U.S. FOOD & DRUG ADMINISTRATION

Regulatory Perspectives on Structural Characterization of Gene Therapy Products

Andrew Byrnes, Ph.D.
Director, Division of Gene Therapy 1
Office of Gene Therapy
Office of Therapeutic Products, FDA CBER

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OUTLINE

Overview of Cell and Gene Therapy Products

Full and empty adeno-associated virus (AAV) capsids

Deamidation of AAV capsid proteins

Diversity of products regulated by CBER's Office of Therapeutic Products

Gene therapies (GT)

- Ex vivo genetically modified cells
- Non-viral vectors (e.g., plasmids)
- Replication-deficient viral vectors (e.g., adenovirus, adeno-associated virus, lentivirus)
- Replication-competent viral vectors (e.g., measles, adenovirus, vaccinia)
- Microbial vectors (e.g., Listeria, Salmonella)

Stem cells/stem cell-derived

- Adult (e.g., hematopoietic, neural, cardiac, adipose, mesenchymal)
- Perinatal (e.g., placental, umbilical cord blood)
- Fetal (e.g., neural)
- Embryonic
- Induced pluripotent stem cells (iPSCs)

Products for xenotransplantation

Functionally mature/differentiated cells (e.g., retinal pigment epithelial cells, pancreatic islets, chondrocytes, keratinocytes)

Combination products

- Engineered tissues/organs

Therapeutic vaccines and other antigen-specific active immunotherapies

Blood- and Plasma-derived products

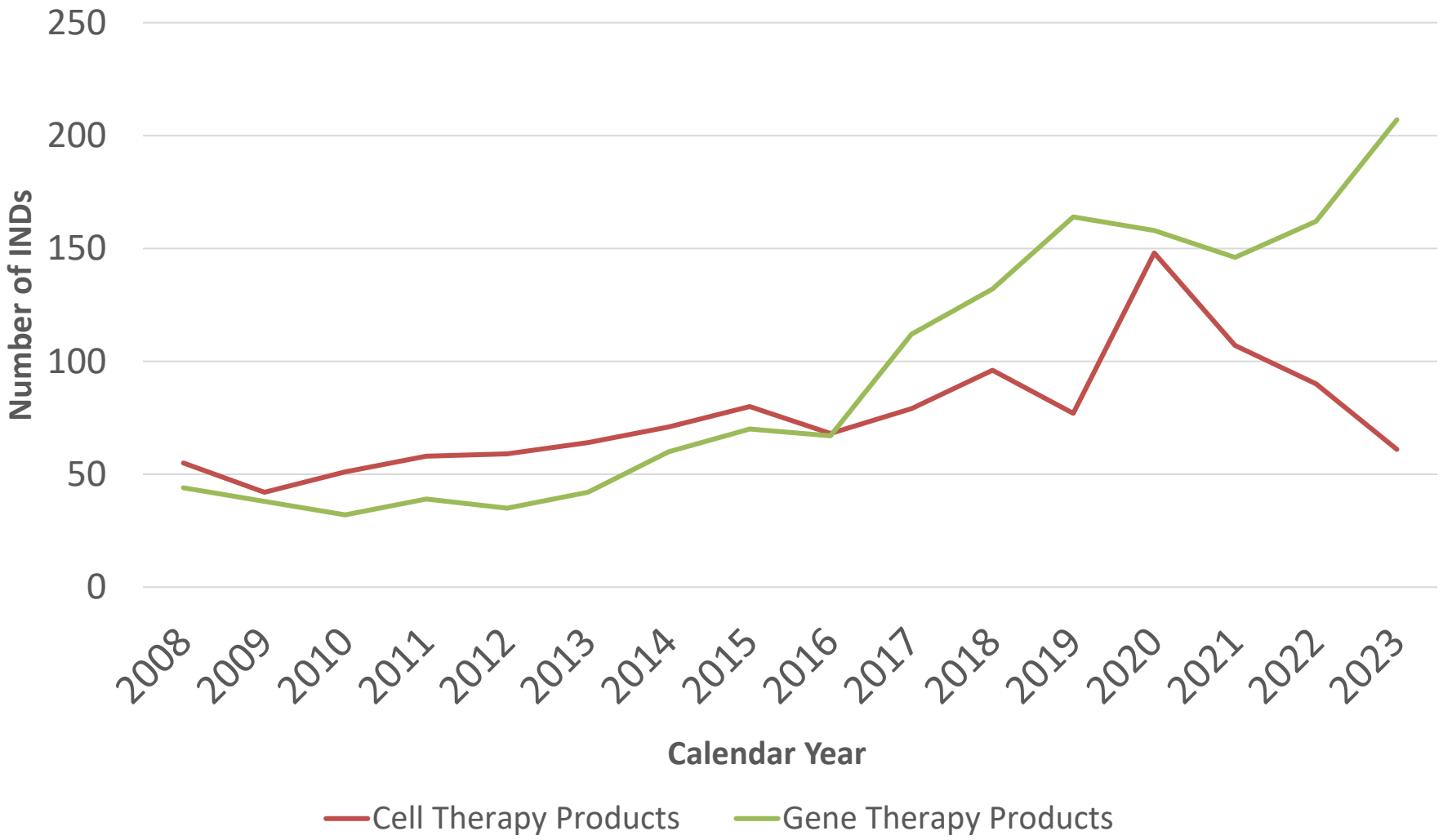
- Coagulation factors
- Fibrin sealants
- Fibrinogen
- Thrombin
- Plasminogen
- Immune globulins
- Anti-toxins
- Venom antisera for scorpions, snakes, and spiders

