COMBINED IN VITRO/IN VIVO METHODS FOR SELECTING VNARs THAT SHUTTLE LARGE THERAPEUTIC MOLECULES ACROSS THE BLOOD BRAIN BARRIER

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BACKGROUND: Penetration of blood brain barrier (BBB) remains a significant hurdle in the development of biologic therapeutics for central nervous system related diseases. These predominantly large antibody-based molecules typically do not cross the BBB in amounts required for therapeutic efficacy.

RESULTS: The amount of Clone C in the brain was 14-fold higher than most of the VNAR sequences found by NGS as amplified in the brain during *in vivo* selection. Brain fractionation showed that clone C was in both the capillary and parenchymal fractions, but preferentially accumulated in the brain parenchyma. To assess carrier activity, clone C was fused to a therapeutic antibody to CD20 (Rituximab) at various positions to create 10 different bispecific formats. The best formats increased brain concentrations over 10-fold that of naked rituximab. A bivalent and monovalent formats were equally effective and brain uptake did not correlate with binding affinity. Fusions of clone C with a therapeutically relevant enzyme iduronidase (IDUA) also showed dramatically improved brain penetration indicating flexibility of the transporter module.

CONCLUSION: Ossianix developed a phage display-based method for functional selection of small VNAR antibodies to TfR1 that when fused to the therapeutic biologics can shuttle large molecules across the BBB with high efficiency and species cross-reactivity.

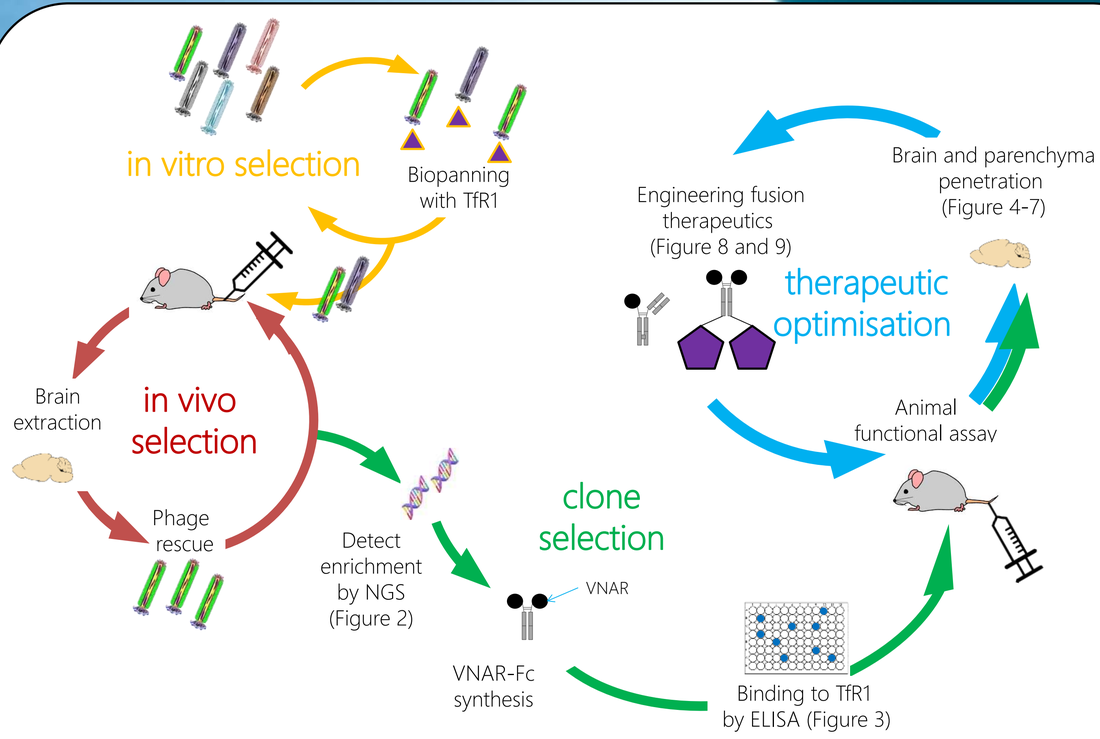

Figure 1. Schematic of the selection using synthetic VNAR phage library, clone selection and therapeutic optimisation.

