Challenges in Development of *In Vivo* Gene Editing Therapeutics

Committee for Advanced Therapies (CAT) Workshop on Gene Editing

Kristy M. Wood, Ph.D.

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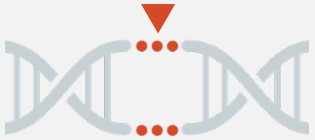
Gene Editing Technology has the Potential to Develop Differentiated Medicines for Patients from Existing Treatment Options



Treat patients
at the **root cause**
of their disease

Single dose treatment
with potential
lifelong benefit

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Gene Editing Can Encompass a Wide-Variety of Permutations Based on the Desired Therapeutic Approach

Therapeutic Approaches	Editing Tools	Editor Formats	Delivery Systems	Manufacturing
In vivo	Cleavase	Nucleic acid	LNP (non-viral)	Synthetic
Ex vivo (autologous)	Base Editor	Protein	Vector (viral)	Biologic
Ex vivo (allogeneic)	DNA Writer	Combination (RNP)	Electroporation	Cellular

- ✓ Any combination of editing tools, editor format, and delivery system may be selected
- ✓ Requirements for non-clinical safety and efficacy studies will ultimately vary
- ✓ Editing tool classification could range from starting material to drug substance and/or drug product

A single regulatory guidance covering all potential options for gene editing therapeutics is challenging → Focus on risk-based guidance, allowing for flexibility as technology matures

RNP: ribonucleoprotein **LNP**: lipid nanoparticle

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Three Ongoing Phase 3 Studies

Hereditary Angioedema (HAE)

Transthyretin (ATTR) Amyloidosis with Cardiomyopathy (CM)

Transthyretin (ATTR) Amyloidosis with Polyneuropathy (PN)

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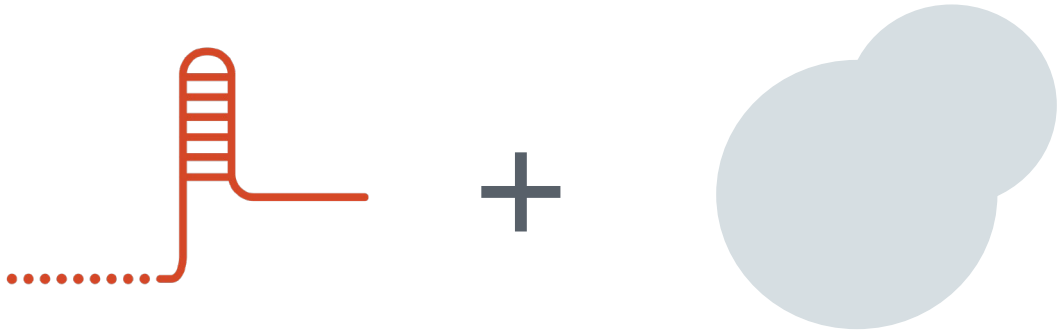
Gene Editing Starts with CRISPR/Cas9, a Two-Part, Programmable System

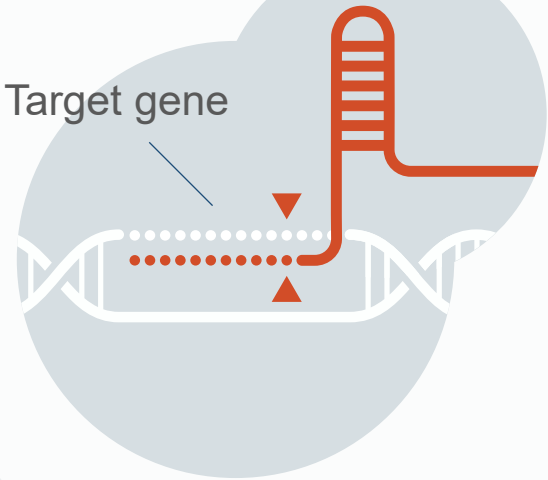
FOUNDATIONAL CRISPR MACHINERY



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 ✓ Potentially address any site ✓ Target multiple DNA sites

