Immunogenicity Assessment of Gene Therapy Compounds: Discussion on Current and Future Concepts

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14 May 2019
**Gene Therapy Principle**

**GENE THERAPIES:**
ideal tool to treat monogenic diseases, by correcting the dysfunction of a single gene

**OVER- or ECTOPIC EXPRESSION**
Corrected by silencing gene expression using non-coding nucleic acid cargos (ASOs, miRNAs, siRNAs)

**NO or FAULTY EXPRESSION**
Corrected via a nucleic acid encoding the missing or non functional (truncated, mutated...) protein

Beyond correcting genetic deficiencies, gene therapy can also provide a cell with capabilities not present in its natural state:

**“ex-vivo GT”**
Most notable example of adoptive cellular therapy: genetically engineered T cells (not discussed today)

Glossary ASO: antisense oligonucleotide; miRNA: micro RNA; siRNA: small interfering RNA; GT: gene therapy; AAV: adeno-associated virus; LNP: lipid nanoparticle

[Link to clinical trials](https://www.barthsyndrome.org/research/clinicaltrials/generereplacementtherapy.html)
### Some Landmarks in Gene Therapy History

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>Friedmann and Robin first suggest gene therapy as a treatment for genetic diseases</td>
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<tr>
<td>1984</td>
<td>The human genome project</td>
</tr>
<tr>
<td>1990</td>
<td>First experimental gene therapy in the USA for a 4-year-old child with immuno-deficiency</td>
</tr>
<tr>
<td>1999</td>
<td>SAEs after gene therapies (Immune reaction; leukemia)</td>
</tr>
</tbody>
</table>
| 2003 | Intensive research to increase vector safety: (SIN vectors, introduction of insulator sequences…)
- First gene therapy approved in Europe for a rare blood disorder |
| 2009 | First gene therapy approved in the world (China) for head and neck cancer |
| 2012 | More regulatory approvals expected |
| 2017 | First gene therapy approved in US for an inherited form of vision loss |
| 2019 | Gene therapy for human genetic disease? Friedmann and Robin (1972)
Science 175 (4025) |
Nature, Vol 461 |
| 2019 | Cancer fears cast doubts on future of gene therapy
Nature, Vol 423 |
Science 359 (6372) |
Gene Therapies: Multiple Component Therapeutics

**Delivery Vehicle**

- **Viral**
  - Adenovirus
  - Adeno-associated virus (AAV)
  - Gamma-retrovirus
  - Lentivirus

- **Non-viral**
  - Lipid nanoparticles (LNP)
  - Cationic polymers

- **None**
  - Cells* (autologous or heterologous; ex-vivo gene therapy)
  - Electroporation
  - Hydrodynamic injection

**Nucleic Acid Cargo**

- **DNA**
  - Plasmids
  - Coding
  - Electroporation
  - Antisense oligo-nucleotide (ASO)
  - Hydrodynamic injection
  - Aptamer

- **RNA**
  - Coding (mRNA or retroviral RNA genome)
  - Interfering RNAs
    - siRNA
    - miRNA

*Cells* refers to autologous or heterologous cells used in ex-vivo gene therapy.
Immunogenicity Assessment: More Than Meets the Eye

**ADAPTIVE IMMUNITY**

**CELL-MEDIATED**

**ANTIBODY-MEDIATED**

- T-dependent antigens (T-D)
- T-independent antigens (T-I-1 and TI-2)

**INFLAMMATION AND INNATE IMMUNITY**

**CELLULAR COMPONENT**

**HUMORAL COMPONENT**

Not only about addressing antibody presence
Inflammation and Innate Immunity

- Chronologically the first line of defense
- Selective but not antigen-specific pattern recognition receptors
- Involves cells such as:
  - tissue-resident macrophages and circulating monocytes which extravasate in the tissues where they differentiate,
  - dendritic cells, NK, NK.T and Tγδ cells
- No memory cells
- Cytokine synthesis
- Multiple bridges between innate and adaptive immunity
  - Activated macrophages act as antigen presenting cells (APC)
Cell-Mediated Adaptive Immunity

Requires T cells

Responds to any cell displaying aberrant MHC markers

Self cells or APCs display foreign antigens
- Self-cells invaded by pathogens, tumor cells, or transplanted cells → MHC I
- APC: MHC II + exogenous antigen fragments
  And bind to T cells

Interleukins (secreted by APCs or helper T cells) co-stimulate activation of T cells
Adaptive Immune Response – MHC Class I

