Successful treatment of a PNH patient non-responsive to eculizumab with the novel complement C5 inhibitor coversin (nomacopan)

Saskia Schols[1], Miles A Nunn[2], Ian Mackie[3], Wynne Weston-Davies[2], Jun-ichi Nishimura[4], Yuzuru Kanakura[4], Nicole Blijlevens[1], Petra Muus[1,5] & Saskia Langemeijer[1]

[1]Department of Hematology, Radboudumc, Nijmegen, The Netherlands,

[2]Akari Therapeutics, London, United Kingdom,

[3]Haemostasis Research unit, Department of Haematology, University College London, London, United Kingdom,

[4]Department of Haematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan

[5]King's College Hospital, Department of Haematology, London, UK

Corresponding author:

Saskia Langemeijer, Department of Hematology, Radboudumc, Geert Grooteplein Zuid 10, 6525GA, Nijmegen, The Netherlands. Saskia.langemeijer@radboudumc.nl

Running title

Coversin (Nomacopan) in paroxysmal nocturnal haemoglobinuria

Summary

Paroxysmal Nocturnal Haemoglobinuria (PNH) is an acquired bone marrow disease characterized by haemolysis, thrombosis and bone marrow failure. The complement C5 inhibitor eculizumab reduces haemolysis, improves symptoms and decreases the risk of PNH-related complications. Failure to respond to eculizumab has been reported in patients with an Asian ancestry who have a polymorphism at p.Arg885His in their C5 protein. This polymorphism interferes with the binding of eculizumab to C5. We describe a different genetic variant of C5 (p.Arg885Ser) in a PNH patient with no Asian ancestry and report on the efficacy, safety, pharmacokinetics and dynamics of treatment with the novel subcutaneous complement inhibitor coversin (nomacopan)

Keywords

Paroxysmal nocturnal haemoglobinuria, complement, coversin, nomacopan, haemolysis, thrombosis

Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare acquired life-threatening disease characterized by complement induced haemolysis and a high incidence of thrombosis. The monoclonal antibody eculizumab binds to complement C5 and prevents its activation and cleavage into C5a and C5b. Treatment of PNH patients with eculizumab decreases haemolysis, transfusion requirements and the risk of thrombosis(Brodsky*, et al* 2008, Hillmen*, et al* 2004, Hillmen*, et al* 2007, Hillmen*, et al* 2006, Kelly*, et al* 2011)**.** Unusually, failure to respond to eculizumab has been reported in a subgroup of Asian PNH patients(Nishimura*, et al* 2014). These patients had a C5 polymorphism which occurs in approximately 3.5% of Japanese and 1% of Chinese Han populations. The polymorphism interferes with binding of eculizumab to C5(Nishimura*, et al* 2014). For PNH patients with this polymorphism there is no effective treatment. A new small (17 kDa) protein complement inhibitor named coversin is now in Phase 2 clinical development. Like eculizumab, coversin prevents cleavage and activation of C5, but it binds C5 at a different site (Jore*, et al* 2016). Administration to healthy volunteers proved safe and demonstrated inhibition of terminal complement activation. We report safety, efficacy, pharmacokinetic and pharmacodynamic data from the first ever PNH patient treated with coversin.

The patient, a 30-year old Caucasian man (BMI 21 kg/m^2) with a PNH granulocyte clone of 96%, suffered from severe haemolysis. LDH and hemoglobin levels since presentation in our center are shown in Figure 2a. There were several episodes of very severe hemolysis with LDH levels >2500 U/L. Three episodes were accompanied by a transient deterioration in kidney function. During one episode (LDH 2813 U/L) eGFR decreased to 24 ml/min. Main symptoms were extreme fatigue and muscle dystonia. He had no history of thrombosis, no prior transfusions and no underlying aplastic anemia or myelodysplastic syndrome. He started treatment with eculizumab 4 years after diagnosis. Unexpectedly, he remained severely haemolytic despite adequate trough drug levels and no antidrug antibodies. Other causes of haemolysis were excluded. Treatment was discontinued when, several months into treatment, the patient experienced increased haemolysis (LDH 9x ULN) and macroscopic haemoglobinuria one day after receiving 900 mg eculizumab. DNA analysis of the coding region of C5 showed a heterozygous genetic variant in C5, c.2653C>A, which predicts p.Arg885Ser (**Fig 1a**). The amino acid substitution is different from that described previously, a missense mutation leading to c.2654G>A(Nishimura*, et al* 2014), but occurs at the same amino acid of the C5 protein. The same genetic variant was demonstrated in the DNA of the patient's healthy father and not in his healthy mother. *In vitro* studies confirmed a diminished response to eculizumab (measured by inhibition of CH50) compared to a normal control (NC), whereas coversin was equally effective in the patient and normal control, reducing CH50 to less than 8 U Eq/ml (**Fig 1b**)(the lower limit of quantification).

