Critical Attributes Analysis & Method Lifecycle Management for Biologics & ATMPs

Selection of Assay Platforms & Data Processing Methods for Potency Assurance

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BioAgilytix

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Learning Objectives

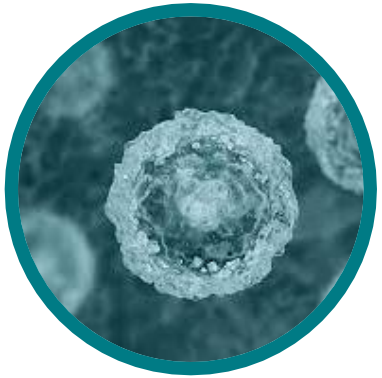
SUMMARY

- Understand the analytical control strategy for various ATMPs and key attributes (CQAs)
- Techniques applied to the control of ATMPs and how they are utilized; flow cytometry, ddPCR, qPCR, and immunoassays (including ELISA and MSD); HRMS, HPLC, CE and other
- Phase appropriate considerations for method utilization and qualification
- Understand the significance of potency assays in potency assurance, control strategies and the regulatory expectations for gene therapies
- Learning objectives will be supported by case studies to demonstrate the analytical technologies and approaches applied to potency assay development for gene therapies

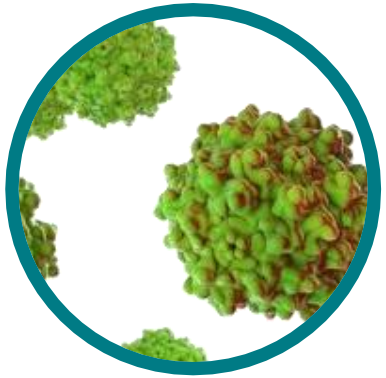
Control Strategies of ATMPs and the Attributes to be Controlled

Advanced Therapy Medicinal Products (ATMPs)

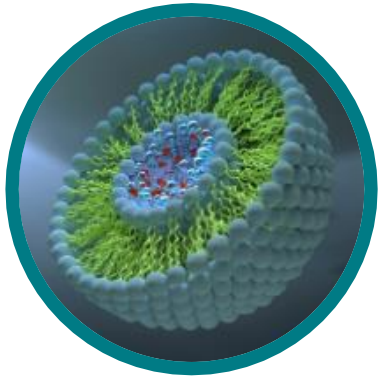
Gene and Cell Therapies (GCT)



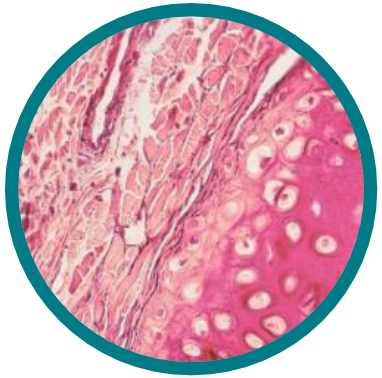
Stem Cells



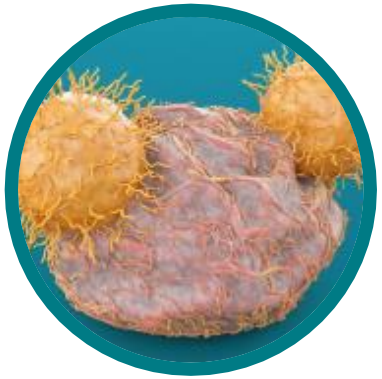
AAV



Lipid Nanoparticles



Tissue Products



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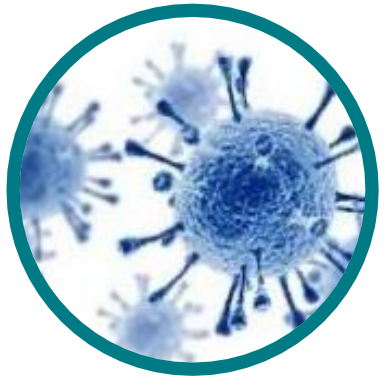
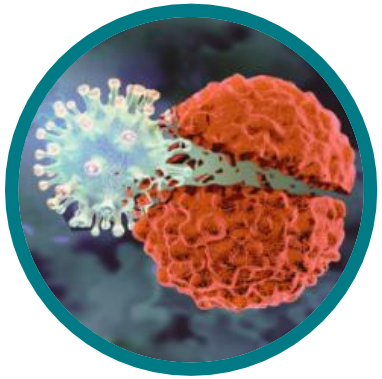


Lentivirus

Naked or Paired Nucleic Acid



Engineered Oncolytic Virus

What is a CMC Analytical Control Strategy?

CMC – Chemistry, Manufacturing and Controls

- Section of regulatory filing: analytical testing is the controls aspects
- Characterization, tests and specifications designed to assure SISPQ (Safety, Identity, Strength, Purity and Quality) for a product or substance
- Addresses and monitors CQAs (critical quality attributes)
- Contains “control strategy” which is typically 10’s of tests driven by: required safety, manufacturing process, formulation and matrix, drug properties, and development data
  - A combination of GMP and characterization assays, combination of PAT and release tests

What Drives a Control Strategy?

Development Phase, CQAs, and SISPQ

Clinical Phase of Development

The phase of development will affect the rigor and demands of the assays and the dimensions of the control strategy as the processes, methods, and product evolve

SISPQ: Safety, Identity, Strength, Purity and Quality

The guiding principles and foundation of the cGMP control and monitoring of a drug substance/API or drug product

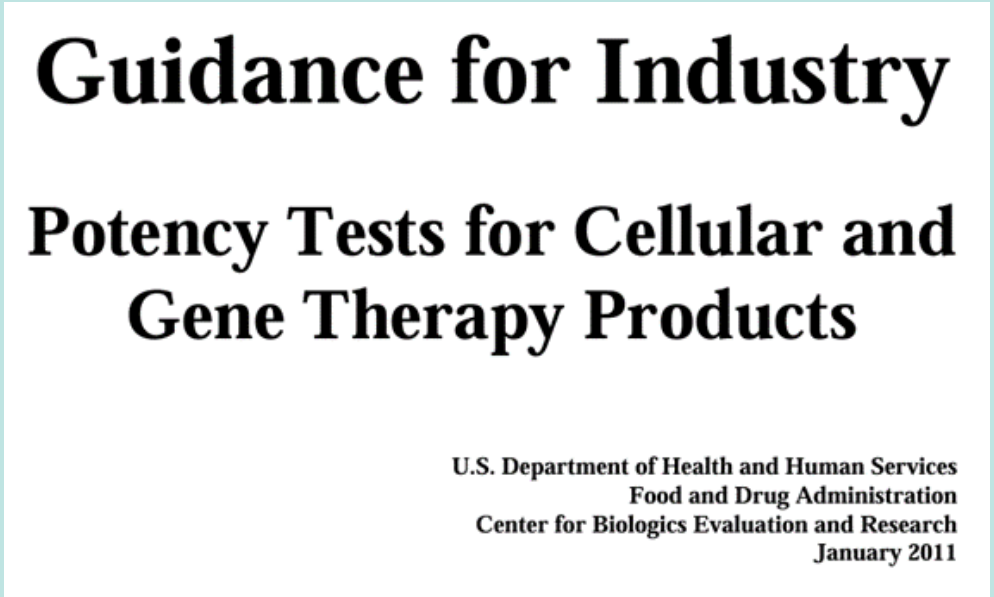
CQA: Critical Quality Attribute

"Physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality."

- ICH Guideline Pharmaceutical Development Q8(R2)

Regulatory Guidance

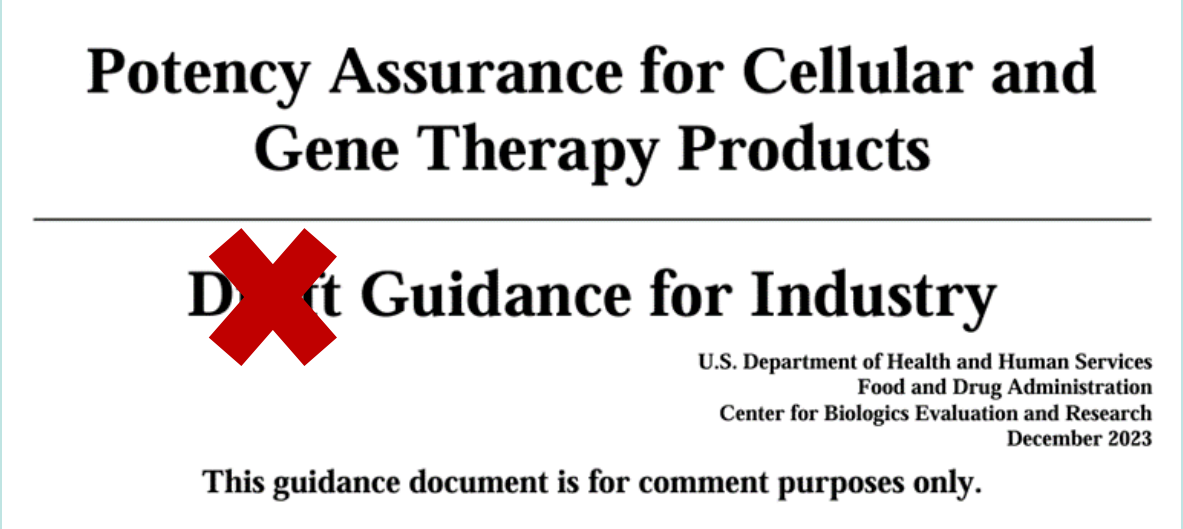
What has changed? What is different between these two Guidance Documents?



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



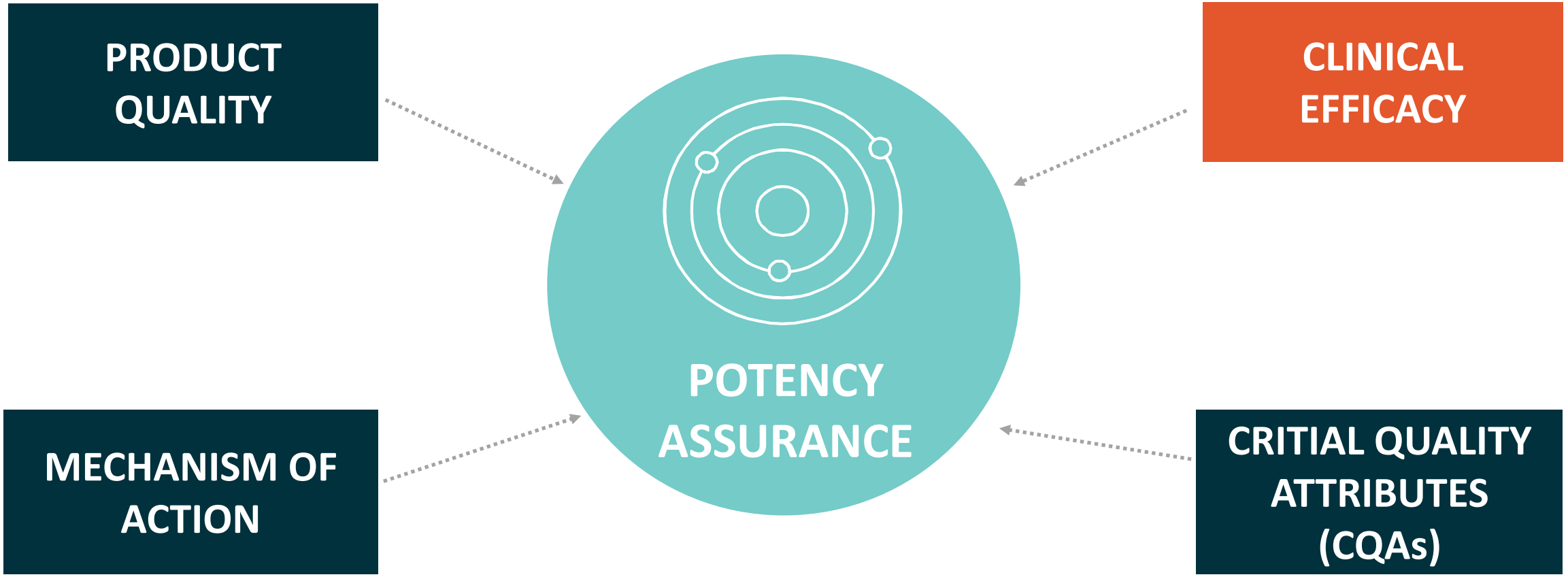
Potency Assurance for Cellular and Gene Therapy Products

Draft Guidance for Industry

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

This guidance document is for comment purposes only.

Potency Assurance Strategy

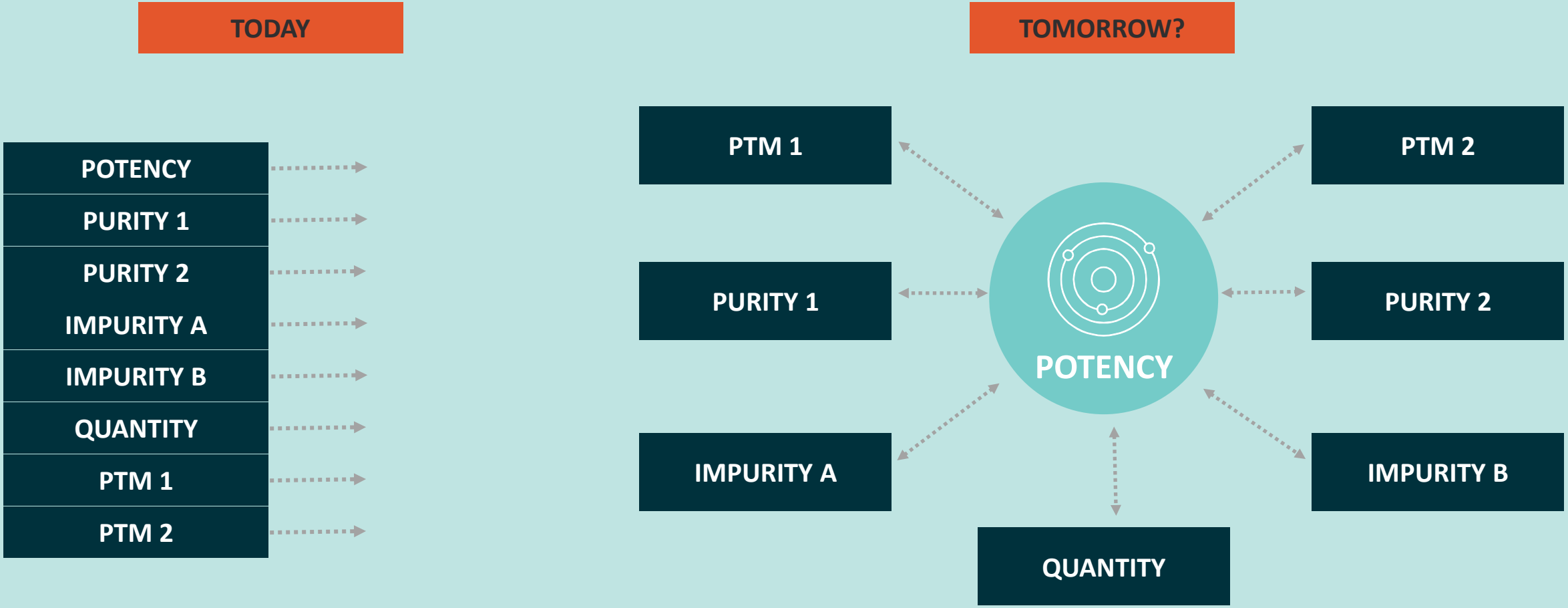


How will the “Potency Assurance” impact future potency assay development?

