Approaches to Potency Assays for CRISPR Genome Editing Therapeutics

CASSS CGTP

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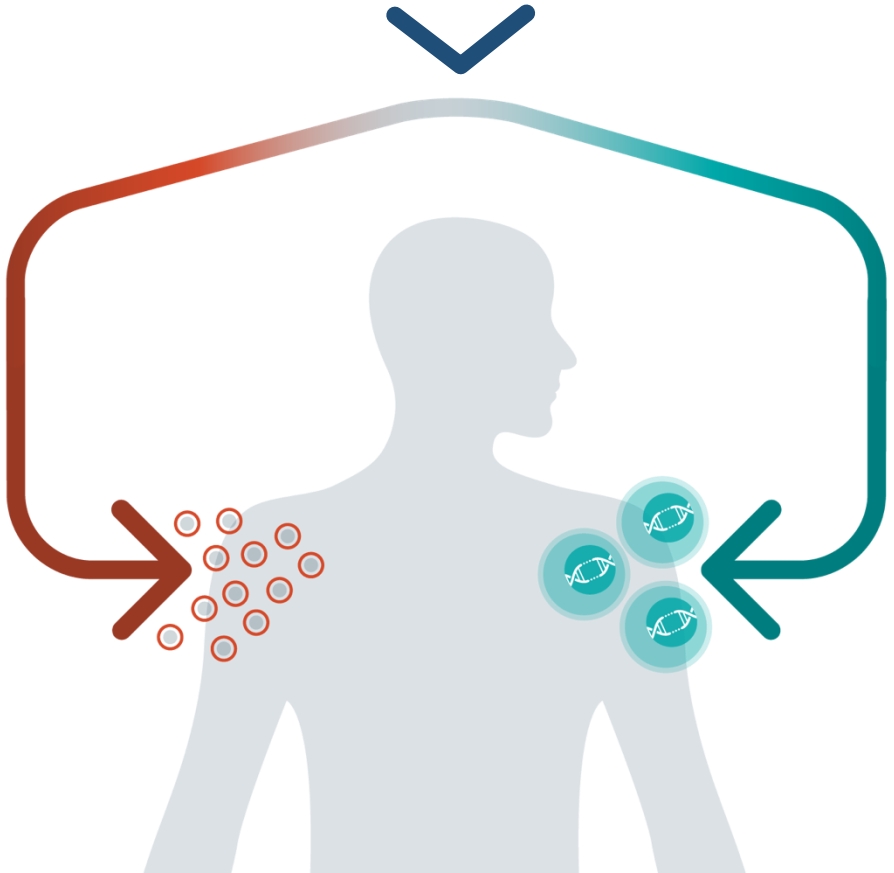
Advancing a Full-Spectrum Genome Editing Company

CRISPR-Based Modular Platform

EMPLOY NOVEL EDITING AND DELIVERY TOOLS



In Vivo

CRISPR *is* the therapy

FIX THE TARGET GENE

Genetic diseases

Ex Vivo

CRISPR *creates* the therapy

REWIRE & REDIRECT CELLS

Immuno-oncology

Autoimmune diseases

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CRISPR/Cas9 and Derivative Gene Editing Technologies Can Be Used to Make Any Type of Edit

INTELLIA'S EDITING TOOLS

CRISPR/ Cas9

DERIVATIVE TECHNOLOGIES

+ Base Editor

+ DNA Writer

+ Other Technologies

KNOCKOUT

Inactivation/deletion of disease-causing DNA sequence

INSERT

Insert new DNA sequence to manufacture therapeutic protein

REPAIR

Correction of "misspelled" disease-driving DNA sequence

INTELLIA SELECTS THE BEST TOOL FOR EACH THERAPEUTIC APPLICATION

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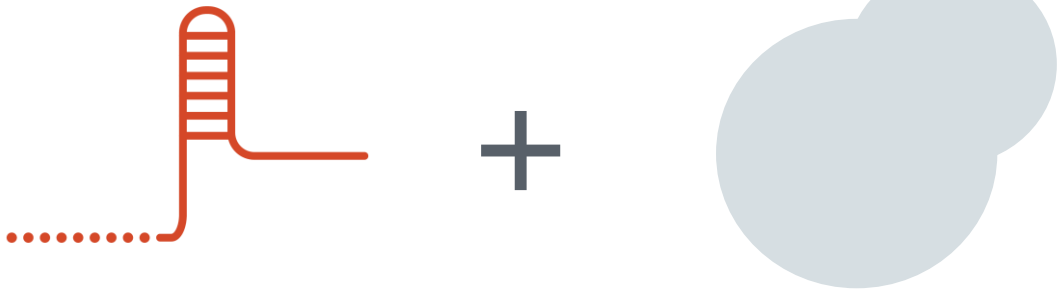
Gene Editing Starts with CRISPR/Cas9, a Two-Part, Programmable System

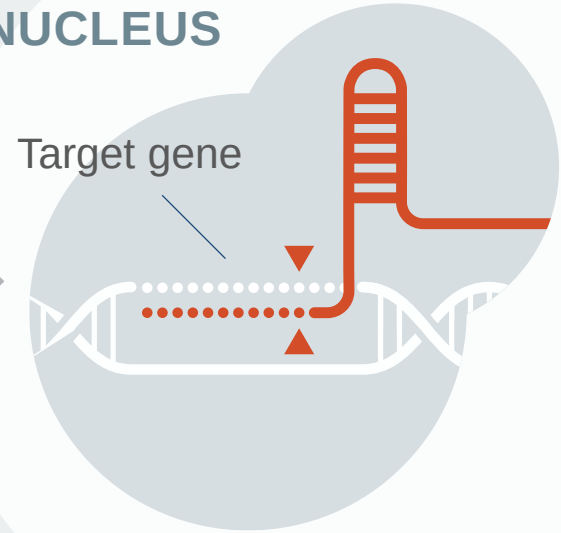
FOUNDATIONAL CRISPR MACHINERY



1 **Guide RNA (gRNA)**
Identifies genetic target

2 **Cas Protein**
Responsible for the targeted DNA editing and provides platform for other enzymatic activities

