ASGCT and FDA Liaison Meeting

February 23, 2024
1:30-3:30 PM ET

American Society of Gene + Cell Therapy

Comprehensive On- and Off-target Analysis for Genome Editing

Presenters:

Daniel Bauer, MD, PhD

Jessica Seitzer

Nate Manley, PhD

American Society of Gene + Cell Therapy

Introduction

Daniel Bauer, MD, PhD
Harvard Medical School

Outline

1. Introduction
2. Comprehensive on-target assessment and characterization
3. Comprehensive genotoxicity evaluation in support of CRISPR/Cas9 FIH trial applications: A case study
4. CMC considerations for off-target genome editing analysis
5. Conclusion
6. Q&A

Goals of the Presentation

ASGCT members are active at all levels of genome editing product development, from early discovery to commercialization. The goal of this presentation is to share our members' perspectives on areas that would benefit from FDA's public insights.

We hope this presentation is helpful to inform the Agency's higher-level thinking about expectations of sponsors, acceptable methodologies for off-target assessments, and a regulatory approach that protects patient safety while also keeping therapeutic development on an efficient and attainable path.

ASGCT recommends that FDA consider ways to share information (via new guidances, revisions to existing draft guidances, FAQs, workshops, etc.) about the issues discussed in this presentation.

We want to thank FDA for finalizing the guidance *Human Gene Therapy Products Incorporating Human Genome Editing*.

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Definitions

For this presentation, therapeutic genome editing technologies are defined as those that enable programmable DNA sequence modifications in human cells with a therapeutic goal.

There are nuances between various types of genome editing technologies. For this presentation, we have used nuclease genome editing technology as the primary example. Other genome editing technologies have some shared challenges and considerations, which we have tried to emphasize here. Additionally, each genome editing technology may have some unique challenges and considerations.

Comprehensive On-target Assessment and Characterization

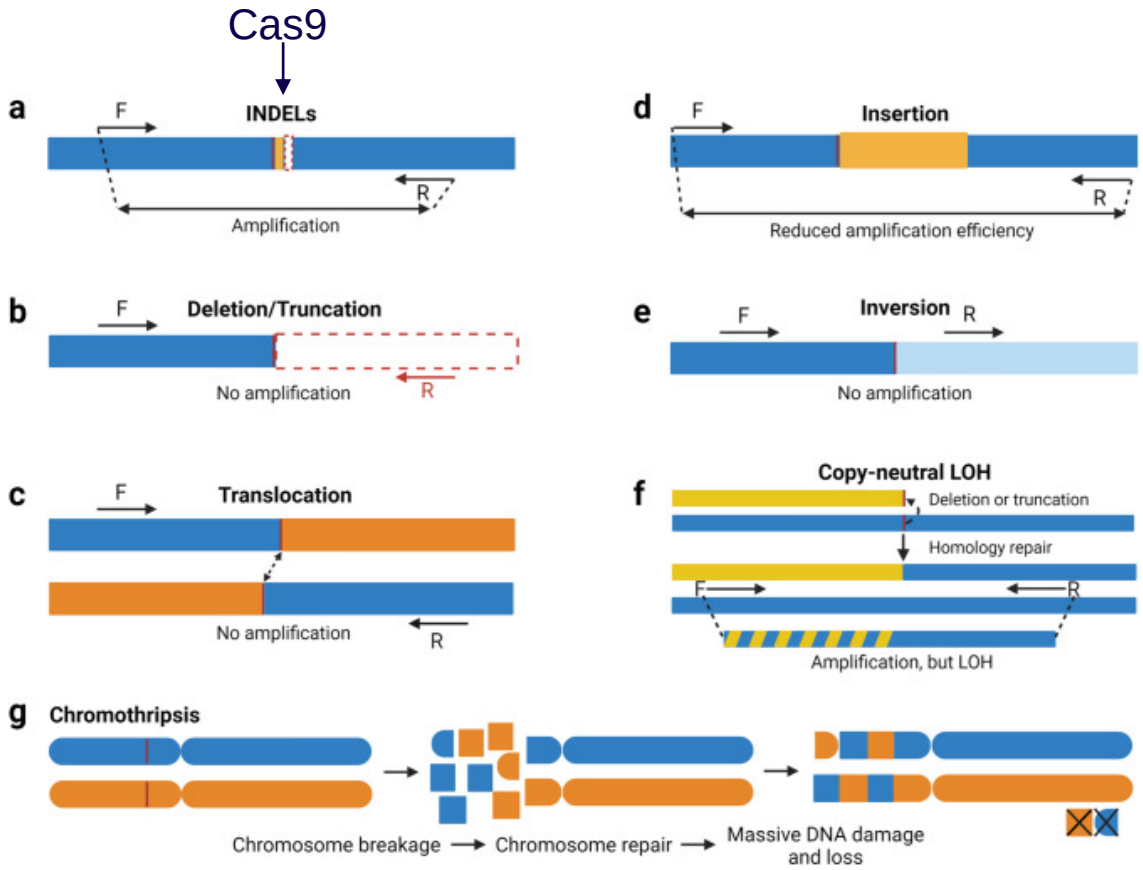
Daniel Bauer, MD, PhD
Harvard Medical School

Considerations for Productivity and Potency

- *Challenge*: Both the nature and frequency of minimally acceptable and ideal productive edits will vary by program. Examples:
    - For disrupting a noncoding regulatory element or creating a null mutation, numerous edit alleles could be considered productive.
    - For correcting a point mutation, only a single allele might produce the intended coding sequence result.
  - *Consideration*: Scientifically justified, fit-for-purpose genotyping methods should be acceptable to quantify productive edits.

- *Challenge*: Productive gene edit composition within the cell product is a highly predictive feature of product potency.
  - *Consideration*: Comprehensive assessment of gene edits, when scientifically justified, should be considered in product potency assurance. In certain cases, the gene edit profile itself might have advantageous features as compared to alternative bioassays for evaluating potency.

On-target Edits May be Heterogeneous, Including Short Indels and Structural Variants



- Standard short-amplicon sequencing cannot capture structural variants.
- Biological significance of any individual structural variant may vary and may be negligible.

Hunt *et al.* Hum Genet (2023) 142:705.