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In Vivo

CRISPR is the therapy

GENETIC DISEASES

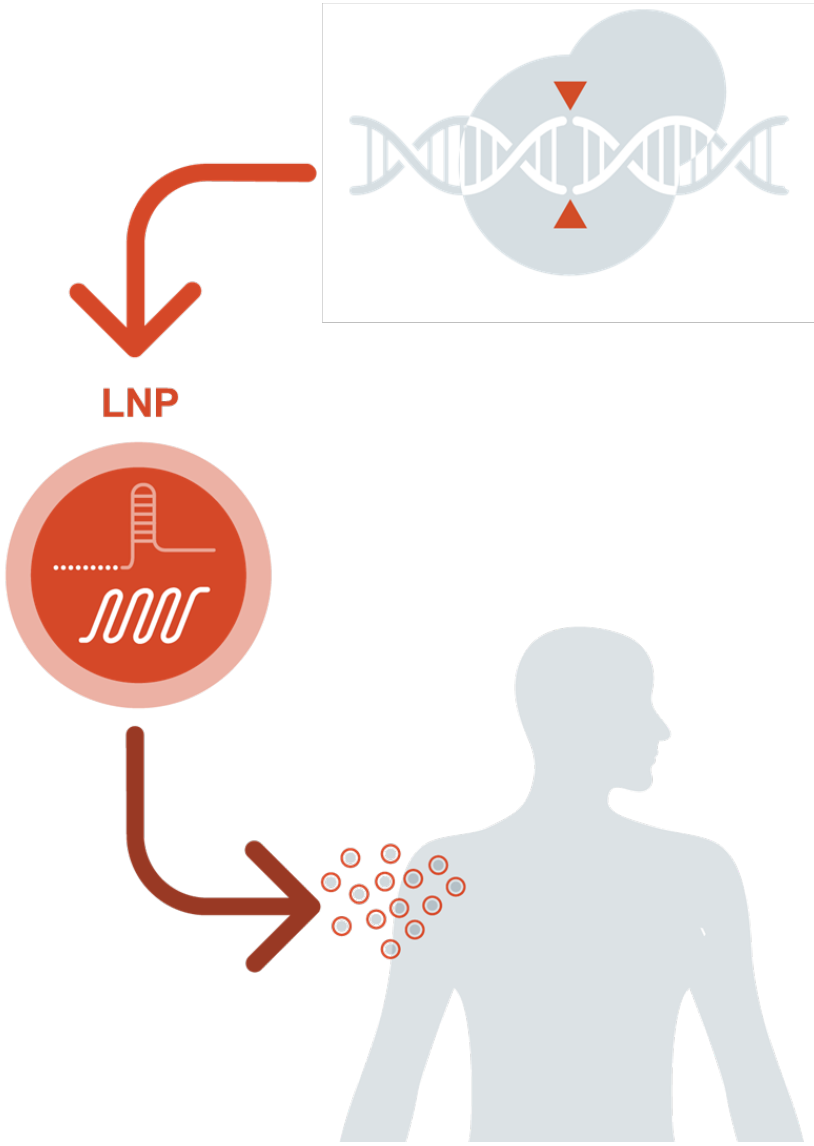
Potential Strategic Advantages:

Potential single dose treatments

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

Potential for permanent gene knockout or gain of function by selecting the appropriate editing tool

Capable of delivering to multiple tissue types for various therapeutic applications



LNP: lipid nanoparticle

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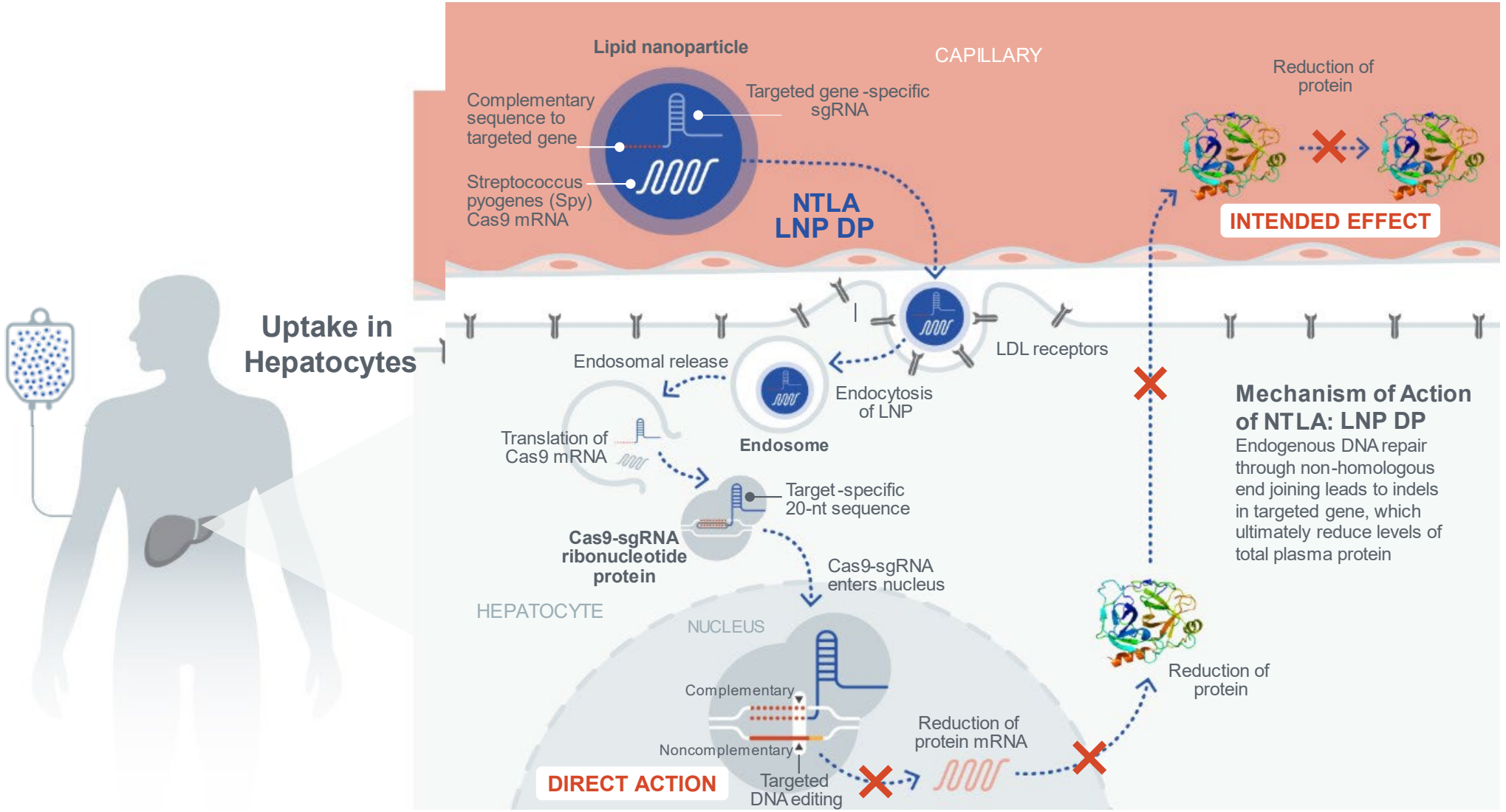
Liver LNP Programs: *In Vivo* Knockout CRISPR Mechanism of Action

