Advancing genomic medicine: Navigating challenges in CMC potency assay development throughout the product life-cycle

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Phillip Ramsey (Presenting)

CASSS CGTP Summit
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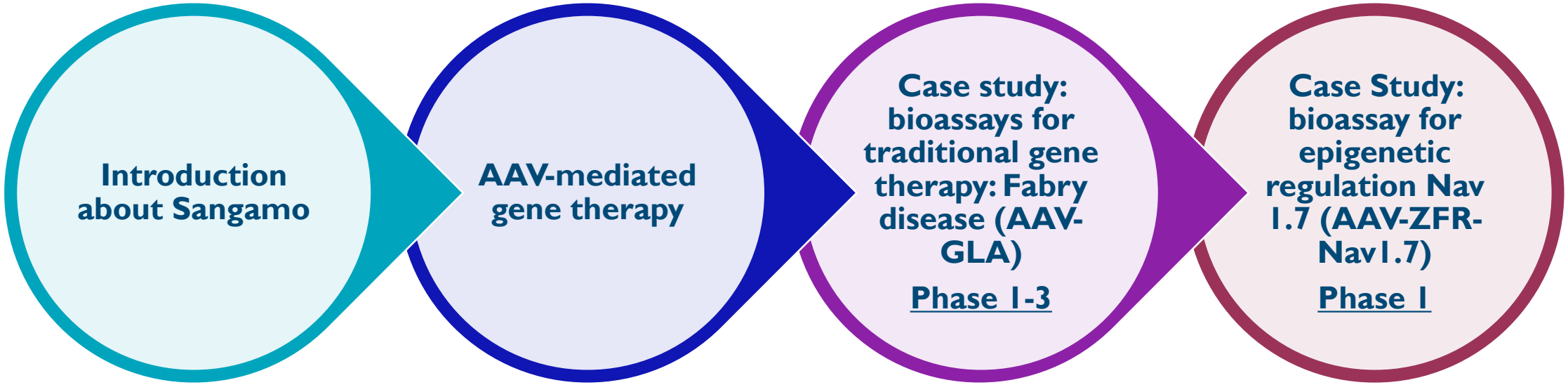
Outline


Introduction about Sangamo
AAV-mediated gene therapy
Case study: bioassays for traditional gene therapy: Fabry disease (AAV-GLA)
Phase 1-3
Case Study: bioassay for epigenetic regulation Nav 1.7 (AAV-ZFR-Nav1.7)
Phase 1

Sangamo is a differentiated genomic medicine company focused on treating debilitating neurological and rare genetic diseases

Sangamo THERAPEUTICS

Potent zinc finger epigenetic regulation technology, with neurology programs advancing towards the clinic

Industry-leading AAV capsid discovery platform has demonstrated non-invasive intrathecal and intravenous delivery to the brain

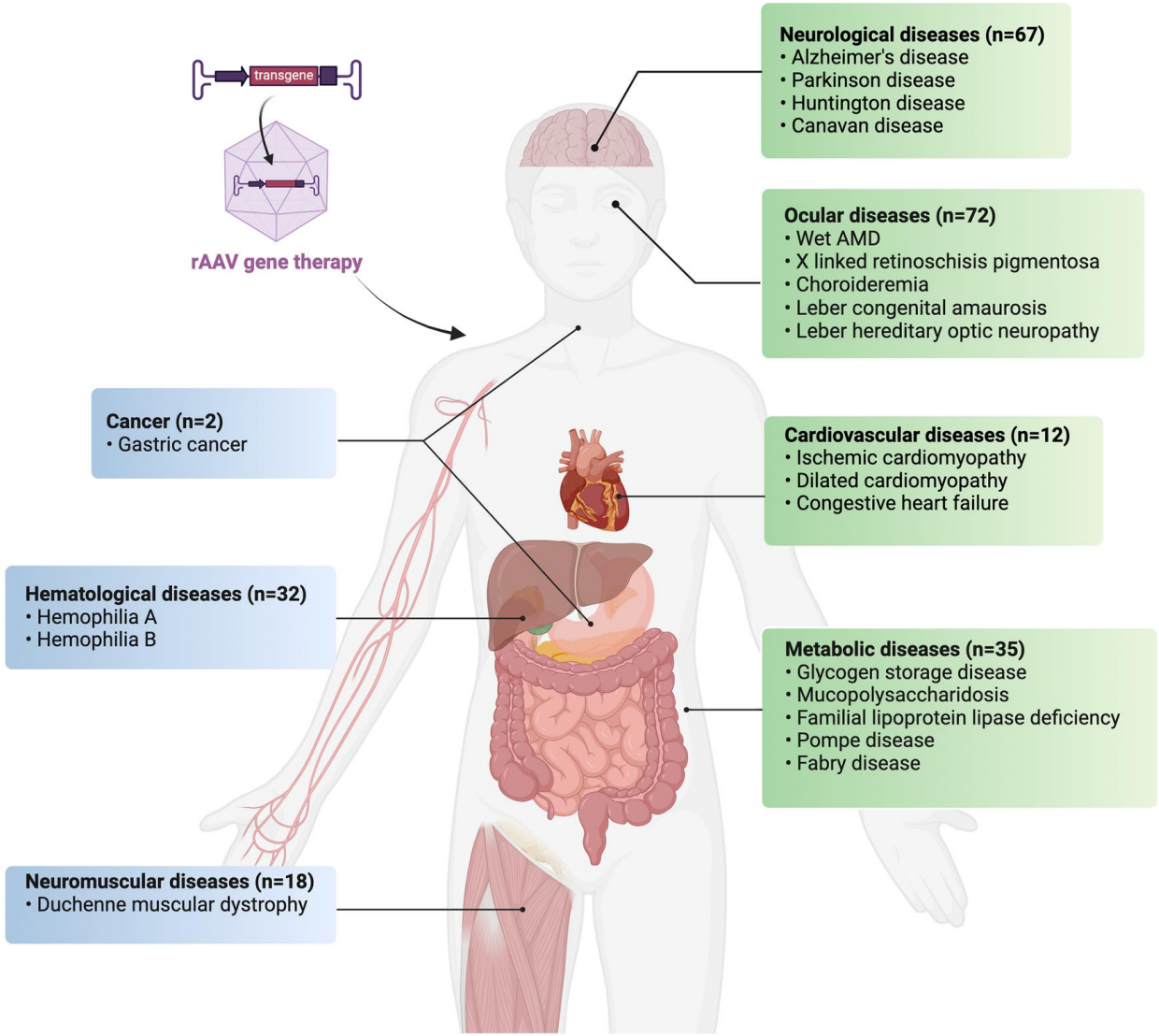
Powerful research platform **continually innovates in new modes of genome modulation** to support value creation opportunities for both wholly owned programs and potential partners

SHARP STRATEGIC FOCUS IN NEUROLOGY

Strong roster of current partners and a **clear regulatory pathway to Accelerated Approval agreed with U.S. FDA in Fabry disease, with partner negotiations ongoing**

OPTIMIZING ASSET VALUE

Adeno-associated virus as a delivery vector for gene therapy of human diseases



- Discovered in 1965
- 3 approvals:

  Luxturna, Zolgensma and Hemophilia B
- 238 clinical trials
- Advantages of AAV
  - ✓ Safety profile
  - ✓ Immunogenicity
  - ✓ Tissue tropism
  - ✓ Long-lasting effects
  - ✓ Versatility
  - ✓ Established clinical Use
  - ✓ Low toxicity

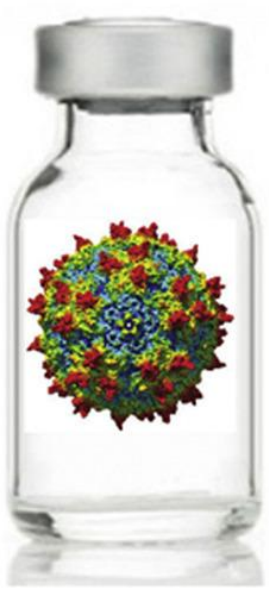
Sangamo THERAPEUTICS

Jiang-Hui Wang *et al.* "Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduction and Targeted Therapy (2024): ISSN 2059-3635

Analytical assays to characterize AAV gene therapy product

Quantity
Capsid Titer (ELISA, DLS, SEC HPLC)
Vector Genome Titer (ddPCR/qPCR)

Identity
Immunophoretic (WB, ELISA)
Peptide Mapping, PTMs (LC-MS)
Molecular weight (LC-MS)
Capillary IEF, SDS-PAGE



