Pozelimab, a Human Antibody Against Complement Factor C5, Demonstrates Robust Inhibition of Alternative Complement Activity Both in Normal Human Serum and in Phase I Normal Healthy Volunteers

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Background and Introduction

- Blockade of complement factor C5 has demonstrated benefit in paroxysmal nocturnal hemoglobinuria,[1] atypical hemolytic uremic syndrome,[2] generalized myasthenia gravis,[3] and neuromyelitis optica.[4]
- We have completed a phase I study of pozelimab, a fully human anti-C5 immunoglobulin G4 (IgG4), in healthy volunteers (NCT03115996).[5]
  - Pozelimab was well tolerated and resulted in dose-dependent inhibition of hemolytic activity through the classical complement pathway in normal healthy volunteers.[5]
  - Complete inhibition of classical pathway hemolytic activity was maintained over a 4-week dosing period by a weekly subcutaneous (SC) regimen following an intravenous (IV) loading dose.[5]

Objectives

- To further characterize the impact of pozelimab on the activity of the alternative complement pathway, we investigated the effect of pozelimab on alternative pathway-mediated hemolysis using an AH50 assay in the completed first-in-human (FIH) study.[5]
- In addition, we compared the effect of pozelimab in both alternative and classical pathway hemolysis assays with those of in-house eculizumab and in-house ravulizumab in pooled normal human serum (NHS) samples, *ex vivo*.

Methods

- In total, 56 subjects were randomized (42 received pozelimab; 14 received placebo) to 4 sequential ascending IV single-dose cohorts plus 2 sequential ascending SC single-dose cohorts followed by 1 multiple-dose cohort (consisting of an IV loading dose and weekly SC doses).
- Each cohort consisted of 8 subjects randomized to receive pozelimab or placebo (6 active; 2 placebo). Serum collected at multiple time-points was used to assess the effect of pozelimab on alternative pathway activity.
- In the FIH study, the alternative pathway (AP) and classical pathway (CP) hemolysis assays were performed based on lysis of rabbit red blood cells (RBCs) and sensitized sheep RBCs, respectively; both assays measure the amount of hemoglobin released from RBCs at 412 nm.
- The pharmacodynamic analysis set included all treated subjects who received any study drug and who had at least 1 non-missing analyte measurement following the first dose of study drug.
- For *ex vivo* spike experiments, pooled NHS was used to compare the hemolytic function of pozelimab, in-house eculizumab and in-house ravulizumab (in-house eculizumab and in-house ravulizumab were synthesized from published sequences).
  - Pozelimab, in-house eculizumab, and in-house ravulizumab were spiked into 10, 25, or 48% pooled NHS for AP hemolysis assays, and into 5, 10, or 25% pooled NHS for CP hemolysis assays.
  - The effect of magnesium concentration (0, 1, 2, and 4 mM $MgCl_2$) on AP hemolysis assays was conducted in 10% NHS.

Results

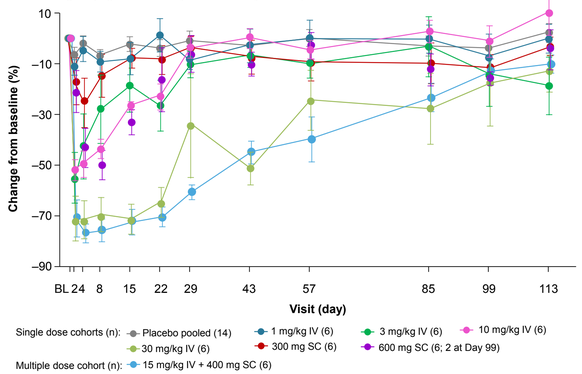
- Baseline characteristics of subjects in the FIH study are summarized in **Table 1** according to treatment group.
- In the FIH study, baseline AH50 was comparable across treatment groups, with a mean ± standard deviation (SD) of 110 ± 19 U/mL (n=56).
- Pozelimab exposure led to dose-dependent inhibition of AH50 (**Figure 1**). In all 4 IV dosing cohorts, peak suppression of hemolysis was observed at the end of the infusion.
- Maximal suppression of hemolysis was approximately –85% change from baseline. This was achieved with the 30 mg/kg IV group and the repeat-dose 15 mg/kg IV + 400 mg SC once weekly group. In the 2 SC cohorts, peak suppression of hemolysis was observed 3–7 days post dosing, which was consistent with observed peak concentrations of pozelimab in serum.

