Strategies for Clinical Assessment of Immunogenicity for Multidomain Therapeutics and Gene Therapy

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Global Product Development

European Bioanalysis Forum
22Nov2019

Breakthroughs that change patients’ lives
Disclaimer

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Agenda

• Emerging modalities
• Immunogenicity Assessment Roadmap
• Case Studies
• Take Home Message
Beyond Antibodies…

- Rare diseases - SMA, DMD, Sickle Cell Anemia, Hemophilia etc.
- Neurological diseases
- Inherited retinal diseases
- Cardiovascular and Metabolic

Innovation of Science and Technology

- Gene Therapies: Primarily targets rare disease caused by DNA mutations
- Cell Therapies: Immuno-Oncology (Targeted immunotherapies), Chimeric Antigen Receptor-T (CAR-T)
- Therapeutic Oligonucleotides
- Nano Particles
- Liposomes
- Antibody-Drug Conjugates
- Bi/Tri Specific antibodies

Immunogenicity evaluation will require creative approaches
New Modalities New Challenges

- Scope of Work
  - Lead Time

- Critical Reagents
  - Reference Material
  - Positive Control

- Technologies
  - Beyond Traditional Assays

- Assay Expectations
  - Regulatory Component Evolving
Immunogenicity Assessment Roadmap

**Risk Evaluation**
- Patient related factors
- Product related factors
- Resources
- Time commitment
- Drug

**Reagent Generation**
- Platform selection
- Multi-Tiered vs Unique assays
- Matrix selectivity
- Cut point calculation

**Assay Development**
- IM guidance/EMA guidance
- Scientific judgement for Unique assays
- Generate acceptance criteria for production

**Validation/Sample Testing**
- Track controls
- In study cut points if warranted

Ownership of Risk evaluation report?
Significant Lead Time
Method development/Qualification report
Working SOP/Method validation plan/report
Final SOP/sample analysis report

Clinical Impact
PK/PD Relationship
Unique Points to Consider for Risk Evaluation

**Antibody Drug Conjugates**

- Dose and frequency of administration
- Patient level of immunocompetence
- Antigen presentation of cell surface
- Degree of homology with endogenous counterpart

**Gene Therapy**

- Size, type and molecular structure of virus
- Physical properties like aggregation
- Route of administration
- Genetic status of patients; each patient will react differently
- Physical factors e.g. storage conditions
- Pre-existing antibodies
Six ADCs have market approval

**Antibody Drug Conjugates**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Maker</th>
<th>Condition</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemtuzumab ozogamicin</td>
<td>Pfizer</td>
<td>relapsed acute myelogenous leukemia (AML)</td>
<td>Mylotarg</td>
</tr>
<tr>
<td>Brentuximab vedotin</td>
<td>Millennium/Takeda</td>
<td>relapsed HL and relapsed sALCL</td>
<td>Adcetris</td>
</tr>
<tr>
<td>Trastuzumab emtansine</td>
<td>Genentech, Roche</td>
<td>HER2-positive metastatic breast cancer (mBC) following treatment with trastuzumab and a taxane</td>
<td>Kadcyla</td>
</tr>
<tr>
<td>Inotuzumab ozogamicin</td>
<td>Pfizer</td>
<td>relapsed or refractory CD22-positive B-cell precursor acute lymphoblastic leukemia</td>
<td>Besponsa</td>
</tr>
<tr>
<td>Moxetumomab pasudotox</td>
<td>AstraZeneca</td>
<td>adult patients with relapsed or refractory hairy cell leukaemia (HCL) who have received at least two prior systemic therapies</td>
<td>Lumoxiti</td>
</tr>
<tr>
<td>Polatuzumab vedotin- piiq^{18}</td>
<td>Genentech, Roche</td>
<td>relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL)</td>
<td>Polivy</td>
</tr>
</tbody>
</table>

**Immunogenicity Assay list for ADC**
- Anti-drug Ab (ADA)
- ADA specificity to Antibody
- ADA specificity to Payload
- Neutralizing Ab to Antibody
- Neutralizing Ab to Payload
Besponsa® Immunogenicity Evaluation