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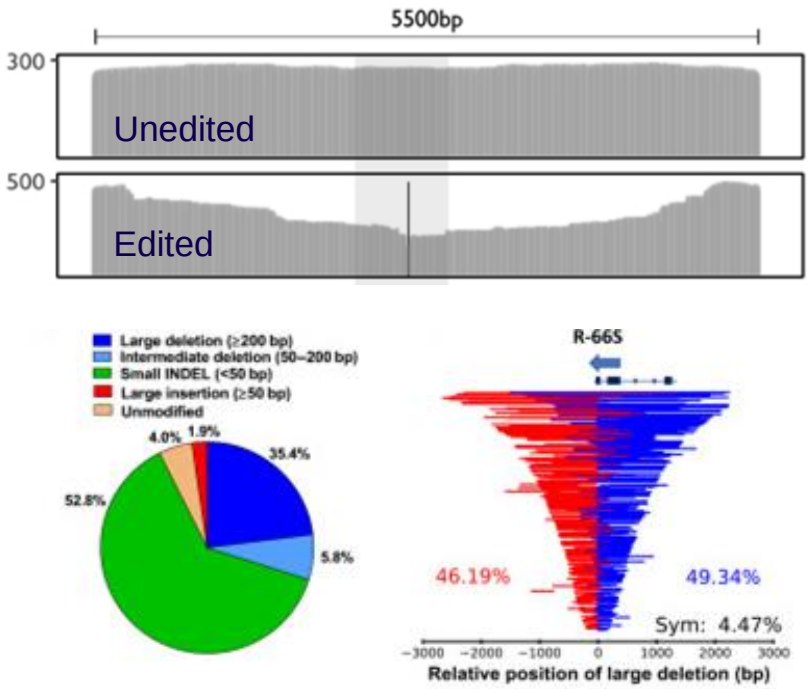
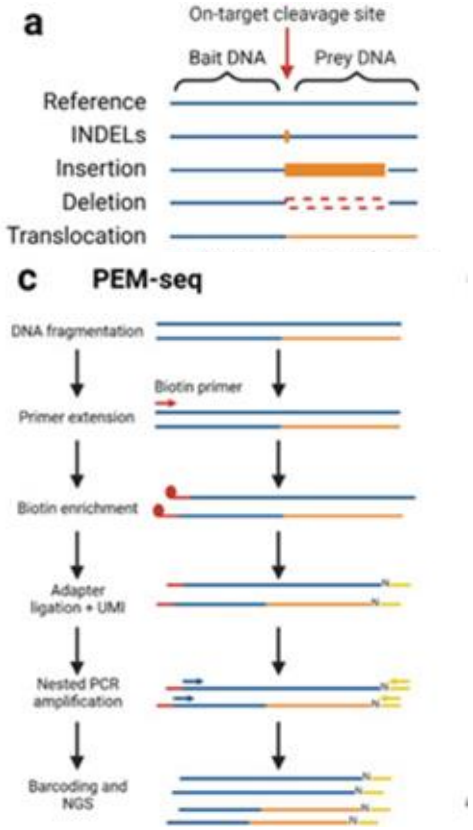
Multiple Assays for Comprehensive Detection of On-target Structural Variants

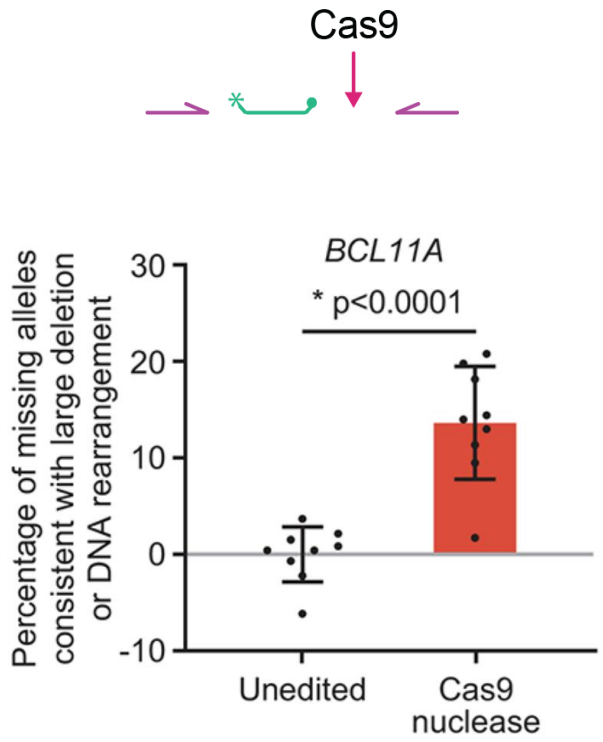
Long-read sequencing



Single-primer amplification



Droplet digital PCR



Numerous assays exist to detect on-target structural variants, although no single assay may fully characterize all classes

Kosicki *et al.* Nat Biotechnol (2018) 36:765. Park *et al.* Sci Adv (2022) 8:7676. Hunt *et al.* Hum Genet (2023) 142:705. Newby *et al.* Nature (2021) 595:295.

Considerations for Structural Variants

- *Challenge*: On-target edits may be heterogeneous and include short edits (like short indels or single nucleotide variants, SNVs) and structural variants (SVs).
  - *Consideration*: On-target edit characterization should be scientifically justified and fit-for-purpose to be capable to detect small indels/SNVs and SVs at the target locus.

- *Challenge*: Karyotype analysis is low-resolution, low-sensitivity, not specifically designed to capture risk of on-target locus SVs, and indirect in that cells must be cultured *ex vivo* which may lead to bias and artifact.
  - *Consideration*: Fit-for-purpose SV assessment may not necessarily require karyotype analysis if higher resolution, higher sensitivity, more targeted, and/or more direct assays are employed instead.

- *Challenge*: Structural variants could have uncertain biological significance.
  - *Consideration*: Risk assessment should include the biological relevance of structural variants.

Comprehensive Genotoxicity Evaluation in Support of CRISPR/Cas9 FIH Trial Applications

