Bringing innovation to global health

Crucell
Challenges of Wanted and Unwanted Immunogenicity Assays

Stefan Kostense
Clinical Immunology, Crucell
Imunogenicity: wanted or unwanted

- Vaccines
  - Wanted immunogenicity:
    - Neutralization and elimination of pathogens / malignancies
  - Unwanted immunity:
    - Enhancement of infection
    - Cross reactive antibodies to self proteins
    - Vector neutralization

- Protein therapeutics
  - Unwanted immunogenicity:
    - Adverse events: autoimmunity, hypersensitivity
    - Drug neutralization

**Crucell**
Challenges of immunogenicity assays

- True references and surrogate references
- Relevant and irrelevant immune responses
- Existing and non-existing guidelines
Challenges of immunogenicity assays

- True references and surrogate references
- Relevant and irrelevant immune responses
- Existing and non-existing guidelines
Positive controls for new biologics

- Humans have generally not been exposed to new biologics

- True reference or positive control for immune responses against new biologics are not existing

- Surrogate antibody controls:
  - Animal serum, affinity purified antibodies, idiotypic moabs
  - No true assay sensitivity, no assay comparison possible
  - Generally accepted by regulatory authorities
## Positive controls for new vaccines

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Antibody assays</th>
<th>T cell assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ serum</td>
<td>+ T cells</td>
</tr>
<tr>
<td></td>
<td>donors from endemic regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>recombinant protein / peptide</td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td>√</td>
<td>- Single sample</td>
</tr>
<tr>
<td></td>
<td>Not in sufficient</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quantity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peptides</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>√ donors from endemic regions</td>
<td>- Single sample</td>
</tr>
<tr>
<td></td>
<td>recombinant protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not in sufficient</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quantity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peptides</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>√ donors from endemic regions,</td>
<td>~ cohort studies</td>
</tr>
<tr>
<td></td>
<td>cohort studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>recombinant protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not in sufficient</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quantity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peptides</td>
<td></td>
</tr>
<tr>
<td>Ebola</td>
<td>- Not existing</td>
<td>- Not existing</td>
</tr>
<tr>
<td></td>
<td>recombinant protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Not existing</td>
<td></td>
</tr>
<tr>
<td>Pandemic</td>
<td>- Not existing</td>
<td>- Not existing</td>
</tr>
<tr>
<td>Influenza</td>
<td>- Not existing</td>
<td>~ consensus peptides</td>
</tr>
</tbody>
</table>

### Source

Crucell
Enzyme Linked ImmunoSpot assay (ELISpot)

T cells stimulated with a pool of 15mer peptides (→) of the Malaria antigen (11 aa overlapping)

Specific T cells secrete IFNγ (▼) and are defined as Spot Forming Units per million cells (SFU/10^6)
Elispot development and validation strategy

- Positive control in Elispot: CEF response
  - CMV/EBV/Flu T cell epitope mix. Most donors are positive for at least one of those peptides
- Prepare and freeze large batch of healthy donor T cells at -160°C
- Determine CEF response of donor → set acceptance criteria
Elispot validation performance

- CEF+ controls used as validation samples and assay controls
- Overall intermediate precision = 29% for CEF positive samples
Alternative control sample strategy

T cell assays

- **Assumption:** Malaria-ELISpot and CEF ELISpot behave similarly
  - Do the controls accurately reflect the clinical trial samples?
    (Spot size, stimulus threshold, background)

Serology assays

- **Assumption:** reference serum and trial samples behave similarly
  - Do the controls accurately reflect the clinical trial samples?
    (Parallellism, stability, affinity, isotype composition)
Challenges of immunogenicity assays

- True references and surrogate references
- Relevant and irrelevant immune responses
- Existing and non-existing guidelines
Which immune responses are clinically relevant

- Unwanted immunogenicity:
  - Which immune response, and what level causes adverse events (autoimmunity, hypersensitivity, etc)
  - Do antibodies neutralize the drug/vector

- Wanted immunogenicity:
  - Which immune response is effective against the pathogen / malignancy
  - What level of immune response confers protection against infection / disease
Which immune responses are clinically relevant

- **Safety aspect:**
  First goal is to detect any anti drug immune response
  → qualitative assay, sensitivity
  
  - How to investigate *clinical relevance of observed immunogenicity*?

- **Efficacy aspect:**
  Aim is to investigate mode of action and protective level
  → functional assays, assay range
  
  - How to investigate *which immune responses are protective*?
Correlates of protection for vaccines

- **B cells**
  - Antibodies capture the pathogen
    - Opsonisation
    - Neutralization
    - Can prevent infection

- **T cells**
  - T cells kill infected cells
    - Eliminate infected or malignant cells
    - Cytokines inhibit replication/proliferation
    - Can clear the infection
Correlates of protection for infectious diseases

- Most current vaccines are based on protective antibody titers
  - Influenza: 1/40 HI
  - Hepatitis B: 10 mIU/ml
  - Haemophilus Infl B: 0.15 ug/ml

Vaccines need to reach the protective antibody titer to get registration
Correlates of protection: one or multiple immune mechanisms

- Several pathogens lack a correlate of protection
  - HIV
  - Tuberculosis
  - Malaria

Antibodies required?

T cells required?

A combination of both?
Intracellular Cytokine Staining (ICS)

- Antigen specific T cell detection and characterization at single cell level
- Simultaneous measurement of 18 parameters possible
- Different marker panels:
  - Activation: CD3, CD8, CD4, HLA-DR, Ki67, BcL-2, CCR5, CCR7, CD38, CD27, viability
  - Memory: CD3, CD8, CD4, CD57, CD103, CD45RO, CD28, CCR5, CCR7, CD27, viability
  - Effector: CD3, CD8, CD4, CD57, CD107, CD62L, Perforin, Granzyme B, IL-2, IFNγ, TNFα

[Image of cytokine staining process]
Investigation of protective immune response

Choices between assays, functionality, location

- Antibodies
  - Quantity (ELISA)
  - Quality (Neutralization assay)
  - Characteristics (Affinity, isotyping)

- T cells:
  - Quantity (tetramers)
  - Quality (Elispot, ICS)
  - Characteristics (phenotype, activation, maturation markers)

- Location:
  - Peripheral blood
  - Target tissue

Too many variables to validate
• Selection and validation of 1 antibody assay and 1 T cell assay as standard read out of immunogenicity: secondary end point

• Selection of several exploratory assays to investigate correlate of protection: exploratory end point
  \(\rightarrow\) challenge studies, field studies

• If an exploratory assay proves to be correlate of protection
  \(\rightarrow\) CoP immunoassay validated as primary endpoint
Challenges of immunogenicity assays for vaccines

- True references and surrogate references
- Relevant and irrelevant immune responses
- Existing and non-existing guidelines
existing Guidelines for assay validation

- ICH (analytical assays for product testing)
- FDA (bioanalytical method validation)
- FDA (immunogenicity of protein therapeutics)
- EMA (immunogenicity of protein therapeutics)
- EMA (bioanalysis of drug concentrations)
- White papers on unwanted immunogenicity
- White papers on bioanalysis, biomarkers

➢ Vaccine immunogenicity = biomarker? (fit for purpose)
Challenges and Opportunities

- Alternative references and positive controls
- Investigation into the Correlates of Protection
- Unwanted Immunogenicity guidelines

- Write our own white paper on vaccine immunogenicity
EBF Topic Team on Vaccines

- Discussions on immunogenicity for vaccines are ongoing
- Other EBF members working on vaccines are invited to join
- White paper planned for 2013