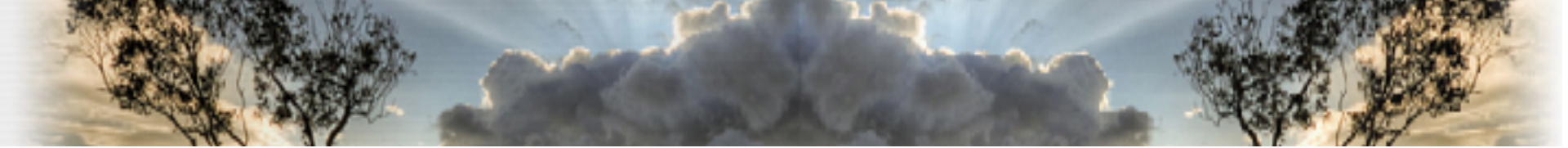
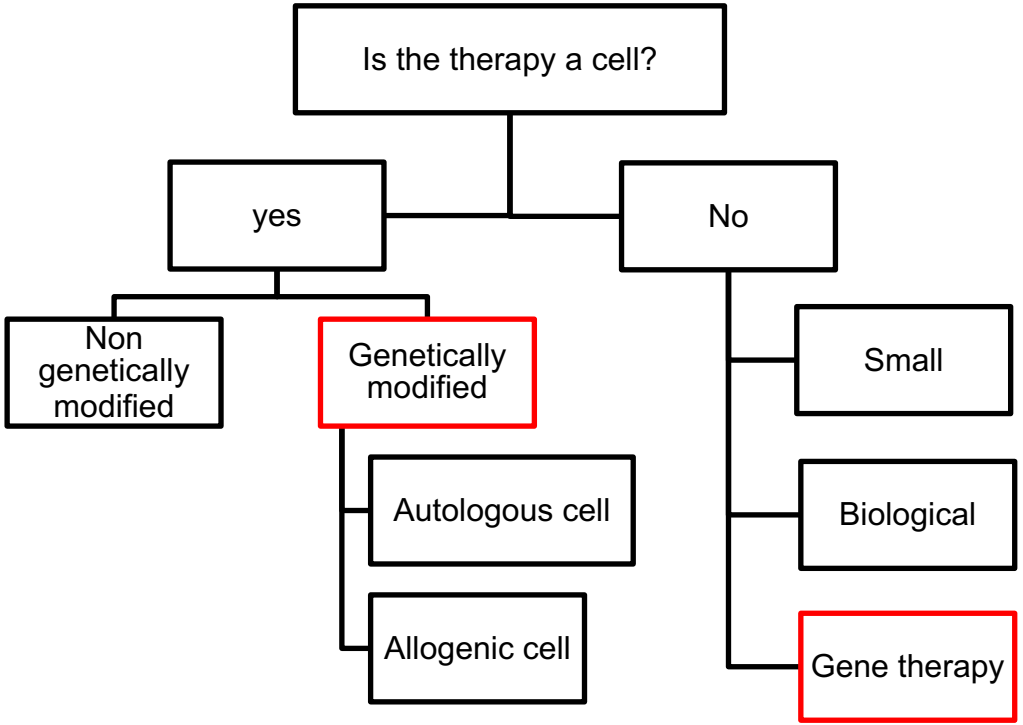


Bioanalytical approaches to assess the immunogenicity in Cell & Gene Therapies

Arno Kromminga (on behalf of the EBF C&GT team)

Cell & Gene Therapy Training Day
15-17 September 2020:

Cell *"vs"* gene therapies



In vivo and ex vivo Gene Therapies

Gene delivery to cells outside the body

Delivery vectors

Lentivirus
Electroporation

Cargo / Payload

Nucleic Acid

Genome editing tool

Collect
(from **patient** (autologous) or **donor**) (allogenic))