Self-cells invaded by pathogens, tumor cells, or transplanted cells

- Body cell
- Infected tissue cell presenting antigenic peptide recognized by cytotoxic T cell
- Viral antigen
- Processed viral antigen (peptide) presented in combination with MHC I protein
- MHC I protein
- CD8 protein
- Immunocompetent cytotoxic T cell
- T cell receptor (TCR)

Clone formation
- Cytotoxic T Memory Cell
- Mature cytotoxic T cells

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Adaptive Immune Response – MHC class II

Antigen presenting cells displaying exogenous antigen fragments on their membrane

Cell-mediated immunity
(attack on infected cells)

Humoral immunity
(secretion of antibodies by plasma cells)

- Helper T cells facilitate both cell-mediated and antibody-mediated immune responses.
- Cytotoxic T cells function similarly to NK cells, however, they only see specific MHC I+ antigen complexes.
Assessing **Cell-Mediated** Immune Response

Infected cells expose epitopes from viral or transgene at their surface (MHC-I)

Recognition by CD8+ T-cells

Activation and / or proliferation

Killing of the transduced (infected) cells by CD8+ T-cells

Assays mainly based on the following underlying biology:

Shin-Ichi et al., (2013)
Assessing Cell-Mediated Immune Response: Cytokine Synthesis

- **ELISpot**: technique of choice to assess activation or proliferation of T-cells
- Customized for the specific Ag of interest
- Investigative purposes: identification of the sequence of presented antigen peptide to ultimately guide mutagenesis to de-risk the immunogenicity potential
- Expertise required in cell preparation and handling
Is there a difference in immune cell population ratios before and after administration of the compound?

- CD19-CD20 (B cells)
- CD4-CD8-CD3 (T cells)
- CD16-CD56 (NK cells)
- HLA DR (activated T cells)
- CD127 (activated T regs)
- CD69-CD207 (activated granulocytes)
Two categories of antigens regarding their recognition and the induction of humoral immune response:

**T dependent (T-D) antigens:**
- Endocytosed by APCs (antigen presenting cells)
- Presentation to Th cells
- T cell activation
- T cell-dependent B cell activation and IgG secretion.

**T independent (T-I) antigens:**
- Directly recognized by B cells
- Cross-linking of the B cell receptors
- IgM secretion through T cell-independent B cell activation.

Sylvain et al., (2012). Biomolecules. 2. 435-466
Tiered Approach for **Antibody Response Assessment**

Classical tiered approach depicted above may not be totally suitable for all gene therapeutics.
Immunogenicity Risk Assessment:
Factors To Be Considered

- Route of Administration
- Nature of the Transgene
- Nature of the Therapeutic Protein
- Nature of the Vector
Route of Administration

Non-invasive routes of administration for gene delivery systems

- Intranasal administration to target the brain
- Topical administration on the surface of the eye to treat retinal inherited diseases
- Aerosolized formulations for inhalation for the treatment of pulmonary diseases.

<table>
<thead>
<tr>
<th>Route</th>
<th>Target</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>intra-arterial</td>
<td>Liver</td>
<td>Naked plasmid DNA</td>
</tr>
<tr>
<td>intra-arterial</td>
<td>Pancreas</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>intra-arterial</td>
<td>Hind limb</td>
<td>Naked plasmid DNA</td>
</tr>
<tr>
<td>intra-arterial</td>
<td>Cecum</td>
<td>AAV</td>
</tr>
<tr>
<td>intra-arterial</td>
<td>Brain tumor</td>
<td>Adenovirus + Lipoplex</td>
</tr>
<tr>
<td>intraportal</td>
<td>Liver</td>
<td>Lipoplex</td>
</tr>
<tr>
<td>retrograde intravenous</td>
<td>Kidney</td>
<td>Naked plasmid DNA</td>
</tr>
</tbody>
</table>

Fumoto et al., IntechOpen, 2013
Nucleic Acids Can Be **Immunogenic**

- Can be recognized as danger-associated molecular patterns (DAMPs) / pathogen-associated molecular patterns (PAMPs) by the innate immune system
- Some DNAs are TI-1 antigen which can **directly activate B cells without T-cell involvement**
- **Lupus:** disease where antibodies against dsDNA are produced

Rosin and Okusa (2011)
# Immunogenicity Risk Assessment
of the Therapeutic Protein