INSIDE CELL NUCLEUS



KEY FEATURES OF CRISPR/CAS9 SYSTEM

✓ Selectivity ✓ High potency ✓ Address any site ✓ Target multiple DNA sites

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In Vivo

CRISPR is the therapy

GENETIC DISEASES

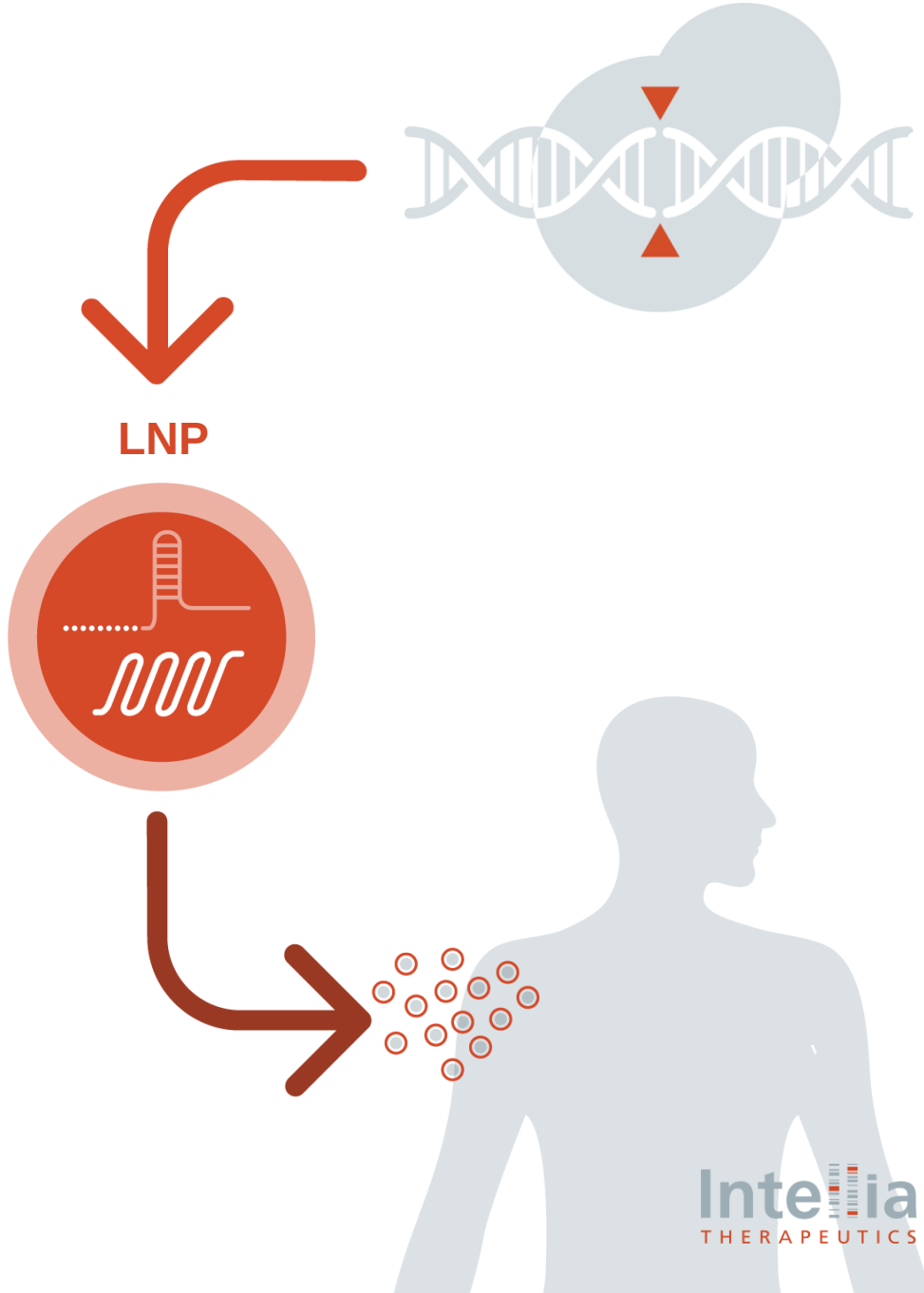
Strategic Advantages:

Potential curative therapy from a single dose

Systemic non-viral delivery of CRISPR/Cas9 provides transient expression and potential safety advantages

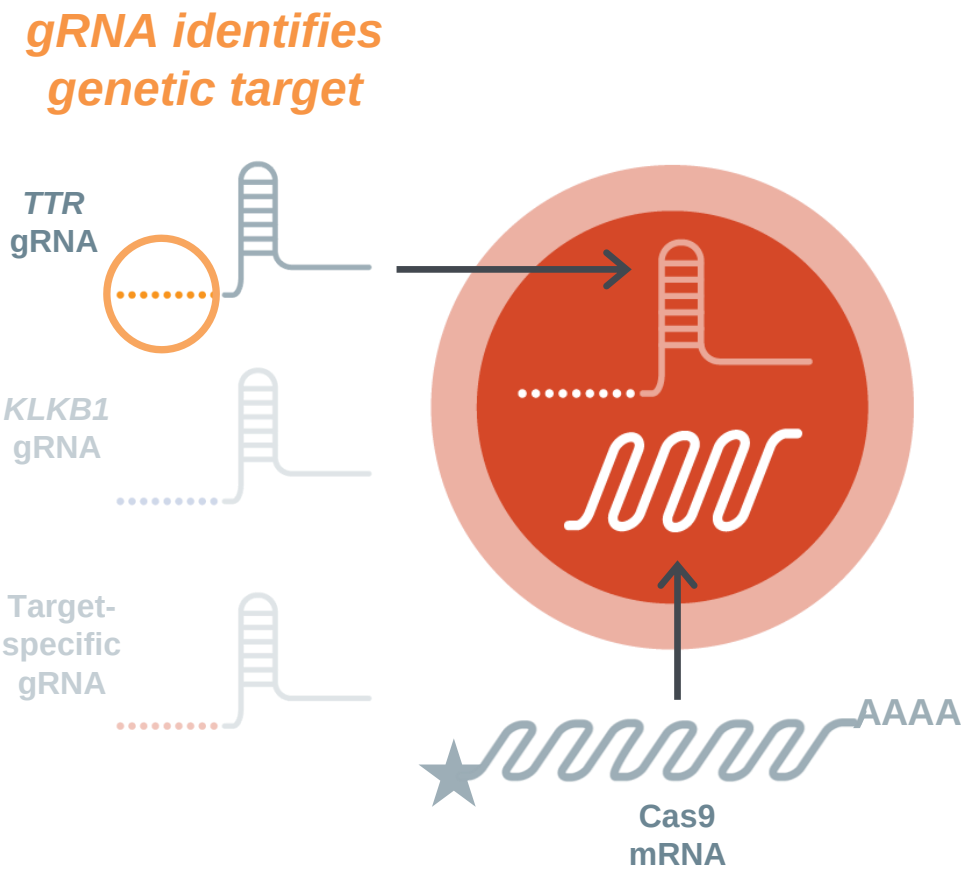
Potential for permanent gene knockout or gain of function by targeted insertion

Capable of delivering to multiple tissue types for various therapeutic applications



Modular Delivery Platform Enables Rapid and Reproducible Path to Clinical Development

LNP Delivery System:



- ✓ LNP drug product is a multi-component lipid formulation with 2 drug substances and a novel lipid excipient
- ✓ gRNA target site specificity defined by 20mer at 5'end
- ✓ mRNA provides transient Cas9 protein expression

