Innate and adaptive immune responses to Gene Therapy Products

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Immunogenicity of Biopharmaceuticals
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Gene Therapy Product definition

- **The Food and Drug Administration (FDA)** describes gene therapy as **products seeking to modify or manipulate the expression of a gene or to alter the biological properties of living cells** for therapeutic use. It generally considers human gene therapy products (GT) to be **all the products** that mediate their effects through the **transcription** or the **translation of transferred genetic material**, or by specifically **altering host (human) genetic sequences** [1]

- **The European Medicines Agency (EMA)** defines gene therapy medicinal products (GTMP) as a biological medicinal product with an **active substance** that contains or **consists of a recombinant nucleic acid** used in or administered to human beings to **regulate, repair, replace, add or delete a genetic sequence**; and mediates its therapeutic, prophylactic or diagnostic **effect** through the **recombinant nucleic acid sequence** it contains, or through the product of genetic expression of this sequence [2]

[1] Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products; Notice Federal Register1993 [53248-51]. Available from: https://fda.report/media/76647/Application-of-Current-Statuatory-Authorities-to-Human-Somatic-Cell-Therapy-Products-and-Gene-Therapy-Products.pdf

[2] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A02001L0083-20190726

Particularities of Gene Therapy Products (GTx)

✓ Diverse group of biotherapeutics
- *ex vivo* (genetically modified donor cells)
- *in vivo* (delivery of genetic material by different types of vectors)

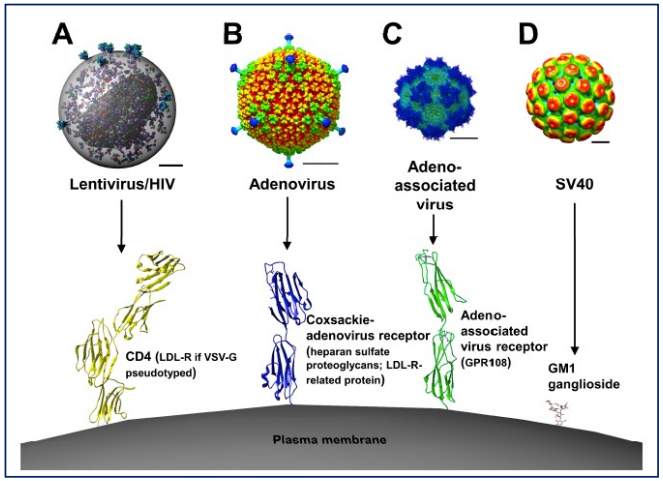
✓ Generally developed to treat conditions for which there are limited or no effective treatments (mostly rare diseases, oncology)

✓ Limitations to the development programs compared to other biotherapeutic products
- Limited number of patients (Orphan diseases)
- Pediatric use
- Single dose treatment
- Production scale: one batch – one patient

Salazar-Fontana LI, Front Med, Regulatory Science section, 2022

Immune responses to virus-mediated GTx

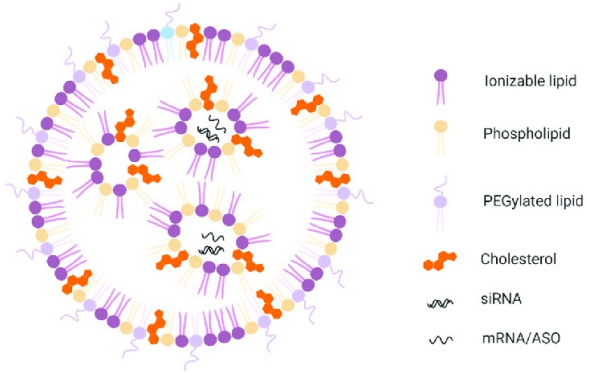
Types of delivery vectors



	AAV Vector	Lentiviral vector	Non-viral vector
Protein expression	Permanent	Permanent	Transient
Target cells	Differentiated cells	Stem cells/proliferating cells	Immune system, others
Applications	In vivo GT[(1)]	Ex vivo GT[(1)]	Vaccines, in vivo GE[(2)]
Manufacturing	Biological	Biological	Synthesis
Disease type	Genetic disease	Genetic disease/Cancer	Genetic disease/Immunotherapy

[(1)] GT: Gene therapy; [(2)] GE: Gene editing

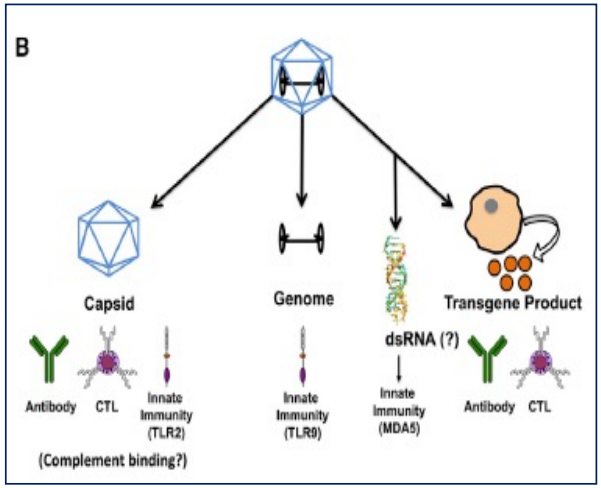
Moscoso and Steer, Genes, 2020; Wahane et al., Molecules, 2020



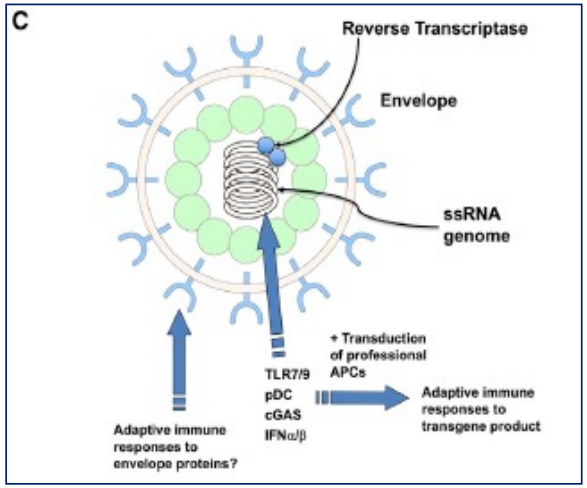
- ✓ The **drug product (DP)** is composed of the delivery vector (viral or non-viral) and the genetic material (DNA, RNA)
- ✓ Immune responses to **both components** need to be considered

