Successful rabbit mAb CDR-grafting: The first step toward clinically suitable monoclonal antibodies

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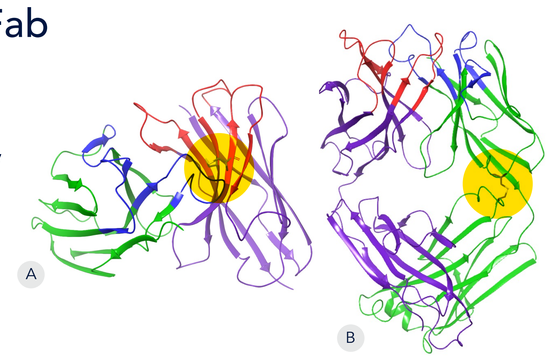
Introduction

Rabbit monoclonal antibodies are of high interest for therapeutic lead development due to their ability to recognize unique epitopes with relatively good affinity. These include epitopes which are poorly immunogenic in other species like mice. However, compared to murine-derived antibodies, humanization and subsequent clinical translation of rabbit antibodies to pharmaceutically relevant molecules might be more challenging due to the presence of additional disulfide bridges in the rabbit Fab, which are not present in the natural murine and human IgG repertoire. Here, we show successful humanization by both sequence-based and structure-based CDR-grafting. Designed CDR-grafted variants of the parental rabbit antibody were well produced and showed antigen binding potency similar to the chimeric version of the parental antibody.

Subsequently, *in silico* and *in vitro* developability analytical tools can be applied to guide the selection of human or humanized therapeutic mAb final lead candidates for further clinical development. Assessment of several key developability parameters will be exemplified for some benchmark reagents that possess good or compromised developability profiles.

Rabbit Fab versus human Fab

The rabbit Fab often contains additional disulfide bridges compared to the human Fab. Specifically, a disulfide bridge in the variable domain of the heavy chain between CDR1 and CDR2 is commonly observed. This heavy chain variable domain disulfide bridge, facing toward the light chain variable domain, may be of importance for correct conformation and stability, but corresponding residues are likely not directly involved in antigen binding as they are minimally solvent exposed. Next, an additional cysteine present in the variable domain of the most prominent rabbit light chain isoform forms a disulfide bridge with a cysteine in the constant region of the light chain.



Rabbit PDB id #5V6L. (A) Fv domain showing the additional disulfide bridge between CDR1 and CDR2 in the variable domain of the heavy chain (yellow circle). (B) Fab domain showing the additional disulfide bridge between the variable domain and constant domain in the light chain (yellow circle). CDR regions in VH and VL are coloured red and blue respectively, while framework regions are coloured purple and green respectively.

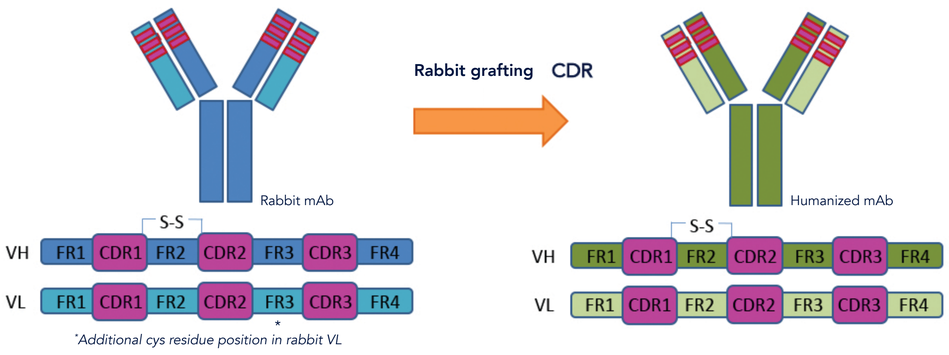
Rabbit humanization approach and empirical validation of the humanized mAb candidates