Purity
HPLC SEC, IEX, RP
SDS-PAGE
Capillary IEF, SDS
Densitometry
Carbohydrate Content

Safety
Endotoxin USP<85>
Bioburden USP<61>
Sterility USP<71>
Particulate Matter <USP788>

Impurities
Host-Cell DNA
Host-Cell Protein
Baculovirus DNA
Baculovirus Protein
Process Residuals

Potency
Bioassays
Cell-based (FACS etc.)
MOA reflective bioassays

General
pH
Osmolality
Appearance
Volume

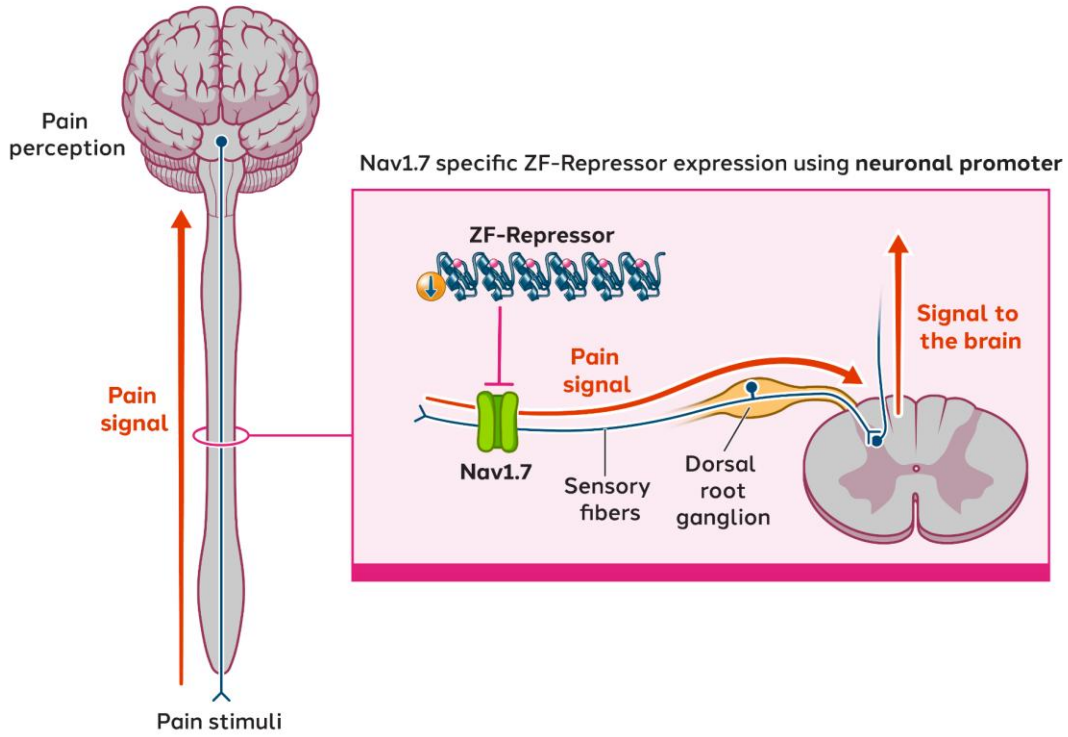
Ramsey, J. Phillip, *et al.* "Overview of analytics needed to support a robust gene therapy manufacturing process." Current Opinion in Biomedical Engineering 20 (2021): 100339.

Case Study: Bioassay for epigenetic regulation: Nav 1.7 (AAV-ZFR-Nav1.7)

Potency assay for Phase I

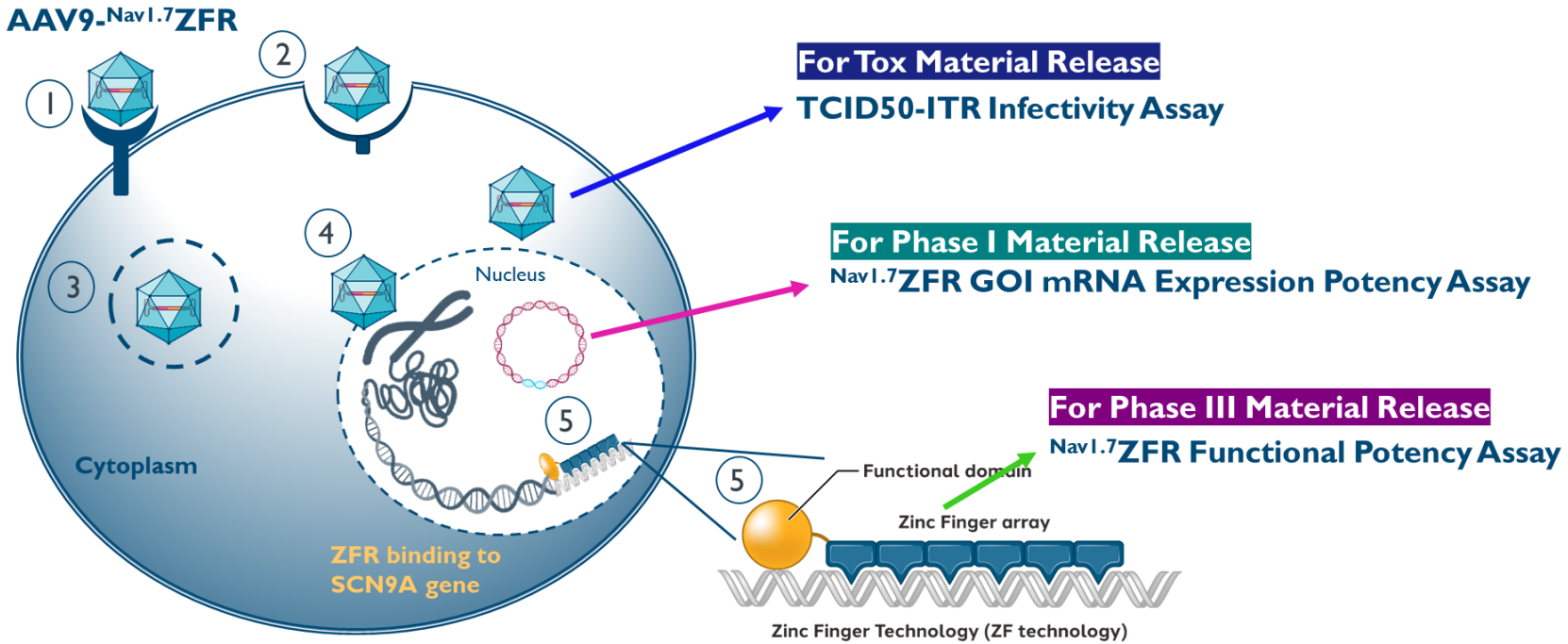
Nav1.7 as a therapeutic target for pain

ST-503 targets a gene validated by human genetics and leverages an AAV delivery capsid already in the clinic



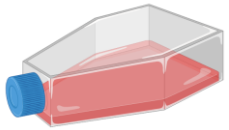
- A significant body of evidence implicates **sodium channels** in mediating the **pathophysiology of neuropathic pain**
- **Nav1.7** is a voltage gated sodium channel expressed in the Dorsal Root Ganglion (DRG)
- Blocking Nav1.7 in the DRG is expected to prevent the **transmission of nociceptive pain signals** to the brain
- This allows us to target multiple **neuropathic pain indications**, regardless of the cause of the pain
- Reducing pain by inhibiting Nav1.7 is not predicted to be associated with **any neurological side effects**
- Administered **intrathecally via AAV9**, a well-established, well-tolerated capsid

An incremental approach to develop potency assays based on the mechanism of action (MOA) of AAV9-$^{Nav1.7}$ZFR