<table>
<thead>
<tr>
<th>Protein Encoded by the Transgene</th>
<th>Immunogenicity Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>None: transgene inhibits the expression of an endogenous protein</td>
<td>Low</td>
</tr>
<tr>
<td>Increased expression of a protein normally expressed at lower levels in the patients</td>
<td>Low to moderate</td>
</tr>
<tr>
<td></td>
<td>Break of immune tolerance</td>
</tr>
<tr>
<td>Functional version of a protein which is mutated in the patient</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Introduction of a single amino-acid change or a conformational epitope</td>
</tr>
<tr>
<td>Functional version of a protein which is truncated in the patient</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Additional domain can be recognized as foreign by the immune system</td>
</tr>
<tr>
<td>Protein not expressed in the patient</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>The whole therapeutic protein can be recognized as non-self by the immune system</td>
</tr>
</tbody>
</table>
• Immunogenicity related reactions

Unwanted immunogenicity could be observed, for example, due to the persistent gene expression. The consequences of such immune reactions range from transient appearance of antibodies or cell mediated immunity without any clinical significance to severe life threatening conditions.

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.

Immediate hypersensitivity reactions would be noticed in the clinical trial, however delayed reaction such as antibodies to the gene expression protein might occur.

Antibodies interfering with the activity of the gene vector or expression protein might lead to a lack of efficacy (in case it is desired to have continuous gene expression) and they can cross-react with the endogenous protein in cases where endogenous protein is still produced. In this case the consequence would be autoimmunity.
Vectors Used in Gene Therapy in Clinical Trials

Over 2,900 clinical trials for gene therapy approved globally 1989-2018

- 64% use viral vectors,
- 15% use naked DNA,
- 4% use lipofection

Lipid Nanoparticles (LNPs) as Non-Viral Vectors

- Class of non-viral vectors formulated with synthetic or naturally derived lipids containing hydrophilic heads and hydrophobic tails.
- Typical components:
  1. A cationic or ionizable amino lipid to complex the nucleic acid and enhance endosomal escape
  2. A helper phospholipid to support the bilayer structure and facilitate cell uptake;
  3. Cholesterol to enhance the stability and promote membrane fusion
  4. Polyethylene glycol (PEG) conjugated lipid to reduce aggregation, avoid reticuloendothelial clearance, and decrease non-specific uptake
- Cationic lipids are currently the most popular non-viral delivery system in clinical trials for genetic drugs.

Xiong et al., (2018)
August 2018: First-Ever FDA approval of an RNAi therapeutic: ONPATTRO™ (Patisiran) formulated using LNP technology for the treatment of the polyneuropathy of hereditary transthyretin-mediated (hTTR) amyloidosis in adults.
Immunogenicity of **ONPATTRO** (Patisiran)

Anti-drug antibodies specific to **PEG2000-C-DMG**

- Antibodies specific to a lipid component exposed on the ONPATTRO surface

Clinical studies (placebo-controlled and open-label)

- 7 of 194 (3.6%) patients with hATTR amyloidosis developed anti-drug antibodies during treatment
- One additional patient had pre-existing anti-drug antibodies.

Available data

- No evidence of an effect of anti-drug antibodies on clinical efficacy, safety, or the pharmacokinetic or pharmacodynamic profiles of the drug.

Source: ONPATTRO prescribing information
Main **Viral Vectors Used** in Gene Therapy

<table>
<thead>
<tr>
<th>Viral vector</th>
<th>Integrative/ Episomal</th>
<th>Utility</th>
<th>Impediments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Episomal</td>
<td>- Very efficient transfection in most tissues</td>
<td>- Induces inflammatory response</td>
</tr>
<tr>
<td>Adeno-associated virus (AAV)</td>
<td>Episomal (&gt;90%)</td>
<td>- Not inducing inflammatory response</td>
<td>- Limited nucleic acid carrying capacity (&lt;5 kb)</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>Integrative</td>
<td>- Persistent gene expression</td>
<td>- Only transfects dividing cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Risk of insertional mutagenesis</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Integrative</td>
<td>- Broad tropism</td>
<td>- Risk of insertional mutagenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Persistent gene expression</td>
<td></td>
</tr>
<tr>
<td>Herpes Simplex Virus-1 (HSV-1)</td>
<td>Episomal</td>
<td>- Large nucleic acid carrying capacity (up to 150 kb)</td>
<td>- Induces inflammatory response</td>
</tr>
</tbody>
</table>