Intended DNA Repair Leads to Indels in a Targeted Gene and Reductions in Levels of Total Plasma Protein



LNP: lipid nanoparticle; **sgRNA**: single guide RNA; **LDL:** low-density lipoprotein

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Case Study 1

Clinical Considerations

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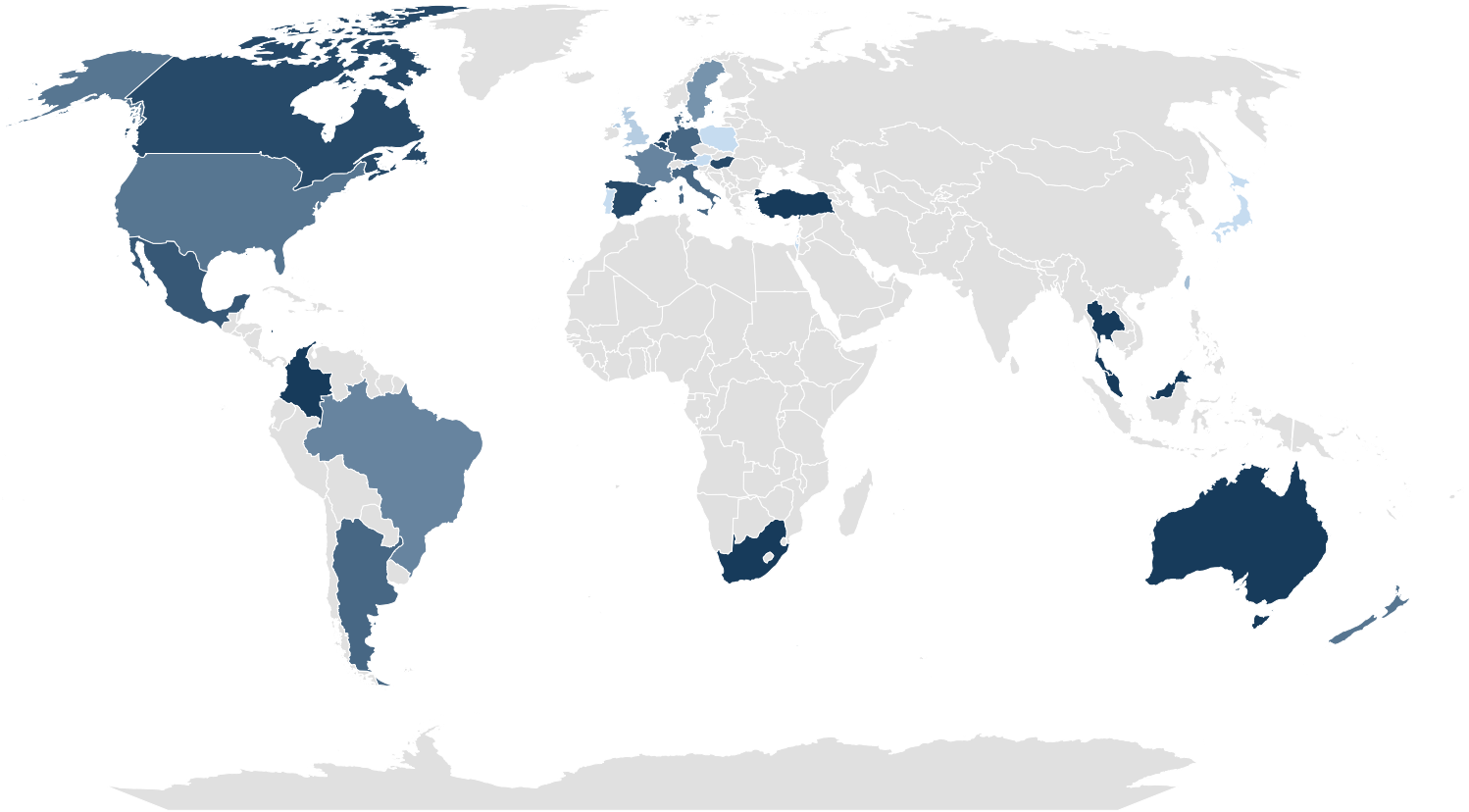
Global CTA Submissions Covering Three Phase 3 Trials



