313 Tick-derived Polypeptide Complement Alternative Pathway Inhibitor, CIRp-A

Asset Overview

Product Type	Polypeptide isolated from tick saliva
Indication	Immunology
Current Stage	Discovery
Target(MoA)	Inhibition of AP initiation by binding to properdin
Brief Description	• The novel glycoprotein isolated from tick saliva is around ~170 residues long, is readily soluble and is chemically and proteolytically stable • Specifically inhibits initiation of the Alternative Pathway (AP) • Specifically binds to properdin which is a positive regulator of complement activation that stabilizes the alternative pathway convertases in human serum
Organization	University of Oxford

Differentiation

□ **Targeting alternative pathway (AP)**

- Therapeutic strategies that target the AP amplification loop or the C3 protein itself offer a robust mechanistic basis for inhibiting complement in PNH, with potentially broader therapeutic coverage by blocking both intravascular and extravascular haemolysis.

□ **Novel mechanism: Inhibition of AP initiation by binding to properdin**

- Only recently had studies begun to link properdin to other complement-related diseases, including renal diseases.
- Properdin is known as the only positive regulator of the complement system. Properdin promotes the activity of this defense system by stabilizing its key enzymatic complexes: the complement alternative pathway (AP) convertases. Besides, some studies have indicated a role for properdin as an initiator of complement activity.
- Though the AP is a powerful activation route of the complement system, it is also involved in a wide variety of autoimmune and inflammatory diseases, many of which affect the kidneys. The role of properdin in regulating complement in health and disease has not received as much appraisal as the many negative AP regulators, such as factor H.
- In the light of the upcoming complement-inhibiting therapies, it is interesting whether properdin can be a therapeutic target to attenuate AP-mediated injury.

□ **Related clinical pipelines:**

- Complement C3 inhibitor NGM-621 (NGM Biopharmaceuticals, mAb) in phase 1
- Complement C3 inhibitor AMY-101 (Amyndas Pharmaceuticals, peptide) in phase 2a
- Complement C3 inhibitor APL-2 (Apellis Pharmaceuticals, synthetic cyclic peptide conjugated to PEG, Long acting) in phase 3

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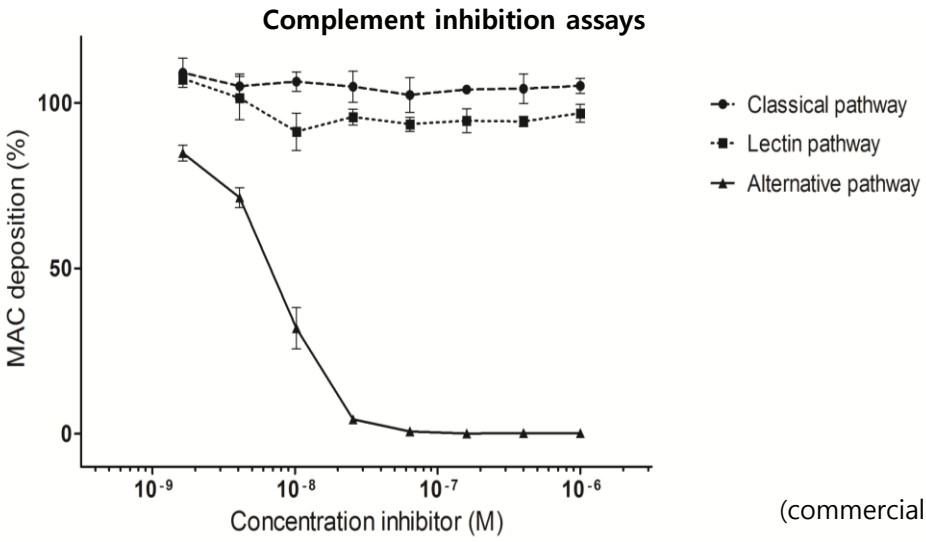
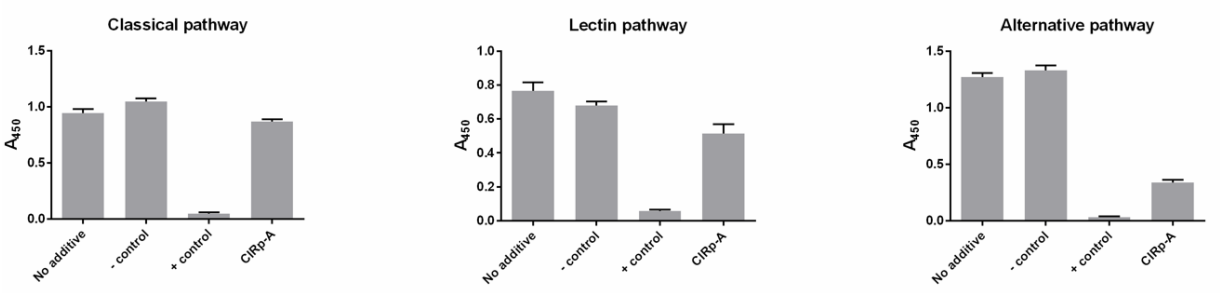
Key Data

