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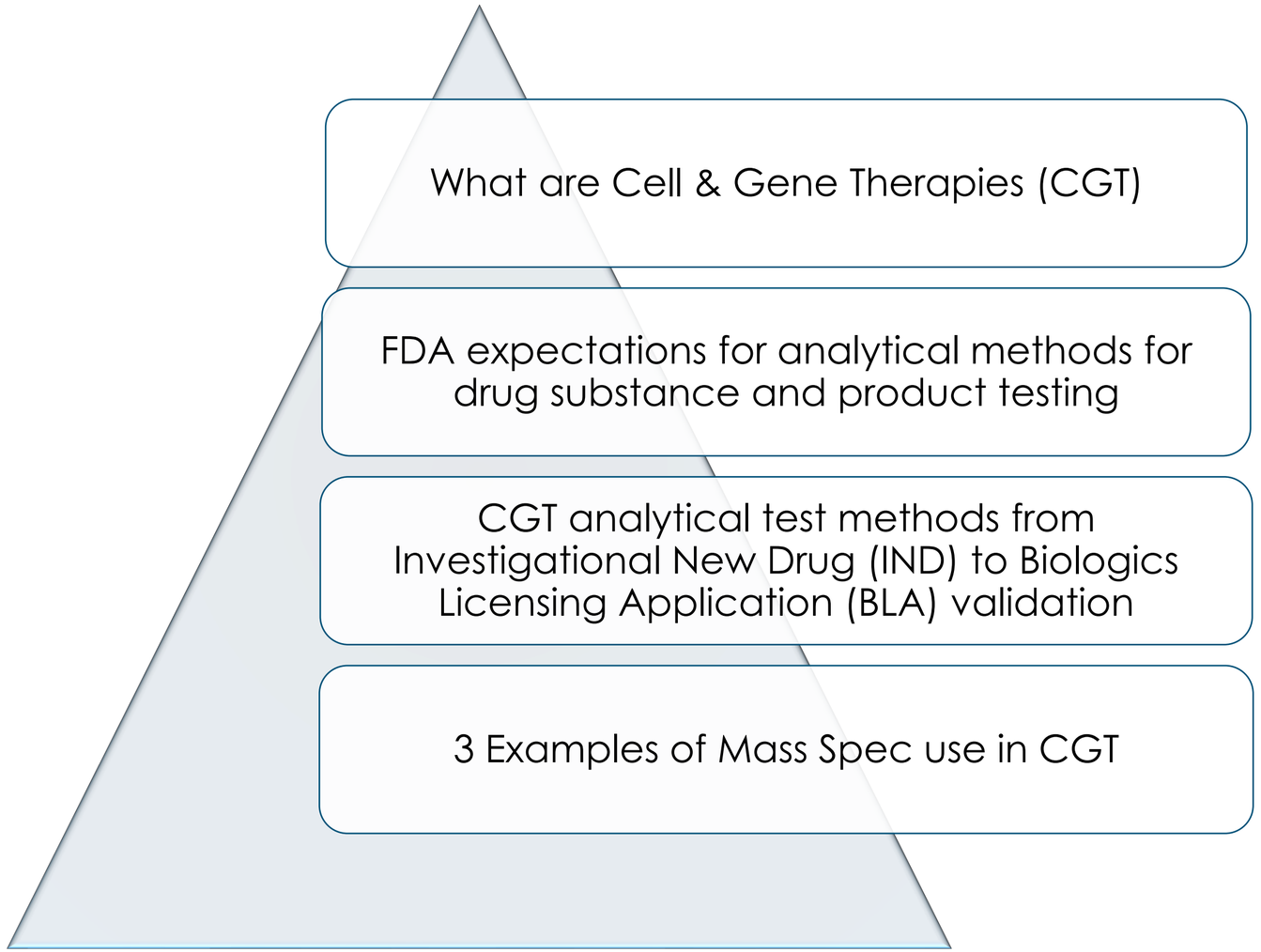
Leveraging Mass Spectrometry Analytical Methods in FDA Cell & Gene Therapy Regulatory Submissions:
Current Uses and Future Opportunities

Tiffany Lucas, PhD
Principal Consultant

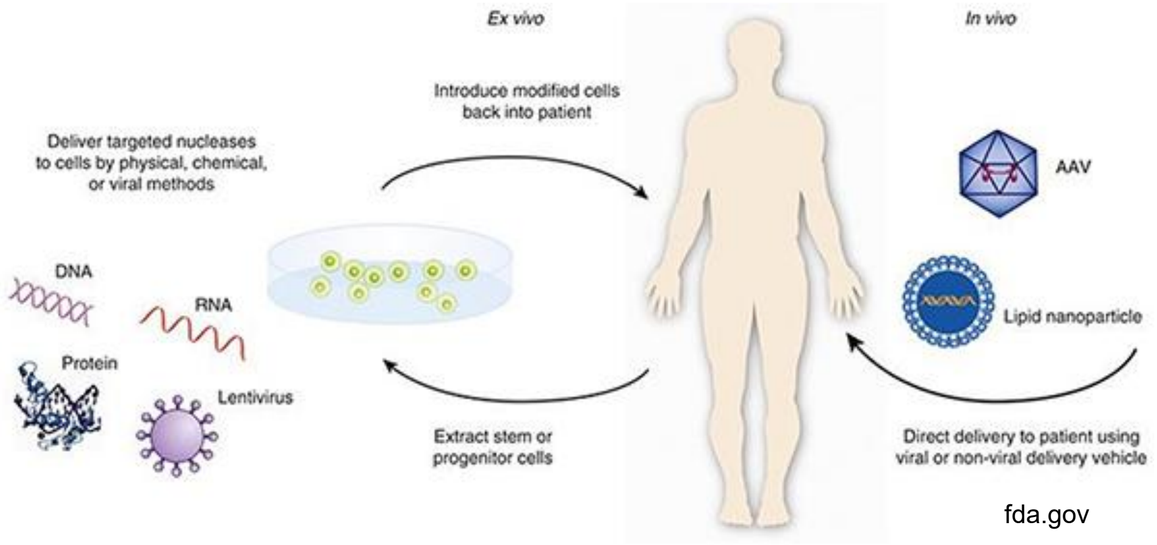
Regulatory Clarity –
thought to finish.

Overview: FDA Regulatory Cell & Gene Therapy

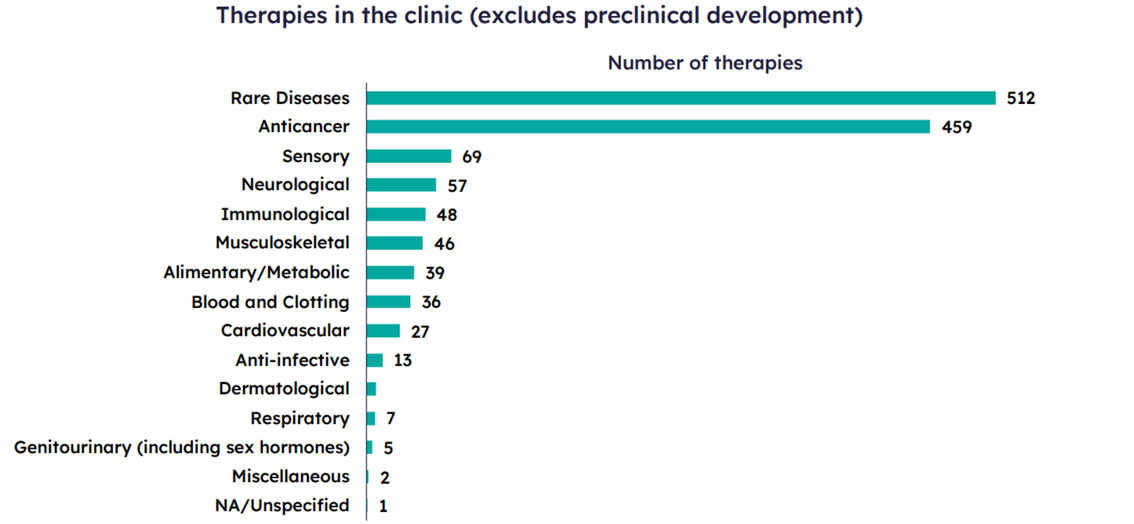


Cell & Gene Therapy is Diverse

- **Cells, viral vectors, gene editing enzymes, mRNA**
- **No single method for making CGTs**



fda.gov

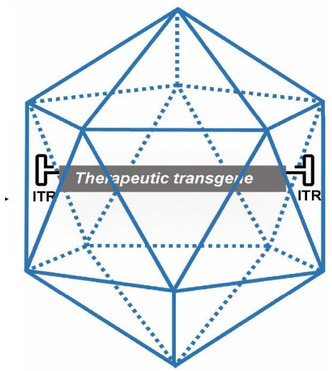


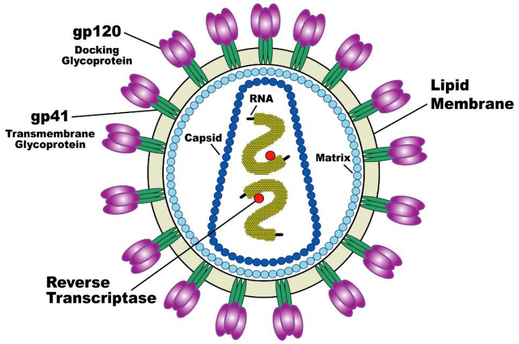
Figures based on indications in pipeline development only for each therapy

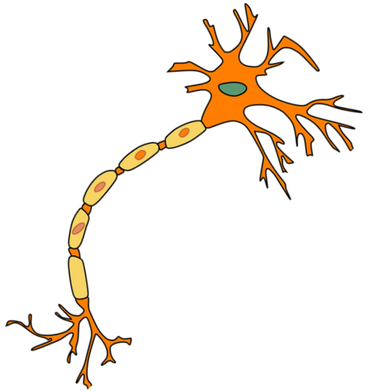
Source: Pharmaprojects | Citeline, July 2025

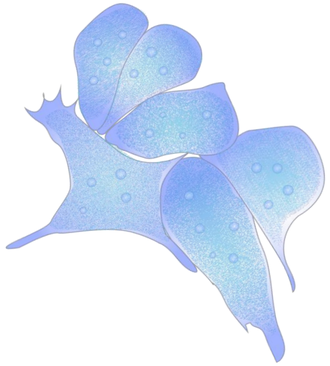
Cell & Gene Therapies: Opportunities to Explore

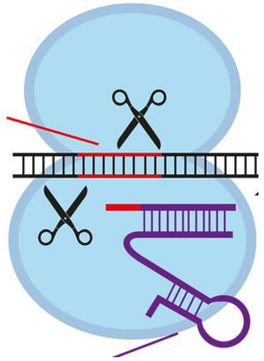
- Complex and often poorly understood biologic products











Adeno-associated viruses
(AAV)

3 Proteins
DNA genome

Lentiviruses Retroviruses
(LVV, RVV)