Jessica Seitzer
Intellia Therapeutics

Intellia is Building a Full-Spectrum Genome Editing Company

CRISPR-based Modular Platform

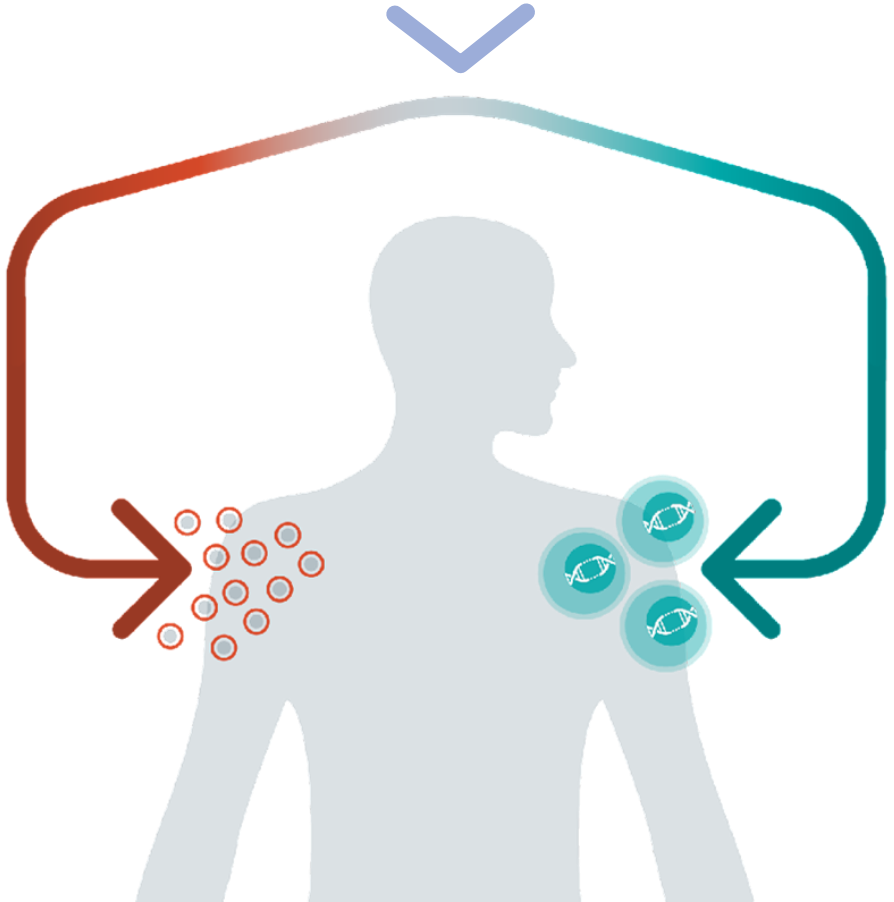
EMPLOY NOVEL EDITING AND DELIVERY TOOLS

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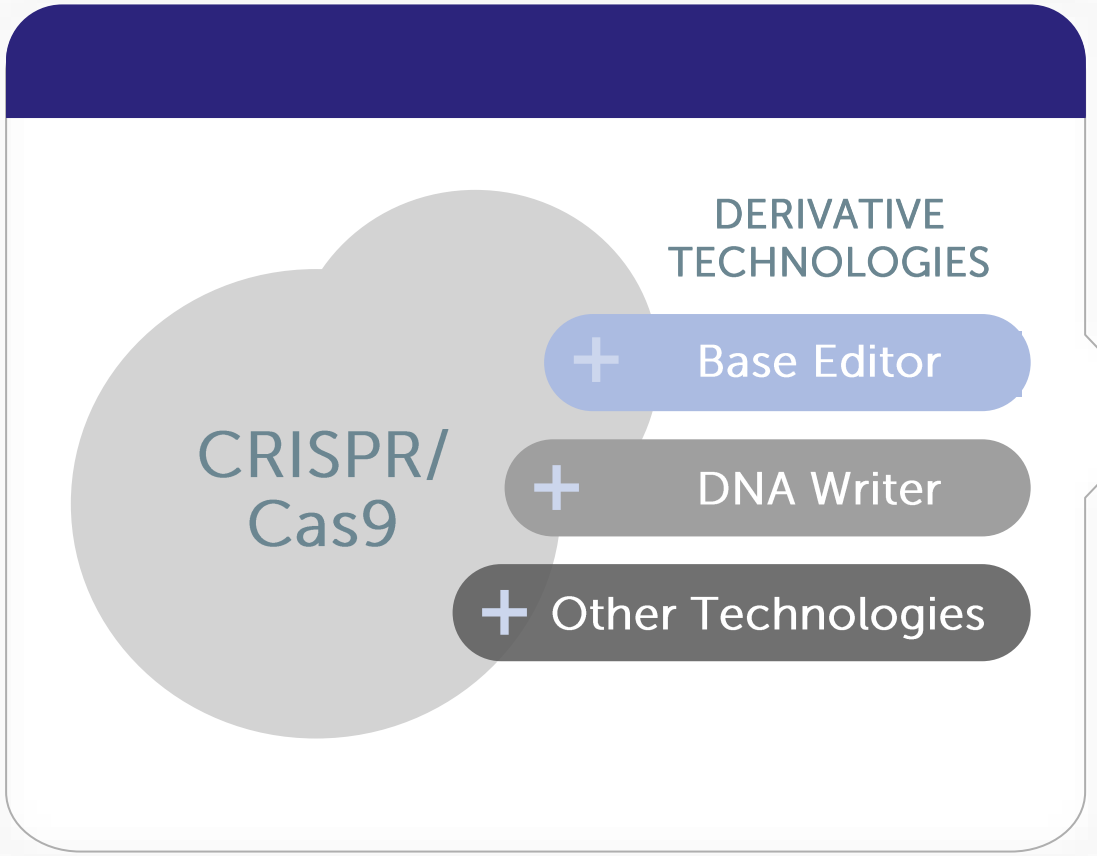
CRISPR/Cas9 and Derivative Gene Editing Technologies Can Be Used to Make Any Type of Edit



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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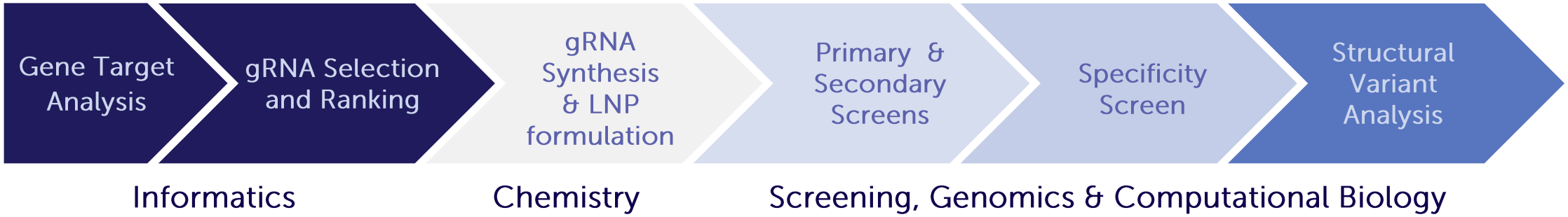
Comprehensive Genotoxicity Evaluation in Support of FIH Trial Applications

Case Study : NTLA-2002

Intellia's gRNA Selection and Qualification Platform



Goal is to select gRNAs with the highest on-target editing activity and no detectable off-target potential at multiples of intended human therapeutic dose.

NGS: Next-Generation Sequencing

Incorporating Genomic Diversity in gRNA Selection and Characterization

On-Target

Pathogenic SNPs (ClinVar[1]) and common SNPs (≥ 1% allele frequency; gnomAD[2]) within target and PAM regions are identified computationally.