Devices

Tissues

Cell and gene therapies: new INDs per year

Excluding expanded access



FDA-approved cellular and gene therapy products

Full list:

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

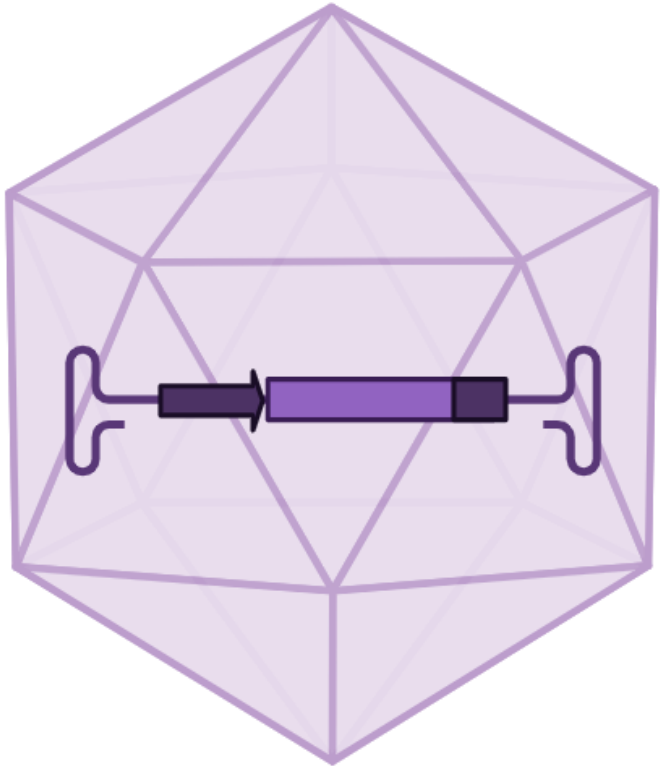
Examples:

Luxturna – AAV vector **Imlygic** – oncolytic HSV **Adstiladrin** – adenovirus vector **Vyjuvec** – HSV vector
Kymriah – CAR T cell **Zynteglo** – hematopoietic stem cell gene therapy **Casgevy** – genome-edited hematopoietic stem cell gene therapy
Hemacord – hematopoietic progenitor cells from cord blood **Stratagraft** – allogeneic cultured keratinocytes and fibroblasts in bovine collagen **Lantidra** – allogeneic pancreatic islets **Amtagvi** – tumor-derived autologous T cell immunotherapy

Adeno-associated virus (AAV) vectors

Protein capsid

- 25 nm diameter protein shell, 3.8 MDa
- Just 3 proteins, no lipids
- A variety of post-translational modifications
  - Deamidation, phosphorylation, N-terminal truncation, others



DNA genome

- Single-stranded DNA genome, up to 4.7 kB
  - For very short genes, AAV vectors can be designed to form a half-length "self-complementary" dsDNA genome
  - For long genes, there are some clever ways to split into 2 or 3 vectors
- All viral genes are deleted and replaced with a transgene cassette
  - AAV vectors cannot replicate
- Inefficient genome packaging into capsids
  - High percentage of empty AAV capsids, with no DNA inside
  - Some capsids contain truncated genomes, host cell DNA, or plasmid DNA

FULL AND EMPTY AAV CAPSIDS

Empty capsid impurities

The vast majority of AAV capsids are empty – no DNA inside

- Most manufacturing methods include some removal of empty capsids

Potential for empty capsids to interfere with cell-based assays

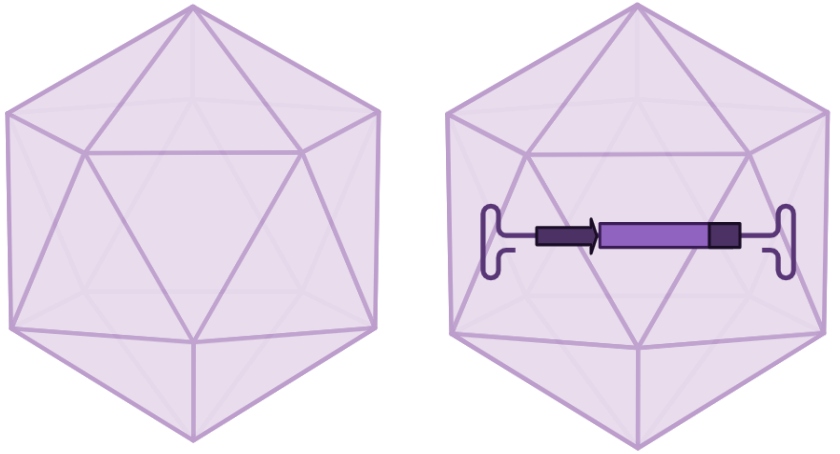
AAV capsids (both full and empty) can be toxic in large amounts

- Short-term toxicities due to complement activation
  - Thrombotic microangiopathy, a few days after administration
- Medium-term toxicities due to T cell responses against capsid proteins
  - Hepatotoxicity, including liver failure, a few weeks after administration
- These toxicities have been very difficult to model in animals
  - And unpredictable manifestation in clinical trials

Which physical properties of capsids can be exploited to improve AAV capsid purity?

