Coversin Blocked in vitro Hemolysis in an Eculizumab-Resistant PNH Patient with the C5 Polymorphism (c.2654G>A)

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Background

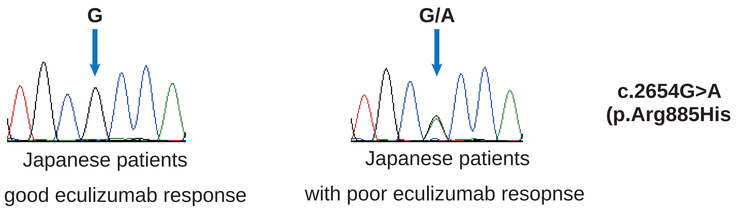
Paroxysmal Nocturnal Hemoglobinurea (PNH) is a rare stem cell disease caused by the expansion of *PIGA* mutated clone(s). PNH-type cells are deficient in the expression of GPI-anchored proteins including DAF and CD59, which protect red blood cells (RBC) from complement-mediated intravascular hemolysis.

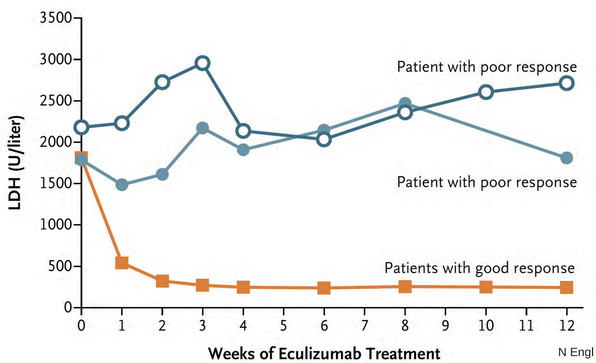
Eculizumab (Soliris®, Alexion Pharmaceuticals) is a humanized monoclonal antibody against C5 which efficiently inhibits hemolysis by blocking the terminal complement cascade. Eculizumab dramatically ameliorates several clinical symptoms, and improves the prognosis in PNH patients. However, among 345 Japanese PNH patients who were treated with eculizumab, 11 patients showed poor response. All the poor responders had a single missense C5 heterozygous mutation, c.2654G>A, which predicts the polymorphism p.Arg885His (Nishimura et al, N Engl J Med. 2014 Feb 13;370(7):632-9) (Fig. 1).
Two of those patients have already passed away due to severe complications related to PNH, and rest of them are still suffered from various clinical symptoms including hemolytic episodes and RBC transfusion. In these circumstances, multiple new anti-complement drugs are under development in Japan.

Coversin (Akari Therapeutics Plc) is a recombinant protein (16,740 Da) derived from a secreted protein in the saliva of the Ornithodoros moubata tick, and it blocks complement-mediated hemolysis at C5 level by binding to the epitope different from the eculizumab binding one (Fig. 2).

In this study, we examined this new anti-complement agent to a PNH patient with C5 polymorphism c.2654G>A, as well as those without the polymorphism.

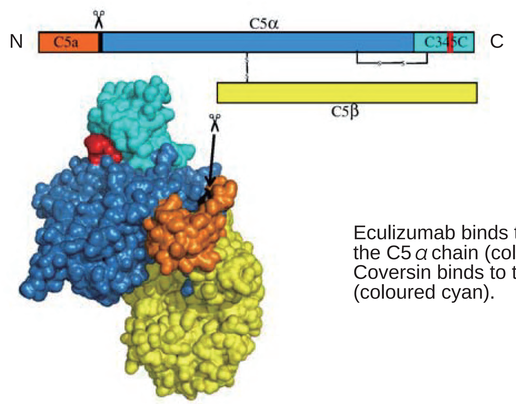
Fig 1 Sequence of C5 variants





N Engl J Med. 2014 Feb 13;370(7):634

Fig. 2 A structural model of C5



Eculizumab binds to the N-terminal region of the C5 α chain (coloured blue), while Coversin binds to the C345C domain (coloured cyan).

