Predictive Immunogenicity in lead discovery
Immunogenicity

‘Immunogenicity is the ability of an antigen to provoke an immune response’
**Immunogenicity** = The intrinsic capacity of a product to induce an immune response in a target population

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Therapeutic proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designed to mount a maximal protective immune response</td>
<td>Designed to exert therapeutic function (biological activity) in vivo</td>
</tr>
<tr>
<td>- Raise Effector T-cell function (HTL/CTL) for fighting disease</td>
<td>- Avoid T-cell activation (HTL) for helping antibody generation</td>
</tr>
<tr>
<td>- Raise broadly neutralising antibodies to prevent new infection and/or further spread</td>
<td>- Avoid neutralising antibodies that prevent the protein from exerting its therapeutic function</td>
</tr>
</tbody>
</table>

**WANTED IMMUNOGENICITY**

**UNWANTED IMMUNOGENICITY**
# Observed Immunogenicity

## Non-antibody protein therapeutics:

<table>
<thead>
<tr>
<th></th>
<th>Abs</th>
<th>Nabs</th>
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<tbody>
<tr>
<td>IFN-α</td>
<td>± 50%</td>
<td>± 25-30%</td>
</tr>
<tr>
<td>IL-2</td>
<td>± 50%</td>
<td>± 5%</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>± 70-95%</td>
<td>± 0-50%</td>
</tr>
<tr>
<td>F-VIII</td>
<td>± 40%</td>
<td>± 30%</td>
</tr>
</tbody>
</table>

anti-idiotypic networks are an example of Abs against self proteins
## Observed Immunogenicity

<table>
<thead>
<tr>
<th>Therapeutic protein</th>
<th>Type</th>
<th>Target</th>
<th>Indication</th>
<th>Assay</th>
<th>AR (%)</th>
<th>Pop</th>
<th>Sup</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT3</td>
<td>murine</td>
<td>CD3</td>
<td>Graft rejection</td>
<td>ELISA</td>
<td>54</td>
<td>82</td>
<td>+</td>
</tr>
<tr>
<td>Bexxar/tositumomab</td>
<td>murine</td>
<td>CD20</td>
<td>Non-Hodgkin's lymphoma</td>
<td>ELISA</td>
<td>9</td>
<td>55</td>
<td>+</td>
</tr>
<tr>
<td>Reopro/abciximab</td>
<td>chimeric</td>
<td>GPIIb/IIIa</td>
<td>Coronary angioplasty</td>
<td>ELISA</td>
<td>21</td>
<td>500</td>
<td></td>
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<tr>
<td>Remicade/infliximab</td>
<td>chimeric</td>
<td>TNFα</td>
<td>Crohn's disease</td>
<td>ELISA</td>
<td>9</td>
<td>199</td>
<td>+</td>
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<tr>
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<td>chimeric</td>
<td>TNFα</td>
<td>Crohn's disease</td>
<td>ELISA</td>
<td>61</td>
<td>125</td>
<td>+</td>
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<tr>
<td>Remicade/infliximab</td>
<td>chimeric</td>
<td>TNFα</td>
<td>Rheumatoid arthritis</td>
<td>ELISA</td>
<td>8</td>
<td>60</td>
<td>+</td>
</tr>
<tr>
<td>Rituxan/rituximab</td>
<td>chimeric</td>
<td>CD20</td>
<td>Non-Hodgkin's lymphoma</td>
<td>ELISA</td>
<td>0</td>
<td>37</td>
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<td>chimeric</td>
<td>CD20</td>
<td>Systemic lupus</td>
<td>ELISA</td>
<td>65</td>
<td>17</td>
<td>+</td>
</tr>
<tr>
<td>Rituxan/rituximab</td>
<td>chimeric</td>
<td>CD20</td>
<td>Systemic lupus</td>
<td>RIA</td>
<td>27</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td>Raptiva/efalizumab</td>
<td>humanised</td>
<td>CD11a</td>
<td>Psoriasis</td>
<td></td>
<td>2.3</td>
<td>501</td>
<td></td>
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<tr>
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<tr>
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<td>Psoriasis</td>
<td>ELISA</td>
<td>6</td>
<td>1063</td>
<td></td>
</tr>
<tr>
<td>Campath/alemtuzumab</td>
<td>humanised</td>
<td>CD52</td>
<td>Rheumatoid arthritis</td>
<td></td>
<td>63</td>
<td>40</td>
<td></td>
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<tr>
<td>Campath/alemtuzumab</td>
<td>humanised</td>
<td>CD52</td>
<td>Rheumatoid arthritis</td>
<td></td>
<td>29</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Campath/alemtuzumab</td>
<td>humanised</td>
<td>CD52</td>
<td>Rheumatoid arthritis</td>
<td></td>
<td>53</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Campath/alemtuzumab</td>
<td>humanised</td>
<td>CD52</td>
<td>B-cell lymphoma</td>
<td></td>
<td>1.9</td>
<td>211</td>
<td></td>
</tr>
<tr>
<td>Humira/adalimumab</td>
<td>human</td>
<td>TNFα</td>
<td>Rheumatoid arthritis</td>
<td>ELISA</td>
<td>5</td>
<td>1062</td>
<td>+</td>
</tr>
</tbody>
</table>