The AK578 study (ClinicalTrials.gov, NCT02591862) was an open label Phase 2 study to investigate the safety and efficacy of coversin in PNH patients with a genetic variant of C5 rendering them unresponsive to treatment with Eculizumab (**supplementary methods**). The study was approved by the independent ethics committee of the Radboudumc and was conducted in agreement with Good Clinical Practice guidelines and according to the Declaration of Helsinki.
Coversin was administered by subcutaneous injection at an ablating dose of 0.57 mg/kg/day on day 1, followed by a maintenance dose of 0.14 mg/kg per day thereafter. Protocol defined blood samples were drawn for CH50 activity, free Coversin levels and anti-drug antibodies (ADA). The primary efficacy endpoint was reduction in serum LDH from day 0 (pre-dose) to day 28. Secondary endpoints were haemoglobin at day 28, 90 and 180 compared to baseline, LDH at day 90 and 180, haptoglobin level at day 28, 90 and 180 compared to baseline, transfusion independency and quality of life

assessed by the FACIT and EORTC QL-C30 instruments on days 0, 28, 90 and 180. Haemoglobin and LDH levels before start of coversin are shown in Figure 2a. The AK578 was designed to include a small number of PNH patients with a genetic variant in C5. When the inclusion period closed, the patient described here was the only participant in this trial.

The patient started with the ablating dose of 0.57 mg/kg/day . There was a good initial response with CH50 decreasing from 100 U Eq/ml to <8 U Eq/ml (**Fig 2b**). He continued with the maintenance dose of 0.14 mg/kg every 24 hours. Clinical symptoms and laboratory markers of haemolysis improved during the first 5 days of treatment. However, on day 6, the patient experienced haemolysis-associated symptoms with dark urine, hours before the next injection, and no further decrease of his LDH occurred (**Fig 2b**). This was accompanied by an increase in CH50 and decrease in free coversin (**Fig 2c and 2d**). According to the protocol the dose was doubled to 0.29 mg/kg/day (a total once daily dose of 19 mg). However, this dose was not sufficient to cover the 24 hr interval between injections. Therefore a dose of 0.14 mg/kg every 12 hours was used which provided long-term stable complement inhibition with CH50 levels <8 U Eq/ml and trough coversin levels of 25-78 ng/mL (**Fig 2**). LDH at day 28 was 474 U/L (ULN 250 U/L) compared to 1400 U/L immediately before the first dose of coversin. LDH levels at day 90 and 180 were 404 and 373 U/l respectively. Haemoglobin remained stable (12.6 g/dL on day 0 and 12.1, 12.6 and 12.9 g/dL after 28, 90 and 180 days). The relatively high haemoglobin level in this patient, even before start of coversin, is most likely caused by the high number of reticulocytes. Reticulocyte counts did not significantly decrease after start of coversin (Supplemental Fig 1). This may be caused by extravascular haemolysis as is seen more often in patients treated with a complement C5 inhibitor. Clinical symptoms improved substantially with only mild complaints of fatigue remaining. His quality of life improved (data not shown). Treatment was well tolerated. The patient once had a transient skin reaction grade 2 at the injection site. Adverse events were CTCAE grade 1 otherwise. (**Supplementary Table S1**). ADA were detected from day 15 onwards but did not result in decreased levels of free coversin or loss of complement inhibition (Fig 2).