~3-12 proteins
Lipid membrane (from cell)
Kissing-double strand RNA genome

Healthy Donor Cells
Patient Cells

T cells, natural killer cells, B cells, stem cells

Manufacturing Cells

Produce gene therapy viruses
HEK293 lines, insect cell lines

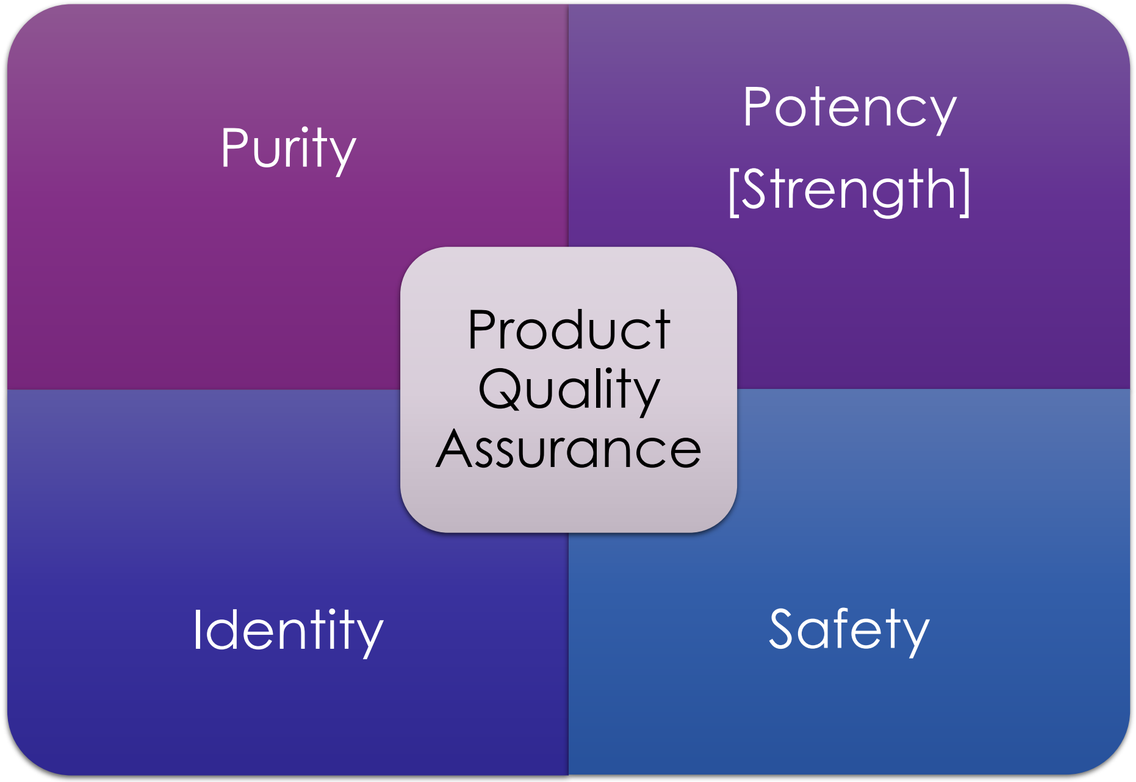
Gene Editing or Modifying

Components
guideRNA, enzymes, LNPs, mRNA

B4fa.org

Four Key Product Requirements

- Required for IND and BLA
- "*Although **in each phase** of the investigation **sufficient information is required to be submitted to assure the proper identification, quality, purity, and strength** of the investigational drug*"
- 21 CFR 312.23(7)(i) and 21 CFR 610.1 for BLA requirements
- Generally analytical method agnostic for CGT



* FDA generally accepts USP methods with verification (sterility, mycoplasma, color)

The Good Science Problem

- Sponsor: “*Please tell me what analytical methods to do*.”

- FDA: Please select analytical methods appropriate for and sufficient to assess the specific attributes that will assure the quality of the product, including safety and efficacy.

Regulatory Notes:

When compendial methods exist (i.e., USP), then non-compendial methods must demonstrate “equal to or greater than” than performance 21 CFR 609.10

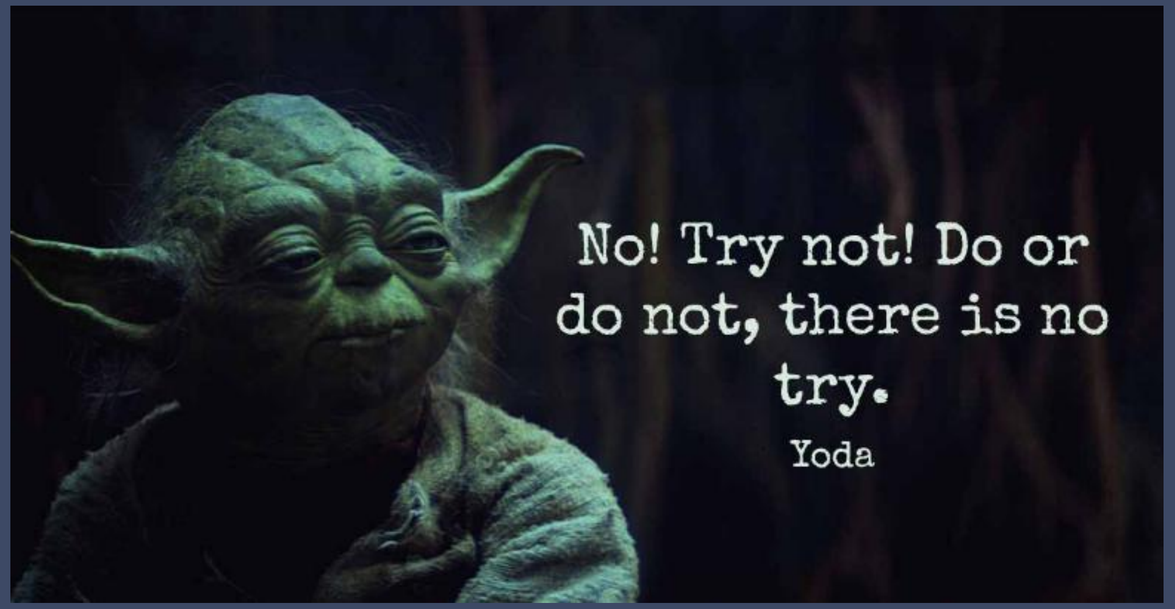
a Lucasfilm Ltd. production ; a Bad Robot production ; produced by Kathleen Kennedy, J.J. Abrams, Bryan Burk ; written by Lawrence Kasdan & J.J. Abrams and Michael Arndt ; directed by J.J. Abrams. (2016). Star wars. Episode VII, The Force awakens. [San Francisco, CA] :Lucasfilm Ltd.

Yes, FDA CGT Encourages New Analytical Methods!

But you must *do them right*.

Mass Spec is well established in mAbs, but newer to CGT.

Most assays are new to CGT.



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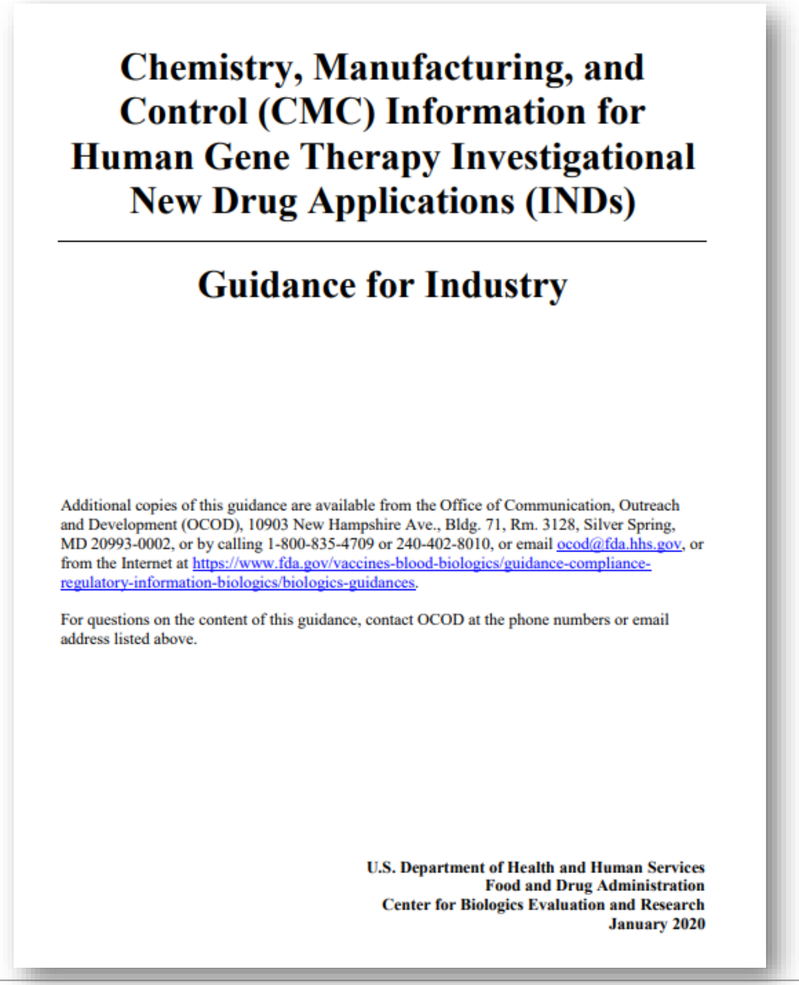
20th Century Fox ; Lucasfilm Ltd. ; directed by Irvin Kershner ; produced by Gary Kurtz ; screenplay by Leigh Brackett and Lawrence Kasdan ; story by George Lucas. (2013). Star wars. Episode V, The empire strikes back. Beverly Hills, Calif. :20th Century Fox Home Entertainment

New Analytical Methods: IND Submission

- **Method qualified for use = suitable for intended use**
  - Clearly stated by FDA CGT CMC 2020 IND guidance and draft 2024 draft FAQ guidance
  - Qualification varies by test type, risk
  - Prior knowledge can be leveraged
- **Method information to include**
  - General method description
  - Rationale for assay design and performance
  - Description of samples/test articles, controls, and reference materials
  - Equipment, reagents
  - Results to demonstrate the assay is suitable for use, including performance across the intended conditions
- **Level of detail required**
  - Novelty of assay
  - Criticality of assay
  - Experience with assay
  - Validation not required until BLA

1st Stop for FDA GT Guidance for CMC in IND



Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances.