The effect of each SNP in disrupting editing is evaluated with CFD score.[3]

Off-Targets

SNPs with ≥ 0.1% allele frequencies (gnomAD) are incorporated into the human reference genome *hg38*

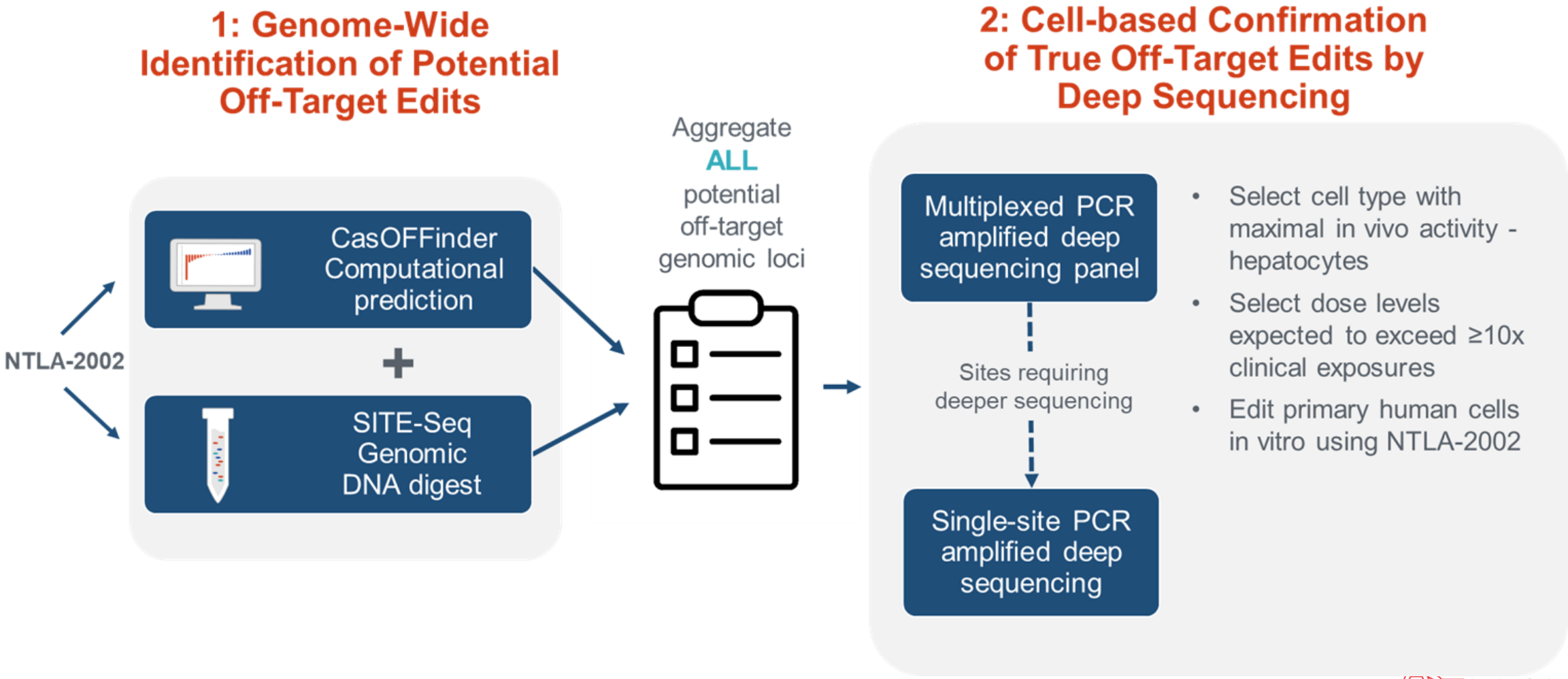
Common indels with ≥1% allele frequencies (gnomAD) are incorporated into the human reference genome *hg38* iteratively to create 30+ genomes.

Potential off-targets are discovered using updated CasOFFinder with the degenerate SNP genome and the indel genomes with up to 4 mismatches allowed.

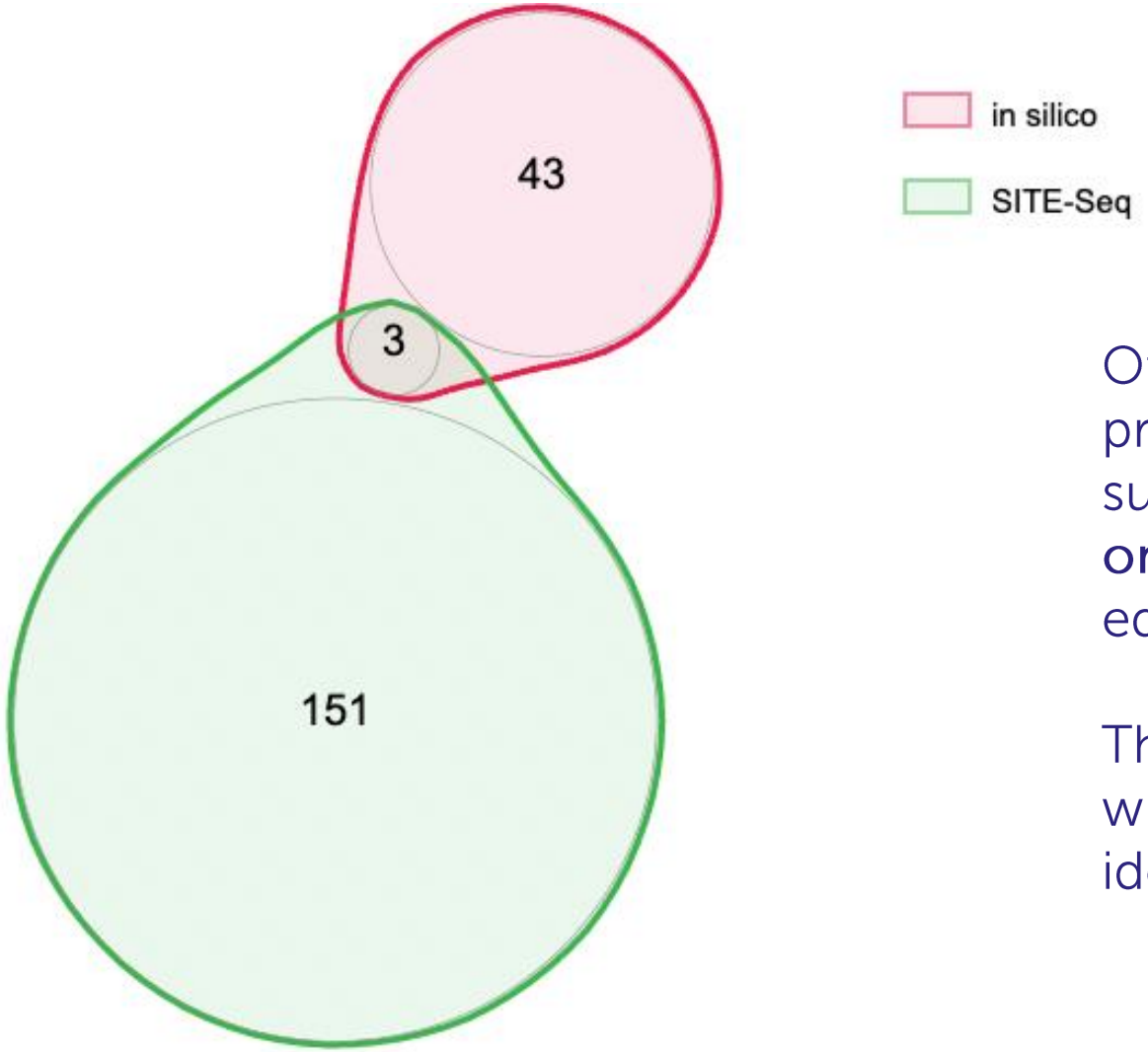
Novel off-targets overlapping with exonic regions are inspected manually considering positions of remaining mismatches in the target region, position of off-target in gene, and expression profile of the mRNA.

