# C5 polymorphism in a Dutch Patient with Paroxysmal Nocturnal Hemoglobinuria and no Asian Ancestry, Resistant to Eculizumab, but in Vitro Sensitive to Coversin

*Saskia Langemeijer[1], Jun-Ichi Nishimura[2], Wynne Weston-Davies[3], Miles A Nunn[3], Yuzuru Kanakura[2], Ian J Mackie[4], Petra Muus[1]*

[1]Department of Hematology, Radboudumc, Nijmegen, Netherlands; [2]Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Suita, Japan; [3]Akari Pharmaceuticals (UK) Plc, London, United Kingdom;
[4]Haemostasis Research Unit, University College London, London, United Kingdom

## Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is rare acquired bone marrow disease characterized by a clone of cells lacking glycosyl phosphatidylinositol (GPI)-anchored proteins. Deficiency of the GPI-anchored complement inhibitors CD55 and CD59 on erythrocytes leads to intravascular hemolysis upon complement activation. In addition to hemolysis, a high risk of thrombosis is a major problem in PNH patients. Eculizumab (Soliris®) is a humanized monoclonal antibody that targets complement factor C5 and inhibits the production of C5a and formation of the terminal complement membrane attack complex. It is registered for the treatment of PNH and atypical hemolytic uremic syndrome (aHUS). Eculizumab results in significant reduction of hemolysis in PNH, improves symptoms and quality of life and reduces the incidence of PNH related thrombosis. A poor response, defined as sustained high levels of LDH during treatment with Eculizumab irrespective of improvement of clinical symptoms, has been reported in a subgroup of PNH patients (Nishimura *et al*, NEJM 2014;370:632-639). These patients had a genetic variant of C5 which occurs in approximately 3.5% of the Japanese and 1% of the Chinese Han populations and interferes with binding of eculizumab to C5.
We describe the first patient with no known Asian ancestry with a poor response to Eculizumab.

## Patient

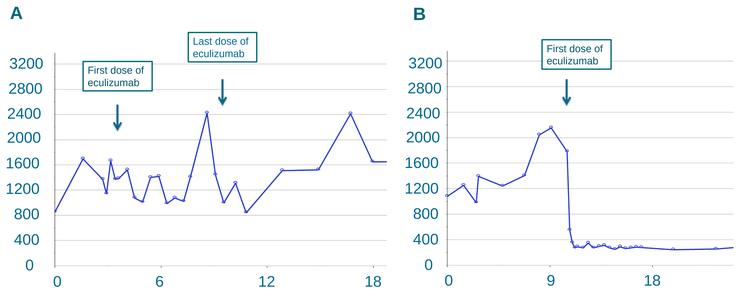
A 30-year old male Caucasian patient commenced treatment with Eculizumab because of PNH (granulocyte clone size: 90%), severe hemolysis (LDH 3-6x upper limit of normal, and peak values of 17xULN), transient renal failure, extreme fatigue and erectile dysfunction. He had no history of thrombosis and no underlying bone marrow disease. During eculizumab treatment (dosed 600 mg iv every 7 days, weeks 1-4 and 900 mg biweekly starting in week 5) he felt better, seemed less fatigued and experienced less erectile dysfunction. However, laboratory examination showed sustained elevated markers of hemolysis (Figure 1). Other causes of hemolysis were excluded. Underdosing of eculizumab was ruled out by demonstrating sustained high LDH levels at different time points in between subsequent eculizumab infusions and by measuring trough levels of eculizumab (>100ug/ml). In vitro terminal complement complex blockage by eculizumab through antibody-coated chicken red blood cell lysis was indicative of ongoing active hemolysis in our patient's serum. The presence of Human Anti-Drug Antibodies was excluded using an illuminescent MSD® assay. Treatment was discontinued when the patient experienced increased hemolysis (LDH 9x ULN) and macroscopic hemoglobinuria one day after receiving a dose of 900 mg eculizumab. As expected, discontinuation did not result in further increase of hemolysis parameters or clinical change.

Contact information

Saskia Langemeijer
Radboud University Medical Centre
Nijmegen, The Netherlands

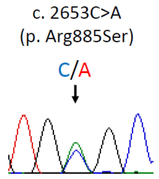
Phone: +31 (0)24 36 14762
Fax: +31 (0)24 35 42080
E-mail: saskia.langemeijer@radboudumc.nl

**Figure 1: LDH levels of index patient (A) and a representative eculizumab-responsive patient (B) before, during and (in index patient) after eculizumab treatment**




Y-axis: LDH level (U/L, ULN 249 U/L)
X-axis: Time (months)

**Figure 2: Sequence analysis of exon 21 of C5 in index patient**

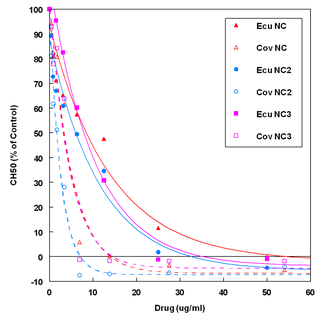



DNA analysis of the coding region of C5 was performed in our index patient. This showed a single C5 heterozygous missense mutation, c.2653C>A, which predicts p.Arg885Ser . The same mutation was demonstrated in the DNA of our patient's healthy father.

**Coversin**

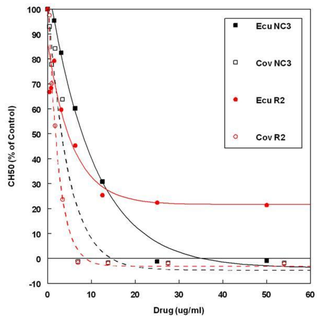
Coversin is a recombinant small protein which is derived from a native protein discovered in the saliva of the *Ornithodoros moubata* tick.
There is a surface active site which binds to the complement C5 molecule with high affinity (Kd 1.85 x $10^{-8}$). Coversin prevents the cleavage of C5 by C5 convertase into C5a and C5b. It is effective in inhibiting terminal complement activity irrespective of the activating pathway. Coversin binds to an epitope on C5 remote from the eculizumab binding site.

**Figure 4: Change in serum complement C5 activity in response to ascending doses of coversin (Cov) and eculizumab (Ecu). NC, NC2, NC3 = normal controls**




Serum samples from 6 healthy controls (3 shown here) were spiked with ascending doses of either eculizumab or coversin and complement activity was measured using a commercially available CH50 Equivalent ELISA (*Quidel Corporation®).*

**Figure 5: Change in serum complement C5 activity in response to ascending doses of coversin (Cov) and eculizumab (Ecu). R2 = patient sample, NC3 = normal control**




Serum samples from a healthy control and our index patient were spiked with ascending doses of either eculizumab or coversin and complement activity was measured using a commercially available CH50 Equivalent ELISA (*Quidel Corporation®).*

## Conclusions

- We describe the first Caucasian patient with no known Asian ancestry who showed resistance to treatment with Eculizumab due to a C5 polymorphism.
- Coversin blocks complement cleavage in vitro by binding to C5 remote of the Eculizumab binding site and blocks complement cleavage in our index patient.
- Coversin may prove a useful alternative to eculizumab for patients with resistance due to C5 polymorphisms.

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