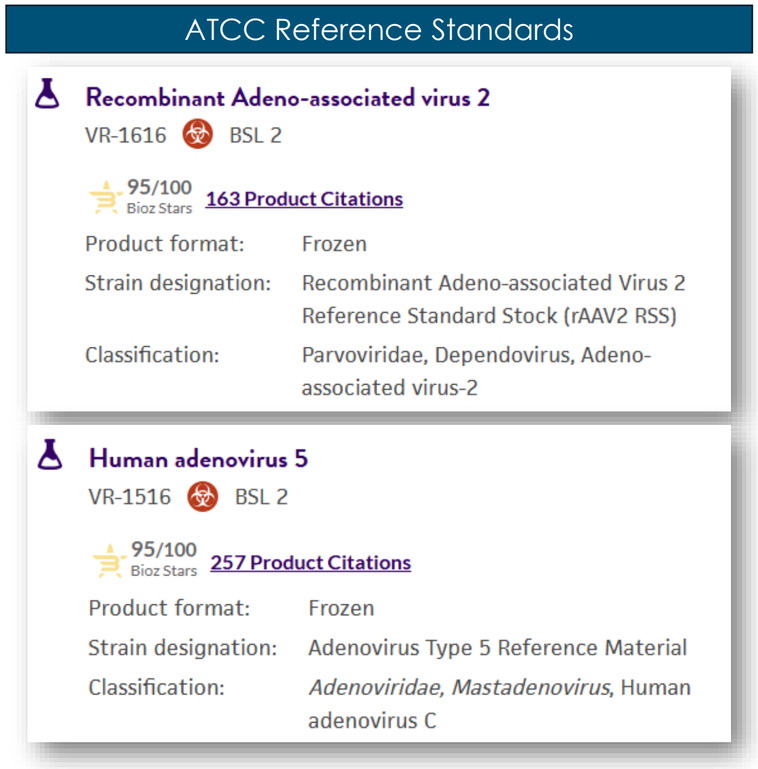
For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
January 2020

https://www.fda.gov/media/113760/download

CGT Challenges in Qualification & Validation

- **Reference materials & standards**
  - Appropriate controls
  - Attributes (COA) should be understood to control assay
  - Bridging studies- planned in advance
  - Some Reference Standards can be purchased
  - Often Reference Materials are developed in-house



Regulatory Notes:

Reference standards are a newer concept to CGT reg. affairs

Responsibility sits with the sponsor

Failure to understand reference lot CQA for assay performance = commercial lot failures

CGT Challenges in Qualification & Validation

- **Range**
  - Cover span of use for assay

- **Limits**
  - Identify necessary level of detection (justification)
  - Examples:
    - Safety related to impurities
    - Accuracy of dosing
    - A clear difference in non-potent product vs the range of potency in potent product

FDA Guidance Q2(R2) March 2024

Technique	Quantitative LC/MS Analysis of Trace Impurities in Product
Performance Characteristic	Validation Study Methodology
Reportable range	Validation of calibration model across the range: Linearity: Experimental demonstration of the linear relationship between analyte concentrations and peak responses (or the ratio of peak response if an internal standard was used) with reference materials at 5 or more concentration levels Validation of lower range limits: DL: Use the CV of responses at the spiking level (with 6 or more repeated injections) as a measure of signal-to-noise. The CV obtained must be less than or equal to a predefined acceptable value QL: The lowest spiking level with acceptable accuracy and precision The range extends from and is inclusive of the QL to the highest spiking level with acceptable accuracy, precision, and response

Regulatory Notes:

Limits must capture the necessary range for the product

Q2(R2) Validation of Analytical Procedures Guidance for Industry. March 2024
https://www.fda.gov/media/177578/download?attachment

CGT Challenges in Qualification & Validation

- **Robustness**
  - Evaluate the system based on real use
  - Lot variability – kits, reagents
  - Sample prep conditions
  - Wait times
- **Often forgotten in BLA with poor outcomes**

FDA Guidance Q2(R2) March 2024

Technique	Quantitative LC/MS Analysis of Trace Impurities in Product
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Deliberate variation of parameters, e.g., LC flow rate, LC injection volume, MS drying/desolvation temperature, MS gas flow, mass accuracy, MS collision energy, stability of test conditions

LC/MS = liquid chromatography/mass spectrometry; MRM = multiple reaction monitoring; CV = coefficient of variation.

Example from BLA: Postmarketing Commitment (PMC)

4. [redacted] commits to perform additional robustness assessments of the (b) (4) assays. The final report will be submitted as a "Postmarketing Commitment - Final Study Report" by May 31, 2024.

   Final Report Submission: May 31, 2024

5. [redacted] commits to perform additional robustness assessments of the (b) (4) assay. The final report will be submitted as a "Postmarketing Commitment - Final Study Report" by October 31, 2024.

   Final Report Submission: October 31, 2024

Regulatory Notes:

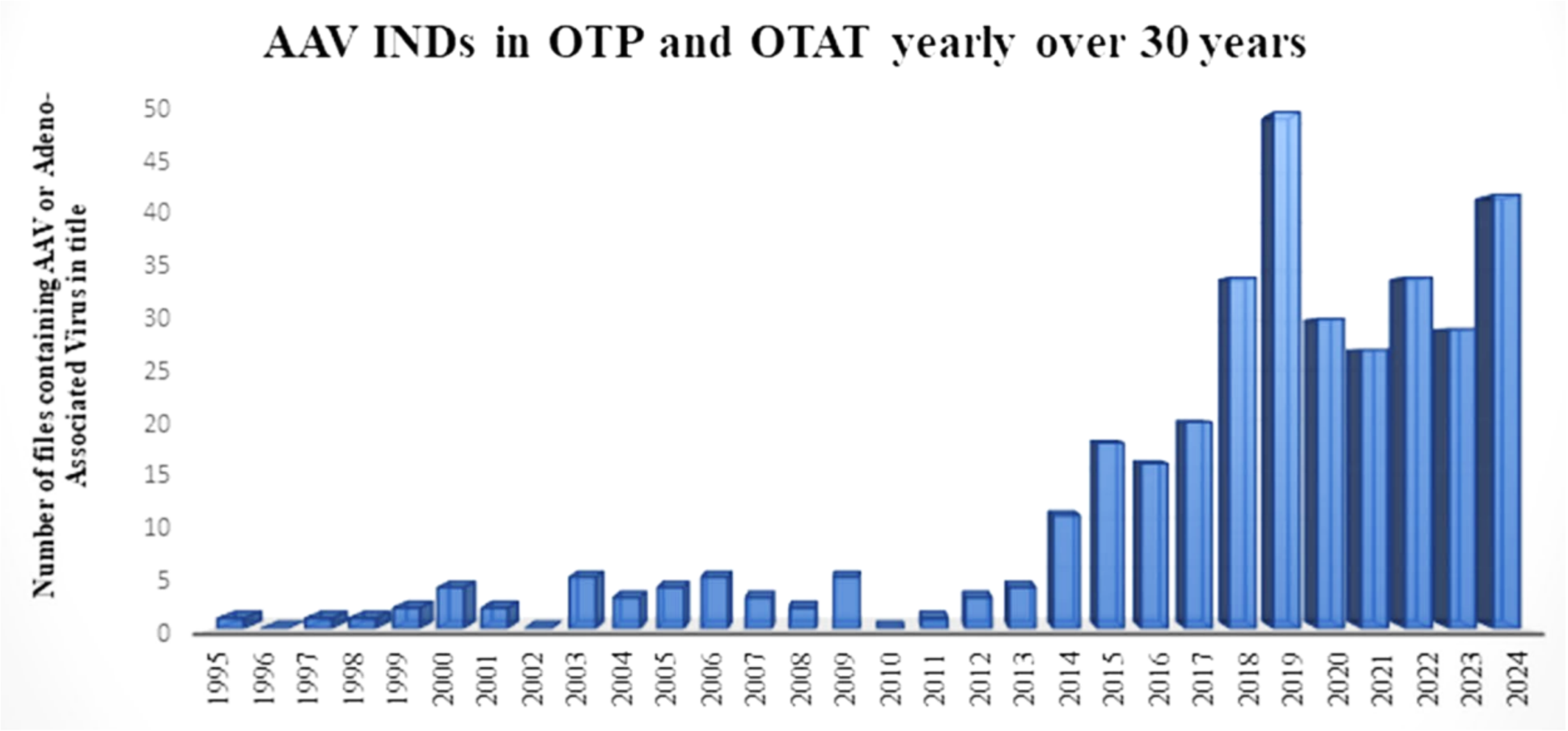
Failure to evaluate robustness can result in Postmarketing Commitments

Or a Complete Response Letter to BLA if risk to quality

Q2(R2) Validation of Analytical Procedures Guidance for Industry. March 2024
https://www.fda.gov/media/177578/download?attachment

Adeno-associated Viruses (AAVs) IND Submissions

- **Seven FDA approved AAV-based therapies by the end of 2024**



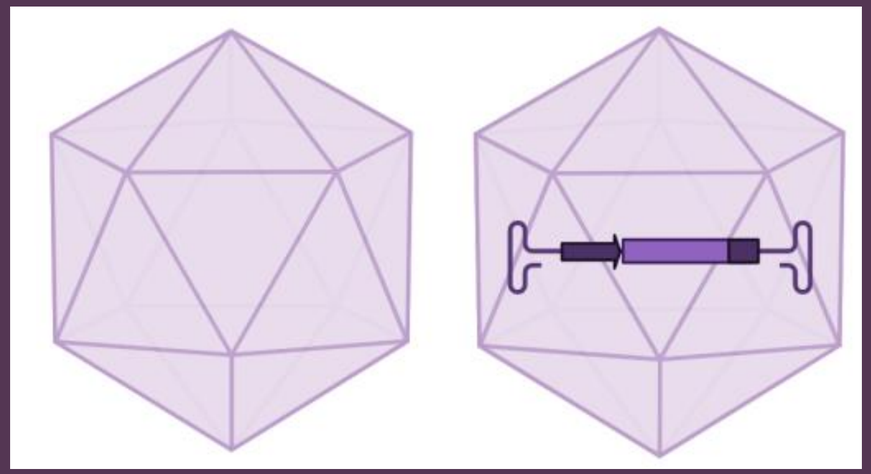
Can Sex-based Variations in the Immune Responses to AAV Gene Therapy Affect Safety and Efficacy? A Review of Current Understanding Leila Abdelhamid1 · Ronit Mazor. AAPS Journal (2025) 27:141 https://doi.org/10.1208/s12248-025-01127-5

*Published by FDA research lab – Mazor lab

Adeno-associated Viruses (AAV)

- **3 proteins with DNA payload**
- **Problems**
  - Tissue tropism
  - High-dose delivery (e.g., 1E14 particles per kg)
  - Delivery through intravenous infusion, injection or pump into central nervous system (brain, spinal cord)
  - Long-lasting (probably)

- **Unintended immune response**
  - Days – innate immune response
    - Complement activation
    - e.g., thrombotic microangiopathy, organ failure: heart, kidney, liver, death
  - Weeks – adaptive immune response
    - T cell response to capsid proteins
    - e.g., hepatotoxicity, liver failure, death



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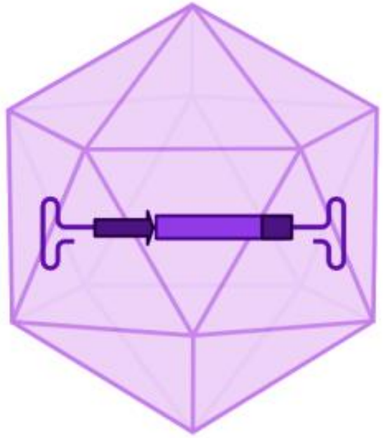
Use 1: AAV Purity