Sequence-based CDR grafting

For the parental rabbit Fv-domain of interest, the specific Fv amino acid residues defining the antibody paratope were unknown. To achieve the highest success rate of the correct antibody paratope conformation in the humanized constructs, CDR grafting was performed with CDR regions defined by various annotation schemes. The CDR residues of the rabbit Fv-domain were grafted on the closest human germline Fv sequence based on sequence homology. The cysteine residues forming a native disulfide bridge between HCDR1 and HCDR2 in the parental rabbit Fv were maintained, while the additional cysteine in the VL domain was removed as result of CDR grafting. *In silico* analysis of the CDR-grafted homology models was performed to identify the potential need for framework back mutations. In summary, 16 variants (4x VH and 4x VL, mAb ID #A-P) were designed for production (with variations in framework back mutations and/or used CDR annotation strategy). *In silico* analysis of the CDR grafted variants demonstrated that the maintained cysteine residues in the HCDR1/HCDR2 are well positioned to form a disulfide bridge.

Structure-based CDR grafting

A homology model of the parental rabbit Fv domain was generated and structurally compared to a library of human Fv-domain models using the *in silico* structure-based humanization tool of BioLuminate (Schrödinger). The human Fv-domain model with the highest overlap in framework regions where the CDR loops join was selected for CDR grafting (mAb ID #Q).

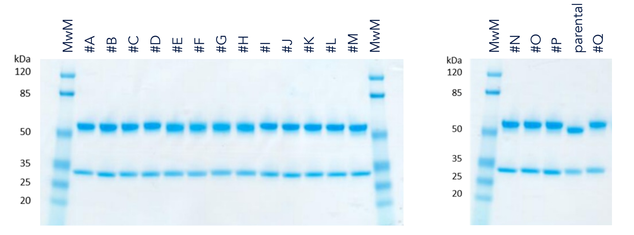


**Additional cys residue position in rabbit VL*

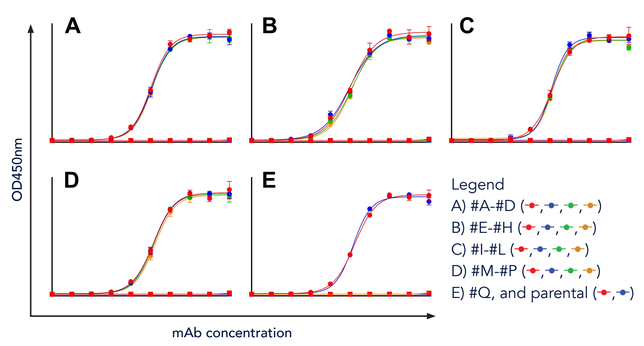
QC of recombinantly expressed humanized mAb variants

Antibody production
Recombinant mAbs #A-#Q showed 3- to 8-fold higher production yields compared to the chimeric version of the parental mAb suggesting good manufacturability of the CDR grafted humanized mAbs.

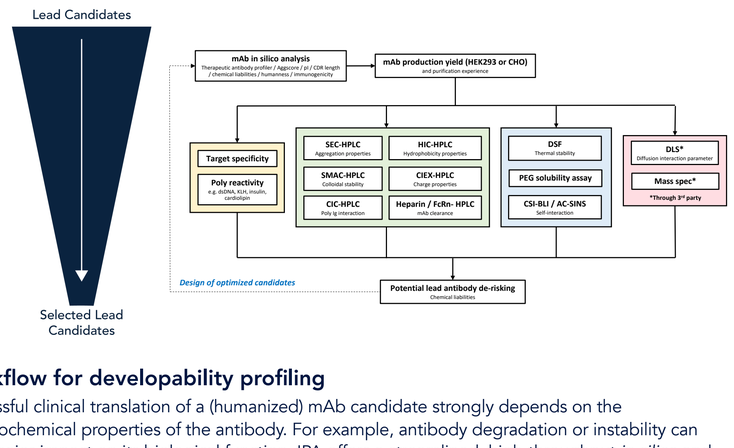
Antibody purity / integrity
Reduced SDS-PAGE of mAbs #A-#Q, the parental chimera, and molecular weight markers (MwM). The data shows pure mAb samples containing a heavy and light chain with bands corresponding to the expected molecular masses. Non-reduced SDS-PAGE showed single bands at 145-155 kDa range, suggesting proper integrity of the mAbs (data not shown).