Distinct cargo (gRNA and mRNA) must be simultaneously delivered to the cytoplasm

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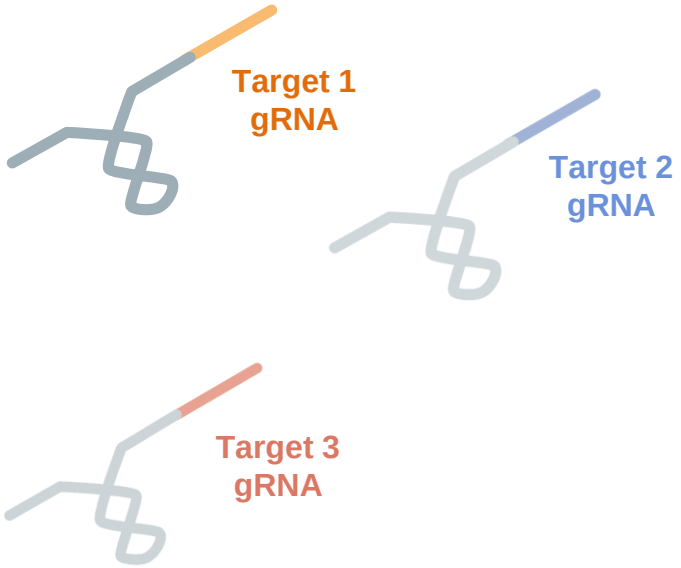
Intellia Leverages Platform Manufacturing for Drug Substances & Drug Product

sgRNA Platform MFG



- 80 to 120 nts in length
- gRNA target site specificity defined by 20mer at 5’ end
- Uses standard solid phase phosphoroamidite oligo synthesis

Cas9 mRNA MFG

Cas9 mRNA

- ~4,400 nts in length, ~1.5 MDa
- Encodes for Streptococcus pyogenes (Spy) Cas9 protein
- Uses enzymatic synthesis approach

LNP Platform MFG

- Combines both mRNA DS and sgRNA DS in an LNP
- LNP composition and formulation process undergoes extensive optimization to ensure a robust manufacturing process

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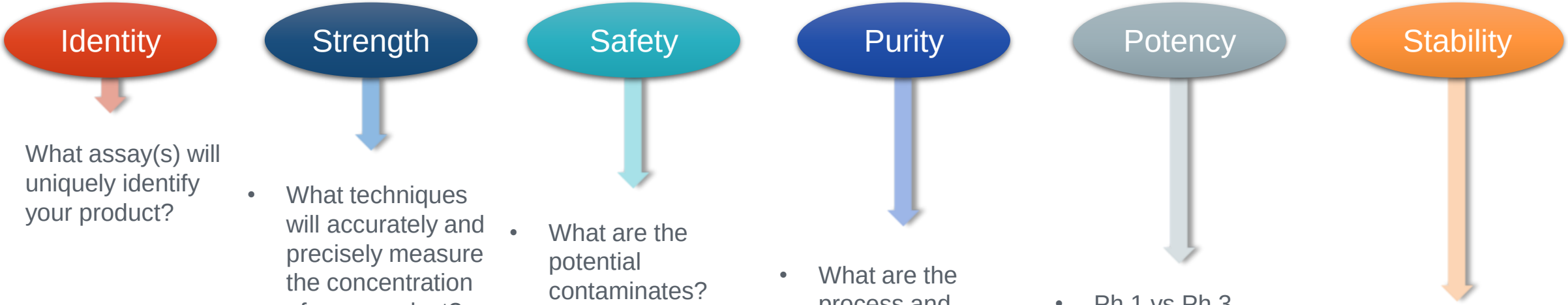
Each Platform Process Must be Supported by a Suite of Analytical Methods

- CRISPR/Cas9 genome editing is regulated as a Cell & Gene Therapy (CGT) product
- CGT manufacturing processes are complex and require equally complex methods to appropriately define and characterize the product



- What assay(s) will uniquely identify your product?
- What techniques will accurately and precisely measure the concentration of your product?
- What are the potential contaminates?
- How are they detected?
- What are the process and product related impurities?
- How are they detected?
- Ph 1 vs Ph 3 assay
- Number and robustness of assay(s)
- Are small changes in strength, purity and potency detectable over time?
- Shelf-life

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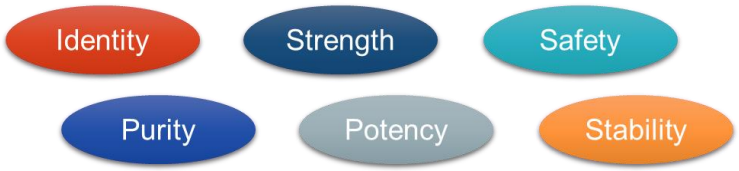
Total Number of Analytical Methods Quickly Multiplies to Support Each Process

sgRNA Platform MFG

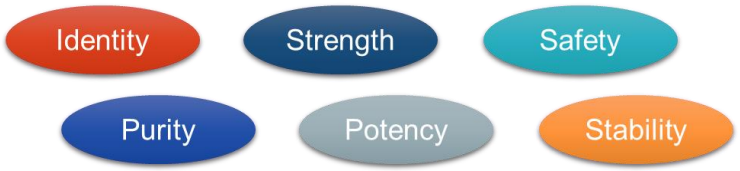
Cas9 mRNA MFG

LNP Platform MFG

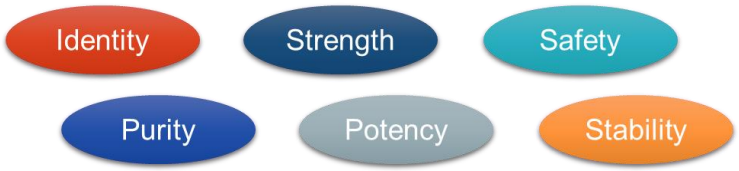
sgRNA Analytics



mRNA Analytics



LNP Analytics



Each manufacturing platform requires 15-20 analytical methods for release, including multiple cell-based potency assays for DS & DP

