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

Dharaben Patel[1], Jane A. Thanassi[1], Guangwei Yang[1], Xuan Yuan[2], Robert A. Brodsky[2], Mingjun Huang[1], and Steven D. Podos[1]

[1]Achillion Pharmaceuticals, Inc., New Haven, CT, USA

[2]Division of Hematology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

INTRODUCTION

- ACH-4471 is an oral complement factor D (fD) 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 convertase formation.
- 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 therapeutic agent eculizumab, a monoclonal antibody directed against the terminal complement component C5, blocks MAC assembly on erythrocyte membranes and so prevents most 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 opsonized erythrocytes[1] and incomplete inhibition of intravascular hemolysis[2], 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.
- To assess this notion directly and also to support a future switch treatment approach, a clinical study is underway to evaluate ACH-4471 co-administration in PNH patients who respond suboptimally 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

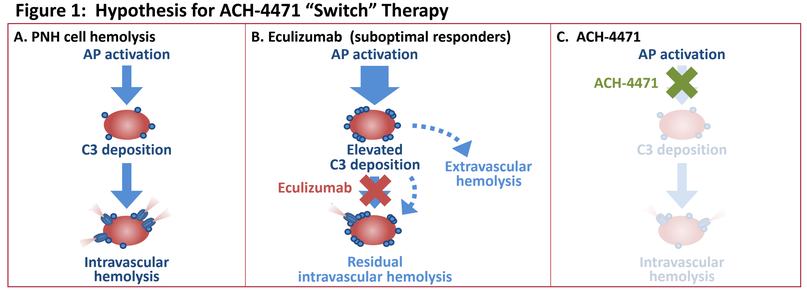
- Concentration series of ACH-4471 were assessed in pairwise combinations with eculizumab. Functional assay for human serum AP activity was done by hemolysis of red blood cells from PNH Patient A (female, 52 years old, blood type B, clone size 88%) using ABO blood group-compatible serum (20%) under conditions of robust AP-specific activation (GVB^0 + MgEGTA, pH7.3). Analyses of interactions were performed using a three-dimensional surface graphing method[3].
- Serum-mediated C3 fragment deposition on erythrocytes from PNH patient A was assessed with ACH-4471 alone and in combination with eculizumab. Physiological conditions were defined as 5 min pre-incubation of serum with inhibitor, 72% ABO blood group-compatible serum, 5×10^7/mL erythrocytes from PNH patient A, GVB^{++} buffer, 37°C for 1 hour, EDTA termination. Hemolysis was assessed from A_{405} of supernatants following centrifugation. C3 fragment deposition on intact and fragmented cells was assessed by flow cytometry using FITC-conjugated anti-C3c (Abcam Ab4212, 1:200), PE-conjugated anti-CD47 (R&D Systems FAB4670P, 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 Fsc-H>20,000 threshold. Intact and fragmented PNH erythrocytes were identified by anti-CD47 (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.

REFERENCES

1. A. M. Risitano et al, Blood, 2009, 113:4094
2. M. J. Harder et al, Blood, 2017, 129:970
3. M.N. Prichard and C. Shipman, Jr. Antiviral Research 1990, 14: 181-205

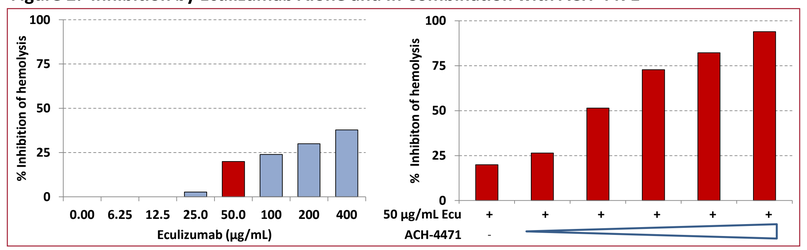
RESULTS

Figure 1: Hypothesis for ACH-4471 "Switch" Therapy



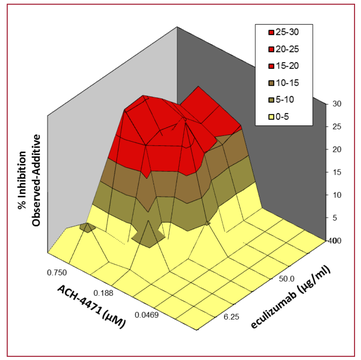
Inhibition of PNH Erythrocyte Hemolysis Under High AP Activation

Figure 2: Inhibition by Eculizumab Alone and In Combination with ACH-4471



Inhibition of PNH erythrocyte hemolysis from one representative experiment.