Van Walle et al, Expert Opin. Biol. Ther., 7(3), in press
Therapeutic antibodies

- rituximab chimeric CD20 NHL 0 37 multiple iv
- rituximab chimeric CD20 SLE 65 17 multiple iv
- rituximab chimeric CD20 RA 27 15 multiple iv

Hwang & Foote, 2005
Generating a risk profile of the potential immunogenicity

- Analysis of the probability to observe immunogenicity
- Analysis of the severity of the observed immunogenicity
According to the EMEA ...

European Medicines Agency

London, 13 December 2007

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS
4.2 Non-clinical assessment of immunogenicity and its consequences

Therapeutic proteins show species differences in most cases. Thus, human proteins will be recognized as foreign proteins by animals. For this reason, the predictivity of non-clinical studies for evaluation of immunogenicity is considered low.

Non-clinical studies aiming at predicting immunogenicity in humans are normally not required.

However, ongoing consideration should be given to the use of emerging technologies (novel in vivo, in vitro and in silico models), which might be used as tools.
Preclinical Strategies

**In vivo strategies**
- Animal studies exploring ADA response
- Transgenic animal studies exploring T-cell responses
- Tolerized animal models/humanized animals

**In vitro strategies**
- T-cell epitope binding assays (HLA binding assays)
- T-cell activation and proliferation assays
- B cell assays

**In silico strategies**
- T-cell epitope mapping tools
Preclinical Immunogenicity screening

- **In vivo strategies**
  - Animal studies exploring ADA response
  - Animal studies exploring T-cell responses
  - Tolerized animal models
- **In vitro strategies**
  - T-cell epitope binding assays
  - T-cell activation and proliferation assays
- **In silico strategies**
  - T-cell epitope mapping tools
In-vivo methods

- Increasingly used to study immunogenicity
  - as predictive tools to assess immunogenicity
  - in studying the mechanisms underlying immunogenicity

- What needs to be predicted
  - neo-epitopes on modified proteins,
  - relative immunogenicity between products
  - breaking of tolerance,
  - immunogenicity in patients,
  - incidence of immunogenicity in patients restricted
  - clinical consequences of antibody development

- However, animal models needs critical evaluation.
  - species differences,
  - predictive value of such models is limited,
  - mechanistic studies can be

In vivo Predictive methods

- Difficult to map observed immunogenicity in animal studies to results in human
- Murine and primate models different from humans
- HLA transgenics
- Evolution towards using mouse models through the grafting of hematopoietic stem cells in immunodeficient mice. Models in Rag2-/- γc-/- and NOD/SCID/IL2r γc-/- mice demonstrated to develop of human DC, B- and T-cells.
- Breaking-tolerance: humanized and/or transgenic mice.
Preclinical Immunoprofiling
Preclinical Immunoprofiling

Clinical trials: ADA responses
Preclinical Immunoprofiling

Clinical trials: ADA responses

HLA binding
T cell activation
Preclinical Immunoprofiling

Clinical trials: ADA responses

B cell analysis

HLA binding
T cell activation
Predict and Reduce Immunogenicity?