Density – separate using ultracentrifugation

- Cesium chloride or iodixanol density gradients
- Extremely efficient separation
  - Can remove empty capsids almost completely
- Challenging to automate and scale up



Charge – separate using anion exchange chromatography

- DNA-containing capsids have slightly different surface charge
- Chromatography is a familiar technology, and easy to automate
  - But separation is inefficient, especially at large scale
    - Diffusion of AAV vectors is slow, so best to use methods that rely on convective mass transfer
      - Monoliths and membrane absorbers, instead of resins
  - Separation parameters need to be fine-tuned and tightly controlled for each product
    - Full and empty capsid pI differences are very small – need shallow elution gradients

Empty capsids – regulatory perspective

Impurities should be identified, controlled, and ideally removed

FDA advisory committee meeting in September 2021 to discuss the safety of AAV gene therapies

https://www.fda.gov/advisory-committees/advisory-committee-calendar/cellular-tissue-and-gene-therapies-advisory-committee-september-2-3-2021-meeting-announcement

Currently, there is no regulatory limit for empty capsids

- Different products have vast differences in dose, route of administration, capsid serotype properties
- Many AAV products have substantial amounts of empty capsids, without undue toxicity
- Not possible to predict safe levels of empty capsids before starting clinical studies

Therefore, regulatory focus is on characterizing and controlling empty capsids

- Different lots of a product should have consistent impurity profiles
  - Empty capsids
  - Non-vector DNA inside capsids (host cell DNA, plasmid DNA)
- Lot release assays should have adequate performance to quantify impurities
- Manufacturing processes should be designed to consistently clear impurities
- After manufacturing process changes, the product should retain a similar (or better) impurity profile
  - Scale up, changes to cells, chromatography changes, etc.

Analytical methods for empty capsids

Transmission electron microscopy

Cryo-electron microscopy

A260/A280 absorbance

Ratio of capsids (e.g., ELISA) to genomes (e.g., qPCR)

Anion exchange chromatography

Analytical ultracentrifugation

Charge-detection mass spectrometry

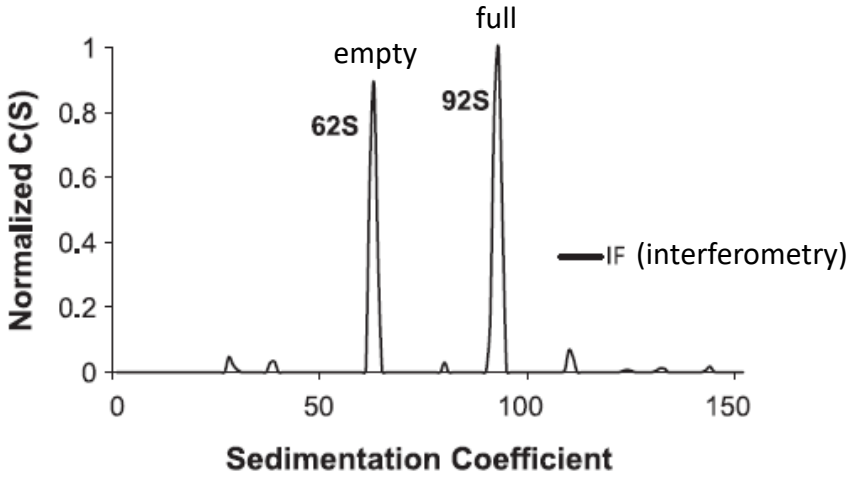
Mass photometry

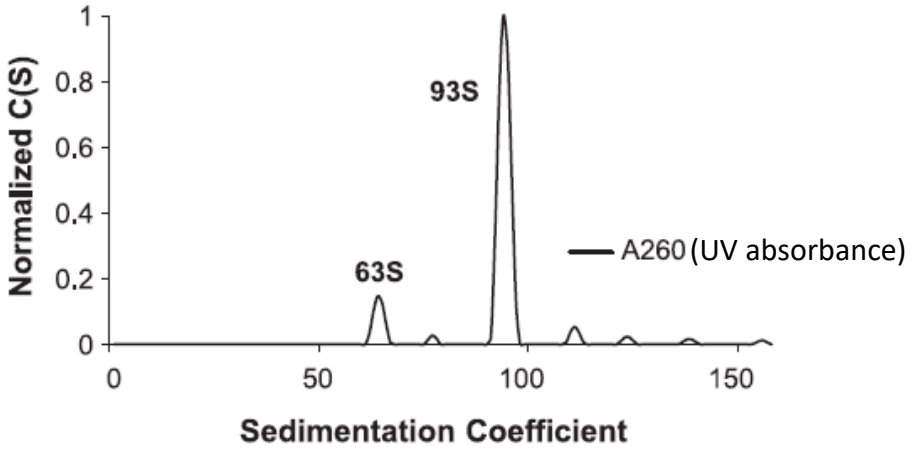
These techniques can distinguish among full, empty, and partially-full capsids