- Potency will continue to be assessed in a progressive manner assays will evolve with the product
- Matrix approaches will apply to complex MOAs BUT will be supported by biophysical methods that measure CQAs related to potency
- Complex matrices may be simplified if the “story” supports they are redundant (perhaps reduce from 4 to 2 biological assays)
- Invest early in your potency assay and assurance strategy
- Tell a story with data and science

Historical and Future Considerations

Potency and Control Strategies



Challenges to CMC from ATMPs

High degree of variability between batches –

- starting materials and their control process changes and scale changes to the product itself (plasmid, vector, promoter, etc)

Identifying and controlling CQAs (Critical Quality Attributes) - complexity of the therapeutics

New CQAs with implications on potency and therapeutic efficacy – risk mitigation and management

CQAs are often less discrete than with more traditional biologics.. Matrix Approach

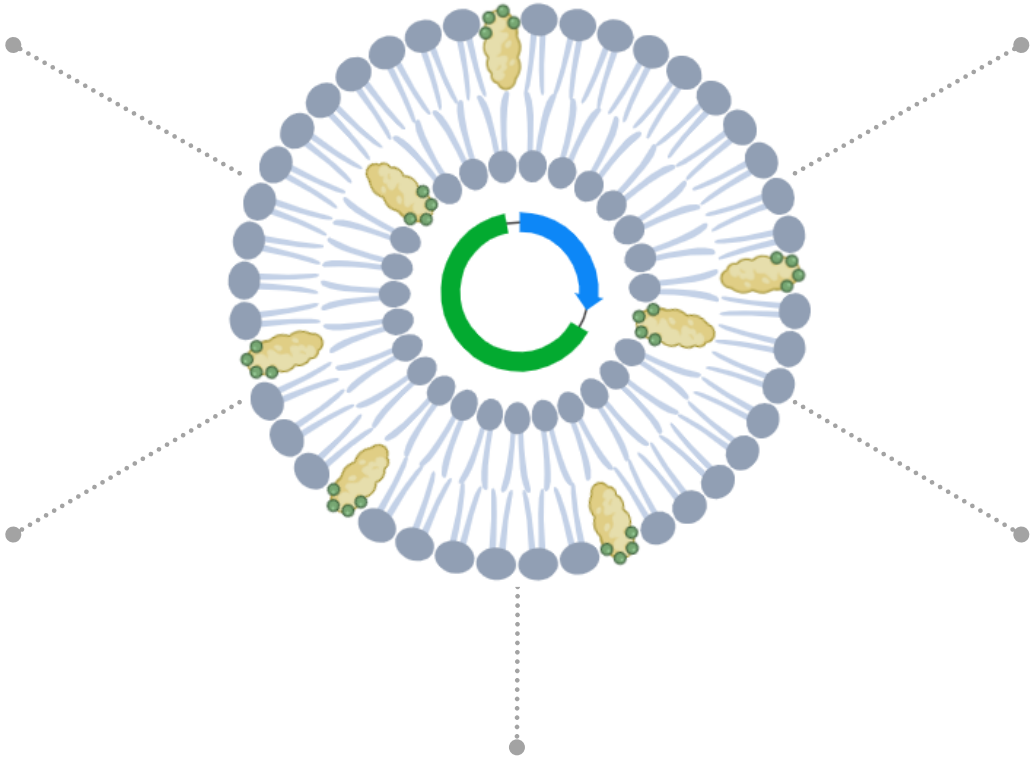
Learning by the Agencies

New analytical methodologies are being employed

Control Strategy for a pDNA or mRNA (LNP) Product

PhysicoChemical
- Physical properties
- Quantity/titer
- Compendial tests

Biological Activity
- Infectivity
- Target gene expression
- MOA relative potency assays



Characterization/ID
- DNA/plasmid ID
- Stability

Safety
- Sterility
- Compendial tests

Purity/Impurity
- Plasmid purity
- HCPs
- HC DNA
- Related impurities and residuals

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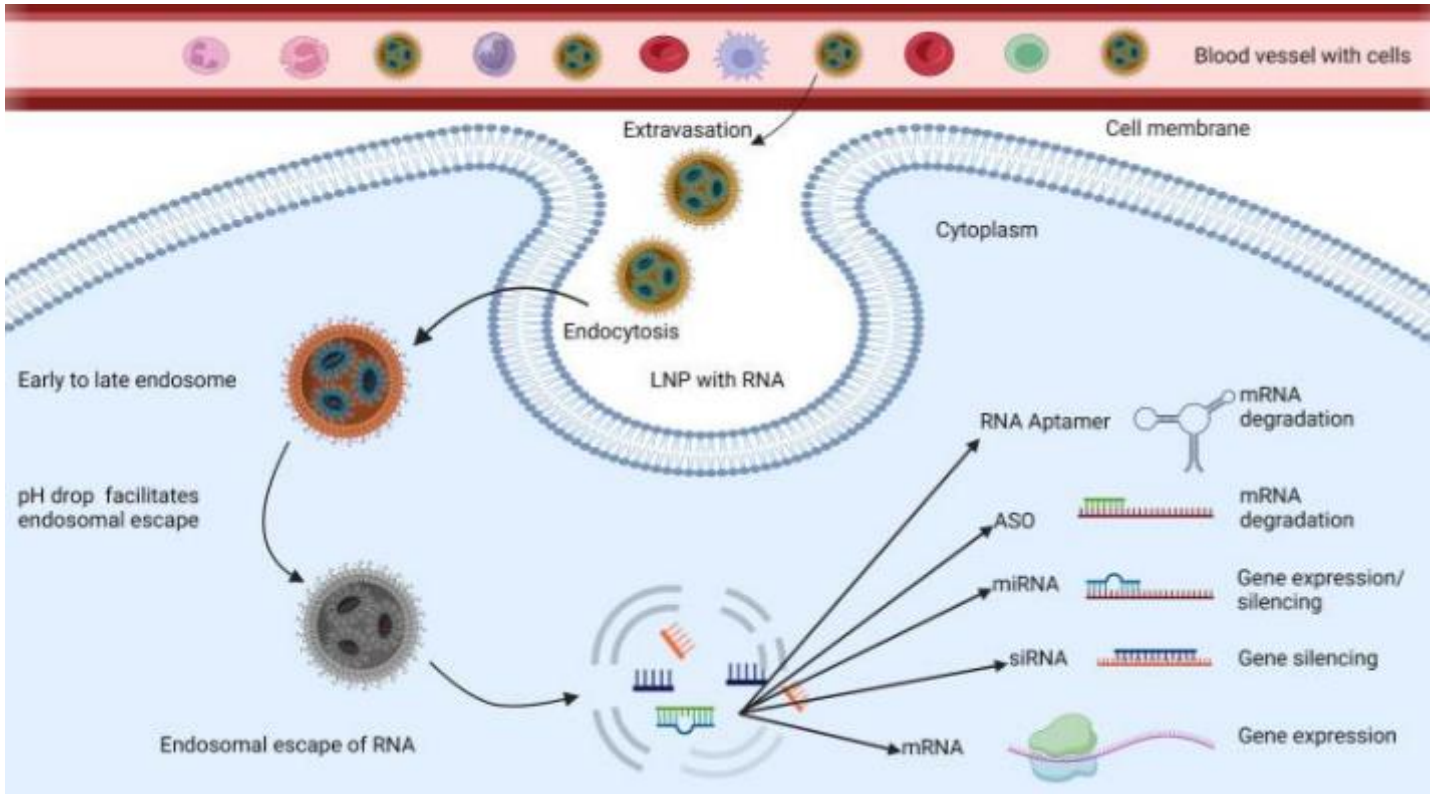
General Mechanism of Action of Lipid Nanoparticle

- This captures the general operating mechanism of any LNP.
- After endocytosis the payload, often mRNA, ASO, RNA aptamer or siRNA, is released and engages with the cellular machinery.
- The nature of the payload will impact the control strategy and methods used.
- The action of the payload will determine the path for a potency and will also impact the general control strategy.
- Engagement with the membrane and endocytosis is highly affected by the lipids in the LNP and any expenditure on them.



Md. Anamul Hauqe, et al, Int J Pharmacuetics: X (2024), 8, 100283.,

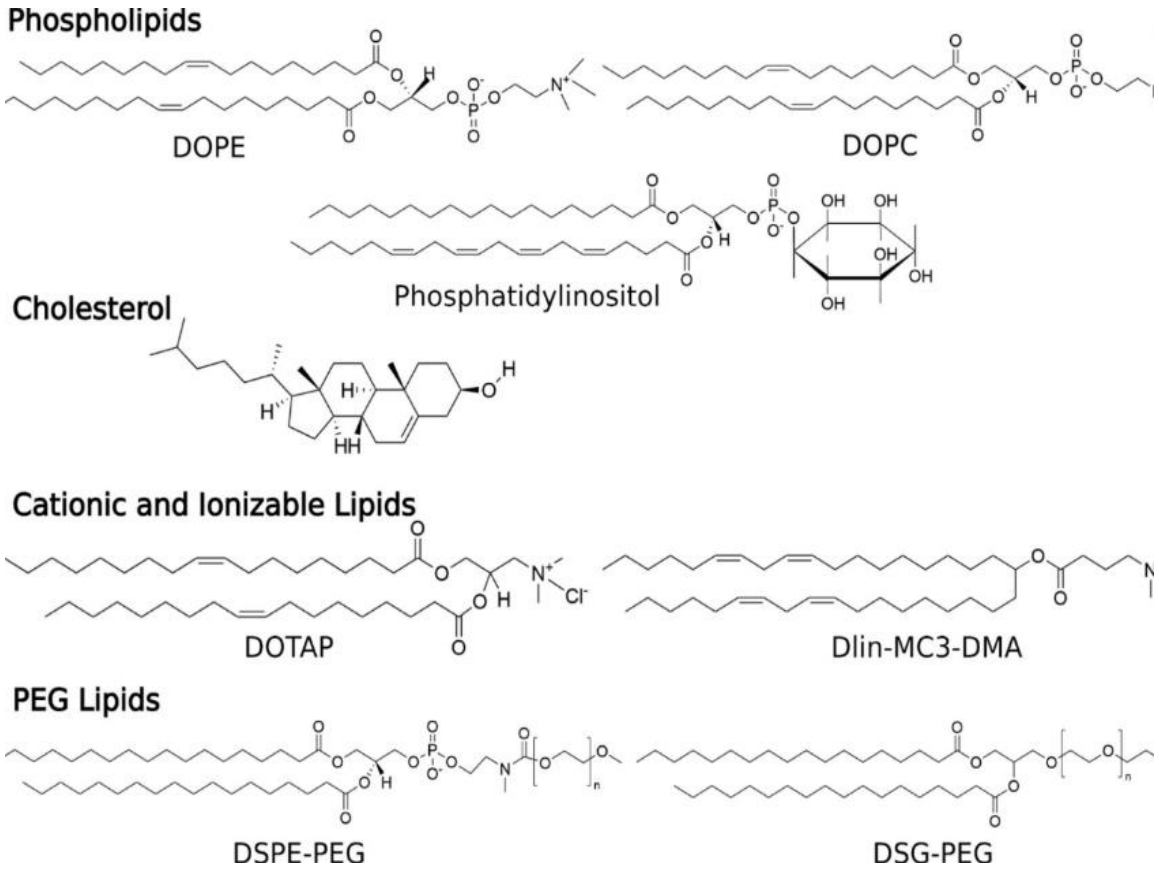
Lipids Content and Stability

Complex mixture of polar lipids; some include "directing molecules" intended to enhance the specificity of uptake in the body

- Representative mixture of lipids to the right
- The relative proportions of the lipids and the process of formation defines the LNP physical characteristics

Lipid content and stability (physical and chemical) can be critical to potency and efficacy

- Protection of the payload (*i.e.,* mRNA) and transfection effectiveness
- Methods exist to monitor this effectively including UPLC/HPLC (often with CAD detection), LC-HRMS and others
- Inset to the right shows a typical data set for the lipids

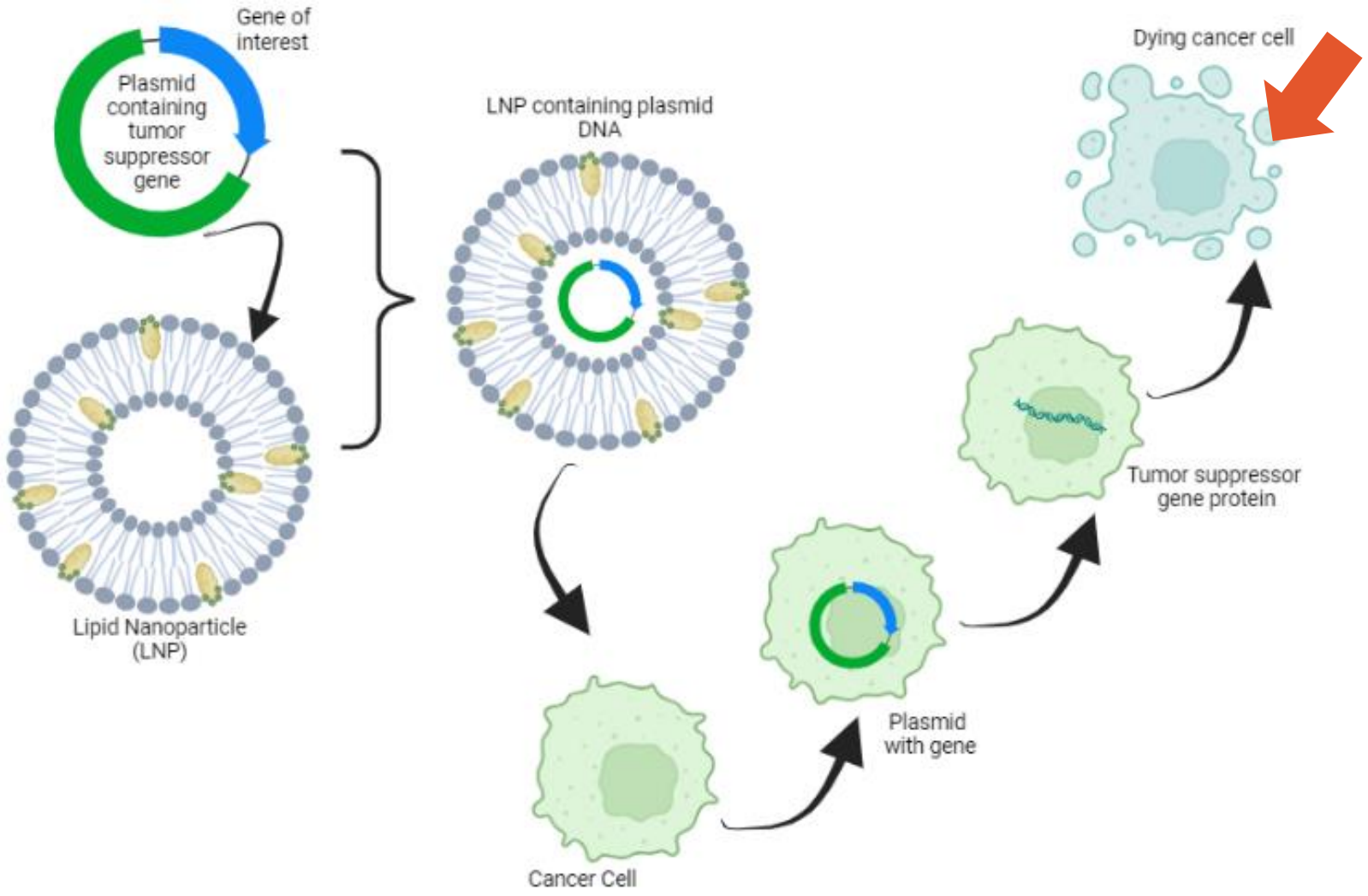


Chemical structures of various components used in LNP formulations. (M. Mehta, et al, Materials Au (2023), 3, 600)