[1] https://www.ncbi.nlm.nih.gov/clinvar/; [2] https://gnomad.broadinstitute.org/; [3] Doench JG et al. 2016 Nature Biotechnology 34 (2): 184; [4] Bae S. et al. 2014 Bioinformatics 30(10):1473-5

Comprehensive 2-Stage Process to First Discover Potential Off-Target Editing Loci Genome-wide, then Sensitively Verify Indel Frequency at All Potential Off-Target Loci in Treated Relevant Cells



NTLA-2002 Potential Off-Target Sites Discovered by Cas-OFFinder and SITE-Seq Exhibit Minimum Overlap



Of the 197 sites tested in multiple lots of primary human hepatocytes treated with supratherapeutic concentrations of NTLA-2002, **only one site exhibited** confirmed off-target editing.

The confirmed off-target site was located within **intron 1 of the *MAPK1* gene** and was identified by SITE-Seq.

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Characterization of All Potential Off-Target Editing Loci Discovered in the Genome-Wide Identification Phase Enables Assessment of Biological Risk Potential

Genomic Location	Description	Biological Risk Potential
Exonic	Protein coding DNA segments	High
Intronic	Non-coding DNA segments within genes	Low
Intergenic	Stretch of non-coding DNA sequence between genes	Very low

Additional Characterization for Risk Potential

- Expression profile in cell type/types of interest
- Cancer Tier Annotation
- Proximity to nearest exonic regions
- Overlap with cis-regulatory elements (cCREs)
- Potential for novel splicing

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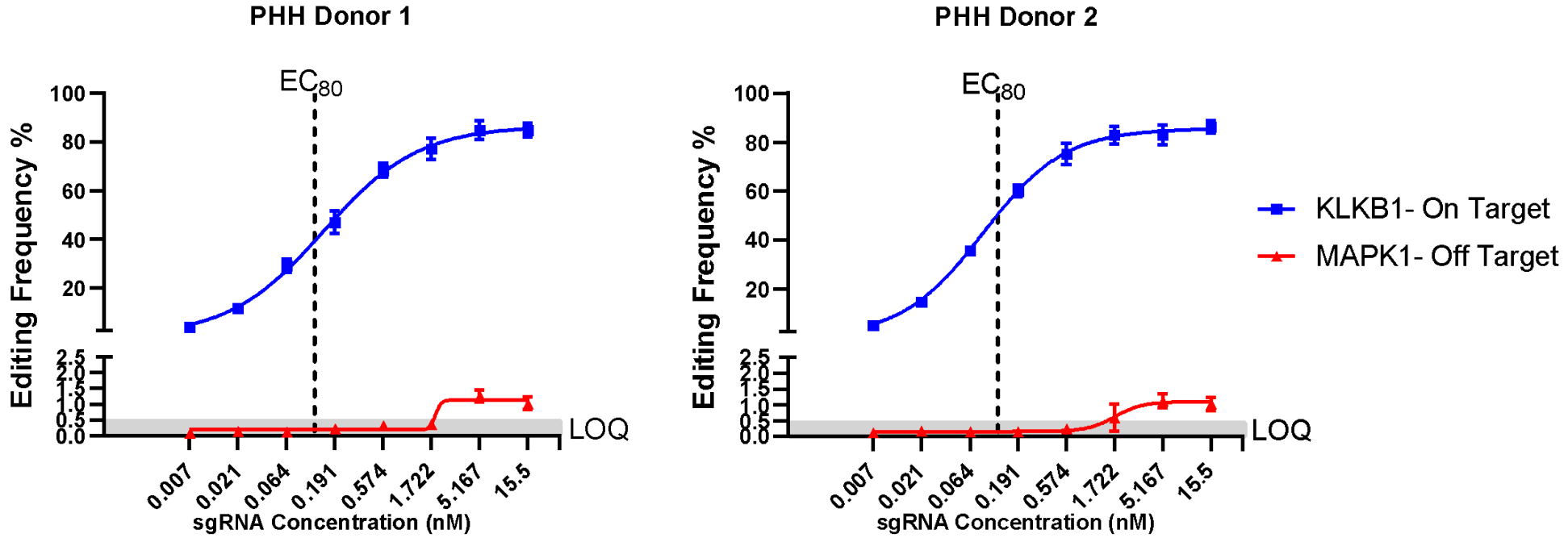
No Detectable Confirmed Off-Targets at Multiples of the Intended NTLA-2002 Human Dose in Primary Human Hepatocytes (PHH)



Dose responsive on-target editing with off-target editing at the *MAPK1* intronic locus was only detectable at supra-pharmacological concentrations (>40-fold above EC_{80}).

EC80, concentration inducing 80% of maximal effect; sgRNA, single guide RNA
The gray boxes indicate values that fall below the level of quantitation (0.5%).

