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

HTRF screening of a protein-protein interaction Targeted-Venom Discovery Array™

TOP SPECS

Number of fractions	640	Novelty factor (1 / published papers)	1
Hit rate	3.4 %	Number of hits	22
Z' of assay	0.78	Hit potency	IC_{50} 16.38 nM
Targeting level 1-5 (1 = general, 5 = specific)	5	Diversity*	3

Immune checkpoint inhibitors have been clinically proven to be 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. To date, only large proteins such as humanised IgG antibodies – for instance, atezolizumab – have been successfully developed, and even these are costly to manufacture and restricted to IV infusion only. Therefore, smaller therapeutic compounds are required.

Venom peptides are a perfect alternative; they are ligands for a large range of receptors and channels in predators and prey, which makes them ideal for disrupting protein-protein interactions in drug discovery. The use of natural protein sources rather than traditional compound libraries results in a higher hit rate – nature has effectively already screened them – which can potentially save time and money in the discovery pipeline. The fact that they are secreted into the lumen of the venom gland, ready for rapid delivery in under a second, also means that they have evolved to be very stable.

This technical note describes the use of venom peptide libraries in a high throughput time-resolved fluorescence (HTRF) format, and the novel identification of venom peptides as inhibitors of PD-1/PD-L1 binding.[1]

METHOD

A protein-protein interaction Targeted-Venom Discovery Array™ (T-VDA^{PPI}) containing 640 venom fractions was engineered from venoms extracted from seven elapid snakes, four arachnids, a lizard and a viper. All venoms were two-dimensionally fractionated using a Thermo Scientific UltiMate™ 3000 UHPLC system, and each T-VDA^{PPI} was standardised, assembled and lyophilised in Echo®-qualified, 384-well plates. The

Figure 1: Method overview.

* Number of taxonomical groups

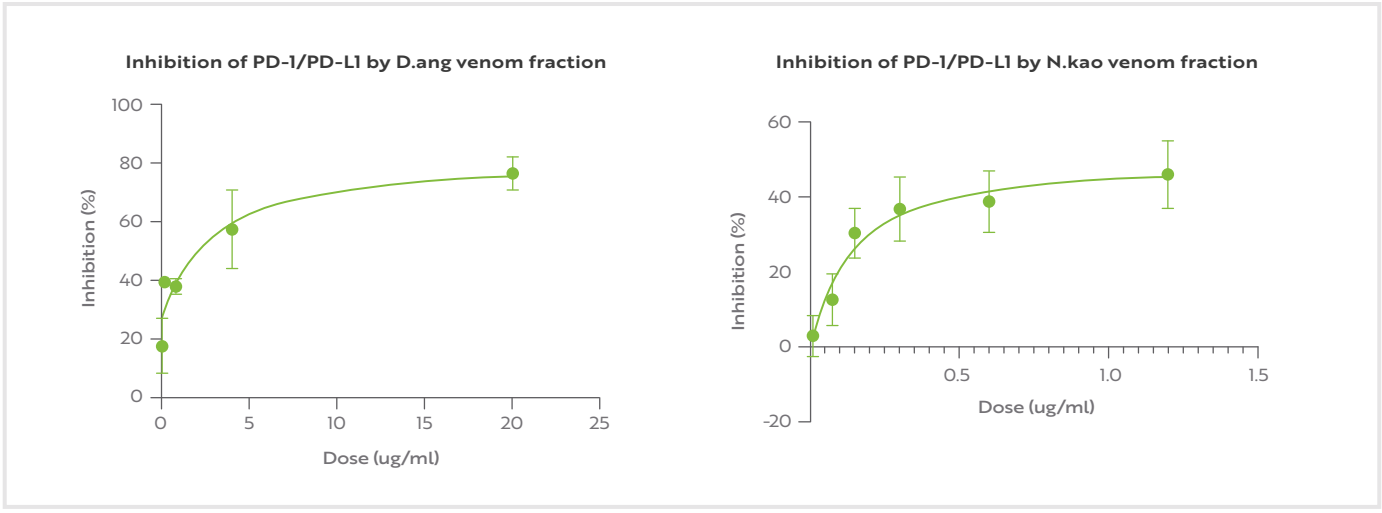

Figure 2: Dose-response confirmation of hits. The green mamba *Dendroaspis angusticeps* (D.ang) fraction (fasciculin-2) has an IC_{50} of 405.44 nM (left), and the monocled cobra *Naja kaouthia* (N.kao cobratoxin) an IC_{50} of 16.38 nM (right).

venom peptides were screened using the HTRF Human PD1/PD-L1 biochemical binding assay (Cisbio). After dissolving in 10 µl assay buffer, 2 µl aliquots were transferred to a 384-well, low volume ProxiPlate™ (PerkinElmer) and time-resolved fluorescence was measured at 25 °C in a CLARIOstar® Plus plate reader (BMG LABTECH), using the Europium TRF filter set with autofocus and dynamic range optimisation. The fluorescence emission ratio (665 nm/620 nm) was determined, and selected hits were identified by intact mass and peptide mapping mass spectrometry.

RESULTS

A mini Z' analysis confirmed the expected assay robustness (Z' = 0.78) and 22 hit fractions were identified (3.4 % hit rate), the majority from elapid snakes (mambas and cobras), but a few from viper and scorpion fractions.

Intact mass and peptide mapping from trypsin/chymotrypsin digests identified elapid three-finger toxins as the top hits. The venom peptides from *Dendroaspis angusticeps* and *Naja kaouthia* were respectively identified as homologous to the fasciculin-2 (6,807 Da), and alpha-cobratoxin (7,819.9 Da). Partial antagonism of PD-1 by venom fractions prevents binding of PD-L1 in a dose responsive manner, as shown in Figure 2. A maximum of 88 % inhibition was observed, although greater inhibition may be achievable.

CONCLUSIONS

This technical note demonstrates the successful screening of venom peptide libraries and the novel identification of venom peptides as inhibitors of PD-1/PD-L1 binding. Venoms are known to modulate protein-protein interactions, and some three-finger toxins have been shown to disrupt PD-L1 binding to PD-1, meaning they could be applied to drug design. The discovery of novel venom actions like this is leading to a better understanding of the actions of these peptides in nature, and the toxins themselves would make excellent tools for PD-1 research.

Venomtech offers a range of T-VDA™ solutions containing purified and freeze-dried venoms from carefully selected species, specifically engineered to maximise the chance of success and deliver the greatest value in the generation of fresh leads. These naturally derived peptides offer increased thermal stability compared to antibodies, and each T-VDA™ is supplied in Echo®-qualified, 384-well plates for high throughput, assay-ready plug-and-play convenience.

ACKNOWLEDGEMENTS

Thanks to Cisbio and PerkinElmer for the use of their images.

[1] Novel biologics for disrupting programmed cell death receptor PD-1 binding to PD-L1. Trim, S.A., McCullough, D., Baker, S. and Grant, P. ELRIG Drug Discovery 2021.

Venomtech Limited
Building 500 East Block,
Ground Floor, Discovery Park
Sandwich, Kent, CT13 9FE

+44 (0) 1304 892694
(Mon-Fri 9am-5pm)

info@venomtech.co.uk

venomtech.co.uk

Venomtech