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> 30 Countries

> 15 RA Meetings

> 300 Queries

RA: regulatory authority

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A Successful Global Regulatory Submission Requires a Strategic, Well-coordinated Approach that Considers the Unique Requirements of Each Country

Country-Specific Regulations

- Each regulatory authority has unique requirements for documentation, review and approvals
- Standards for safety, efficacy and quality may vary from country to country
  - Requests for additional information during CTA review
  - Post-approval safety reporting
- Local advisors play a crucial role

Harmonization and Adaptation

- ICH efforts have harmonized regulations; however, differences still exist
  - GMO Regulations
- Submission documents need to be adapted to local language and cultural context
  - Translation requirements

Time and Resource Management

- Meticulous project management and resources availability is essential to managing multiple simultaneous submissions

CTA: clinical trial application; **ICH**: International Council for Harmonisation; **GMO**: genetically modified organism

Best Laid Plans are Not Always Successful

- Primary follow-up and secondary follow-up protocols reviewed by different divisions within the same health authority which resulted in conflicting guidance
- Prolonged review timelines due to several rounds of informal and formal review of responses and lack of direct communication between Sponsor and reviewer
- Despite approval of the protocol in over 25 countries including US and EU, a significant number of unique changes were requested

Opportunities may exist for mutual recognition for global protocols or enhance regulatory flexibilities to allow country populations to safely access potential life-changing therapies

Case Studies 2 and 3

sgRNA Purity/Impurities and Off-Target Approach

Impurity Control for a Novel Excipient

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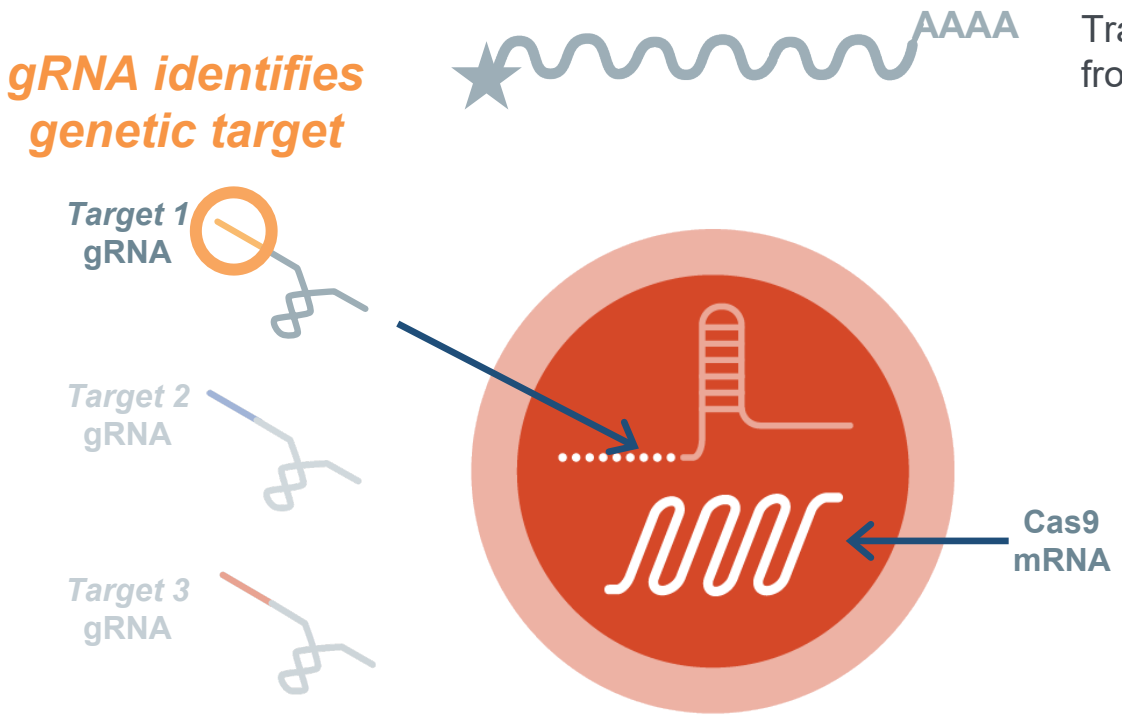
A CRISPR *In Vivo* Knock-out Product Requires Development of Two Drug Substances and One Drug Product with a Novel Excipient

LNP Delivery System:



Transient Cas9 expression from mRNA

gRNA target site specificity defined by 20mer at 5' end

LNP is a multi-component lipid formulation

DS1: Target-gene sgRNA
DS2: Cas9 mRNA
DP: LNP containing RNA cargo and novel lipid excipient

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

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Intellia Leverages Synthetic Manufacturing for Drug Substances and Drug Product

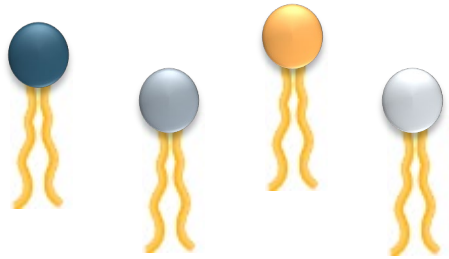
sgRNA

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

Cas9 mRNA

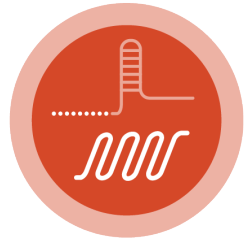
- ~4400 nts in length, ~1.5 MDa
- Encodes for Spy Cas9 protein
- Uses enzymatic synthesis approach

