Pharma&Biotech

Computational Approaches for Prediction of Developability Properties and Risk Mitigation of Biotherapeutics

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Summary

It is estimated that only 1 out of every 1,000 preclinical candidates reaches the commercial market. Most failures are due to a lack of efficacy and safety. Unwanted immunogenic responses can have serious consequences on the efficacy of drugs and potential patient safety. Stability and aggregation issues can affect affinity, function and safety of a product and can greatly increase costs of process development and manufacturing. The ability to assess the "developability" of a therapeutic candidate in early preclinical and clinical phases of development can be a very powerful tool to enhance the probability of success and reduce attrition rates.

In silico and *in vitro* methodologies can be employed to perform a developability assessment to aid selection of optimal leads with improved manufacturability and safety profiles and to highlight potential risks of failure including immunogenicity, lack of chemical stability, aggregation and low productivity.

Focus of Lonza's Developability Assessment Toolbox

- Aggregation
- Chemical Stability
- Immunogenicity

Developability Assessment

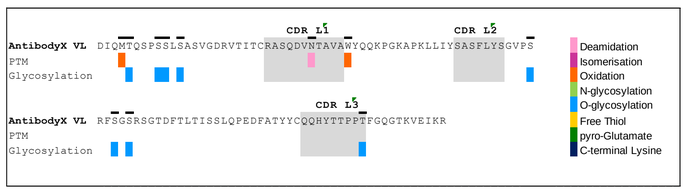
Manufacturability	Safety	Pharmacology & Mode of Action
• **Productivity** • **Quality** o Chemical stability (e.g. oxidation, degradation) o Physical stability (aggregation) o Post Translational Modifications • **Formulability**	• **Immunogenicity** o T cell epitopes o Cellular responses • **ImmunoToxicology** o Cytokine release syndrome • **Specificity** o Off-target interactions	• **PK/PD - Bioavailability** o Delivery / RoA o Half life o Formulability • **Mode of Action** o Immunomodulation o Dosing & patient segmentation o Efficacy

In Silico Manufacturability Assessment

Post Translational Modifications / Chemical Stability Assessment

- Post translational modifications (PTMs) may pose a risk for a candidate's manufacturability and safety by affecting binding affinity and function of the product, impacting productivity and resulting in the presence of different isoforms of the product
- Identify structural and sequence motifs that could result in PTMs and highlight those of highest risk
  - Deamidation sites
  - Aspartate isomerization sites
  - Oxidation sites (Met and Trp)
  - Free-Cysteine Thiol groups
  - N & O-Glycosylation sites

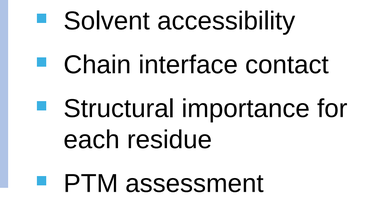
Light chain of a marketed antibody: deamidation in CDR-L1 is a known stability problem



Aggregation prediction / Physical Stability

- Protein aggregation can severely impact the manufacturing process, activity and pharmacokinetics of a product and contribute to immunogenicity
- Lonza's *in silico* model predicts the risk of antibodies aggregating
  - Models were trained and tested on 500 antibodies
  - Sequence and structural algorithms identify motifs with potential to impact on aggregation
  - Rank candidates based on high/low risk (5% aggregates)
  - 84% accuracy is achieved by the model on an external validation set of 49 mabs
  - Profile libraries and select best leads using high-throughput approaches

Diverse set of antibodies



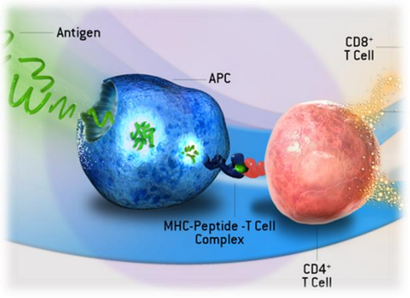
Sentinel APART™ Platform (Aggregation Prediction and Re-engineering Tool)

- Web-based platform to guide protein re-engineering by identifying aggregation-prone residues
- High-throughput screening, suitable for ranking of large sets (thousands) of sequences
- Provides in-depth analysis of residues contributing to aggregation
- Uses heat map to assess changes in aggregation risk due to a single or double mutation in antibody sequence (1-point and 2-point randomisation)
- Design antibody variants with reduced propensity to aggregate

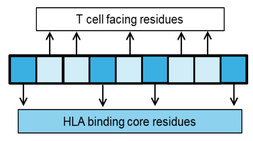
- Incorporates antibody engineering knowledge:
- Positional amino acid frequencies
- Antigen contact
- Solvent accessibility
- Chain interface contact
- Structural importance for each residue
- PTM assessment

In Silico Immunogenicity Assessment

Epibase® *In Silico* (IS) is a T cell epitope screening platform that analyses and predicts the potential immunogenicity of proteins.