Select

Modify

Amplify

Deliver

DOMINANT CURRENT DELIVERY VECTOR: **Lentivirus**

Diseases

Cancer
Infectious diseases
Cardiovascular
Neurological
Ocular
Metabolic
…

In vivo Gene delivery to patient cells

Local injection

Systemic injection

Delivery vectors

AAV **LNP**

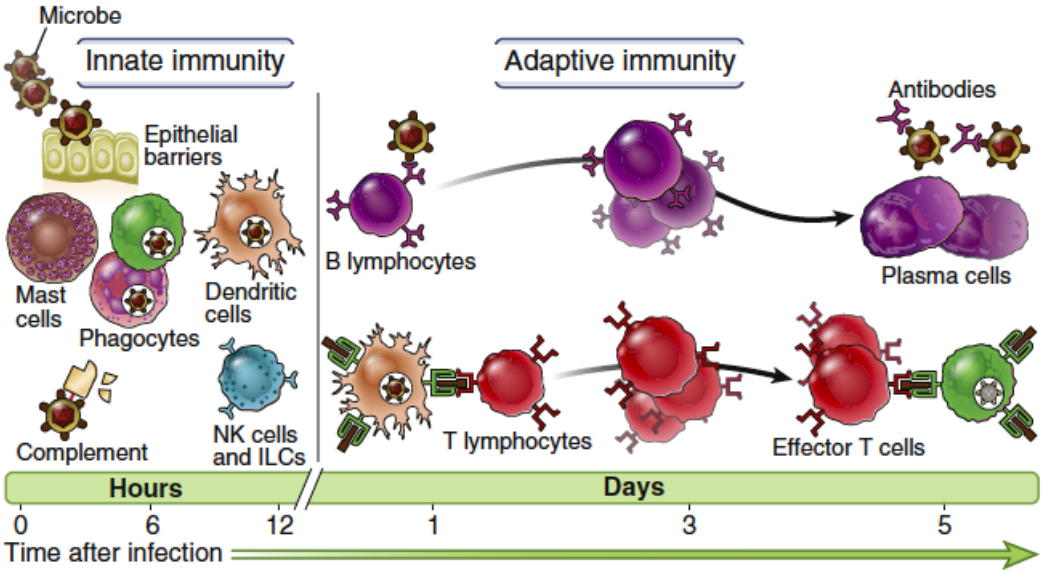
Cargo / Payload

Nucleic Acid

Genome editing tool

DOMINANT CURRENT DELIVERY VECTOR: **AAV**

Induction of Immune Responses

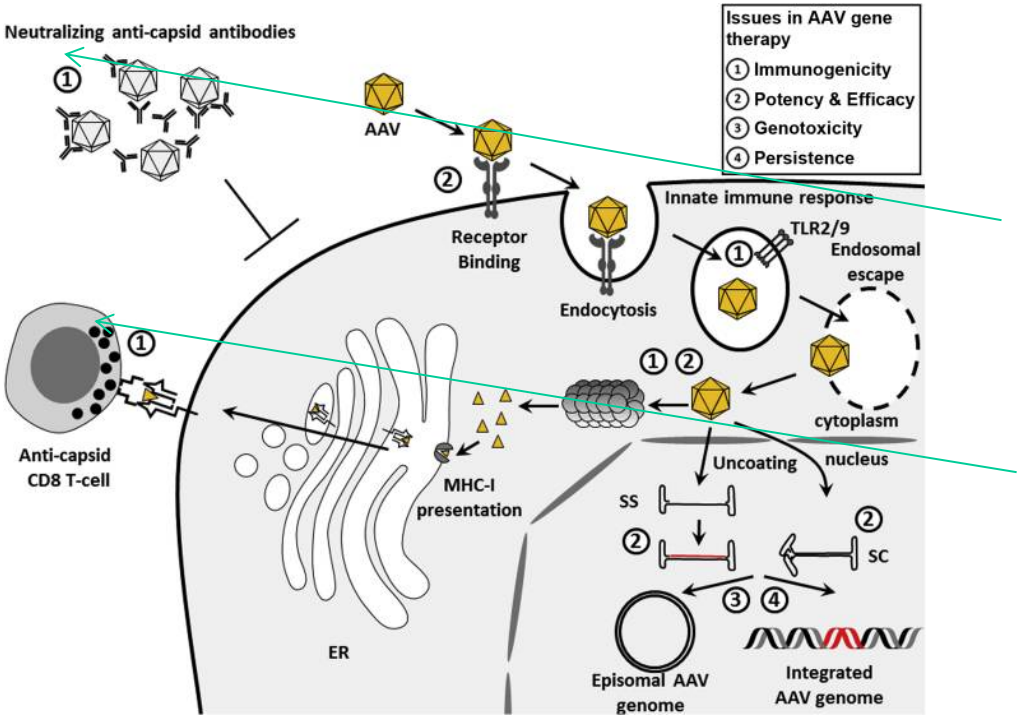


ADA

ELISpot
FACS

Image from: Abbas, Lichtman and Pillai, 2016

Gene Therapy – Capsid immunogenicity

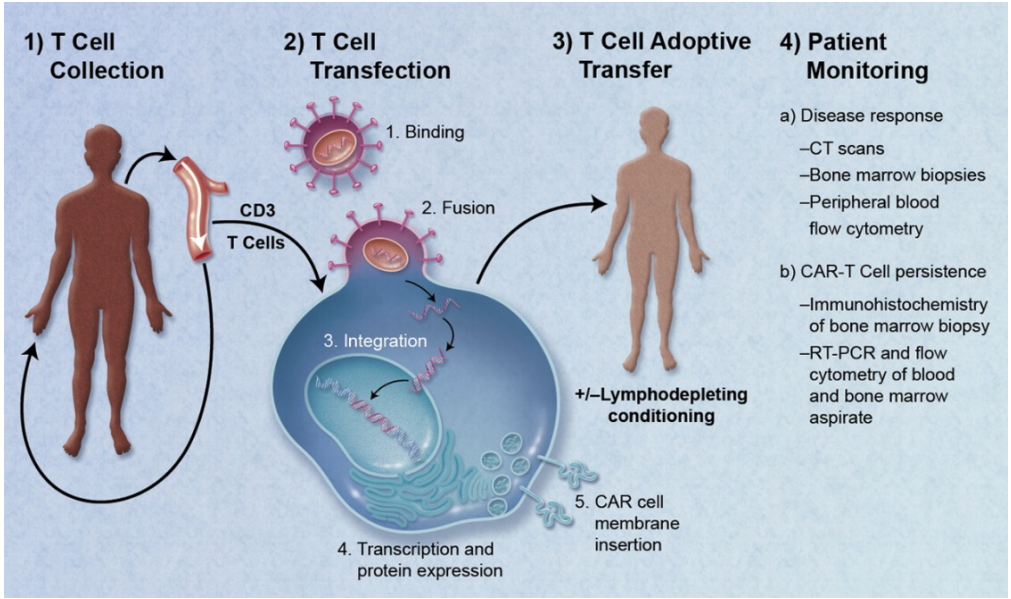


Humoral response:
ADA and/or NAB assay

Cell mediated response:
ELISpot and/or FACS

Image from: Emerging Issues in AAV-Mediated In Vivo Gene Therapy. Pasqualina Colella, Giuseppe Ronzitti, and Federico Mingozzi. Molecular Therapy: Methods & Clinical Development Vol. 8 March 2018

Autologous T Cell Therapies



- CAR-T: current paradigm of cell therapies
- Allogenic cell therapies: cells come from donors => impact on the immunogenicity assessment

Tiered Antibody Measurement (ADA)

Antibodies against:

- ✓ Therapeutic protein
- ✓ Vector
  - ○ Viral
  - ○ Non-viral
- ✓ Nucleic Acid Cargo

- ✓ Screening Assays
- ✓ Confirmatory Assays
- ✓ Titer Assays
- ✓ Characterization Assays
  - ✓ Neutralizing capacity (NAB)
  - ✓ Domain Characterization
  - ✓ Isotyping
  - ✓ Avidity

Consequences of Anti-Transgene Antibodies

Protein Encoded by the Transgene	Likelihood of Antibody Response
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*

Antibodies against Viral Vectors

- Pre-existing antibodies
  - Neutralizing: Prevent viral particle to dock on the cell and infect it
  - Clearing: induce the clearance of the vector by the immune system before it has reached its target cell
  - Destroy the corrected cells which express the transgene
  - No effect
- Induced or boosted AB
  - Same consequences as above
  - Prevent re-administration

