Immunogenicity of biotherapeutics
Immune response

Winslow, 2001
## 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>Supr</th>
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<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>
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<tr>
<td>Bexxar/tositumomab</td>
<td>murine</td>
<td>CD20</td>
<td>Non-Hodgkin's lymphoma</td>
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<td>9</td>
<td>55</td>
<td>+</td>
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<tr>
<td>Reopro/abciximab</td>
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<td>GPIIb/IIa</td>
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<td>TNFα</td>
<td>Crohn's disease</td>
<td>ELISA</td>
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<td>199</td>
<td>+</td>
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<td>ELISA</td>
<td>8</td>
<td>60</td>
<td>+</td>
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<td>17</td>
<td>+</td>
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<td>Raptiva/efalizumab</td>
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<tr>
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<td>CD52</td>
<td>Rheumatoid arthritis</td>
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<td>CD52</td>
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<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>
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*Van Walle et al, Expert Opin. Biol. Ther., 7(3)*
Therapeutic antibodies

<table>
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<tr>
<th>Antibody</th>
<th>Report</th>
<th>Target</th>
<th>Disease</th>
<th>Effect</th>
<th>Dose</th>
<th>Route</th>
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<td>Rituxan/rituximab</td>
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<td>NHL</td>
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<td>CD20</td>
<td>RA</td>
<td>27</td>
<td>15</td>
<td>multiple iv</td>
</tr>
</tbody>
</table>

Hwang & Foote, 2005

Humanized

Mouse

Chimeric

Humira human TNFa RA
COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS
Immunogenicity assessment

LEAD SELECTION & OPTIMIZATION

DEVELOPMENT FORMULATION

PRECLINICAL

CLINICAL TRIALS

POST-MARKETING

IN SILICO T-EPITOPE IDENTIFICATION

IN VITRO T-CELL ASSAYS

ADA SCREENING
4.2 Non-clinical assessment of immunogenicity and its consequences

Therapeutic proteins show species differences in most cases. Thus, human proteins will be recognised 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.
CONCEPT PAPER ON IMMUNOGENICITY ASSESSMENT OF MONOCLONAL ANTIBODIES INTENDED FOR IN VIVO CLINICAL USE

Doc. Ref. EMEA/CHMP/BMWP/114720/2009 (DRAFT)

1. Discussion
Recently developed combinations of in-silico and T-cell based procedures are showing promise for predicting potential immunogenicity with some biologicals including mAbs. Identification of epitopes associated with induction or suppression of immune responses has been possible.

4. Recommendations
The main topics to be addressed include: (6 topics)

...  
- Approaches which may be helpful in predicting unwanted immunogenicity of mAbs.
...
Immunogenicity assessment
Adaptive immunity: Induction of naïve T-cell responses

- Generation/maturation of dendritic cells
- QC of dendritic cells

- Antigen loading of DC
- co-culture DC + T-cells

- Antigen-loaded CD14+
- co-culture CD14+ enriched T-cells
**in vitro T cell assays:**

1. **Blood collection and HLA typing (50 donors)**
   - Healthy PBMCs

2. **PBMC isolation**
   - Monocytes purification
   - QC on viability and polyclonal activation potential
   - Dissect possible noises derived from product formulations (buffers, etc)

3. **Evaluate direct drug effects on PBMC (whether they interfere with cell survival)**

4. **Identify optimal parameters on T cell responses**
   - Time kinetics: *Naive or recall response*
   - Functional assays
   - Optimized kinetics on whole proteins or peptides

5. **Assay characterization on extend donor population**
   - Optimized, multi-parameter measurement
Assessment of recall responses to peptides (CEFT) qualifies the multi-parameter flow cytometry assay and enriched IFN-g Elspot for determining the recall responses to vaccines.