Immunogenicity to virus-mediated GTx – Innate and adaptive responses

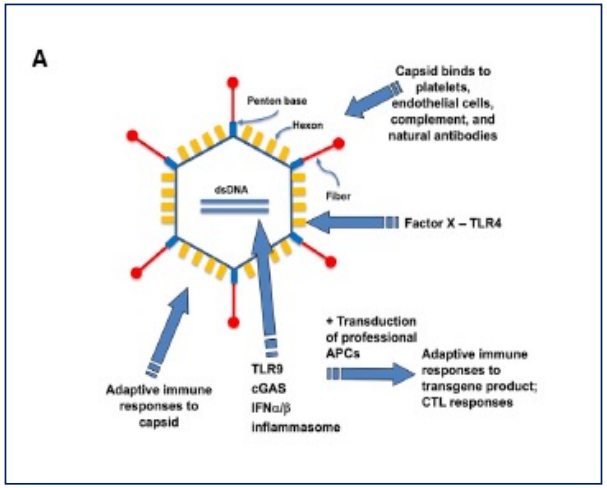
AAV vector



Lentiviral vector (LV)



Adenoviral vector (AV)



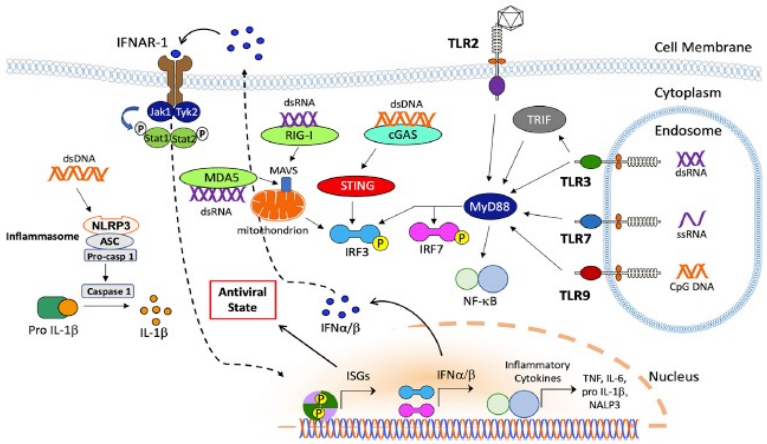
✓ Both the **innate and the adaptive immune systems** can be triggered by GTx components

- Innate immune system responses to the vector and the genetic material – ***Potential clinical consequences****: Cytokine Release Syndrome (CRS) , complement activation, upregulation of adaptive immune responses to GTx components*
- Adaptive immune responses to the vector and the expressed transgene (TG) – ***Potential clinical consequences****: autoimmunity, disease progression, tissue toxicity*

Shirley, JL et al., Mol Therapy, 2020, vol 28, n. 3; Annoni, A et al, Cell Immunol, 2019, vol 342

Molecular mediators of innate immune responses to viral GTx

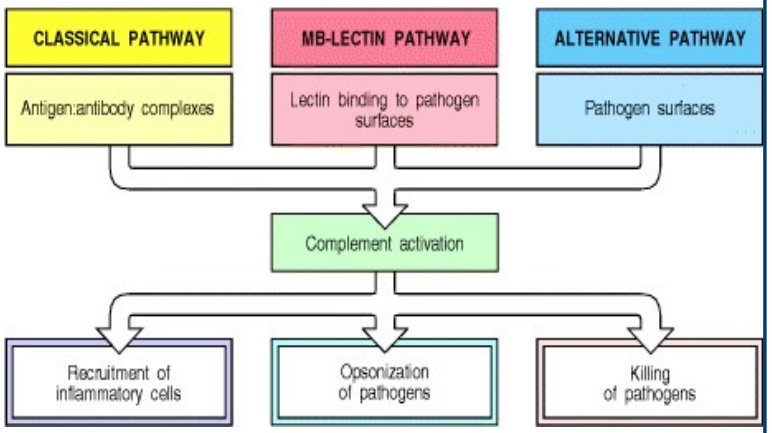
TLR system



Shirley et al., 2020

- **TLR2 -** Envelope glycoprotein
- **TLR3, TLR7** – Uracyl-rich regions present in dsRNA (TLR3) and ssRNA (TLR7)
- **TLR9** – Un-methylated CpG motifs present in the genome DNA (AAV)

The complement system



Janeway, 2001

Initiated by pre-existing antibodies to the virus and natural IgM

Direct binding of complement components to the viral capsid

Reported immune responses to *in vivo* and *ex vivo* GTx

- ✓ **Pre-existing immunity to the delivery vector**
  - Anti-viral vector antibodies (AVA)
  - Anti-PEG antibodies (LNP)
  - CD8+ virus-specific T cells

- ✓ **Innate immune responses to the delivery vector and the genetic material**
  - Toll-Like Receptor activation
  - Complement system activation

- ✓ **Adaptive immune responses to the expressed TG**
  - Anti-TG antibodies
  - CD8+ TG-specific T cells