Non-Viral Delivery of Tumor Suppressor Gene

This system utilizes positively charged nanoparticles that selectively bind to negatively charged cancer cells and enter them through endocytosis

The nanoparticle is designed to deliver the functioning Gene X to cancer cells while minimizing their uptake by normal tissue and elicit apoptosis in cancer cell



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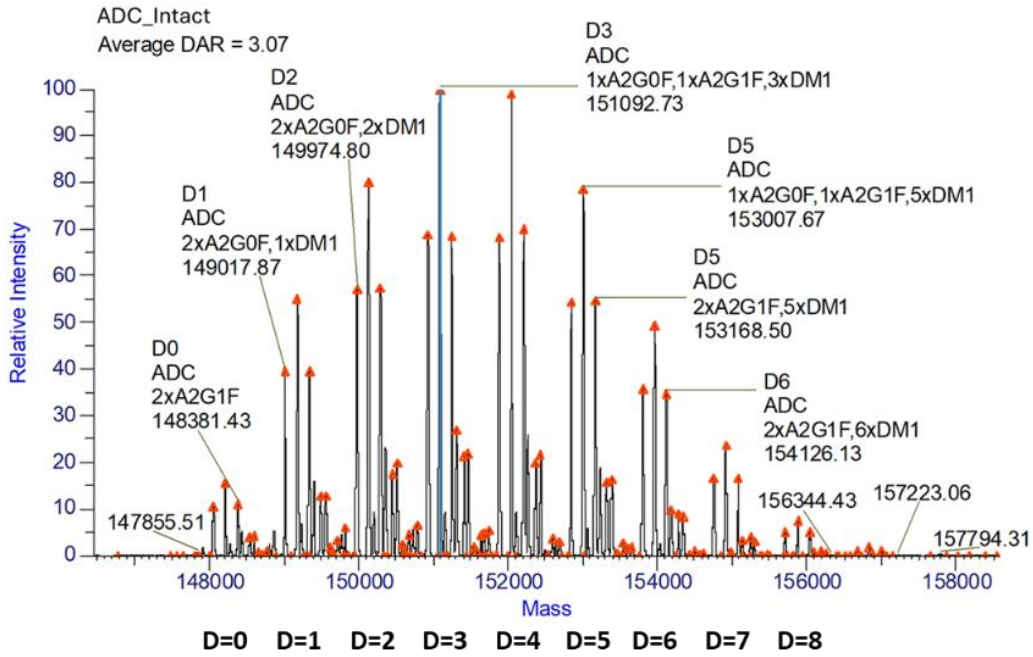
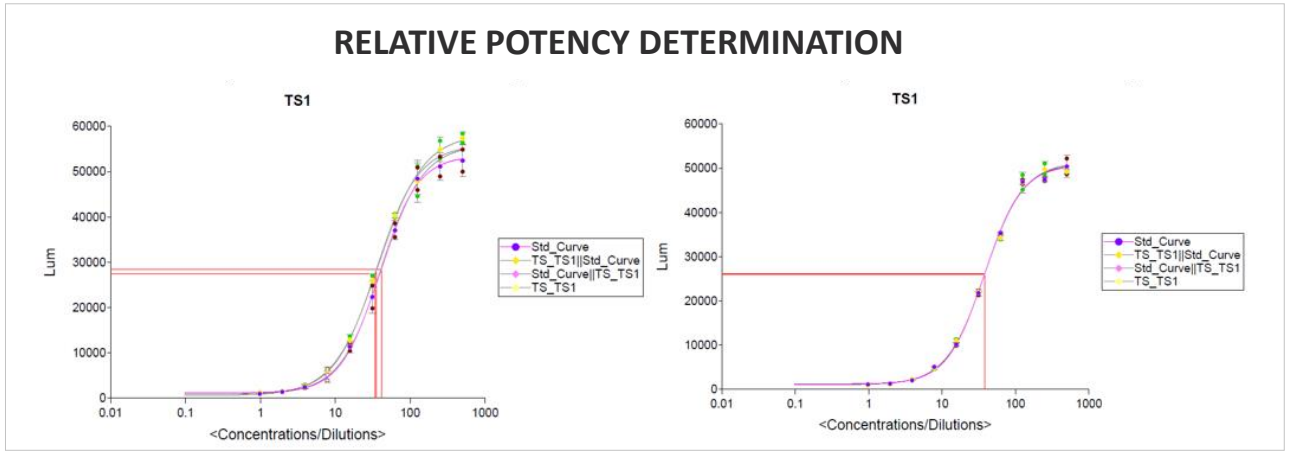
Potency Assurance and Relationship to CQAs

- Potency assurance is critical per recent FDA Guidance
- DAR is a well-established predictor of ADC potency
- Similar attributes will be established (are being established) for ATMPs
- Watch where new correlations get established



Change the DAR or the distribution in DAR and change relative potency



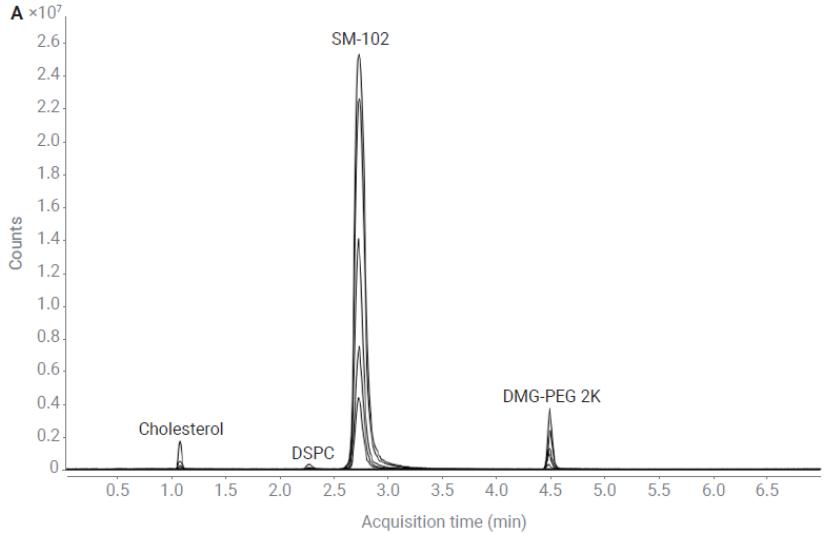
Potency Assurance and Relationship to CQAs - LNPs

Potency assurance is critical per recent FDA Guidance

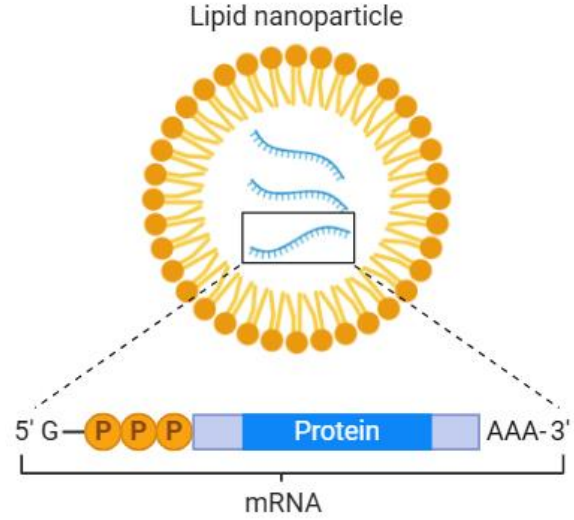
Attributes that may correlate to LNP potency and ultimately efficacy are being determined

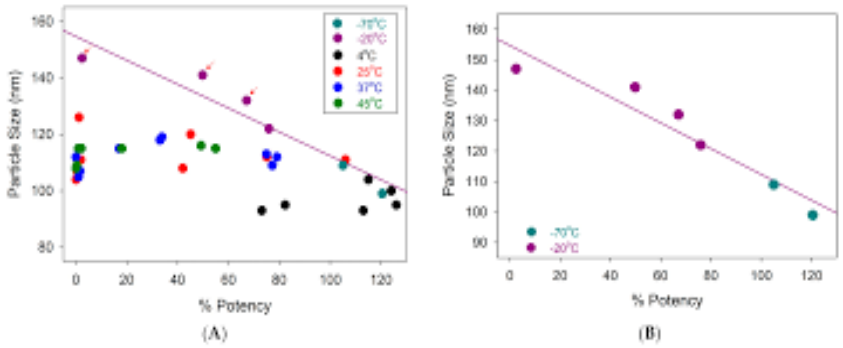
- Lipid composition
- Payload quality
- Particle size and distribution

And similar for AAVs and other



Lipid Composition





Particle Size Distribution

Payload Quality and Composition

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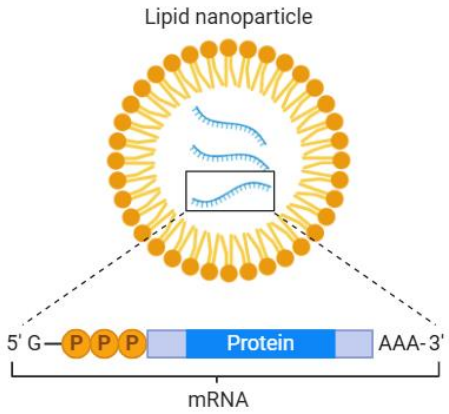
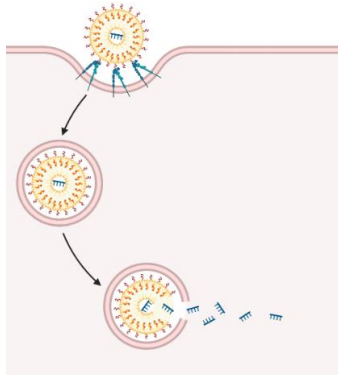
When You Don’t Have Reagents for an Expression Assay



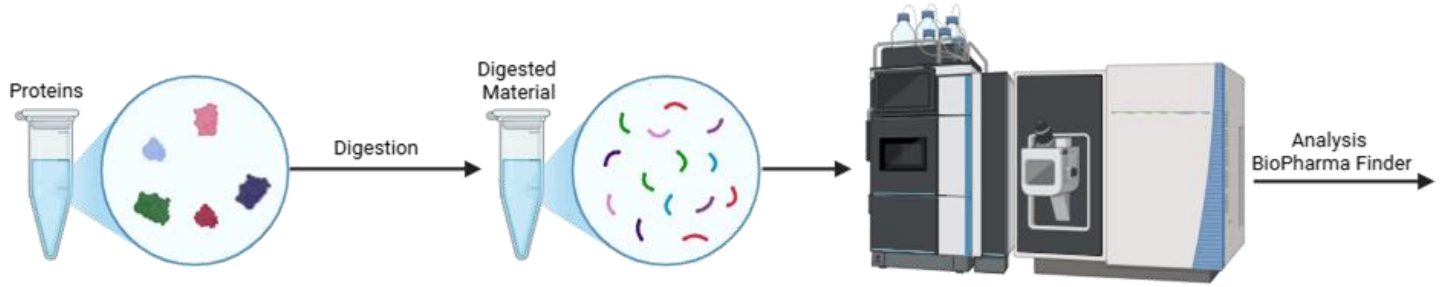
LNP containing mRNA for three antigenic proteins

EXPERIMENTAL CONDITIONS

- Mass spectrometry was performed on a Thermo Exploris Orbitrap in various modes and R=15000.
- HPLC was performed using a Thermo Vanquish Flex system with mobile phases of water, formic acid, trifluoracetic acid and acetonitrile with separation performed on a Peptide BEH C18 column (2.1x100mm) operated at 0.1 ml/min
- Protein expression occurred by transfection in HEK293T cells overnight for ca. 18 hrs.
- 14 Native peptides and 14 heavy-labelled (13C) peptides were monitored
- Extraction from cell lysates occurred using extraction in Urea, Rapigest, CHAPS, non-ionic surfactants or using Mem-PER kits
- Digestion was of 20ug of lysate protein using trypsin in 1M urea/Tris after treatment with TCEP and iodoacetamide
- Desalting was by SPE on a C18 bed



Cells are transfected with the LPNs and proteins translated



Proteins are detected and quantified in disrupted cells as their surrogate peptides using LC-HRMS/MS

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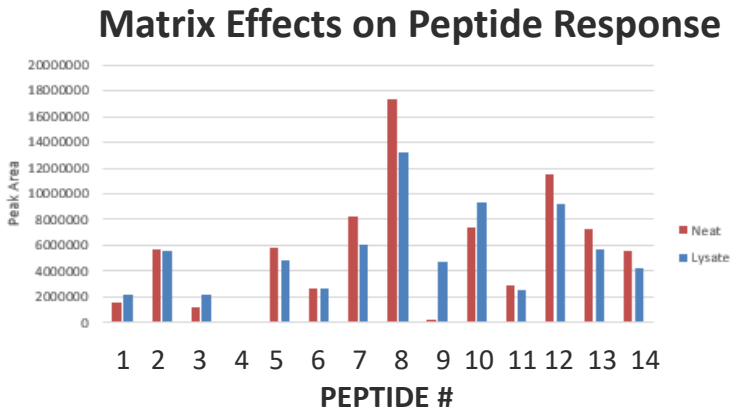
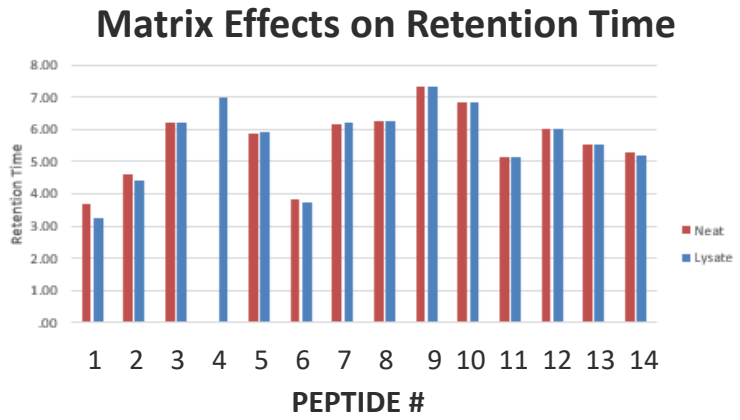
The Development Process for the Assay

Identification of peptides in target proteins in silico. Uniqueness HPLC and MS-friendliness Stability and PTM consideration.