Figure 3: Three Dimensional Surface Graph of Pairwise Combinations



Three dimensional surface graph of one representative experiment.

Table 1: Three Dimensional Surface Graph Volumes

Volume (µM • µg/mL • % inhibition)		Interaction
Synergy	Antagonism	
306 ± 57	-4 ± 6	Strongly Synergistic

Mean + SD from N=2 independent experiments

- Synergy and antagonism volumes were calculated as the summed volumes respectively above and below the Z = 0 plane at 95% confidence limits.
- Interactions were categorized as additive for volumes -25 to 25, slightly synergistic 25 to 50, moderately synergistic 50 to 100, and strongly synergistic above 100 µM•µg/mL•% inhibition.
- Volumes above -25 µM•µg/mL•% inhibition were considered not antagonistic.

➢ **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[2].**

➢ **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

Figure 3: Flow Cytometry Key

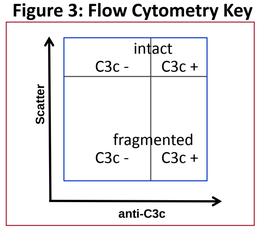


Figure 4: Evaluation of PNH erythrocytes

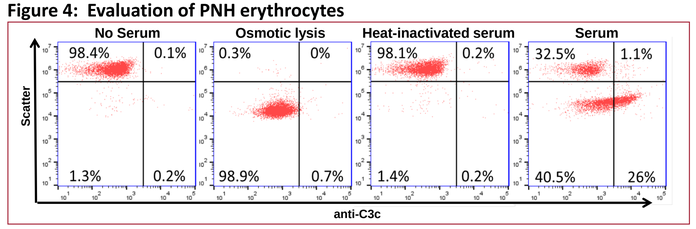
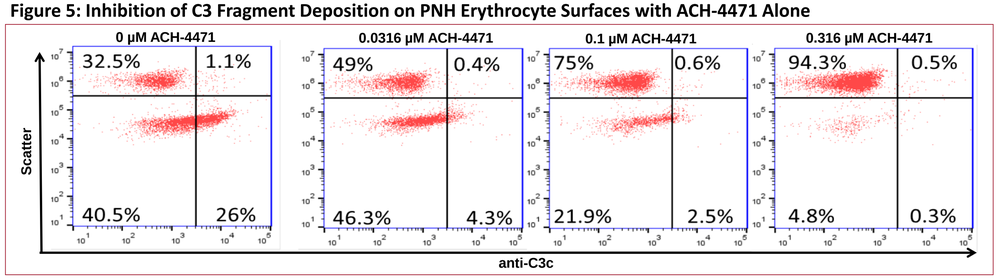


Figure 5: Inhibition of C3 Fragment Deposition on PNH Erythrocyte Surfaces with ACH-4471 Alone



➢ **ACH-4471 inhibited PNH erythrocyte lysis and C3 fragment deposition under physiological conditions.**

Figure 6: Inhibition of C3 Fragment Deposition on PNH Cell Surfaces with ACH-4471 and Eculizumab

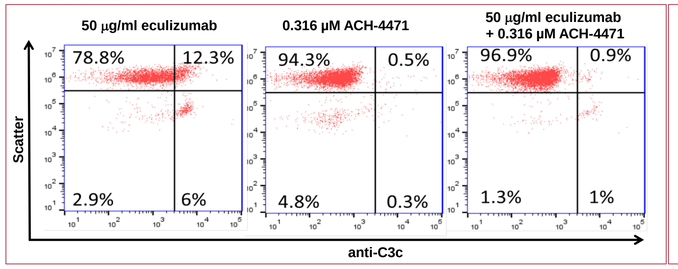
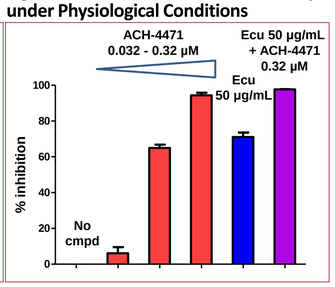


Figure 7: Inhibition of PNH Cell Hemolysis under Physiological Conditions



➢ **Eculizumab 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: RB is on the advisory board of Achillion; DP, GY, JT, SP and MH are employees and share holders of Achillion

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