Clone selection

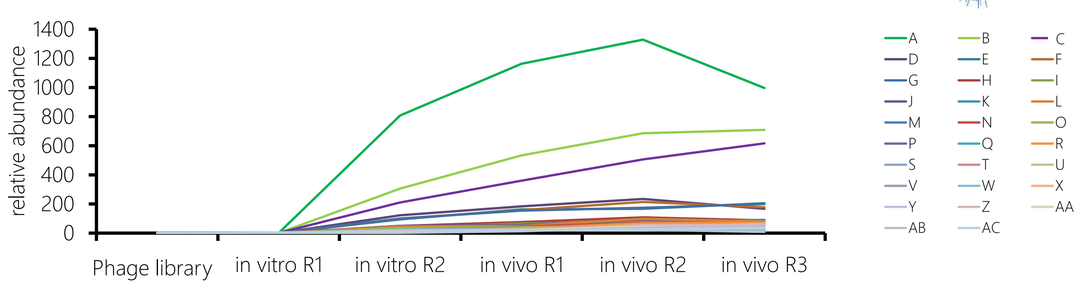

Figure 2. Selection of clones based on NGS and abundancy analysis.

TfR1 binding

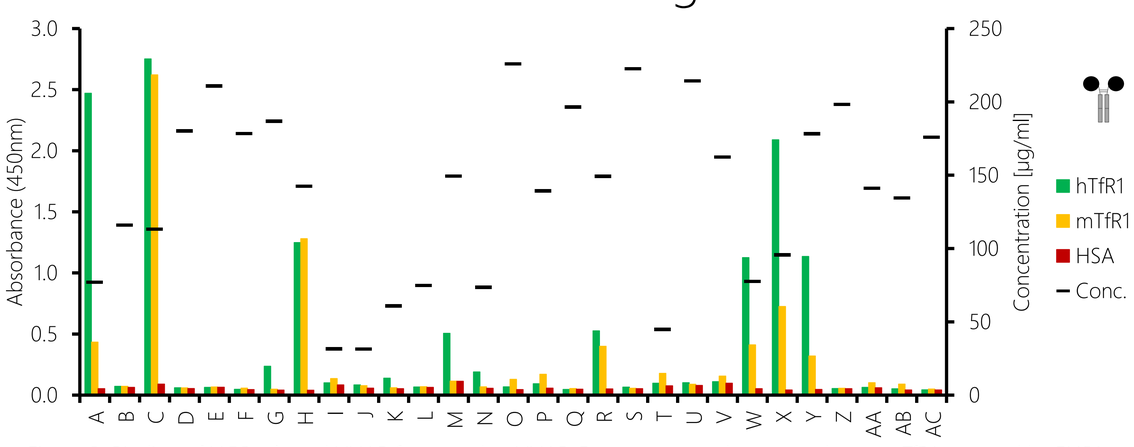

Figure 3. Binding of NGS selected VNAR formatted as VNAR-Fc constructs to human and mouse TfR1 assessed by ELISA.

