sterile water for injection to form a solution of 30mg/mL concentration. After injection of

sterile water into the Coversin vial it should be shaken a few times until no particulate matter remains. After reconstitution vials should be stored between 2 and 6^{o}C until used and in any case for no longer than 24 hours.

Following the switch to lyophilised powder the maintenance dose will be changed to a fixed multiple of 15mg (ie 15, 30 or 60mg) which will be the next dose above the dose that the patient is being maintained on. Thereafter the first ablating dose in all future patients treated under this protocol will be 60mg (2mL) which will divided between two injection sites.

After the first, ablating, dose all subjects will receive fixed daily maintenance doses for the remainder of the trial. The size of the fixed maintenance dose will be determined by the results achieved in the first patient treated under this protocol. As mentioned above there will be some flexibility to allow for adjustment of dose in individual patients if they do not appear to be responding adequately but the overall aim will be to find a fixed dose that will maintain full complement inhibition in all patients treated.

All doses should be given at the same time each day, ideally between 6 and 8 am. Blood samples should be taken immediately pre-dose or as shown in appendix 1.

7.0 STOPPING RULES AND DISCONTINUATION CRITERIA

7.1 Management of individual patients who experience clinically significant toxicity

In the event of a patient experiencing significant toxicity the PI and the Sponsor Medical Director will examine the circumstances. If this is not possible the Principal PI or any other physician having direct responsibility for the patient's treatment may take appropriate action. The patient will be withdrawn from the trial and offered alternative treatment in the event of any of the following:

- An adverse event graded serious or severe which in the opinion of the TSC is probably or definitely related to administration of the trial drug and which would prejudice the patient's well-being if it was to continue or recur.

- Any other reason for which, in the opinion of the Principal PI or the TSC, it would not be in the best interests of the patient to remain in the trial.

- At the request of the patient for any reason.

7.2 Toxicity occurring in two or more patients

In the event of the same or a similar severe adverse event occurring in two or more patients in the trial and which, in the opinion of the PI, is possibly, probably or definitely related to administration of Coversin consideration will be given to halting the trial. A serious adverse event (SAE) occurring in two or more patients which is probably or definitely drug related is

an absolute indication to terminate the trial. Adverse events which are not SAEs but which appear to affect more than one patient will be assessed for their severity, threat to the patients' health and drug relationship and a decision on whether to halt the trial or allow it to continue while keeping the situation under review will be made by the PI and the Sponsor Medical Director.

8.0 ACCOUNTABILITY PROCEDURES

The PI and the hospital pharmacist are responsible for study medication accountability, reconciliation, and record maintenance. Drug accountability records will be maintained during the study, including the amount of study medication received from the Sponsor, the amount distributed to each subject, and the amount of unused drug returned to the Sponsor or destroyed at Sponsors request. In addition, in the event of necessary disposal of opened but wasted medication, the disposal should be documented appropriately (ie, witnessed) in accordance with applicable local regulations, and "Good Clinical Practice" (GCP) procedures.

9.0 SELECTION AND WITHDRAWAL OF SUBJECTS

Trialists will be patients with PNH and known genetically determined resistance to eculizumab who are willing to give informed consent to participate in the trial.

9.1 Inclusion Criteria

- Males or females taking adequate contraceptive precautions if of childbearing potential, 18 – 80 years of age
- Body weight ≥50kg and ≤ 100kg
- The patient has provided written informed consent.
- Willing to avoid prohibited medications for duration of study (see Exclusion Criteria)
- Must agree to take appropriate prophylactic precautions against Neisseria infection
- Must be counselled regarding the possible reproductive risks of using Coversin and be advised to use an adequate method of contraception pending further data on reproductive toxicology.