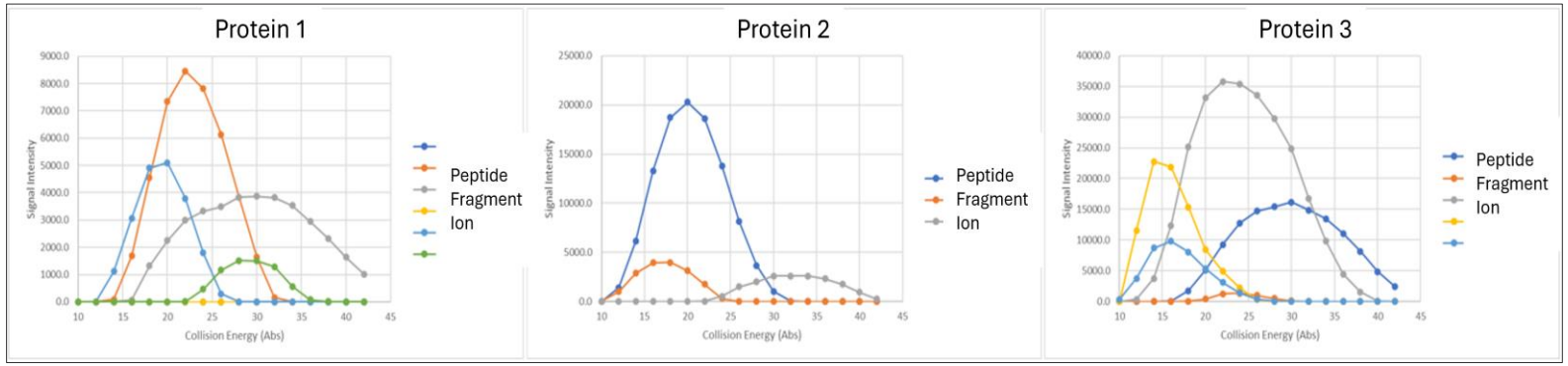
Optimize MS/MS conditions and select transitions. Optimize HRMS resolution and conditions. Map into LC-HRMS method.

Explored matrix effects Optimized LCMS conditions for time and resolution. Selected optimal transitions to maximize sensitivity





Matrix Effects: Recovery and Retention Time Stability



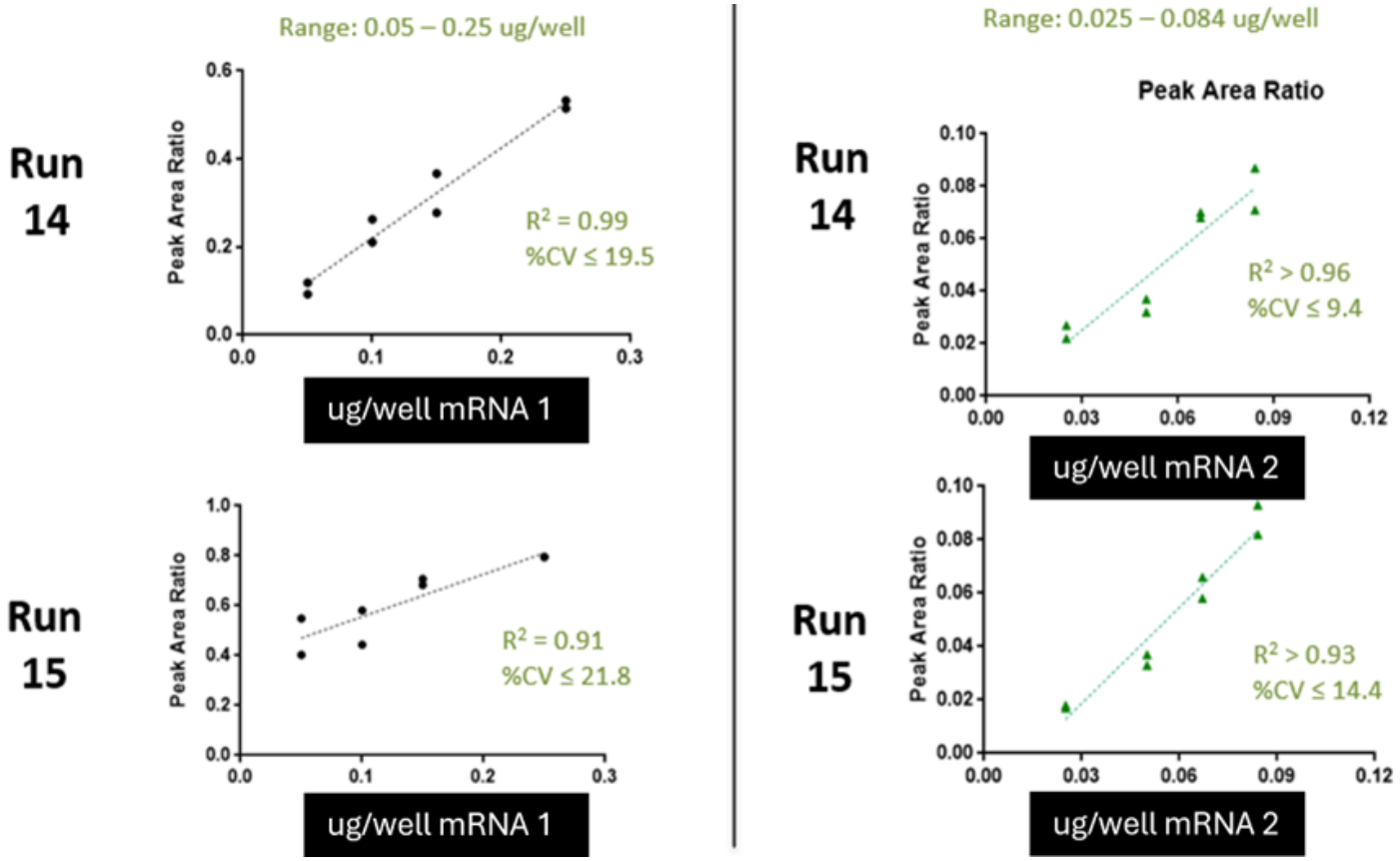
Optimization of MS/MS Conditions for Representative Peptides of All Three Target Proteins

Continued Development…

Conditions for cell culture were optimized. Dosing and timing of Lipid Nanoparticle was optimized.

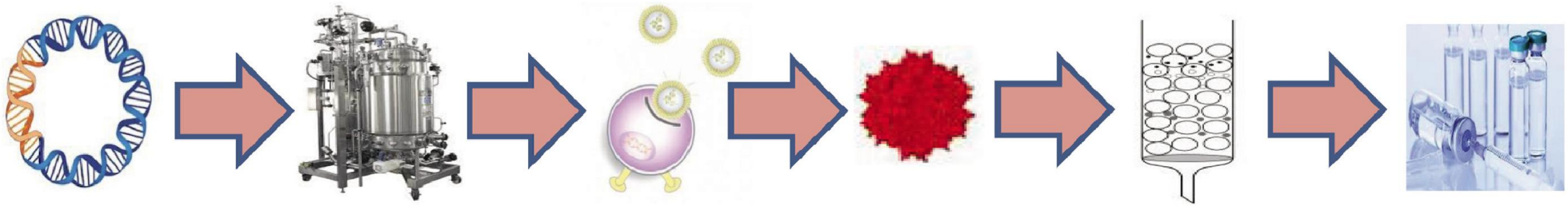
Tested treatment and cleanup methods. Obtain maximum recovery vs total protein and heavy labelled peptides

Clean up methods were compared and evaluated using urea, Triton, CHAPS, Rapigest™, and Mem-PER to achieve the highest recovery for ALL proteins and peptides.



This provides an assay with NO critical reagents and an ability to provide the attributes for an expression-based relative potency assay for three proteins delivered using an LNP

AAV viral vector manufacturing workflow



Plasmid development and production | Cell expansion | Plasmid transfection | Viral vector production | Viral vector purification | Viral vector formulation, fill/finish

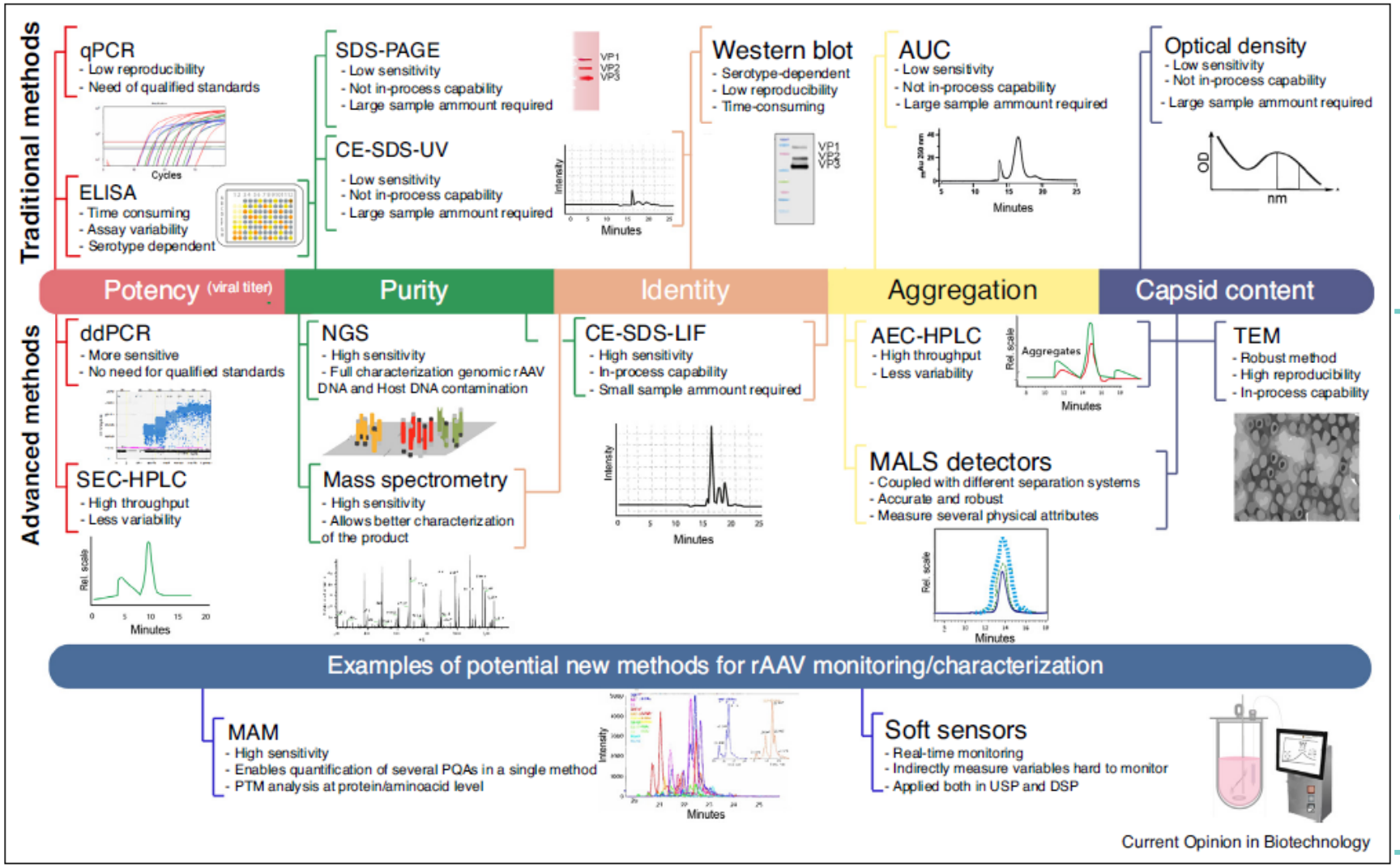
Upstream | Downstream | Formulation, fill/finish

Manufacturing Challenges and Rational Formulation Development for AAV Viral Vectors
Srivastava, Arvind et al.
Journal of Pharmaceutical Sciences, Volume 110, Issue 7, 2609 - 2624

What Might be in a Control Strategy for an AAV (“SISPQ")

SAFETY	IDENTITY	STRENGTH	PURITY	QUALITY (PHYSICAL)
Replication-Competent Virus	GOI Sequence	Vector Titer	Process-related impurities: Benzonase, Resins, etc.	Capsid Occupancy or Empty/Full Evaluation
Adventious Viruses	Pro-viral Sequencing (DNA sequencing)	Gene expression	Plasmid Purity	Osmolality
Endotoxins		Virus/Capsid titer	Expressed Purity	pH
Mycoplasma		Infectious Titer	Residual Plasmid DNA	Aggregates
Adventitious Viruses: Adeno-Associated Virus (qPCR)		Particle to infectivity ratio	Residual HC-DNA (SV40 T-Ag, E1A)	Appearance
Replication Competent Virus		Transgene Expression	Residual HCP	
Adventitious Viruses: In Vitro Adventitious Agents (cell culture)		Enzymatic Activity; Relative Potency	Other Residual Proteins/DNA	
Viral Safety Testing (Cell therapies)		Stability (long term; short term)	Residual Genomic DNA (qPCR)	
Bovine Polyoma virus		Relative Potency (cell culture/vector transduction / ELISA)	p24 Concentration (ELISA)	
Bovine viral contaminants		Relative %CAR Expression (cell culture/vector transduction/flow cytometry)	Pluronic, Tween, Triton	
		Infectious Titer (transduction/cell culture/qPCR)	Residual Benzonase	

Traditional and Emerging Analytical techniques



Mix of physical and biological methods Includes tests for residuals and additives
May also include RM tests

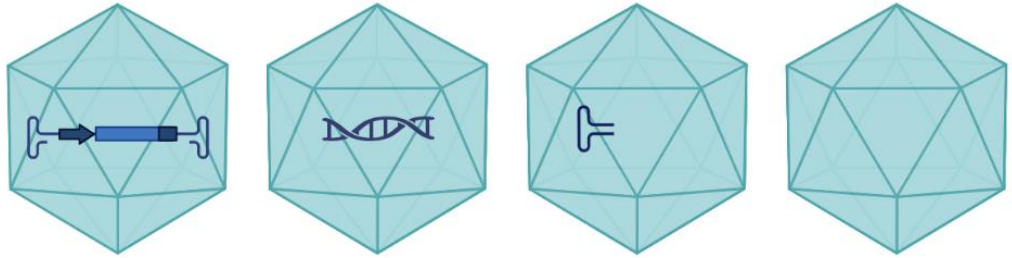
Advanced methods are often used as characterization initially; some do not become control methods

What is Significant About an AAV Viral Vector (CQAs)?