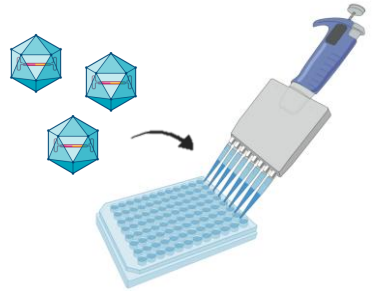


ZFR$^{Nav1.7}$ mRNA expression potency assay process flow

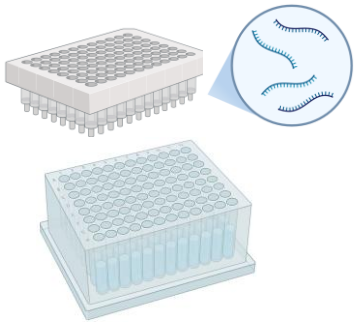
Cell plating



Infect cells with AAV9-$^{Nav1.7}$ZFR

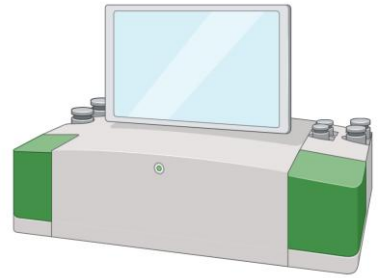


RNA Isolation

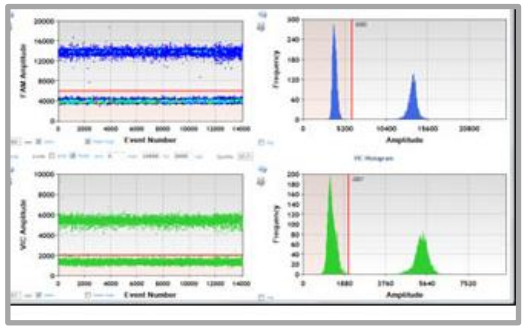


cDNA Synthesis

Droplet Digital PCR (ddPCR)

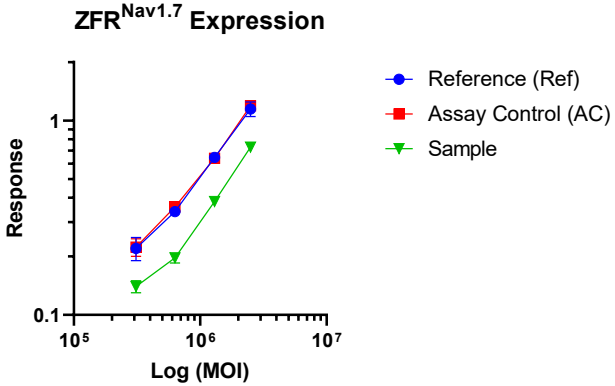


ZFR & Housekeeping Gene (HKG) Expression



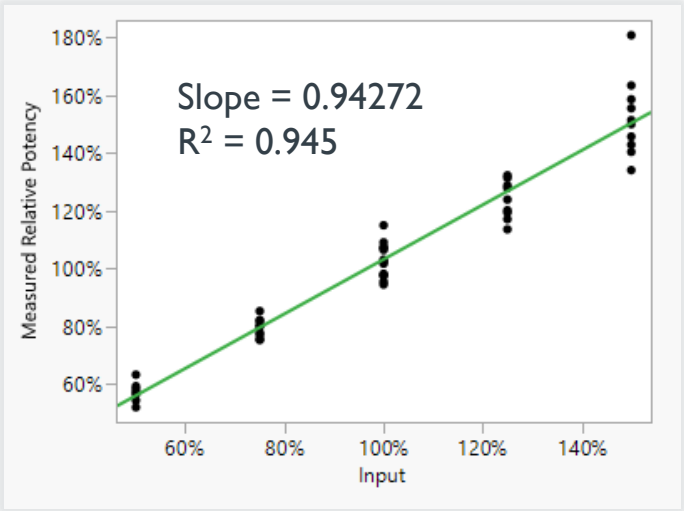
Bioassay Software

Determine Relative Potency



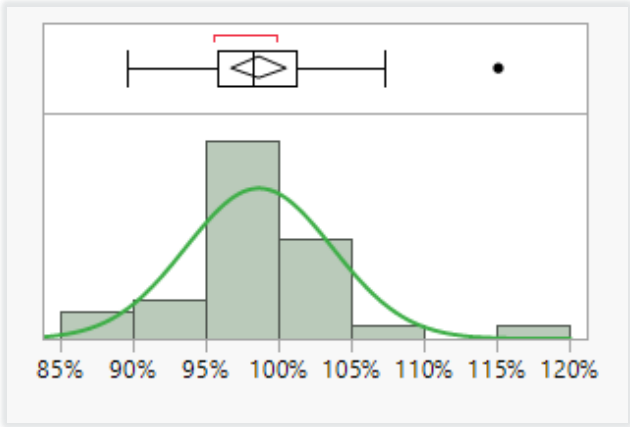
Pre-qualification: Accuracy and Precision studies

Accuracy and Linearity



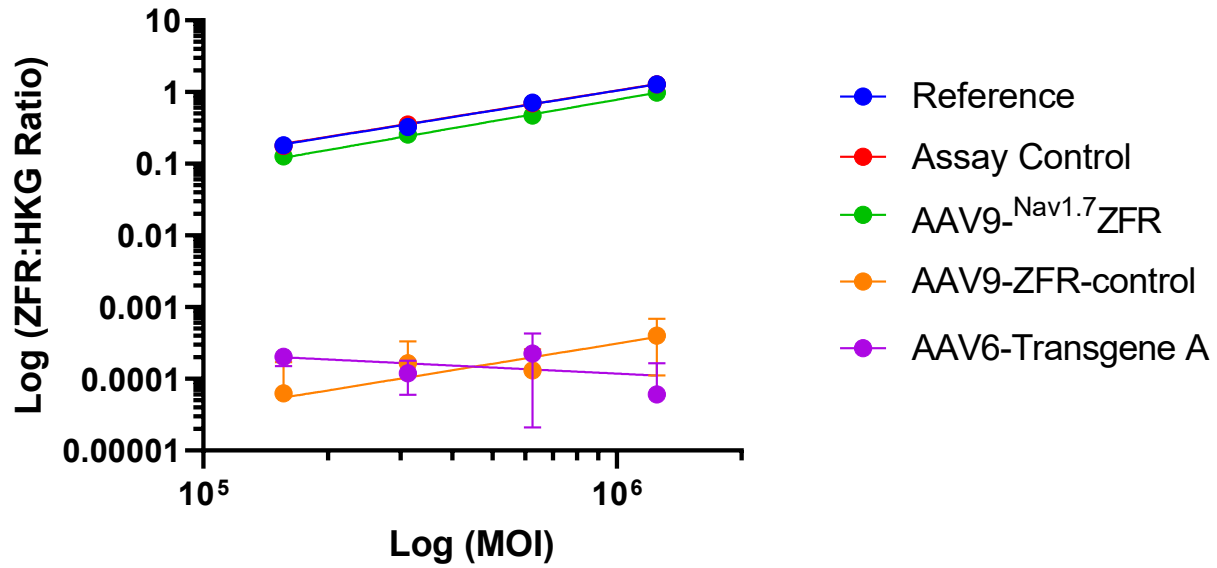
N	Input	Average Measured Relative Potency	CV
10	50%	57.1%	**5.4%**
10	75%	79.7%	**4.0%**
10	100%	102.9%	**5.7%**
10	125%	124.%	**5.4%**
10	150%	152.4%	**8.7%**