Brain penetration screen

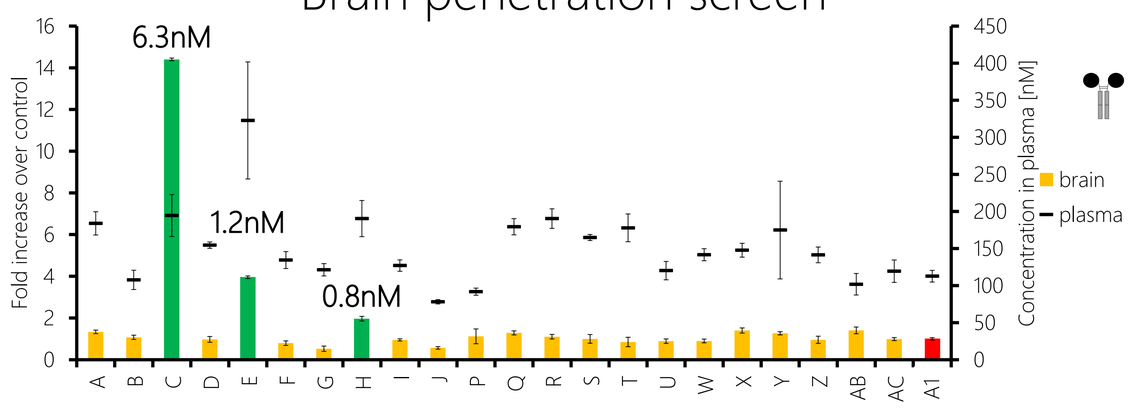

Figure 4. Brain penetration levels of NGS selected VNARs. IV at 25nmol/kg for 18 hours. Data were normalised to the control and presented as fold increase over control, ±RSD; plasma concentration, ±SD.

Brain penetration of clone C

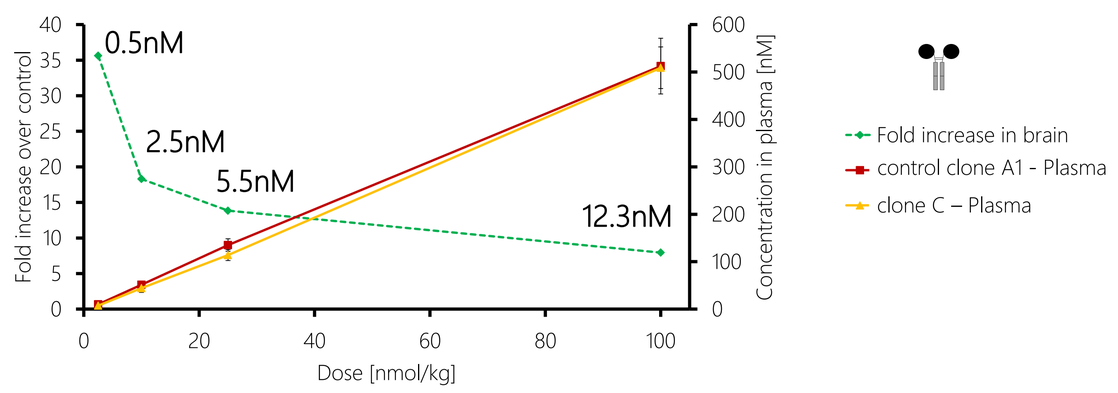

Figure 5. Dose dependent brain penetration levels of clone C as VNAR-Fc at 18 hours post IV injection. Brain penetration data were normalised to the control and presented as fold increase over control (clone A1); plasma concentration, ±SD.

Parenchymal transport of clone C

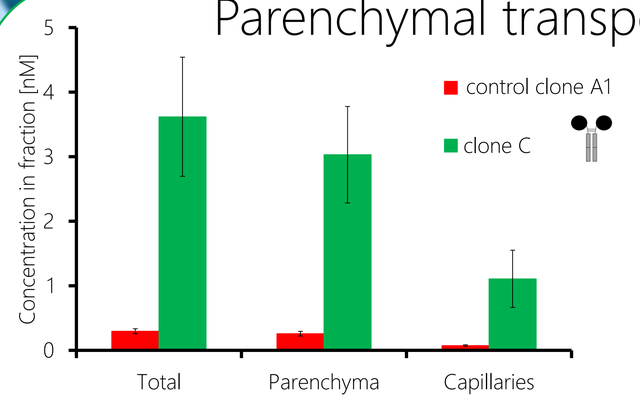

Figure 6. Fractional distribution of clone C as VNAR-Fc in brain. The data presented as average, ±SD.

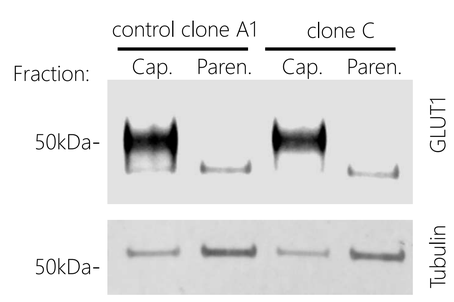

Figure 7. Fractionation quality control with GLUT1 as capillary marker. Brain tissue was fractionated for capillaries (Cap.) and parenchyma (Paren.).

