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Immunogenicity Assessment of Gene Therapy Compounds:

Discussion on Current and Future Concepts

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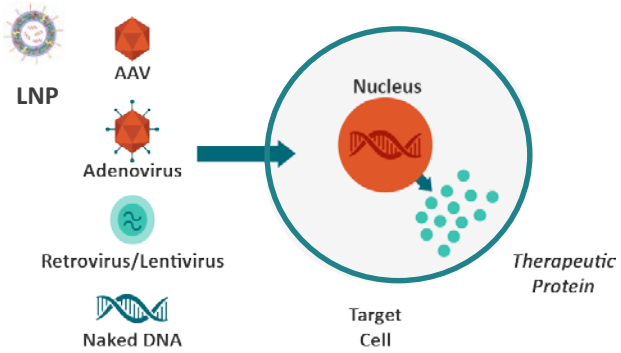
Gene Therapy Principle

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

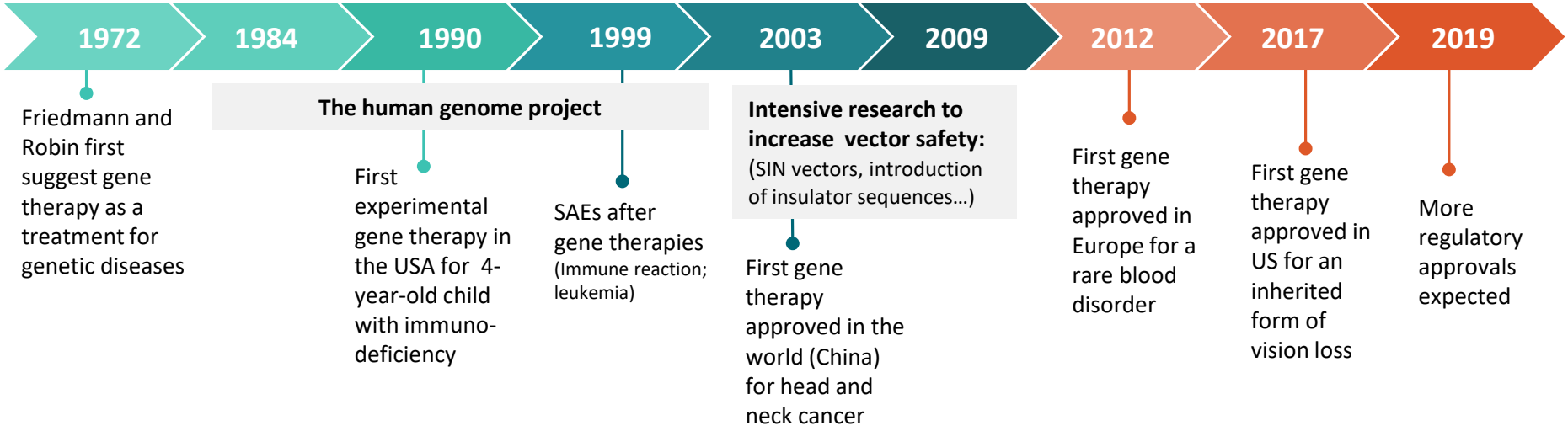
Beyond correcting genetic deficiencies, gene therapy can also provide a cell with capabilities not present in its natural state: **"ex-vivo GT"**
Most notable example of **adoptive cellular therapy :** genetically engineered T cells *(not discussed today)*



https://www.barthsyndrome.org/research/clinicaltrials/genereplacementtherapy.html