Intermediate precision



	Assay Control
N	31
Mean	98.7%
90% CI Interval	96.8% - 100.5%
CV	**5%**

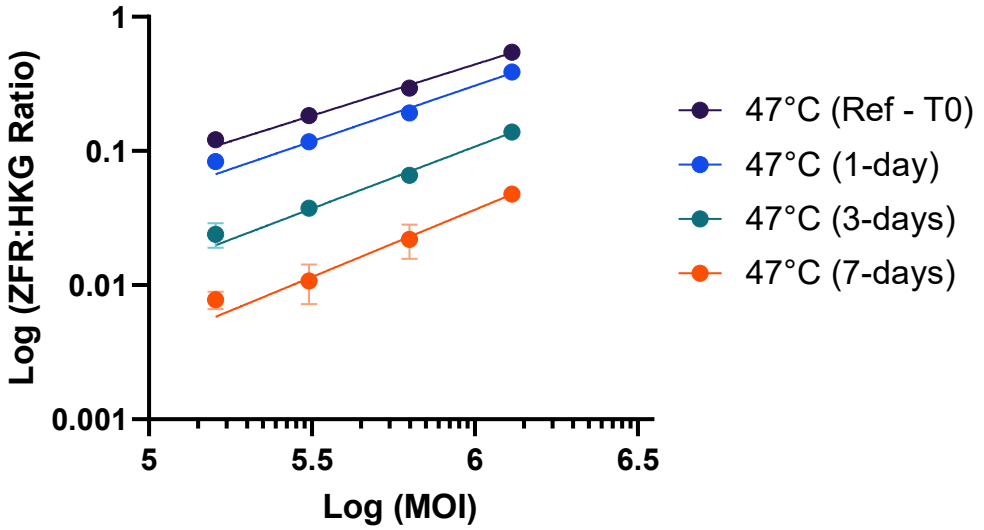
Pre-qualification: Specificity study



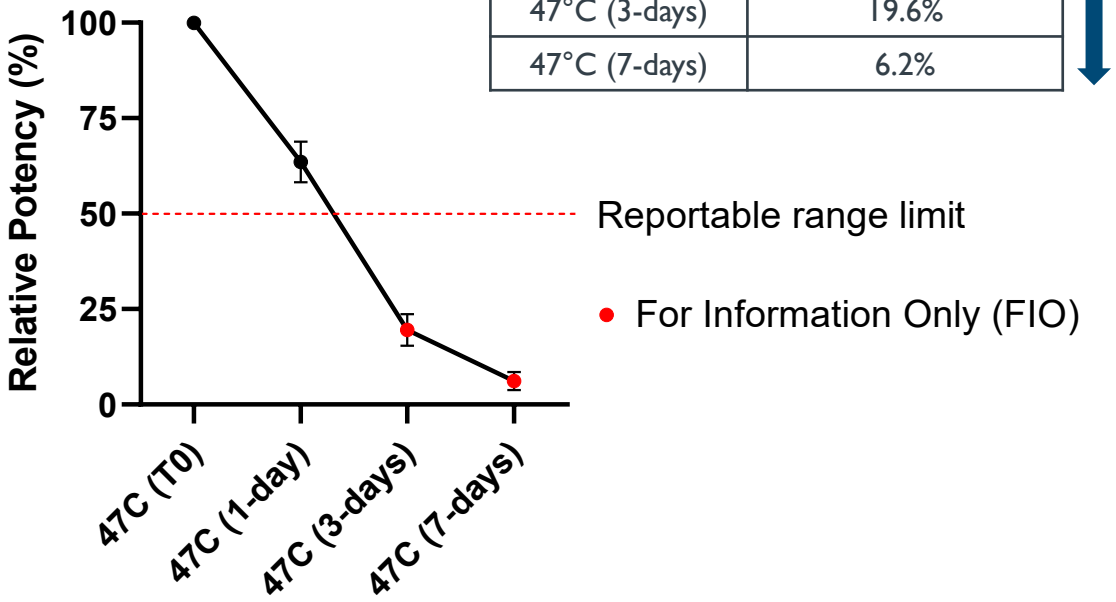
Primers/probe must be able to differentiate between vectors that were designed for different programs

				ddPCR target gene concentration at starting MOI (copies/uL)	
Lot	**N**	**Average Relative Potency**	**CV**	**ZFR$^{Nav1.7}$**	**HKG**
AAV9-$^{Nav1.7}$ZFR	**6**	**74.9%**	**8.8%**	337.36	505.06
AAV9-ZFR-control	6	N/A	N/A	0.13	362.06
AAV6-Transgene A	6	N/A	N/A	0.23	417.26

Pre-qualification: Stability study



Sample	Relative Potency
47°C (Ref - T0)	100%
47°C (1-day)	63.5%
47°C (3-days)	19.6%
47°C (7-days)	6.2%



- Heat stressed samples were prepared by incubating at 47°C
- Timepoints were collected over 7-days at various timepoints
- Targeted reportable range of the assay is between 50% to 150%, relative potency below 50% is reported as "For Information Only (FIO)"

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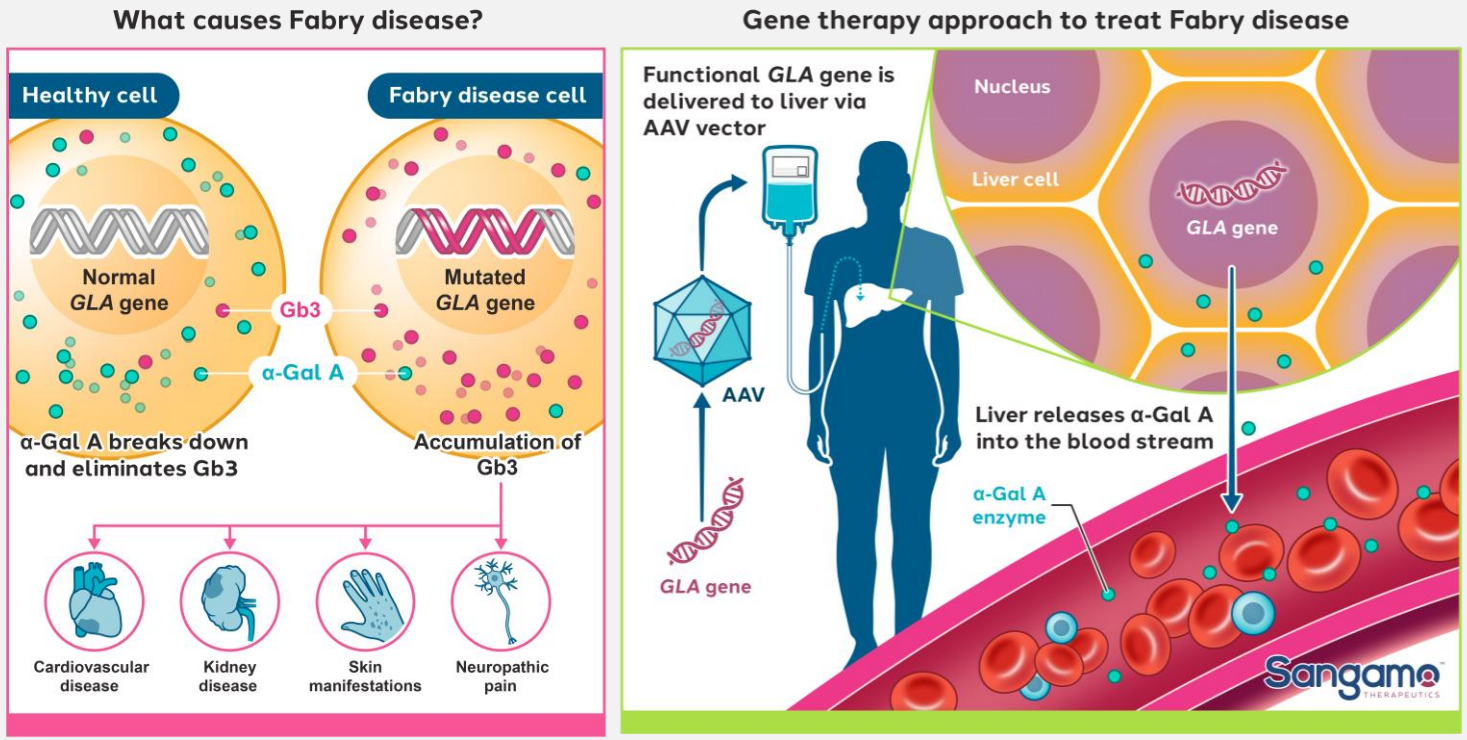
Case study: Bioassays for Traditional gene therapy: Fabry disease (AAV-GLA)

Potency assays for Phase 3

Fabry Disease: Isaralgagene civaparvovec (ST-920)

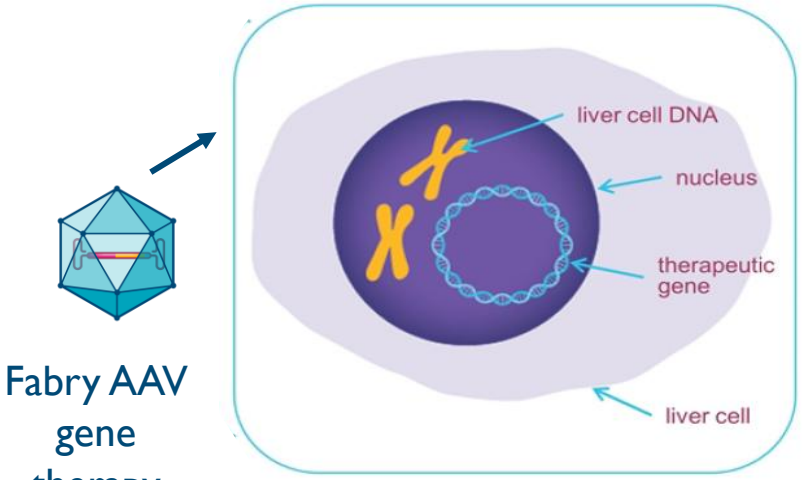
Abbreviated clinical pathway supports BLA submission in Q4 2025