Antibody reactivity
The humanized monoclonal antibodies #A-#Q were tested for antigen reactivity and compared to the chimeric parental mAb control. The various humanized antibodies display a highly similar binding profile to the parental control. Off-target binding of mAbs to BSA coated plates was not observed (data not shown).

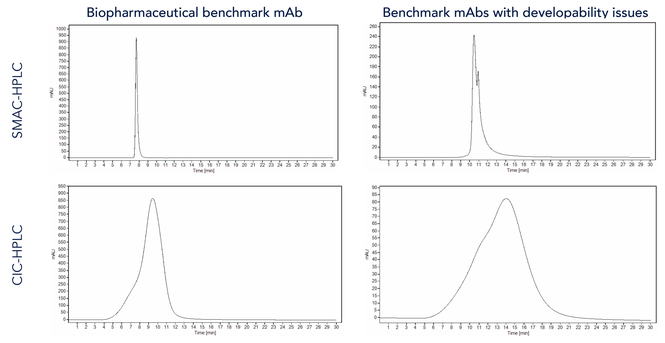


Developability profiling to promote clinical success



Workflow for developability profiling

Successful clinical translation of a (humanized) mAb candidate strongly depends on the physicochemical properties of the antibody. For example, antibody degradation or instability can have major impact on its biological function. IPA offers a streamlined, high-throughput *in silico* and *in vitro* workflow for developability profiling by the assessment of critical mAb quality attributes. I*n silico* analysis include identification of potential chemical liabilities and aggregation prone surface areas. *In vitro* analytical tools allow to study various critical quality attributes like production yield, target- and/or poly-reactivity, colloidal stability, melting temperature, self- and/or cross-interaction, solubility, and fragmentation. Our workflow is based on throughput and parallel efforts to monitor potential liabilities in an early stage. Based on the developability assessment, lead antibodies can be subjected to sequence optimization to enhance success rate for clinical translation.



HPLC-based developability assessment

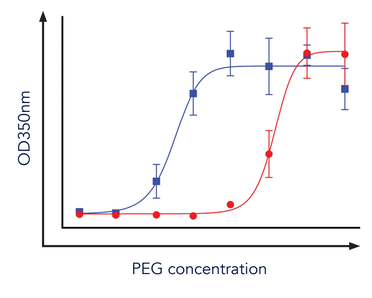
IPA is equipped with a state-of-the-art bio-inert HPLC enabling analytical determination of mAb biochemical and biophysical properties like aggregation levels (SEC-HPLC), colloidal stability (SMAC-HPLC), cross interaction (CIC-HPLC), Asn deamidation and isomerization (CIEX-HPLC), and Met / Trp oxidation (HIC-HPLC). Here, data is presented for SMAC-HPLC (top row) and cross-interaction human poly-Ig CIC-HPLC (bottom row) for a biopharmaceutical control antibody (left) and control mAbs with developability issues (right). The latter antibodies displays either strong column retention on SMAC-HPLC or CIC-HPLC due to a-specific interactions with the column material.

In silico profiling

IPA's *in silico* tool set includes in silico sequence analysis, Fv model-based structure analysis, and Fv model-based surface analysis for antibody lead candidates to identify e.g. chemical liability motifs, aggregation prone regions, and charge properties. These *in silico* engineering approaches facilitate focused antibody de-risking, if required, to improve the mAb developability profile.

Solubility profiling

The PEG solubility assay measures mAb turbidity (OD350nm) formation at increasing concentrations of PEG and facilitates comparison of solubility profiles of lead candidate mAbs as demonstrated here for an antibody with known reduced solubility (blue) and a biopharmaceutical control antibody (red).



Conclusions

In conclusion, leveraging IPA's in-house expertise and *in silico* antibody engineering tool set, rabbit mAbs were successfully humanized without affecting binding potency when compared to the chimeric parental mAb. IPA's analytical capabilities and streamlined developability profiling workflow in combination with sophisticated *in silico* antibody engineering tools facilitate selection and antibody sequence optimization of prioritized (humanized) lead candidate mAbs to promote clinical success.