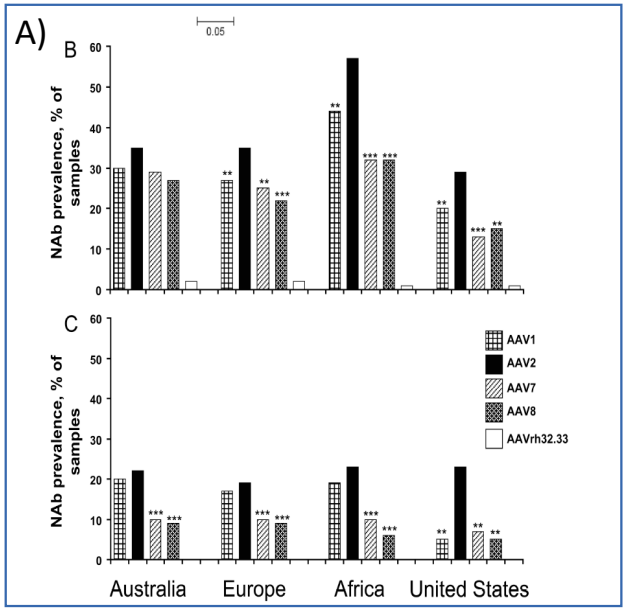
Shirley, JL et al., Mol Therapy, 2020, vol 28, n. 3
Costa Verdera et al, Mol Therapy, 2020
Petrus-Reurer et al, Commun Biol, 2021, 4: 798
Wagner et al., Nat Rev Clin Oncol, 2021, 18(6): 379
Ertl HCJ, Front Immunol, 2022

Pre-existing anti-AAV antibodies in the human population

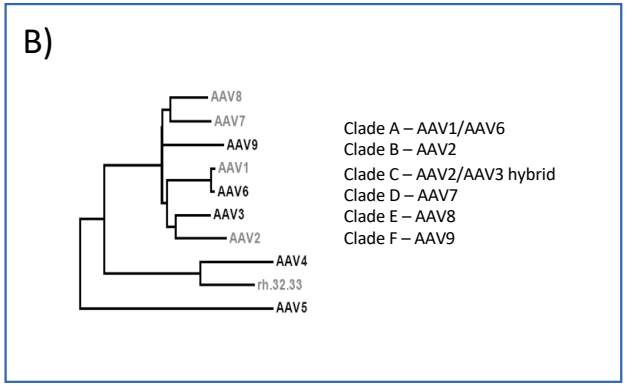
- ✓ Prevalence of anti-AAV antibodies ranges between 30-80% of adults:
  - anti-AAV2 Ab > anti-AAV1 Ab > anti-AAV6 Ab > anti-AAV5 Ab > anti-AAV9 Ab > anti-AAV8 Ab (A)

- ✓ Predominant IgG1 response and low IgG2, IgG3, and IgG4 indicative of an adaptive (mature) immune response. Evidence of increased neutralizing activity with IgG isotype switching
- ✓ High level of cross-reactivity among anti-AAV Abs due to common phylogenetic origin (B)
- ✓ Detected in pediatric population with higher prevalence of anti-AAV2 and anti-AAV8 Ab (C)

A)



B)



Calcedo, R. et al, J Infect Dis, 2009, vol 199
Calcedo, R. et al, Clin Vac Immunol, 2011, vol 18
Gao et al, J Virol, 2004, vol 78
Boutin et al, Hum Gene Ther, 2010

C)

Table 3. Neutralizing antibody (NAb) titers of human pooled IgG in response to different adeno-associated virus (AAV) types.

AAV type	NAb titer
AAV1	1:640
AAV2	1:2560
AAV7	1:640
AAV8	1:320
AAVrh32.33	1:20

TABLE 2. Average prevalence of NAb (titer of ≥ 1:20) by AAV serotype in anonymous serum samples from Children's National Medical Center

AAV serotype	No. of samples: Tested	Positive	% prevalence	Relative prevalence	95% confidence interval	*P* value
2[a]	275	78	22.1			
8	333	62	15.7	0.71	0.53, 0.96	0.025

[a] Reference group for comparisons of relative prevalence.

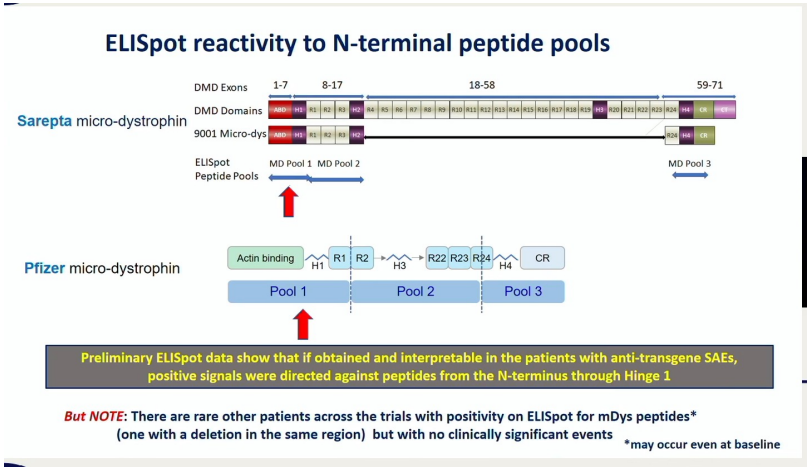
Immune toxicities to *in vivo* AAV-mediated GT