Commonly infect humans => presence of pre-existing antibodies in a large fraction of the population
- Safety
- Distribution
- Efficacy
## AAV Tropism and Antibody Seroprevalence

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Tropisms</th>
<th>Serum antibodies</th>
<th>Neutralizing factor seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV1</td>
<td>Airway, CNS, retina, skeletal muscle</td>
<td>67 % High</td>
<td>50.5 %</td>
</tr>
<tr>
<td>AAV2</td>
<td>Kidney, liver, retina, vascular tissue</td>
<td>72 % High</td>
<td>59 %</td>
</tr>
<tr>
<td>AAV4</td>
<td>CNS, kidney, lung</td>
<td>N/A High</td>
<td>N/A</td>
</tr>
<tr>
<td>AAV5</td>
<td>Airway, CNS, skeletal muscle</td>
<td>40 % Low</td>
<td>3.2 %</td>
</tr>
<tr>
<td>AAV6</td>
<td>Skeletal muscle, T-cells, HSC</td>
<td>46 % High</td>
<td>N/A</td>
</tr>
<tr>
<td>AAV8</td>
<td>Liver, CNS, Retina</td>
<td>38 % Low</td>
<td>19 %</td>
</tr>
<tr>
<td>AAV9</td>
<td>Cardiac, liver, CNS, pancreas, retina</td>
<td>47 % Low</td>
<td>N/A</td>
</tr>
</tbody>
</table>

- **Selection and optimization**
  - Tropism
  - Immunogenicity

- Low titer seroprevalence with limited neutralizing activity: key **advantage** for systemic AAV vector use

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Consequences of Antibodies Against the Viral Vector

Mingozzi & High, 2011
Loss of factor IX expression and transient increase in serum liver enzymes observed in the AAV2 trial for haemophilia B
Zhang et al. (2018): Gendicine (ad-p53) the first approved gene therapy product for cancer 12 years in the clinic

Anti-vector neutralizing antibody titers increased or became positive in all patients after intra-arterial infusion with Gendicine:

- **Group I**: basal level: 176 – 103 -> week 2 of treatment: 2,129 – 1,198
- **Group II**: basal level: 168 – 101 -> week 2 of treatment: 2,137 – 1,173
- Antibody titers increased after administration of subsequent cycles of treatment.

However, the antitumor activity of Gendicine was not affected by these neutralizing antibodies.

- Most patients were previously exposed to adenovirus and thus had **pre-existing neutralizing antibodies**
- The clinical efficacy of Ad5-based therapies **appeared to be effective and was not suppressed by neutralizing antibodies**
- In other clinical trials using adenoviral vector, the majority of patients presented with neutralizing antibodies and **almost all showed a significant increase in viral titer after the initial Ad vector injection**
- Gendicine delivered systemically in the clinic was reported to be safe and effective in enhancing responses to chemo- and/or radiotherapy => **presence of pre-existing Ad immunity and the rapid development of Ad vector immunity did not completely block Gendicine function**
- Clinical study of Gendicine **delivered via intra-external carotid artery infusion**
Suggestion for an Alternative to the Classical Tier-based Approach for Antibodies Against Viral Vectors

Assay Validation
- Confirm by immuno-depletion that the non-drug exposed samples that screened positive contain antibodies specific to the viral epitopes.
- Determine Minimum Significant Ratio (MSR): value from which an increase or decrease in titer is statistically relevant.

Sample Screening
- (Pre-dose) samples are analyzed in the screening assay to get an estimate of the magnitude of the ADA response.
- For assays with a broad working range, there is a good correlation between the signal and the titer (Starcevic Manning et al., 2017).
- Not necessary to establish a screening cut-point: use signal level.

Sample Titration
- Starting at the dilution estimated thanks to the signal level.
- Dilution factor between each dilution step = MSR (e.g., if MSR=6.2, dilute 6 fold between each step).
- Titer cut-point defined as per proposal from Wakshull et al., 2011.

Michaut, Sickert, et al., (manuscript in preparation)
Long-term follow-up (LTFU) required

- EMA Guideline on follow-up of patients administered with gene therapy medicinal products (2009)

We provide the following examples of evidence obtained from investigation of a product that may warrant our recommendation of LTFU observations for delayed adverse events:

- A preclinical toxicology study indicates that expression of the therapeutic gene (the transgene in your product) is associated with delayed toxicity.
- The therapeutic gene provides functional replacement of a host gene that is otherwise not expressed, and the therapeutic protein is potentially immunogenic.
Thank You For Your Attention!