Multiple Lipids



- Includes compendial, non-compendial and a novel lipid excipient

LNP



- Combines both RNA drug substances in an LNP
- Made by self-assembly process with controlled dilution

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

Identity | Strength | Safety | Purity | Potency | Stability

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sgRNA Manufacturing Overview

- Synthetic Molecule (80 to 120-mer)
  - Solid phase synthesis (79 to 119-cycles): "*Upstream*"
  - Work up, solvent exchange, purification: "*Downstream*"

Solid Phase Synthesis → Cleavage & Deprotection → Crude UF/DF → Purification → UF/DF (Concentration, Desalting) → Filtration & Packaging

- Purity/Yield Readout (after Cleavage & Deprotection)
- Purity/Yield & Identification Readout (after Crude UF/DF)
- Purity/Yield Readout (after Purification)
- Release Testing (after Filtration & Packaging)

- Leverage many knowns from oligonucleotide manufacturing used in marketed products (i.e., siRNA, ASOs)
  - Standard phosphoramidite chemistry with similar raw materials
  - Approach to process and control strategy are independent of oligonucleotide length
- An established sgRNA process can be applied across target sequence changes
- Key Challenges
  - ***Process and analytics do require optimization for long-mer oligonucleotides***

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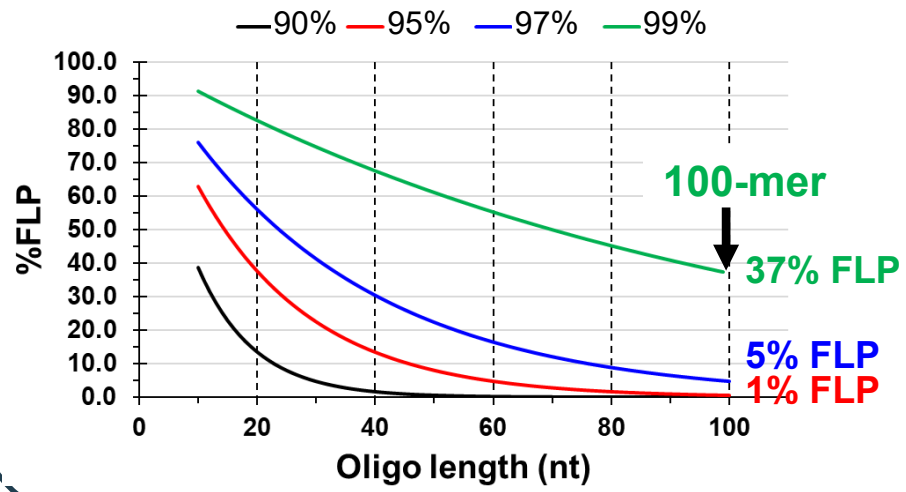
Achieving High Purity Long-mers is Limited by Synthesis Efficiency and Complex Impurity Profile

Theoretical purity is proportional to per-cycle efficiency and exponentially decays with oligo length

%FLP = pre-cycle efficiency $^{(n-1)}$



Traditional 20-mer

19 Cycles x 4 reactions =
76 reactions

For 99% per-cycle efficiency:
- 82% purity
- 18% impurities

2^N Possible distinct impurities:
$\sim 1.0 \times 10^6$

VS

100-mer

99 Cycles x 4 reactions =
396 reactions

For 99% per-cycle efficiency:
- 37% purity
- **63% impurities**

2^N Possible distinct impurities:
$\sim \mathbf{1.3 \times 10^{30}}$

- High-purity crudes at 99% efficiency are readily achieved for 20nt “short-mers” with current analytical methods capable of identifying and characterizing impurities.
- Low crude purity **~37%** at 99% efficiency and complex impurity profile of 100nt “long-mers” presents significant challenges for synthesis optimization, purification, recovery and analysis.
- FDA recommends % FLP purity > 80%; can result in significant impact on final yield