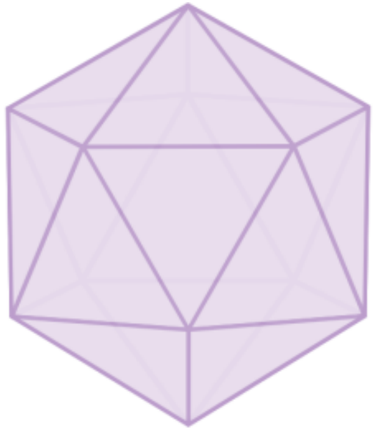
Potency
Delivers Gene

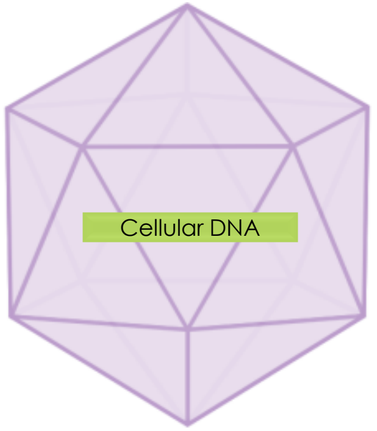
Impurity
Immunogenic

Impurity
Immunogenic

Impurity
Immunogenic







Full

Complete transgene packaged

Empty

No DNA packaging

Partial

Fragment of transgene packaged

Other

Cellular DNA packaged
Plasmid DNA packaged
Double capsids

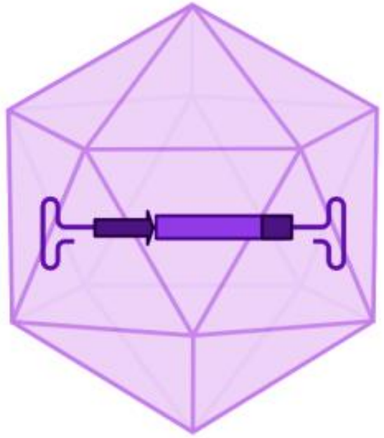
Use 1: AAV Purity

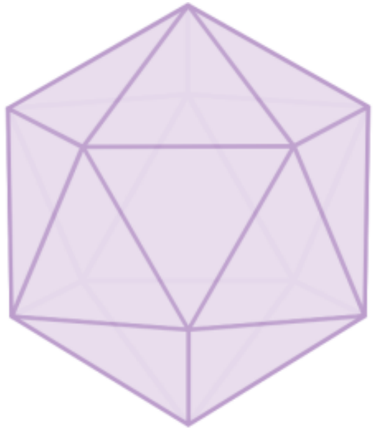
Potency
Delivers Gene

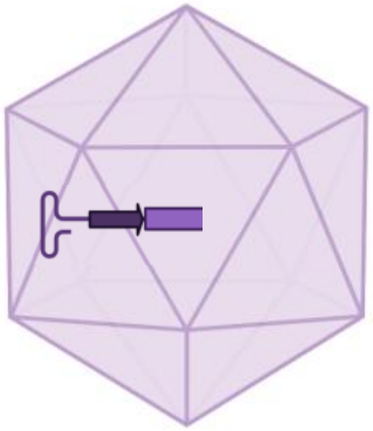
Impurity
Immunogenic

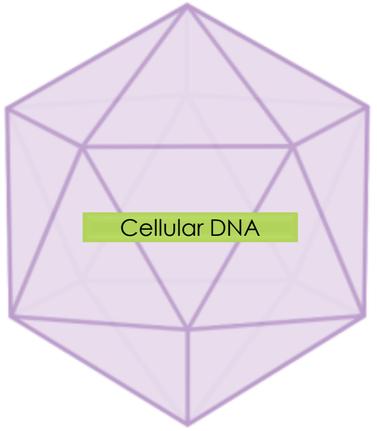
Impurity
Immunogenic

Impurity
Immunogenic







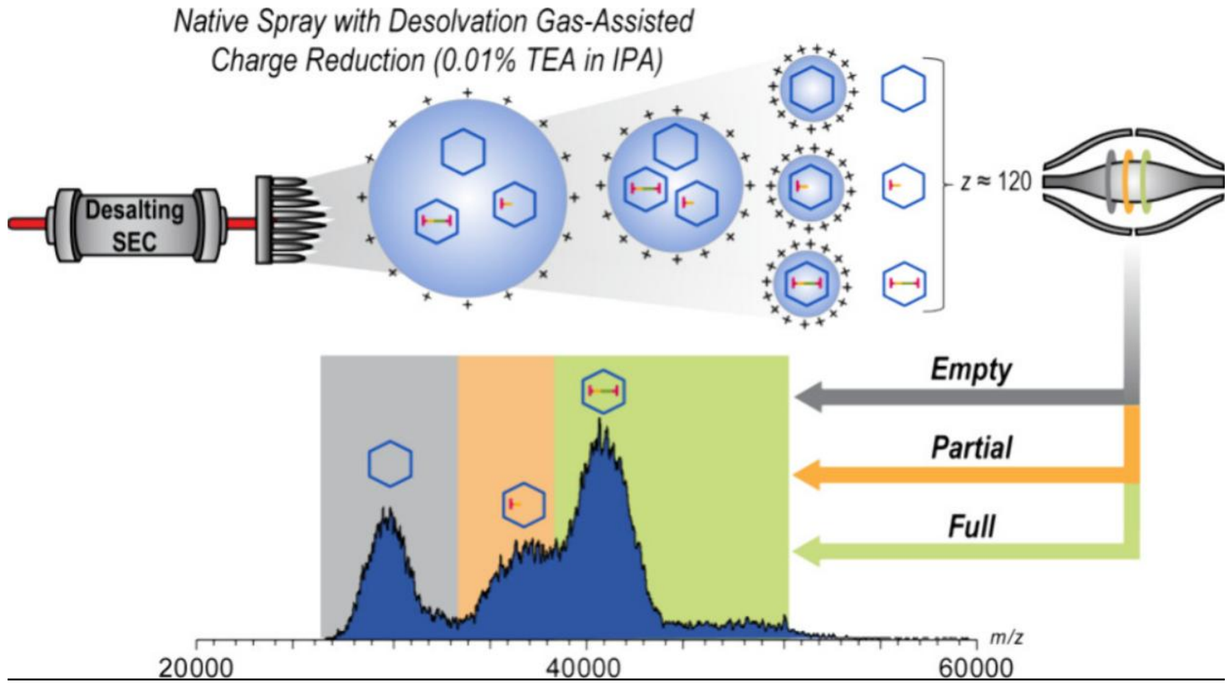


Test product lot mixture at end of manufacturing

Critical to understand capsid fill: Safety and Purity

Use 1: AAV Purity

Test Parameter Measure	Capsid fill ratio Empty: Partial : Full capsids
Quality Attribute Supported	Purity, Safety
Analytical Method	Native Mass Spec Online buffer exchange & charge reduction
What Phase for IND	Qualified method for Phase 1 required
Why Critical	Informs viral particle dose for safety (immunogenicity)& efficacy

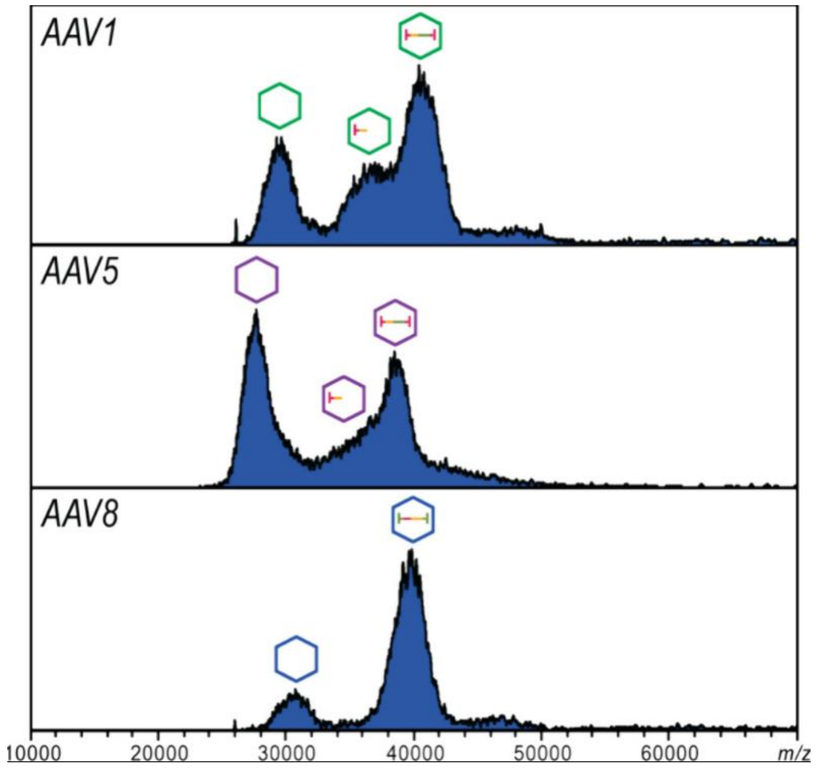


An Online Native Mass Spectrometry Approach for Fast, Sensitive, and Quantitative Assessment of Adeno-Associated Virus Capsid Content Ratios
Victoria C. Cotham, Shunhai Wang, and Ning Li
Journal of the American Society for Mass Spectrometry 2024 35 (7), 1567-1575
DOI: 10.1021/jasms.4c00151