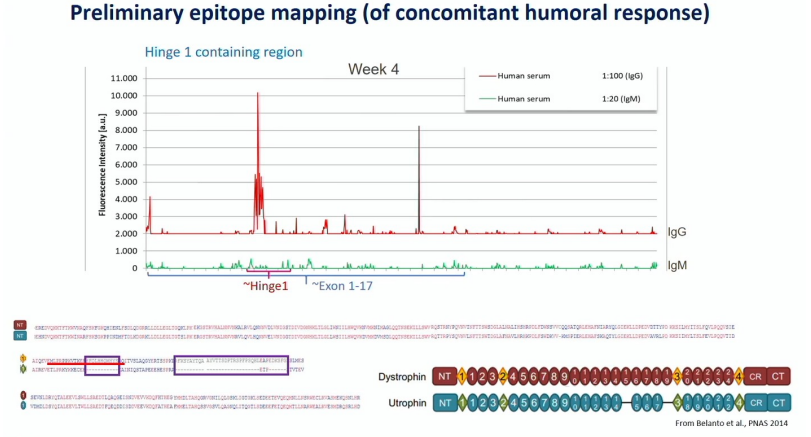
Observed Immune Toxicities	Preclinical	Clinical	Immune response mediators	Immunosuppresive regimens
Hepatotoxicity	NHP	Hemophilia A (F.VIII) Hemophilia B (F.IX) X-linked Myotubular myopathy	• Anti-AAV antibodies • **AAV-specific CD8+ T cells** • TG-specific CD8+ T cells (*) • Treatment-emergent anti-TG antibodies	• Plasmapheresis • Glucocorticoids • Rapamycin (T cell responses) • Ibrutinib (B cell inhibitor) • Eculizumab (Complement system) • Rituxumab (B cell responses)
Thrombotic microangiopathy (TMA)	NHP	SMA DMD	• **Complement activation** • AAV-specific CD8+ T cells (*) • **Pre-existing anti-AAV antibodies**	
Dorsal Root Ganglia (DRG)	Mice Piglets NHP	SMA	• AAV-specific CD8+ T cells • TG-specific CD8+ T cells (*)	
Myocarditis	NHP (?)	DMD	• **TG-specific CD8+ T cells** • Anti-TG antibodies (*)	
NHP: Non-human primates; SMA: Spinal Muscular Atrophy; DMD: Duchenne Muscular Dystrophy; (*) No direct measurement available				

Ertl, HC, Front. Immunol., 12 Aug 2022; Ronzitti, G et al., Front. Immunol., 2020; FDA CTGTAC meeting, Toxicity risks of AAV vectors for GT, Sept 2-3, 2021)

DMD and adaptive immunogenicity

- ✓ Observed in four different clinical trials (Pfizer Inc., Genethon, Sarepta Therapeutics, Solid Bioscience Inc.)
- ✓ AAV-mediated delivery of micro-Dystrophin (doses 1e13 to 2e14 vg/kg)
- ✓ Symptoms onset: 24 – 42 days post dosing – Consistent with time course of TG expression
  - ➢ Muscular weakness
  - ➢ Heart involvement (EF, MRI)
  - ➢ No obvious problems with kidney, liver, platelet, or complement system
- ✓ Patients with N-terminal deletions in the dystrophin protein showed lack of tolerance to the TG (*del Ex. 8-21, 8-44, 5-42, 3-43*):
  - ➢ **N-terminal specific T cell reactivity (ELISpot)**
  - ➢ **Anti-TG IgG**





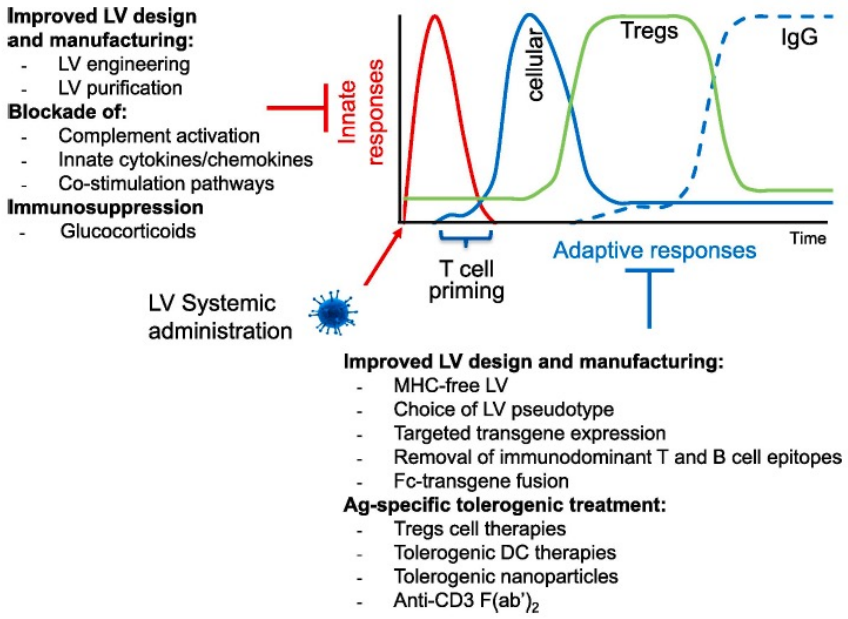
Source: 25th ASGCT conference - Collaborative working group presentation

Immunogenicity to LV vectors

- ✓ **Enveloped** viruses with a **ssRNA genome of 7-10 kb** infecting both non-dividing and actively dividing cells
- ✓ **Less severe immune toxicities** reported for **LV-mediated GT** since they are mostly used as ***ex vivo* GT**.

Reported immunes responses to ***in vivo*** LV-mediated GT:

1. Innate immune responses: Complement and TLR systems
2. Pre-existing antibodies to **LV pseudotype proteins** (not clear preclinical evidence)
3. **Allogeneic/xenogeneic immune responses to residual MHC class I (packaging cell line)**