Partially-full capsids

AUC is the most widely-used technique for resolving partially-full capsids

Typical AAV vector with ssDNA genome





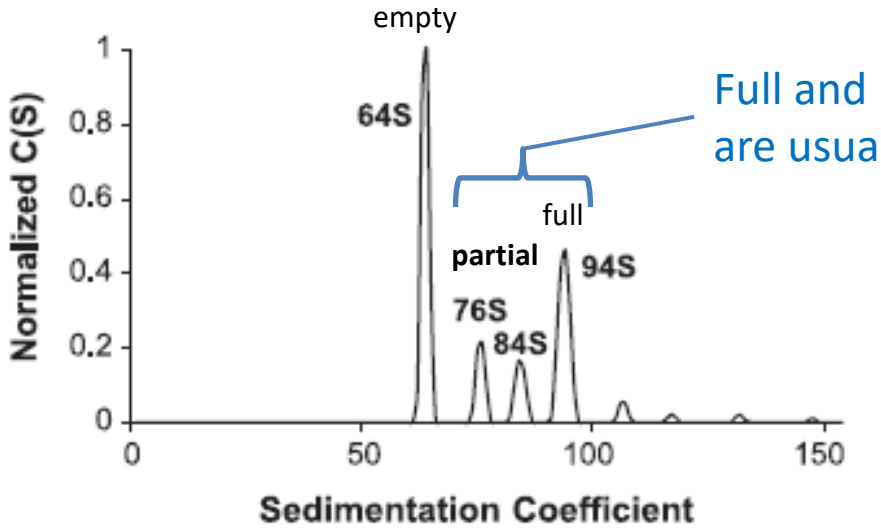
Analytical Ultracentrifugation as an Approach to Characterize Recombinant Adeno-Associated Viral Vectors

Brenda Burnham, Shelley Nass, Elton Kong, MaryEllen Mattingly, Denise Woodcock, Antonius Song, Samuel Wadsworth,† Seng H. Cheng, Abraham Scaria, and Catherine R. O'Riordan*

Gene Therapy, Genzyme, a Sanofi Company, Framingham, Massachusetts.

HUMAN GENE THERAPY METHODS, VOLUME 26 NUMBER 6

Typical self-complementary AAV vector with dsDNA genome



Full and partially-full capsids are usually co-purified

Summary
AAV capsid forms

AAV vectors are heterogeneous

- Most capsids are empty
- Some capsids contain full vector genomes
- Self-complementary AAV vectors can have substantial percentages of partially-full capsid
- Some capsids contain host cell DNA or plasmid DNA

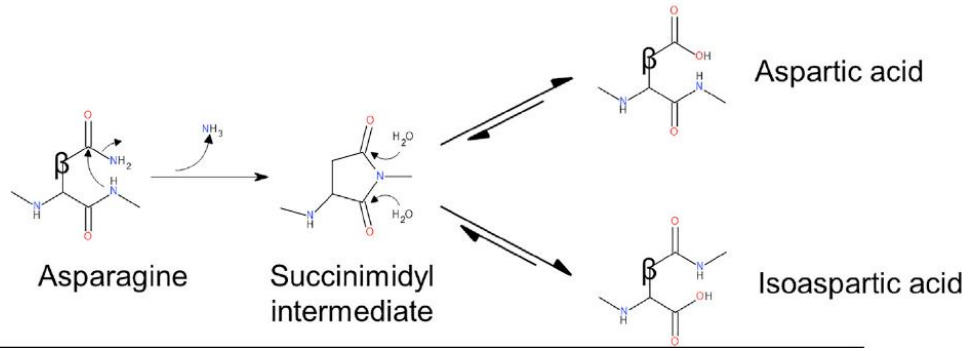
Capsids that do not contain full genomes are impurities

- When possible, use purification methods that reduce empty capsids
- Quantitate and control capsids forms and DNA impurities in capsids

DEAMIDATION OF AAV CAPSID PROTEINS

Protein deamidation and AAV vectors
Decreased potency

Spontaneous deamidation



Amide group of an asparagine (or a glutamine) side chain is lost after nucleophilic attack from an adjacent main-chain amide

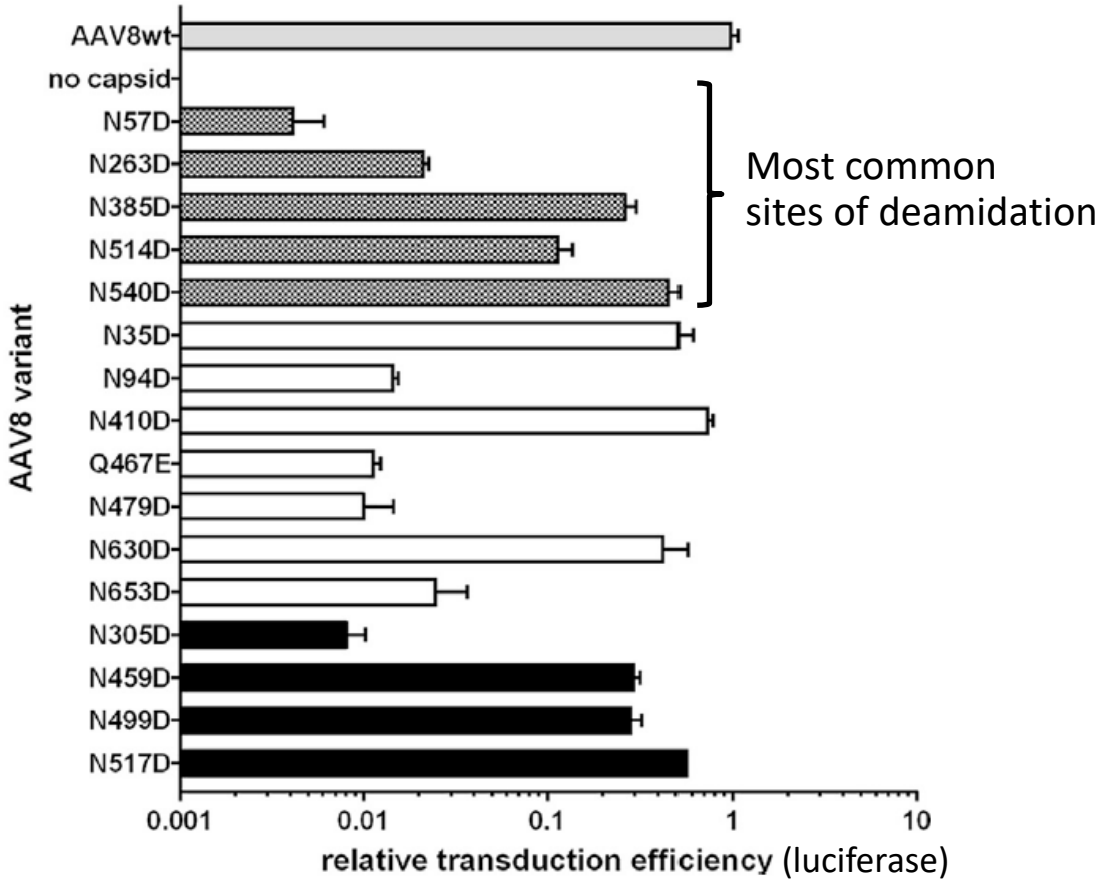
The intermediate succinimidyl undergoes hydrolysis