9.2 Exclusion Criteria

- Body weight <50kg or > 100kg
- Pregnancy

10.0 *NEISSERIA* IMMUNISATION AND ANTIBIOTIC PROPHYLAXIS

It is known that the products of C5 complement activation are important in protection against infection by *Neisseria* species (15). This is believed to be the only known hazard of blocking this stage of the complement cascade and patients taking part in previous trials of complement C5 inhibitors were found to be at greater risk of *Neisseria* infection, particularly meningococcal infection (16). For this reason all patients entering the trial should where possible have received immunization against *Neisseria meningitidis* infection in the past, or must receive a booster shot of quadrivalent vaccine (against meningococci type ACW135Y)) at the time of recruitment into the trial and at least prior to receiving the first dose of medication. In addition all patients should be immunised against Meningitis B using the *Bexsero*® vaccine as soon as this vaccine is available in the country where the patient is treated. This vaccine requires an initial dose followed by a booster 1 month later.

All patients will in addition have throat and nasal swabs taken after entry to the trial and prior to receiving the first dose. Cultures positive for any *Neisseria* species will not be grounds for excluding that subject from the trial but will alert the PI to use extra vigilance during and after induction of complement inhibition. Any patient having positive nasal swabs will be re-tested 7 days later to ensure that the pathogen has been eliminated by ciprofloxacin.

Patients will preferably receive a 5 day course of oral ciprofloxacin commencing the day before the first dose of Coversin and this will either be followed by oral penicillin prophylactic treatment or, at the discretion of the PI and in accordance with local custom, the patient may be provided with high dose ciprofloxacin to be started in the event of an actual or suspected infection. Patients will be instructed to make urgent contact with the PI or her deputy in that event.

Because of the known drug interaction between tizanidine and ciprofloxacin the former drug will be prohibited during this trial. In addition patients will be given a medication leaflet for ciprofloxacin in their native language in which other possible drug interactions (e.g. with antacids) and precautions regarding known or suspected sensitivity to fluoroquinolones will be pointed out. It is the responsibility of the PI and her deputies to make the patient(s) aware of these precautions.

11.0 COMPLIANCE

Whilst patients are in a hospital setting responsibility for compliance will rest with the medical, pharmacy, pharmaceutical science and nursing/technical staff who will be responsible for administering the trial medication. When patients return home, for the first month compliance will be assured by retrieval and counting of all used vials and syringes and completion of a diary to record the time and date of each dose. All empty and unused Coversin vials will be returned to the Sponsor as an additional compliance check.

11.1 Assessment of pharmacokinetics and complement activity

Blood (5ml) samples for CH50 assays and Coversin levels will be collected according to the time schedule presented in Appendix 1. These blood samples will take precedence over all other procedures. Samples will be processed in accordance with Paragraph 20.2.

12.0 ADVERSE EVENTS

12.1 Adverse Events

All adverse events, whether or not considered to be related to the study drug, will be monitored and reported on at all stages of the study. Adverse events occurring between discharge from hospital and the 1 week follow up visit will be reported at that visit and entered on the adverse event form of the case report forms.

12.2 Definition

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product whether or not it is caused by this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Worsening of a medical condition that was present prior to the administration of the study medication will also be considered a new adverse event and reported. Any medical condition present prior to the administration of the study medication which remains unchanged or improved will not be recorded as an adverse event at subsequent visits.

Documentation regarding the adverse event will include the nature, time and date of onset, time and date of resolution, severity, presumed relationship to study medication, action(s) taken, and outcome of any sign or symptom observed by the physician or reported by the subject upon indirect questioning.

12.3 Relationship to study medication

The relationship between the AE and the investigational product will be determined by the PI or Sub-PI on the basis of his/her clinical judgement and the following definitions:

Unrelated: Clinical event with an incompatible time relationship to IMP administration, and that could be explained by underlying disease or other drugs or chemicals or is incontrovertibly not related to the IMP

Possibly related: Clinical event with a reasonable time relationship to IMP administration, and that is unlikely to be attributed to concurrent disease or other drugs or chemicals

Related: Clinical event with plausible time relationship to IMP administration and that cannot be explained by concurrent disease or other drugs or chemicals

The degree of certainty with which an AE is attributed to IMP administration (or alternative causes, e.g. natural history of the underlying disease, concomitant therapy, etc) will be determined by how well the experience can be understood in terms of one or more of the following:

- known pharmacology of the IMP
- reactions of a similar nature have been previously observed with the IMP or this class of drug
- the experience being related by time to IMP administration, terminating with IMP withdrawal or recurring on re challenge
- alternative cause

12.4 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence at any dose that:

- Results in death

- Is life threatening (Note: The term "life threatening" refers to an event in which the subject was actually at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe).