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

Some Landmarks in **Gene Therapy History**



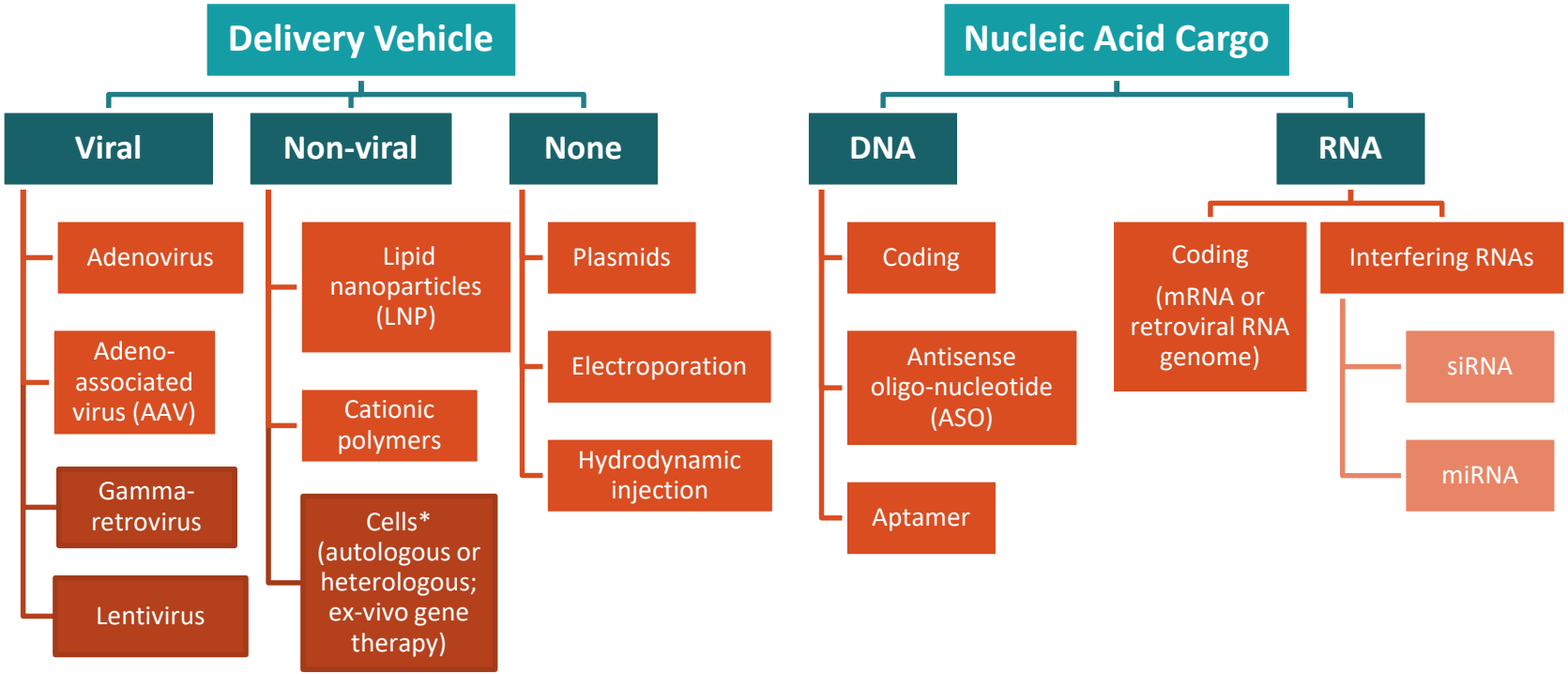
Gene therapy for human genetic disease? *Friedman and Robin (1972) Science 175 (4025)*

Cancer fears cast doubts on future of gene therapy *Editorial (2003) Nature, Vol 423*

Gene therapy deserves a fresh chance. *Editorial (2009) Nature, Vol 461*

Gene therapy comes of age. *Dunbar et al. (2018) Science 359 (6372)*

Gene Therapies: Multiple Component Therapeutics



Immunogenicity Assessment: More Than Meets the Eye

ADAPTIVE IMMUNITY

INFLAMMATION AND INNATE IMMUNITY

CELL-MEDIATED

ANTIBODY-MEDIATED

CELLULAR COMPONENT

HUMORAL COMPONENT

T-dependent antigens (T-D)

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

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:

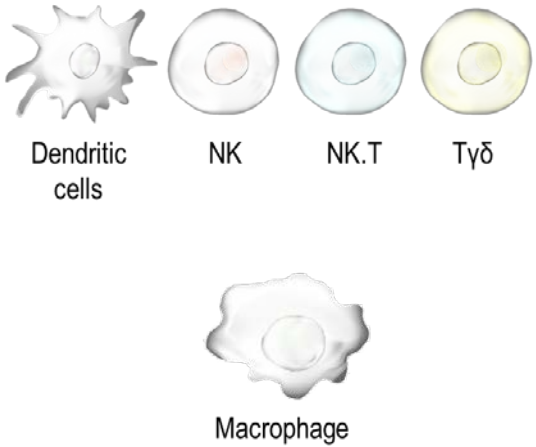
- tissue-resident macrophages and circulating monocytes which extravasate in the tissues where they differentiate,
- dendritic cells, NK, NK.T and Tγδ cells

Cytokine synthesis

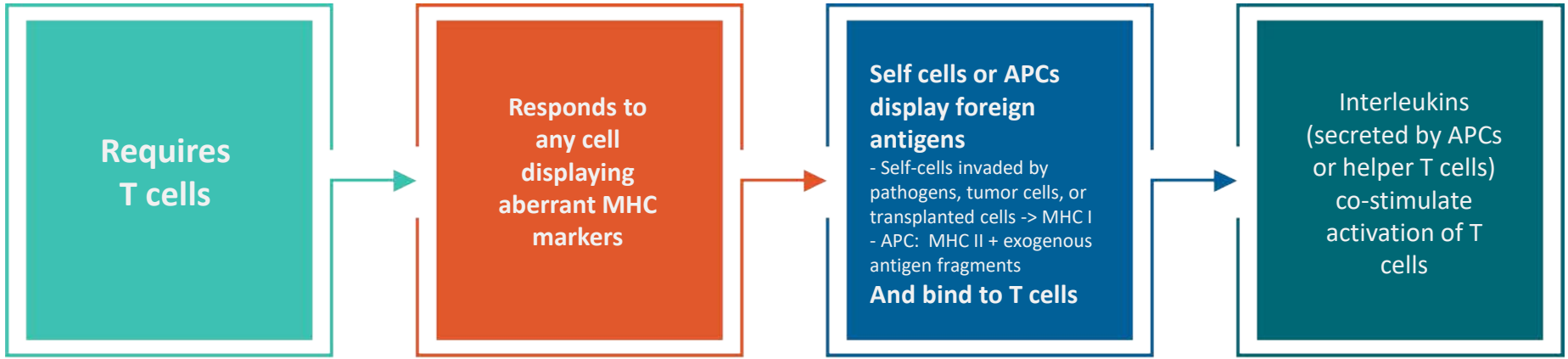
No memory cells

Multiple bridges between innate and adaptive immunity

Activated macrophages act as antigen presenting cells (APC)