**Analysis parameter**: % responsive donors in a set of 15 healthy community donor samples

IFN-\(\gamma\) spotforming units PBMC, \(\Delta\) frequency of activated wells (FCM) \(\text{CD3}^+\text{CD4}^+\)

<table>
<thead>
<tr>
<th>Peptide (properties)</th>
<th>Elispot IFN-(\gamma)</th>
<th>Flow cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative control peptides</strong></td>
<td>AP3 (non-binding self)</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Positive control peptides</strong></td>
<td>CEFT (binding – non self – recall)</td>
<td>100%</td>
</tr>
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</table>
Multiparameter Measurements

**EPIBASE IV Data flow**

- **Raw Data**
  - CTI-ImmuNoSpot® SS Core Analyzer
  - Blank, stimulated

- **Upload data in LIMS database to link experimental design and manipulations to the raw data**

- **Entry of exp design and manipulations in LIMS**

- Generates csv file for statistical analysis
Immunogenicity assessment

- Lead Selection & Optimization
- Development Formulation
- Preclinical
- Clinical Trials
- Post-Marketing

- Epibase
  - In Silico T-epitope Identification
  - In Vitro T-cell Assays
  - ADA Screening
Classical Predictive Methods

- **Position matrices:**
  - Score each position in the peptide for “likelihood” to fit in “pockets”
  - Sum those scores => epitope vs. non-epitope
Epibase®

1. Model building

- Template identification: retrieve HLA subtypes of known 3-D structure that are at least 50% identical to a given HLA subtype
- Build a 3-D structure

2. Run the proprietary FASTER algorithm

- Select relevant part of the receptor
- Include the flexibility of side chains

3. Determine

- Binding affinity
- Promiscuity

EP 1226528, Proteins, 2002

Proteins, 2005
# MHCII Population Frequencies

Population frequencies from Doolan et al. 2000

![Bar chart showing MHCII population frequencies for different populations: Caucasian, Japanese, and Chinese.](chart.png)

<table>
<thead>
<tr>
<th></th>
<th>Epibase® Global</th>
<th>Epibase® Caucasian</th>
<th>Epibase® Oriental</th>
<th>Epibase® Hispanic</th>
<th>Epibase® Afro-Am.</th>
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<tbody>
<tr>
<td>DR</td>
<td>45</td>
<td>27</td>
<td>29</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>DQ</td>
<td>23</td>
<td>14</td>
<td>14</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DP</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Van Walle et al, Expert Opin. Biol. Ther., 7(3) 2007
Case study: Adalimumab

- Human antibody recognizing TNF-α isolated by phage-display technology
- Study performed in collaboration with Sanquin and Genmab.
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 described:
  - 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
Study design

• 109 RA patient enrolled for the study
• Patients were tested for:
  • HAHA response (low, high) determined from the binding of the Humira F(ab’)2 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®
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>
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</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>
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<tr>
<td>DRB1*0404</td>
<td>5.9%</td>
<td>9.2%</td>
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</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>
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<tbody>
<tr>
<td>1</td>
<td>FwR2-HCDR2</td>
<td>DRB1*0701</td>
<td>1</td>
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<tr>
<td>2</td>
<td>FwR2-HCDR2</td>
<td>DQA1*0201</td>
<td>DQB1*0303</td>
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<td>DRB1*0901</td>
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<td>5</td>
<td>FwR3-HCDR3</td>
<td>DRB1*0801</td>
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<td>DQB1*0201</td>
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<tr>
<td>7</td>
<td>FwR3-LCDR3</td>
<td>DRB5*0101</td>
<td>5</td>
</tr>
</tbody>
</table>
Conclusions

- HAHA+ patients can be explained on basis of the critical epitopes as defined by *in silico* analysis.

- RA associated HLA allotypes contribute to HAHA+ response against Humira.

- Research project continued to:
  - Measure the epitopes in this patient group using immunological techniques.
  - Measure epitopes in non-MTX population.
Case study 2: 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:
  - Phase III 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>
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<tbody>
<tr>
<td>DRB1*0401</td>
<td>1 in 35</td>
<td>2 strong</td>
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</tr>
<tr>
<td>DRB1*0404</td>
<td>1 in 20</td>
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<td>no</td>
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<tr>
<td>DRB1*0101</td>
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<td>0401 and 0404</td>
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