- Requires in-patient hospitalisation or prolongation of existing hospitalisation. (Note: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from Baseline is not considered to be an AE.)

- Results in persistent or significant disability/incapacity. (Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, or accidental trauma (e.g. sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.)

- Is a congenital abnormality.

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

All serious adverse events will be reported to the PI, and the Sponsor within 24 hours of learning of its occurrence. The Sponsor will be responsible for reporting the adverse event to the appropriate regulatory authority and the IRB within the legally specified period. It is important to distinguish between SAEs and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria above. An AE of severe intensity need not necessarily be considered serious. For example, a migraine headache that incapacitates a patient for many hours may be considered a severe AE, whereas a stroke that results in a limited degree of disability may be considered mild, but should be reported as an SAE).

12.5 Severity

The following definitions will apply:

Mild: Event is noticeable to the patient, but is easily tolerated, requires no additional therapy and does not interfere with the subject's daily activities.

Moderate: Event causes discomfort or inconvenience, possibly requiring additional therapy, and may interfere with the subject's daily activities.

Severe: Event is intolerable, necessitates additional therapy or alteration of therapy and interferes with the subject's daily activities.

12.6 Adverse event reporting

All Serious or Severe adverse events, whether or not considered to be drug related, will be reported immediately to the PI and the Sponsor who will be responsible for reporting it/them to the regulatory authority (MHRA) and the Ethics Committee within 24 hours of learning of its occurrence.. They will be recorded on the appropriate CRF with a description of the outcome. Non-serious moderate or mild adverse events will be recorded in the CRFs but need not be reported to the Sponsor at the time unless there are unusual circumstances (e.g. the adverse event was of a completely unexpected nature); they should be reported to the Safety Monitoring Committee within sufficient time for decisions regarding dose escalation to be made.

<u>Procedures for reporting a serious adverse event:</u>

Any serious adverse event occurring during the study period should be reported < 24 hours of learning of its occurrence to the Sponsor or its representative listed on the personnel page

and recorded on the appropriate case report forms. All patients experiencing a serious adverse event must be followed up and the outcome reported.

Procedures for reporting a suspected unexpected serious adverse reaction (SUSAR)

It is the responsibility of the Sponsor to determine whether a reported SAE fits the classification of a SUSAR and to notify the PI of their decision as soon as possible.

Expedited Reporting of Events

It is the responsibility of the Sponsor to determine whether an event requires expedited reporting and to notify the PI of their decision as soon as possible.
Where expedited reporting is required, the following procedures should be followed.

Fatal or life-threatening SUSARs

It is the responsibility of the Sponsor to report fatal or life-threatening SUSARs to the MHRA and EMA as soon as possible, but no later than 7 calendar days after they first became aware of the reaction.in accordance with Directive.. or per national reg requeirements in particular countries.

The PI is required to notify the EC of any SUSAR as soon as possible, but no later than 7 calendar days after they first became aware of the reaction. Any additional relevant information should be sent within 8 days of the report.

Other SUSARs
It is the responsibility of the Sponsor to report other SUSARs to the MHRA and EMA as soon as possible, but no later than 15 calendar days after they first became aware of the reaction.

The PI is required to notify the EC of any other SUSAR as soon as possible, but no later than 15 calendar days after they first became aware of the reaction.