AAV Tropism and Prevalence

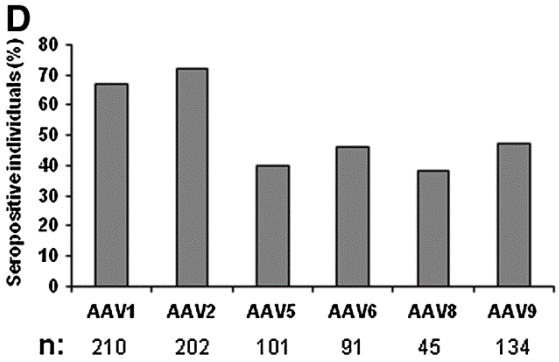
Serotype	Tropisms	Serum antibodies	
		Prevalence	Titer
AAV1	Airway, CNS, retina, skeletal muscle	67 %	High
AAV2	Kidney, liver, retina, vascular tissue	72 %	High
AAV4	CNS, kidney, lung	N/A	High
AAV5	Airway, CNS, skeletal muscle	40 %	Low
AAV6	Skeletal muscle, T-cells, HSC	46 %	High
AAV8	Liver, CNS, retina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

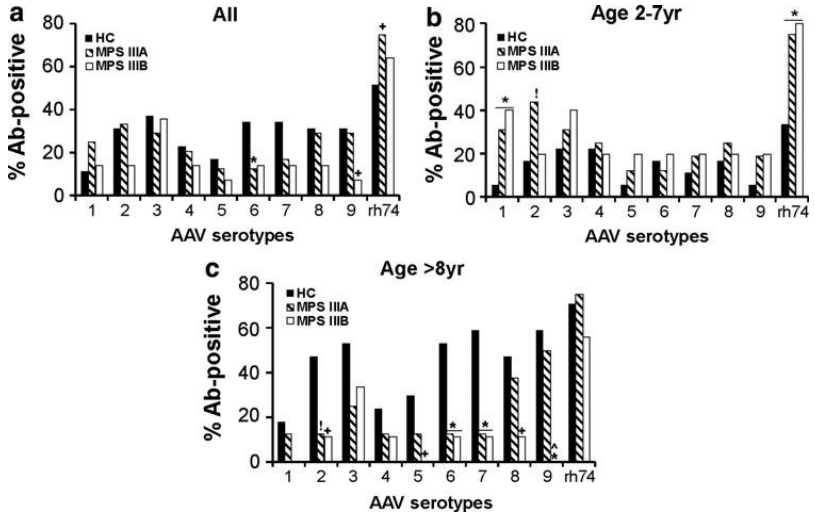
Vectors with
low titer seroprevalence
and **limited neutralizing activity**
have a key advantage
for systemic AAV vector use

Selection and **optimization**
Immunogenicity
Tropism

Kruzik et al. Mol Ther Methods Clin Dev. 2019; Sharon and Kamen, Biotechnology and Bioengineering 2018; Boutin et al. Hum Gene Ther. 2010; Sands, Methods Mol Biol. 2011; Mingozzi and High, Blood, 2013

Prevalence of anti-AAV across age and disease



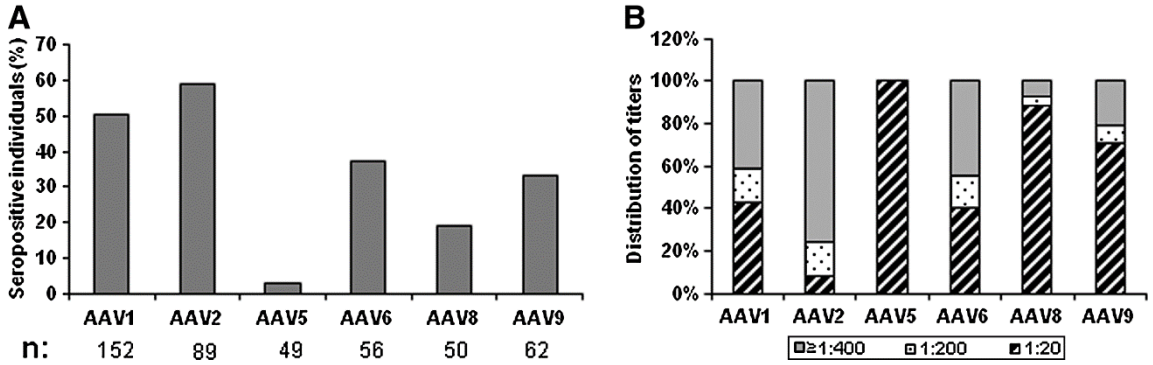


Differential Prevalence of Antibodies Against Adeno-Associated Virus in Healthy Children and Patients with Mucopolysaccharidosis III: Perspective for AAV-Mediated Gene Therapy