FLP: full-length product

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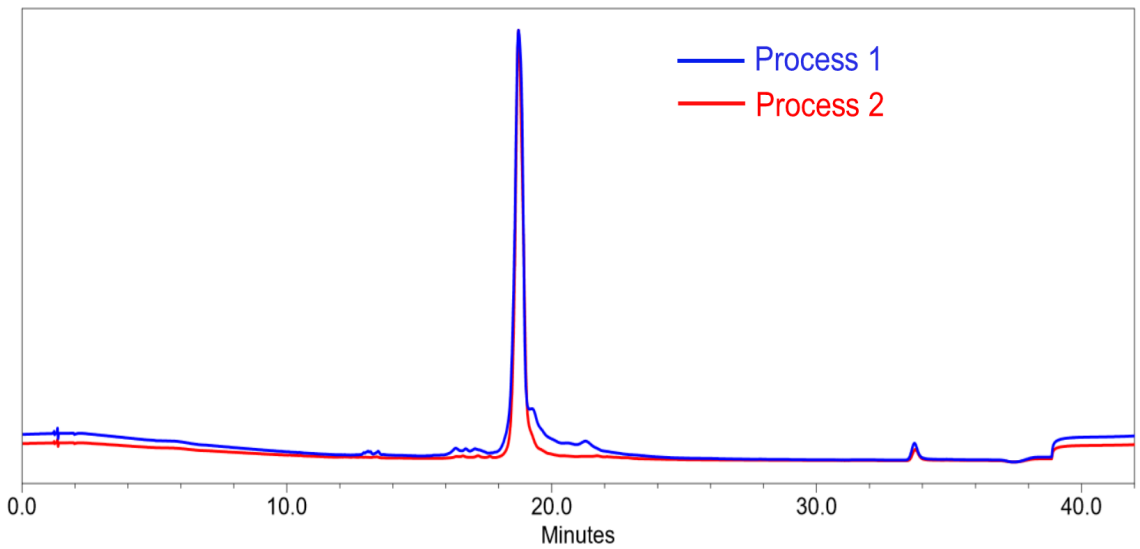
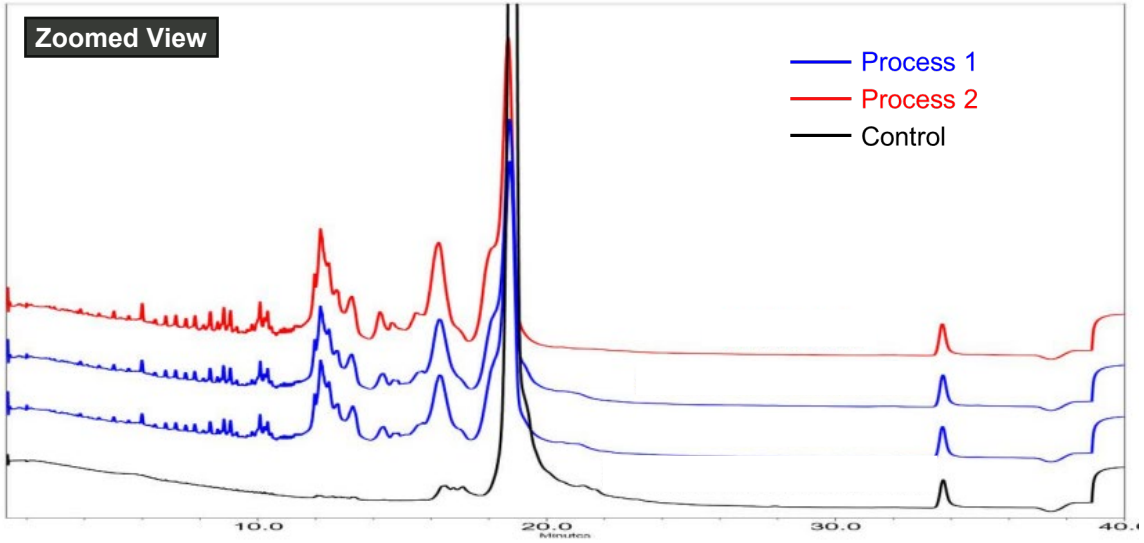
Implementation of Process Changes Requires an Assessment of Comparability

- Comparability included the following assessments:
  - Release data
  - Extended characterization testing (i.e., activity, impurity analysis)
  - Stability comparison (consistency in stability-indicating attributes, including a side-by-side thermal stress)

Purity & Impurity Profile



Side-by-side After Thermal Stress



Overall improved purity and impurity profile for Process 2
Similar degradation pathways and impurity profile after stress conditions