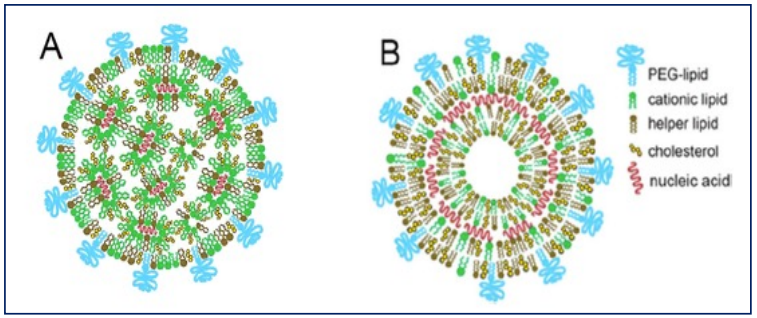
Annoni et al., 2019

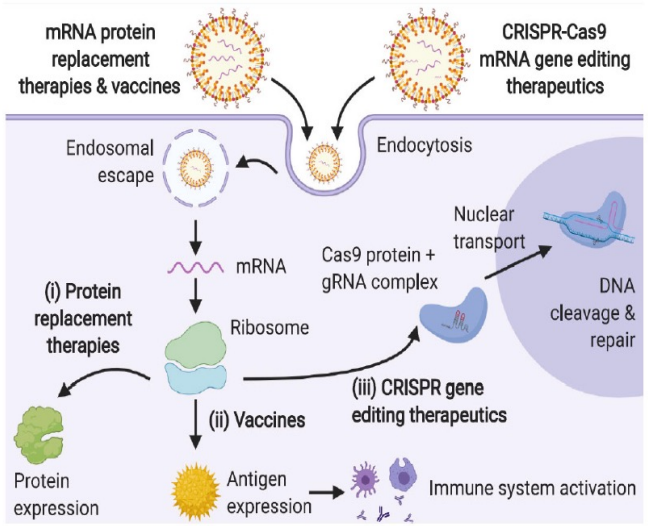
Immune responses to LNP-delivered GTx

Immune responses to lipid nanoparticles (LNPs)

Lipid-based formulations represent the most developed tool for **RNA and Gene Editing delivery**

- ✓ *Lipid nanoparticle*: pH-responsive lipids with tertiary and quaternary amines to encapsulate the RNA. Contains **pegylated lipids** (improves colloidal stability)
- ✓ *Lipid –polymer hybrid nanoparticle*: mono or bilayer. Contains **pegylated lipids**





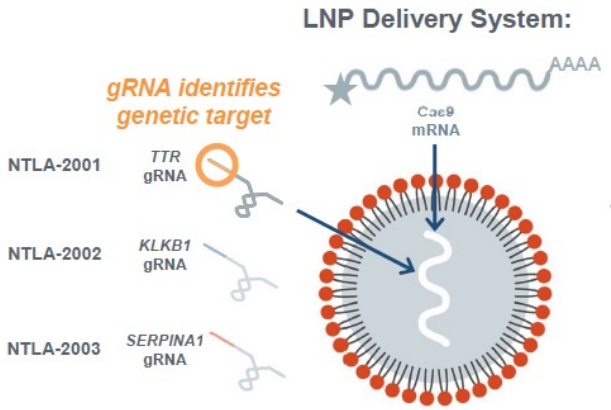
Swingle et al, Trends in Mol Med 2021

Potential clinical immunogenicity risks

- ✓ Anti-PEG antibody-mediated hypersensitivity reactions
- ✓ PK alterations (?)
- ✓ Elimination of transduced cells

Guevara, ML et al., Front Chem, 2020; Zhang, X et al., J Clin Pharm, 2020; Polack, FP et al., N Engl J Med, 2020; Swingle et al., Trends in Mol Med, 2021; Ju et al, Nature Rev Immunol, 2023

Preclinical immunogenicity of Intellia's CRIPR/Cas9 NTLA-2001



Immunogenicity potential

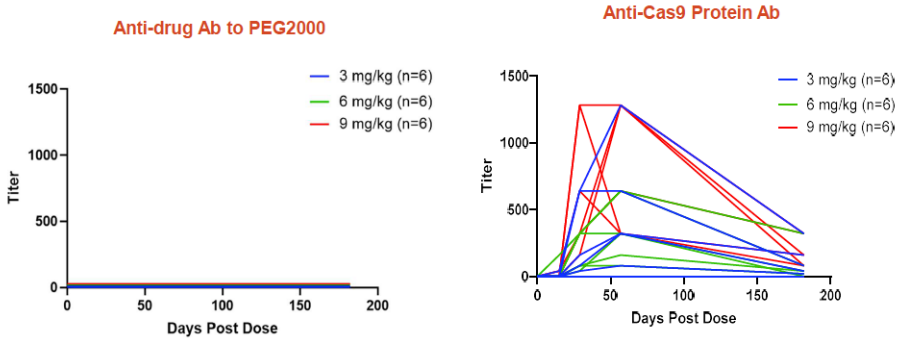
- LNP PEG lipid – systemic exposure (IV)
- Bacterial Cas enzyme - intracellular location

Establish risk-based immunogenicity strategy

- Assess anti-drug antibody (ADA) to LNPs and antibody to Cas9 protein

- **Mild adverse (grade 1) events at day 28 in 3/6 patients with no impact on reduction in serum levels of TTR**
- **Pre-conditioning treatment with glucocorticoids and anti-histamine**

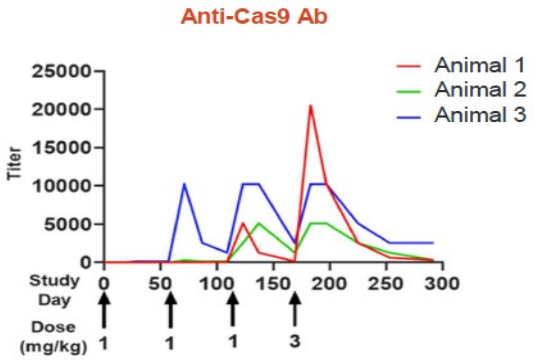
NHP Single IV dose



- Representative of PEG present in LNP

NHP Multiple IV dose

- Transient Ab response
- No impact on PK/PD
- No anatomical findings



Yuanxin Xu, MD, PhD, September 30th, 2022 WRIB presentation; Gillmore et al., NEJM, 2021, vol 385