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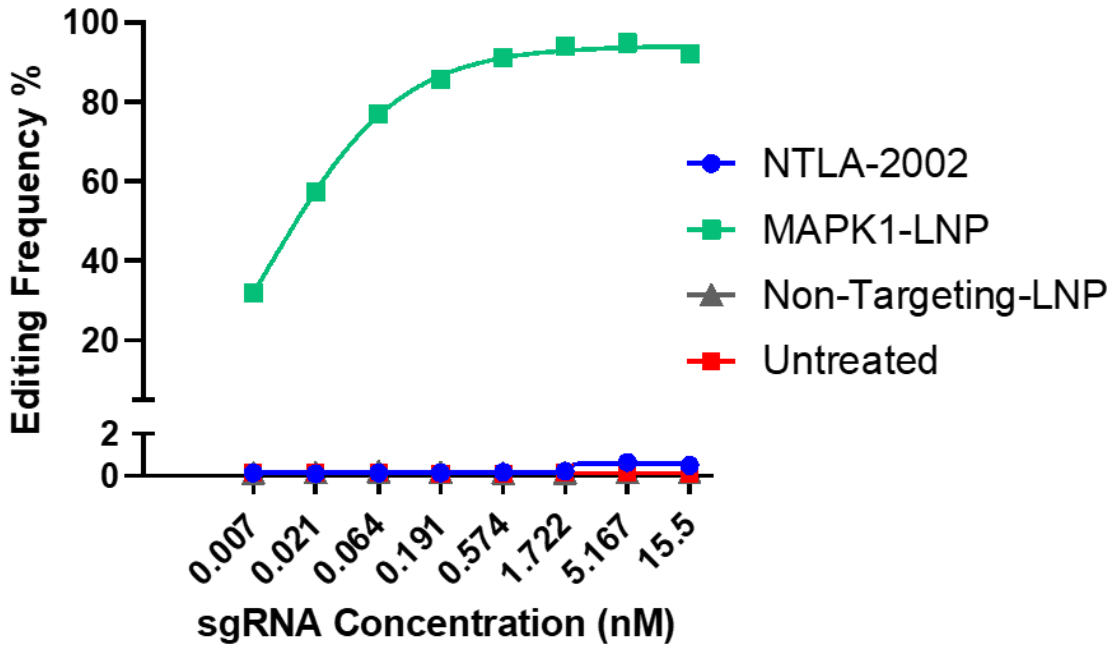
Additional Experiments Performed To Evaluate Any Potential Biological Risk From Off-Target Editing at the *MAPK1* Intronic Locus

- *MAPK1* encodes a kinase involved in proliferation, differentiation, transcription regulation, and development.
- Due to the location within an intronic region, editing at the *MAPK1* locus was not expected to impact *MAPK1* gene expression.
- To maximize *MAPK1* editing and any potential impact to *MAPK1* gene expression, a tool sgRNA with perfect homology to the *MAPK1* intronic locus was designed, synthesized and formulated into an LNP.
- An exaggerated *in vitro* pharmacology study was performed in primary human hepatocytes leveraging a dose response curve treatment followed by NGS to evaluate editing at the *MAPK1* locus as well as ddPCR to quantify *MAPK1* mRNA expression levels.

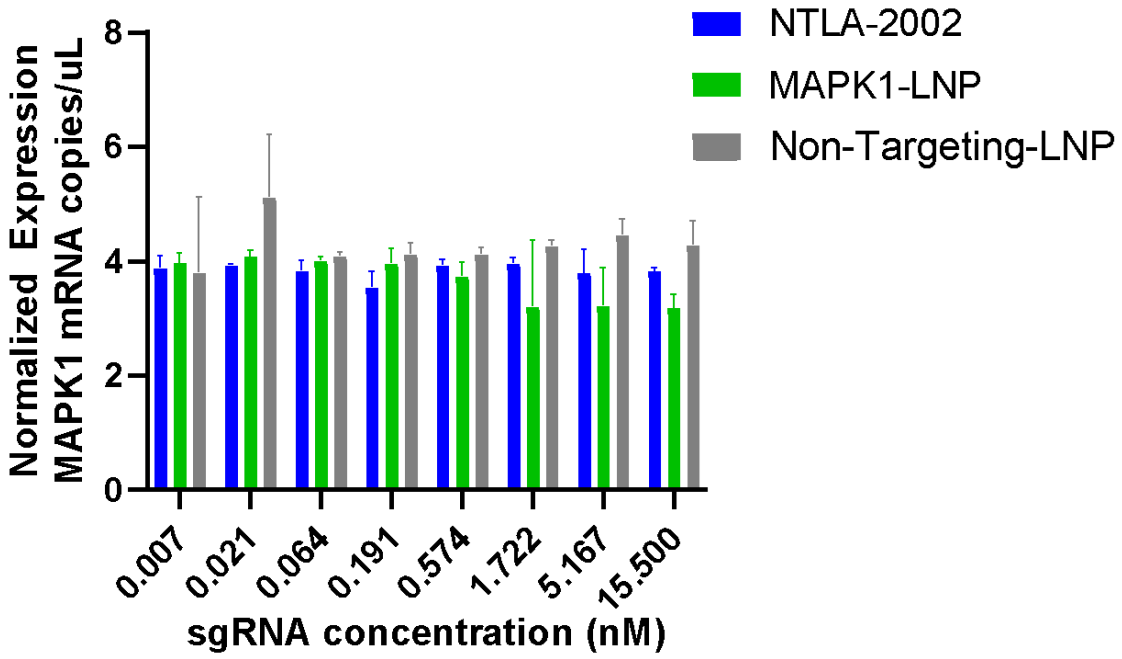
https://www.ncbi.nlm.nih.gov/gene/5594

Full Editing of *MAPK1* Intronic Locus with Tool sgRNA Has No Impact on MAPK mRNA Expression; Supports Low Biological Risk of NTLA-2002

MAPK1-LNP achieves saturating *MAPK1* intronic editing >90%



No statistical difference in *MAPK1* mRNA expression observed across multiple PHH lots at 10- and 14-days post treatment



No impact on MAPK1 mRNA expression coupled with no detectable editing at therapeutically relevant doses supports low biological safety risk.