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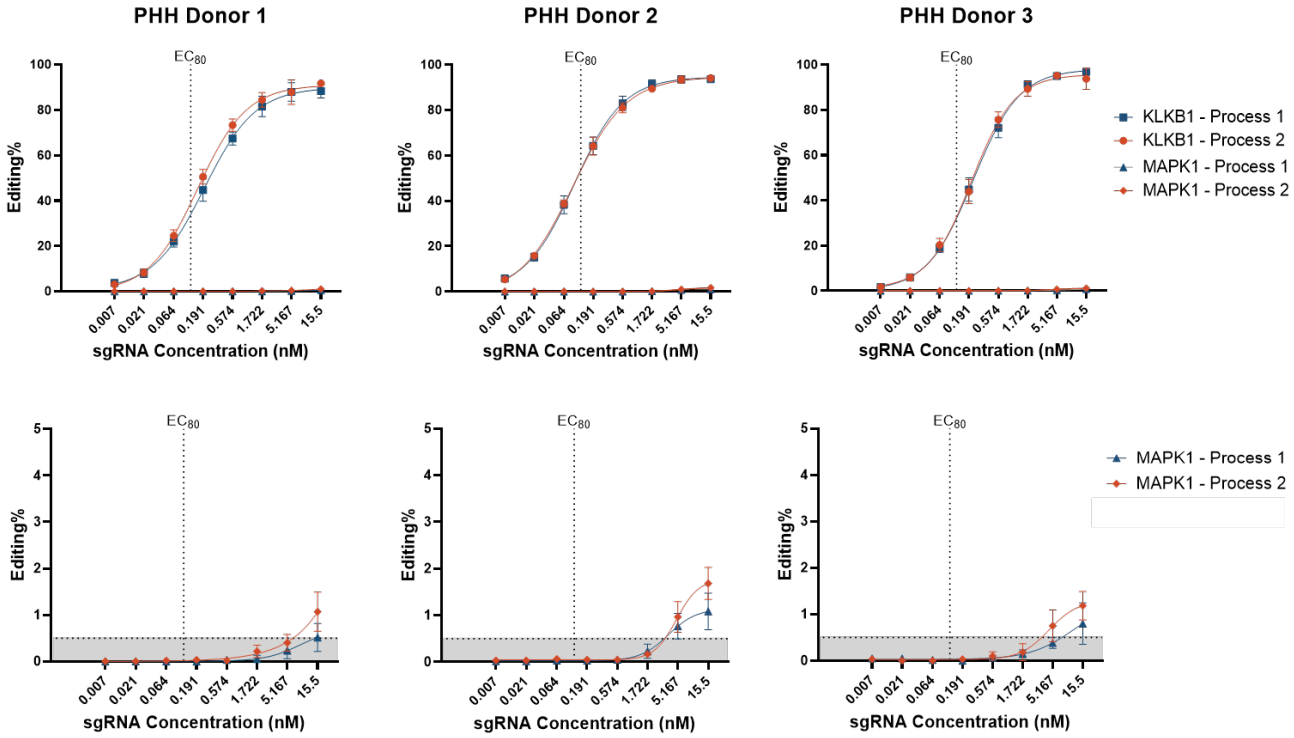
Consistent On-Target and Off-Target Profiles Observed Across Multiple sgRNA Processes and Multiple Donors of Primary Human Hepatocytes



EC_{80}: concentration inducing 80% of maximal effect; **sgRNA**: single guide RNA
The gray boxes indicate values that fall below the level of quantitation (0.5%).

- ✓ Demonstrate batch to batch consistency through analytical controls (in-process, release, & stability)
- ✓ sgRNA product purity and grouped impurities can be varied in on- & off-target analysis by both process improvements & supersaturation
- ✓ Development of impurity classification system helps define potential impact to off-target profile from individual impurities (i.e., 3' truncation vs 5' substitution)

Use a risk-based framework to determine when off-target editing characterization in primary cells should be conducted.
Off-target analysis should not be required for all manufactured batches.

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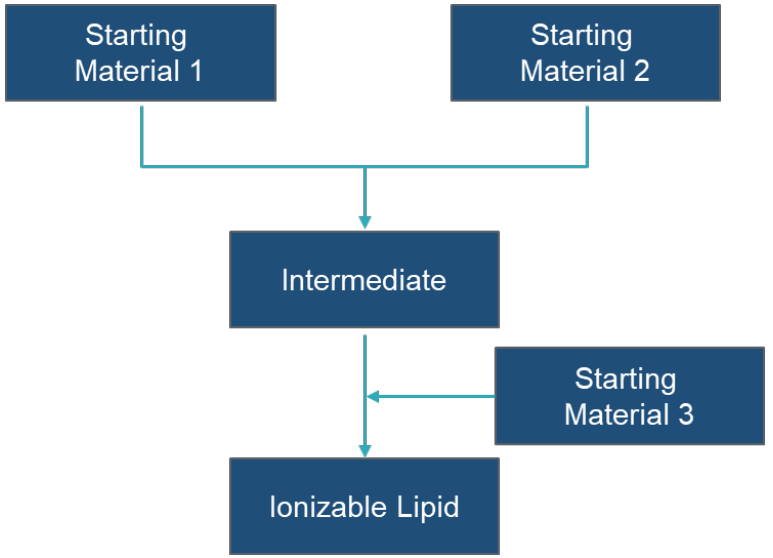
Development of an Impurity Control Strategy for a Novel Excipient Presented Challenges for a Single-Dose Therapeutic

- Ionizable lipid excipient:
  - Synthetically manufactured small molecule
  - Same route of synthesis throughout development
  - One of 4 lipid components used in drug product manufacturing
- Classification as a novel excipient requires same level of rigor as a drug substance
- Application of ICH Q3A for identification and qualification thresholds of impurities has minimal consideration for a single-dose therapeutic
- Health authority feedback throughout development lifecycle on impurity control strategy was inconsistent across agencies



ICH Q3A Thresholds

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

[1] The amount of drug substance administered per day

[2] Higher reporting thresholds should be scientifically justified

[3] Lower thresholds can be appropriate if the impurity is unusually toxic

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Considerations for a Patient-centric Impurity Control Strategy in Relation to ICH Q3A

Based on ICH Q3A Decision Tree

Structure Identified?	• Yes (identified impurities) • No → any known characteristics? (unidentified impurities)
Duration of Use	• Single Dose
Genotoxicity Studies	• Ames Testing (based on ICH M7) • In Vitro Mammalian Gene Mutation Test • In Vitro Micronucleus test
General Toxicology Studies	• Single dose studies to establish NOAEL • Determine safety margin at planned clinical dose for human exposure compared to toxicology species
Serious Adverse Effects	• None observed in Phase 1/2, Phase 3 studies across 2 *in vivo* KO products