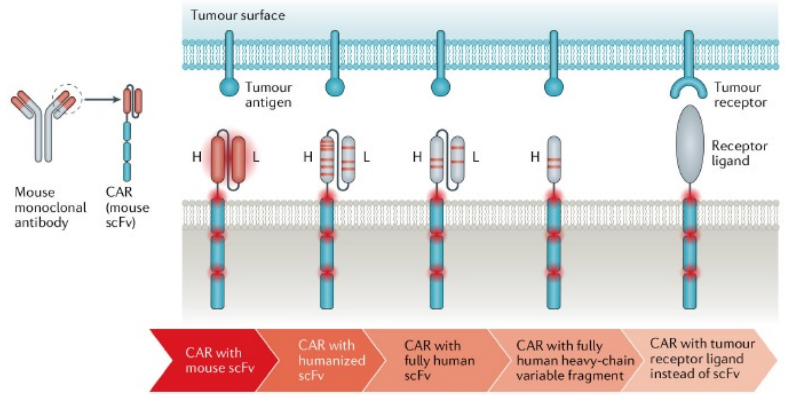
Immune responses to *ex vivo* GTx

Immune toxicities to *ex vivo* GTx (LV-mediated)

Kymriah [3]	CD19 CAR T Autol. *ex vivo* GTMP (L)	Treatment of paediatric and young adult patients up to 25 years of age with B–ALL that is refractory, in relapse post-transplant or in second or later relapse and for the treatment of adult patients with relapsed or refractory DLBCL after >2 lines of systemic therapy	▸ High T-cell activation, CRS, neurotoxicity, murine binding domain, lentivirus vector, GvHD, tumor lysis syndrome ▸ Patient conditioning	▸ *In vitro* cytokine production of CAR+ T cells after stimulation with tumor cells ▸ *In vivo* studies in NOD/Shi-scid IL-2Rγ null (NOG) mice	▸ CRS reported in most patients ▸ Neurotoxicity reported in 21and 40% of DLBCL and ALL patients, respectively ▸ GvHD detected in one patient; 3 % of patients suffered from tumor lysis syndrome ▸ Tocilizumab treatment for severe immunotoxicity ▸ Risk of immunotoxicity to be further studied in a PASS
Yescarta [5]	CD19 CAR T Autol. *ex vivo* GTMP (R)	Treatment of adult patients with relapsed or refractory DLBCL and PMBCL, after two or more lines of systemic therapy.	▸ High T-cell activation, CRS, neurotoxicity, murine binding domain, retrovirus vector, GvHD, tumor lysis syndrome ▸ Patient conditioning	▸ Immunophenotyping (CD3, CD4, CD8 and CD45RA) ▸ Co-culture with CD19-K562 or NGFR-K562 cell lines; analysis of 17 cytokines/chemokines/ ▸ Effector molecules ▸ A homologous syngeneic mouse lymphoma model for on-target/off-tumor toxicity	▸ CRS reported in most patients ▸ Neurotoxicity in 65% of the patients, severe neurotoxicity associated with a panel of cytokines ▸ Binding antibodies against the murine FMC63 binding domain ▸ Tocilizumab treatment for severe immunotoxicity ▸ Risk of immunotoxicity to be further studied in a PASS

Salmikangas,P.; Chamberlain, P. et al. 2019

- ✓ Cytokine Release Syndrome (CRS) (MoA)
- ✓ Neurotoxicity associated to levels of pro-inflammatory cytokines (IL-1β and IL-6) (MoA)
- ✓ Graft-versus Host disease (GvHD) (Allogeneic modality)
- ✓ HAMA responses (non-germline sequences in CAR construct)



Wagber et al., Nat Rev Clin Oncol, 2022

Management of inflammatory toxicities to CAR-T cell therapy products

Pre-clinical development

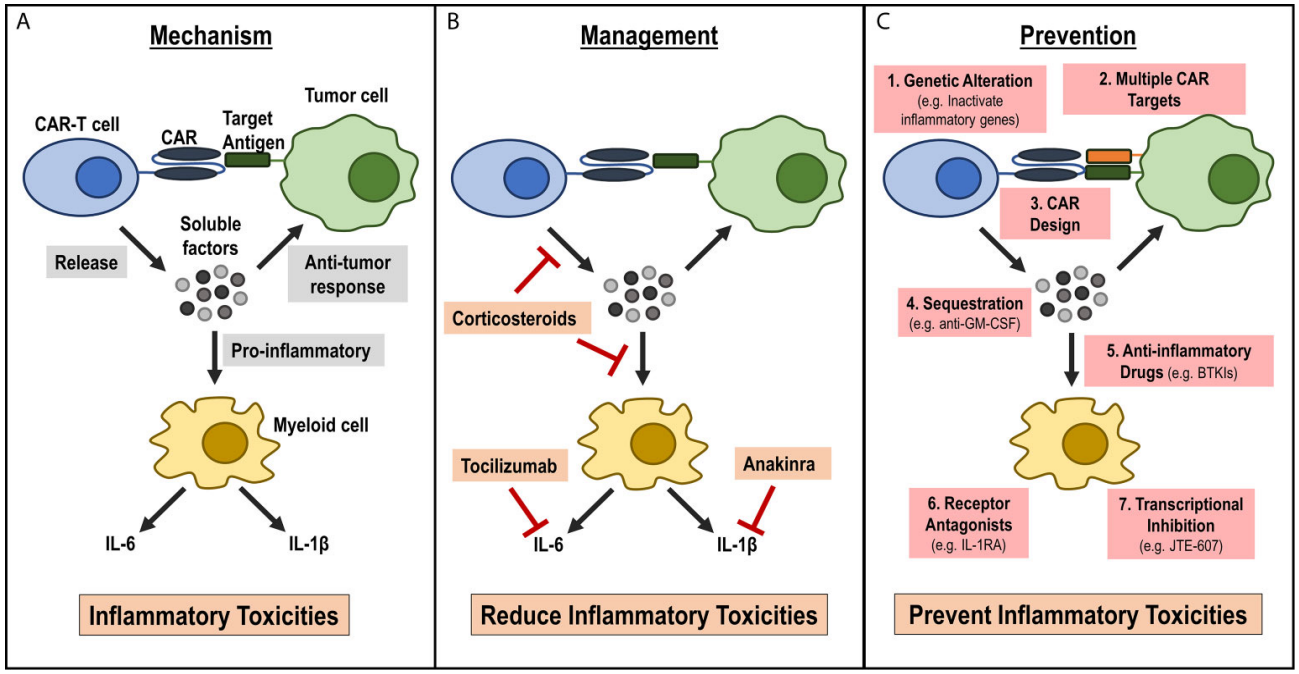
- ✓ Predictive immunogenicity
  - ➢ *In silico* prediction
  - ➢ *In vitro* cytokine release