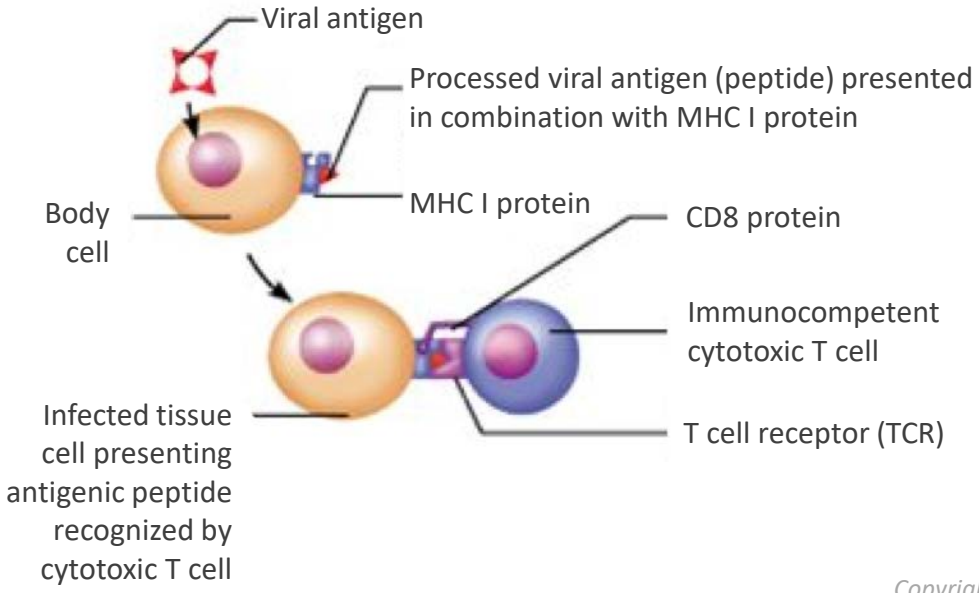


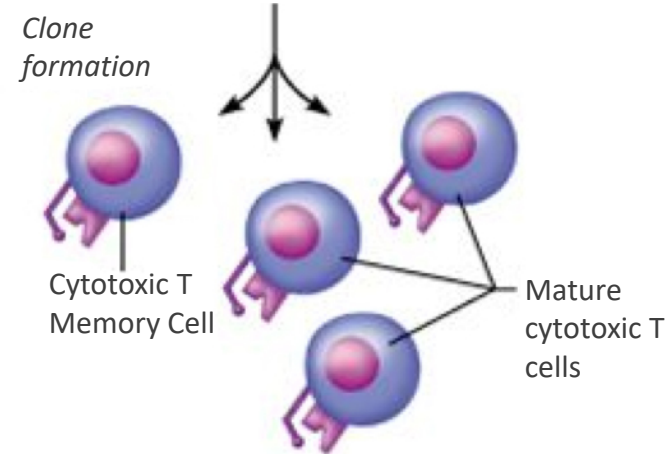
Cell-Mediated Adaptive Immunity



Adaptive Immune Response – MHC Class I

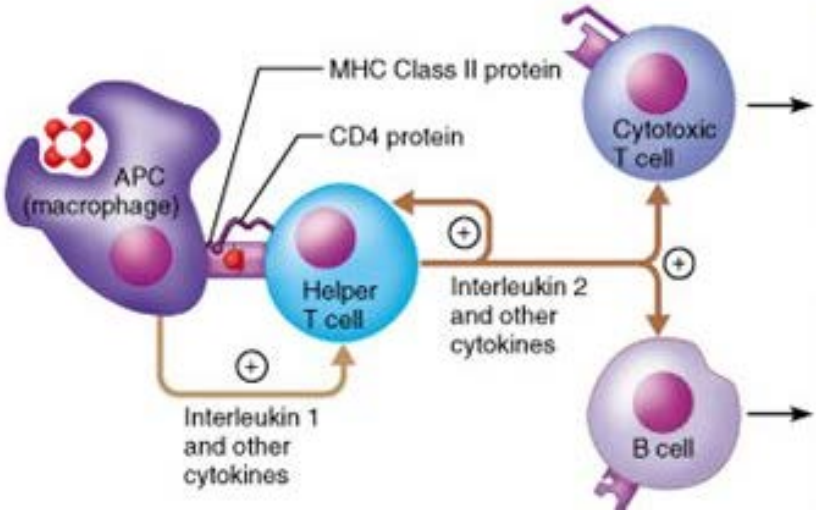
Self-cells invaded by pathogens, tumor cells, or transplanted cells





Adaptive Immune Response – MHC class II

Antigen presenting cells displaying exogenous antigen fragments on their membrane