- Impurity control strategy should ensure acceptable product quality for the intended use with a focus on safety and patient needs
- Use of toxicological justification in alignment with technical capabilities (process and analytical) and acknowledgement of the nature of the manufacturing process
- Applying the spirit of ICH Q3A and taking into account supporting factors:
  a) Existing structural and process knowledge
  b) Duration of use and maximum exposure to the Novel Excipient
  c) Toxicological Assessments
  d) Clinical Exposure

→ *In vivo* gene editing drug products are designed as a one-time dose, which significantly reduces the potential toxicological burden

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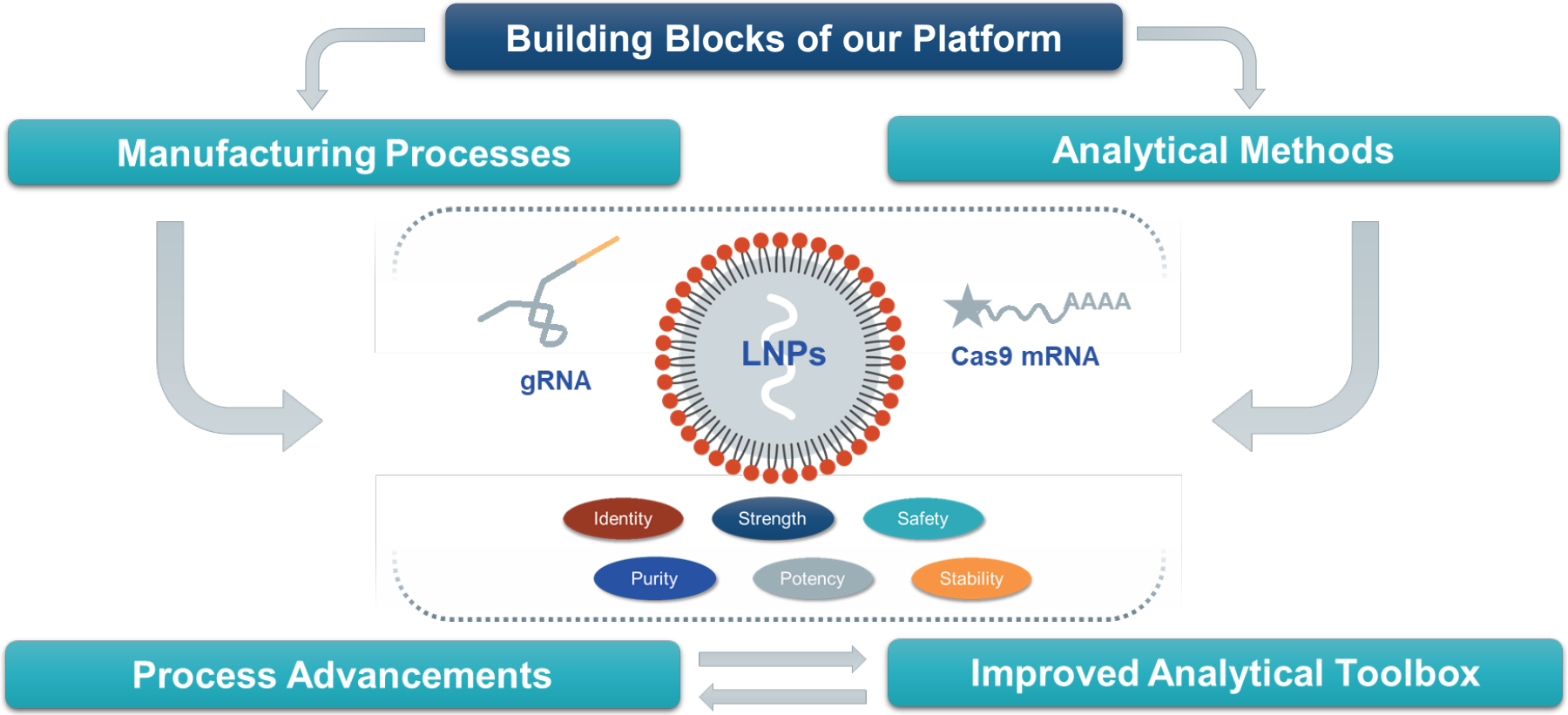
Leveraging Prior Platform Knowledge Over the Development Lifecycle Benefits Current & Future Programs



- ✓ Significant time and resources are used in initial platform development
- ✓ Platform design, process, and analytical knowledge can be quickly deployed for subsequent programs with increased probability of success
- ✓ Future programs benefit from regulatory learnings and interactions around the platform

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KEY TAKEAWAYS

- Gene editing regulatory guidance needs flexibility due to technology diversity
- Global regulatory harmonization remains a challenge to efficiently advancing potentially life-changing therapies
- Single-dose therapeutics may require new approaches to safety assessments and impurity control strategies

Recommendations:

→ Focus on risk-based guidance that allows flexibility as the technology continues to mature

→ Consider specialized reviewers for ATMPs who are educated on complexity of gene editing technologies

→ Establish mutual recognition across countries, especially as programs advance to Ph.3 and become global clinical trials

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Q&A

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