Results in amino acid change from asparagine to aspartic acid or isoaspartic acid (1:3)

Or less frequently: from glutamine to glutamic acid or pyroglutamic acid

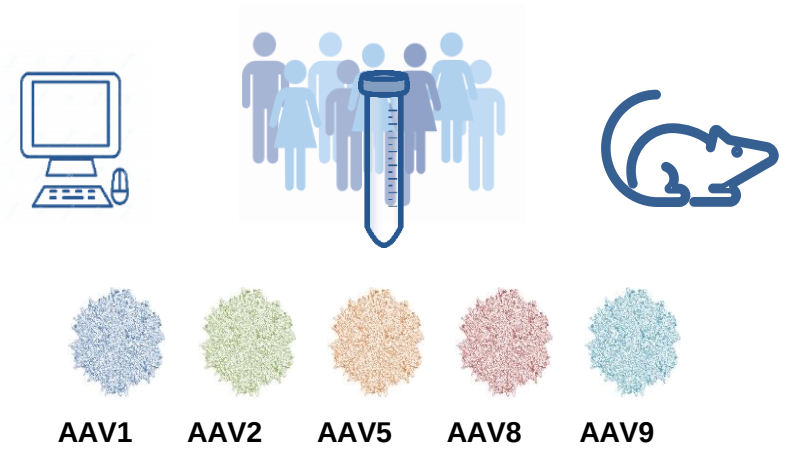
More likely when Asparagine is followed by Glycine (NG sites)

AAV8 vectors
N→D (or Q→E) can decrease vector activity



Giles et al. (2018) *Deamidation of Amino Acids on the Surface of Adeno-Associated Virus Capsids Leads to Charge Heterogeneity and Altered Vector Function.* Mol. Ther. 26:2848

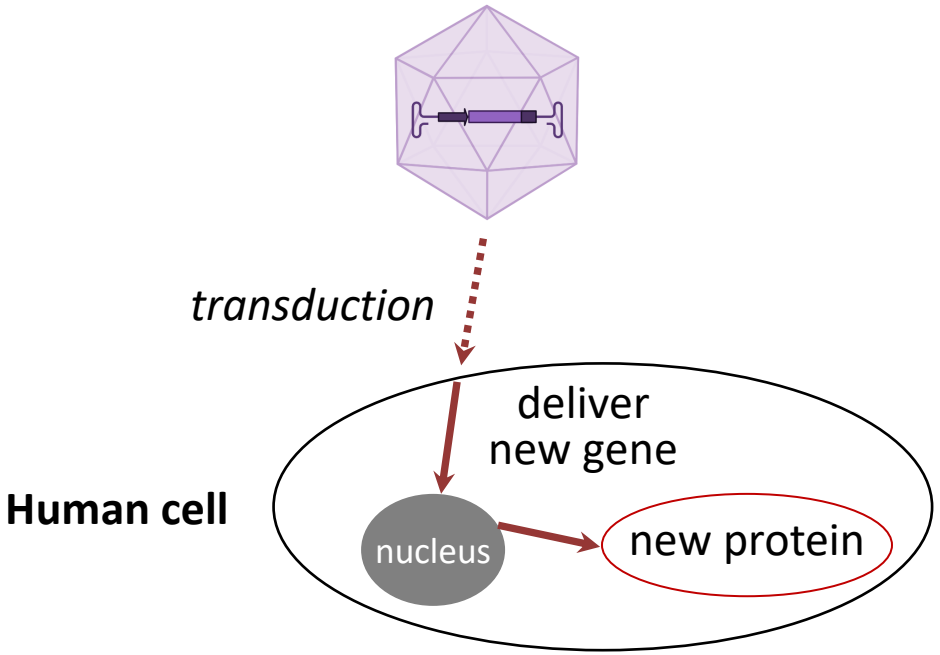
Ronit Mazor laboratory at FDA CBER



What impact does deamidation have on the immunogenicity of AAV vectors?