Binding Antibody Assay

1. Screening assay
   - Samples/control diluted 1:100
   - Mix with biotinylated and sulfo-Tag labeled drug
   - Avidin MSD plates
   - Is the signal ≥cutpoint?
     - Yes
     - No

   Sample is negative for ADA

2. Confirmation assay
   - Is % inhibition with excess signal ≥cutpoint?
     - Yes
     - No

   Sample is negative for ADA

3. Titer assay and 4. Characterization assay

Neutralizing Antibody Assay

Phase 3 pivotal study
- Anti-drug antibodies were detected in 7 of 159 (4%) ALL patients
- Neutralizing antibodies were not detected in any of the 7 patients
Overall, no impact of ADA on PK parameters

- Anti-drug antibodies were detected in (4%) ALL patients
- Neutralizing antibodies were not detected in any of the ADA positive patients
Assessment of clinical immunogenicity of inotuzumab ozogamicin in patients with non-Hodgkin lymphoma and acute lymphoblastic leukemia

Darshana Jani, John Nowak, Ying Chen, Joseph Boni and Boris Gorovits
Gene Therapy - Virus Based

- Defined as the insertion, removal or manipulation of one or multiple genes inside a cell to treat a specific disease
- More frequently, simply means “gene transfer’ and can be accomplished by transient expression or permanent insertion of gene
- Disease caused by absence of functional gene product normally produced by a single gene
- Genes delivered usually upregulated by strong promoter leading to protein expression

https://www.stjude.org/research/initiatives/gene-therapy.html
## Approved Therapies

<table>
<thead>
<tr>
<th>Product</th>
<th>Condition</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gendicine</strong></td>
<td>Head and neck squamous cell carcinoma</td>
<td>Adenovirus vector carrying the p53 tumour-suppressor gene</td>
</tr>
<tr>
<td><strong>Oncorine</strong></td>
<td>Late stage refractory nasopharyngeal cancer.</td>
<td>Oncolytic Adenovirus (H101)</td>
</tr>
<tr>
<td><strong>Glybera</strong></td>
<td>Lipoprotein lipase deficiency (LPLD), a rare inherited disorder which can cause severe pancreatitis</td>
<td>AAV1 viral vector with an intact copy of the human lipoprotein lipase (LPL) gene for delivery to muscle cells</td>
</tr>
<tr>
<td><strong>Imlygic</strong></td>
<td>Melanoma</td>
<td>HSV-1, two genes are removed and one gene is added. gene coding for granulocyte colony-stimulating factor (GM-CSF) is inserted to promote an immune response</td>
</tr>
<tr>
<td><strong>Zalmoxis</strong></td>
<td>Lymphoma or Leukemia patients receiving haematopoietic stem cell transplant</td>
<td>T cells modified with a Retrovirus vector encoding for a human nerve growth factor receptor and the herpes simplex I virus thymidine kinase</td>
</tr>
<tr>
<td><strong>Strimvelis</strong></td>
<td>ADA-SCID (Severe Combined Immunodeficiency due to Adenosine Deaminase deficiency).</td>
<td>Retrovirus containing the human adenosine deaminase gene and then reinfused into the patient.</td>
</tr>
<tr>
<td><strong>Luxturna</strong></td>
<td>Retinal dystrophy</td>
<td>AAV2-based treatment with RPE65 gene</td>
</tr>
<tr>
<td><strong>Kymriah</strong></td>
<td>B-cell acute lymphoblastic leukemia</td>
<td>T-cells engineered to target CD19 receptors on the tumor B cells.</td>
</tr>
<tr>
<td><strong>Yescarta</strong></td>
<td>B-cell acute lymphoblastic leukemia</td>
<td></td>
</tr>
<tr>
<td><strong>Zolgensma</strong></td>
<td>Spinal muscular atrophy</td>
<td>AAV9 viral vector delivers the SMN1 transgene to cell nuclei where the transgene begins encoding SMN protein</td>
</tr>
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**Courtesy of:** Genetherapynet.com  
Gene Therapy Review  
DOI: 10.1056/NEJMra1706910
Understanding Challenges is the Key for Proper Support