J Mol Biol. 2007 Jun 8;369(3):789

Patient

A 41-year-old male with fatigue was diagnosed as aplastic anemia with PNH in 2008, and cyclosporine (CyA) was initiated at the dose of 150mg/day. The PNH clone expanded from 30.6% to 70.2% in granulocytes from 2008 to 2011 with elevated LDH (700 U/L) and the patient was referred to our hospital to undergo eculizumab treatment. CyA was reduced to 100mg/day and eculizumab was initiated in May 2012. Eculizumab treatment did not change the serum LDH level without any improvement of the symptoms: fatigue, abdominal pain, and periodical hemoglobinurea. A heterozygous mutation c.2654G>A was identified as the cause of the failure to eculizumab treatment, and he is still suffered from continuous intravascular hemolysis (LDH > 1,400 U/L) with periodical acute hemolytic episodes, requiring frequent RBC transfusion.

Method

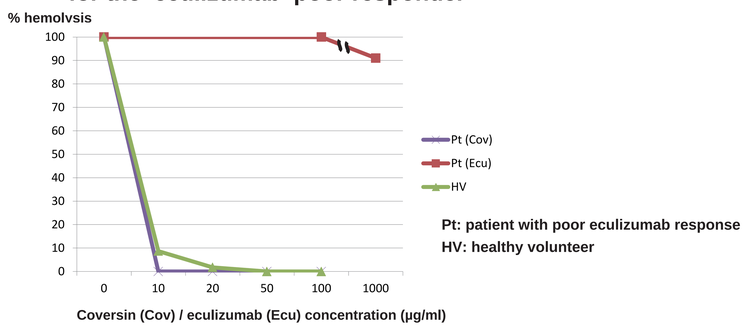
Materials:
Peripheral blood samples were collected from a poor responder to eculizumab, hemolytic PNH patients and healthy volunteers with written informed consent as approved by the Institutional Review Board of Osaka University Hospital.

In vitro hemolytic assay:
RBC from ABO-matched PNH patients off eculizumab treatment were washed 3 times in saline, and subsequently incubated with Mg2+ supplemented serum of the poor responder in the presence or absence of an anti-complement agent. Alternative pathway was activated by adding HCl (22:1 of 0.4M HCl) to the serum. Heat-inactivated (56°C for 30min) serum was used as a negative control. After a 24-hour incubation at 37°C, hemolysis was quantified by measuring the optical density at 405nm (OD405). The hemolytic activity was normalized against maximum hemolysis as induced by HCl (100%) and minimum hemolysis with inactivated acidified serum (0%).

Results

In the hemolytic assay, Coversin completely blocked hemolysis at the concentration of 10ug/ml, similar to the effective inhibition with hemolytic PNH patients without the polymorphisms or healthy volunteers.

Fig. 3 Representative data of in vitro hemolytic assay for the eculizumab poor responder



Discussion

Eculizumab has dramatically improved the quality-of-life in the majority of the PNH patients by blocking intravascular hemolysis, but there are still some concerns; poor response due to C5 polymorphisms, C3b deposition on the RBC, high cost and burden for scheduled infusion. Blocking the complement cascade at C5 level has shown to be relatively safe if meningococcal vaccination is properly performed, but still an extravascular hemolysis remains problematic at least in some cases. Inhibiting C3 amplification would resolve both intra and extravascular hemolysis, but susceptibility to infections remains a major concern.
Our study showed that Coversin efficiently blocked in vitro hemolysis in the eculizumab resistant patient with C5 heterozygous mutation, c.2654G>A. Coversin may be a therapeutic option for the population of C5 polymorphism c.2654G>A in PNH patients. Our results warrant further investigation to explore new anti-complement agents for hemolytic PNH patients.

Conclusions

- Coversin efficiently blocked in vitro hemolysis in the eculizumab resistant patient with C5 heterozygous mutation, c.2654G>A.

- Our results warrant further investigation of new anti-complement agents targeting different epitops of C5 from eculizumab or another factor in the complement cascade for treating PNH patients with poor response to eculizumab.

Conflicts of interests

Yasutaka Ueda receives research funding from Alexion Pharma.
Makiko Osato receives research funding from Alexion Pharma.
Wynne Weston-Davies is an employee of Akari Therapeutics Plc and owns shares in the company.
Miles A Nunn is an employee of Akari Therapeutics Plc and owns shares in the company.
Satoru Hayashi receives research funding from Alexion Phama.
Jun-ichi Nishimura is a member of advisory committees of Alexion Pharma and receives research funding and honoraria from Alexion Phama.
Yuzuru Kanakura is a member of advisory committees of Alexion Pharma and receives research funding and honoraria from Alexion Phama.

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