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Novel biologics for disrupting programmed cell death receptor PD-1 binding to PD-L1

Steven A Trim, Danielle McCullough, Stuart Baker and Paul Grant

Affiliation – Venomtech Ltd, Discovery Park, Sandwich, Kent, UK.

Abstract

Immune checkpoint inhibitors are clinically proven effective treatments for both solid tumours and haematological cancers. However, the large interaction surface of PD-1 with its ligand PD-L1 is one of the many challenges that have prevented small molecules from being effective. Thus, the clinically effective compounds are humanised IgG antibodies such as Atezolizumab, and these large proteins are only administrable via i.v. infusion and have high cost of manufacture. Therefore, smaller therapeutic compounds are needed. Venom peptides are secreted into the lumen of the venom gland ready for rapid delivery in under a second and thus have evolved to be stable. Venom peptides act via protein-protein interactions and are ligands for a large range of receptors and channels in predators and prey. These properties make them ideal for disrupting protein-protein interactions in drug discovery also. In this study a Targeted-Venom Discovery Array™ (T-VDA™) containing 640 venom fractions was screened using the cisbio High throughput Time Resolved Fluorescence (HTRF) Human PD1/PD-L1 biochemical binding assay to detect venom peptides that inhibit PD-1 binding to its receptor. Time resolved fluorescence was measured on the CLARIOstar®*Plus* (BMG LabTech) with the HTFR filter set. Using a mini Z' on each assay plate we confirmed the expected assay robustness (Z' = 0.78) and identified 22 hit fractions (3.4% hit rate). The majority of hits were from elapid snakes (Mambas and Cobras) but a few viper and scorpion fractions were also identified. This is poster will be the first published use of venom peptide libraries in HTRF format known and the novel identification of venom peptides as inhibitors of PD-1/PD-L1 binding is evidenced within.

Results

Figure 1. Hit identification and selection

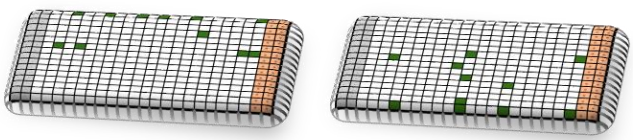


Figure 1: Initial hit finding screen identified 22 hits spread evenly across the two T-VDA© plates. From these the best 9 were selected for dose response confirmation. Eight fractions from Elapid venoms and one viper venom fraction was selected. The mini Z' performed with the control edges of the plates confirmed the expected assay robustness of Z'=0.78.

Figure 2. Dose response confirmation of hits

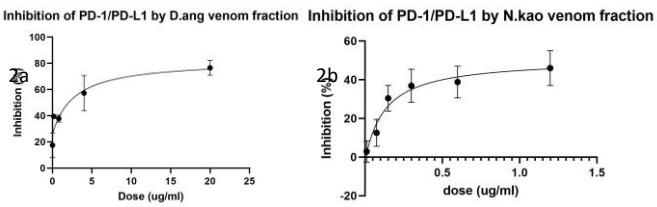


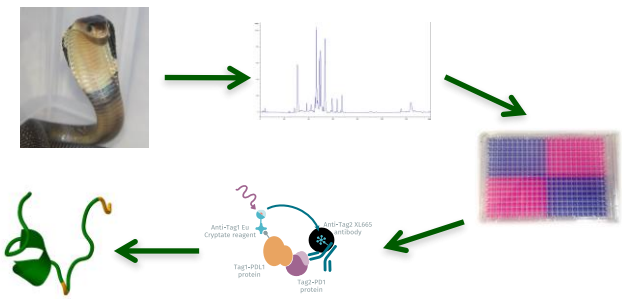
Figure 2: Partial antagonism of PD-1 by venom fractions prevents binding of PD-L1 in a dose responsive manner. In our hands the assay kit standard maxed out at 88% inhibition such that these peptide may achieve greater inhibition than seen in our hands. Figure 2a is a fraction from the green mamba *Dendroaspis angusticeps* and 2b is from the monocled cobra *Naja kaouthia* as pictured in visual method. D.ang fraction (Fasciculin -2) has an IC50 of 405.44nM and N.kao cobratoxin has an IC50 of 16.38nM.

Conclusions

- Venom peptide libraries can be screened in HTRF assays
- Venoms modulate protein-protein interactions
- Some 3-finger toxins interact with PD-L1 binding to PD-1 thus sequences from 3-finger toxins could aid drug design
- Discovery of novel venom actions like this could further aid our understanding of the actions of such peptides in nature
- As three finger toxins are more stable than antibodies they could make superior tools for PD-1 research

Method

Visual Method



Method

- Protein-protein interaction Targeted-Venom Discovery Array© (T-VDA^{PPI}) designed
- Venoms collected from seven elapid snakes, four arachnids, a lizard and a viper
- T-VDA^{PPI} standardized, assembled and lyophilized in echo qualified 384-well plates
- Venoms two dimensionally fractionated, using in-line system on UltiMate 3000
- PD1/PDL1 High-Throughput Time Resolved Fluorescence (HTRF) assay kit (cisbio)
- T-VDA^{PPI} dissolved in 10µl assay buffer, 2µl aliquoted into 384-well low volume proxiplate (Perkin Elmer).
- Plates read at 25°C in the CLARIOstar^{plus} plate reader (BMG Labtech) using the Europium TRF filter set with autofocus and dynamic range optimisation.
- Data was analysed as ratio of emission at 665nm/620nm.
- Selected hits identified by intact mass and peptide mapping mass spectrometry at Peak Proteins Ltd

Figure 3. Identification of active peptides

3a Fasciculin-2 (UniProtKB) 3b α-cobratoxin (UniProtKB)

Figure 3: Using intact mass and peptide mapping from trypsin/chymotrypsin digests the top hits were identified as elapid three finger toxins. The venom peptide from *Dendroaspis angusticeps was* identified as homologous to Fasciculin-2(3a) 6807Da and the venom peptide from *Naja kaouthia* identified as alpha-cobratoxin (3b) 7819.9Da.

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D.ang Fasciculin 32 KMVLGRGCGCPPGDDYLEVKCCTSPDKCN  60
                      ++ LG    CP      ++++CC++ D CN
N.kao cobratoxin 36 RVDLGCAATCPTVKTGVDIQCCST-DNCN  63
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Sequence analysis: The two identified three finger toxins share no significant sequence homology with PD-1 or PD-L1 and therefore potentially represent new binding interactions to disrupt PD-L1/PD-1 interaction. Nor is there any sequence homologue with the nicotinic acetylcholine receptors these proteins and known to inhibit. They share nine amino acids and a similarly charged region which is where further investigation will be focused.

Acknowledgements

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