12.7 Follow-up after Adverse Events

Adverse events which have not resolved by the time of anticipated discharge from the clinical unit will be followed up until resolution. If, in the opinion of the PI, they are sufficiently clinically important to necessitate continued observation in a hospital or clinic setting the Sponsor will be informed and arrangements made for continued in-patient care in a suitable facility or referred to their GP. If the subject is able to be discharged they will be followed up in the same way as other subjects in the study or more frequently at the discretion of the PI. All AEs will be recorded in the appropriate pages of the CRFs until there has been full resolution. Any medications (e.g. analgesics) that may be required at the time of discharge will be supplied. Subjects who are discharged from the clinical unit but who have not had full

resolution of the AE (e.g. a skin rash) will continue their own observations at home and make daily records in the diary card, including any worsening or resolution of the AE. Diary cards on which AEs are recorded will be signed by the subject and the PI and will subsequently become part of the CRF record.

13 STATISTICS

13.1 Statistical Methods

The statistical approach taken will depend on the number of patients recruited.

If less than 4 patients are recruited, each patient will be analysed separately. Changes in the primary efficacy outcome during the period Day 0 to Day 28 will be analysed using linear regression using all measurements in the analysis. Secondary efficacy outcomes measured multiple times will be analysed in an equivalent manner.

If 4 or more patients are recruited, the patients will be analysed as a single group. Differences between specific timepoints (e.g. Day 0 and Day 28) for the primary and secondary efficacy endpoints will be performed using the paired t-test.

Additionally the change in the primary efficacy outcome over time will also be examined using all measurements. Mixed model will be used to allow for repeat measurements from the same patients, with the patient considered as a random effect.

13.2 Number of Subjects

A total of 6 patients may be enrolled under this protocol if they present.

13.3 Significance Level

For all comparisons $p<0.05$ will be considered significant.

13.4 Missing, Unused or Spurious Data

Missing data that cannot be retrieved from source records or other repositories will be recorded as such in the CRFs and will not be entered into the statistical analysis. Spurious data will be examined by the Sponsor's monitor, medical or statistical advisors and a decision made as to how it should be handled. If there is an obvious transcription or data entry error such as a misplaced decimal point in a biochemical parameter this will be discussed with the CRO or the laboratory and, if all parties agree, it will be corrected and endorsed by both the PI and the Sponsor. Outlying data may be treated appropriately after consultation with the Sponsor's statistical advisors (e.g. isolated, out of range biochemical parameters where there may have been a recording error may be treated by removal of highest and lowest observations and re-analysis. In such an event both the fact will be recorded in the statistical report and data will be presented showing the full data set and after removal of paired outliers).

Unused data (e.g. superfluous blood pressure recordings or haematological results in addition to those required by the protocol will remain part of the source documentation and only be incorporated into the trial documents and analysis if there is reason for them to be (e.g. an unexpected fall in blood pressure that might constitute an AE).

13.5 Deviations from the Statistical Plan

It is not envisaged that there should be any deviations from the statistical plan since this is primarily a safety study. Any unexpected deviations (eg a requirement for a trend analysis not foreseen in planning the trial) will be discussed with the PI and the statistical advisor and the rationale for such an analysis will be included in the trial report.

13.6 Subjects to be Included in the Analysis

All patients in the study will be included in the safety analysis whether completing the trial according to the protocol or not. The efficacy analysis will include all measurements made whilst the patient(s) correctly followed the trial protocol.

14 DIRECT ACCESS TO SOURCE DOCUMENTS

The CRO, hospitals and PIs carrying out this study will allow the Sponsor direct access to all source documents and will permit trial-related auditing of clinical, pharmacy and laboratory facilities.

15 QUALITY CONTROL AND QUALITY ASSURANCE

Hospitals/departments taking part in the trial are responsible for maintaining their own SOPs and QA/QC procedures. The Sponsor will also maintain its SOPs, QA and QC procedures and will, at its discretion, carry out a full or partial audit of the trial site facilities and SOPs. The Sponsor will be responsible for monitoring the trial and carrying out full or representative source data verification.

