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Novel shark antibodies engineered to characterise and improve delivery of therapeutics across the blood-brain barrier

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Introduction: The blood-brain barrier (BBB) prevents >98% of therapeutics from reaching the brain, posing a major problem for treatment of central nervous system afflictions. Novel shark antigen receptor variable domains - termed VNARs - can be humanized and used as molecular Trojan Horses to deliver therapeutics to the brain. This study aims to identify new VNARs, more efficient at crossing the BBB than existing Transferrin Receptor 1 (TfR1) targeted VNARs, isolated by selection on the purified receptor (1).

Methods: A library pre-selected against human TfR1 was used in an *in vitro* transcytosis model for functional selection of phage clones that cross the BBB. After 3 rounds of selection using the hCMEC/D3 (human BBB) cell line, positive TfR1 binding clones were expressed as VNAR-Fc with a human Fc scaffold. These were re-tested for TfR1 binding before use in *in vivo* mouse studies. 6-12week-old female BALB/c mice were injected with VNAR-Fc via the tail vein. After 18 hours, vasculature was flushed by perfusion with saline, before brains were harvested. All *in vivo* experiments were carried out within the framework of the Animals Scientific Procedures Act (1986).

Results: After selection, clones were sequenced and ~70 unique clones were identified. Twenty-four were formatted as VNAR-human-Fcs based on their binding (as phage) to both human and mouse TfR1, relative to a control VNAR-Fc (found by *in vivo* phage display). Nine were selected for *in vivo* studies in mouse, based on binding to TfR1 as VNAR-Fcs. None of the 9 clones have been found in brain at concentrations comparable to the positive control VNAR-Fc. However, 2 clones show potential. Clones 3 and 13 were detected in brain at higher concentrations than the negative control VNAR-Fc (Table 1). This difference is statistically significant for clone 13 ($p=0.005$) and is tending towards significance for clone 3 ($p=0.07$)(Student's t test).

Table 1: Concentrations of VNAR-Fc clones in BALB/c mouse brain

	Negative Control (n=6)	Clone 3 (n=4)	Clone 13 (n=4)
Brain Concentration (nM)	0.145±0.012	0.227±0.059	0.308±0.050

Conclusions: This method identified unique clones, not found previously using dish/plate *in vitro* selection. It identified 2 clones found using *in vivo* phage selection, which validates the selection method. We believe the "functional" aspect of the assay will provide more VNARs that cross the BBB and transcytose to the brain, rather than just binding the TfR1 target.

References:

1. Häsler, J., et al.(2014). S*pecies cross-reactive single domain antibodies (VNARs) to the transferrin receptor 1 (TfR-1) that cross the BBB*. Poster session presented at 4th Cold Spring Harbor Blood-Brain Barrier Conference, New York