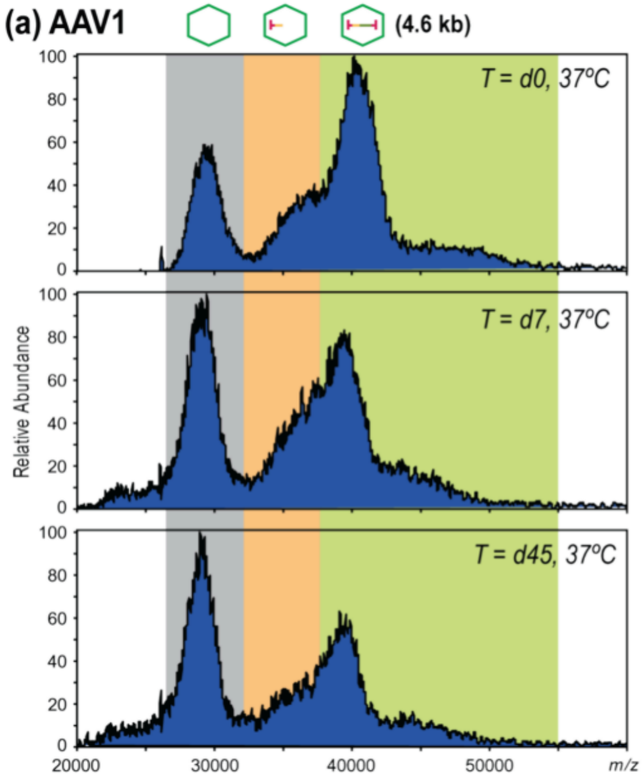
Use 1: AAV Purity

Two Examples Investigating AAV Packaging

Different AAV Serotypes Vary in Packaging Efficiency



Use in Stability Study



Regulatory Note:
Stability study plan required for all IND studies

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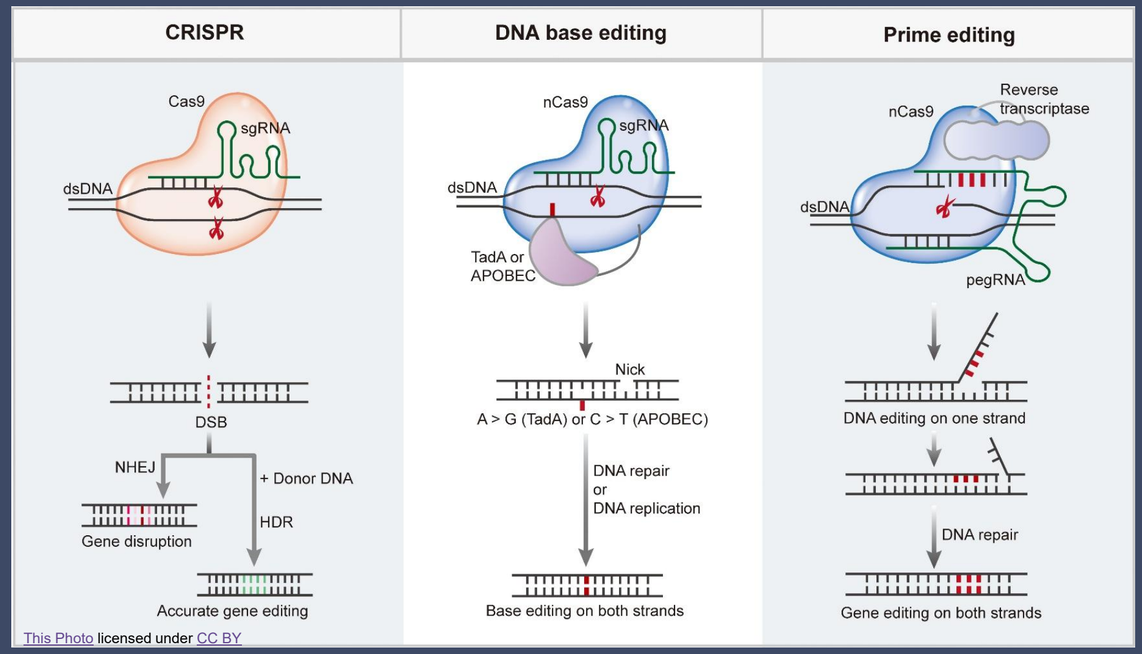
Use 2: RNAs for Gene Editing & Cancer Vaccines

- **RNA can be chemically or biologically synthesized**
- **Examples**
  - CRISPR-based gene editing
    - RNA-guide that locates the genomic site (e.g., gRNA, sgRNA, pegRNA)
    - Enzyme that can cut or nick (e.g., Cas9)
  - LNP-packaged RNA expressing tumor-specific antigenic signatures
  - mRNA electroporated into cells ex vivo



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Use 2: Genome Editing

- **US FDA**
  - CGT RNA are critical components
  - Treated as Drug Substance
  - Potency, purity, safety, and identity testing
- **CDMO Testing**
  - No guarantee COA will meet your IND's FDA requirements
- **Product type & Center**
  - RNA testing requirements depend on FDA Center (CBER vs CDER)
  - Testing is risk dependent
  - Ex vivo vs in vivo
  - Gene editing vs transient expression
- **Safety is assured across all Phases**

Example of CDMO CRISPR Products and Testing

Product Specifications

	GMP Gene Editors (SpCas9 & AccuBase)	GMP sgRNA	INDe sgRNA
Regulatory Compliance	Complies with FDA and ICH cGMP regulations including Chemistry, Manufacturing and Controls (CMC) for gene editing components used in cell and gene therapies	Complies with FDA and ICH cGMP regulations including Chemistry, Manufacturing and Controls (CMC) for gene editing components used in cell and gene therapies	Complies with equipment, facility, material controls in 21 CFR part 58 and provides comparability for nonclinical vs clinical materials

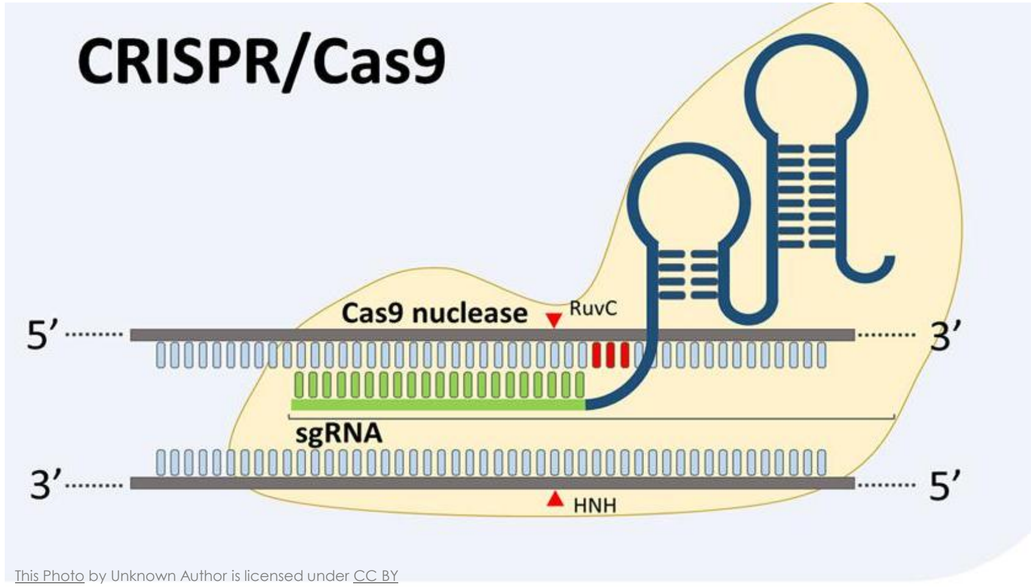
GMP sgRNA Available Quality Tests	GMP Gene Editor Quality Tests
• Appearance	• Appearance
• Concentration by UV-Vis	• Concentration by UV-Vis
• pH	• pH
• Purity by HPLC-UV	• Identity by electrophoresis
• Identity by ESI-MS	• Purity by RP-HPLC
• NGS	• Purity by SEC-HPLC
• Residual water content	• Purity by NR-CE
• Residual solvent content	• Purity by R-CE
• Elemental impurities	• Activity
• Endotoxin Testing	• Residual DNase
• Bioburden Testing	• Residual RNase
• Sterility Testing	• Residual Host Cell Protein
• CCIT	• Residual Host cell DNA
	• Endotoxin Testing
	• Sterility Testing

Regulatory Note:
Rarely a one-size-fits-all COA for all products

RNA must meet potency, safety, purity, identity

Use 2: Sequence Identity

- **Identity requirement**
  - RNA sequence matches the intended sequence
- **Purity/Impurity**
  - Fragments, missing Poly-A tail, truncations, etc.
- **Poor synthesis, mix-ups at CDMO or at manufacturing site**
  - Sequence matters
  - No oops in gene editing peoples' cells



