Humanization of functional antibodies against membrane spanning proteins

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ImmunoPrecise
Formerly ModiQuest Research

Introduction

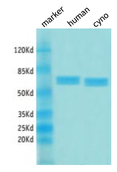
Immunized animals are still a key source for antibodies generated within therapeutic campaigns to obtain high affinity antibody drug candidates. Therefore, humanization and de-risking of these antibodies is an important antibody engineering step in the development of therapeutic antibodies. However, humanization is still characterized by a high risk as affinity and specificity can be lost during the humanization process. Therefore, ImmunoPrecise (formerly ModiQuest) developed a full antibody engineering platform (ModiTune™) to obtain humanized antibodies with similar affinity and specificity as the original antibody.

Methods & Results

Phase I

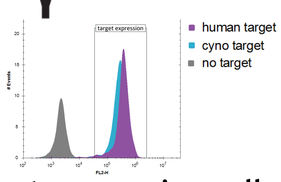
Antigen Generation

ModiXpress™



fusion proteins

ModiVacc™



Target-expressing cells

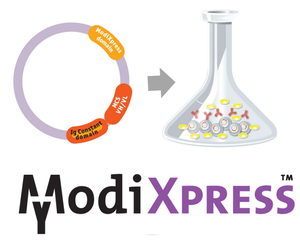
Biotinylated Fc-fusion proteins (>95% purity) and stable >2log target-overexpressing ModiVacc (MV) cells were generated.

CDR Grafting

ModiTune™

Cartoon of grafting

Production

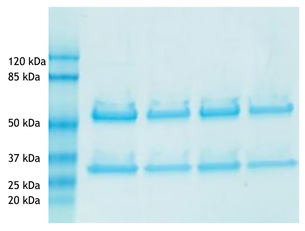


ModiXpress™

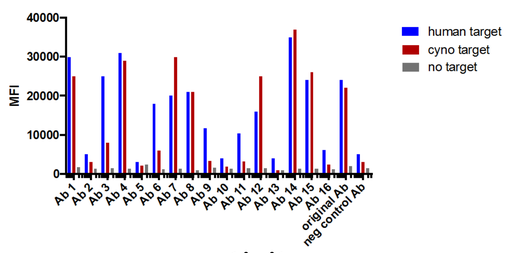
After CDR grafting of the mouse V-domains to the closest human germline gene using in-silico modelling, framework residue analysis and chemical liability analysis (e.g. deamidation sites), 16 variants (4x VH and 4x VL) were selected for production and characterization.

Full Characterization

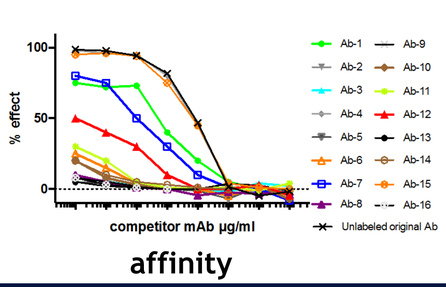
One representative image



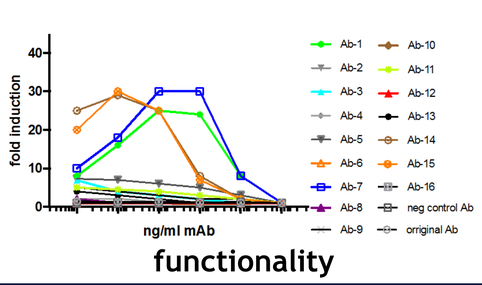
purity



reactivity

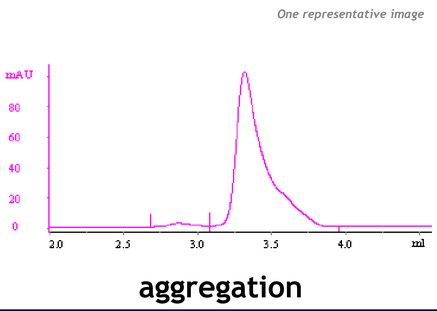


affinity



functionality

One representative image



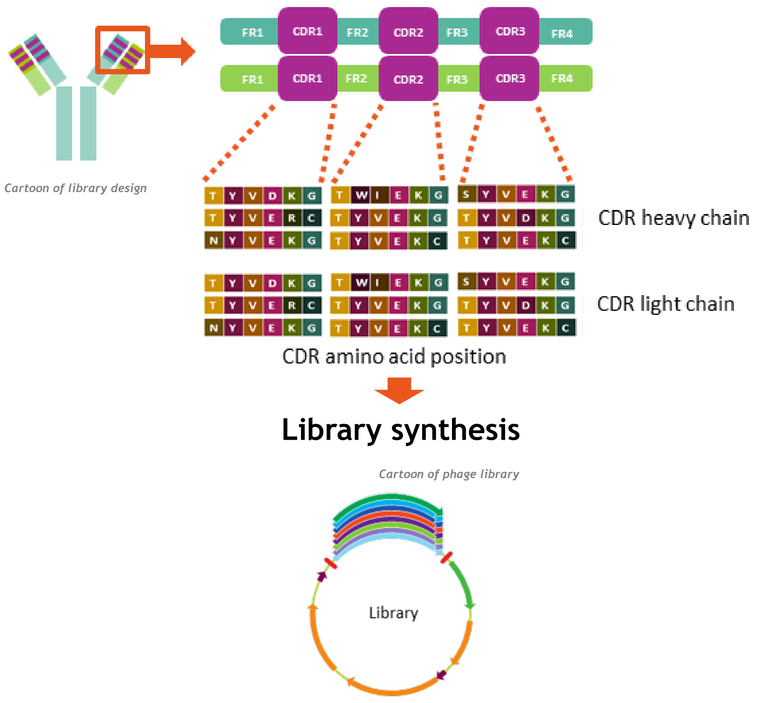
aggregation

Full characterization of produced antibodies for purity (SDS-PAGE), reactivity (FACS), affinity (HTRF), functionality and aggregation (SEC). Clone 7 was selected as lead antibody for library generation based on functionality and cross-reactivity to human cyno cell-expressed target protein.

