

Generation of functional antibodies against membrane spanning proteins

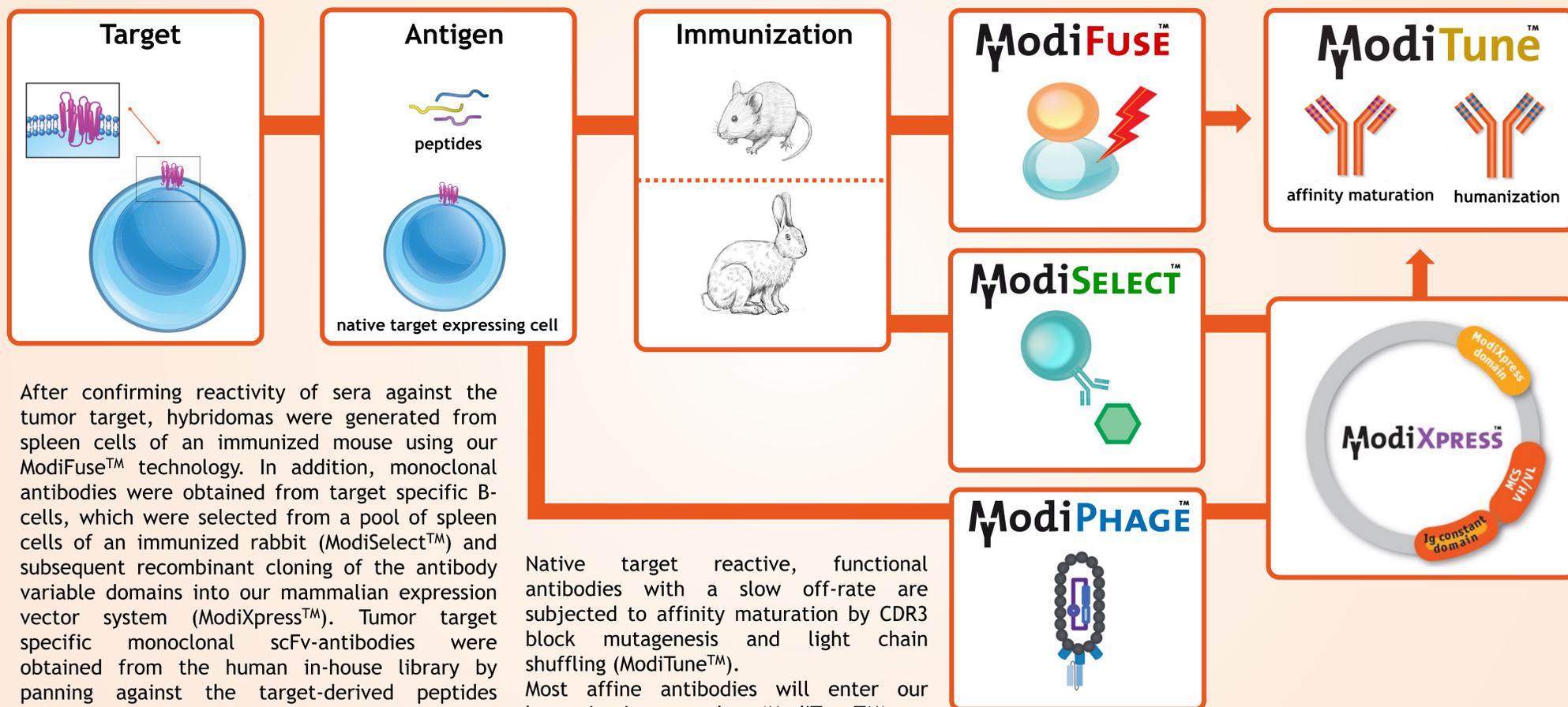
Nienke Smits, Ilse Roodink, Debby Kruijsen and Jos M.H. Raats

Introduction

There is a substantial interest in high-quality, functional antibodies against membrane spanning proteins. Generating antibodies against the native conformation of these intractable drug 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. In order to generate these tumor target specific antibodies we applied different technologies of our antibody generation platform.

