DeepDisplay™: a ground-breaking and influential human lead antibody discovery platform utilizing OmniAb® animals.

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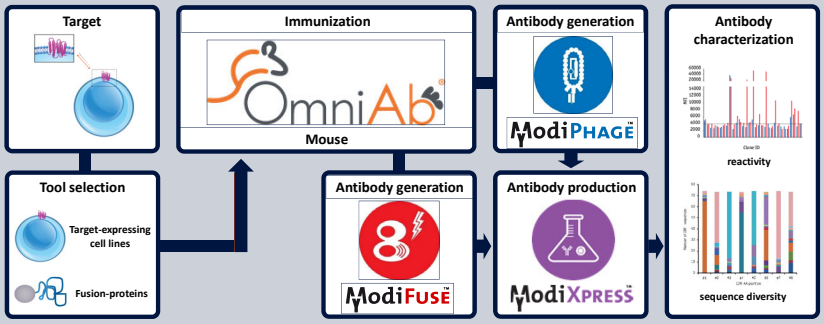
Introduction

Preclinical lead antibody selection out of a diverse pool of antibodies generated via immunization of animals often results in high affinity, target-reactive antibodies that need further humanization afterwards. To reduce timelines of antibody discovery programs without loss of quality of the original lead molecules, transgenic animals expressing human antibody variable domains are frequently used for immunization. This poster introduces DeepDisplay™, a powerful platform for discovery of a sequence diverse human antibody panel with broad species cross-reactivity (e.g. mouse, cyno, human), which is specifically directed against a cell-associated transmembrane target. DeepDisplay™, a unique combination of OmniMouse® immunization and phage display technology, was compared to hybridoma-based lead antibody discovery.

Materials & Methods



To obtain therapeutic lead antibody candidates with mouse, cyno and human target cross-reactivity, two groups of OmniMouse® were immunized using different alternating immunization strategies. Subsequently, target-specific titer against recombinant human, cyno, and mouse target-ECD protein and cell-expressed human and cyno target was analyzed. Top 3 best responder mice were selected for hybridoma generation via ModiFuse™ (proprietary electrofusion) and/or immune scFv phage library generation and subsequent selections using ModiPhage™ (phage display). To increase the number of multiple species cross-reactive molecules in the phage display selection outputs, 4 different selection strategies using recombinant target-ECD proteins and/or recombinant target-expressing cell lines were applied. Obtained outputs from both ModiFuse™ and ModiPhage™ strategies were screened for target-specificity, species cross-reactivity (analyzed on recombinant target-ECD and cell-expressed target) and sequence diversity.

Results

Immunization

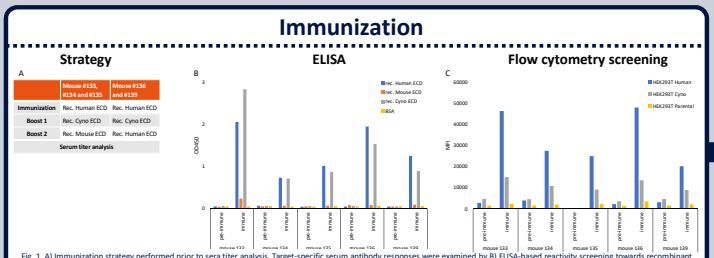


Fig. 1. A) Immunization strategy performed prior to sera titer analysis. Target-specific serum antibody responses were examined by B) ELISA-based reactivity screening towards recombinant human, cyno, and mouse target-ECD protein and C) flow cytometry-based reactivity screenings against human and cyno target-expressing HEK cell lines and corresponding parental HEK cells using iQue.

ModiFuse™

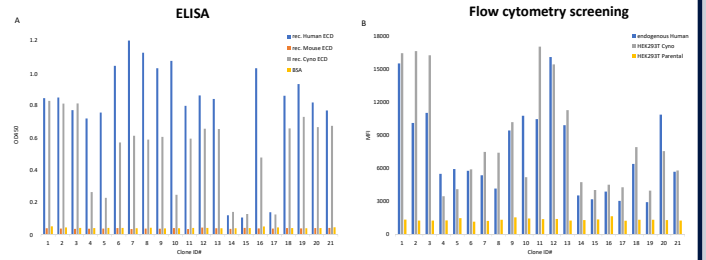


Fig. 2. Out of 13,000 primary hybridomas screened, 125 were selected for subcloning. Following reactivity analysis of all outgrown subclones, 21 subclones were selected for cryopreservation and target-reactivity rescreening by ELISA (A) and flow cytometry (B) to identify human/mouse/cyno target (cross-)reactive clones.