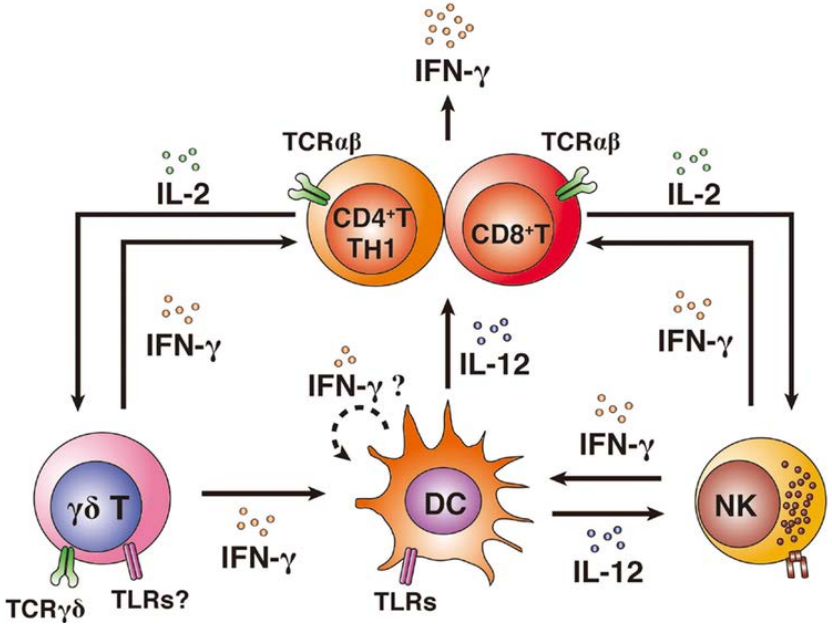


Cell-mediated immunity
(attack on infected cells)

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



Shin-Ichi et al., (2013)

Assays mainly based on the following underlying biology:

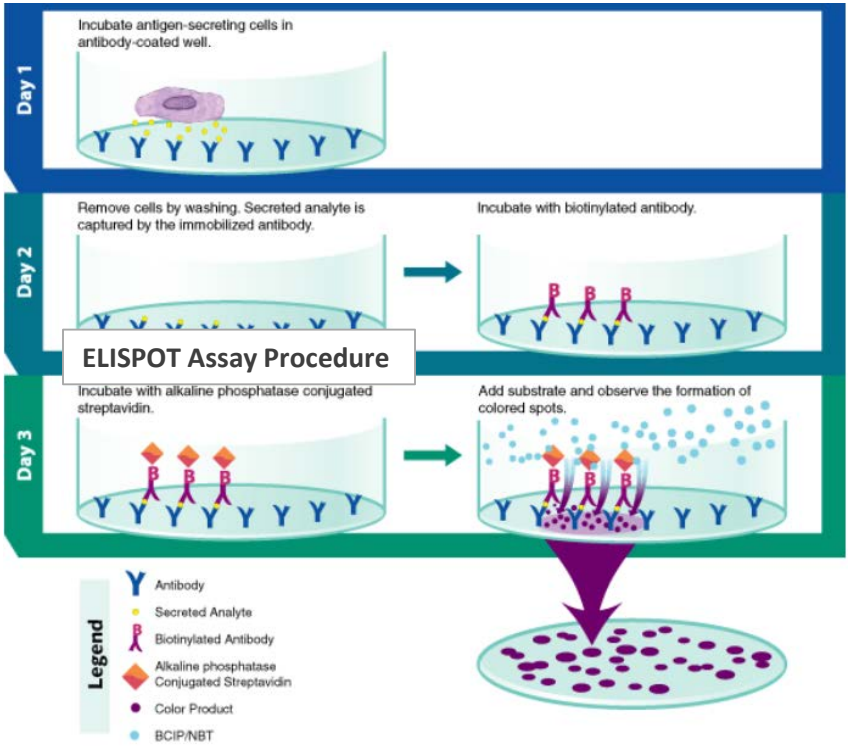
Infected cells expose epitopes from viral or transgene at their surface (MHC-I)

→ Recognition by CD8+ T-cells

→ Activation and / or proliferation

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

Assessing Cell-Mediated Immune Response:
Cytokine Synthesis

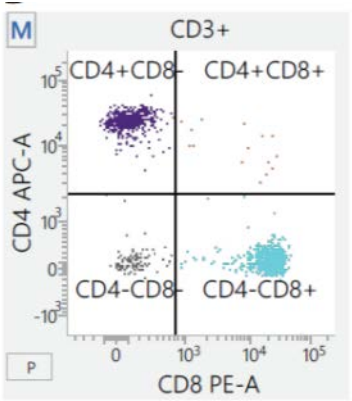


- **ELISpot:** technique of choice to assess activation or proliferation of T-cells
- Customized for the specific Ag of interest
- Investigative purposes: identification of the sequence of presented antigen peptide to ultimately guide mutagenesis to de-risk the immunogenicity potential
- Expertise required in cell preparation and handling

Assessing Cell-Mediated Immune Response:
Immuno-phenotyping

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)