- B cells need T-cell help to produce high affinity antibodies
- Eliminate T-helper epitopes will potentially reduce / diminish immunogenicity

**B-cell epitopes:** The surface patches required for B-cell response

**T-cell epitopes:** Peptide sequence of the protein needed to ensure effective T-cell help
**In Silico Immunoprofiling**

![Diagram showing the process of lead discovery, lead selection, lead optimization, process development & formulation, preclinical, and clinical trials]

- **In silico T-Epitope Identification**
- **In vitro T-cell and B-cell Assays**
- **Anti-Drug Antibody Screening**

**T-cell Epitope Prediction**
- Lead ranking and selection
- Deimmunization in combination with the support of protein modelling
In Silico Immunoprofiling

Clinical trials: ADA responses

In Silico: HLA binding
In Vitro: T cell activation
T-cell epitope identification

- **In silico methods**
  - Low cost
  - High throughput

- **Previous generation methods:**
  - Methods by inference
  - Sequence based methods
    - Based on known epitopes and sequences comparison
    - Use of different kinds of learning based algorithms
    - Bias towards known “peptide motives” and “anchor residues”

- Inference based methods tend to become better as more experimental data exists and fail on less studied HLA subtypes
Previous Generation tools

1. Public Database
2. Sequence Analysis
3. Target Sequence
4. Motif Found?
5. Epitope Motif (XVXXXXXXXL)
6. Epitope List
7. Test Peptides in vitro
8. Peptide confirmed?
Epibase™ for epitope prediction

- Analyze the antibody sequence
- Explore whether 10-mer peptides can bind to the MHC receptor
- Predictive tool driven by structural bioinformatics in conjunction with experimental data

Usage:
- Project Basis: compare a limited set of lead candidates in a program to explore which drug to proceed.
- Compute server: to screen libraries or high volume selection techniques
Population frequencies from Doolan et al. 2000

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>Japanese</th>
<th>Chinese</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>27</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>DR3</td>
<td>14</td>
<td>14</td>
<td>N/A</td>
</tr>
<tr>
<td>DR7</td>
<td>8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DR8</td>
<td>7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DR9</td>
<td>24</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Epibase™ and MHCII Population Frequencies**

- **DR1**: (DBR1*0101)
- **DR3**: (DBR1*0301)
- **DR7**: (DBR1*0701)
- **DR8**: (DBR1*0802)
- **DR9**: (DBR1*0901)
Case Study 1: Ofatumumab and Rituximab

- Targeting CD20, a B-cell differentiation antigen
- Treatment of
  - Cancer: e.g. Follicular lymphoma.
  - Inflammatory disease: e.g. Rheumatoid arthritis, SLE
- Observed immunogenicity of Rituximab
  - <1% in B-CLL
  - 35-60% in SLE
  - 4.3-23% in RA
  - Chimeric antibody
- Ofatumumab
  - BLA in B-CLL
  - Phase III in RA
  - Fully human antibody
Immunoprofile: Ofatumumab and Rituximab

- Ofatumumab is very clean in epitopes as compared to rituximab

- Ofatumumab contains no epitopes for HLA allotypes associated with RA

<table>
<thead>
<tr>
<th>HLA class II gene</th>
<th>RA Risk ratio</th>
<th>Epitopes in rituximab</th>
<th>Epitopes in ofatumumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0401</td>
<td>1 in 35</td>
<td>2 strong</td>
<td>no</td>
</tr>
<tr>
<td>DRB1*0404</td>
<td>1 in 20</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>DRB1*0101</td>
<td>1 in 80</td>
<td>4 strong</td>
<td>no</td>
</tr>
<tr>
<td>0401 and 0404</td>
<td>1 in 7</td>
<td>2 strong</td>
<td>no</td>
</tr>
</tbody>
</table>
Case Study 2: Adalimumab

- Human antibody recognizing TNF-α isolated by phage-display technology

- 109 RA patients enrolled for the study (collaboration with Sanquin and Genmab)

- Patients were tested for:
  - HAHA response (low, high)
  - determined from the binding of the Humira Fab fragment to protein A absorbed patient IgG

- DQ, DR high resolution typing
  - no DP typing was done as no strong epitopes were identified by Epibase®
Immunoprofiling of Adalimumab

- **Epibase profiling**
  - Epitope identification on full sequence
  - Removal of epitopes present in the human germline
  - Critical epitopes are identified as the strong and medium binders to DRB1, and the strong binders to DRB3/4/5, DQ and DP

- **7 strong epitopes found**
  - 5 strong epitopes in the VH
    - 2 in the FwR2-HCDR2 region
    - 3 in the FwR3-HCDR3 region
  - 2 strong epitopes in the VL:
    - LCDR1 and FwR3-LCDR3
Patient Data

