Generation of functional antibodies against membrane spanning proteins

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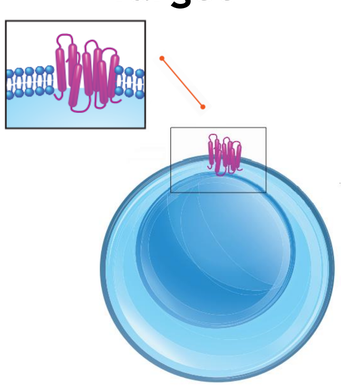
Introduction

There is a substantial interest in high-quality, functional antibodies against membrane spanning proteins, especially those considered intractable targets. Generating antibodies against these targets is very challenging, and crucial for therapeutic and diagnostic antibody development. ModiQuest Research has developed novel tumor specific antibodies with high therapeutic and diagnostic potential.

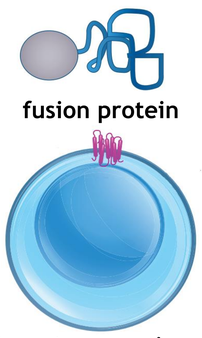
Materials & methods

Target



transmembrane tumor target

Antigen & tool selection

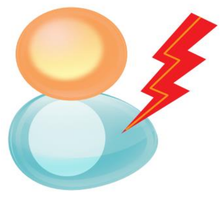


stable target expressing cell line

Immunization

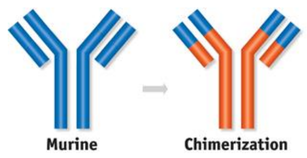
mouse immunization

Antibody generation



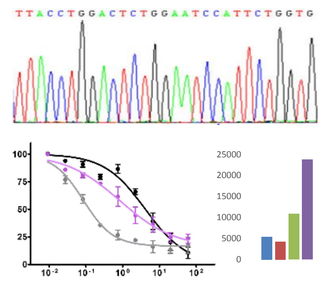
ModiFuse™

Chimerization of lead clones



ModiTune™

Antibody characterization



Based on *in silico* modeling of the transmembrane tumor target, fusion proteins representing the extracellular domain of the human and cynomolgus (cyno) form of the target were designed, produced and purified. These fusion proteins were used for immunization of mice. After confirming serum cross-reactivity against both forms of the cell-expressed tumor targets (ModiVacc™, MV cells), hybridomas were generated from spleen cells of the best responder mouse using ModiQuest's ModiFuse™ technology. Sequences of the variable domains of the target reactive hybridomas were obtained, and subsequently recloned into our mammalian human IgG expression vector system (ModiXpress™). Chimerized antibodies were further characterized for internalization capacity, inhibition of cytokine production upon target cell stimulation and ADCC/CDC.

Results

Antigen & tool selection

Fig.1. Purity analysis of tumor target fusion protein on SDS-PAGE

Fig.2. Validation of target expression on generated ModiVacc cells

Succesfully generated target overexpressing MV cells with >2 log overexpression of full length target and >95% pure recombinant proteins

Immunization

Fig.3. Serum analysis on the human tumor target expressing MV cells and cyno tumor target expressing MV cells

Cyno and human cell-expressed target protein cross-reactivity was induced in 6 out of 7 animals