Table 1. Baseline characteristics (safety analysis set)

		Pozelimab						
	Placebo[a] (n=14)	1 mg/kg IV (n=6)	3 mg/kg IV (n=6)	10 mg/kg IV (n=6)	30 mg/kg IV (n=6)	300 mg SC (n=6)	600 mg SC (n=6)	15 mg/kg + 400 mg SC[b] (n=6)
Age, years, mean (SD)	36.5 (8.9)	35.5 (7.1)	36.7 (10.7)	39.3 (12.1)	35.3 (12.3)	24.5 (4.1)	32.5 (9.1)	40.0 (6.8)
Male, n (%)	9 (64.3)	2 (33.3)	3 (50.0)	3 (50.0)	1 (16.7)	3 (50.0)	2 (33.3)	2 (33.3)
Race, n (%)								
White	12 (85.7)	4 (66.7)	4 (66.7)	5 (83.3)	6 (100)	5 (83.3)	4 (66.7)	5 (83.3)
Black or African American	2 (14.3)	1 (16.7)	1 (16.7)	1 (16.7)	0	0	1 (16.7)	0
Asian	0	0	0	0	0	1 (16.7)	1 (16.7)	1 (16.7)
Other	0	1 (16.7)	1 (16.7)	0	0	0	0	0
Weight, kg, mean (SD)	73.9 (10.7)	69.9 (16.8)	67.8 (8.6)	68.8 (12.7)	64.5 (3.1)	74.7 (11.6)	75.5 (21.1)	71.2 (6.7)

[a]Pool of all administration types. [b]Multiple dose study drug administration given as single dose of 15 mg/kg IV + 400 mg SC once weekly for 4 weeks. IV, intravenous; SC, subcutaneous; SD, standard deviation.

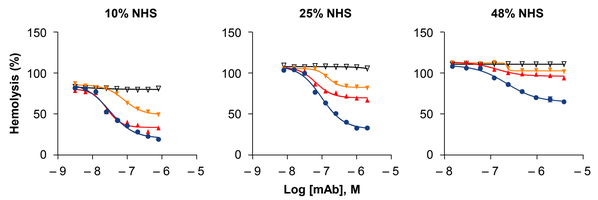
Figure 1. Mean (± SE) percentage change from baseline in AH50 versus nominal time by treatment group (pharmacodynamic analysis set)



BL, baseline; IV, intravenous; SC, subcutaneous; SE, standard error.

- The *ex vivo* AP hemolysis assays showed that, for a given concentration of spiked antibody, the maximal suppression of hemolysis for all the antibodies decreased with increased percentage of serum (**Figure 2**).
- The maximal suppression of hemolysis was consistently greater (32–169%) for pozelimab than for either in-house eculizumab or in-house ravulizumab. In-house ravulizumab had less suppression than pozelimab and in-house eculizumab at all serum percentages tested (**Figure 2**).
- Increasing magnesium concentration decreased the percentage maximum suppression of AP hemolysis for in-house eculizumab and in-house ravulizumab, but not for pozelimab (**Figure 3**).
- The results from CP hemolysis assays showed that, although the maximal suppression of hemolysis was similar across all NHS concentrations for all antibodies tested, in-house ravulizumab was required to be at least a log higher in concentration to achieve a similar effect as the other 2 anti-C5 antibodies (**Figure 4**).
- The binding kinetics of all 3 anti-C5 antibodies are presented in **Table 2**.