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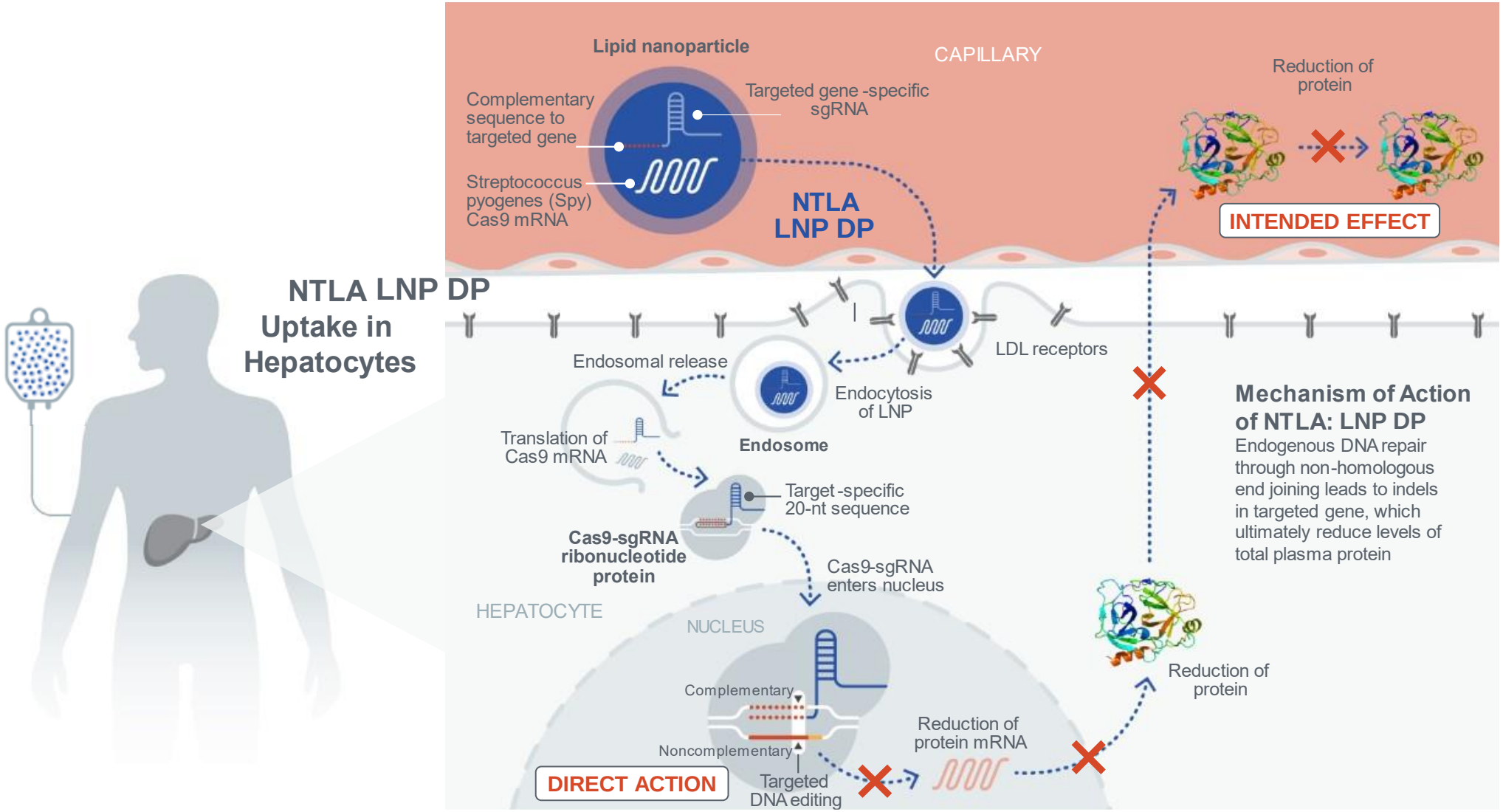
In Vivo CRISPR Mechanism of Action



LNP: lipid nanoparticle; **sgRNA**: single guide RNA

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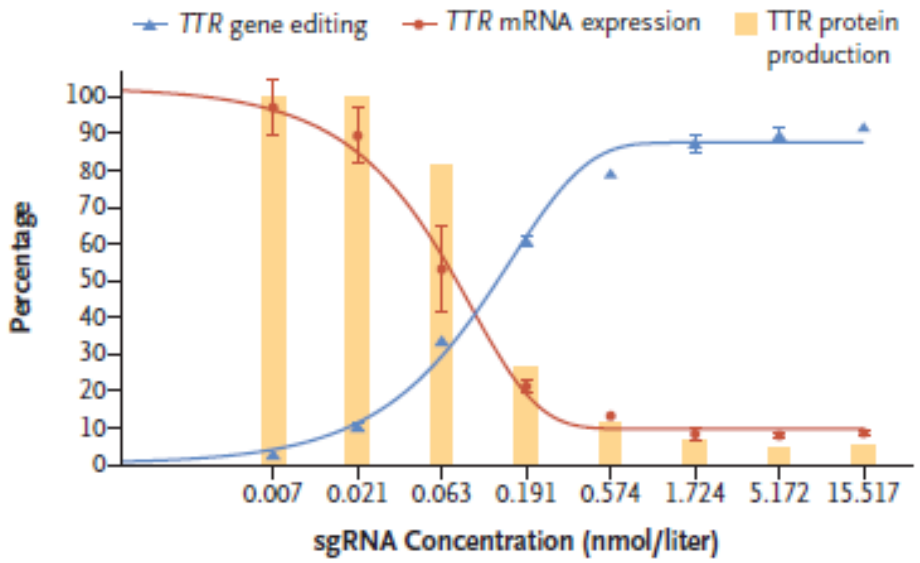
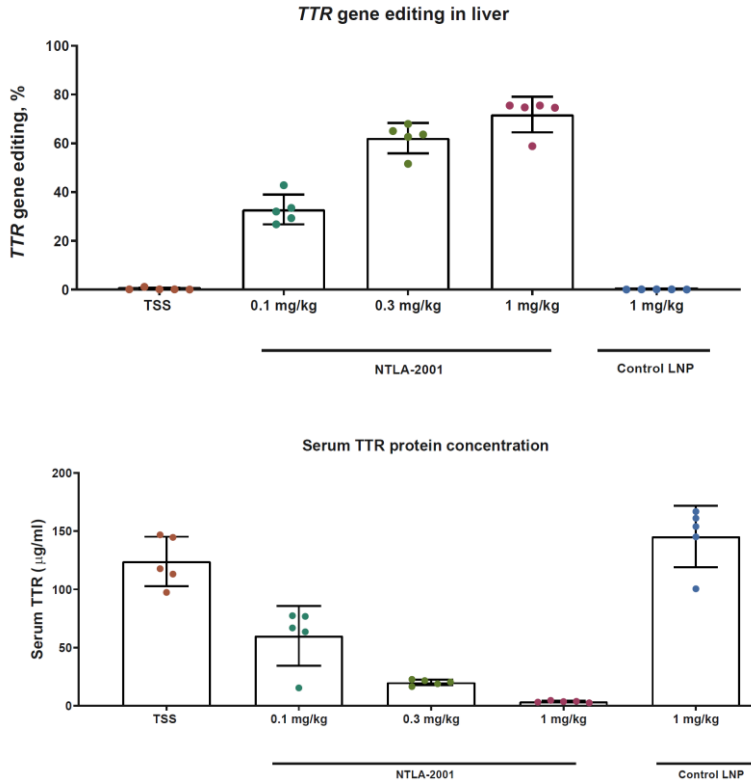
Initial Potency Assay Design is Based on Primary Pharmacology

- Primary pharmacology *in vitro*
  - Primary human hepatocytes



- Primary pharmacology *in vivo*
  - Humanized transgenic mice



Development work is required to convert primary pharmacology observations into assays that can be used for routine drug product potency testing

