JOINT CASE STUDY

Combining the Triple vector technology from FairJourney Biologics and the Membrane Proteome Array platform from Integral Molecular for the discovery of highly specific anti-human CXCR4 antibody molecules in full therapeutic format

Introduction

Phage display selected antibody fragments are often converted to immunoglobulins or to Fc fusion proteins and expressed in mammalian cells. The resulting final molecules are bivalent, possess Fc effector function and a half-life comparable to natural immunoglobulins.

For antibody fragments as, single chain Fvs (scFvs) or single domain antibodies (sdAbs), the conversion to Fc fusion proteins and expression in mammalian cells might result in loss of affinity and/or properties.

To circumvent these drawbacks, FairJourney Biologics has developed a technology dubbed "The triple vector", based on a vector containing the elements necessary for phage display, production of scFvs and sdAbs fused to human Fc in bacteria and for production in mammalian cells. Ultimately the selection of immunological effector function bearing bivalent molecules, bypassing the cloning into mammalian expression vectors, will accelerate the drug discovery process.

One of the key factors to address during the selection of the antibody leads candidates is specificity. Integral Molecular has developed a platform dubbed "The membrane Proteome Array" (MPA) in which the candidate antibodies are tested for binding to over 5300 human membrane proteins including GPCRs, ion channels and transporters.

Here we describe an efficient generation of highly specific anti-human CXCR4 VHH-huFc molecules by combining the Triple vector technology from FairJourney Biologics and the MPA platform from Integral Molecular.

Results

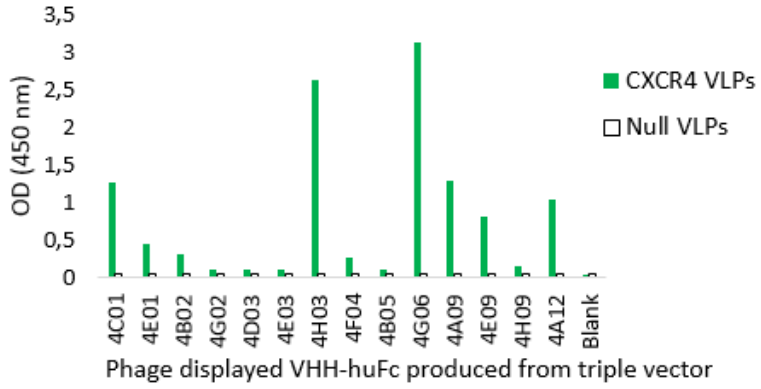

Figure 1 – ELISA results for binding to human CXCR4 and Null lipoparticles or virus-like particles (VLPs) from Integral Molecular of phage displayed VHH-huFc molecules selected from the VHH-huFc Naïve Library derived from the triple vector of FairJourney Biologics.

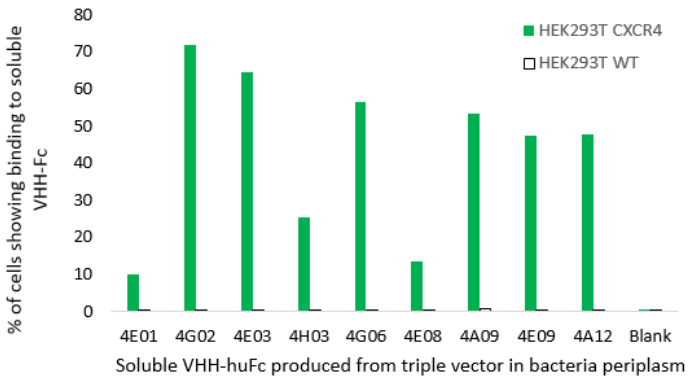

Figure 2 – FACS results for binding to HEK WT and to HEK cells transfected with human CXCR4 of soluble VHH-huFc molecules selected from the VHH-huFc Naïve Library derived from the triple vector of FairJourney Biologics and expressed in bacteria.