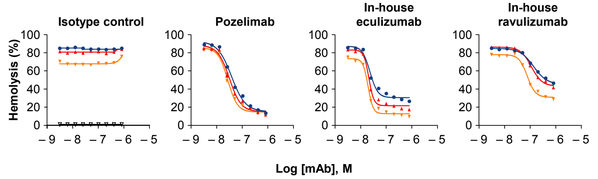
Figure 2. Maximum suppression of hemolysis with pozelimab, in-house eculizumab, and in-house ravulizumab in AP hemolysis assays in 10, 25, or 48% NHS



Antibody	Maximum % suppression		
	10%	25%	48%
Pozelimab	77.06	70.81	41.17
In-house eculizumab	58.39	35.15	15.29
In-house ravulizumab	42.95	21.86	8.46
Isotype control	N/A	N/A	N/A

AP, alternative pathway; mAb, monoclonal antibody; NHS, normal human serum

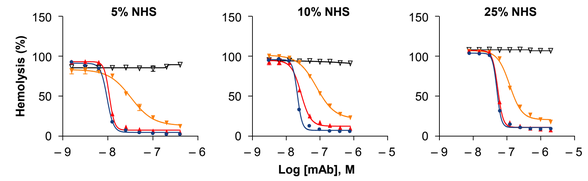
Figure 3. Effect of increasing magnesium concentration on maximum suppression of hemolysis with pozelimab, in-house eculizumab, and in-house ravulizumab in AP hemolysis assays in 10% NHS



Antibody	Maximum % suppression		
	1 mM $MgCl_2$	1.5 mM $MgCl_2$	2 mM $MgCl_2$
Pozelimab	82.58	82.75	83.40
In-house eculizumab	82.61	74.06	64.93
In-house ravulizumab	60.14	49.71	46.52
Isotype control	N/A	N/A	N/A

AP, alternative pathway; mAb, monoclonal antibody; NHS, normal human serum.

Figure 4. Maximum suppression of hemolysis with pozelimab, in-house eculizumab, and in-house ravulizumab in CP hemolysis assays in 5, 10, or 25% NHS



Antibody	Maximum % suppression		
	5%	10%	25%
Pozelimab	95.05	92.83	89.24
In-house eculizumab	92.23	87.15	89.31
In-house ravulizumab	84.41	78.84	80.45
Isotype control	N/A	N/A	N/A

CP, classical pathway; mAb, monoclonal antibody; NHS, normal human serum.

Table 2. Binding kinetics of pozelimab, in-house eculizumab, and in-house ravulizumab with C5

	25°C		37°C	
Antibody	K_D (M)	$t_{1/2}$ (min)	K_D (M)	$t_{1/2}$ (min)
Pozelimab	1.37E-10	167	3.50E-10	58
In-house eculizumab	2.73E-10	226	8.14E-10	57
In-house ravulizumab	1.07E-08	8	5.52E-09	6
Isotype control	–	–	–	–

K_D, dissociation constant; $t_{1/2}$, half-life.

Conclusions

- The phase I healthy volunteer study of pozelimab demonstrated dose-dependent and significant inhibition of AP hemolysis, with the maximal suppression of hemolysis approximately –85% change from baseline.
- *Ex vivo* studies with pooled NHS demonstrate that pozelimab robustly blocks both CP and AP hemolysis.
- In-house ravulizumab appeared to be less effective than in-house eculizumab in both CP and AP hemolysis assays.
- Although pozelimab and in-house eculizumab demonstrated similar effectiveness in CP hemolysis assays, pozelimab showed greater maximal suppression in AP hemolysis assays.
- As expected, all 3 antibodies provided stoichiometric inhibition of CP hemolysis; however, stoichiometric inhibition was not observed for AP hemolysis.

References

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Disclosures

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