Use 2: Sequence Identity

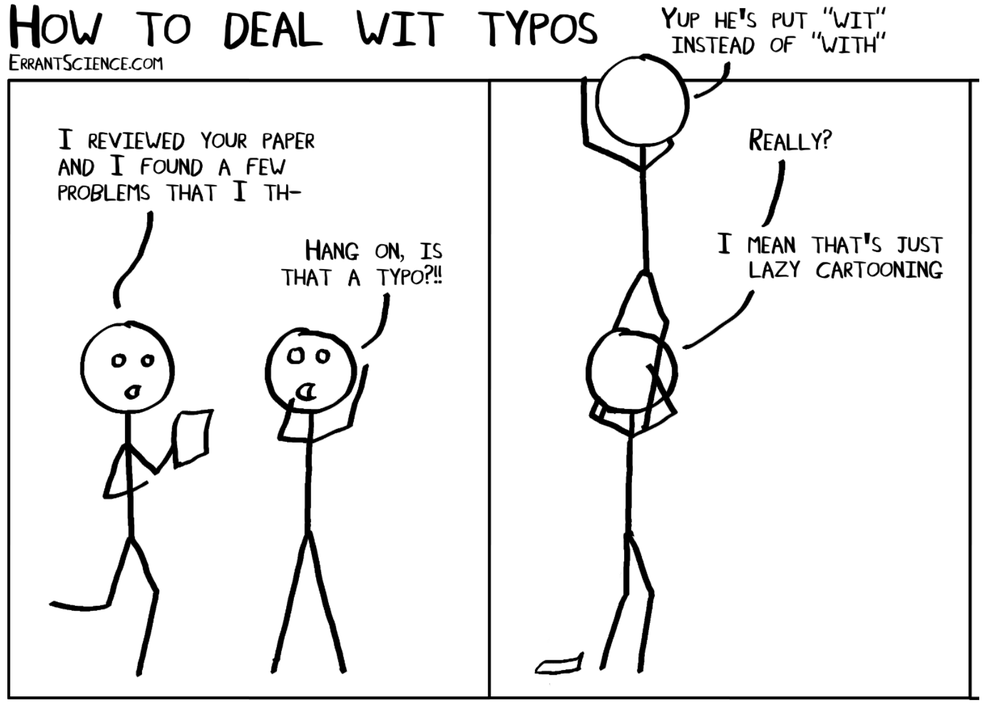
Sequence

HOW TO DEAL WITHTYPOS

HOW TO DEALHWIT TYPOS

TYPOS

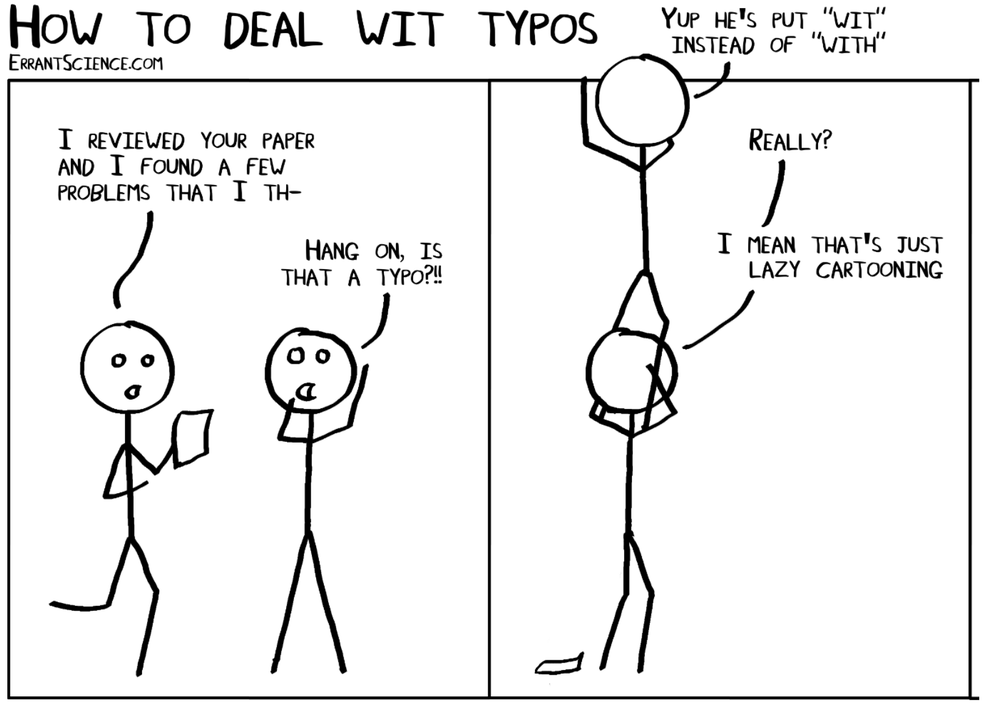
HOW TO DEAL WIT TYPOS



Use 2: Sequence Identity

Identity & Purity
Identical & Pure
Impurity
Impurity
Impurity

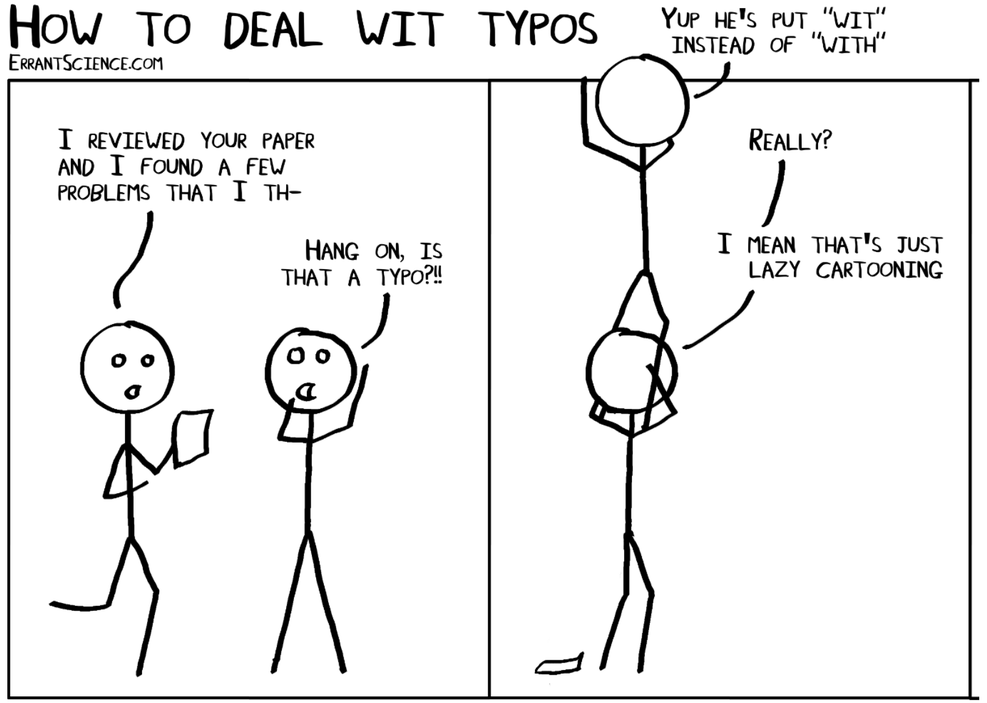
Sequence
HOW TO DEAL WITHTYPOS
HOW TO DEALHWIT TYPOS
TYPOS
HOW TO DEAL WIT TYPOS



Use 2: Sequence Identity

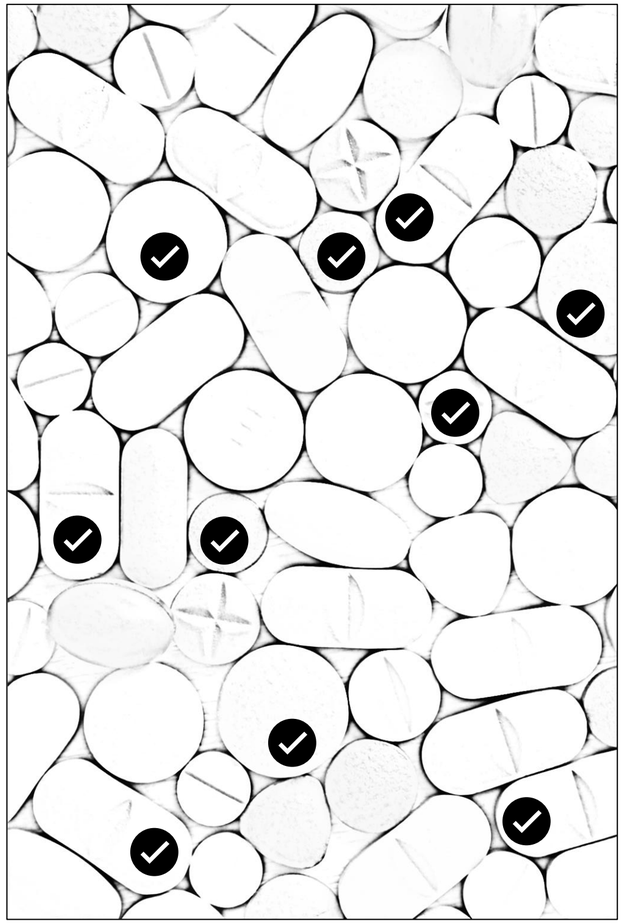
Identity & Purity	Mass Spec Result	Sequence
Identical & Pure	Pass	HOW TO DEAL WITHTYPOS
Impurity	Pass	HOW TO DEALHWIT TYPOS
Impurity	Fail	TYPOS
Impurity	Fail	HOW TO DEAL WIT TYPOS

A sequencing-based method is still required to determine identity
Mass Spec useful in combination for impurities



Use 3: Characterization - Powerful Unknowns

Why do some patients respond well to some product lots?



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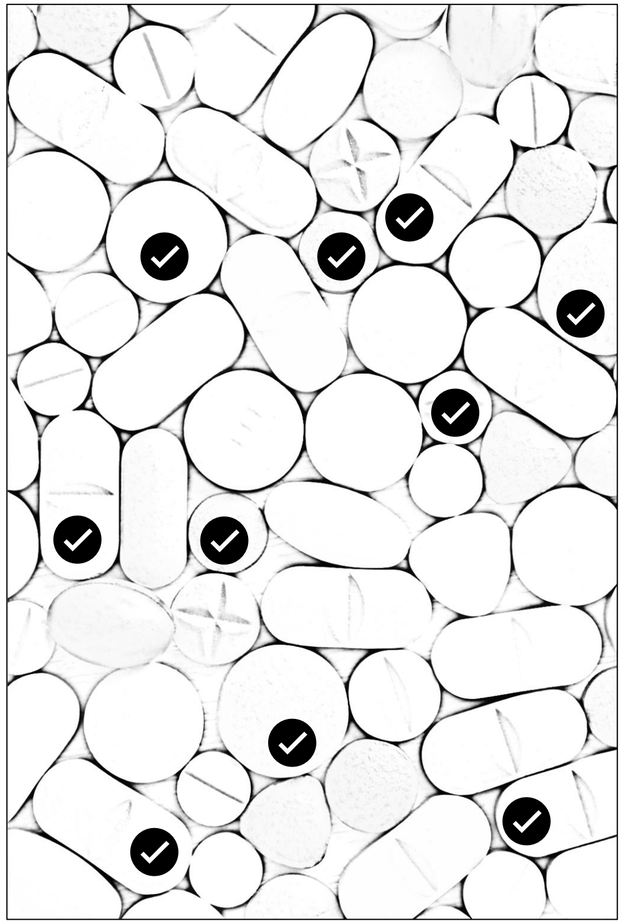
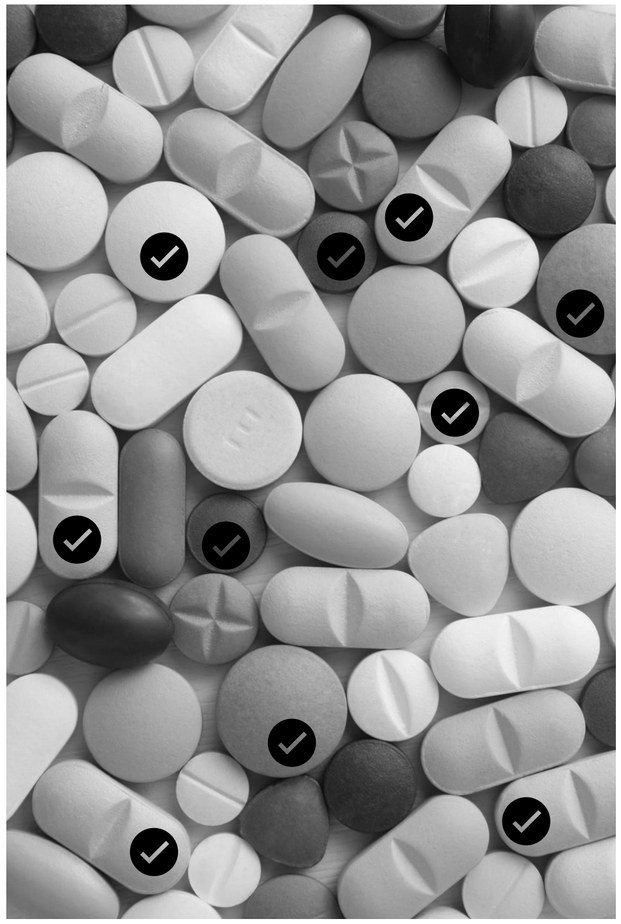
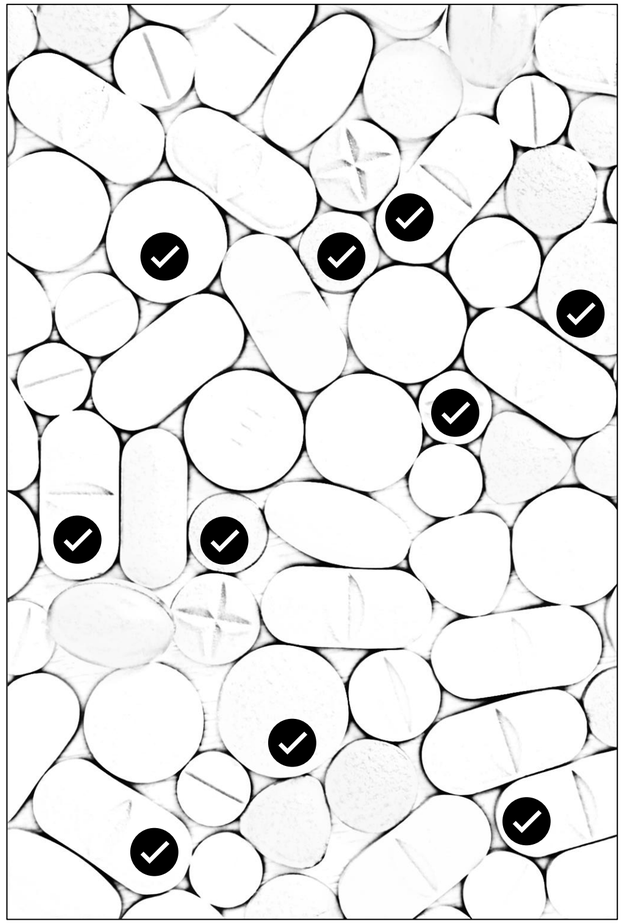