Sequence Diversity Analysis

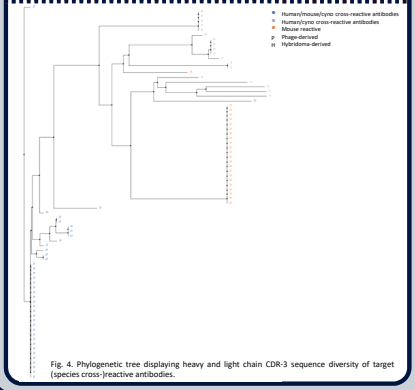
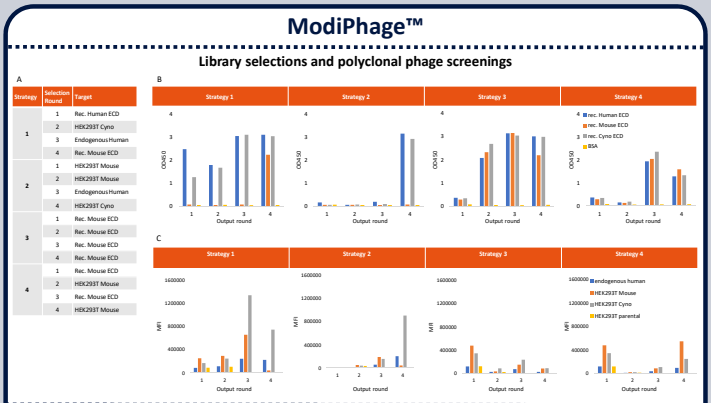


Fig. 4. Phylogenetic tree displaying heavy and light chain CDR-3 sequence diversity of target (species cross-)reactive antibodies.

ModiPhage™



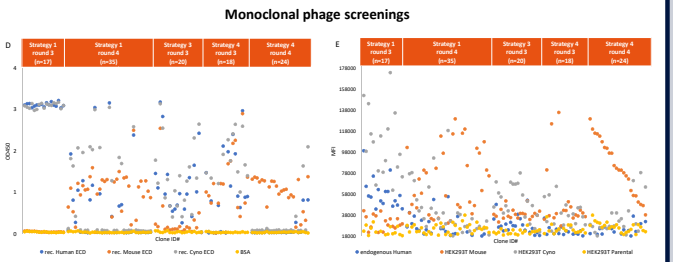


Fig. 3 Selection and identification of lead antibody candidates was performed by phage display scFv immune library generation and subsequent phage display selections. The immune OmniMouse® phage display library was used in 4 different selection strategies against recombinant target-ECD protein and/or cell-associated target (A). Polyclonal phage binding profiles of the various polyclonal phage outputs were examined by B) ELISA-based reactivity screening towards recombinant human, cyno, and mouse target-ECD protein and C) flow cytometry-based reactivity screenings against endogenously human target-expressing cells, recombinant cyno and mouse target-expressing HEK cell lines and corresponding parental HEK cells using iQue. Based on these data, outputs were selected for screening monoclonals. Phage supernatants of randomly picked clones of selected outputs were screened for their reactivity towards recombinant human, cyno, and mouse target-ECD protein by ELISA (D) and against endogenously human target-expressing cells, recombinant cyno and mouse target-expressing HEK cell lines and corresponding parental HEK cells by flow cytometry using iQue (E). Out of 360 monoclonals screened, the displayed 114 clones were identified as target-

Eight OmniMouse® were immunized with recombinant target-ECD proteins using two different alternating immunization strategies (Fig. 1A). Sera reactivity analysis revealed that 5 out of 8 immunized OmniMouse® showed a good immune response against both human and cyno recombinant target protein (Fig. 1B) and human and cyno cell-expressed target protein (Fig. 1C) as determined by ELISA and flow cytometry, respectively. Low serum reactivity against recombinant mouse target protein was observed for mouse #133 and was not detectable in the remaining mice (Fig. 1B). Based on sera analysis, top 3 best responder mice were selected for monoclonal antibody selection by ModiFuse™ (hybridoma generation by electrofusion, Fig. 2) and ModiPhage™ (scFv phage display selection, Fig. 3). Reactivity screening of the generated hybridomas against plate-immobilized target-ECD proteins by ELISA (Fig. 2A) and cell-associated target protein by flow cytometry (Fig. 2B) revealed that 21 hybridoma subclones could be identified as human and cyno target cross-reactive clones. No mouse target protein (cross-)reactive hybridomas were obtained. In addition to the hybridoma approach, a phage display strategy was designed to obtain full human, mouse and cyno target cross-reactive antibodies. Four different selection strategies against recombinant target-ECD proteins and/or cell-associated target were performed (Fig. 3A). Subsequent reactivity screening of the various polyclonal phage outputs revealed that 3 out of 4 selection strategies resulted in selection of plate-immobilized (Fig. 3B) and cell-associated (Fig. 3C) human, mouse and cyno target recognizing phages. Screening of phage supernatants of 360 randomly picked monoclonals from top 5 selection outputs resulted in a panel of antibodies with diverse target-reactivity profiles. Approximately 20% of the screened monoclonals showed full cross-reactivity towards plate-immobilized (Fig. 3D) and, importantly, cell-associated (Fig. 3E) human, cyno and mouse target. Sequence analysis of 7 selected hybridoma subclones and top 90 phage clones revealed 5 hybridoma-derived and 75 phage-derived unique antibody variable domain sequences (Fig. 4).

Conclusions

By applying ModiQuest® Research's fully integrated antibody discovery platform in combination with OmniMouse® immunization, a panel of sequence diverse, human lead antibodies specifically directed against a transmembrane protein were selected in a relatively short time span. Importantly, despite the initial very low mouse serum reactivity towards the mouse target protein, various antibody leads with broad species cross-reactivity (e.g. human, cyno and mouse target), which is required for preclinical validation, were obtained by applying DeepDisplay™, the powerful and unique antibody discovery platform.