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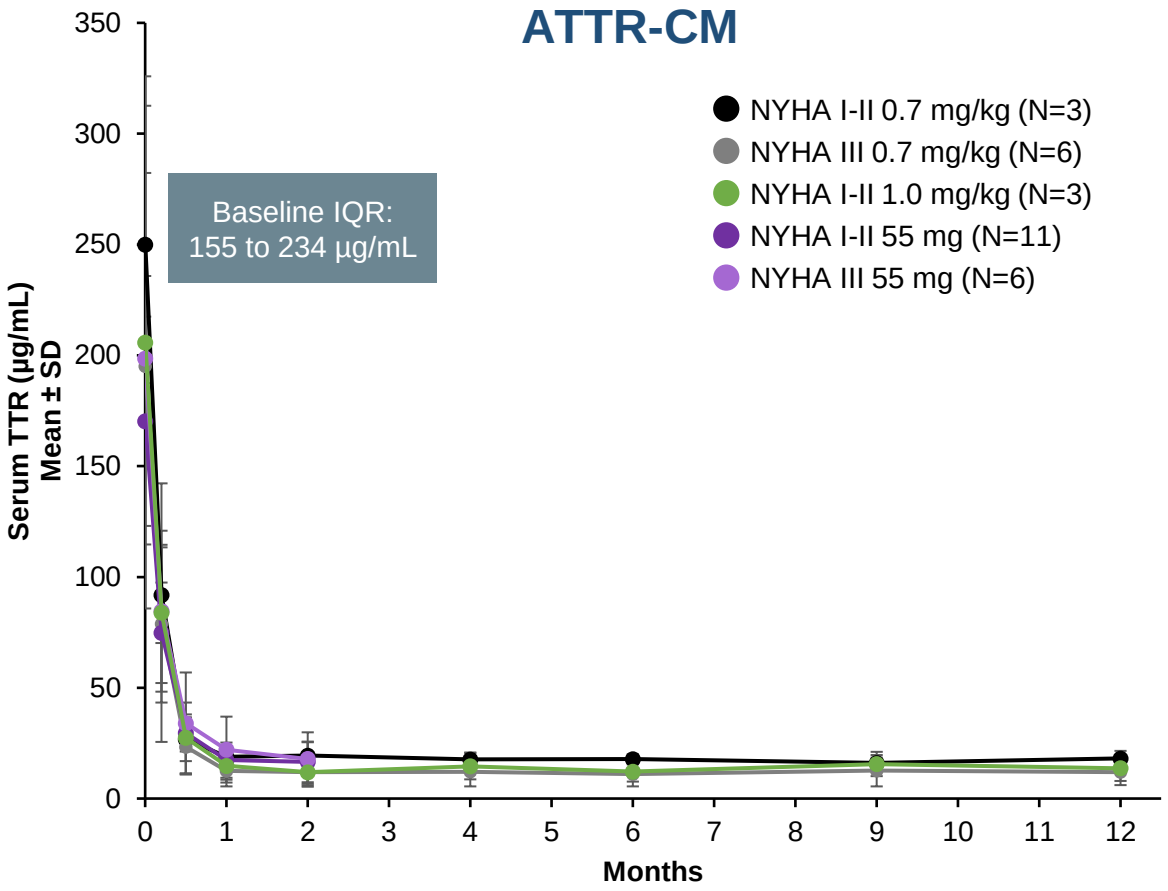
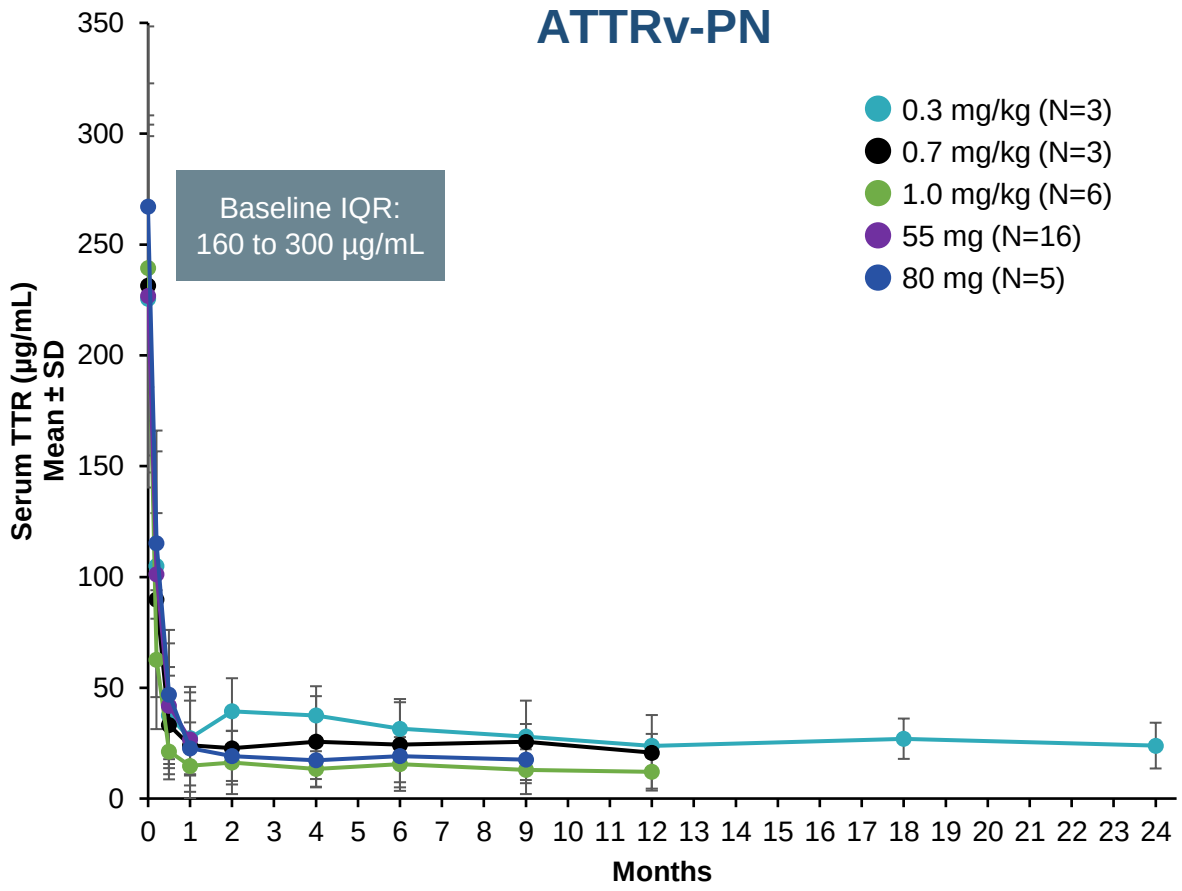
NTLA-2001 Led to Consistently Low and Sustained Absolute Serum TTR in All Patients





Median (IQR) Serum TTR at Day 28 (n=62)	Residual absolute TTR concentration at day 28	17 µg/mL (11 to 24 µg/mL)
	% Change from baseline in serum TTR at day 28	-91% (-88 to -94%)

Data cutoff May 11, 2023.
Figure notes: Results for each dose level are shown out to the last time point with complete follow-up for the entire cohort. Interim data presented excludes the 0.1 mg/kg cohort from the dose-escalation of the polyneuropathy arm. The three patients in the 0.1 mg/kg cohort have been re-dosed at 55 mg and results will be shared in a future presentation. The 55 mg and 80 mg doses are the fixed doses corresponding to 0.7 mg/kg and 1.0 mg/kg, respectively.