Haiyan Fu,[1,4,*] Aaron S. Meadows,[1] Ricardo J. Pineda,[1] Krista L. Kunkler,[1] Kristen V. Truxal,[1,3,4] Kim L. McBride,[2–4] Kevin M. Flanigan,[1,4,5] and Douglas M. McCarty[1,4]

[1]*Center for Gene Therapy,* [2]*Center for Cardiovascular Research,* [3]*Division of Molecular and Human Genetics, Research Institute at Nationwide Children's Hospital, Columbus, Ohio;* *Department of* [4]*Pediatrics and* [5]*Neurology, School of Medicine The Ohio State University, Columbus, Ohio.*

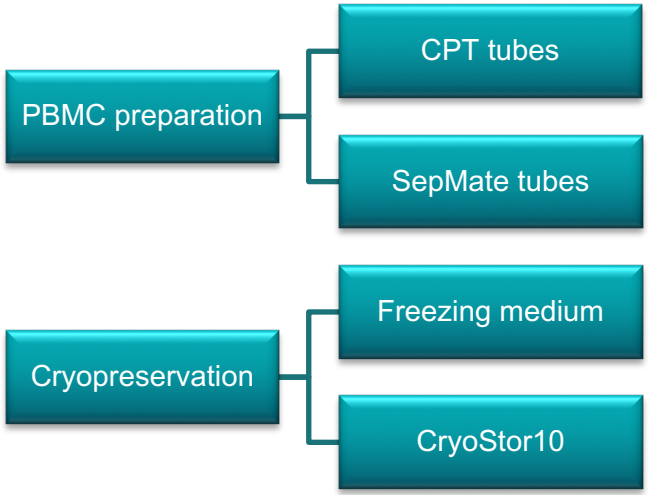
Neutralizing Antibodies to AAV

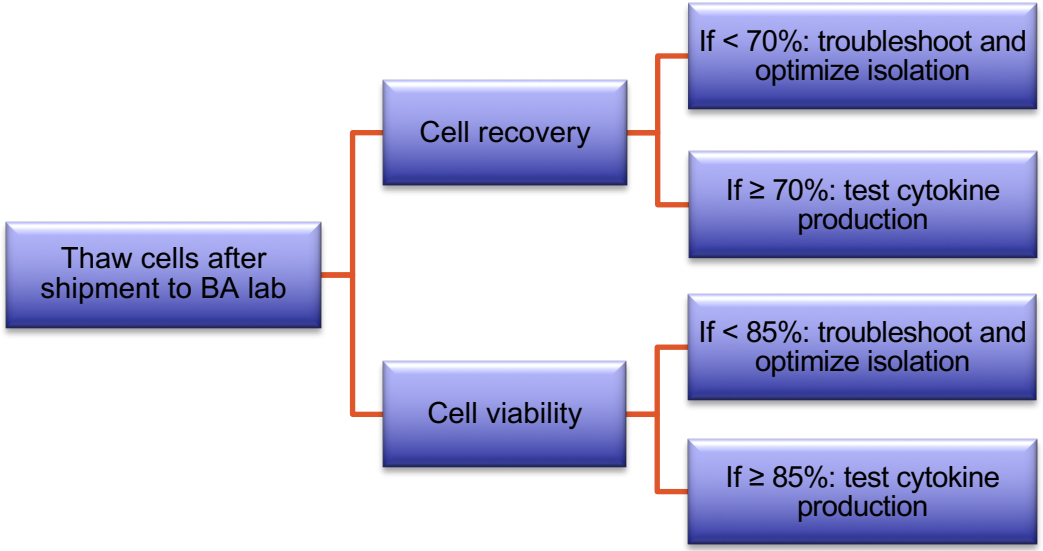


Prevalence of Serum IgG and Neutralizing Factors Against Adeno-Associated Virus (AAV) Types 1, 2, 5, 6, 8, and 9 in the Healthy Population: Implications for Gene Therapy Using AAV Vectors

Sylvie Boutin,[1] Virginie Monteilhet,[1] Philippe Veron,[1] Christian Leborgne,[1] Olivier Benveniste,[2] Marie Françoise Montus,[1] and Carole Masurier[1]

Cellular Analysis: PBMC storage and shipment





To discuss for cellular analysis

- Duplicate or triplicate analysis?
- Is one read-out sufficient to assess T-cell responses?
- Qualification level?

Acknowledgement

The EBF CGT group:

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James **M**unday Johannes **S**tanta, Robert **N**elson

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