- **Largest known gene therapy program in Fabry disease**
  - Dosing complete in Phase 1/2 STAAR study
- **Compelling clinical data**
  - Continue to amass encouraging clinical data, including preliminary analysis of a positive mean eGFR slope in all 32 patients treated >1yr
  - Pivotal readout expected later in Q2 2025
- **FDA alignment on Accelerated Approval pathway**
  - FDA confirmed that eGFR slope data at one year across all Phase 1/2 patients can serve as a primary basis for accelerated approval
  - Potential BLA submission expected as early as 1Q 2026
- Has FDA RMAT, EMA PRIME eligibility and UK MHRA ILAP **designations**

eGFR: estimated glomerular filtration rate

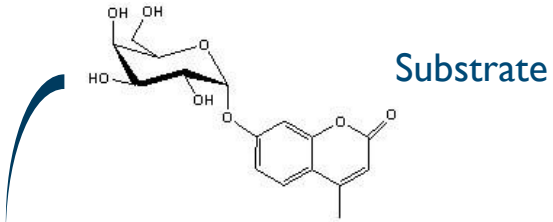
Potency assay matrix for the Fabry program

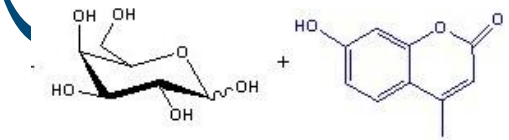


Fabry AAV gene therapy

α-GAL A enzyme

Activity

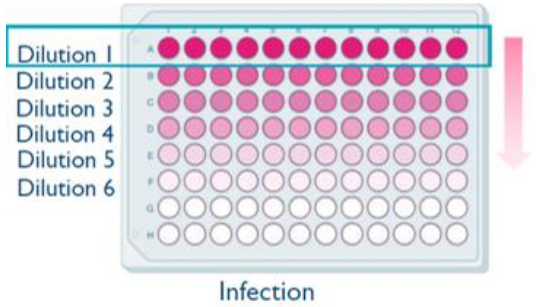




4-MU, Fluorescent product

GLA activity assay

Infectivity



TCID50 (50% Tissue Culture Infectious Dose)

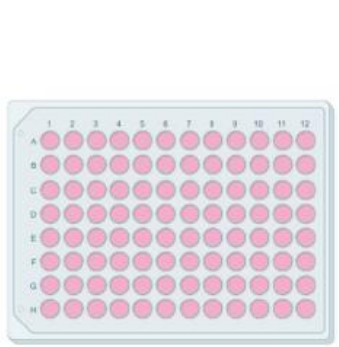
Expression

Signal detection

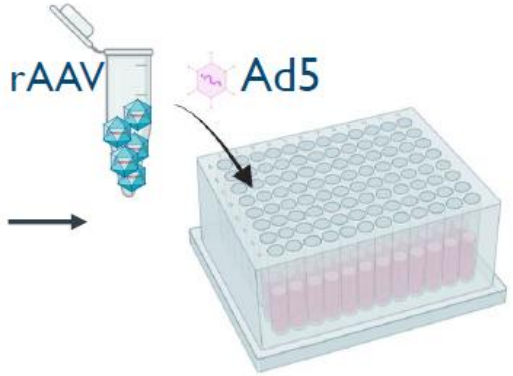
GLA protein expression assay

α-GAL A: Alpha-galactosidase

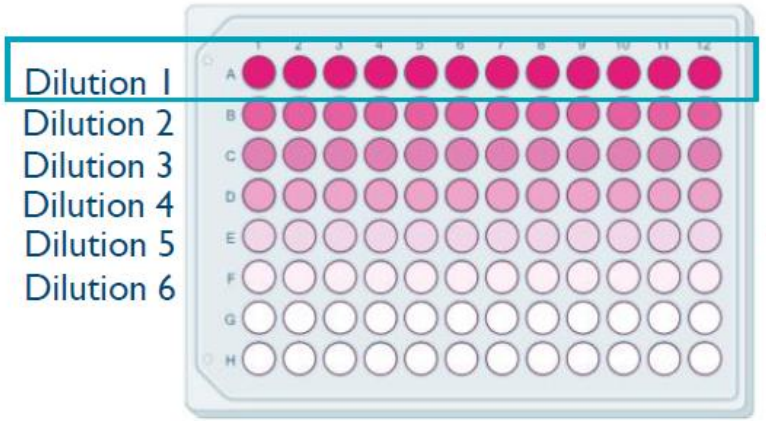
$TCID_{50}$ infectivity assay workflow



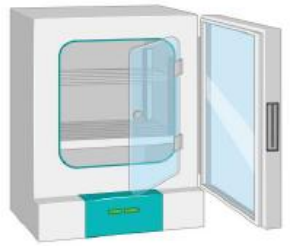
Cells plating

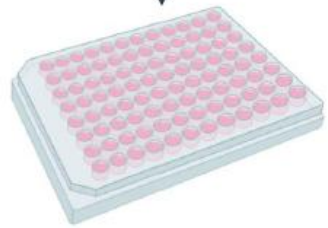


Viral serial dilution



Infection

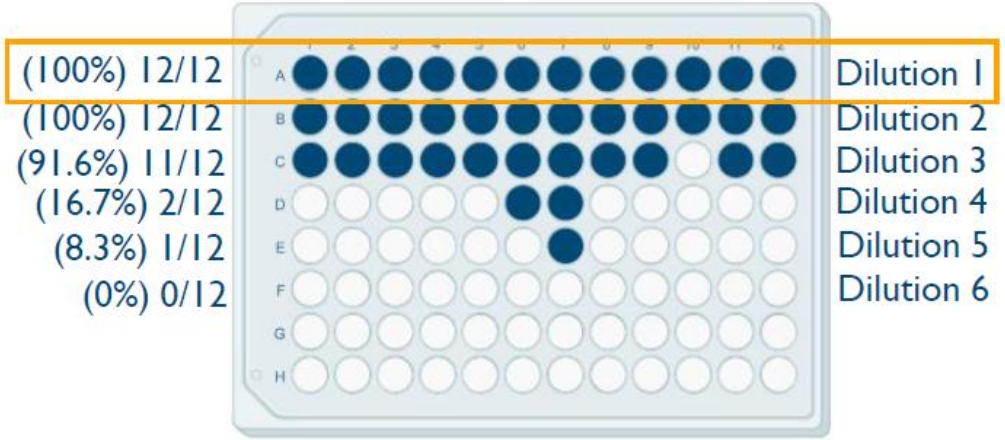




DNA extraction

Vector genome (Vg) quantification

Successful infection = Final Vg > Input Vg



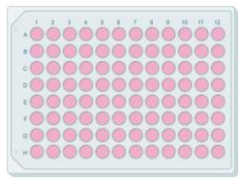
● Positive well
○ Negative well

Spearman-Kärber formula

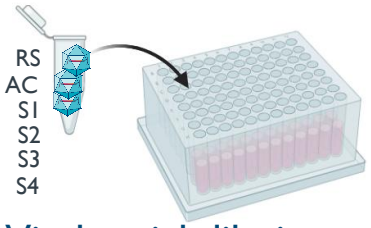
1. **$TCID_{50}$ titer (Vg/mL)** by Spearman-Karber method
2. **Vg: IU ratio** (lower IU, lower infectivity, higher Vg: IU ratio)

Credit: Qian Zhang

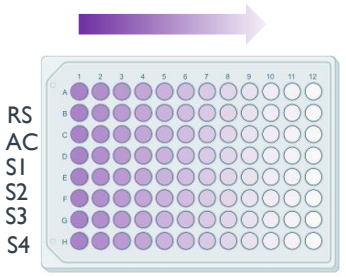
Relative expression and relative activity assays for Fabry program



