In Vitro Combination Studies of ACH-4471 with Eculizumab to Assess a Potential “Switch” Treatment Approach for Paroxysmal Nocturnal Hemoglobininuria

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INTRODUCTION

• ACH-4471 is an oral complement factor D (CFD) inhibitor that has that has been shown to provide clinically meaningful improvement in an ongoing phase 2 study in naïve patients suffering from paroxysmal nocturnal hemoglobinuria (PNH). ACH-4471 blocks both fluid and solid phase C3 conversion.

• PNH is a rare clonal blood disorder in which erythrocytes lacking the GPI-anchored complement regulators CD55 and CD59 are susceptible to destruction by MAC following the normal low-level activation of the complement alternative pathway (AP) in the fluid phase. The therapeutically agent eculizumab, a monoclonal antibody directed against the terminal complement component C5, blocks MAC assembly on erythrocyte membranes and so prevents intravascular hemolytic destruction.

• Eculizumab treatment however leads to increased deposition of complement C3 fragments on PNH membranes, which can result in extravascular phagocytic elimination of osmotic erythrocytes and incomplete inhibition of intravascular hemolysis.1, and consequently to the continued anemia and transfusion dependence observed in a significant subset of patients.

• As an AP inhibitor, in contrast to eculizumab, ACH-4471 prevents both MAC assembly and C3 fragment deposition on PNH cell surfaces and therefore is expected to address the medical needs of these suboptimal responders to eculizumab.

• In the present study, we conducted in vitro combination experiments to explore the potential pharmacological consequences when ACH-4471 is added on to eculizumab, using functional AP assays that included hemolysis and C3 fragment deposition on erythrocytes from a PNH patient.

METHODS

• Concentration series of ACH-4471 were assessed in pairwise combinations with eculizumab. Functional assay for human serum AP activity was done by hemolysis of red blood cells from PNH Patient A (female, 52 years old, blood type B, clone size 88%) using ABO blood group-compatible serum (20%) under conditions of robust AP-specific activation (GVP + MEGESTA, pH 7.3). Analyses of interactions were performed using a three-dimensional surface graph method.2

• Serum-mediated C3 fragment deposition on erythrocytes from PNH patient A was assessed with ACH-4471 alone and in combination with eculizumab. Physiologically relevant conditions were defined as 5 min pre-incubation of serum with inhibitor, 72% ABO blood group-compatible serum, 5×10^6/mL erythrocytes from PNH patient A, GVB buffer, 37°C for 1 h, EDTA termination. Hemolysis was assessed from A_540 of supernatants following centrifugation. C3 fragment deposition on intact and fragmented cells was assessed by flow cytometry using FITC-conjugated anti-C3 (Abcam Ab4322, 1:200), PE-conjugated anti-C4D (R&D Systems FAB8470P, 1:50), and APC-conjugated anti-CD59 (Abcam Ab187769, 1:200 dilution) following dilution of reaction mixtures in FC buffer (PBS + 15 mM EDTA, 1% BSA). After incubation at room temperature for 30 min, samples were diluted to final 1:20 in FC buffer and examined by flow cytometry (BD Accuri C6) with a Fc-H+20,000 threshold. Intact and fragmented PNH erythrocytes were identified by anti-C4D (positive) and anti-CD59 (negative) staining; intact and fragmented cells were distinguished from each other by size (FSC-A); C3 fragment deposition was assessed by anti-C3c staining.

RESULTS

Inhibition of PNH Erythrocyte Hemolysis Under High AP Activation

- ACH-4471 and eculizumab showed strong synergy and no antagonism in the inhibition of AP-mediated hemolysis of erythrocytes from a PNH patient.
- Eculizumab alone showed incomplete inhibition under conditions of high AP activation, likely due to the high density of C3b deposits.
- Addition of ACH-4471 reduces the density of C3b deposits and enables eculizumab to inhibit completely, accounting for the observed synergy.

Inhibition of C3 Fragment Deposition on PNH Cells Under Physiological Conditions

- ACH-4471 inhibited PNH erythrocyte lysis but allowed increased C3 fragment deposition on intact cells.
- ACH-4471 inhibited PNH erythrocyte lysis and protected against increased C3 fragment deposition.

CONCLUSIONS

- ACH-4471 and eculizumab show strong synergy against hemolysis of PNH patient erythrocytes under high AP activation.
- Under physiological conditions, eculizumab prevents lysis of PNH erythrocytes but allows increased deposition of complement C3 fragments. ACH-4471 inhibits lysis of PNH erythrocytes and protects against elevated C3 fragment deposition, with or without eculizumab.
- These results support ongoing clinical evaluation of a “switch” strategy for ACH-4471 in PNH patients with suboptimal response to eculizumab.

Disclosures: RP is on the advisory board of Achillion; DE, OY, IT, SP and MH are employees and share holders of Achillion

REFERENCES

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