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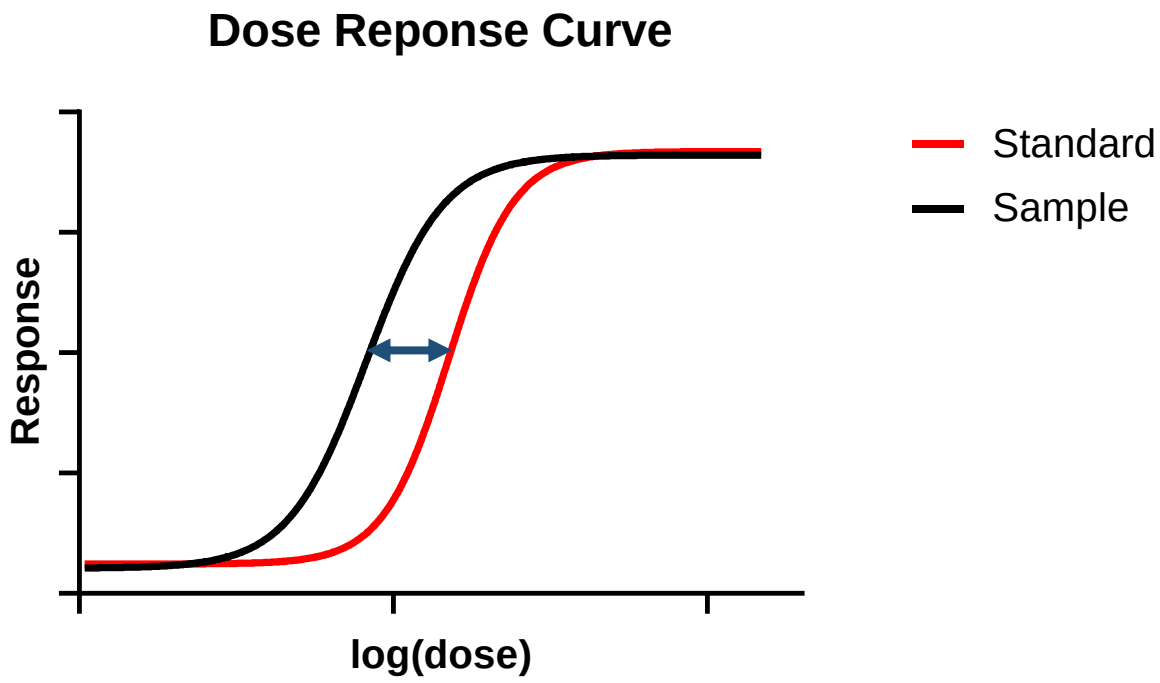
What is a relative potency assay?

- A relative potency assay looks at a shift in a biological response between a sample in comparison to a reference
- Some assays employ a dose-response curve for the sample and the reference standard



Why use a relative potency assay?

- Biological systems may respond different day-to-day, plate-to-plate or analyst-to-analyst
- The reference standard/test sample will shift together on the plate
- This helps with the robustness of the assay

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Developing Relative Potency Assays at the Drug Product Level

Challenges

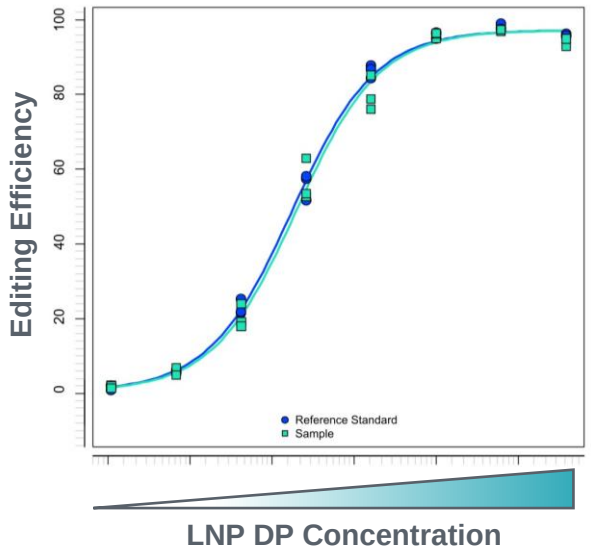
- Primary human hepatocytes
- Genomic editing readout by NGS
- Protein reduction readout by western blot
- Absolute readouts

Solutions

- ✓ Liver cell line
- ✓ PCR-based sequencing readout
- ✓ ELISA or MSD/ECL readout
- ✓ Convert to relative potency, establish early reference standards

