Preclinical evaluation of RA101495, a potent cyclic peptide inhibitor of C5 for the treatment of paroxysmal nocturnal hemoglobinuria

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Abstract # 939

Abstract

Regulation of the terminal phase of the complement component pathway is a clinically validated approach for the therapeutic treatment of complement disorders. Inhibition of complement activation at the C5 level with the monoclonal antibody eculizumab has successfully been used for the treatment of rare disorders such as PNH and aHUS. However, even in these settings there remains a continued unmet need primarily due to need for intravenous administration, lack of activity in patients with C5 mutations and lack of universal access.

Ra Pharmaceuticals has developed a macrocyclic synthetic peptide, RA101495, which binds complement C5 with subnanomolar affinity and allosterically inhibits its cleavage into C5a and C5b upon activation of the classical, alternative or lectin pathways. *In vitro* studies also demonstrated that RA101495 is capable of preventing MAC assembly after thrombin mediated complement activation and is a potent disruptor of the interaction in between C5b and C6.

Inhibition of complement activity was evaluated in cynomolgus monkeys following single- and multi-dose subcutaneous (SC) administration. RA101495 exhibited high SC bioavailability and low, single doses fully inhibited complement-mediated hemolytic activity (>95%). Repeat dosing was well tolerated in monkeys and rats at high multiples of the projected human therapeutic dose and resulted in sustained and predictable inhibition of complement activity.

RA101495 fully inhibited the hemolysis of erythrocytes from PNH patients after activation of the alternative pathway. The synthetic peptide offers a novel therapeutic approach for inhibiting C5 for the treatment of disorders caused by or associated with complement dysregulation. As a product designed for convenient self-administration, RA101495 should provide an attractive option over monoclonal antibody therapy for patients with PNH and aHUS, including those with C5 polymorphisms and other complement disorders, especially those associated with hypercoagulable states.

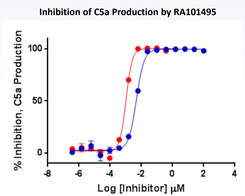
RA101495 binds human C5 with high affinity

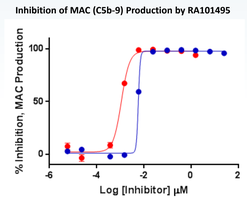
RA101495 a 15 aa macrocyclic peptide binds Complement C5 with a subnanomolar affinity as evaluated by Surface Plasmon Resonance (SPR)

Protein	ka (1/Ms)	kd (1/s)	KD (M)
C5	$9.15x10^{5}$	$1.8x10^{-4}$	$1.97x10^{-10}$

RA101495 inhibits C5 cleavage

RA101495 binds to C5 and allosterically inhibits its cleavage into C5a and C5b by the C5 convertase as determined by using an ELISA assay (C5b-9 was measured as surrogate of C5b). This mechanism of complement inhibition is similar to the one displayed by the monoclonal antibody Eculizumab.

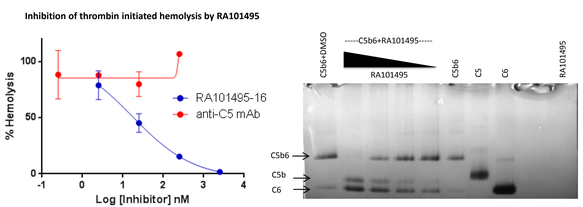




Inhibitor	C5a ELISA IC_{50} (nM)	MAC ELISA IC_{50} (nM)
RA101495	4.8	5.9
Anti-C5 mAb	1	1.1

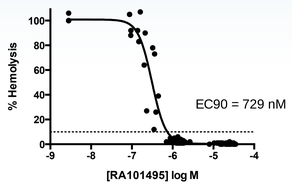
RA101495 inhibits thrombin initiated MAC formation by interfering with C5b6 formation

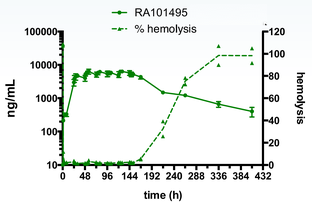
RA101495 is also able to prevent the formation of MAC initiated by proteases of the coagulation cascade (Thrombin shown) as determined by a hemolytic assay. In addition, RA101495 is capable of disrupting pre-formed C5b6 complex in a dose dependent manner. This mechanistic differentiation over Eculizumab may offer clinical advantages in diseases where hypercoagulative states are present



RA101495 exhibits excellent PK/PD correlation in single and repeat dose studies in NHPs

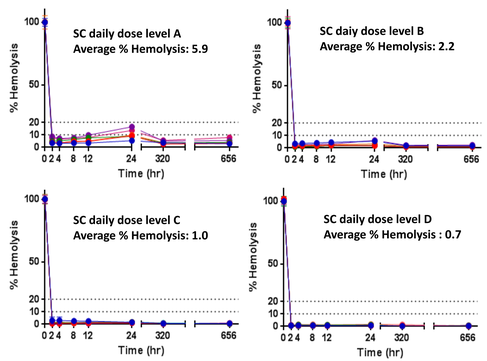
RA101495 inhibits complement activation in human and non-human primates. Single and repeat dose in cynomolgus monkeys demonstrated a clear correlation in between circulating drug levels and inhibition of complement mediated hemolysis (determined using an *ex vivo* sRBC hemolysis assay)





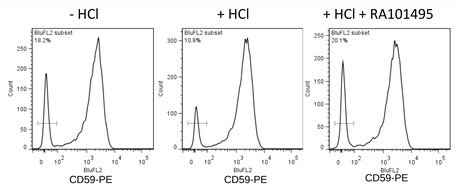
RA101495 provides sustained inhibition of complement activity in a dose dependent manner

Repeat dosing studies in cynomolgus monkeys resulted in fast suppression of complement mediated hemolytic activity (>95%). Sustained inhibition was observed at all dose levels evaluated in the study.



RA101495 inhibits hemolysis of PNH erythrocytes upon activation of the complement alternative pathway

The ability of RA101495 to prevent the hemolysis of PNH erythrocytes was evaluated using a modified Ham test[1,2]. PNH Blood samples were collected at the Cleveland Clinic under protocol (CCF IRB 5024). Acidification of ABO-matched serum resulted in the activation of complement alternative pathway and hemolysis of the PNH erythrocytes. Addition of RA101495 to the acidified ABO-matched serum completely blocked complement mediated hemolysis of these cells



Preclinical safety data supports the use of RA101495 in human clinical studies

- The safety of RA101495 has been evaluated in repeat dose GLP toxicology studies. RA101495 is safe and well tolerated in non-human primates and rodents at high multiples of anticipated human therapeutic dose
- A Phase I randomized, double-blind study designed to test the safety of single, escalating doses of RA101495 in humans has been initiated. This study will also be used to monitor the pharmacokinetics and pharmacodynamics after subcutaneous administration in healthy volunteers

Acknowledgements

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References

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