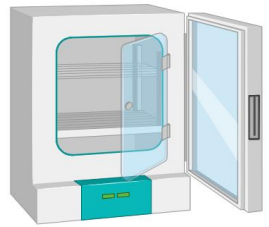
Cells plating



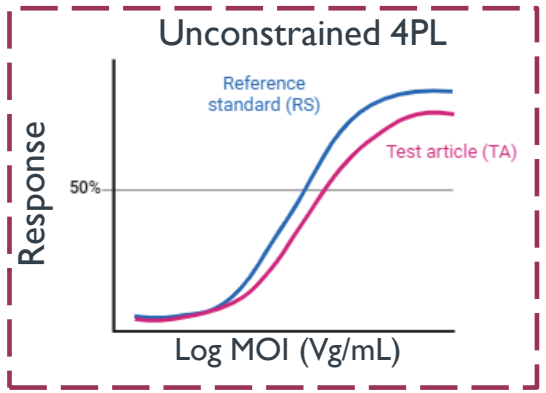
Viral serial dilution

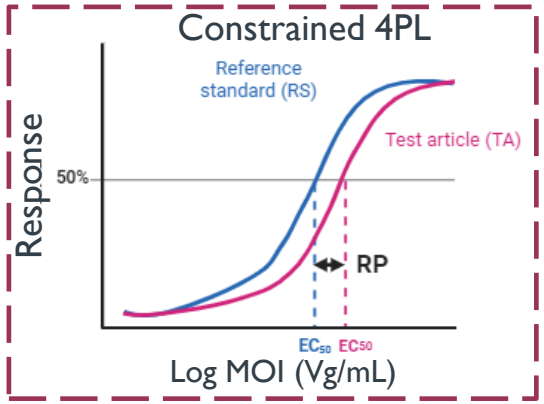


Infection

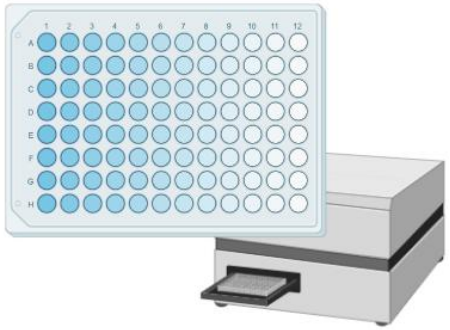


Sample collection





Data analysis



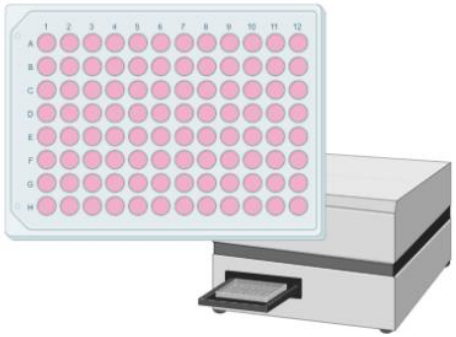
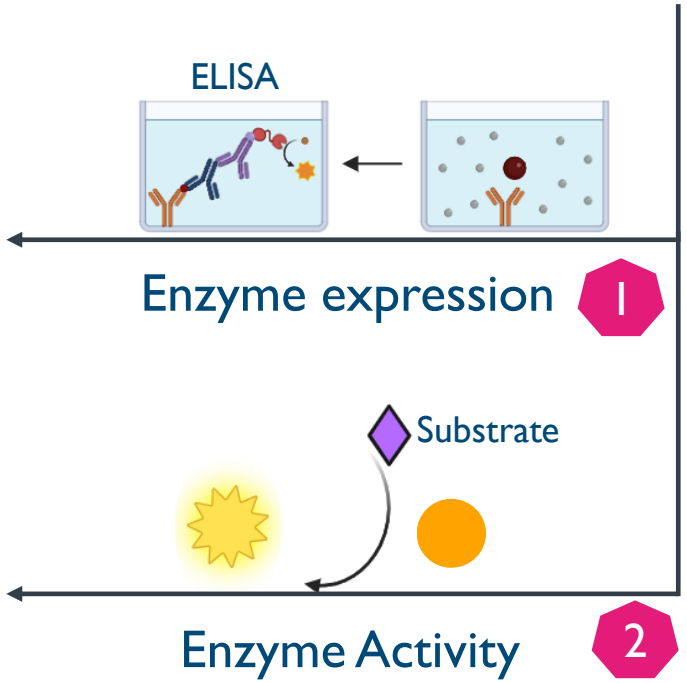
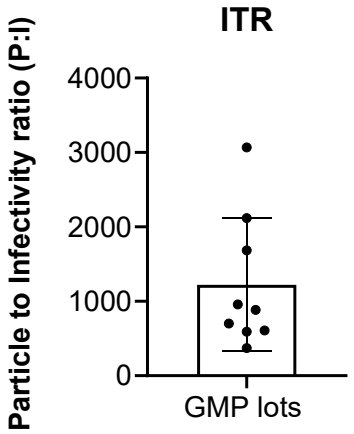


Plate reading



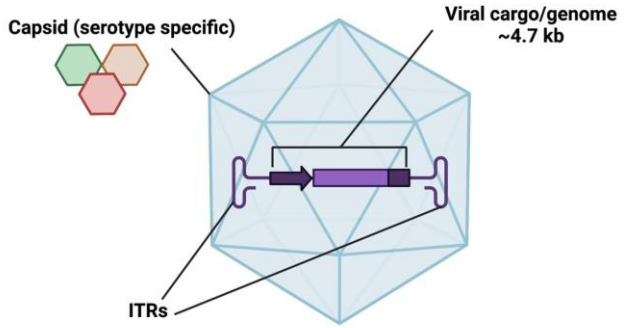
Phase-appropriate bioassays

Infectivity of the vector TCID50 assay



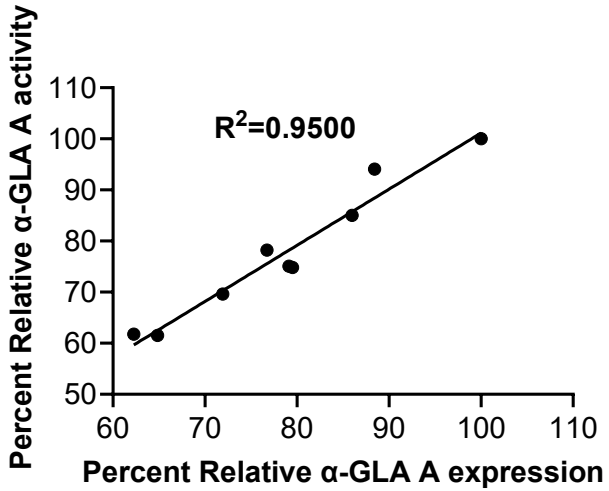
1

Structure of AAV genome

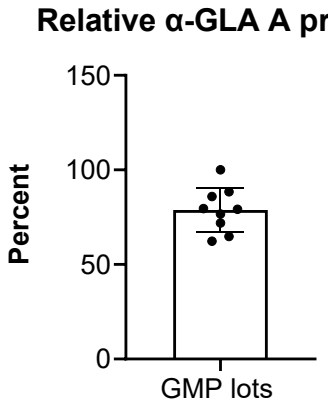


https://blog.addgene.org/an-introduction-to-aav

Correlation of GLA protein to GLA activity assay

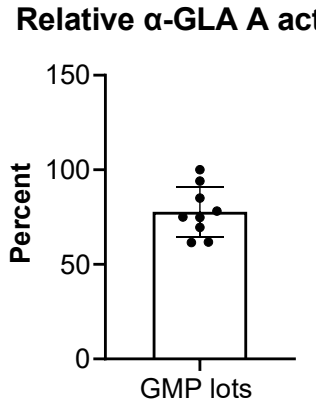


GLA protein expression assay



2

GLA activity assay

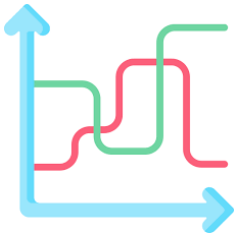


3

Sangamo THERAPEUTICS

Summary of current challenges in potency assay method development in gene therapy

Complex mechanism of action (MOA)



High variability

Lack of standardization

Emerging technologies

Evolving regulatory expectations

Thank you all !!!