• Risk assessment based on vector/therapeutic area – high risk for humoral immunity
• Immunogenicity evaluation requires
  • multiple assays, reagents and lead time
  • use of novel assays such as ELISPOT and other technologies
• Scientific and technical judgement is critical while regulatory environment is evolving.
Regulatory Guidance on Immunogenicity Assessment of Gene Therapy Products

EMA: Guideline on follow-up of patients administered with gene therapy medicinal products, 2010:

*If it is clinically relevant,* antibody and cell mediated immunity testing shall be a part of the clinical trial and the observation period should be sufficient to detect a signal. *If the antibody is a non neutralising antibody, not targeting epitopes linked to the activity of the protein, and therefore without any impact on the efficacy of the GT medicinal product, then screening tests are not needed*

FDA: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, Draft Guidance, 2013:

*If immunogenicity is a concern,* then each subject’s immune response to the product should be evaluated. This evaluation may include monitoring for evidence of both cellular and humoral immune responses.”
Three Components play Role in Immunogenicity for Virus based Gene Therapy

- Vector Viral
- Nucleic Acid
- Therapeutic Protein encoded by nucleic acid

Immune Response

- Humoral
- Cellular

Safety Efficacy
## Example: Immunogenicity Assays Using AAV Vector

<table>
<thead>
<tr>
<th>Assay</th>
<th>Commonly Used Assay Platform</th>
<th>Assay Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Capsid (e.g. AAV00) antibodies</td>
<td>Immunoassay (MSD) Bridging format</td>
<td>Tiered approach of screen, confirm, titer; End Point Titer</td>
</tr>
<tr>
<td>Anti-Transgene antibodies</td>
<td>Immunoassay to measure antibodies against transgene–protein using surrogate recombinant protein</td>
<td>Tiered approach of screen, confirm, titer</td>
</tr>
<tr>
<td>Anti-Capsid (AAV00) NAB</td>
<td>Cell based assay</td>
<td>Inhibition of transduction</td>
</tr>
<tr>
<td>Anti-Capsid or anti-transgene T cell response measured by cytokine production</td>
<td>ELISPOT</td>
<td>Relative index</td>
</tr>
<tr>
<td>Binding Antibodies: IgG and IgM isotyping</td>
<td>Immunoassay Multiplex</td>
<td>Isotype positive/negative Semi Quantitative approach</td>
</tr>
<tr>
<td>Anti-Capsid or Anti-Transgene immune cell population ratio before and after treatment</td>
<td>Flow Cytometry</td>
<td>Relative index</td>
</tr>
</tbody>
</table>
Commonly available assays to evaluate cytokine production for immunogenicity assessment

- Enzyme-Linked Immunosorbent Assay (ELISA)
- Luminex & Cytometric Bead Array (CBA)
- Enzyme-Linked Immunospot Assay (ELISpot)
- Fluorescence-Activated Cell Sorter (FACS)
  - Intracytoplasmatic Cytokine Staining (ICS)
  - Tetramer staining
  - Pentamer staining
  - Surface marker staining
Desirable features of assays for immunogenicity assessment in regulated environments

• Performs identical with fresh and previously frozen samples
• Works with genetically diverse populations
• Uses the least amount of cells and clinical sample material
• Scalable to accommodate large-volume testing with hundreds of samples in later stage clinical trials
• Can be standardized; to enable, for example, inter-study comparisons to make data more robust for multicenter or large international clinical trials
New Modalities are an exciting area of drug discovery/development

Immunogenicity assessment will continue to evolve
Novel techniques emerge.
Novel algorithms may be needed

Assay approaches will need thinking
Approach can be simple or complex depending upon the needs of the project

Design, execution and reporting of data from these assays have not been standardized.

A combination of Gold standard methods and new creative approaches generate confidence in assay performance and data.
Acknowledgement

• Many Pfizer scientists
• AAPS/APA/PBSS/EBF Scientists