16 ETHICAL CONSIDERATIONS

The application will be reviewed and approved by appropriate Ethics Committees prior to the trial commencing. All ethical aspects of the study will be in accordance with ICH Good Clinical Practice (GCP) guidelines and the requirements of the Declaration of Helsinki.

17 DATA HANDLING, RECORD KEEPING AND SAMPLE COLLECTION

The CRO and hospitals will supply and complete the source documentation and all related documentation (eg ECG traces, laboratory data) and at the conclusion of the trial will transfer these to the CRO who will be responsible for data entry and analysis. The original documentation will be returned to the hospitals for archiving (at least 20 years after completing of the trial) and the Sponsor will also keep duplicates for the same purpose.

Biological samples may only be stored indefinitely for the purpose of additional research related to this protocol if the patient has given informed consent for additional scientific research (directly related to this protocol). If no informed consent was obtained, samples must be destroyed after the patient has completed all protocol treatment and procedures.

Storage of biological samples on site is subject to the site's guidelines. When samples are shipped to another facility (e.g. a central laboratory), they should be stripped from any identifying information and labeled with a code (trialname, patient study number, date and time of collection).

18 FINANCING AND INSURANCE

The Sponsor will have a commercial contract in place with the hospital and the PI and the hospitals/universities who will be responsible for any payments to subjects and for payment of travel and other expenses.

The Sponsor has a clinical trials insurance policy in place, underwritten by AON Limited. A copy of the policy/certificate of insurance will be supplied separately.

19 PUBLICATION POLICY

The trial will be registered with the U.S. National Institutes of Health ClinicalTrials.gov website and the results will be posted there when available.

The PI of the clinical units has independent rights of publication but will agree to discuss any intended publications or presentations with the Sponsor and to allow the Sponsor reasonable time to make comments, file or add to patent applications or request changes to the manuscript.

20.0 SUPPLEMENTS

20.1 CH50 Eq ELISA Assay

Coversin acts as an inhibitor of the terminal common pathway of complement activation by binding to C5 in a 1:1 ratio and preventing its cleavage to C5a and the Membrane Attack Complex proteins. The Quidel CH50 Eq ELISA provides a direct measure of the total complement activity in serum by quantifying the amount of MAC generated under standard conditions. The MAC present in the sample binds to the monoclonal antibodies coating the surface of the microassay wells. The microassay plate is then loaded with a Horseradish Peroxidase (HRP) conjugate which binds to the bound MAC. After washing, the microplate is loaded with a chromogenic enzyme substrate. The chromophore building reaction is then halted by addition of a stop solution.

The amount of chromophore, which will be detected at λ = 405nm, correlates with the concentration of MAC. An increased amount of Coversin in the sample is accompanied by a corresponding decrease in MAC formation which can therefore be detected by a reduced absorption at λ = 405nm.

This analysis will be performed by the Sponsor at the Haemostasis Research Laboratory (see Page 1) and the results will be relayed to the PI and to the CRO for entry into the subject records as soon as they are available.

20.2 Handling of samples for complement (CH50) analysis

1. Equipment

For Blood Collection:
Butterfly Cannula, needles (Abbott), Vacutainer Collection set (Becton Dickinson), luer adaptors, as appropriate. Alternative, equivalent, collection equipment may be used, as available at site.

CH50 Complement Pathway Assays:
5-6ml plain glass, or plastic tubes containing silica clot additive (e.g. Vacutainer Ref 367837, Red tube), or SST type tubes with plastic block (e.g. Vacutainer SST II, Ref 367954, Gold tube), suitable for **serum** preparation, should be used.

Complement Proteins and Complement Activation Markers (e.g. C3, C5, C3a, C5a, SC5b9):
4-5ml EDTA tube (e.g. Vacutainer K2E tubes, Ref 367839, Lavender tubes), for **plasma** preparation.

For Sample Separation:
Plastic Transfer Pipettes - disposable polypropylene pipettes with a bulb at the end (e.g. Elkay 1ml Liquipettes, product No. 127-P511-000).