CIRp-A specifically inhibits initiation of the AP



(commercial Wieslab assay)

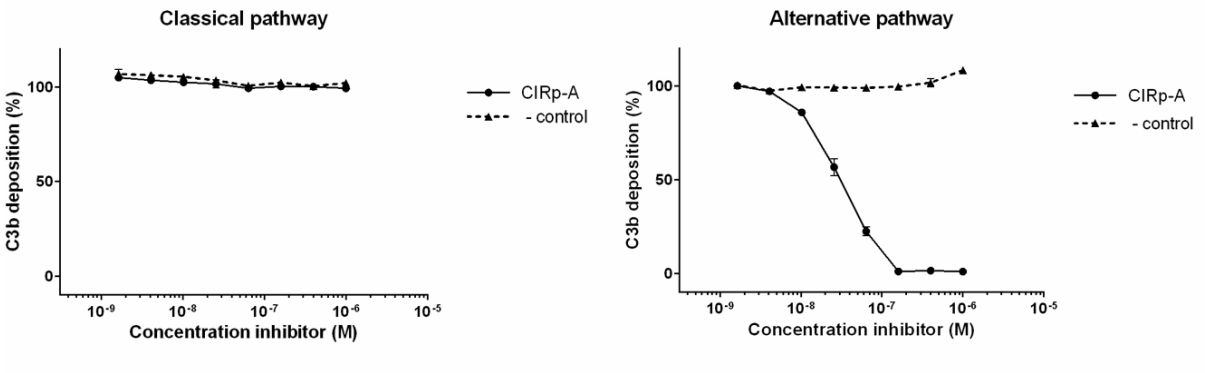
CIRp-A significantly inhibits C3a formation through the AP



The above figure shows C3a levels in the supernatants of the Wieslab assay at 1 µM inhibitor depicted in the previous slide. Levels were measured with a Microvue C3a kit (Quidel, USA). CIRp-A can be seen to significantly inhibit C3a formation through the alternative pathway - $p < 0.0001$ by unpaired two-tailed t test, with PBS as a reference. N=3 and error bars represent s.e.m.

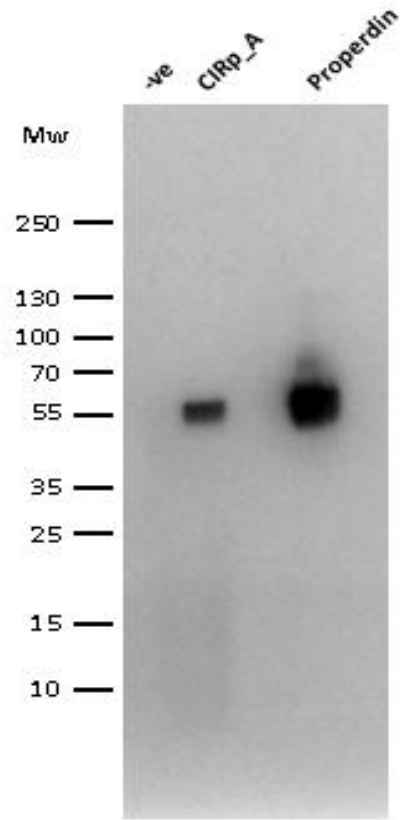
Tick-derived Polypeptide Complement Alternative Pathway Inhibitor

CIRp-A significantly inhibits C3b deposition through the AP



The figure shows C3b deposition in an ELISA based assay for Classical and Alternative pathway inhibition. 96 well plates were coated with IgM (classical pathway) or LPS (alternative pathway). Diluted normal human serum was added in the presence or absence of the CIRp-A. Plates were incubated for 1 hour at 37°C and C3b deposition was detected using an anti-C3 antibody coupled to HRP (Amsbio, UK). N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% activity) and no additive (100% activity).

CIRp-A specifically binds to properdin in human serum



The figure shows the results of a western blot with a polyclonal antibody against properdin and shows that CIRp-A binds properdin, while empty beads (-ve control) do not bind properdin. CIRp-A was coupled to NHS-activated magnetic beads (Thermo Scientific, UK) following the manufacturer's instructions. The beads were incubated with normal human serum followed by three wash steps. Bound proteins were eluted by heating the beads in non-reducing SDS-loading buffer and resolved on a SDS-PAGE gel. Proteins were transferred to a PVDF membrane which was blotted for properdin using polyclonal anti-properdin antibodies (Comptech, USA). Pure properdin (Comptech, USA) was loaded as positive control.

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Intellectual Property

Patent No.	PCT-GB2018-052180
Application Date	2018.07.31
Status	Application Pending
Country	

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