CGT products often highly variable
Complex MOAs
Quality Attributes insufficient

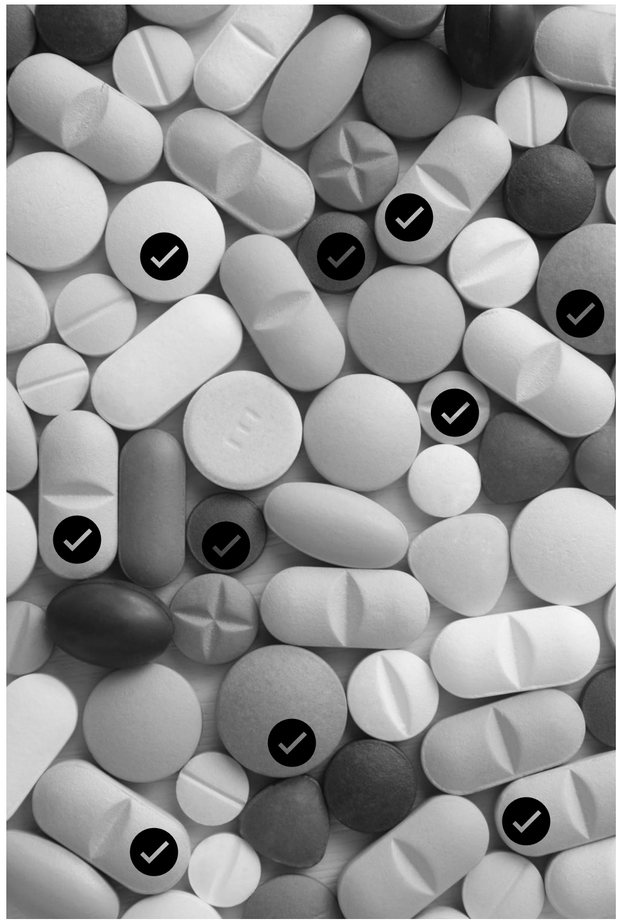


Use 3: Characterization - Powerful Unknowns

Why do some patients respond well to some product lots?



CGT products often highly variable
Complex MOAs
Quality Attributes insufficient



Characterization data can elucidate how a cell or gene therapy works

Use 3: AAV Safety & Unknowns

Table 3. AAV-based therapies clinical holds

Sponsor/Program/Trial identifier #	Target disease	Dates of hold	Reason for hold	Status/Conclusions
Pfizer[45,46] PF-06939926 NCT03362502	Duchenne muscular dystrophy	Dec-21 – Apr-22	Death of a young patient.	Cause of death was assumed to be more advanced disease and underlying cardiac dysfunction. FDA requested a potency assay and a protocol amendment, and the sponsor did both. Protocol amendment consists of closer monitoring of patients; there will be a 7-day hospitalization period after patients receive the therapy. Still determining strategy for non-ambulatory patients.
Pfizer[47,48] giroctocogene fitelparovec NCT04370054	Hemophilia A	Dec-21 – Mar-22	Symptomatic elevations in liver transaminases and normal factor levels which increases the risk of blood clots.	Adjusted protocol. Voluntary pause was [illegible] (Sept 22) until [illegible] had met [illegible] necessary conditions, including [illegible] regulatory authorities.
Selecta[49,50] SEL-302, (MMA101 plus ImmTOR) N/A	Methyl-malonic acidemia (MMA)	Nov-21 – Mar-22	Trial has not been initiated yet—no clinical or preclinical issues.	FDA requested further data on CMC linked to the MMA-101 product candidate.
BioMarin[51] BMN 307 NCT04480567	Phenyl-ketonuria	Sep-21 – Ongoing as of Dec-2022	[illegible] safety findings from a preclinical non-GLP pharmacology study. Several mice developed tumors. Lower doses were administered in clinical trials. No issues in humans to date (over 3,000 patients treated).	FDA asked for more animal studies. Delay is expected to last "several quarters."
Astellas[52] AT132 (bilparvovec) NCT03199469	X-linked myotubular myopathy	Sep-21 – Ongoing as of Dec-2022	A total of four patient deaths. The first three received the higher dose and developed liver dysfunction and eventual liver failure. Most recent patient died who received a lower dose; cause of death has not been disclosed.	Patient who received this dose had abnormal liver tests despite a normal liver ultrasound and tests prior to dosing. This is the second time this therapy has been put on clinical hold. Hold was lifted in January 2023.
Rocket Pharma[53,54] RP-A501 (AAV9.LAMP2B) NCT03882437	Damon disease	May-21 – Aug-21	FDA requested modifications to the trial protocol and revised guidelines for patient selection and management.	Protocol modifications only.

Clinical holds for cell and gene therapy trials: Risks, impact, and lessons learned. Wills, Carolyn A. et al., Molecular Therapy Methods & Clinical Development, Volume 31, 101125

Cell and gene therapies potentially powerful in benefit and harm

Field is looking forward towards safety

Inside Precision Medicine › Precision Medicine › Reporter's Notebook: Child Dies in Brain-Targeting AAV Gene Therapy Trial

Reporter's Notebook: Child Dies in Brain-Targeting AAV Gene Therapy Trial

Capsida voluntarily halted the trial for CAP-002—an IV-administered gene therapy for a rare neurological condition—to determine the patient's death

By Jonathan D. Grinstein, PhD September 15, 2025 https://www.insideprecisionmedicine.com/topics/precision-medicine/reporters-notebook-child-dies-in-brain-targeting-aav-gene-therapy-trial/

The NEW ENGLAND JOURNAL of MEDICINE

CURRENT ISSUE SPECIALTIES TOPIC

ORIGINAL ARTICLE | BRIEF REPORT

Death after High-Dose rAAV9 Gene Therapy in a Patient with Duchenne's Muscular Dystrophy

Authors: Angela Lek, Ph.D., Brenda Wong, M.D., Allison Keeler, Ph.D., Meghan Blackwood, Kaiyue Ma, M.Phil., Shushu Huang, M.D., Ph.D., Katelyn Sylvia, M.A., +17, and Terence Flotte, M.D. Author Info & Affiliations

Published September 27, 2023 | N Engl J Med 2023;389:1203-1210 | DOI: 10.1056/NEJMoa2307798
VOL. 389 NO. 13 | Copyright © 2023

Summary

We treated a 27-year-old patient with Duchenne's muscular dystrophy (DMD) with recombinant adeno-associated virus (rAAV) serotype 9 containing dSaCas9 (i.e., "dead" *Staphylococcus aureus* Cas9, in which the Cas9 nuclease activity has been inactivated) fused to VP64; this transgene was designed to up-regulate cortical dystrophin as a custom CRISPR–transactivator therapy. The dose of rAAV used was 1×10^{14} vector genomes per kilogram of body weight. Mild cardiac dysfunction and pericardial effusion developed, followed by acute respiratory distress syndrome (ARDS) and cardiac arrest 6 days after transgene treatment; the patient died 2 days later. A postmortem examination showed severe diffuse alveolar damage. Expression of transgene in the liver was minimal, and there was no evidence of AAV serotype 9 antibodies or effector T-cell reactivity in the organs. These findings indicate that an innate immune reaction caused ARDS in a patient with advanced DMD treated with high-dose rAAV gene therapy. (Funded by Cure Rare Disease.)

FDA Investigating Deaths Due to Acute Liver Failure Following Treatment with Sarepta's AAVrh74 Gene Therapies

FDA Safety Communication - July 18, 2025

Summary of the Issue

As of July 18, 2025, the Food and Drug Administration (FDA) has received three reports of fatal acute liver failure following treatment of patients with Sarepta AAVrh74 gene therapies that appear to have been caused by the gene therapy products as a result of acute liver failure. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/fda-investigating-deaths-due-acute-liver-failure-following-treatment-sareptas-aavrh74-gene-therapies

Patient dies in Rocket's Phase II Danon disease gene therapy trial

The FDA ordered a clinical hold on the Phase II trial after the adverse event was first disclosed.

Abigail Beaney May 27, 2025

A patient has died in a pivotal Phase II trial of Rocket Pharmaceuticals' gene therapy for Danon disease after suffering a serious adverse event (AE).

Rocket said that the patient received a dose of RP-A501 in the Phase II study (NCT06092034) and experienced clinical complications related to capillary leak syndrome. The patient later died after suffering an acute systemic infection.

https://www.clinicaltrialsarena.com/news/patient-dies-rocket-danon-disease-gene-therapy-trial/

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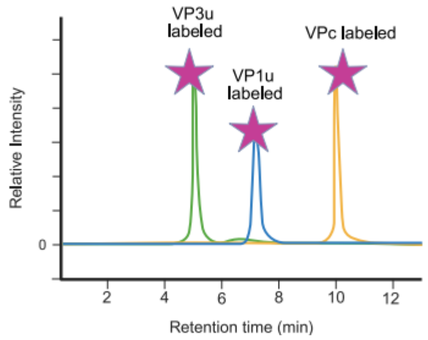
Use 3: AAV Unknowns

- Viruses = Nature's original cellular delivery system for DNA and RNA
- Many unknowns



Histone H4
Nucleolin
Heat shock cognate protein 71 kDa
Heat shock protein 70 kDa 1A
Heat shock protein 70 kDa 1B
Putative elongation factor 1-alpha-like 3
Elongation factor 1-alpha 1
Chromobox protein homolog 1
Chromobox protein homolog 3
Polyadenylate-binding protein 1
Y-box-binding protein 1
Ubiquitin 40S ribosomal protein S27a

How they put 3 proteins together

"**observed ratio of 1 : 5.5 : 7.3 deviates from the widely assumed AAV capsid stoichiometry of 1 : 1 : 10**"

Previous work demonstrates ratio impacts potency

What DNA they package

Host cell DNA
Specific sequences

The consequence of delivering DNA from the virus-producing cells to a patient is unknown

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Development of LC-MS methods for AAV capsid protein quantification and host cell protein profiling. Kontogiannis, Theodoros et al. Molecular Therapy Methods & Clinical Development, Volume 33, Issue 3, 101562

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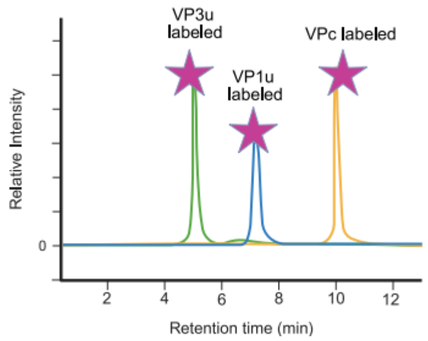