Clinical development

- ✓ Levels of serum pro-inflammatory cytokines
  - ➢ Adaptive immune responses to allogeneic cells and CAR-T molecule

Clinical intervention

- ✓ anti-inflammatory drugs , receptor agonists, transcriptional inhibitors



Fischer and Bhattarai, Front. Immunol., 2021

Immune responses to approved GTx

Immunogenicity evaluations in approved *in vivo* virus-mediated GTx

Name	Indication	Vector type, dose, route of administration	Immunogenicity	Immune mitigation
Zolgensma	Spinal Muscular Atrophy (SMA)	AAV9; 1.1e14 vg/kg; single IV infusion	• Pre-existing and treatment-emergent anti-AAV9 Abs in all treated patients (min 1: 102,400) • Thrombotic microangiopathy (TMA) • Systemic Immune response	Glucocorticoids (preconditioning and 30 day pos-administration) Exclusion of patients with anti-AAV9 Ab titers > 1:150
Luxturna	Leber's congenital amaurosis; retinitis pigmentosa	AAV2; 1.5e11 vg; sub-retinal	• Not available	Corticosteroids
Roctavian [2]	Hemophilia A	AAV5 (FVIII); ; single IV infusion	• CDx anti-AAV5 Tab • AAV5-cellular responses (ALT elevations) • Anti-FVIII NAb	Exclusion of patients positive for anti-AAV5 Ab (above 29 ng/mL LOD CDx) or NAbs to FVIII
Hemgenix [1]	Hemophilia B	AAV5; 2e13 gc/kg; single IV infusion	• Treatment-emergent anti-AAV5 NAbs (non validated assay) [3] • PD marker: Factor IX activity • 33% infusion reactions • 14%Flu-like symptoms	Corticosteroids
Adtiladrin [1]	Bladder cancer	Non-replicating Ad-TG-hIFNalpha2b; intravesical instillation; 3e11 vp/4 doses/3 mo apart	• Not available	None

[1] Only in the US; [2] Only in the EU; [3] First detected at week 3 and persist up to 24 months

Sources: ASGCT and Pharmaprojects Citeline Gene, Cell, & RNA Therapy Landscape report Q2 2023; CBER: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

Immunogenicity evaluations in approved genetically modified cell therapy products (*ex vivo* GTx)

24 approved GTx worldwide from which 19 are genetically modified cell therapies including CARs, oncolytic viruses, and modified somatic and stem cells [(*)]

- ✓ All CAR therapies monitor for CRS and neurotoxicity
  - ➢ Immunogenicity reported as presence of anti-CAR antibodies
  - ➢ T cell responses (only Kymriah)

- ✓ Oncolytic virus (immune modulators): only one approved IMLYGIC
  - ✓ No evaluation of anti-TG antibodies or pro-inflammatory cytokines
  - ✓ Report manifestations of immune responses under Adverse Events (i.e. flu-like symptoms, autoimmunity)

- ✓ Genetically and minimally-modified autologous cells (HSC[1,2], MNC[3]):
  - ✓ GvHD (engraftment) [1, 2]
  - ✓ Anti-TG antibodies [1]
  - ✓ Adverse events reported: infusion reactions, influenza-like illness, cytopenia, autoimmunity [2,3]

[(*)] *ASGCT and Pharmaprojects Citeline Gene, Cell, & RNA Therapy Landscape report Q2 2023;* [1] *LIBMELDY;* [2] *SKYSONA;* [3] *PROVENGE*

De-risking new GTx drug candidates

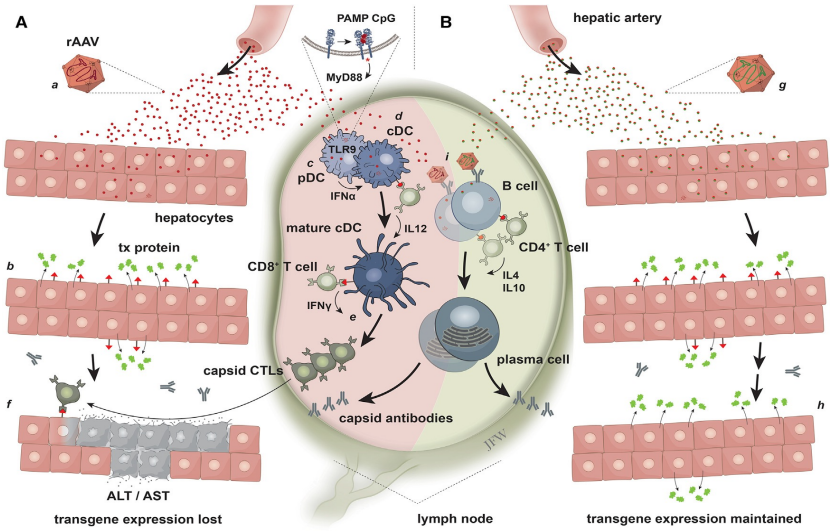
AAV GTX

- ✓ Removal of CpG motifs from ORF and non-coding regions of the TG expression vector to reduce TLR9 responses
- ✓ Engineered viral capsids proteins to reduce impact of pre-existing antibodies
- ✓ Manufacturing process impurities:
  - ➢ residual unpackaged plasmid DNA
  - ➢ empty and partially loaded capsids
  - ➢ Residual packaging cell line DNA and HCP
  - ➢ Residual Benzonase