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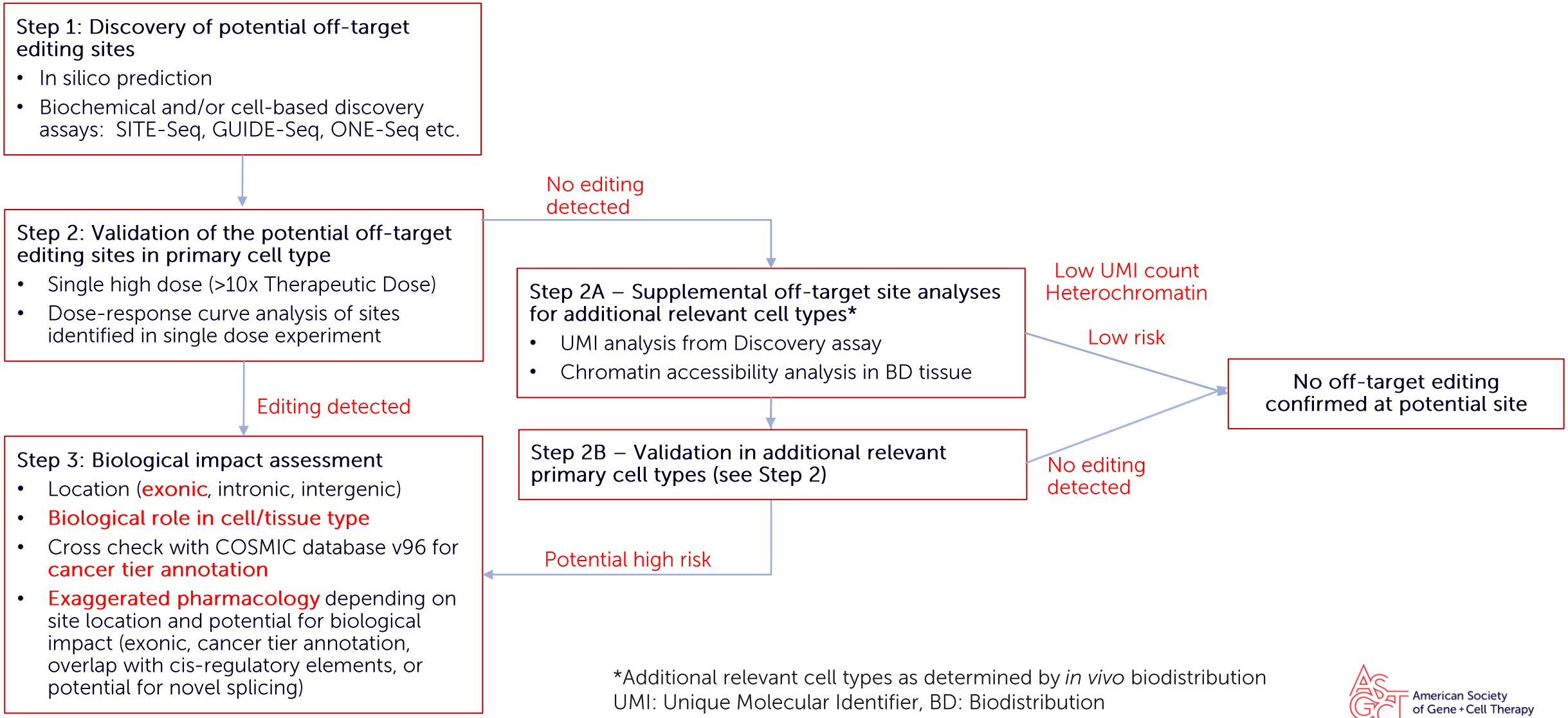
Consideration: Testing Strategy To Characterize Off-Target Editing Risk Potential



*Additional relevant cell types as determined by *in vivo* biodistribution
UMI: Unique Molecular Identifier, BD: Biodistribution

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Key Takeaways

Consideration: Testing strategy can be leveraged to characterize off-target editing risk potential

- Off-target genome-wide discovery and validation workflow in conjunction with a tiered approach to biological impact assessment can be applied to assess biological risk of confirmed off-targets in the primary cell type

- A tiered approach for additional relevant cell types (as determined by *in vivo* biodistribution) leveraging UMI count from the genome-wide discovery assay and chromatin accessibility can be implemented to assess risk of off-target editing potential in additional relevant cell types

CMC Considerations for Off-target Genome Editing Analysis

Nate Manley, PhD
Dark Horse Consulting

How do we ensure that editing reagent sourcing and the off-target analytical pipeline are properly aligned with product development?

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Overview of Preclinical CMC Activities for Gene-Edited Products

Editing reagents sourcing and off-target editing analysis play a key role during preclinical product development

Stages of product development & key CMC activities

Discovery | Preclinical | Clinical | Commercial

Process development

- Manufacturing Unit Operations
- Equipment Selection
- Raw Material Sourcing
- ★ sgRNA
- ★ Nuclease/enzyme
- ★ Donor sequence

Ph1 Process Lock

Analytical development

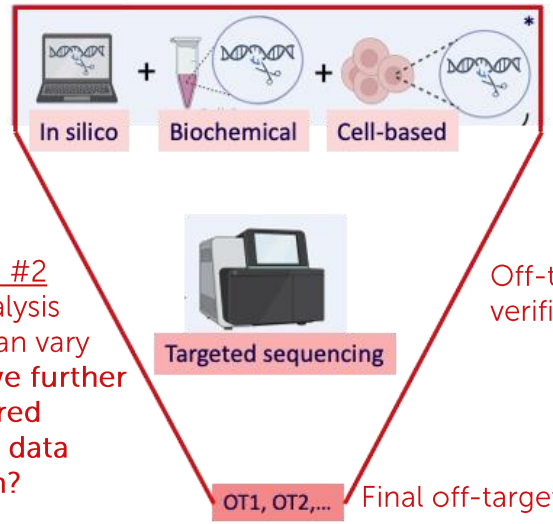
- Assay Development — Assay Qual
- Tracking & Trending
- Off-Target Editing Analysis
- ★ Off-target analysis
- ★ Translocation analysis
- ★ Whole genome integrity

DP Testing Plan

Consideration #1
Changes in editing reagent quality or manufacturing process can occur during development. **How does this impact off-target editing analysis?**

Consideration #2
Off-target analysis approaches can vary widely. **Can we further define preferred methods and data interpretation?**

General off-target analysis pipeline



★ Implementation of editing reagent sourcing and off-target analyses in a phase-appropriate manner can be challenging given their potential to gate other key CMC activities and overall product development.

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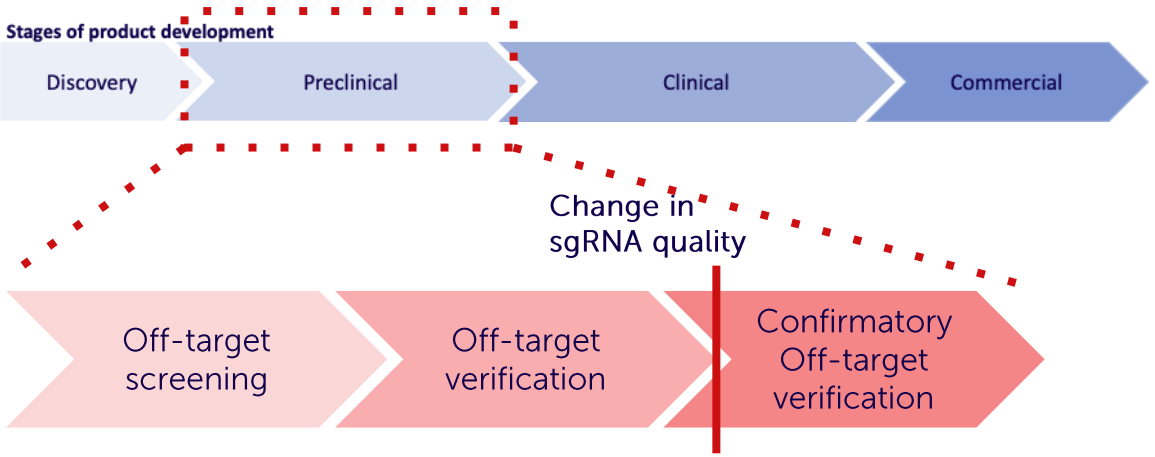
** Image adapted from Cromer et al. 2023*