Immune responses to AAV vectors

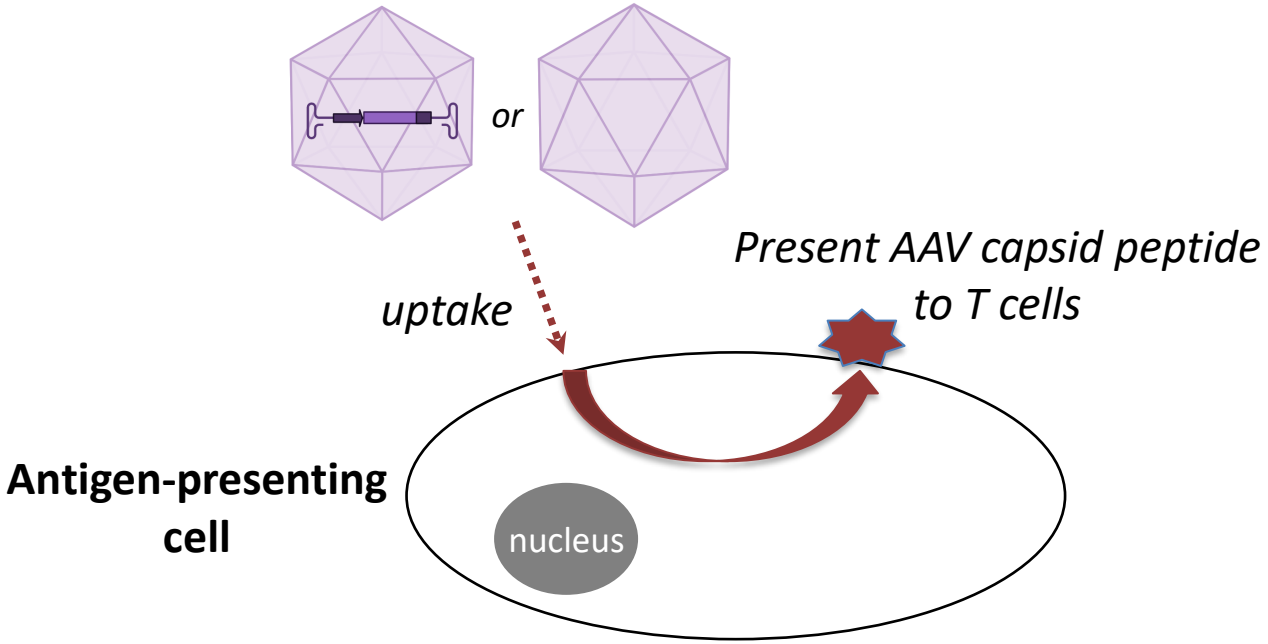
Immunity to transgene protein



T cell responses to new protein:
Cytotoxicity, loss of protein expression

B cell responses to new protein:
Neutralization of protein, loss of activity

Immunity to AAV capsid protein

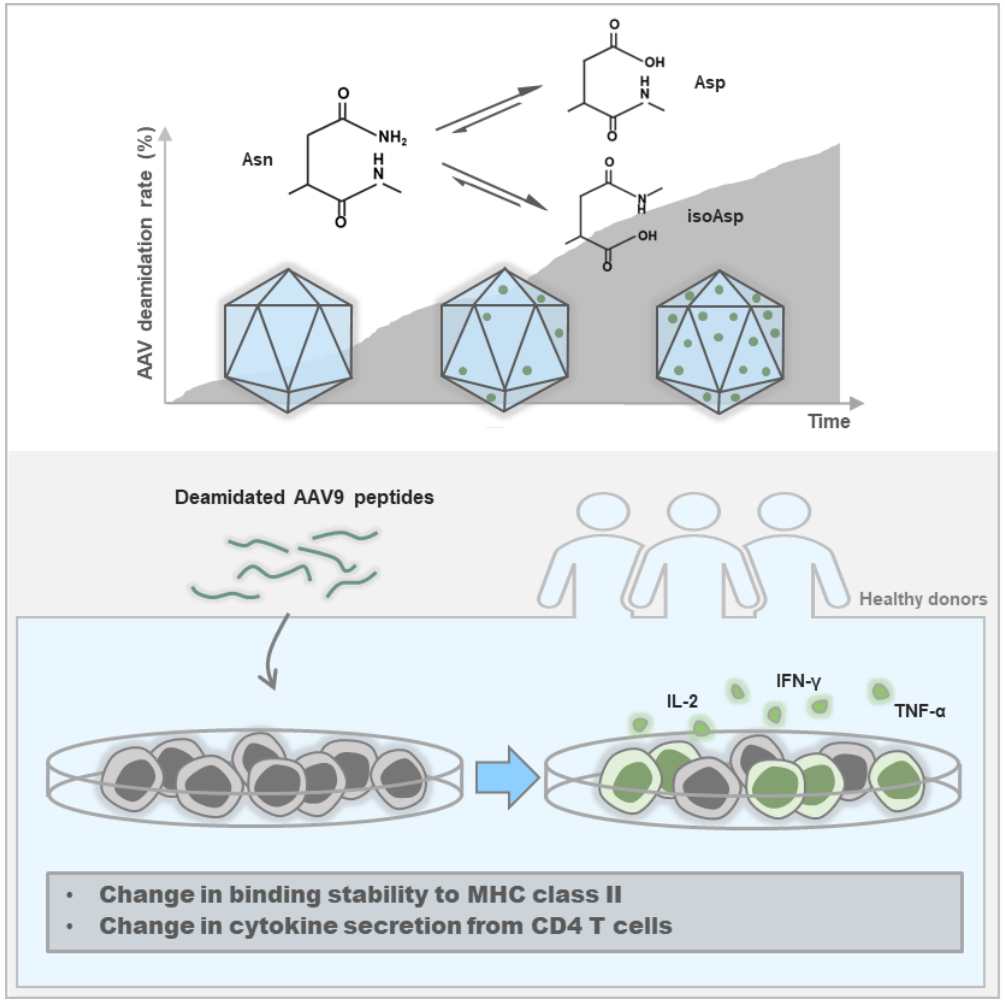


T cell responses to capsid:
Cytotoxicity, loss of expression from vector

B cell responses to capsid:
