Generating potent and selective inhibitors of Kv1.3 ion channels by fusing venom derived mini proteins into peripheral CDR loops of antibodies

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Pathogenic T cell effector memory (TEM) cells drive many autoimmune disorders and are uniquely dependent on the Kv1.3 channel. A number of venom derived knottin (cysteine-rich mini-protein) inhibitors of Kv1.3 are being developed as potential drug candidates, but can suffer from manufacturing difficulties, short half-lives and a lack of specificity. We have developed a novel molecular format wherein a peripheral CDR loop of an antibody has been replaced by a knottin. In this novel KnotBody™ format, the knottin benefits from the improved therapeutic functionality of an antibody and the antibody gains additional diversity by the addition of a scaffold which is pre-disposed to blockade of ion channels.

A proof-of-concept fusion protein of one structural domain within another was initially achieved by inserting a trypsin inhibiting knottin (EETI-II) flanked by diverse repertoire of short linker sequences into the CDR2 position of naïve antibody light chain sequences. Functional KnotBody™ molecules were selected from this library using phage display technology on the basis of retained trypsin binding, with the correct folding of both domains confirmed using X-ray crystallography.

To further demonstrate the benefits of this novel format, the modular nature of the KnotBody™ binding surface was exploited to: (i) improve existing knottin binding by introducing additional V_H contacts; (ii) create a bispecific molecule by introducing a V_H chain that binds to a different target; (iii) substitute the proof-of-concept knottin (EETI-II, a trypsin inhibitor) with ShK, a Kv1.3 ion channel blocking toxin; (iv) develop a panel of low-nM Kv1.3 inhibitors with selectivity exceeding 3000-fold over the Kv1.1 channel, a closely related Kv family member.