There are key attributes in AAVs that also affect their quality and potency (*i.e.,* efficacy)

THESE INCLUDE:

- **Capsid attributes including**
  - Full/Empty
  - Contaminant (Host) DNA
- **Capsid viral protein attributes**
  - Degradation
  - Impurities
  - Glycoforms (*i.e.,* sialic acid)
- **Serotype**
  - Alignment with cell line and/or tissue

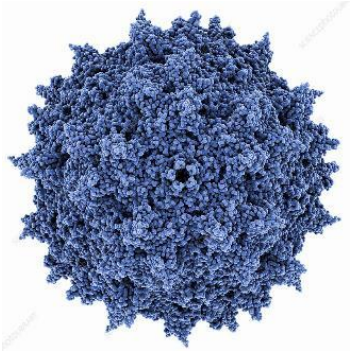


FULL GENOME | DNA OF CONTAMINANTS | PARTIAL AAV GENOME | EMPTY CAPSID

The origin of common AAV isolates, their receptors and tissue tropism

Serotype	Origin	Receptor and co-receptors[a]
AAV1	Human or NHP	N-linked sialic acid
AAV2	Human	HSPG, FGFR1, HGFR, LamR, CD9, integrin $\alpha_V\beta_5$, $\alpha_5\beta_1$
AAV3	Human	HSPG, FGFR, HGFR, LamR
AAV4	NHP	O-linked sialic acid
AAV5	Human	N-linked sialic acid, PDGFR
AAV6[c]	Human	N-linked sialic acid, EGFR
AAV7	Rhesus macaque	?
AAV8	Rhesus macaque	LamR
AAV9	Human	LamR, N-linked glycans
AAVrh10[d]	Rhesus macaque	LamR

AAV Serotypes and Receptors (L. Lisowski, et al, Curr Opin Pharmacol (2015), 24, 59



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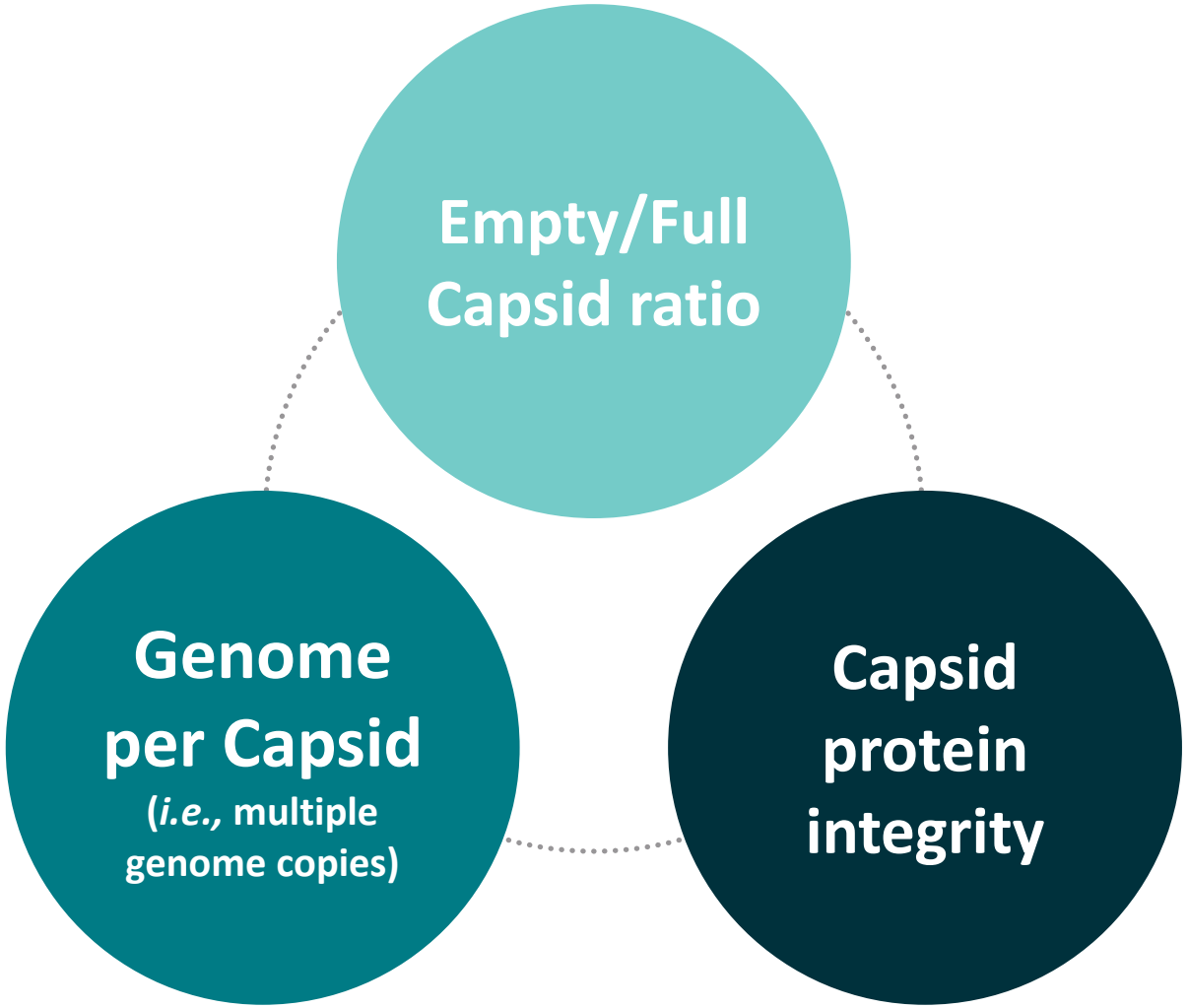
Key Considerations in Potency Assurance for AAVs



These attributes may be affected by

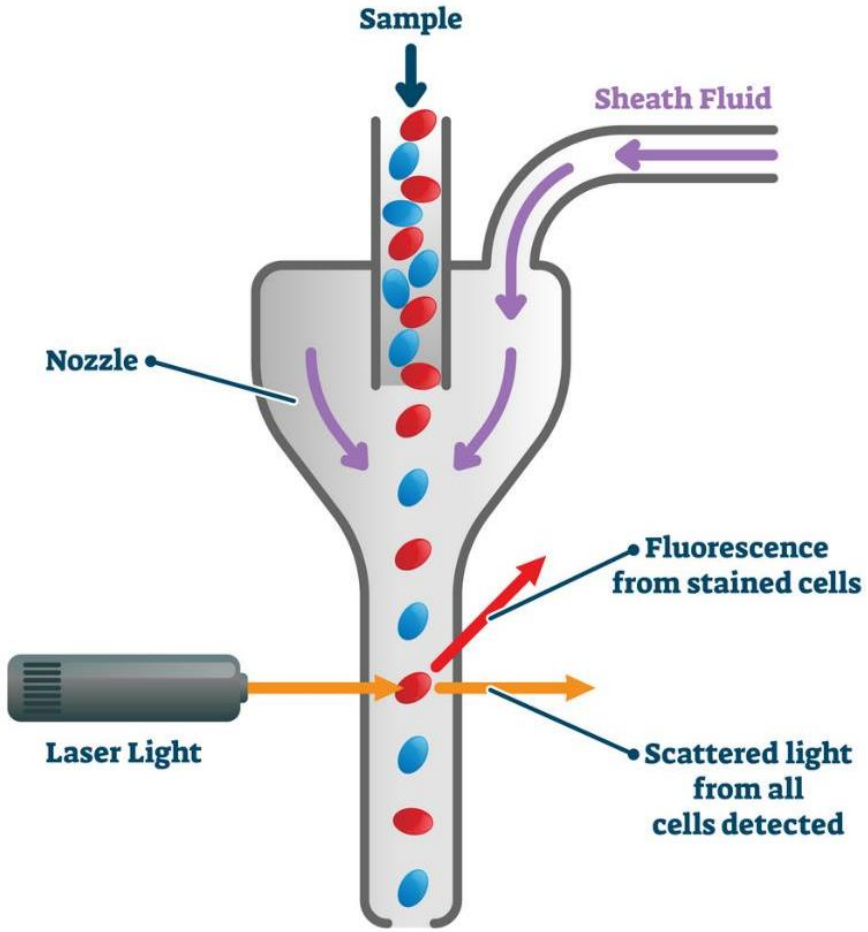
- Host Cell Line
- Cell Culture conditions
- Isolation/Purification Process

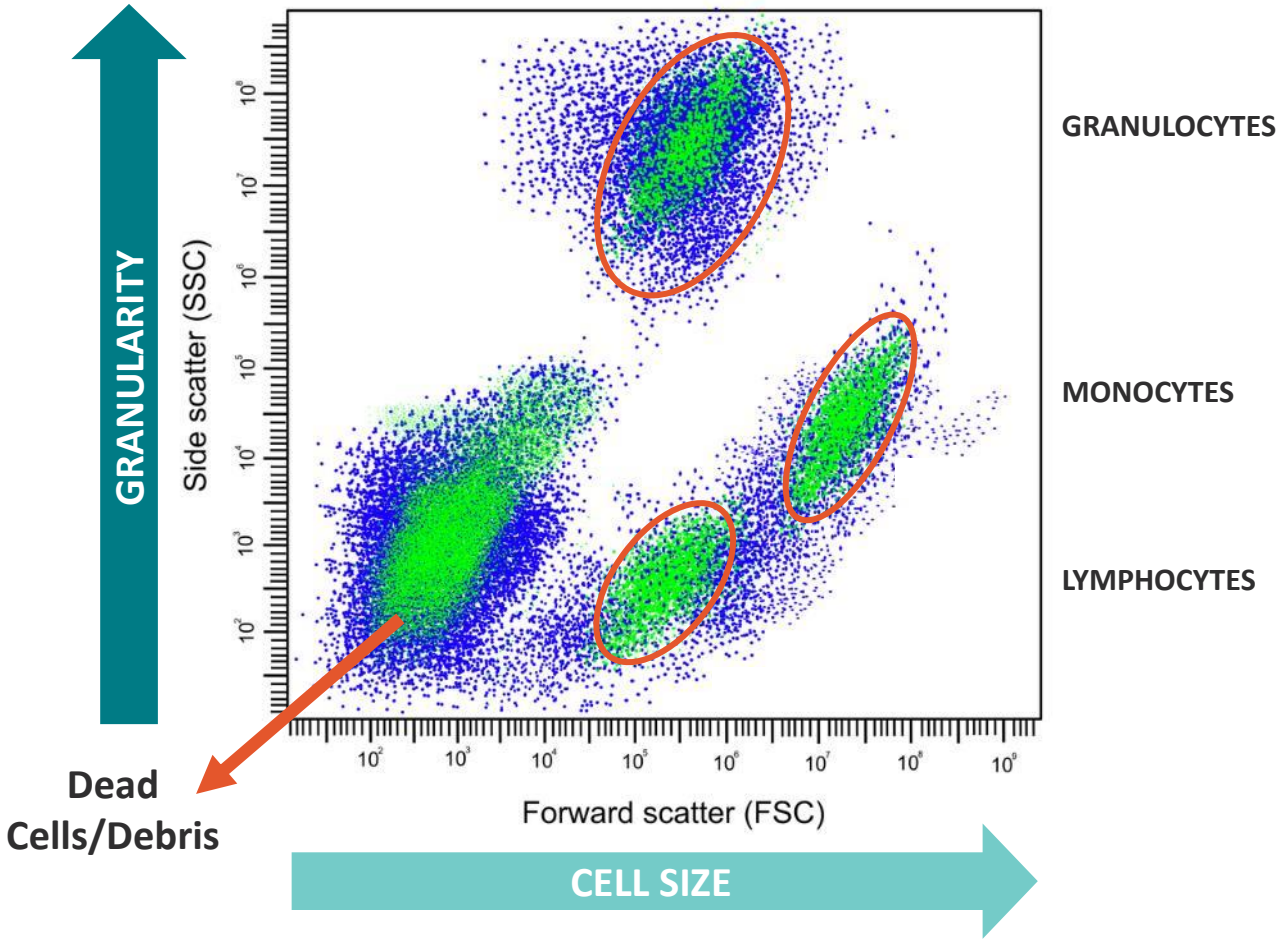
These attributes may and will vary from batch to batch and during scale up

Other Techniques and Technologies Significant to Control Strategies and Potency Assays

Flow Cytometry

Flow Cytometry – Platform Basics





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Advantages of Flow Cytometry in ATMP Assays

1. High-Throughput: Flow cytometry allows for the rapid analysis of a large number of cells

2. Multiparametric Analysis: Flow cytometry allows for the simultaneous measurement of multiple parameters in a single experiment and provides a more comprehensive understanding of cellular characteristics compared to some cell-based assays

3. Quantitative Measurements: Flow cytometry provides quantitative data for multiple attributes.

4. Cell Sorting Capability: Flow cytometers equipped with cell sorting capabilities can isolate specific cell populations based on their characteristics

5. Single-Cell Analysis: Flow cytometry can analyze and sort individual cells, enabling the study of cellular heterogeneity within a population

6. Real-Time Analysis: Flow cytometry provides real-time data acquisition allowing analysis of fast kinetics, transient changes, or dynamic interactions within a cell population

7. Automated Data Analysis: Flow cytometry data analysis can be automated, facilitating the handling of large datasets using a range of software tools

8. Transferability: The movement or transfer of experiments between instruments or even between platforms is relatively robust

9. Flexibility: All the above attributes makes flow a highly flexible and adaptable technique amenable to cell-based determination

Snapshot for Assay (Phase I) - CAR-NK

BACKGROUND

- Development with GMP Phase I Qualification
- **Modality** – Irradiated uAPC cells (CAR-NK therapy)
- **MOA** – Ki-67 is absent in resting cells (G0) but present in all active phases of the cell cycle.
- **Cells** – irradiated uAPC and non-irradiated K-562
- **Readout** – Flow cytometry to demonstrate the lack of proliferation in irradiated uAPC cells over time

DESCRIPTION OF ASSAY

This procedure demonstrates the lack of Ki-67 staining in the nucleus of irradiated uAPC cells compared to the control non-irradiated K-562 cells cultured over a period of 21 days.

FIGURE 2: Linearity

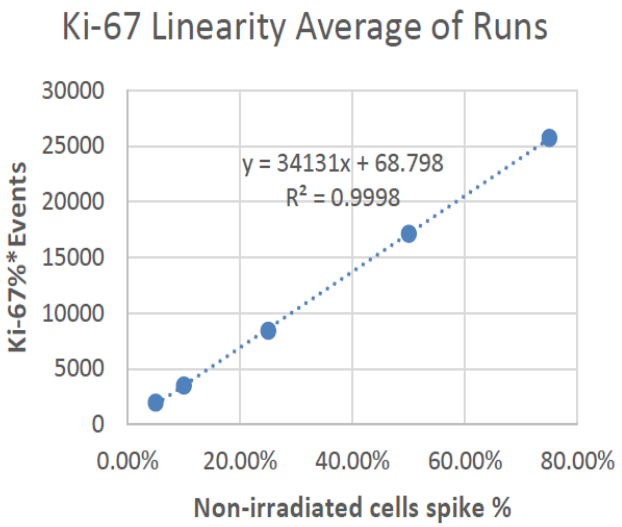
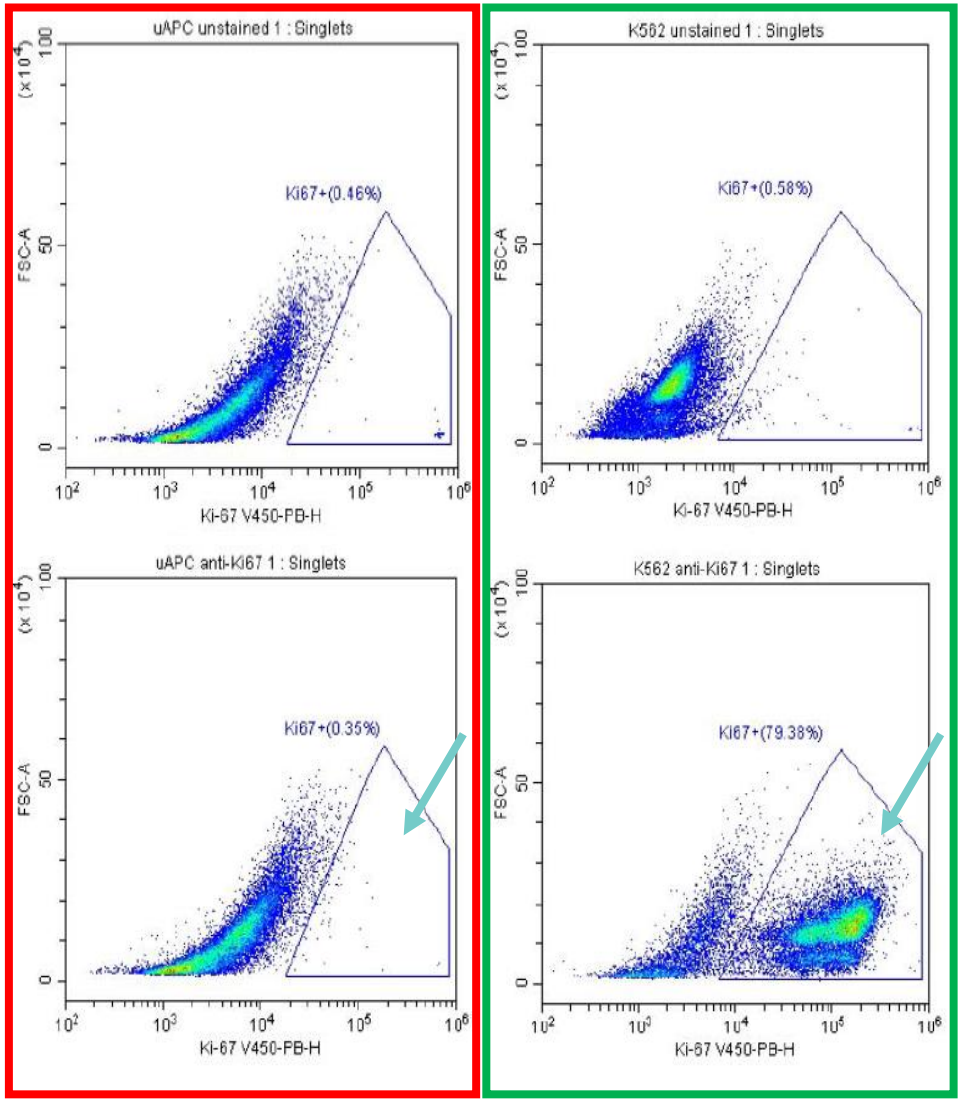


FIGURE 1: Flow Cytometry Raw Data



Snapshot for Assay (Phase I) – MPC

BACKGROUND

- Development with GMP Phase I Qualification
- **Modality** – Mesenchymal Precursor cells (MPC)
- **Cells** - Bone marrow derived MPC
- **Readout** – Flow cytometry to demonstrate positive STRO4 and CD146 expression, with negative CD31, CD45, CD80, CD85, HLA-DR expression.