Special thanks to

Alicia Villegas, Sr. Res. Assoc: Analyst on Nav 1.7 potency program

Keith Cheung PhD, Scientist III: Lead on Nav 1.7 potency program

Hyosuk Cho PhD, Scientist II: Lead on cell line development

Phillip Ramsey, CTO, SVP: Head of Technical Operations

Analytical Development Team

Bioinformatics & Research Team

Sequencing and Molecular Biology Team

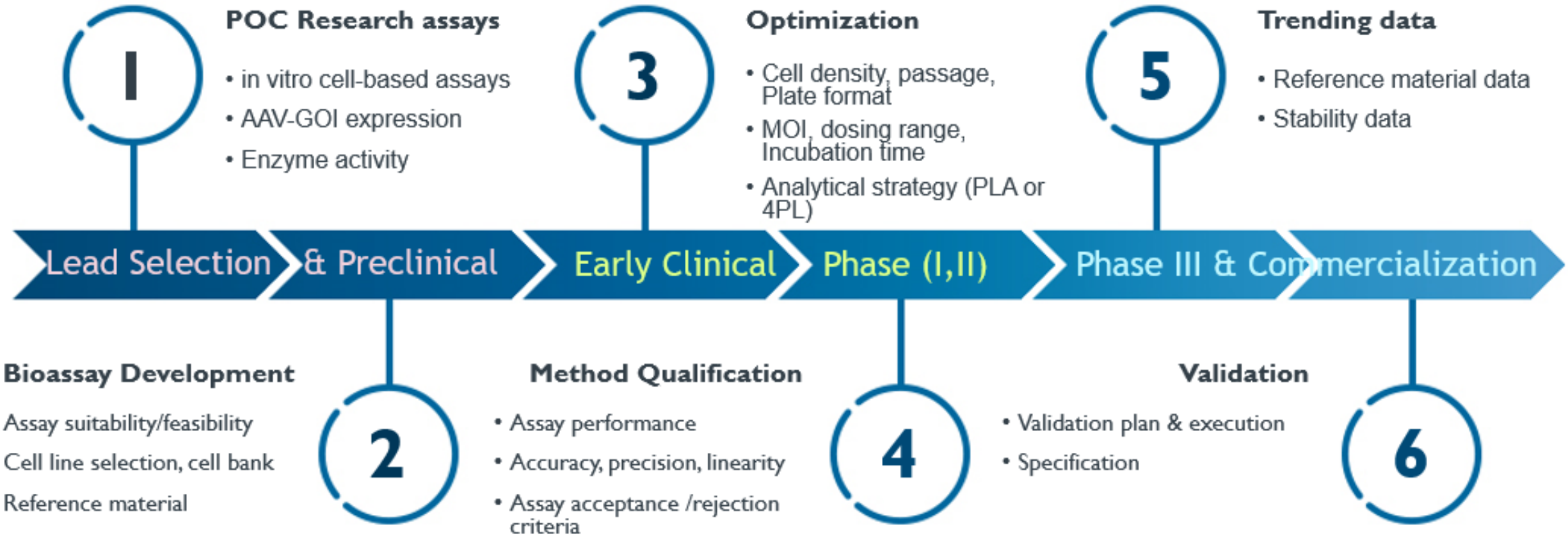
Technical Operations

Sangamo Scientific Review and Legal Team

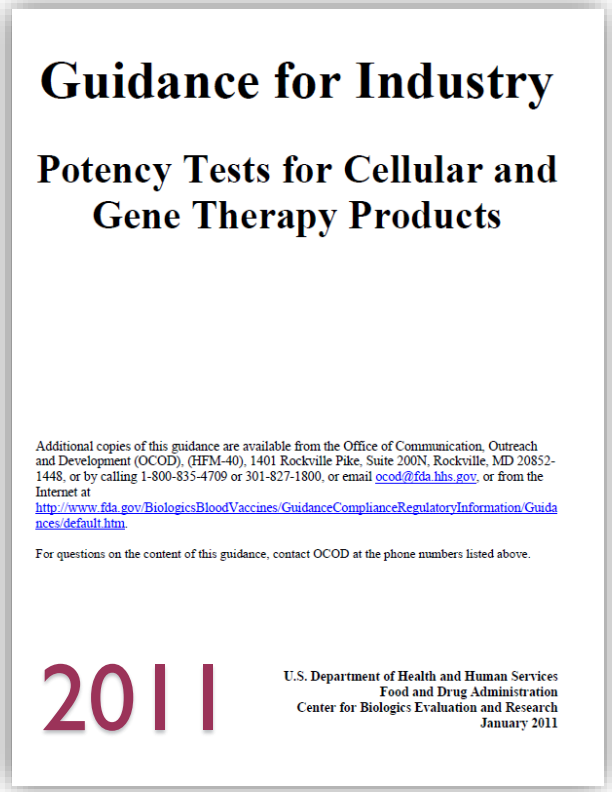
Sangamo THERAPEUTICS

Questions?

Potency Assay Development Life Cycle



Regulatory guidance documents to measure potency of cell and gene therapy products



Guidance for Industry

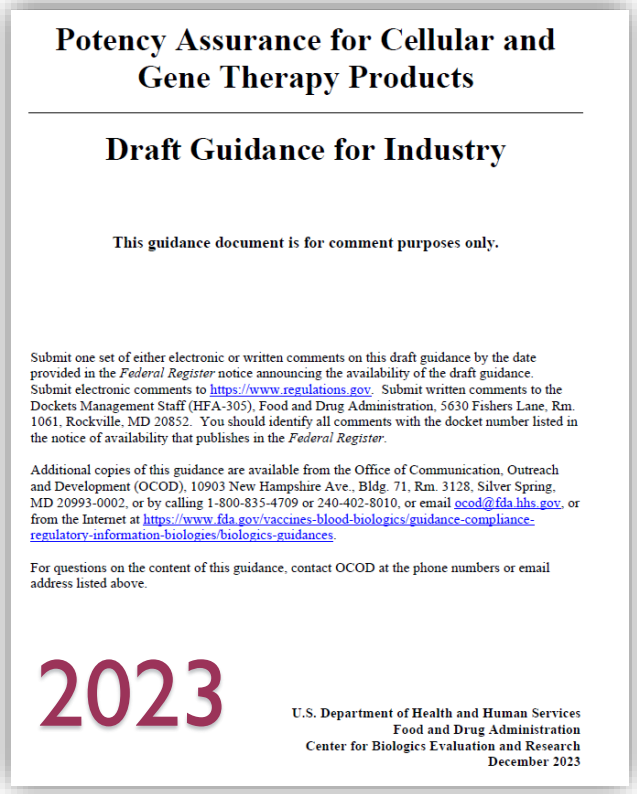
Potency Tests for Cellular and Gene Therapy Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

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

2011

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
January 2011



Potency Assurance for Cellular and Gene Therapy Products

Draft Guidance for Industry

This guidance document is for comment purposes only.

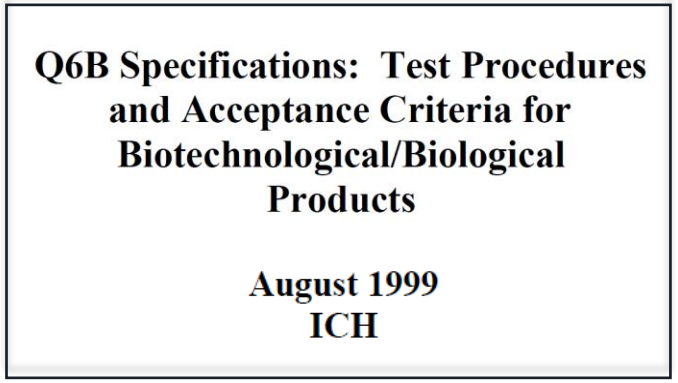
Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

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-biologies/biologics-guidances.

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

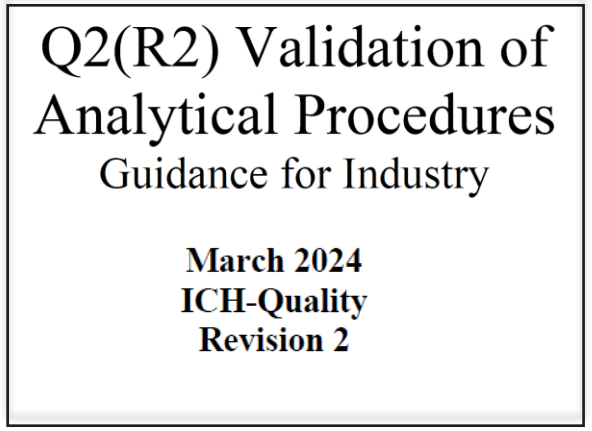
2023

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
December 2023



Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

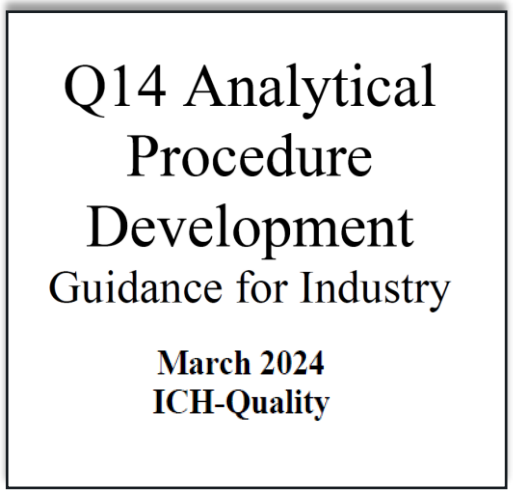
August 1999
ICH



Q2(R2) Validation of Analytical Procedures

Guidance for Industry

March 2024
ICH-Quality
Revision 2



Q14 Analytical Procedure Development

Guidance for Industry

March 2024
ICH-Quality

CMC Bioassay or Potency Assay

Guidance for Industry

Potency Tests for Cellular and Gene Therapy Products

U.S. Department of Health and Human Services

Food and Drug Administration
Center for Biologics Evaluation and Research

January 2011

What Is a Potency assay?