Materials & methods

Based on *in silico* modeling of the transmembrane tumor target, peptides representing exposed epitopes of the extracellular domain were designed. These peptides, as well as native expressing cells were used for immunization of mice and rabbits, and in addition, for screening of our in-house human phage display library.



Results

Using the different antibody generation technologies a large panel of monoclonal antibodies against the tumor target were obtained. These antibodies were further characterized by immunostainings on cells overexpressing the tumor target (fig. 1) and human tumor tissue (fig. 2), Octet-based off-rate ranking (fig. 3) and *in vivo* targeting studies (fig. 4).

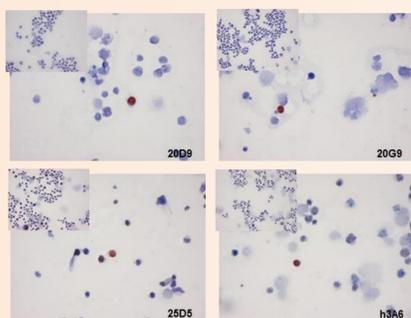


Fig. 1 Analysis of staining pattern of a subset of the anti-tumor target antibodies on tumor target transfected CHO-s cells. Insets show staining pattern on untransfected cells.

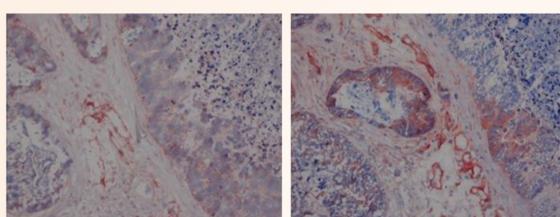


Fig. 2 Immunostaining on human brain metastasis of an adenocarcinoma of two of the generated anti-tumor target antibodies.

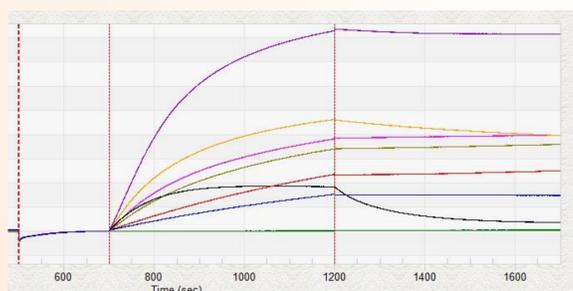


Fig. 3 Off-rate ranking of a subset of ELISA positive anti-tumor target antibodies.

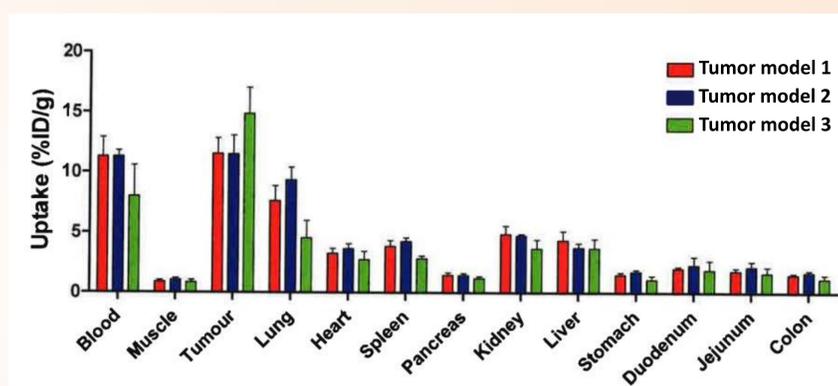


Fig. 4 Biodistribution of ¹¹¹In-labelled anti-tumor target antibodies, 3 days after injection.

Conclusions

Using different technologies of our antibody generation platform we obtained a large panel of native tumor target recognizing antibodies. Therapeutic and diagnostic value of the most affine humanized antibodies will be further evaluated by extended *in vivo* tumor targeting studies.