- Level of HAHA response
  - 19 patients show a HAHA response, i.e. 17.6% of the patients are HAHA +

- RA associated HLA allotypes:

<table>
<thead>
<tr>
<th>Allotype</th>
<th>Caucasian</th>
<th>RA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0101</td>
<td>17.2%</td>
<td>28.4%</td>
</tr>
<tr>
<td>DRB1*0401</td>
<td>9.8%</td>
<td>52.3%</td>
</tr>
<tr>
<td>DRB1*0404</td>
<td>5.9%</td>
<td>9.2%</td>
</tr>
</tbody>
</table>
Epitopes and HAHA response

- The 7 strong epitopes explain 17/19 HAHA+ patients
- Epitopes are directed against the RA associated allotypes

<table>
<thead>
<tr>
<th>Epitopes</th>
<th>Region</th>
<th>HLA allotypes</th>
<th>HAHA+ patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FwR2-HCDR2</td>
<td>DRB1*0701</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>FwR2-HCDR2</td>
<td>DQA1*0201</td>
<td>DQB1*0303</td>
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<tr>
<td></td>
<td></td>
<td>DQA1*0401</td>
<td>DQB1*0402</td>
</tr>
<tr>
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<td>DQB1*0301</td>
</tr>
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<td>DRB1*0401</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DRB1*0405</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DRB1*0407</td>
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<td>6</td>
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<td>DQA1*0501</td>
<td>DQB1*0201</td>
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<td>7</td>
<td>FwR3-LCDR3</td>
<td>DRB5*0101</td>
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</tr>
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</table>
Case study 3: Anticalin®

- Anticalins® are engineered human proteins with prescribed binding properties derived from the lipocalin fold

- Lipocalins
  - Highly-conserved family of structural proteins
  - Optimized by evolution to perform diverse binding and physiological functions
  - Function in human tissues and body fluids in the presence of the human immune system
  - Low molecular weight, non-glycosylated, monomeric human proteins

- Pieris AG has pioneered the design of Anticalins® from lipocalins by advanced protein engineering
Potential immunogenicity of anticalins

- Epibase® profiles were generated for a human lipocalin and four derived, target-specific Anticalin® lead candidates

Results:
- Number of mapped epitopes in lipocalin and Anticalins® ery limited

<table>
<thead>
<tr>
<th></th>
<th>DRB1</th>
<th>DRB 3/4/5</th>
<th>DP</th>
<th>DQ</th>
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<tbody>
<tr>
<td></td>
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<td>medium</td>
<td>strong</td>
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<tr>
<td>lipocalin</td>
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<td>Anticalin® 1</td>
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In Vitro Immunoprofiling

An Indication of External Factors on Drug Immunogenicity

- Formulation
- Aggregates
- Degradation products
- Production contaminants
- Biosimilar / Innovator comparisons

In silico T-Epitope Identification

In vitro T-cell and B-cell Assays

Anti-Drug Antibody Screening
**In Vitro Immunoprofiling**

- *In silico* T-Epitope Identification
- *In vitro* T-cell and B-cell Assays
- Anti-Drug Antibody Screening

**Characterize T cell epitopes guided by in silico**
- At individual donor and population level

**Comparison of immunogenicity between biosimilar/second generation products and reference products**
- Healthy population/Patient population
Cellular Immunoprofiling

Chirino et al., DDT, 2004
Identify T-Cell Responses: Overview Read Out Parameters

- Cell surface markers (CD3/CD4)
- **Proliferation (EDU)**
- Cytokine analysis
  - Intracellular cytokine staining
  - CBA
  - ELISA
- Antigen-specific T cells
  - HLA class I tetramers
  - HLA class II tetramers

CD4/CD8 T-cell ELISPOT
**Screening Strategy**

1. **Donor**
   - HLA typing
   - 50 PBMC preparation
   - Assay development
   - Screening
     - 50 donor cell preps
     - Check by independent reviewer
     - Check quality PBMC by polyclonal T-cell activation assay

2. **Peptides/proteins**
   - Assay development
   - Screening
     - 50 donor cell preps
   - Training of operators

3. **Technology platform**
   - Flow cytometry
   - ELISpot
     - Using statistical program « R »
     - QC/QA of raw data and manipulations
     - Following project-specific flow charts
   - Readout & data management
   - Data analysis

**Final report**
Donor Population

Frequency (%)