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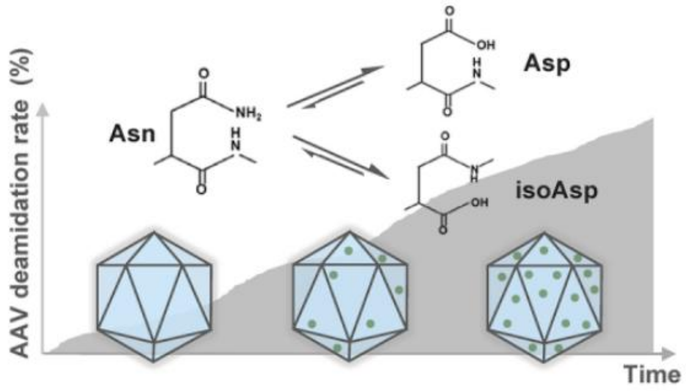
Y-box-binding protein 1
Ubiquitin 40S ribosomal protein S27a



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How they trigger immune responses

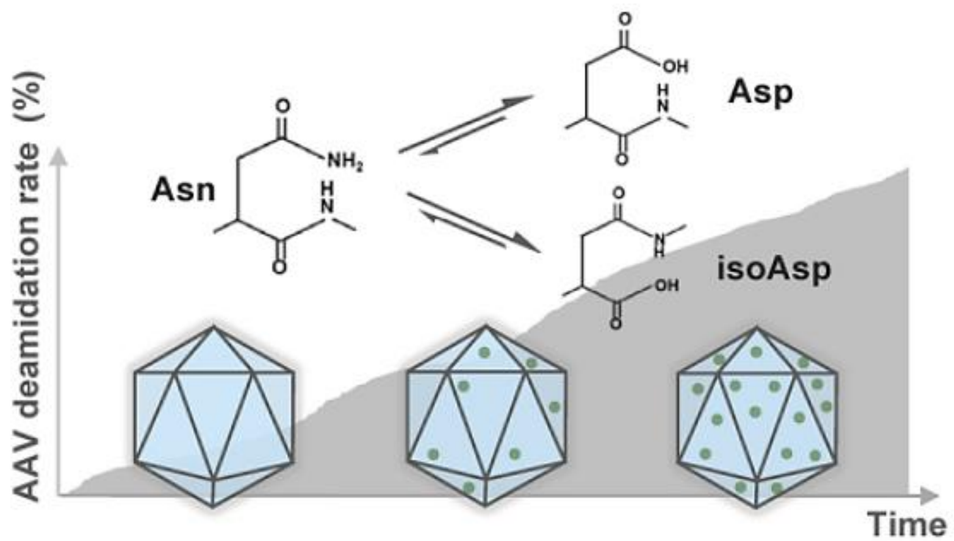
Post-translational protein signatures alter immune responses

*Work done at FDA

Development of LC-MS methods for AAV capsid protein quantification and host cell protein profiling. Kontogiannis, Theodoros et al. Molecular Therapy Methods & Clinical Development, Volume 33, Issue 3, 101562

Bing SJ, Justesen S, Wu WW, Sajib AM, Warrington S, Baer A, Thorgrimsen S, Shen RF, Mazor R. Differential T cell immune responses to deamidated adeno-associated virus vector. Mol Ther Methods Clin Dev. 2022 Jan 18;24:255-267. doi: 10.1016/j.omtm.2022.01.005. PMID: 35211638

Use 3: AAV Spontaneous Deamidation and Immunogenicity



rAAV9 produced, viral capsid was denatured, separated by gel electrophoresis 11, 25, and 83 days after purification

Excised gel bands subjected to in-gel tryptic digestion

Tryptic peptides extracted from gels, filtered, and analyzed for deamidation by **liquid chromatography-mass spectrometry**

LC-MS

*Work done at FDA

Regulatory Notes:

Research learnings translate to clinical safety, efficacy

Slow shifts in regulatory perspectives

Bing SJ, Justesen S, Wu WW, Sajib AM, Warrington S, Baer A, Thorgrimsen S, Shen RF, Mazor R. Differential T cell immune responses to deamidated adeno-associated virus vector. Mol Ther Methods Clin Dev. 2022 Jan 18;24:255-267. doi: 10.1016/j.omtm.2022.01.005. PMID: 35211638

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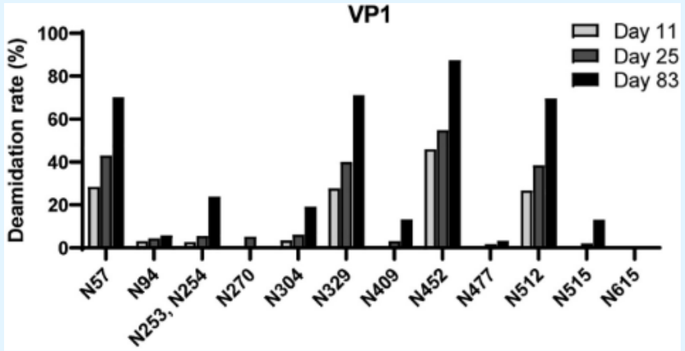
Use 3: AAV Spontaneous Deamidation and Immunogenicity

Experiment: Measure deamidation at sites over time after purification

Result: Time impacts frequency of deamidation



Future Considerations:
- Limit hold and storage times
- Optimize formulation

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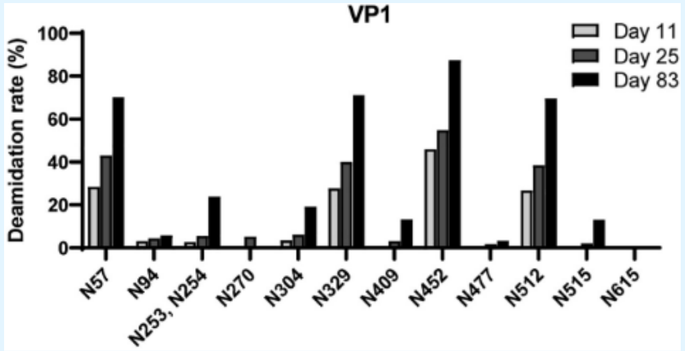
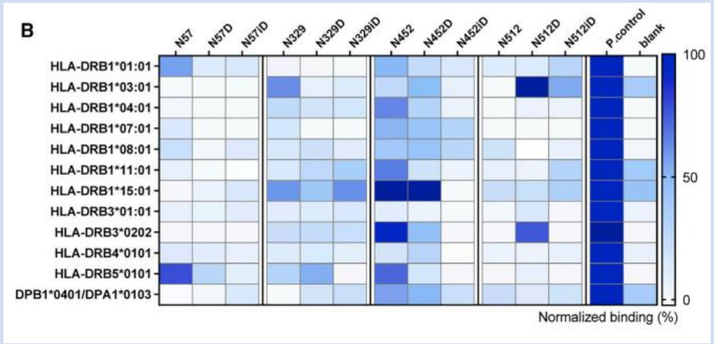


Future Considerations:
- Limit hold and storage times
- Optimize formulation

Experiment: Identify binding between deamidation sites and human leukocyte antigen types (HLA/ major histocompatibility class II)

Result: Recognition of deamidated sites varies across HLA types

*HLA allele types vary by person (organ rejection)



Future Considerations:
- Tailoring of drugs to personal HLA genotypes
- Patient inclusion/exclusion

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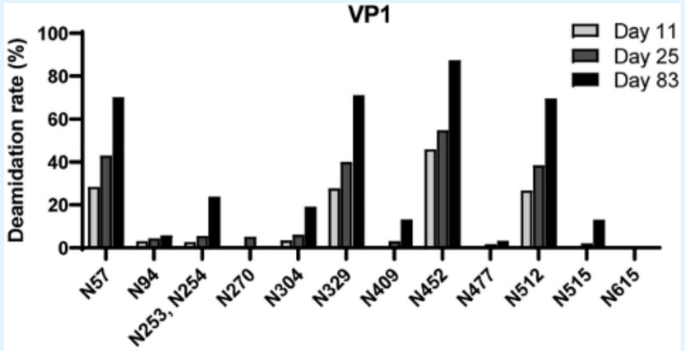

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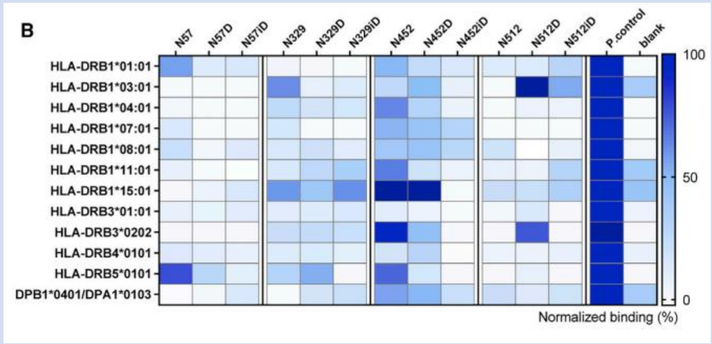
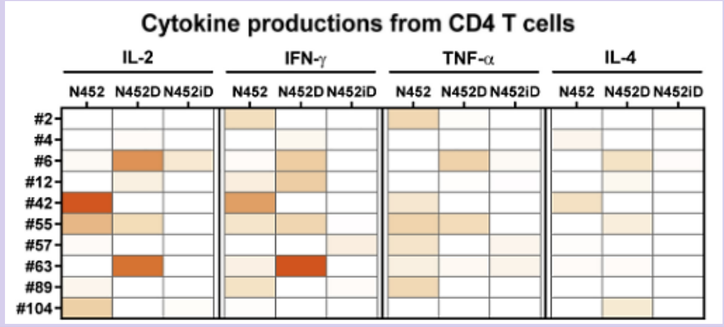


Future Considerations:
- Possible tailoring of drugs to personal HLA genotypes
- Patient inclusion/exclusion

Experiment: Stimulate donor CD4 T cells with variants at deamidation sites

Result: Secretion of immune signaling varied by donor and site modification



Future Considerations:
- Testing AAV deamidation as a Critical Quality Attribute

Regulatory Notes:

Products with improved performance & safety profiles can differentiate from other products

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*Work done at FDA

Summary

- Field of CGT is open for new approaches via Mass Spec
- FDA CGT is open to novel approaches – everything is new
- Develop Mass Spec methods with focus on qualification, then validation

- Discovering unknowns in biological complexity can unlock the future of safe & effective drugs

Analytical methods reveal the world around us

References

- Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) FDA Guidance. January 2020.
- Q14 Analytical Procedure Development, FDA Guidance for Industry. March 2024.
- Q2(R2) Validation of Analytical Procedures, FDA Guidance for Industry. March 2024.
- Bioanalytical Method Validation, FDA Guidance for Industry. May 2018. (CDER, CVM)
- Analytical Procedures and Methods Validation for Drugs and Biologics, FDA Guidance for Industry. July 2015.

Non-exhaustive list

See FDA CGT for a comprehensive guide including

FAQ for Cell and Gene Therapy. Draft Guidance

Potency Assurance Draft Guidance

CAR T Guidance

Gene Editing Guidance

See also OTPs Town Hall Series recordings

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