DESCRIPTION OF ASSAY

This is an immunophenotyping assay for MPC, demonstrating the high expression of positive markers STRO4 and CD146 on MPC cells while control Jurkat cells display high expression of CD31 and CD45 and control Raji cells express CD45, CD80, CD86, and HLA-DR, which are all negative markers for MPC cells.

FIGURE 1: Flow Cytometry Raw Data

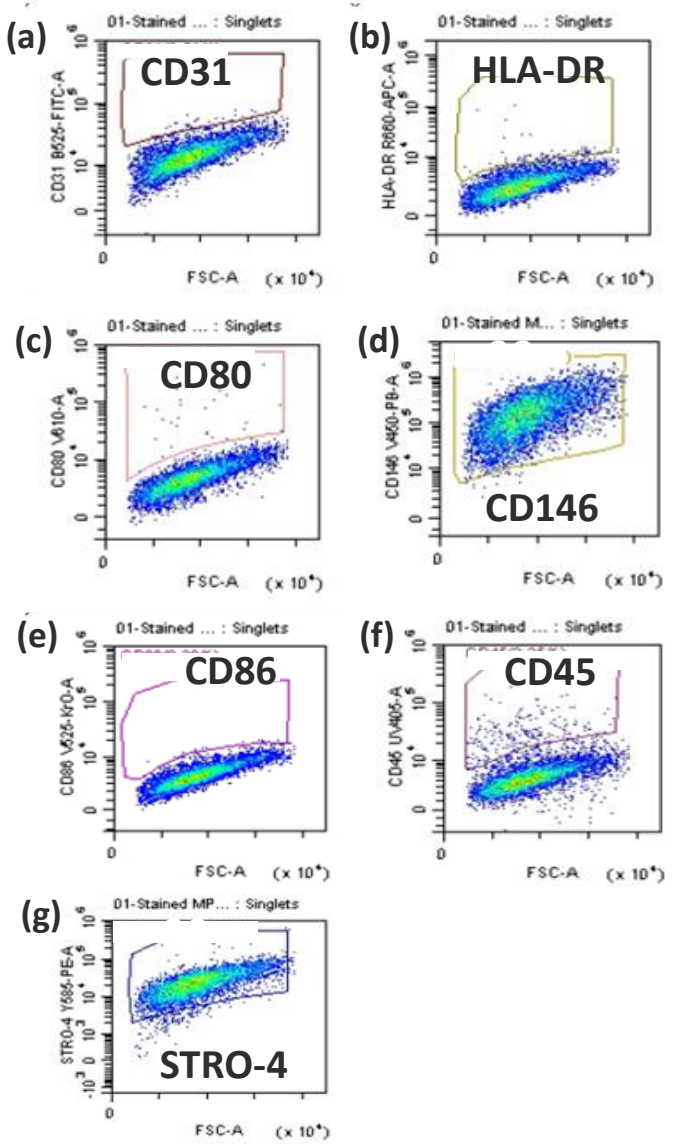


FIGURE 2: CD146 Linearity

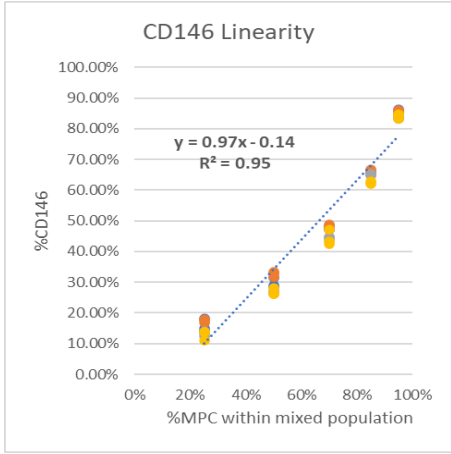
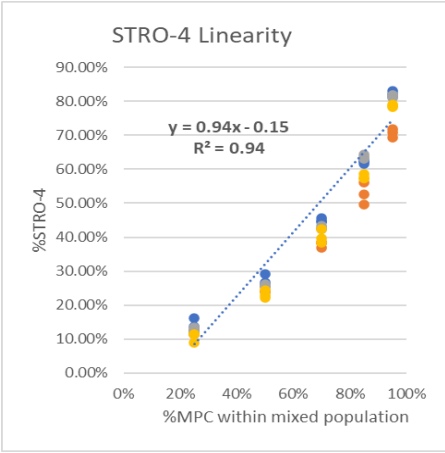


FIGURE 3: STRO-4 Linearity



Snapshot for Assay (Phase I) – mRNA-LNP

BACKGROUND

- Development with GMP Phase1 Qualification
- **Modality** – mRNA-lipid nanoparticle vaccine contains 4 constructs
- **Readout** – Flow cytometry to confirm the expression and the relative potency for each antigen

DESCRIPTION OF ASSAY

HEK293T cells are plated and treated with mRNA-Lipid drug at 5 doses. Cells are stained and fixed with individual primary antibodies to 4 antigens and followed with secondary antibody for detection. %Expression/MFI is used to confirm the antigen expression and to determine the relative potency.

FIGURE 2: Relative Potency Plots for Antigen1

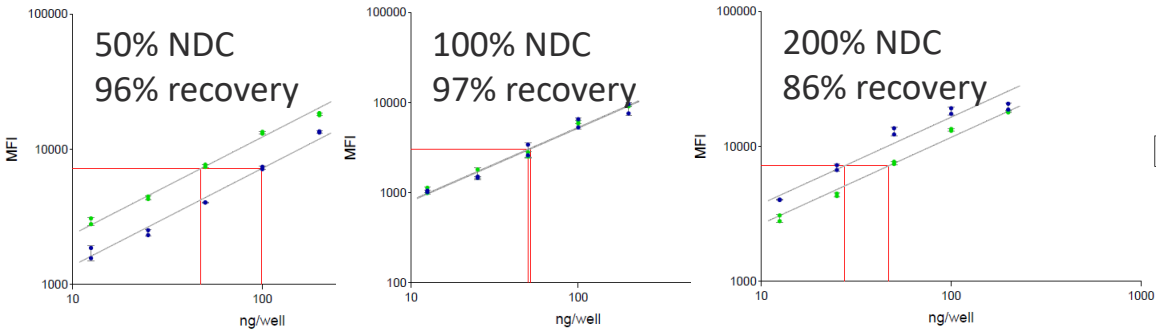
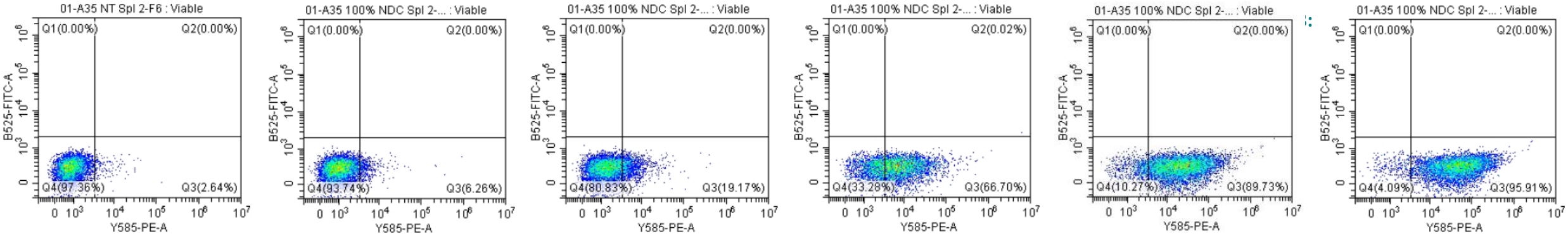


FIGURE 1: Flow Cytometry Raw Date for Antigen1



Non-transfected (NT) Control | 12.5 ng | 25 ng | 50 ng | 100 ng | 200 ng

mRNA LNP dose/well

ddPCR and qPCR

PCR Applications in ATMP Drug Development

CHEMISTRY, MANUFACTURING & CONTROLS (CMC)

- Vector Copy Number (VCN)
- Replication Competent Lenti/Retrovirus (RCL/RCR)
- Replication competent AAV
- Residual plasmid
- Host cell DNA
- Adventitious agents
- Potency Readout

Currently >30 potency or other assays at various stages of development

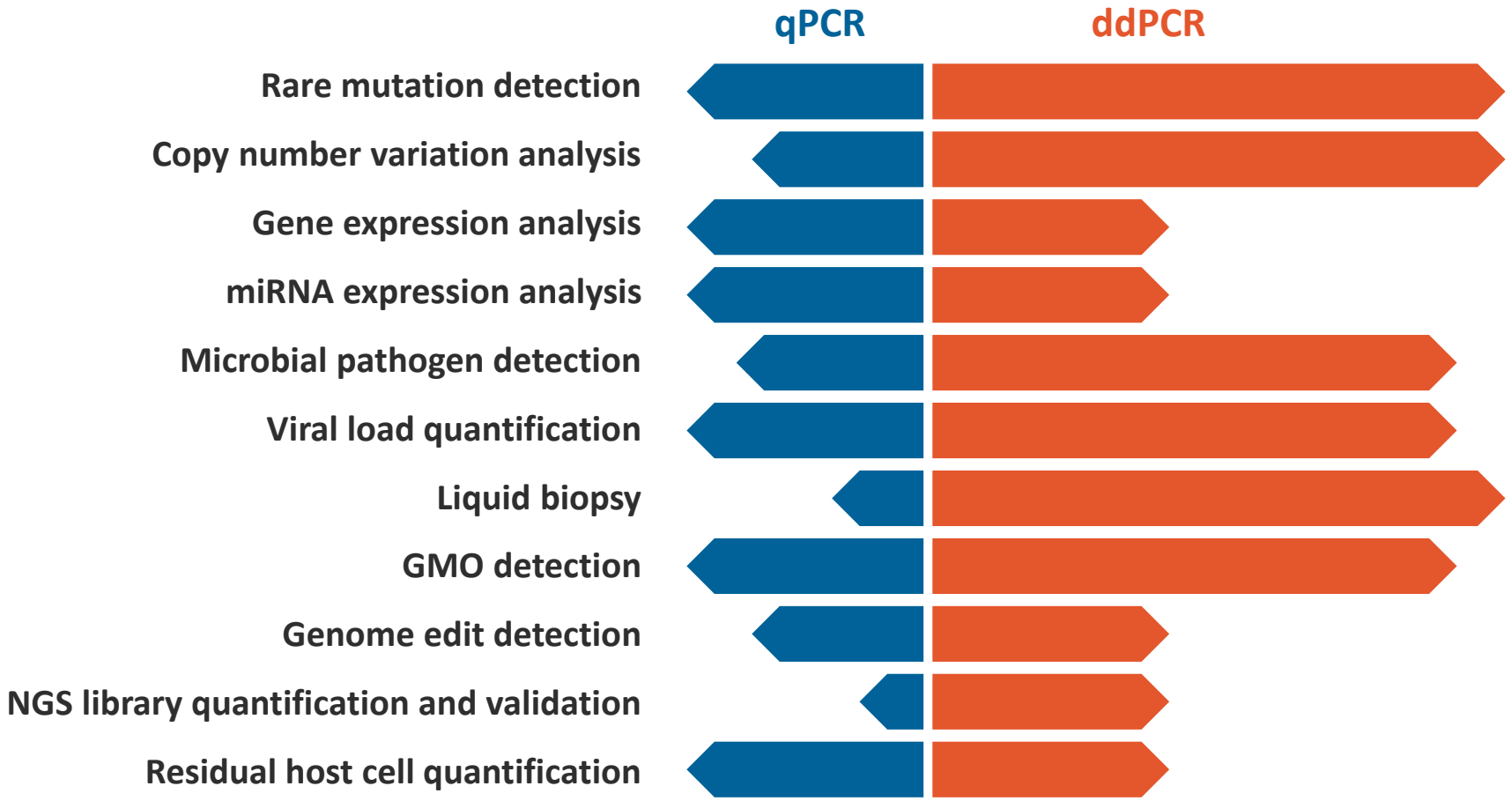
ddPCR and qPCR Technology Platforms

ddPCR

qPCR

	ddPCR	qPCR
Quantitative (with standard curve)	+	+
Absolute quantitation	+	-
Susceptible to interferents/inhibitors	Low	Variable/high
Multiplex capable	+/++	++
Sensitivity	++	++
Precision for rare events	++	+
Reverse transcriptase-incorporated workflow	+	+
Cost of instrumentation	$$$	$/$$
Cost of reagents/consumables	$$$	$$
Average run throughput	5h	1.5h
Reactions per plate	96	96/3 84

Applications of ddPCR vs qPCR



Snapshot for Assay (Pre-IND) - AAV

BACKGROUND

Development of Vector Copy Number

Modality – AAV

Readout – ddPCR (QX One)