Recently, treatment with coversin was shown to suppress complement activity in the setting of post HSCT thrombotic microangiopathy in a 1- year old patient suffering from HSCT associated TMA. This patient carried the c.2654 G>A (p.Arg885His) polymorphism in C5, rendering him resistant to eculizumab treatment(Goodship*, et al* 2017). Our PNH patient has been stable for more than 1,5 years (**Fig 2**) and continued treatment with coversin in the AK580 extension study (ClinicalTrial.gov, NCT03588026). After 2 years, he experienced one transient episode of break through hemolysis during an influenza B infection for which no additional coversin administration was necessary. Based on our results we conclude that coversin may be a useful s.c. therapy for PNH patients who are resistant to eculizumab therapy due to genetic variants of C5.

Author contributions:

S.S. performed research, analyzed data and wrote the manuscript. M. N. performed research, analyzed data and contributed vital analytical tools. I. M performed research, analyzed data and contributed vital analytical tools. W.W. designed research. J.N. designed and performed research and analyzed data. Y.K designed and performed research and analyzed data. N.B. designed research. P.M

designed and performed research, analyzed data and wrote the manuscript. S.L. designed and performed research, analyzed data and wrote the manuscript.

Conflict of interest:

S.S, N.B. and S.L. report no conflicts of interests. M. N. and W.W. are employed by Akari Therapeutics. I.M. has received research funding from Akari Therapeutics. P.M. has received travel support and fees for consultancy from Akari Therapeutics. Y.K. and J.N. have participated in advisory committee meetings for Alexion Pharma.

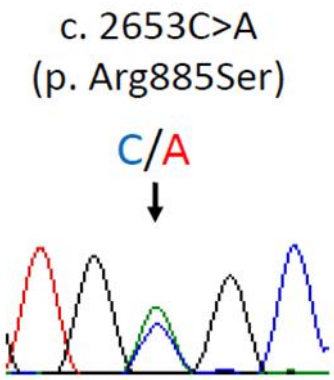
Footnotes

P.M. and S.L. contributed equally to this study

Figure 1: C5 mutation detection in index patient and in vitro sensitivity to eculizumab and coversin

a. DNA analysis of the coding region of C5 showed a single C5 heterozygous polymorphism, c.2653C>A, which predicts p.Arg885Ser. **b**. Serum samples from a normal control (NC) and our index patient were spiked with ascending doses of either eculizumab or coversin and terminal complement activity was measured using a commercially available CH50 Equivalent ELISA (Quidel Corporation®).

a.



b.

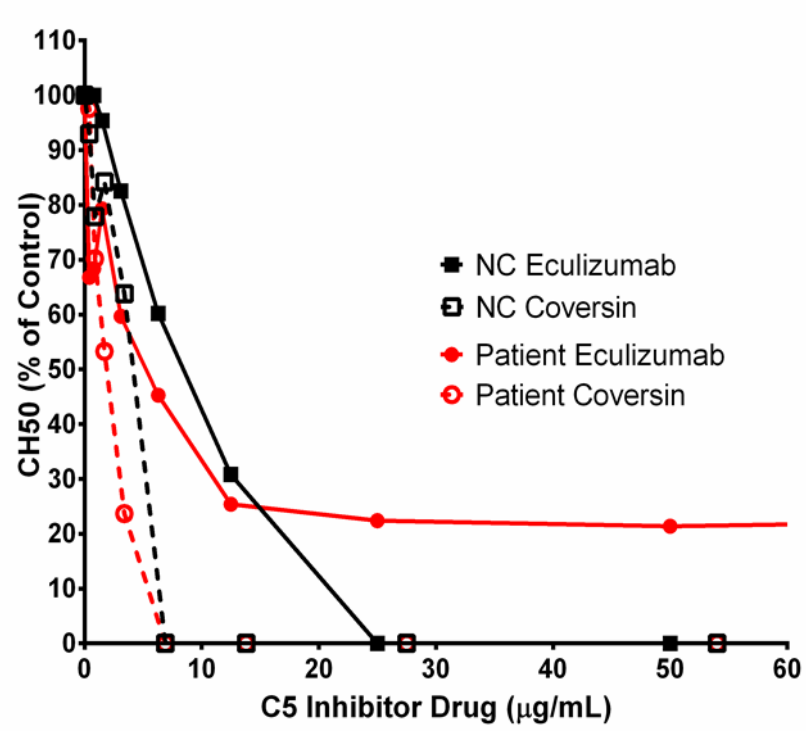
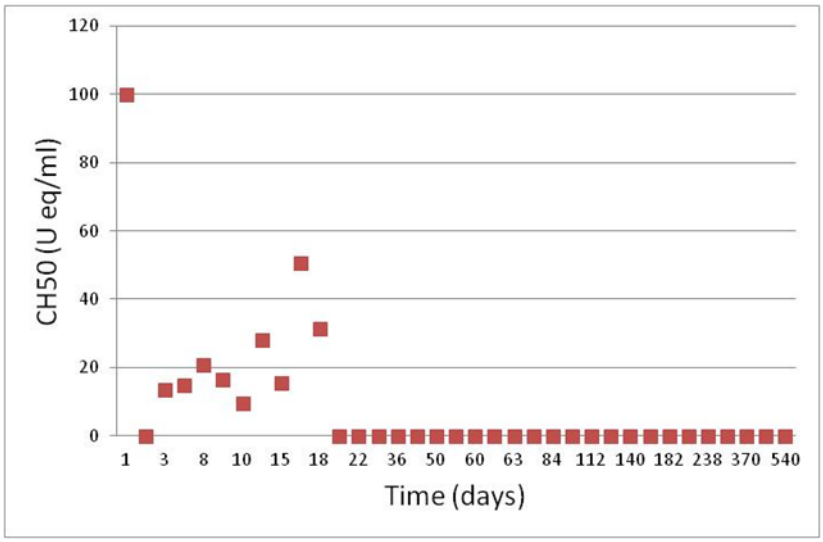


