Tools and Technologies for Immunogenicity and Allergenicity Risk Management

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Immunogenicity and Allergenicity

• **Immunogenicity**
  - A broad term covering the general ability of an antigen (protein, organism) to induce an immune response; wanted or unwanted

  **Immunogenicity can occur with exposure to:**
  - native proteins
  - new biologics or consumer products containing novel protein content

• **Allergenicity**
  - A specific term relating to IgE-mediated Type 1 hypersensitivity reaction to an antigen

• **T cell responses drive immunogenicity / allergenicity**
CD4⁺ T Cell Help Drives Allergenicity

**SENSITIZATION**

- APC
- Th2 CD4⁺
- IL-4, IL-10
- IFNγ
- B

**ELICITATION**

- FcεR1 receptor
- Mast cell
- Histamine
- Prostaglandin
- Proteases
- IL-4, IL-5, IL-6

Clinical effects:
- Hay fever
- Asthma
- Eczema
- Anaphylaxis

Adapted from Roitt *et al.*
T cell Epitope Antigenicity Profiling

Antigen Processing

T cell Function

MHC-peptide Binding
• Characterizing immune responses to protein content is vital to manage risks of:
  
  – allergenicity
  
  – loss of biologic efficacy
  
  – cross-reactive immune responses leading to serious adverse events
REVEAL™ Immunogenicity Case Study

Humira® (adalimumab): anti-TNFα

- Indication: RA, Crohn’s disease, Ulcerative Colitis, Ankylosing spondilitis
- IgG1 antibody with human derived heavy and light chain variable regions and human IgG1:k constant regions generated by phage-display
- Immunogenicity: 5%-20% of patients develop low titre neutralizing Abs (depending on indication)

Project Aim: to identify and characterize immunogenic epitopes of Humira®
Humira® Sequence Information

Variable heavy domain (122 amino acids)

1 EVQLVESGGG LVQPGRSLRL SCAASGFTFS DDYAMHWVRQ APGKGLEWVS AITWNSGHID
61 YADSVEGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCAKV SYLSTASSLD YWGQGTLVTV
121 SS

Variable light domain (107 amino acids)

1 DIQMTQSPSS LSASVGDRVT ITCRASQGIR NYLAWYQQKP GKAPKLLIYA ASTLQSGVPS
61 RFSGSGSGTD FTLTISSSLQP EDVATYYCQR YNARPYTFGQ GTKVEIK

NB. Full sequence information for Humira® is not available in the public domain.
ProPresent™: Antigen Presentation Assay

- ProPresent™ directly measures **MHC-peptide presentation** on DCs cultured with protein of interest using tandem Mass Spectrometry

- Key tool for understanding immunogenicity:
  - *De novo* identification of presented epitopes from protein of interest – can address mode of action
  - Evaluate the impact of protein modifications
  - Assess impact of allelic variants of proteins (e.g. replacement factors)
ProPresent™ identifies naturally processed and presented peptides on MHC
Experimental Summary

• 20 x healthy donors; fully HLA typed; global HLA distribution

• Monocyte-derived Dendritic cells

• Quality Control:
  – Routine viability / phenotypic testing of cells by flow cytometry, MHC recovery, Peptide recovery, detection of endogenous proteins, identification of nested sequences

• >95% confidence based exclusion search

• < 1% False discovery rate

• Match to actual protein sequence of Humira®
## Summary of Identified Humira® Peptides (with Allele Association and Anchor Analysis)

<table>
<thead>
<tr>
<th>Unique Peptides</th>
<th>Amino Acid Start/End</th>
<th>Protein Domain</th>
<th>DRB1* Alleles Present with Detected Peptide</th>
<th>Likely Allele Association Based on known Anchors</th>
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<tr>
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<tr>
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Alignment of detected Humira® peptides to CDR regions

4 unique regions identified in ProPresent™ are highlighted in green

Peptides are located around the CDR-2 and CDR-3 of Humira® heavy chain and CDR-2 of the light chain

No Humira® peptides were discovered in either unloaded controls or cells loaded with an isotype matched and functionally similar human antibody

None of these sequences were previously described in the literature
Do these presented peptides, identified by Mass Spectrometry, elicit a functional T cell response in healthy donors?
Functional confirmation of putative Humira® epitopes

- **CD8**⁺ **depleted** PBMC from ~40 x HLA-typed individuals representing tissue type distribution in the general population: HLA-DR, -DP and –DQ; specific HLA distributions can be requested

- Generate overlapping peptides for protein of interest: 15-mers offset by 3 amino acids

- Co-culture of PBMC with synthetic peptides

- T cell proliferation is measured over 7 day period by ultra-sensitive CFSE flow cytometry assay in sextuplicate analysis
CFSE Naïve T Cell Proliferation Assays

- Flow cytometry assay that measures only live CD4⁺ cells

\[
\text{Cell Division Index} = \frac{\text{C}/(\text{C}+\text{D})}{\text{A}/(\text{A}+\text{B})}
\]

% proliferating to peptide / % proliferating in negative control

- A significant response is determined as \( p<0.05 \) by ANOVA analysis, \( \text{CDI}>2 \) and 2 SEM above background
T cell Proliferation – Humira® Variable Heavy Chain

CDR1
CDR2
CDR3

Percentage Antigenicity

Peptide Number

DNAKNSLYLQMNSLRAEDTA
Peptides identified in ProPresent™ are confirmed to have functional response

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What is the global immunogenicity impact of each epitope identified in Humira®?

Requires confirmation of HLA association of these epitopes
REVEAL® HLA-peptide Binding Assays

• High-throughput physical HLA-peptide binding assays
  — Eliminate inaccuracies associated with in silico approaches
  — >90% global coverage of HLA-DR, -DP and -DQ
  — Rapidly identifies the precise HLA restriction of functional T cell epitopes

• Understand immunogenicity risk in specific target populations, especially with HLA-linked diseases

• Assess the impact of mutations on HLA-binding
ProImmune REVEAL® HLA Binding Assay

2 components:
- MHC-peptide Binding Assay
- MHC-peptide Stability Assay (24h, 37°C)
**HLA restriction of functional T cell epitopes**

**Analysis of Peptide 102 (SLYLQMNSLRAEDTA) from Humira® Heavy Chain CDR3**

<table>
<thead>
<tr>
<th>Donor</th>
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Summary

• Integration of assays
  – Rapid characterization of immune response
  – Naturally processed and presented epitopes
  – Post-translational modifications
  – Healthy versus allergic patients

✓ Unlimited by protein / allergen size

✓ Specific immune monitoring of allergic patients or at-risk individuals

✓ Immunotherapy design – QbD strategy
Thank you for your attention

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