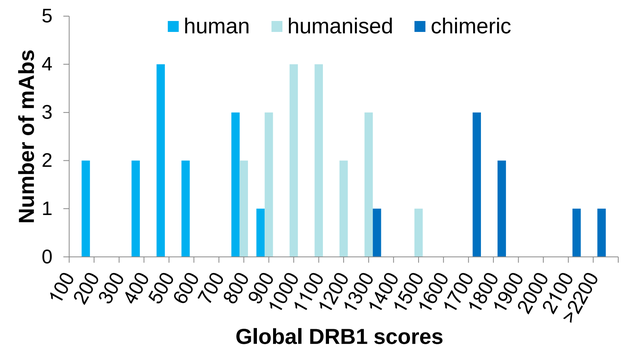
- The Epibase® tool predicts peptide/HLA binding
- Uses 3D structural characteristics of HLA receptors
- Statistical modelling based on experimentally determined binding affinities of peptides
- Epibase® utilises population frequencies and covers 99% of the global population (85 HLA allotypes)
- Applies self-peptide filtering (e.g. human antibody germline)
- Enhanced filter recognising 'self' on the level of T Cell Receptor (TCR) facing residues



Ranking of Proteins and Epitopes by Immunogenicity Risk

- DRB1 score combines multiple ranking criteria and reflects potential immunogenicity risk
- Relative ranking of protein leads by immunogenicity risk
- Identify and rank high risk T cell epitopes
- Guide re-engineering to remove immunogenic epitopes - deimmunisation

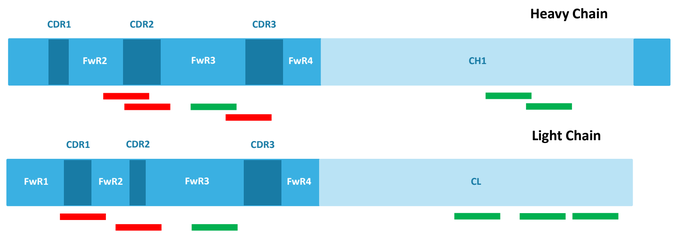
Immunogenicity risk scores for marketed antibodies



In Vitro Immunogenicity: MAPPs Case Study

- MHC-Associated Peptide Proteomics (MAPPs)
- Accurate identification of potential T cell epitopes
- Takes into account the protein uptake, cleavage and processing within the dendritic cells
- MAPPs peptides correlate with Epibase® *in silico* predictions

Human anti-TNFα monoclonal antibody known to induce ADA in the clinic



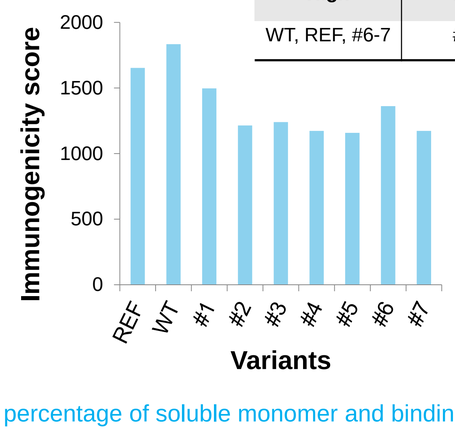
Red bars - High risk HLA binders identified by MAPPs
Green bars - Low risk binders (human germline regions)

Developability Assessment for Lead Selection

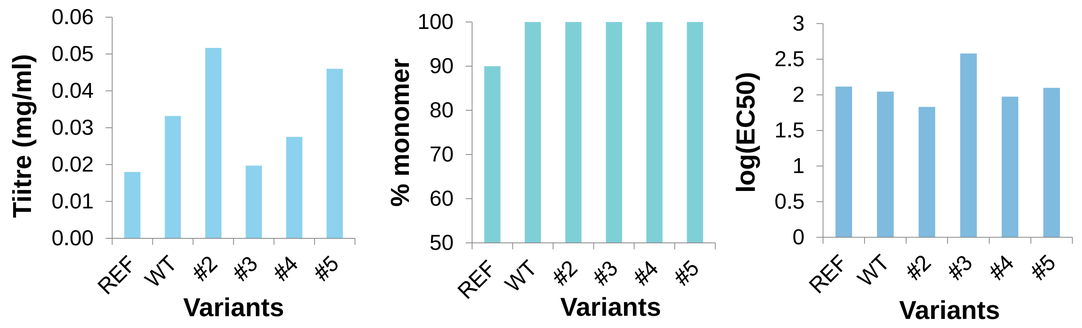
- Engineered variants of anti-IFNγ antibody
- Variants were profiled *in silico* for the aggregation risk and the immunogenicity potential using the Epibase® platform
- Four variants (#2-5) with good overall profile were progressed to *in vitro* expression and characterization
- Variants #2, #4 and #5 have better overall developability profiles: titre, aggregation, binding affinity and predicted immunogenicity
- The tool can be used to support candidate selection, reducing the number of molecules which need to progress to *in vitro* stages

Predicted aggregation risk and immunogenicity score

Predicted Aggregations Risk	
High	**Low**
WT, REF, #6-7	#1-5



In vitro characterization of the anti-IFNγ variants: titre, percentage of soluble monomer and binding affinity



Conclusion

- *In silico* and *in vitro* screening tools offer rapid, cost effective methods for lead selection and optimisation during biotherapeutic development
- Developability assessment reduce attrition in later preclinical and clinical development and increase the probability of success, increase product integrity and quality, increase aspects of clinical safety