Clone C-Rituximab

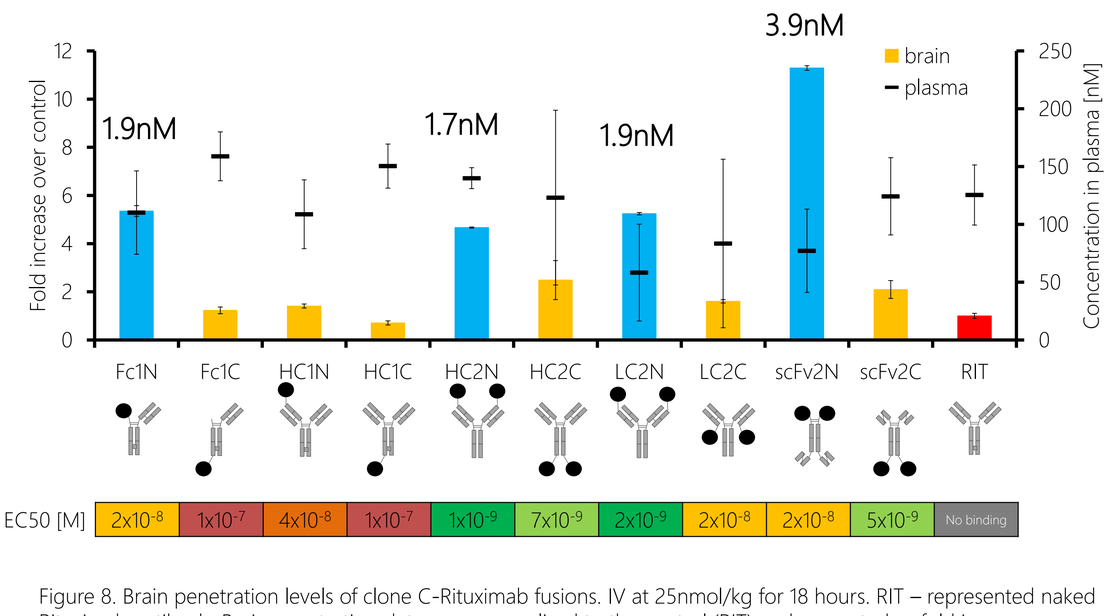

Figure 8. Brain penetration levels of clone C-Rituximab fusions. IV at 25nmol/kg for 18 hours. RIT – represented naked Rituximab antibody. Brain penetration data were normalised to the control (RIT) and presented as fold increase over control, ±RSD; plasma concentration, ±SD. EC50 results of ELISA with mouse TfR1.

Clone C-IDUA

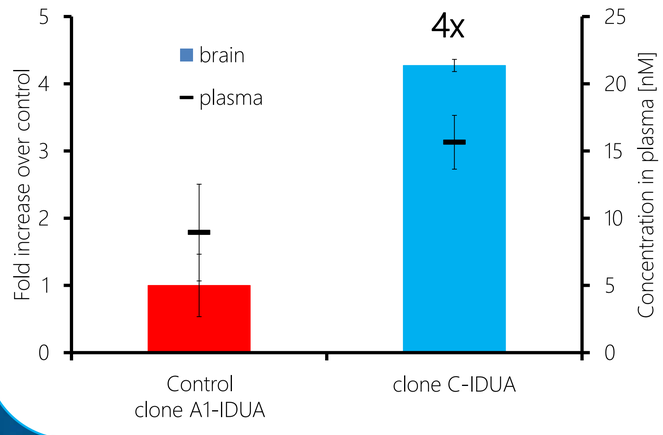

Figure 9. Clone C fusion to iduronidase (IDUA) improves brain penetration of the enzyme. IV at 20nmol/kg for 18 hours. Brain penetration data were normalised to the control and presented as fold increase over control, ±RSD; plasma concentration, ±SD.