Direct Action

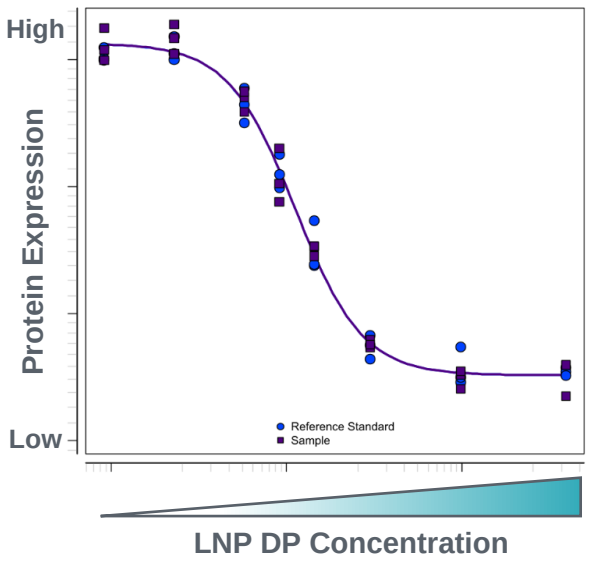
Genomic Editing



Less optimization per target

Intended Effect

Protein Reduction



More optimization per target

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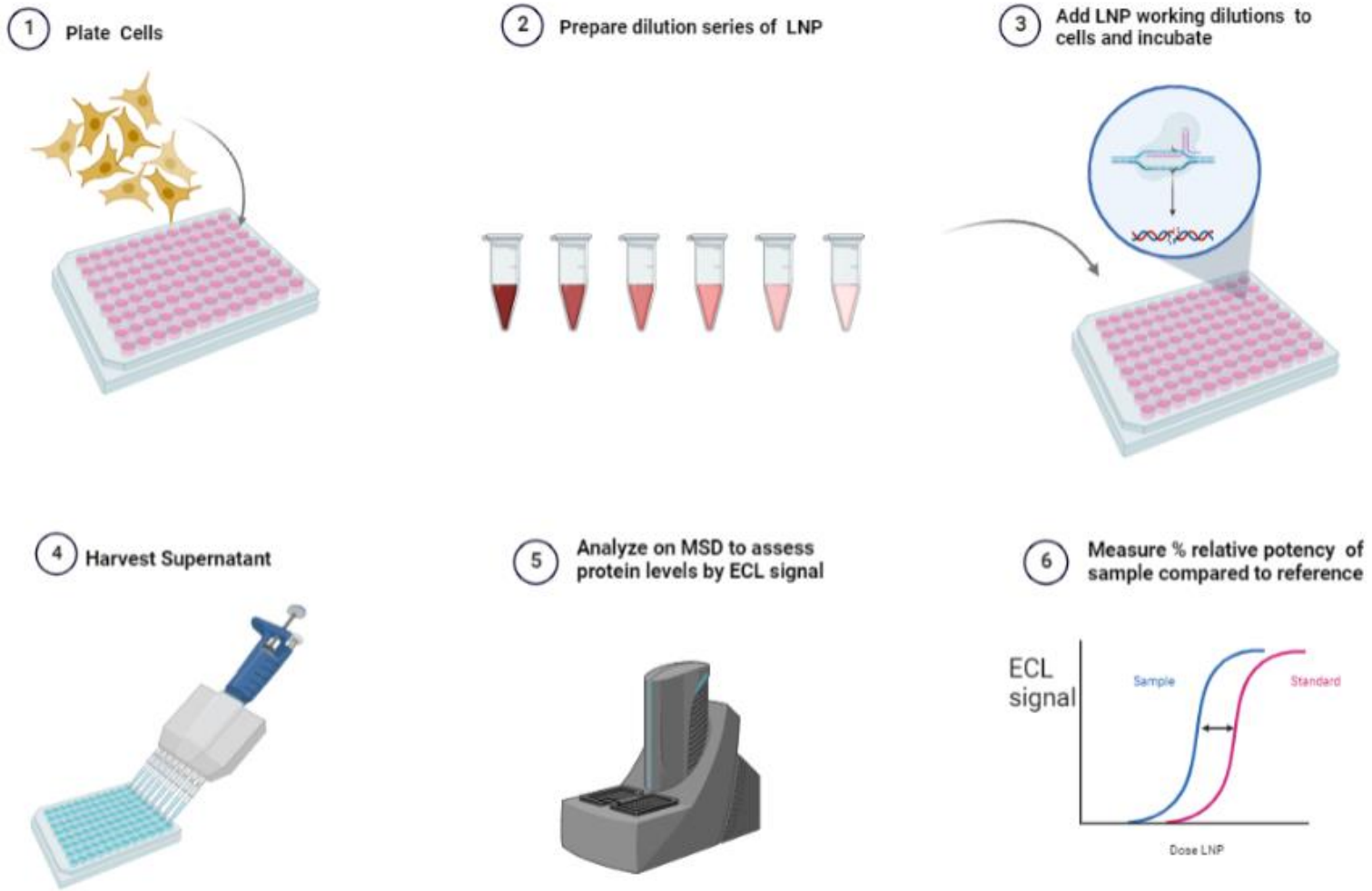
Example Cell Based Assay for Protein Reduction



Optimization Parameters
Cell Seeding Density
Plate Type
Cell Incubation Time
Selection of Media
Media Exchange
Sample Preparation & Addition
Supernatant Harvest Time
Dose Response Curve
MSD/ECL readout

Each step must be optimized to ensure the accuracy, sensitivity, specificity, and reproducibility of the assay

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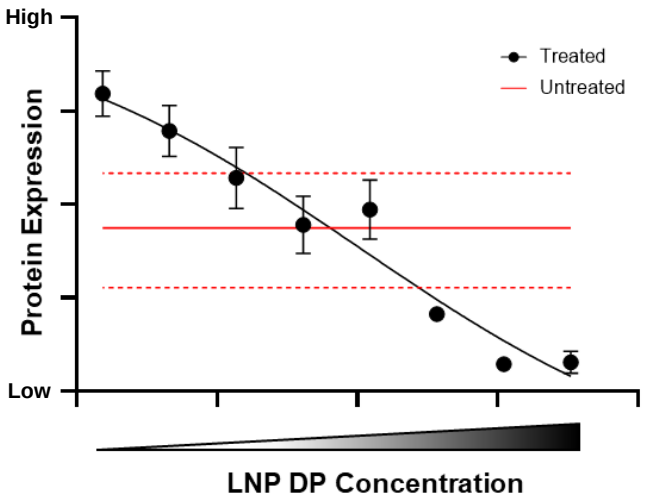
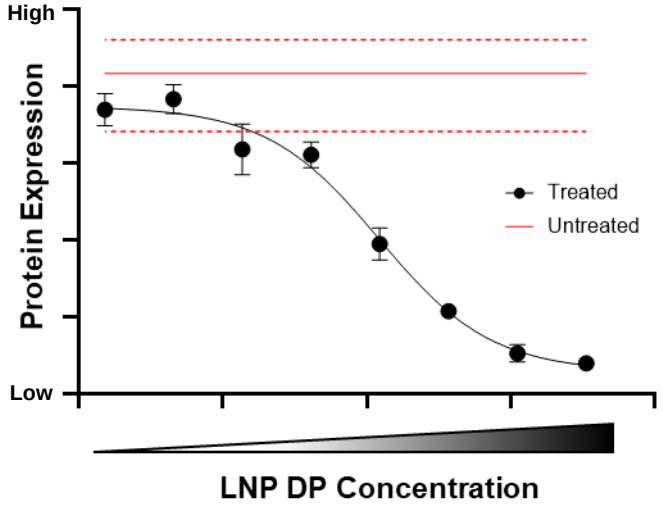
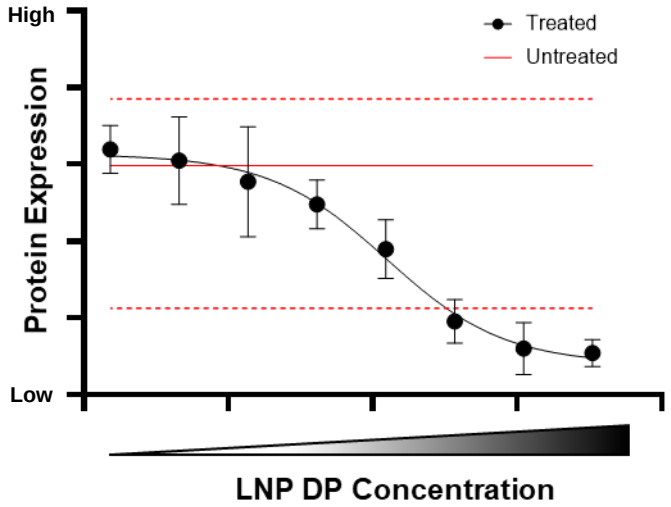
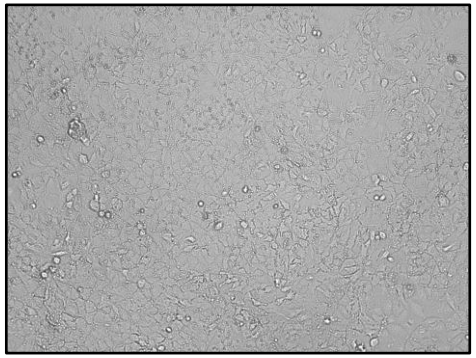
Development of Protein Reduction Assays Is Challenging as Cells Must Remain in Culture for Several Days Post-Sample Treatment