Figure 1: Primer/probe Screening

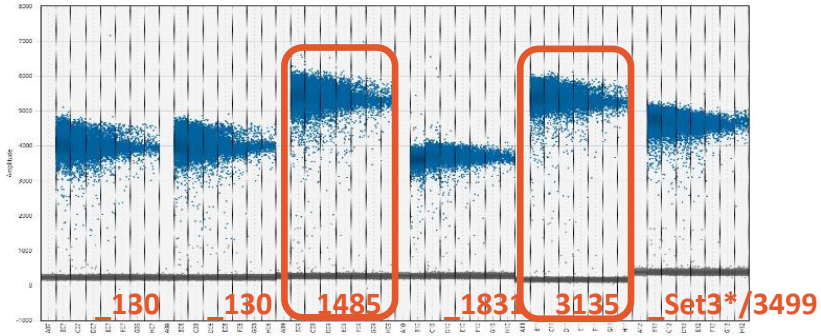
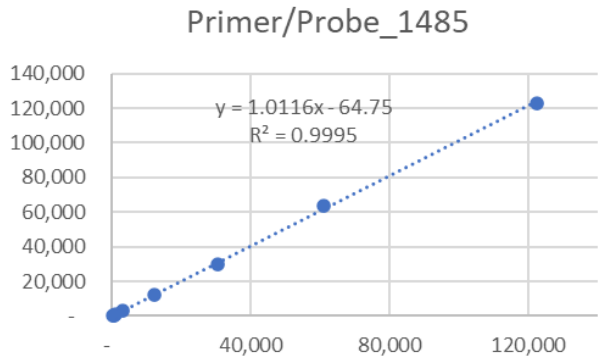
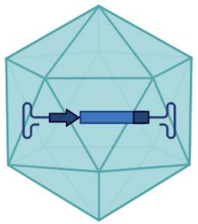


Figure 2: Linearity of Plasmid





Method 1

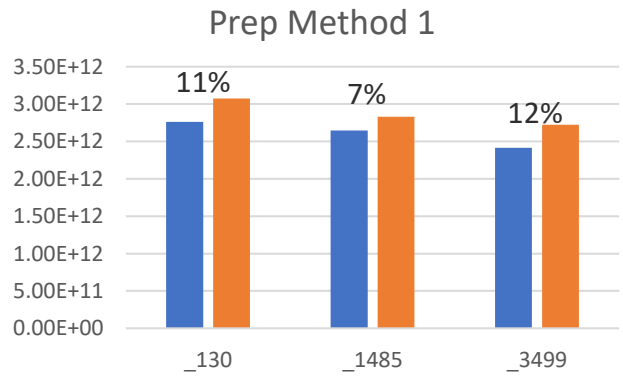
Method 2

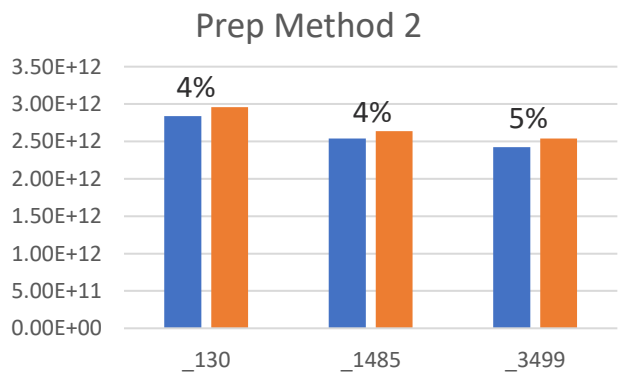
DNase Digestion → Capsid Disruption (Heating) → Dilution

DNase Digestion → Dilution → Capsid Disruption (Heating)

Sample preparation method:

AAV sample was prepared using two methods. In each method, sample was diluted to 2 levels in triplicates. Each sample preparation was tested with three sets of primer/probe.





BioAgilytix

Snapshot for Assay (Phase I) – Plasmid DNA

BACKGROUND

- Development and Phase 1 qualification (Relative Potency Assay)
- **Modality** – Plasmid DNA
- **MOA** – Protein replacement
- **Cells** – HEK293T
- **Readout** – qPCR to measure transgene expression at mRNA level

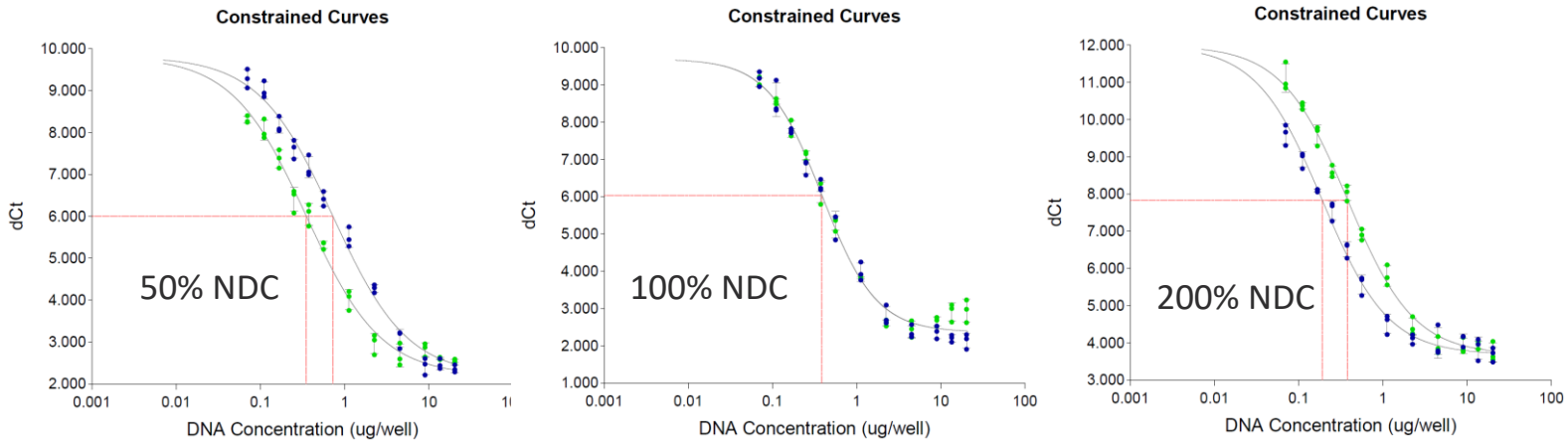
FIGURE 1: Transfection Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Untreated Cells			Lipofectamine								
B	REF1	REF2	REF3	REF4	REF5	REF6	REF7	REF8	REF9	REF10	REF11	REF12
C	REF1	REF2	REF3	REF4	REF5	REF6	REF7	REF8	REF9	REF10	REF11	REF12
D	REF1	REF2	REF3	REF4	REF5	REF6	REF7	REF8	REF9	REF10	REF11	REF12
E	SPL1	SPL2	SPL3	SPL4	SPL5	SPL6	SPL7	SPL8	SPL9	SPL10	SPL11	SPL12
F	SPL1	SPL2	SPL3	SPL4	SPL5	SPL6	SPL7	SPL8	SPL9	SPL10	SPL11	SPL12
G	SPL1	SPL2	SPL3	SPL4	SPL5	SPL6	SPL7	SPL8	SPL9	SPL10	SPL11	SPL12
H												

DESCRIPTION OF ASSAY

HEK293T cells are seeded at 7e4 cells/well in a 96-well plate overnight and transfected with 12 concentrations of plasmid DNA. The mRNA extracted from cell lysates is then analyzed for the expression of transgene normalized to the housekeeping control. Relative potency is determined from 4-PL dose response curve (dCT vs Plasmid DNA concentration).

FIGURE 2: 4-PL Curve (dCT vs DNA concentration)

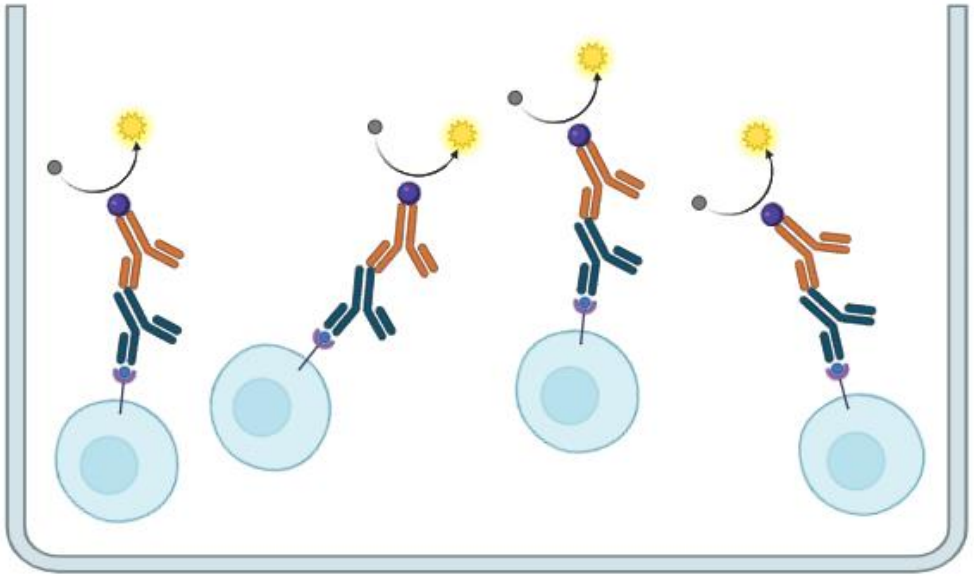


MSD (Meso Scale Discovery)

MSD (Meso Scale Discovery)

How do we leverage MSD capabilities?

- Sensitivity
- Dynamic range
- Custom compatibilities
- Multiplex capabilities
- Off-the-shelf “Plex” panels (developed for biomarkers but useful to CMC applications)



MSD-ECL Imager 600

MSD SQ120 Quickplex

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MSD Utilization in HCP Analysis

BACKGROUND

- Development/Optimization with GMP Phase II Validation
- Modality – Fusion Protein
- MOA – Toxin fragments are delivered to cells to inhibit protein synthesis
- Readout – MSD ELISA measuring the amount of process related impurities (Host Cell Proteins, HCP) present in samples, measured by luminescence

ADVANTAGES OF MSD APPROACH

- Broad dynamic range
- High sensitivity to preserve material and detect lower levels of protein impurities
- Relatively Robust method

LIMITATIONS

Uniquely MSD

FIGURE 1:

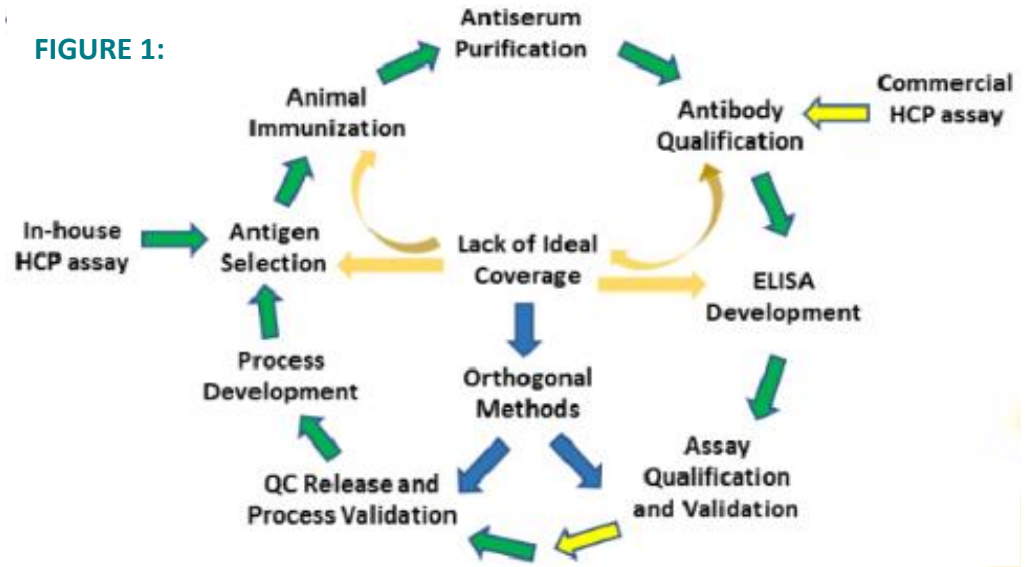


FIGURE 2:

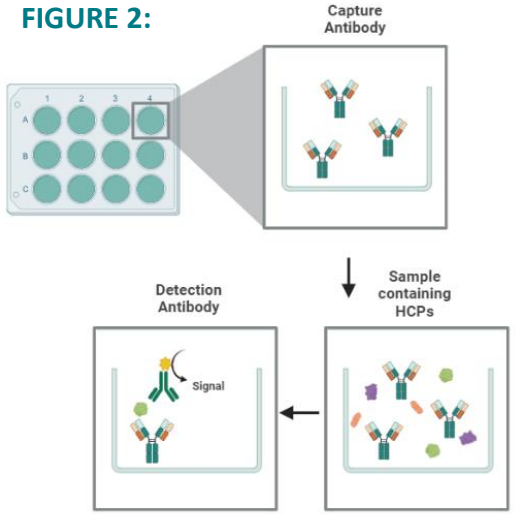
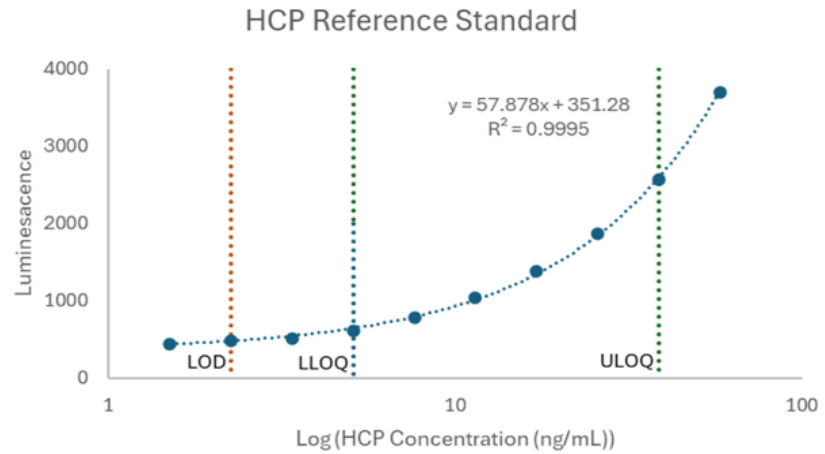


FIGURE 3:



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Snapshot for Assay (Phase I) – Bispecific Abs

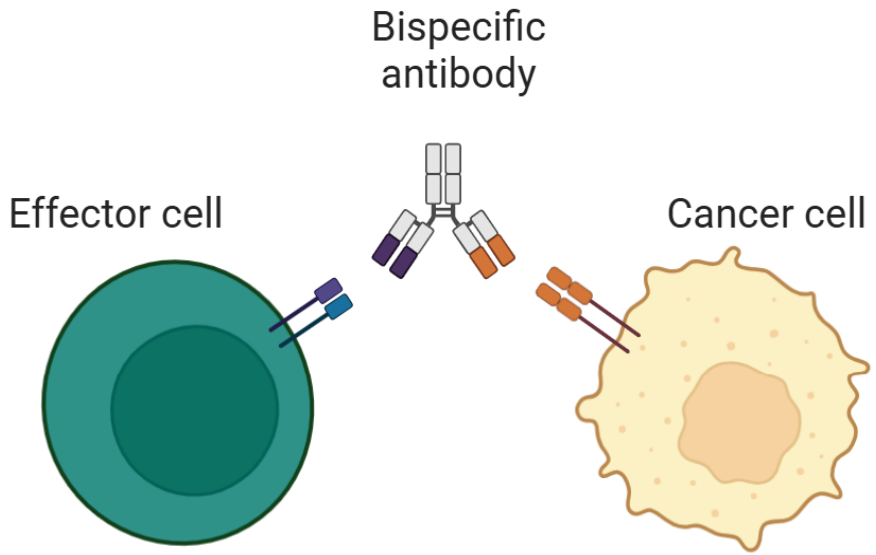
BACKGROUND

- Development and Phase 1 qualification (Relative Potency Assay)
- **Modality** – Bispecific Abs
- **MOA** – Antibody X contains two antigen binding sites, targeting a cell surface protein X (CSPX) on T-cells and a cell surface protein Y (CSPY) on certain tumor cells (Figure 1). CSPX plays a vital role in T-cell activation while CSPY is a tumor-associated antigen expressed in various solid tumor types. Therefore, Antibody X promote T-cell activation and the subsequent lysis of tumor cells.
- **Readout** – Target binding using MSD

DESCRIPTION OF ASSAY

To align with the mechanism of action (MOA) of Antibody X, two potency assays were developed to measure the binding of the BsAb to each target antigen independently.