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Clone ID	Final amount (mg)	Yield (mg/L)
4G06	5.9	120
4A09	1.1	22

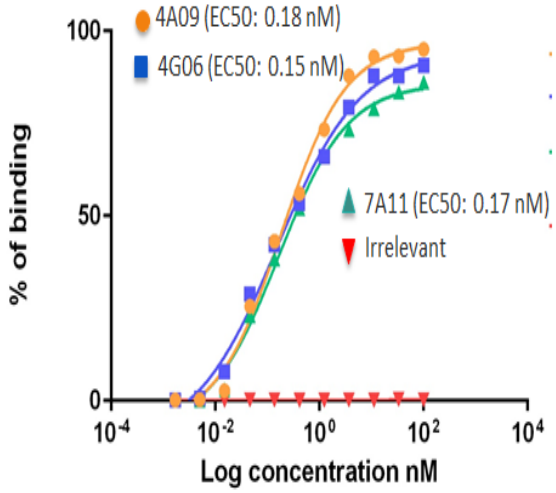

Figure 3 – Table showing amounts and yield values of anti-human CXCR4 VHH-huFc produced in ExpiCHO cells and purified via protein A. Molecular weight and purity of the produced proteins were analysed by SDS-PAGE. FACS results for titration of binding to HEK cells transfected with human CXCR4 of purified anti-human CXCR4 VHH-huFc molecule 4A09 and 4G06, selected from VHH-Fc Naïve Library derived from the triple vector of FairJourney Biologics and the calculated EC50 values. For comparison the results obtained with 7A11 clone, selected as VHH from another library and then formatted to VHH-huFc, and with an irrelevant VHH-huFc molecule are shown.

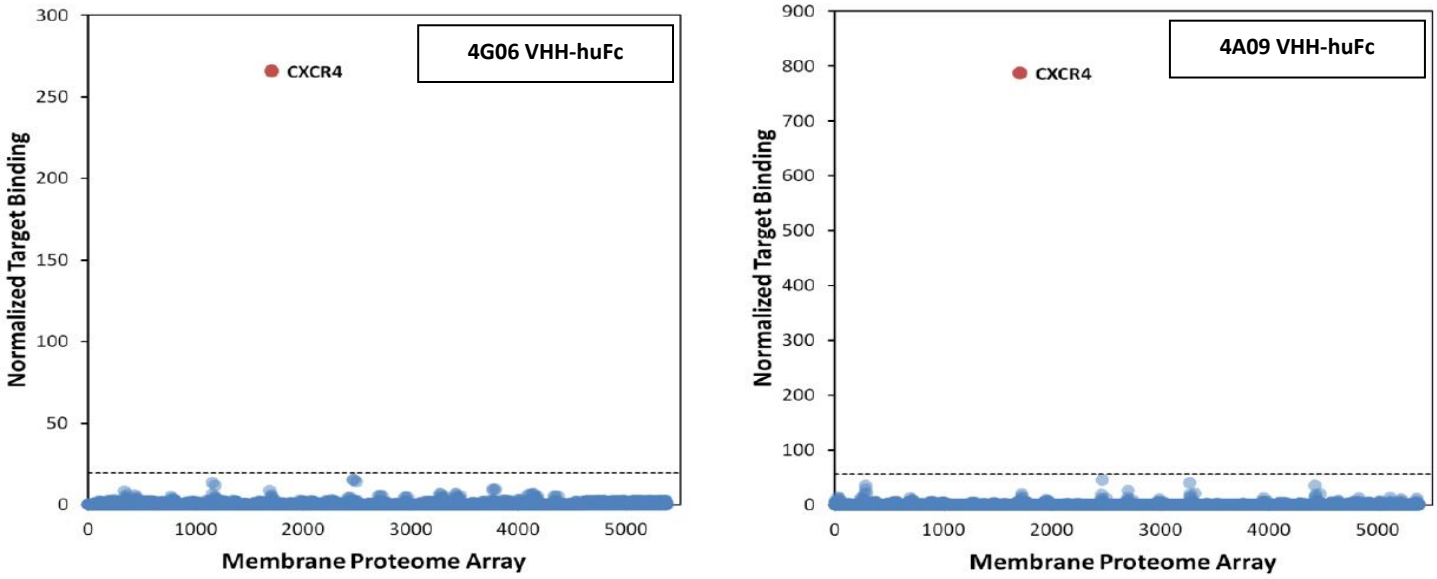

Figure 4 – Identification of membrane protein binding targets for 4G06 VHH-huFc and 4A09 VHH-huFc at 0.5 µg/ml selected from the VHH-huFc Naïve Library derived from the triple vector of FairJourney Biologics using the MPA platform of Integral Molecular. Non-specific fluorescence was determined to be any value below 3 standard deviations above noise (dotted line). Accordingly, targets that showed increased antibody binding are displayed above the dotted line and denoted in red.

Conclusions

The successful isolation of highly specific anti-human CXCR4 VHH-huFc shown here demonstrates the capacity of FairJourney Biologics in providing VHH-huFc in an efficient and fast manner by using the Triple vector technology as well as the capacity of Integral Molecular in offering its MPA platform to evaluate the specificity of antibodies against a wide panel of human membrane receptors.