Study Cohort
World Population

Donor Population

10-Feb-12
EIP Course - Introduction to Immunogenicity
PBMC Quality Control

Blood collection
  ➢ within 3 hours after collection

PBMC isolation

Counting
  ➢ Automated cell counting by Guava

Freezing
  ➢ Automated cell freezing by Nicool+

Liquid nitrogen storage
  ➢ Sample storage management in LIMS
Epibase IV: PBMC Data Recording

Frozen cell preparations overview

- **Name:** CP00903
- **Donor:** AIV00221
- **Type:** PBMC
- **Source:** whole blood
- **Protocol:**
- **Preparation date:** 2009-01-06
- **Prepared by:** sarah
- **Total prepared batches:** 2
- **Total prepared tubes:** 152 x 10^6
- **Total prepared cells:** 152 x 10^6
- **Total reserved cells:** 0 x 10^6
- **Total available tubes:** 4
- **Total available cells:** 71 x 10^6
- **Project:** PRJ00009
- **Experiments:** 4
- **Reference:** ALG-00280-20090106

QC:
- Yes

Comment:
- Derived preparations: 3

Attributes:
- % monocytes in PBMC pool: 26.8
- % CD4+ in CD3+ pool: 82.1
- % CD8+ in CD3+ pool: 13.5
- % CD14+ in PBMC pool: 24.6
- % CD3+ in PBMC pool: 49.3
T Cell Assays: whole PBMC formats

PBMC
+ Antigen
Day 0
Day 1-7
Analyze T cells

PBMC/mono
CD14 isolation
Day 7 + Antigen
Day 9-11
Analyze T cells

+ Antigen
Day 0
T Cell Assays: DC/CD4+ format

1. CD14 isolation

GM-CSF
IL4

Day 0

1. Antigen
2. Maturation cocktail

Day 4-5

Wash, count & incubate

Day 6-7

2. CD4 isolation

count & incubate

Day 12-15 Readout

Analyze T cells
Analyze T Cells: ensure quality

PARTICIPATE IN PROFICIENCY PANELS
ELISPOT PROFICIENCY PANEL IV CVC-2009, REPORT FOR LAB 22, DATE: September 20, 2009

Figure 6. Overall Results – Donor 2, CEF

Reference value

Quality Controlled using Immunspot 5.0.3
Case Study: Background

Half-life extension by Albumin binding

Serum albumin an ideal carrier
- Long half-life (17-19 days)
- Favorable dosing
- Convenience
- Safety
- Wide distribution
- Present in plasma (40%) and tissues (60%)
- Albumin Binding Domain (ABD)
  - high affinity for HSA
  - Small size (5 kDa)
Case Study: objective

- Originally naturally occurring bacterial protein: $\text{ABD}_{\text{wt}}$ with known T-cell epitope (Goetsch 2003)
  - Affinity maturation to femtomolar affinity (Jonsson 2008)
  - Structure analysis and modelling of ABD variants
- Protein engineering for stability, expression yield and reduced immunogenicity
  - Validation by Algonomics/Lonza
    - T-cell epitope mapping in silico
    - T-cell proliferation assay of selected deimmunized mutants

\[\text{ABD}_{\text{wt}}\]

Bacterial

Johansson02

\[\text{ABD}_{035}\]

Affinity matured

Jonsson08

\[\text{ABD}_{094}\]

Deimmunized

unpublished
Epibase™ *In Silico* Profile of ABD and variants

- **Epibase™ screening**
  - In silico T cell epitope mapping and ranking of 131 variants, selected based on their stability, affinity and predicted antigenicity / immunogenicity
  - Rational selection of best candidate for in vitro testing
Epibase™ *In vitro* Testing of ABD and variants

Compare the immunogenic potential of wild type ABD and variants based on:

- number of responsive donors
- mean SI over the population
- Relative response
Epibase™ *In vitro* Testing of ABD and variants: results

No significant immunogenicity was detected with ABD094

*Significantly different compared to buffer alone.*
Conclusion

- In contrast to wildtype ABD, no significant immunogenicity was detected with ABD094.

- Combined *in silico* and *in vitro* approach used for testing of mutants allowed for discrimination of molecules differing in only one amino acid.