Phase II

Library Design

ModiTune™



Cartoon of library design

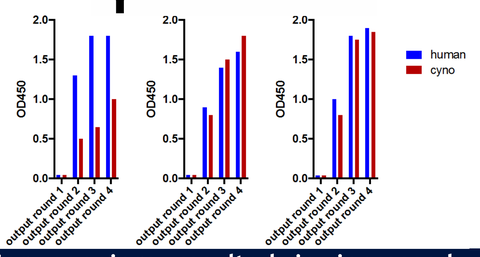
Library synthesis

Cartoon of phage library

Library (10^9) based on lead sequence was designed and synthesized using mutagenesis (random/directional/CDR only). Rescue of the library resulted in a library with a complexity of $3.5x10^8$ and full-length insert ratio of 86%.

Library Selection

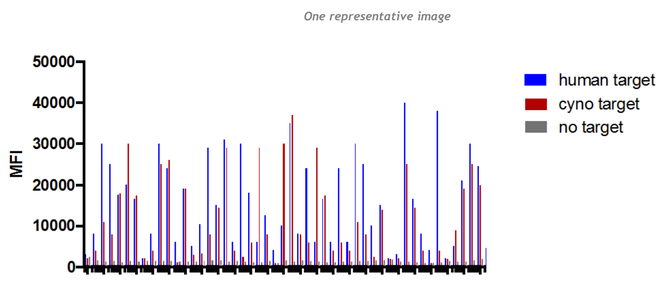
ModiPhage™



Alternating pannings resulted in increased output titers in subsequent selection rounds (data not shown), which represented enrichment of human/cyno cross-reactive target-specific clones.

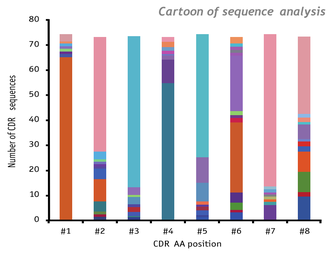
High-Throughput Monoclonal Phage Screening

One representative image



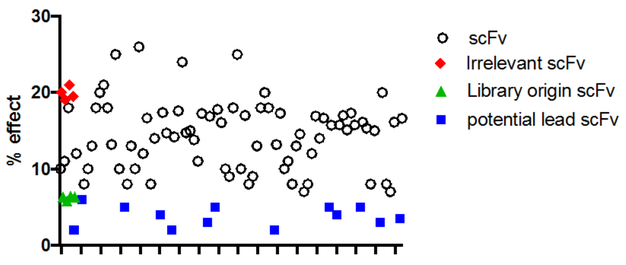
reactivity

Cartoon of sequence analysis



sequence diversity

One representative image

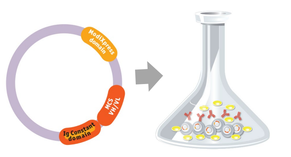


relative affinity

Monoclonal output screenings of ~1200 clones on target-expressing cells in FACS, HTRF and sequence diversity revealed ~100 unique target-reactive, good affinity clones of which 12 lead clones were selected for production and full characterization.

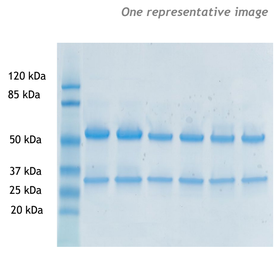
Phase III

Full Lenght IgG Production

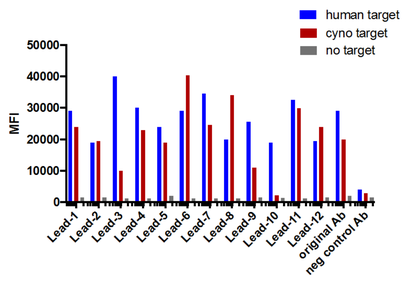


ModiXpress™

One representative image

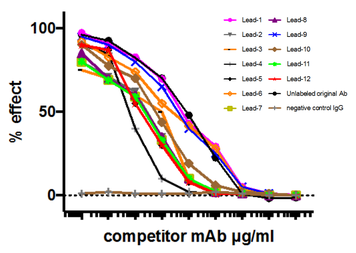


purity

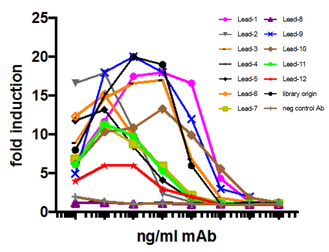


reactivity

Characterization

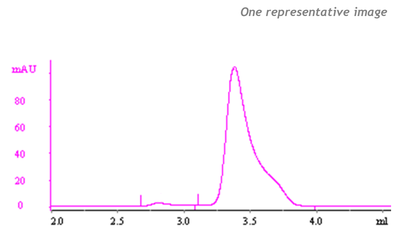


affinity



functionality

One representative image



aggregation

12 lead clones were selected for full-length hIgG1 production and screening for purity (SDS-PAGE), reactivity (FACS), affinity (HTRF), functionality and aggregation (SEC).

Conclusions

IPA's ModiTune™ humanization approach resulted in the humanization of a mouse antibody without affinity and specificity loss and the selection of 3 fully characterized therapeutic lead candidates to be taken forward into pre-clinical studies in less than a year.