Antibody-Mediated Immune Response

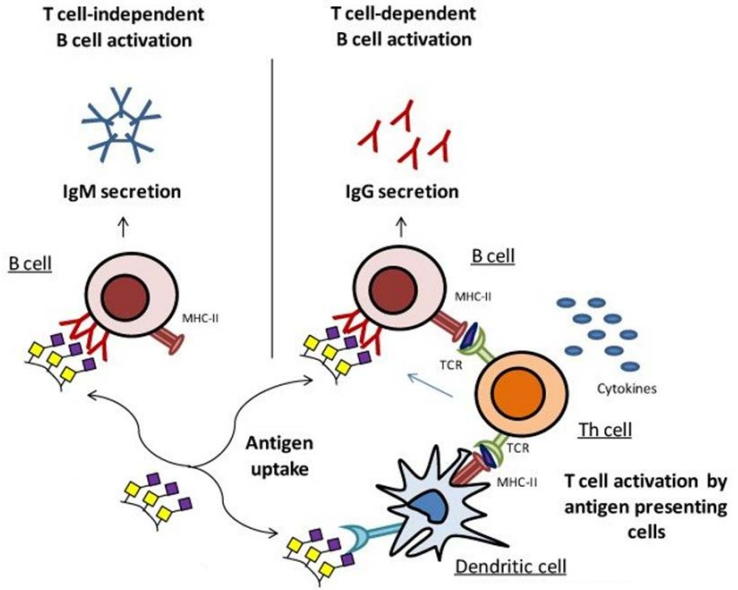
Two categories of antigens regarding their recognition and the induction of humoral immune response:

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.

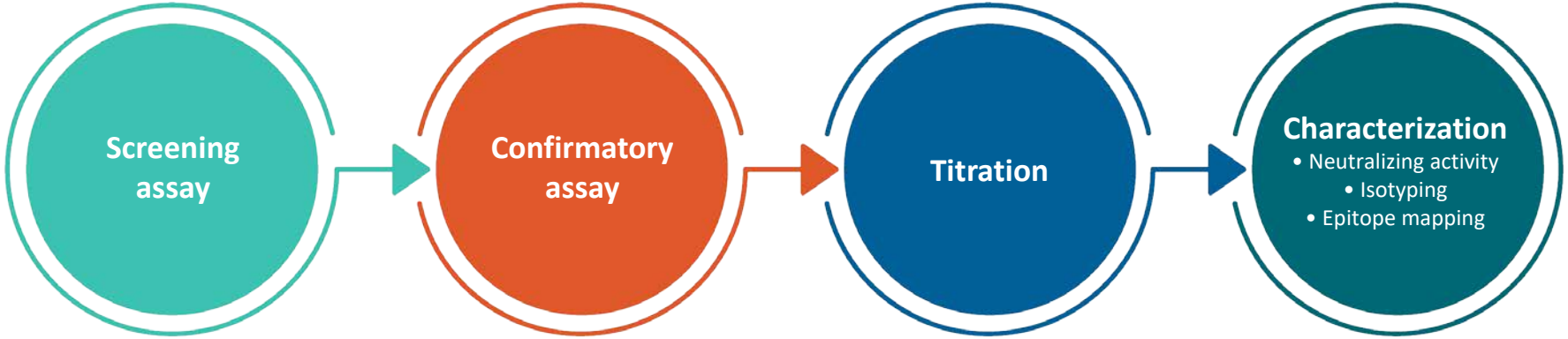
T independent (T-I) antigens:

- ✓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

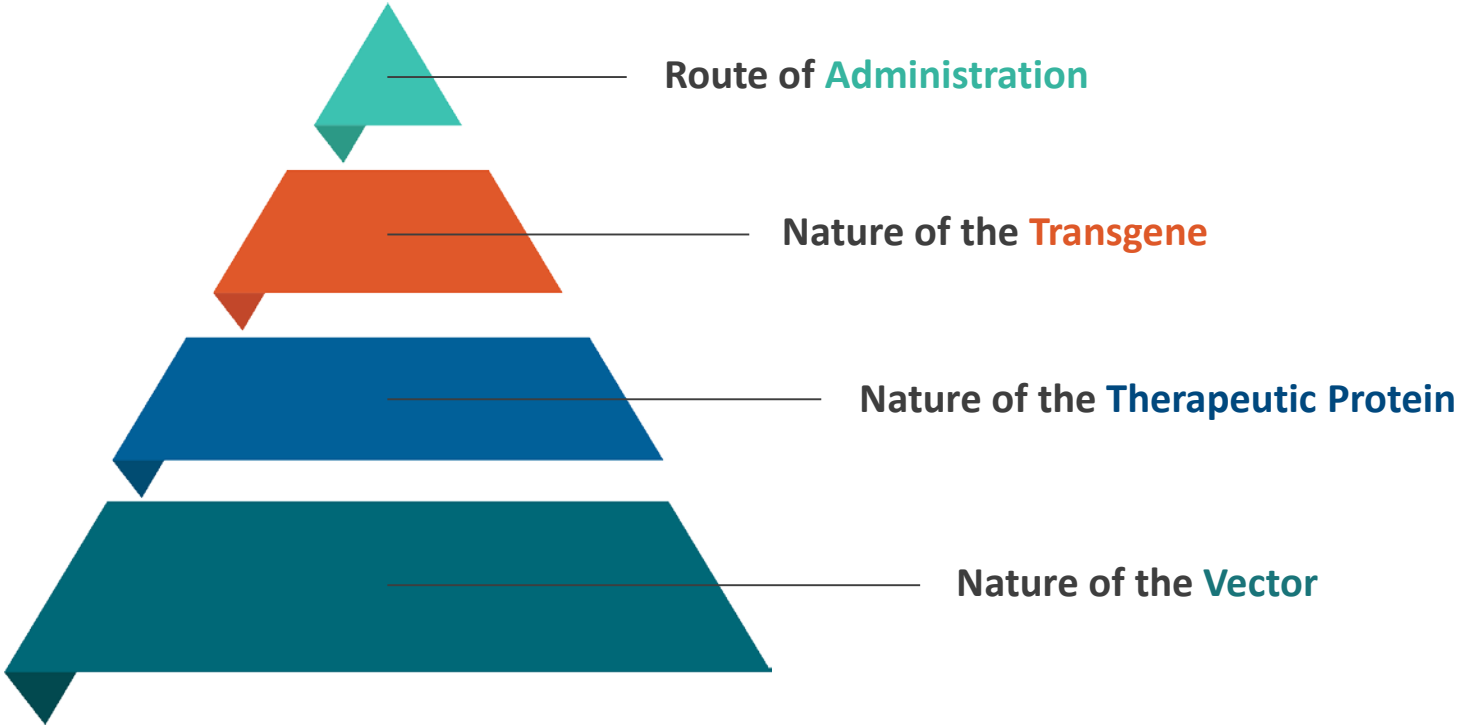
Tiered Approach for **Antibody Response Assessment**



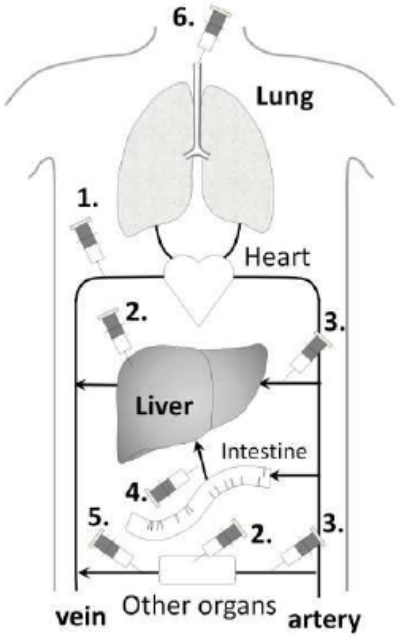
Classical tiered approach depicted above may not be totally suitable for all gene therapeutics

Immunogenicity Risk Assessment:

Factors To Be Considered



Route of **Administration**



1. Intravenous injection
2. Direct injection
   Organ surface instillation
3. Intra-arterial injection
4. Intraportal injection
5. Retrograde intravenous injection
6. Intratracheal administration
   Inhalation

Fumoto et al., IntechOpen, 2013

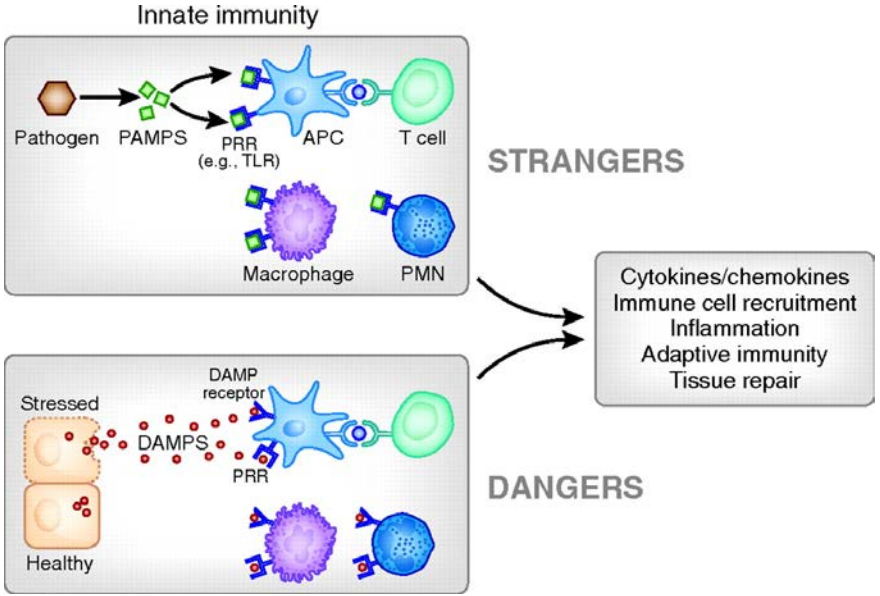
Route	Target	Vector
intra-arterial	Liver	Naked plasmid DNA
intra-arterial	Pancreas	Adenovirus
intra-arterial	Hind limb	Naked plasmid DNA
intra-arterial	Cecum	AAV
intra-arterial	Brain tumor	Adenovirus + Lipoplex
intraportal	Liver	Lipoplex
retrograde intravenous	Kidney	Naked plasmid DNA

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.