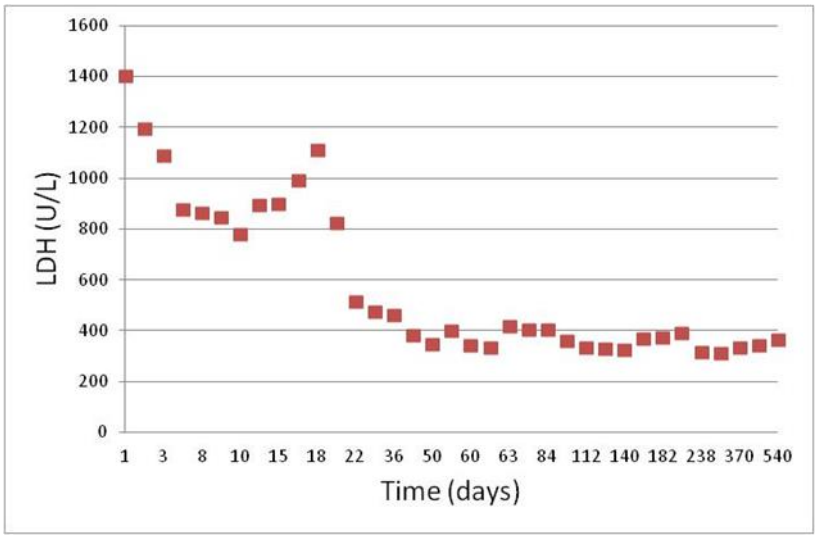
Figure 2: Response to treatment with eculizumab and coversin in index patient

LDH (ULN 250 U/L) and Hemoglobin levels before, during and after eculizumab treatment (**a**), LDH and hemoglobin levels after start of coversin (**b**), Terminal complement activity measured by CH50 after start of coversin (lower limit of quantification 8 U Eq/mL) (**c**), and free coversin levels (**d**) since start of treatment with coversin. Dosing schedule as described in the results section: *Day 1:* 0.57 mg/kg single dose, *Day 2-7*: 0.14 mg/kg once daily, *Day 8-17*: 0.29 mg/kg once daily, *day 18 onwards* 0.14 mg/kg twice daily. The treatment is ongoing. Note, the x-axes in these plots are non-linear and have been presented this way to illustrate the response to Coversin over the first few weeks, while the efficacious dose and dose interval for this patient was established, and during maintenance therapy.