- 21 CFR 600.3(s):"**The specific ability or capacity of the product**, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, **to effect a given result**."

Why is Potency assay necessary?

- Potency measurements are crucial in clinical trials to ensure product identity, purity, strength (potency), and stability. They provide consistent manufacturing in all clinical study phases, aiding in product characterization, comparability studies, and stability assessment for reliable trial outcomes.

What are the expectations from a Potency assay?

- ✓The *in vitro* bioassay should be quantitative and be able to mimic the product's mechanism of action.
- ✓The potency (biological activity/activities) tested should be specific to the product.
- ✓Meet pre-defined specifications or acceptance criteria (Conformance testing) during all stages of clinical investigation and following market approval.
- ✓Include appropriate reference materials, standards, and/or controls.
- ✓Demonstrate stability indicating properties.
- ✓Establish and document assay development, qualification and validation.

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2023 FDA guidance for CGT based on Quality risk management framework

A potency assurance strategy is a comprehensive approach to ensure that every lot of a product has the potency necessary to achieve the intended therapeutic effect

QTPP: Quality target product profile (QTPP) should be developed based on your understanding of the product's mechanism of action (MOA), the intended clinical indication, and the route of administration

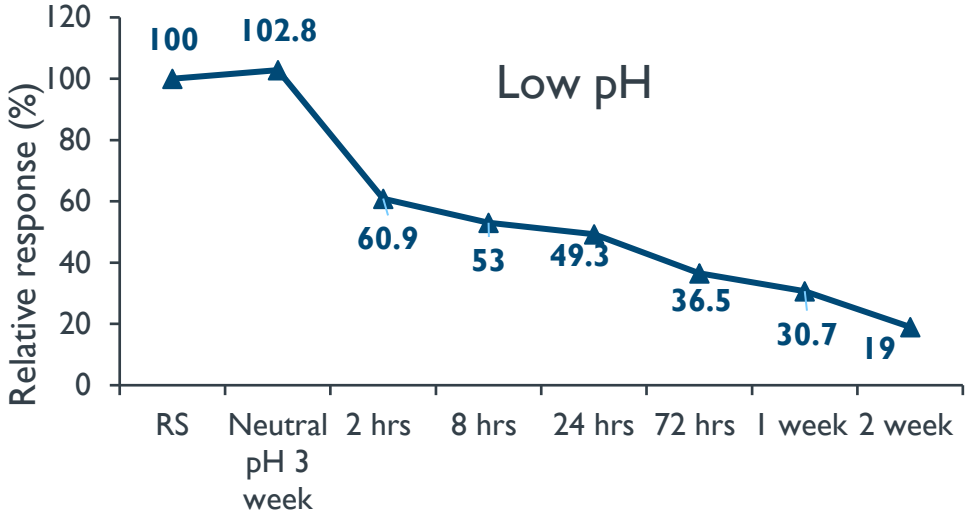
Control strategy: Monitor manufacturing process parameters, in-process testing, material testing or examination, lot release tests, and associated acceptance criteria.

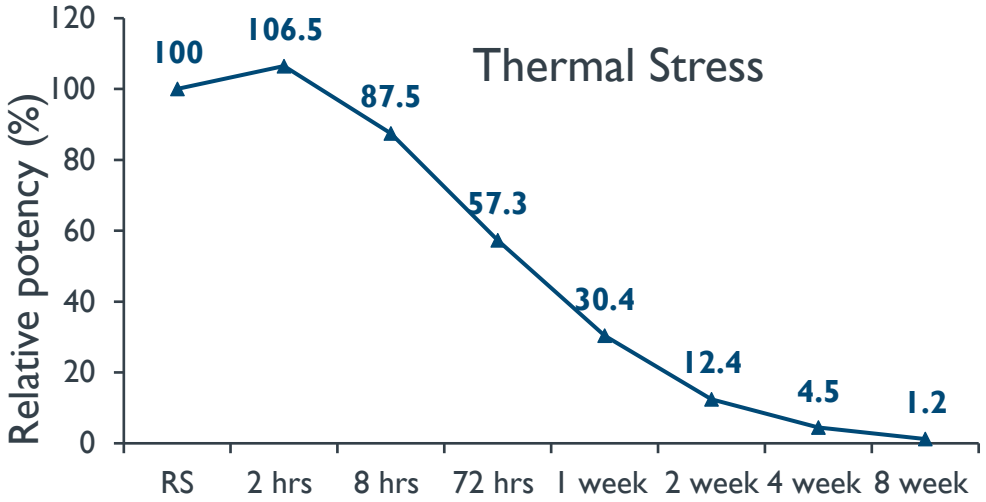
Critical quality attribute (CQA): Ensure Potency-related attributes that are vital for achieving the intended therapeutic effect meet appropriate acceptance criteria for lot release testing.

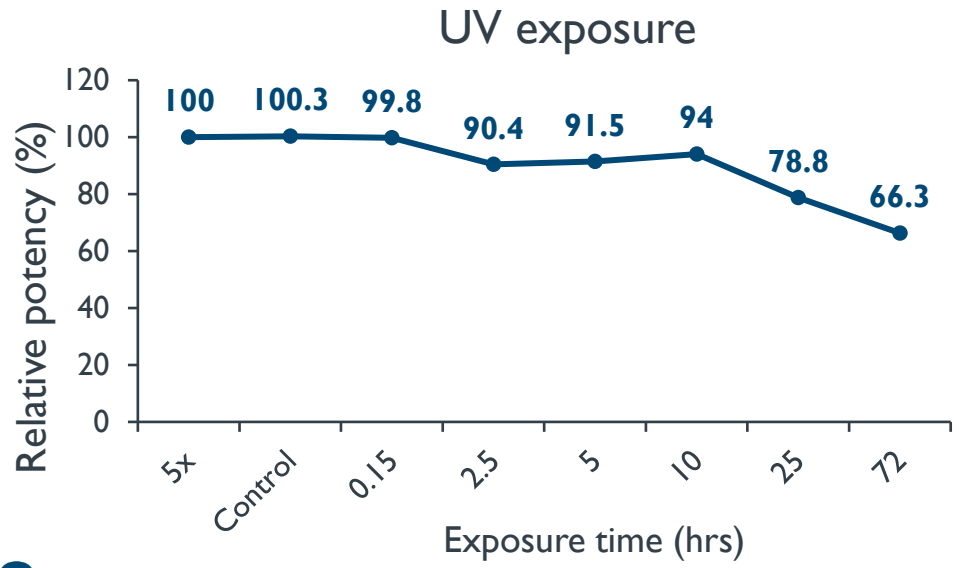
Critical process parameter (CPP): Monitor CPPs that influence potency-related CQAs within appropriate pre-determined limits.

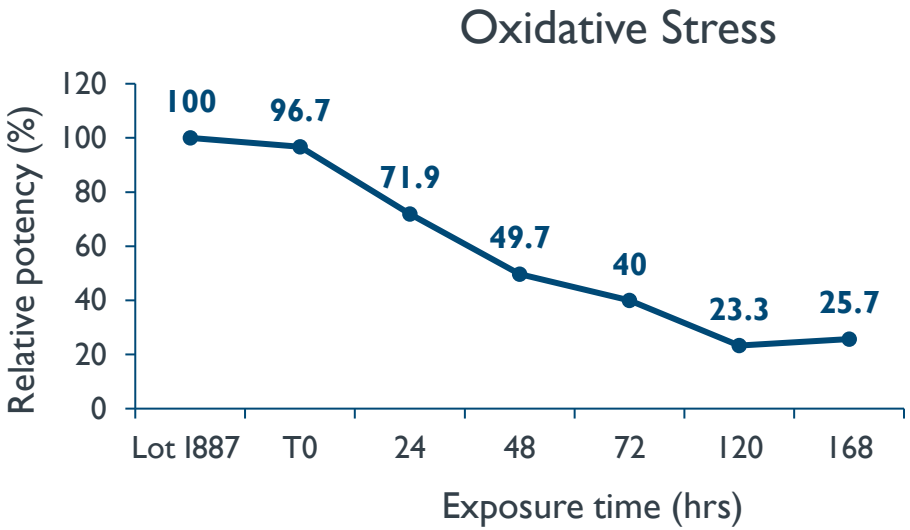
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/potency-assurance-cellular-and-gene-therapy-products

Stability indicating potency assay









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