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

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

European Medicines Agency (2009)

Guideline on follow-up of patients administered with gene therapy medicinal products

- Immunogenicity related reactions

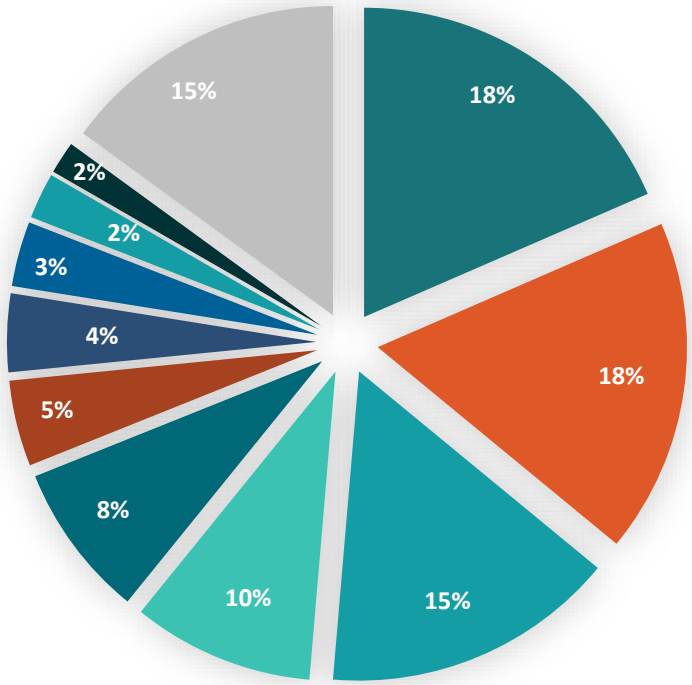
Unwanted immunogenicity could be observed, for example, due to the persistent gene expression. The consequences of such immune reactions range from transient appearance of antibodies or cell mediated immunity without any clinical significance to severe life threatening conditions.

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



- Adenovirus (19%)
- Retrovirus (18%)
- Naked plasmid DNA (15%)
- Lentivirus (10%)
- Adeno-associated virus (8%)
- Vaccinia virus (5%)
- Lipofection (4%)
- Herpes simplex virus (3%)
- Poxvirus (2%)
- RNA transfer (2%)
- Other (15%)

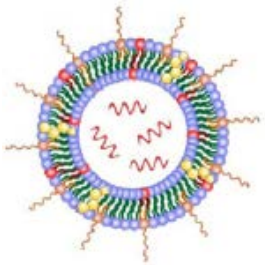
Over 2,900 clinical trials for gene therapy approved globally 1989-2018

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

Source: Gene therapy clinical trials worldwide. The Journal of Gene Medicine Clinical Trial site http://www.abedia.com/wiley/vectors.php (assessed 09-May-2019).

Lipid Nanoparticles (LNPs) as **Non-Viral Vectors**

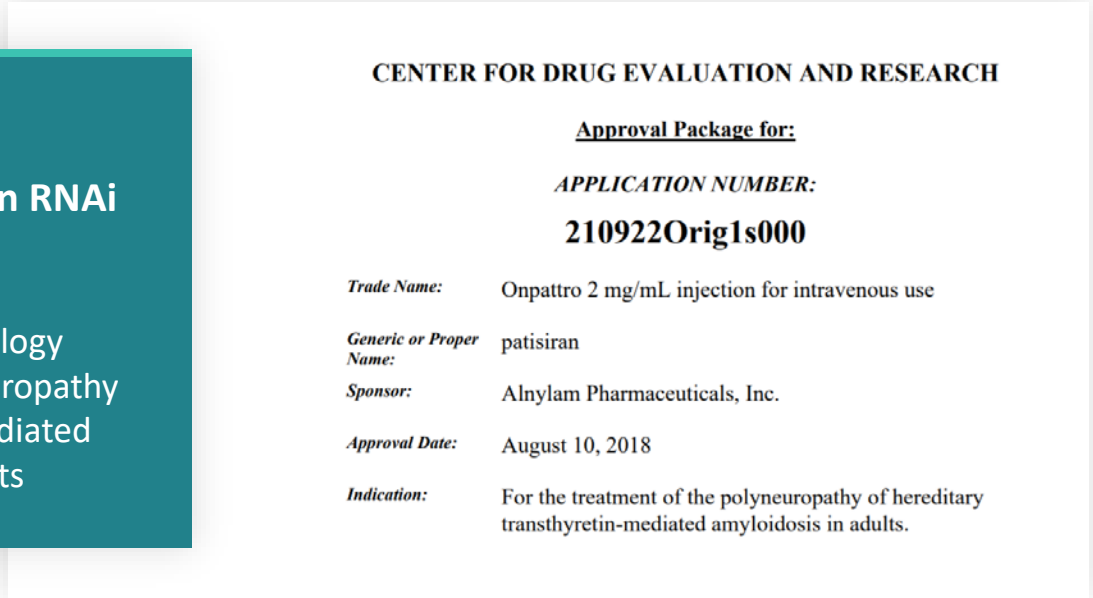
- Class of non-viral vectors formulated with synthetic or naturally derived lipids containing hydrophilic heads and hydrophobic tails.
- Typical components:
  1. a cationic or ionizable amino lipid to complex the nucleic acid and enhance endosomal escape
  2. a helper phospholipid to support the bilayer 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)