LV GTx

- ✓ Pseudo typing virus
- ✓ Manufacturing process impurities
  - ➢ Residual antibody-coated beads used in expansion (CGTx)
  - ➢ Residual vector envelopes
  - ➢ Residual packaging cell line DNA and HCP
  - ➢ Residual Benzonase

High PAMP CpG content | **Low PAMP CpG content**



Faust, S.M., et al, J. Clin. Invest. 2013; Fraser Wright, J., Mol. Therapy, 2020; Merten et al., Molecular Therapy-Methods & Clinical Development, 2016; *Perry and Rayat, Viruses, 2021*

Challenges to the current immunogenicity paradigm

Re-evaluating of the immunogenicity concept

- ✓ The evaluation, management, and mitigation of anti-drug antibodies (ADA) established for therapeutic proteins **is not sufficient** to address the **immunogenicity risk** of this new product modality in their overall **benefit:risk analysis**

- ✓ Important to distinguish between **immunogenicity evaluation** (ADA formation and innate immunity) and **immune toxicity monitoring**
  - Immune toxicity seems to be directly linked to the viral vector load (i.e. hepatotoxicity) and the route of administration (i.e. systemic >> intramuscular)

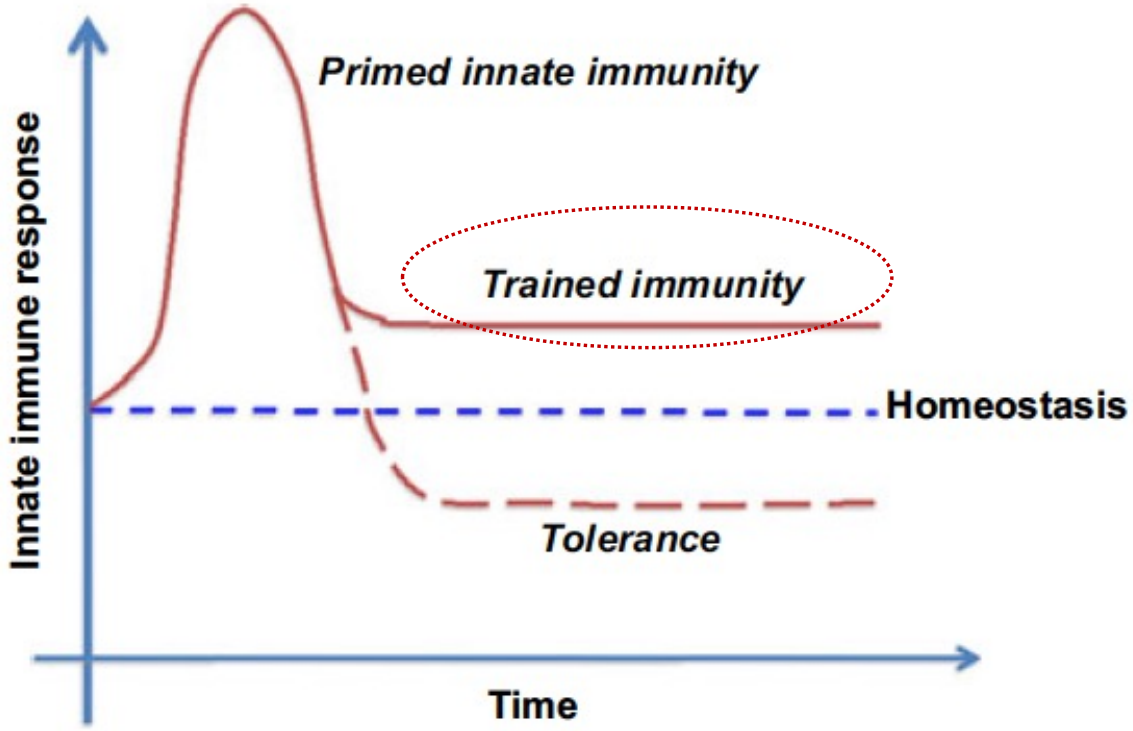
- ✓ Principles of the **risk-based approach** used to evaluate the **immunogenicity of therapeutic proteins** can be adapted to define a **robust clinical immunogenicity plan**

Predictive immunogenicity in GTx development

Predictive immunogenicity is crucial to understand the potential:

- ✓ Risk to CRS
- ✓ Risk of complement activation (pre-existing immunity to viral and non-viral vectors)
- ✓ Risk of TLR activation (candidate selection)
- ✓ Risk of long-term consequences of adaptive immune responses (humanized animal models and disease models)

Trained immunity – Long-term impact in safety and efficacy



Netea, 2013

- ✓ Most GTx induce transient innate immune responses evidenced by elevations of IL-1β, IL-6, IL-8, CXCL10 and type I IFNs
- ✓ The contribution of pro-inflammatory cytokines to the magnitude of adaptive immune responses **is currently unknown**
- ✓ Evaluation of **trained immunity thresholds** seems to be **crucial to interpret the magnitude and potential consequences of adaptive immune responses over time**

Immunogenicity findings and benefit:risk analysis

Clinical Concern	Clinical Outcome
Safety	• Immune toxicity (i.e. hepatotoxicity) • Complement-mediated reactions • TLR-mediated pro-inflammatory responses (i.e. type I IFN secretion, CXCL10)
Efficacy	• Loss of efficacy due to increased vector clearance (pre-existing and treatment-induced responses) • Elimination of transduced cells (treatment-induced cellular responses)
Insignificant	• Transient treatment-emergent immune response (single-dose treatment) • Immunosuppressive treatments

Applying a **question-based approach** that interrogates your product from **multiple perspectives** is the best approach to:

- define **a tailored bioanalytical and testing strategy**
- **interpretation of clinical findings** leveraging the contribution of **innate responses in long-term adaptive immunogenicity** (i.e. autoimmunity)

THANK YOU!

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