Hybridoma generation and lead selection based on 1st screening round

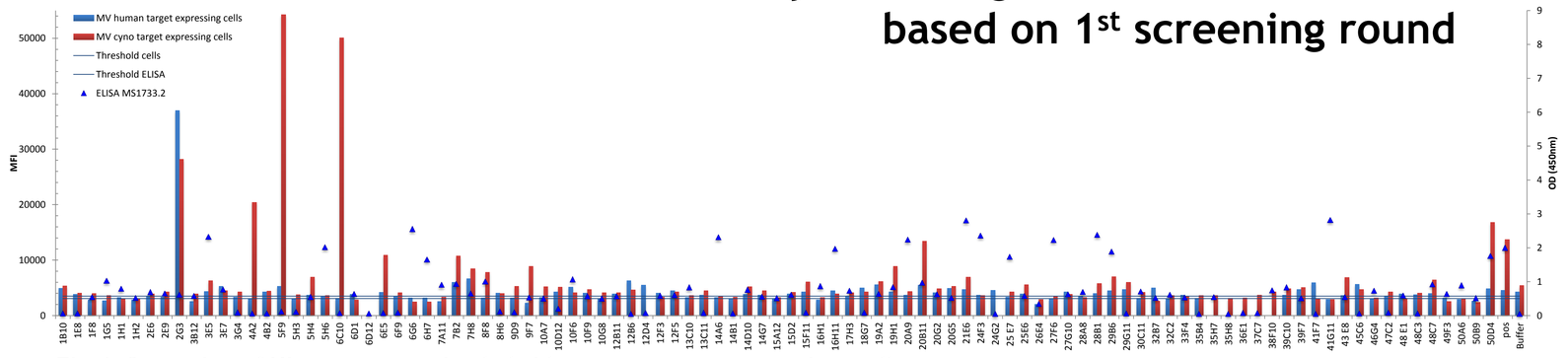


Fig.4. Screening of His-target reactive hybridomas on target expressing cells

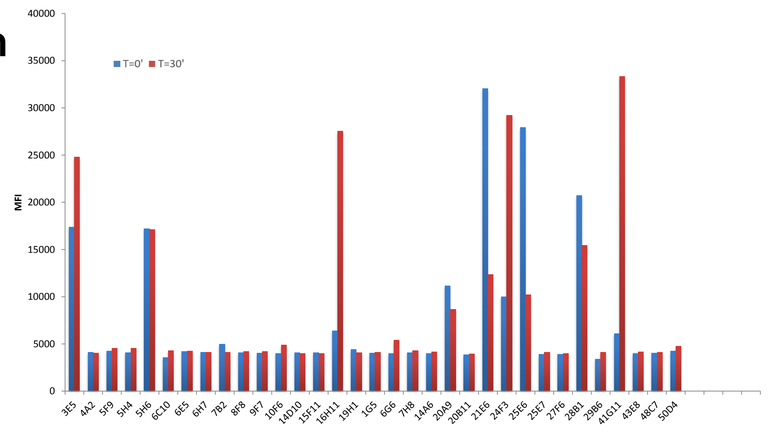
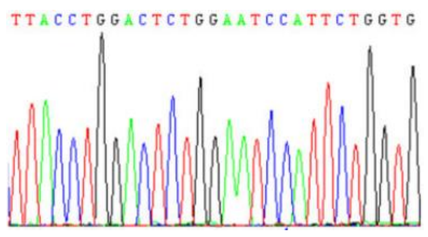


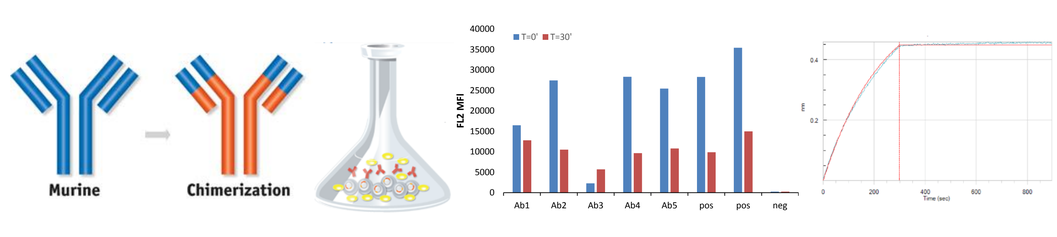
Fig.5. Internalization assay using target expressing cell reactive antibodies

126 recombinant target reactive hybridomas were screened on target expressing MV cells revealing 34 target expressing cell reactive antibodies, which have been screened for internalization properties. 12 lead antibodies were further characterized in the second screening round.

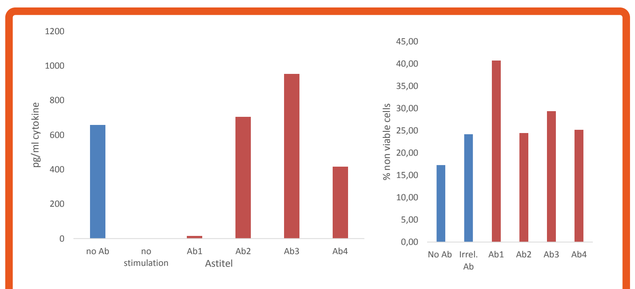
Lead characterization based on 2nd screening round



Sequence analysis of 12 lead antibodies



5 unique sequences were expressed as mouse/human chimeric antibodies and screened for internalization properties and ranked for relative affinity



4 antibodies were selected to analyze the ability to inhibit cytokine production of activated target cells and ADCC/CDC

Conclusions

By exploiting ModiQuest's proprietary technology platform and subsequent high-throughput screening, we successfully generated and fully characterized a panel of lead antibodies to a therapeutically relevant transmembrane protein in less than a year.