Lipid Nanoparticle **Application**

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



CENTER FOR DRUG EVALUATION AND RESEARCH

Approval Package for:

APPLICATION NUMBER:

210922Orig1s000

Trade Name:	Onpattro 2 mg/mL injection for intravenous use
Generic or Proper Name:	patisiran
Sponsor:	Alnylam Pharmaceuticals, Inc.
Approval Date:	August 10, 2018
Indication:	For the treatment of the polyneuropathy of hereditary transthyretin-mediated 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

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

Fumoto et al., (IntechOpen, 2015); from Thomas, Ehrhardt and Kay (2003)

Commonly infect humans => presence of pre-existing antibodies in a large fraction of the population

- Safety
- Distribution
- Efficacy

AAV Tropism and Antibody **Seroprevalence**

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

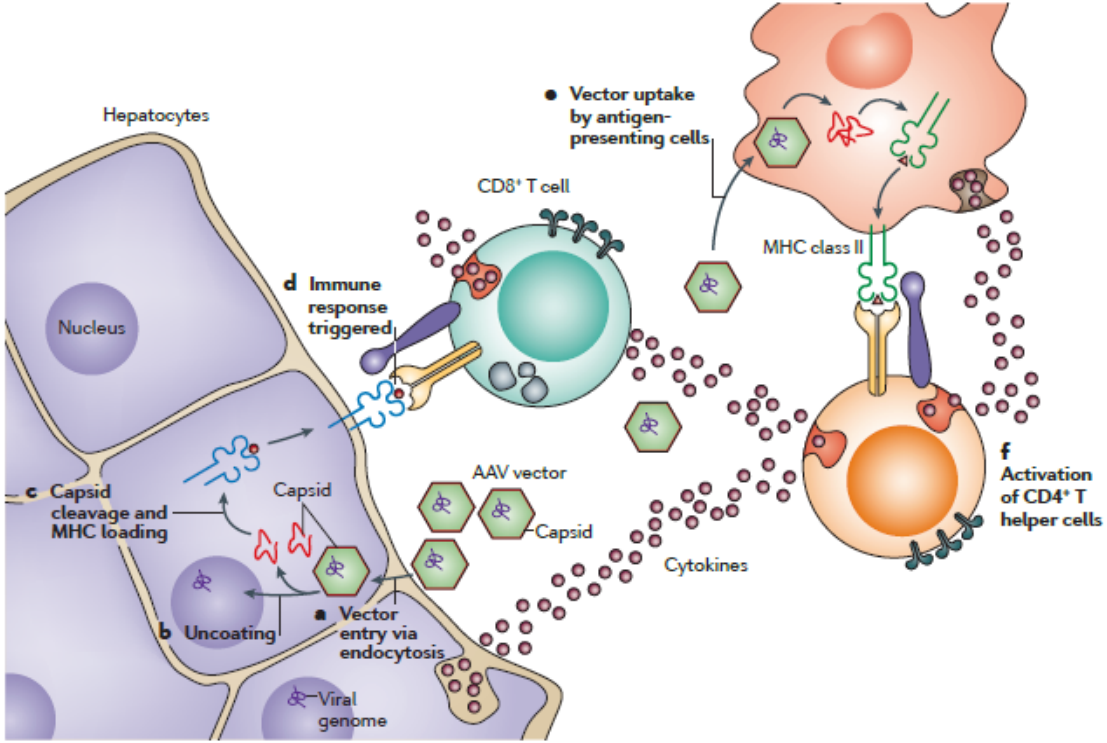
Sharon and Kamen, Biotechnology and Bioengineering 2018; Boutin et al. Hum Gene Ther. 2010; Sands, Methods Mol Biol. 2011; Mingozzi and High, Blood, 2013

- **Selection** and **optimization**
  - Tropism
  - Immunogenicity
- Low titer seroprevalence with limited neutralizing activity: key **advantage** for systemic AAV vector use

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



Consequences of Antibodies Against the Viral Vector

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)

Regulatory **Requirements**

Long-term follow-up (LTFU) required

- EMA Guideline on follow-up of patients administered with gene therapy medicinal products (2009)
- FDA Draft Guidance for Industry (2018) : Long Term Follow-up After Administration of Human Gene Therapy Products

338 We provide the following examples of evidence obtained from investigation of a product
339 that may warrant our recommendation of LTFU observations for delayed adverse events:
340
341 • A preclinical toxicology study indicates that expression of the therapeutic
342 gene (the transgene in your product) is associated with delayed toxicity.
343
344 • The therapeutic gene provides functional replacement of a host gene that
345 is otherwise not expressed, and the therapeutic protein is potentially
346 immunogenic.
347

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Thank You For Your Attention!