FIGURE 1: Bispecific Antibody X with two antigen binding sites



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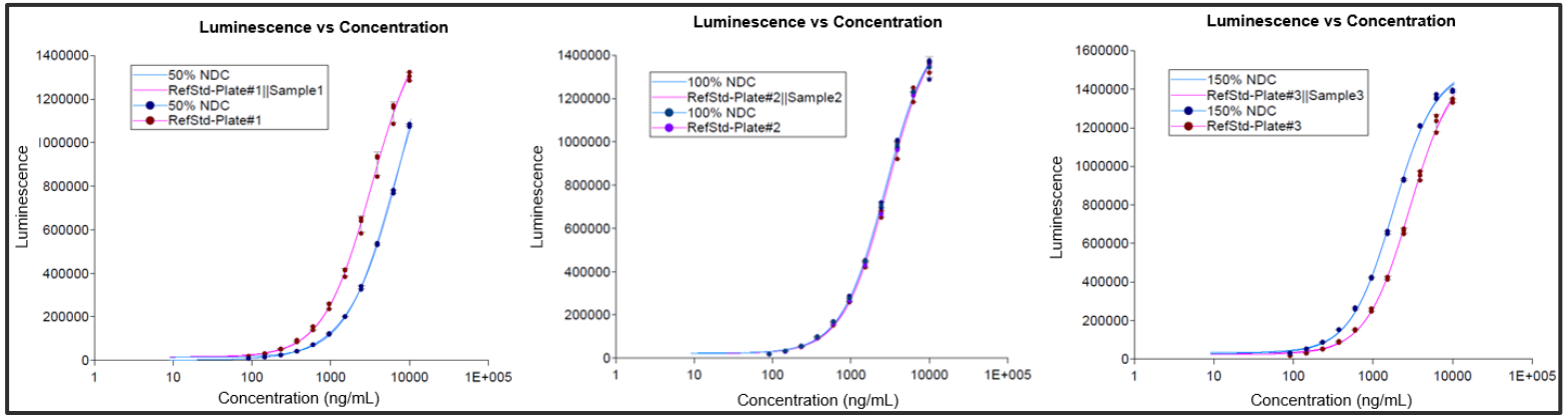
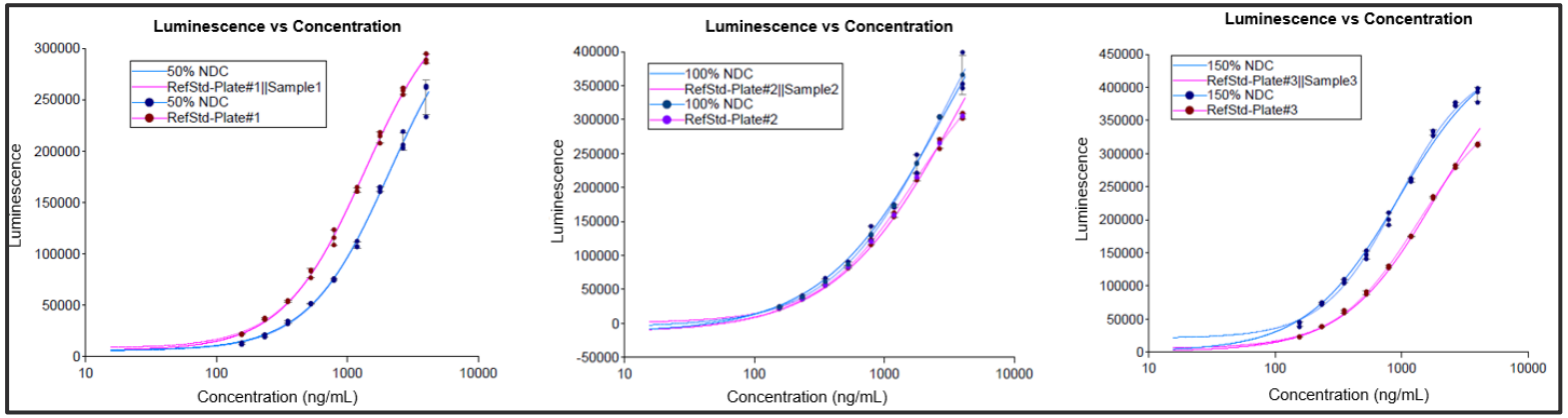
Snapshot for Assay (Phase I) – Bispecific Abs

- Capture protein (CSPX or CSPY) immobilized on standard Meso Scale Discovery (MSD) multi-array plates.
- Serial dilutions of BS Antibody X (analyte) and a negative control were added to the plates.
- Ruthenylated secondary Ab bound to BS Antibody X for detection (electrochemiluminescence (ECL)) on MSD
- Mean, R^2, %CV, and Relative Potency (REP) were calculated using a 4-parameter nonlinear logistic (4PL) curve using Gen5 Secure, version 3.02 (Agilent Technologies).
- Capture protein concentration and ratio of capture protein to primary antibody were explored and optimized.
- BS Antibody dose concentrations were optimized for each target antigen independently



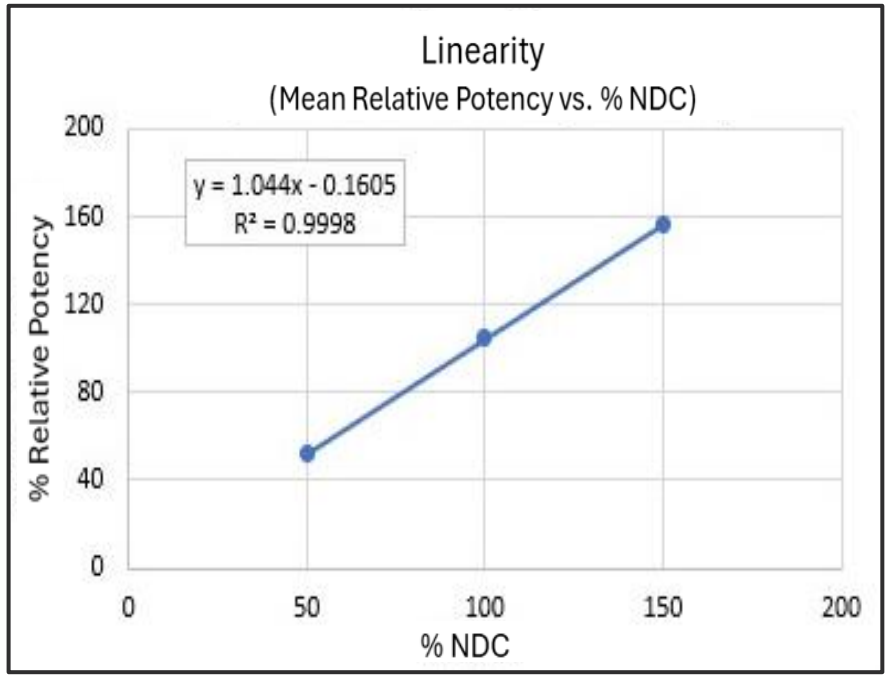
CSPX dose-response curves at (A) 50%, (B) 100% and (C) 150% NDC



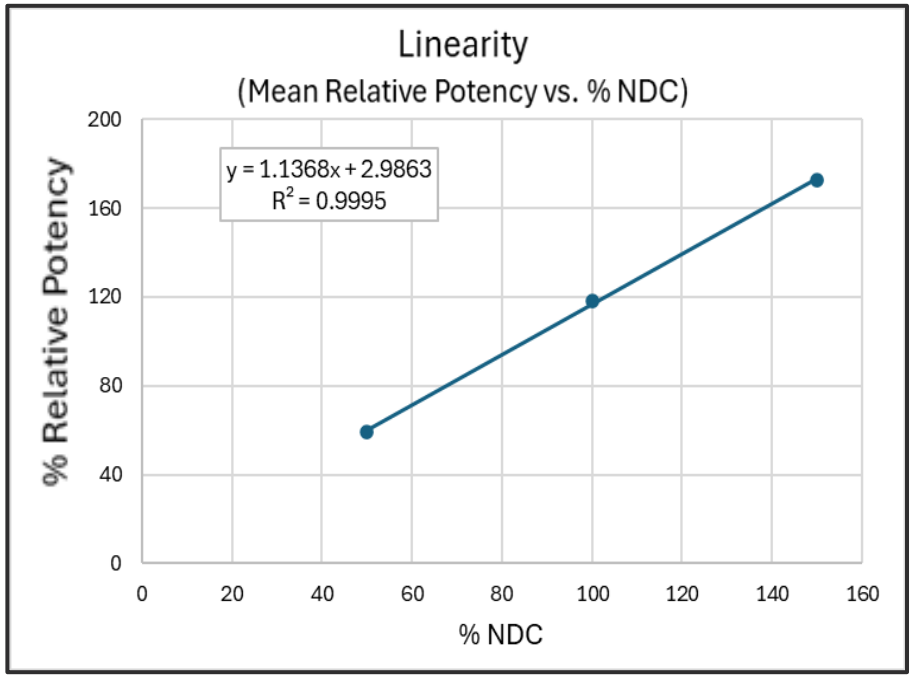
CSPY dose-response curves at (A) 50%, (B) 100% and (C) 150% NDC

Snapshot for Assay (Phase I) – Bispecific Abs

- Good linearity across necessary range
- Very different curve characteristics but similar performance
- MSD utilization needed to provide lower end sensitivity on the curves and characterize curves accurately
- Early phase qualification… more development and correlation with CQAs and other attributes to provide most robust potency assurance strategy



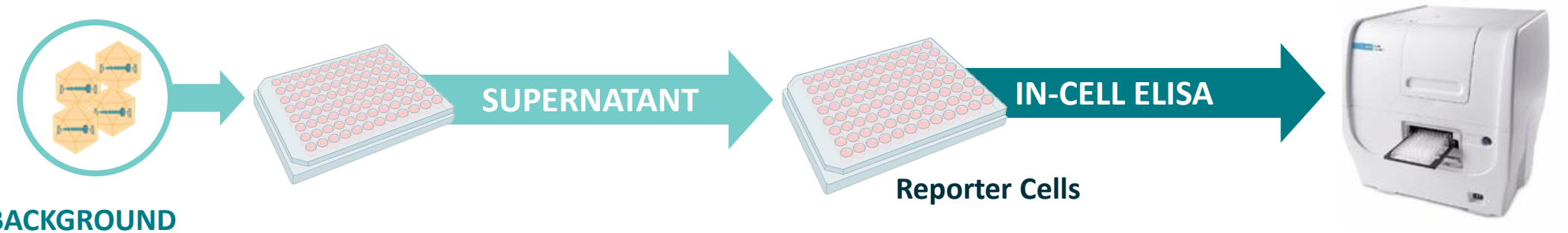
Linearity for CSPX at (A) 50%, (B) 100% and (C) 150% NDC



Linearity for CSPY at (A) 50%, (B) 100% and (C) 150% NDC

Cell Imaging Reader

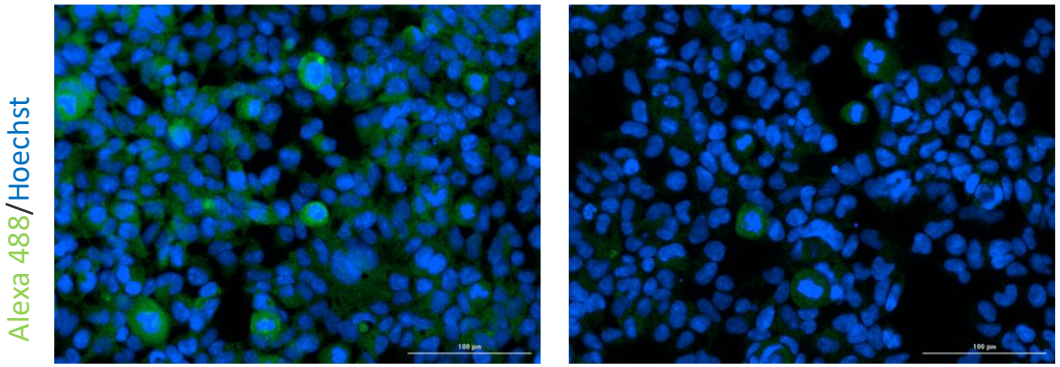
Snapshot for Assay (Phase I) – AAV



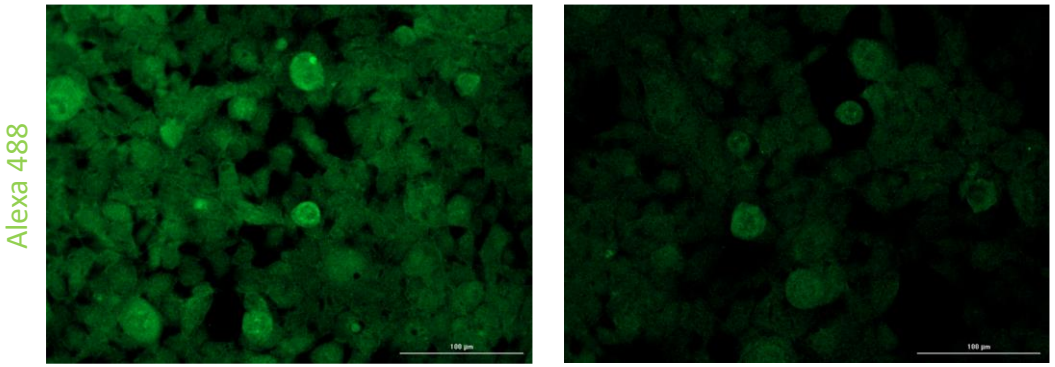
BACKGROUND

- Method Development (functional based potency assay)
- **Modality** – AAV
- **MOA** – Transgene expression trigs a downstream signal cascade.
- **Cells** – HT1080 and Neuro-2a **Readout** – In-cell ELISA for Protein X

Target Protein X and Cells



Target Protein X only



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Summary

Reviewed LNPs, their attributes and examples or potency assurance approaches

- Development and Implementation of Early phase assay using LC-HRMS
- Using Apoptosis as a determination of anti-cancer

Reviewed attributes of AAVs and how these might impact potency assurance

Reviewed tools used in the control of ATMPs

- Flow Cytometry for potency
- ddPCR and qPCR
- MSD
- Cell Imaging Reader

Conclusions

Highlights and recommendations for gene therapy potency assurance strategies and control strategies

- **Get started early and explore options**
- **Leverage guidance documents and time with regulators**
- **Select analytical technologies based on CQAs and MOA**
- **Explore relationships with other CQAs**
- **Matrix approach offers solutions but also challenges**
- **Take phase appropriate approach**
- **Changes will happen and assays must evolve**

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