Epibase™: Platform for Immunogenicity Assessment of Therapeutic Proteins
Olga Obrezanova, Andreas Arnell, Thomas Gallagher, Yannick Gansemans, Younes Mokrab, Sofie Pattijn, Yvette Stallwood, Philippe Stas
Applied Protein Services, Lonza Biologics plc, Granta Park, Great Abington, Cambridge, CB21 6DS, UK

Summary
Most therapeutic proteins are, to a variable extent, immunogenic. When developing protein therapeutics, it is essential to consider the possibility of immunogenicity from the beginning of a project. This risk is assessed by a number of in silico tools available these days. Epibase™ is a comprehensive platform for the assessment of immunogenicity risk at early stages, which is very useful for deimmunization and deimmunization of proteins. Epibase™ can be used as a cost-effective tool for assessing immunogenicity risk for therapeutic proteins.

Overview of MHC class II allotypes covered by Epibase™

<table>
<thead>
<tr>
<th>Allotype group</th>
<th>Epibase™ Western African</th>
<th>Epibase™ Eastern African</th>
<th>Epibase™ Caucasian</th>
<th>Epibase™ Oriental</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWEI</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>DBB/3.2/S</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>DP</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Criteria for Immunogenicity Ranking

- Ranking at the level of individual epitopes
- De-immunocontext
- Binding strength
- Filtered out by human antibody germlines or not (for antibodies)
- Promiscuity (binders to multiple HLA allotypes contribute more to the immunogenic potential than binders which only affect a single allotype)
- Importance (frequency) of affected allotypes in population
- DRB1 score for individual epitopes based on frequencies of affected DRB1 allotypes
- Ranking for whole proteins
- Lead selection context
- Counts of critical epitopes
- Risk score based on critical epitopes and frequencies of DRB1 allotypes

De-immunization Heat Map

- Assessment of an increase / decrease in immunogenicity potential due to a single mutation in protein sequence
- Enables de-immunization
- Available as part of Epibase™ servers

Case Study: De-immunization of VB6-845® Fab

- Vivenia’s VB6-845® is a anti-EpCAM recombinant immunotoxin
- Humanized Fab fragment fused to a deimmunized toxin (bougainvillea)
- Fab was de-immunized in silico using Epibase™
- De-immunized Fab showed reduced T cell activation potential in vitro

In Silico De-immunization

- Screening for T cell epitopes using Epibase™
- Antibody structure modelling
- Substitutions to eliminate T-cell epitopes while retaining affinity for target
- 14/19 (74%) of proposed mutations retained expression and binding affinity for EpCAM

In Vitro Verification of De-immunization

- Screening for T helper cell responses using PBMCs from healthy donors
- Detection methods covering immune responses to antigenic determinants

Case Study: Immunogenicity Screening for Lead Selection

- In silico Epibase™ screening
- In silico T cell epitope mapping and ranking of 131 A00 (albumin-binding domain) variants
- Rational selection of best candidates for in vitro testing based on their affinity, stability, and predicted immunogenicity

Epibase™ IV Testing

- Compare the immunogenic potential of wild type A00 and variants
- No significant immunogenicity was detected with A00D94

Conclusion

As shown by the VB6-845® and A00 case studies, combined in silico and in vitro testing provides a cost-effective and rapid solution to further reducing or avoiding potential immunogenic risk of therapeutic proteins and facilitates the selection of the best leads.

in silico T-cell epitope screening platform that analyses and predicts the potential immunogenicity of protein leads. Epibase™ IV evaluates immunogenicity potential of therapeutics by directly measuring T and B-cell responses in human population using PBMCs. Epibase™ version 3.0 has recently been released featuring increased prediction accuracy, updated allele population frequencies, improved speed of prediction and novel heat maps of variants to enable protein de-immunization. It provides efficient capabilities for high-throughput in silico immunogenicity profiling, lead selection and optimization. Combined in silico and in vitro testing provides a cost-effective and rapid solution to further reducing or avoiding potential immunogenic risk of therapeutic proteins and facilitates the selection of the best leads.

Early Stage Immunogenicity Assessment at Applied Protein Services

Most therapeutic proteins are, to a variable extent, immunogenic. When developing protein therapeutics, it is essential to consider the possibility of immunogenicity from the beginning of a project. This risk is assessed by a number of in silico tools available these days. Epibase™ is a comprehensive platform for the assessment of immunogenicity risk at early stages, which is very useful for deimmunization and deimmunization of proteins. Epibase™ can be used as a cost-effective tool for assessing immunogenicity risk for therapeutic proteins.