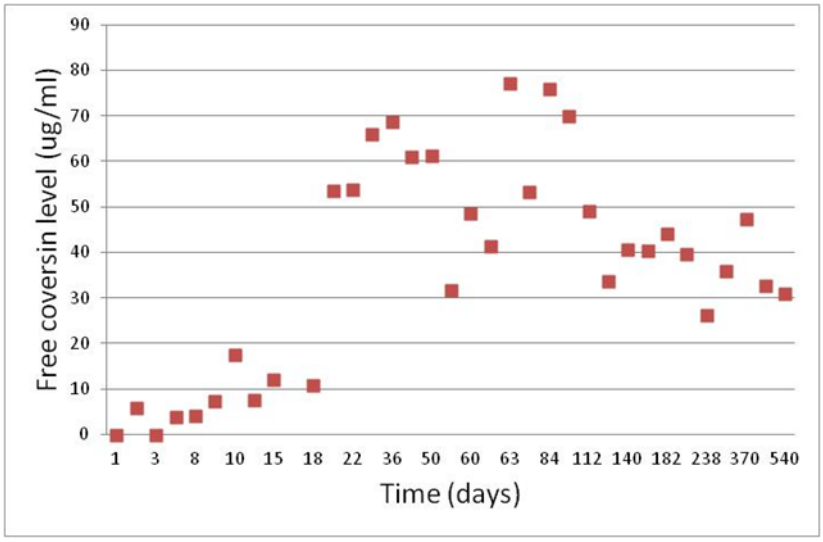
a.



b.



c.


Free coversin level (ug/ml)
90
80
70
60
50
40
30
20
10
0
1 3 8 10 15 18 22 36 50 60 63 84 112 140 182 238 370 540
Time (days)

References

Brodsky, R.A., Young, N.S., Antonioli, E., Risitano, A.M., Schrezenmeier, H., Schubert, J., Gaya, A., Coyle, L., de Castro, C., Fu, C.L., Maciejewski, J.P., Bessler, M., Kroon, H.A., Rother, R.P. & Hillmen, P. (2008) Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood,* **111,** 1840-1847.

Goodship, T.H.J., Pinto, F., Weston-Davies, W.H., Silva, J., Nishimura, J.I., Nunn, M.A., Mackie, I., Machin, S.J., Palm, L., Pryce, J.W., Chiesa, R., Amrolia, P. & Veys, P. (2017) Use of the complement inhibitor Coversin to treat HSCT-associated TMA. *Blood Adv,* **1,** 1254-1258.

Hillmen, P., Hall, C., Marsh, J.C., Elebute, M., Bombara, M.P., Petro, B.E., Cullen, M.J., Richards, S.J., Rollins, S.A., Mojcik, C.F. & Rother, R.P. (2004) Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med,* **350,** 552-559.

Hillmen, P., Muus, P., Duhrsen, U., Risitano, A.M., Schubert, J., Luzzatto, L., Schrezenmeier, H., Szer, J., Brodsky, R.A., Hill, A., Socie, G., Bessler, M., Rollins, S.A., Bell, L., Rother, R.P. & Young, N.S. (2007) Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood,* **110,** 4123-4128.

Hillmen, P., Young, N.S., Schubert, J., Brodsky, R.A., Socie, G., Muus, P., Roth, A., Szer, J., Elebute, M.O., Nakamura, R., Browne, P., Risitano, A.M., Hill, A., Schrezenmeier, H., Fu, C.L., Maciejewski, J., Rollins, S.A., Mojcik, C.F., Rother, R.P. & Luzzatto, L. (2006) The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med,* **355,** 1233-1243.

Jore, M.M., Johnson, S., Sheppard, D., Barber, N.M., Li, Y.I., Nunn, M.A., Elmlund, H. & Lea, S.M. (2016) Structural basis for therapeutic inhibition of complement C5. *Nat Struct Mol Biol,* **23,** 378-386.

Kelly, R.J., Hill, A., Arnold, L.M., Brooksbank, G.L., Richards, S.J., Cullen, M., Mitchell, L.D., Cohen, D.R., Gregory, W.M. & Hillmen, P. (2011) Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood,* **117,** 6786-6792.

Nishimura, J., Yamamoto, M., Hayashi, S., Ohyashiki, K., Ando, K., Brodsky, A.L., Noji, H., Kitamura, K., Eto, T., Takahashi, T., Masuko, M., Matsumoto, T., Wano, Y., Shichishima, T., Shibayama, H., Hase, M., Li, L., Johnson, K., Lazarowski, A., Tamburini, P., Inazawa, J., Kinoshita, T. & Kanakura, Y. (2014) Genetic variants in C5 and poor response to eculizumab. *N Engl J Med,* **370,** 632-639.