- *In silico* mapping provides a cost effective and rapid solution to further reducing or avoiding potential immunogenicity risk of therapeutic proteins.
Case study: CHemotaxis Inhibitory Protein

- 14 kDa protein
- High affinity for the C5aR ($K_D \sim 1 \text{nM}$)
- Blocks the binding of C5a to the C5aR
- Potent inhibitor of C5a-induced activation of phagocytes \textit{in vitro}
- Promising drug candidate for acute indications
Material of non-human origin

The majority of the human population has circulating anti-CHIPS antibodies

Phase I:
Adverse effects due to immunocomplexes

→ To develop CHIPS as an anti-inflammatory drug, this must be taken into consideration.
Removal of B cell epitopes

Aim:
Remove antigenic epitopes in CHIPS while retaining function

Methods:
Truncation, random mutagenesis, in vitro evolution, site directed mutagenesis, ELISA using human anti-CHIPS antibodies, Ca+ flux, ELISA using receptor peptides, FACS
Reduce interaction with pre-existing ADA

CHIPS variants screened for decreased interaction with human anti-CHIPS IgG.....
Reduce interaction with pre-existing ADA

The new, less antigenic CHIPS variants are mutated in one or several of the amino acid positions shown.
Reduce interaction with pre-existing ADA

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Reducing Immunogenicity

Study each of the 12 substitution sites
At each site, consider wt and 19 mutants

All strong epitopes overlapping with substitutions can be removed

Only one strong DQ/DR epitope left
CHIPS: Conclusions

CHIPS variants with drastically decreased interaction with human anti-CHIPS antibodies and with retained function have been identified.

No major differences between variants and Wt with respect to T cell epitopes.

Optimal clones with respect to minimized T cells epitopes were designed – under evaluation.
Thank you for your attention
Any Question to: Philippe Stas
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Lonza
Applied Protein Services
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Case Study: De-immunization of VB6-845®

**Background**

- Viventia’s anti-EpCAM recombinant immunotoxin
  - Humanized Fab fragment fused to a deimmunized toxin (bouganin)
- Targets and mediates cell death in EpCAM-positive solid tumors
- First-in-man Phase I trial assessed the safety of VB6-845 in 13 patients with various EpCAM-positive cancers
  - Low or no antibody responses against deimmunized bouganin portion
  - Observed immune response to Fab moiety

**Objective**

- Minimize the potential immunogenicity risk of the fusion protein by deimmunizing the Fab portion
Case Study: De-immunization of VB6-845®

- **In silico de-immunization**
  - Screening for T cell epitopes using Epibase™
  - Antibody structure modelling
  - Substitutions to eliminate T cell epitopes based on structure integrity

- **In Vitro verification and testing of deimmunized protein**
  - Screening for T helper cell responses using PBMCs from healthy donors
  - Individual and population responses
De-immunization of VB6-845® Fab

- **Epibase™ screening**
  - Epitope identification on Fab sequence
  - Filtering of epitopes present in human germlines
  - Critical epitopes are identified as strong binders to DRB1 and DRB 3/4/5

- **Identification of mutations that remove epitopes**
  - Prevention of novel epitopes
    - For other allotypes
    - In overlapping frames
  - Respecting structural integrity of the protein
    - Stability
    - Function (e.g. affinity for ligand)
De-immunized VB6-845® Fab

- **Proposed changes**
  - 19 mutations (11 in VH and 8 in VL) removed critical epitopes or decreased the affinity of remaining epitopes
  - 14 out of 19 proposed mutations (10 in VH and 4 in VL) retained expression and affinity for EpCAM: 74% success rate

- De-immunized Fab has a similar binding affinity to wild type

  Binding Affinity

  \[
  \text{De-Fab: } K_D = 1.31 \times 10^{-9} \\
  \text{WT: } K_D = 1.56 \times 10^{-9}
  \]
In vitro Testing of De-immunized VB6-845® Fab

Compare the immunogenic potential of de-immunized Fab to wild type based on:

- Number of responsive donors
- Mean SI over the population
- Relative response
In vitro Testing: single donor and population level

De-immunized Fab shows a substantial and significant reduction in its ability to raise T cell responses
Conclusion

- De-immunized anti-EpCAM Fab showed reduced T cell activation potential in vitro, compared to wild type

- A 2nd generation VB6-845 molecule has been engineered and is now ready for testing in Phase I trials

- In silico deimmunization provides a cost effective and rapid solution to further reducing or avoiding potential immunogenicity risk of therapeutic proteins