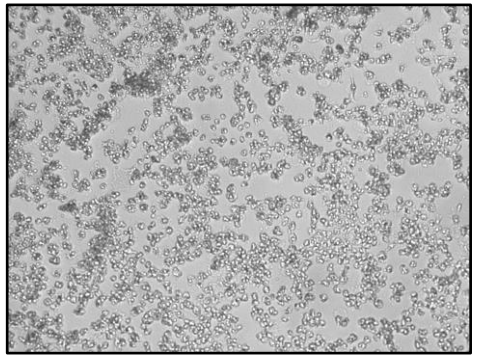


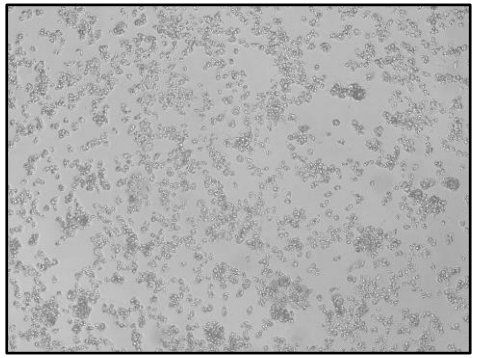




Shorter — Incubation Time (Days) — Longer







- ✓ Longer incubation time improves dose response curves, but cell health is poor
- ✓ Variability of untreated samples still remains high

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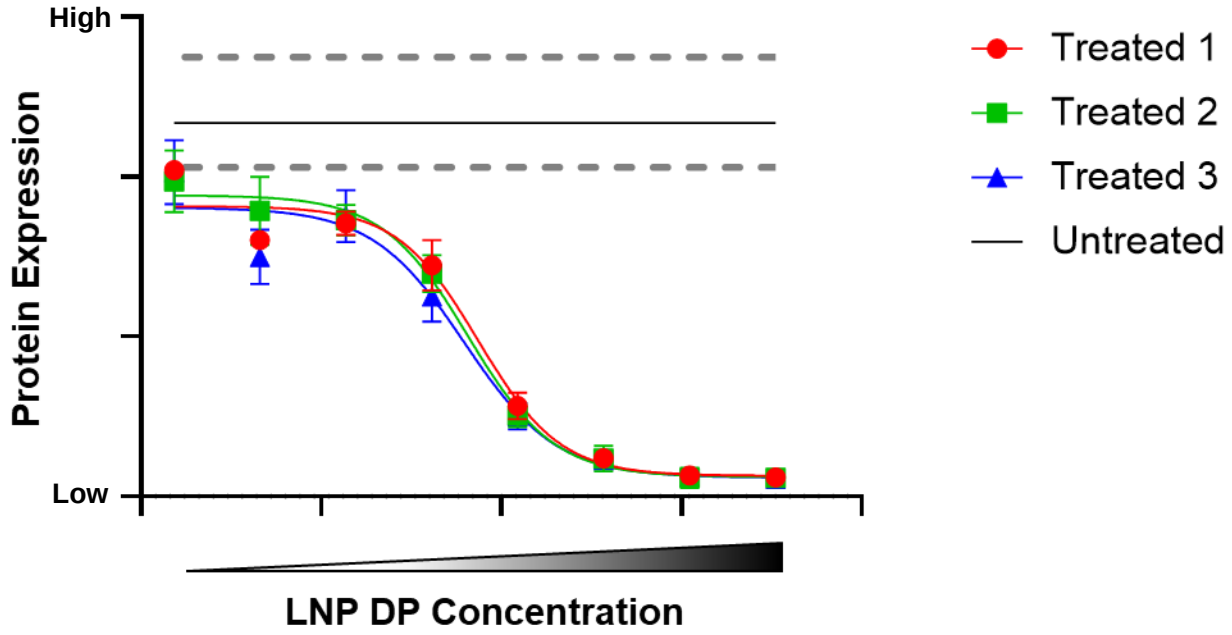
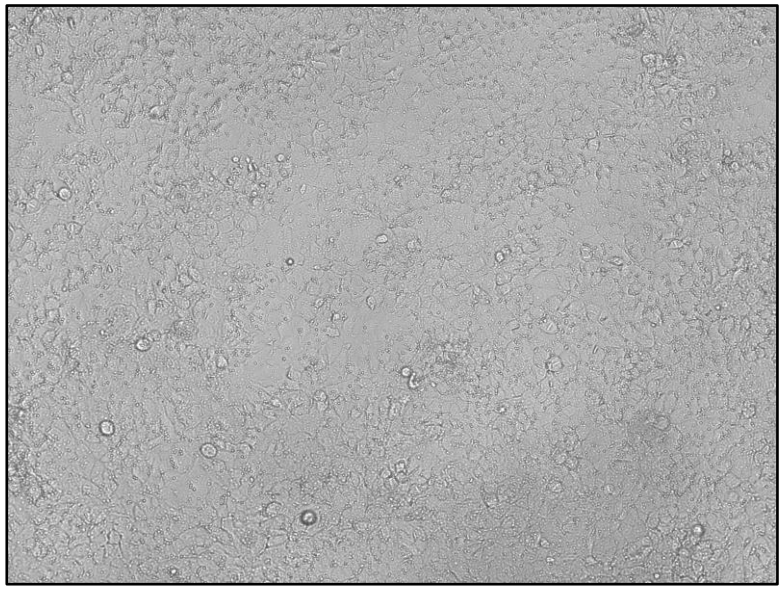
Additional Assay Optimization Identified Suitable Conditions for Continued Development





- ✓ Improved dose response curves with upper and lower asymptote
- ✓ Reduced variability of untreated samples
- ✓ Cell health is maintained during the assay

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Supporting Potency Assurance at the Drug Substance Level

Target-specific sgRNA

- Target region provides genomic specificity
- Scaffold region interacts with Cas9 protein in RNP formation
- sgRNA has no biologic activity on its own
- Example activity assays
  - Biochemical or cell-based to measure binding or DNA cleavage
  - Cas9 protein & DNA substrate must be incorporated in assay design for cleavage
- Paired with complementary assays to determine sequence identity, purity, molecular weight, and chemical modifications

Cas9 mRNA

- mRNA ORF encodes Cas9 protein
- 5' cap, UTRs, and polyA tail are required for efficient protein translation
- mRNA has biologic activity on its own and expresses Cas9 protein
- Example activity assays
  - Cell-free translation or cell-based
  - Measure protein expression or nuclease function (i.e. cleavage)
- Paired with complementary assays to determine sequence identity, purity, 5' capping, and polyA tail length

Activity of the drug substances and CRISPR mechanism is also confirmed by both drug product potency assays

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Key Takeaways

- A modular approach allows Intellia to target multiple indications with modifications at a specific location on the sgRNA while utilizing the same mRNA and LNP
  - Enables a more rapid path to clinic
  - Improves familiarity with complex CMC packages
- Developed a potency assurance strategy for Intellia's *in vivo* gene knockout programs
  - Drug product potency assays are used to measure the direct action (genomic editing) and the intended effect (protein reduction)
  - Protein reduction assays will require more optimization on a target-by-target basis
  - Assays at the drug substance level help support potency assurance

Q&A

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