Development of Next-Generation Compstatin Analogs with Enhanced Target Residence Time Enables New Applications

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The compstatin family of cyclic peptide C3 inhibitors have emerged as a pillar in modulation of complement activation. Pegcetacoplan, a 40-kDa PEGylated compstatin analogue, has been approved for clinical use, although potential limitations associated with high-MW PEG polymers encourage further development. More recently, sequence-optimised non-PEGylated analogs have shown promise in clinical development (e.g. Cp40, AMY-101). Despite the progress already achieved by compstatin optimisation, some positions within the peptide sequence have not been fully explored. In this work, we investigated the structure-activity relationships (SAR) of the relatively ill-explored positions 3 and 5-8. In particular, we identified a highly advantageous V3I modification that increases the target affinity of compstatin analogues by more than 10-fold.

One of the new analogues, Cp01-3I, represents the most potent compstatin to date consisting entirely of canonical amino acids (C3b K_D = 20 nM). This enabled the grafting of the peptide onto an antibody Fc domain (Peptibody) with retained complement inhibtion *in vitro*. Meanwhile, the V3I modification can be combined with late-generation analogs, resulting in a low picomolar affinity derivative (C3b K_D = 30 pM), termed Cp50, with improved *in-vitro* complement inhibition compared to the clinical candidate Cp40. Finally, we demonstrate the application of the ultra-high affinity Cp50 peptide as a diagnostic and biochemical tool for the detection of opsonised C3 fragments on biosurfaces and endothelial cells. Overall, this work highlights the importance of vigorous optimisation of de novo peptide inhibitors to achieve ultra-high target affinities and to develop new peptide-based applications.

[1] Ricklin D, et al.: Nat. Rev. Nephrol. **2017**; 14: 26-47.
[2] Lamers Ch, et al. Nat. Commun. **2022**; 13: 5519.