Anti-AAV antibodies prevent AAV re-administration

Protein deamidation and AAV vectors

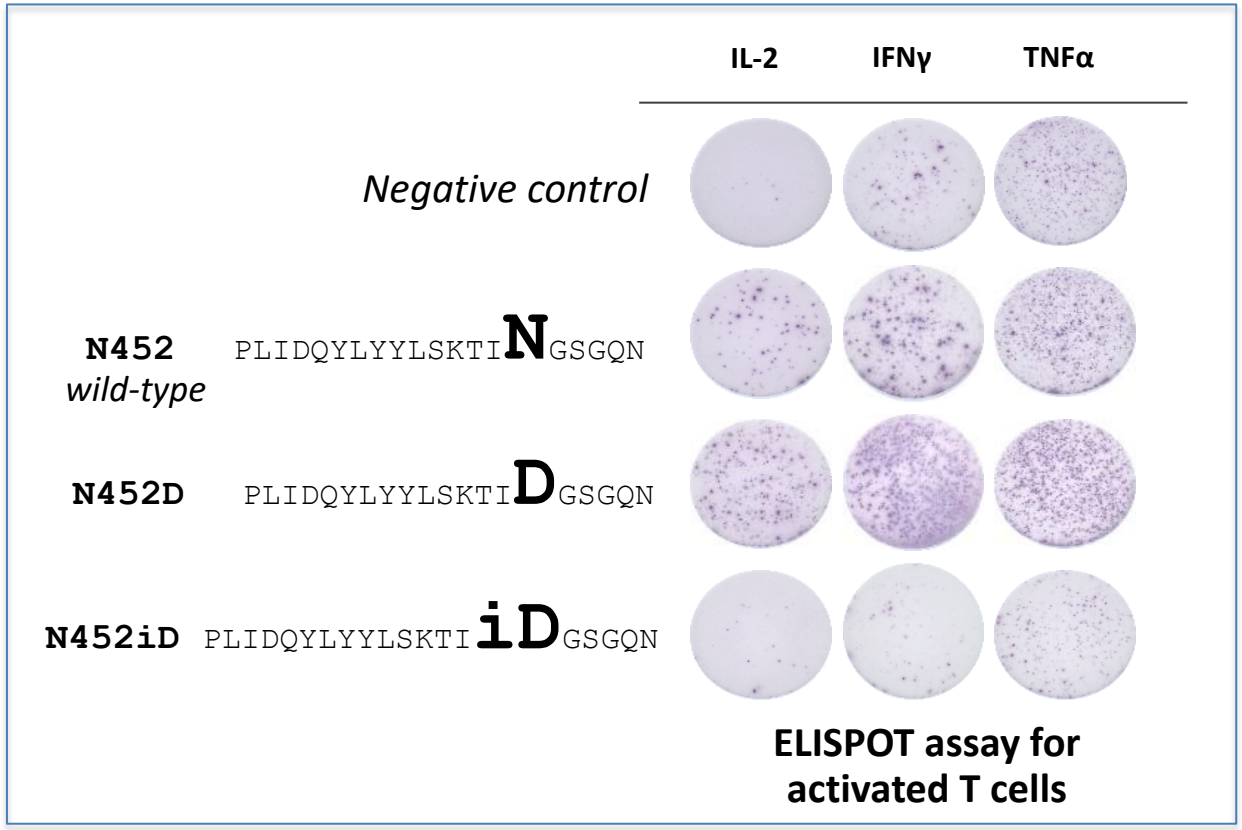
Altered human T cell response to capsid



Mazor lab: Bing et al. (2022) *Differential T cell immune responses to deamidated adeno-associated virus vector.* Mol. Ther. Meth. Clin. Devel. 24:255

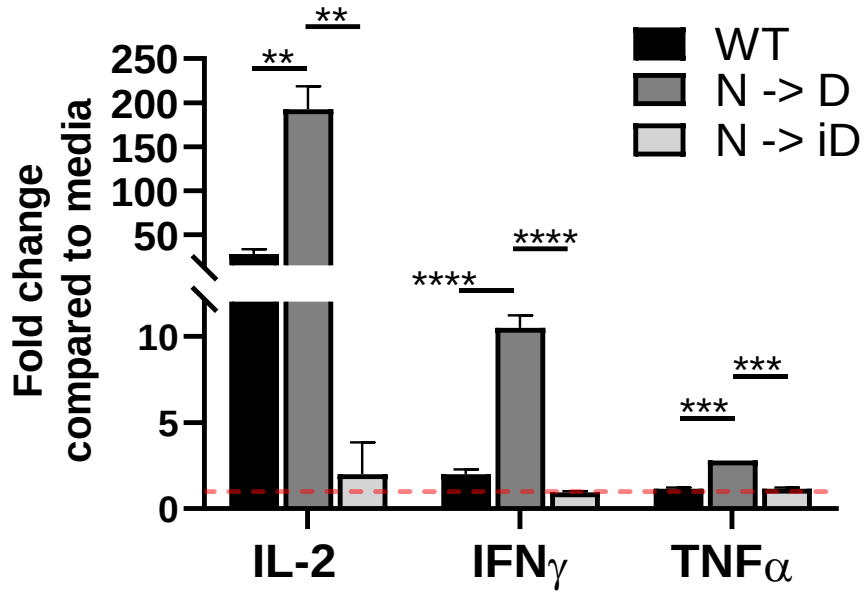
Protein deamidation and AAV vectors
Altered human T cell response to capsid