Intermediate Plastic Tubes for Sample Handling – Polypropylene 75x12mm test tubes capable of withstanding centrifugation and suitable push fit stoppers (e.g. Sarstedt, Cat No. 55.526.005 & 65.719). Alternatively, cryotubes as specified below may also be used as intermediate tubes.

Cryotubes for Sample Storage – 1.8ml screw-cap polypropylene tubes suitable for low temperatures, with an ‘o’ ring in the lid (e.g. Sarstedt Cat. No. 72/694.007, Micro tube SC 2ml SP).

Indelible Marker - Water insoluble permanent marking pen with fine tip, for labelling cryotubes, or suitable, water resistant labels (e.g. Brady THT Freezerbondz B492 25mm x 12.7mm labels).

2. Blood Collection

Blood should be collected from a single, clean venepuncture with minimal stasis, using a 19 or 21 gauge Butterfly needle. Where the Vacutainer system, or other evacuated containers are used, a luer adaptor may be required.
The first tube of blood that is drawn should not be used for markers of complement activation; where appropriate, it is usually convenient to collect a plain tube for CH50 or other tests. The blood for complement activation markers should be collected into an EDTA tube. The anticoagulated blood must be immediately mixed by gentle inversion 5-6 times. The samples should be maintained upright, at room temperature (18-22°C) until centrifugation.

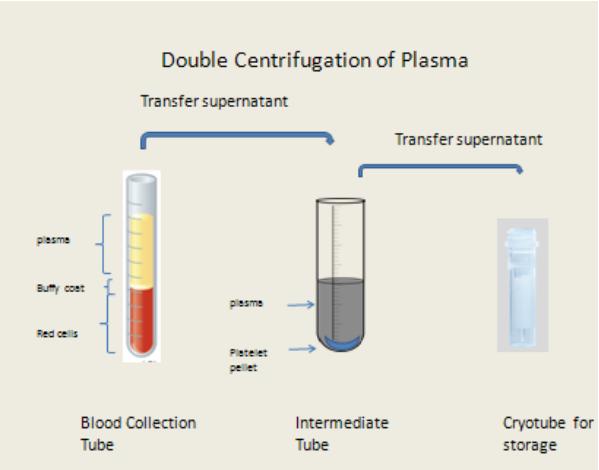
3. Centrifugation and Separation of Serum and Plasma for Complement Tests

Plain Tubes for Serum (CH50 samples):
Maintain at ambient temperature (18-20°C) for 1 hour to allow clot formation. Centrifuge at 1500g for 10 minutes to separate serum. Carefully remove the supernatant serum, avoiding disturbance of any cells, with a polypropylene transfer pipette, and deliver into at least 2 x 0.5ml aliquots using screw-cap cryotubes.

Store at –80°C (temporary storage at –20°C for 24 hours is possible, with subsequent transfer to –80°C, or shipment on dry ice).

EDTA Tubes (Complement Activation samples):
Centrifuge EDTA tubes at 2000g (approximately 3800 rpm in a typical laboratory bench-top centrifuge), at room temperature (18-20°C), for 15 minutes, within 2 hours of collection. Carefully remove the supernatant plasma, avoiding disturbance of the buffy coat, with a plastic transfer pipette, and deliver into intermediate plastic tubes. Repeat the centrifugation step above, to remove any residual platelets. Save at least 2 x 0.5ml aliquots of EDTA plasma in screw-cap cryotubes.

Ensure that the tubes are labelled throughout the separation process and clearly identified during freezing!



4. Storage of Serum and Plasma Samples

The aliquots of serum, EDTA and citrate plasma must be frozen rapidly at below –40°C (dry ice or freezer), and transferred to a -80°C freezer as soon as possible, where they should be maintained until shipping for assay. When placed in the freezer, the samples must be allowed to freeze rapidly, preferably in a metal rack (they must not be placed in polystyrene racks).

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