Altered T cell response after substituting Asn with Asp / iso-Asp in an AAV9 capsid peptide



This particular donor's T cells show:

- Higher response with aspartate in peptide
- Reduced response with isoaspartate



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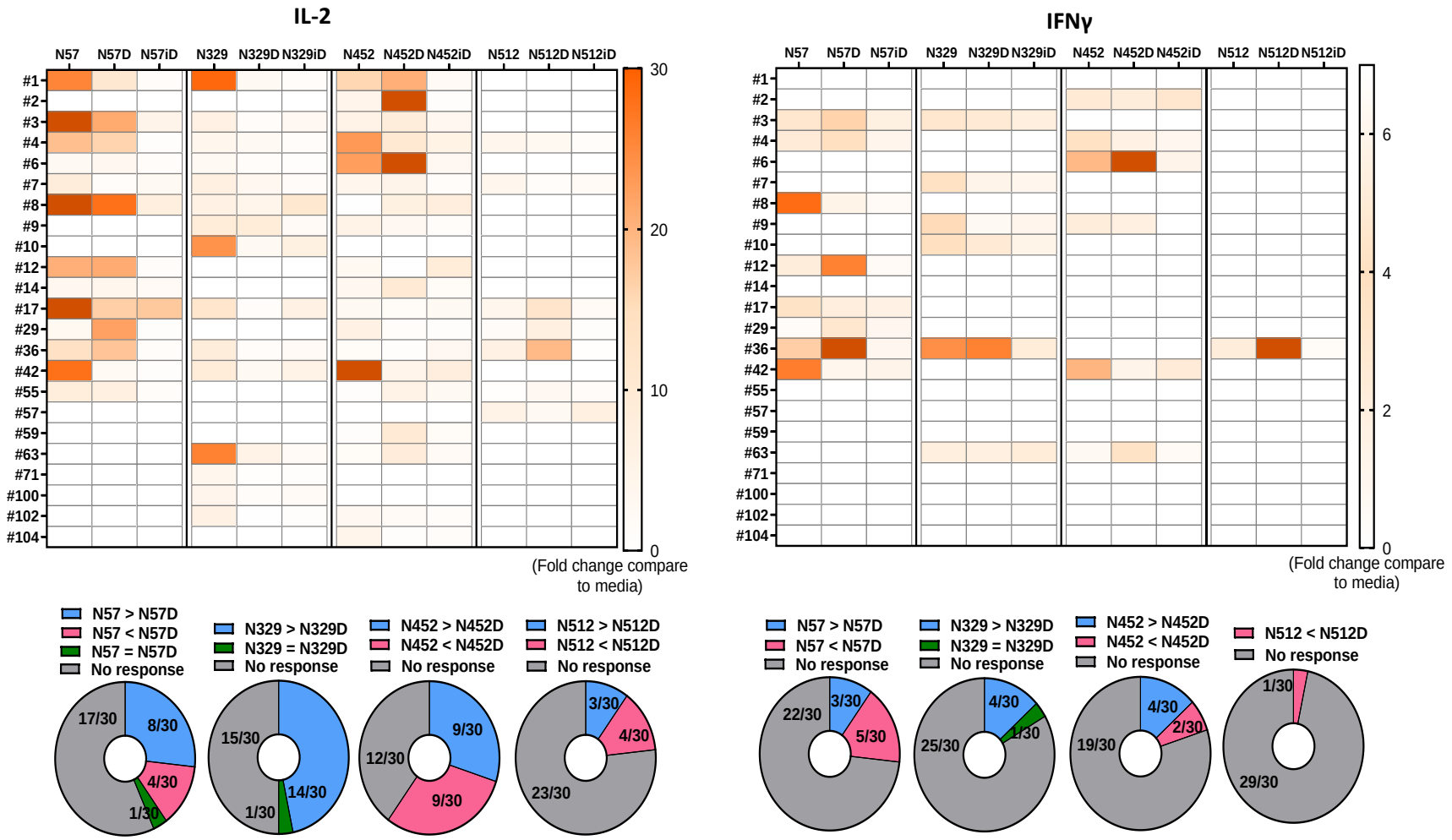
Protein deamidation and AAV vectors

Deamidation increases T cell responses in some donors, decreases in others



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Protein deamidation and AAV vectors

High level of deamidation in AAV vectors

- More deamidation over time during storage
- Residue-dependent variability

Deamidation may decrease AAV vector activity

Deamidation may increase or decrease the immunogenicity of AAV vectors

T cell assays that do not include deamidated peptides may underestimate T cell responses to vectors

Summary

AAV vectors have complex heterogeneity

- Full, empty, and partial capsids
  - Empty capsids have no activity, need to be controlled
- Post-translational modifications
  - The impact of PTMs on AAV vector quality is not completely understood
  - PTMs may affect vector activity and immunogenicity

We recommend:

- Develop manufacturing processes that yield vectors with consistent impurity profiles
- Develop assays to quantitate and control capsid forms
- Characterize post-translational modifications and seek to understand their impact on product quality

Contact Information

- **Andrew Byrnes**

  Andrew.Byrnes@fda.hhs.gov
- **Regulatory Questions:**

  OTP Main Line – 240 402 0685

  Email: OTPRPMS@fda.hhs.gov
- **OTP (OTAT) Learn Webinar Series:**

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