CMC Consideration 1: Sourcing of Editing Reagents

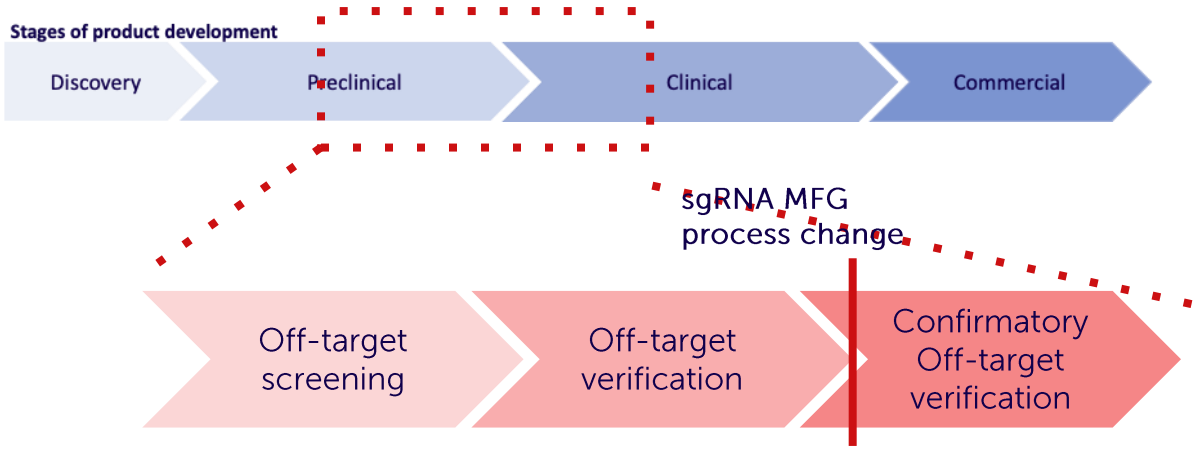
Use of phase-appropriate editing reagents for off-target editing analyses

Scenario 1



1. Initial off-target screening and verification performed with non-GMP sgRNA and nuclease.
2. Switch to GMP sgRNA and nuclease for Ph1 process.
3. Only confirmatory off-target verification performed with GMP sgRNA and nuclease.

Scenario 2



1. Initial off-target screening and verification performed with GMP sgRNA and nuclease prior to Ph1 clinical initiation.
2. Manufacturing process change during Ph1 to improve sgRNA yield.
3. Only confirmatory off-target verification performed with new sgRNA.

Consideration: Providing that the pre-and post-change editing reagents are appropriately characterized and shown to have:

- ✓ Similar purity profiles
- ✓ Similar % editing efficiency
- ✓ Similar indel profiles

It should be acceptable to forgo off-target screening with post-change editing reagents.

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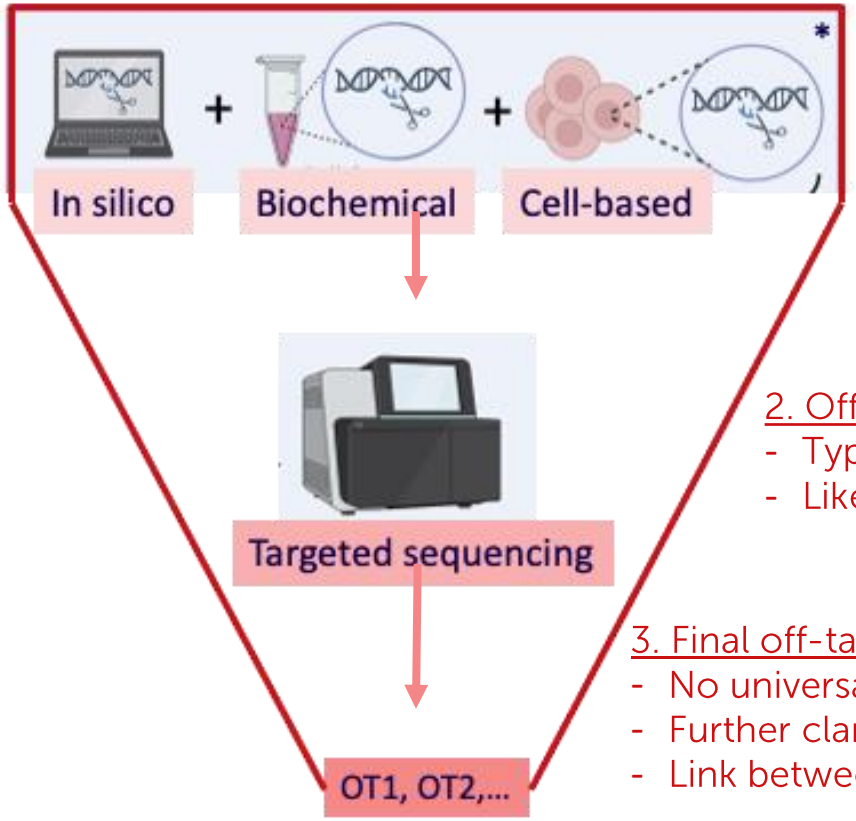
CMC Consideration 2: Off-target Analysis Pipeline & Results

Off-target editing analyses vary widely among programs; interpretation lacking standardization

General off-target analysis strategy is outlined in the 2024 finalized FDA guidance:

- Use of multiple methods, including at least one genome-wide approach
- Verification of bona fide off-targets using a sensitive method and target cells from multiple donors
- Appropriate controls to ensure integrity of results

However, recommended off-target analysis methods and best practices for data interpretation remain undefined



1. Off-target screening
- Many available methods
- Can result in a large number of candidate hits, especially if combining results of different methods
- Assay parameters not standardized (e.g., allowance for mismatches and gaps)

2. Off-target verification
- Typically small n, further impacted by donor variability
- Likely to get multiple hits at or near assay LLOQ

3. Final off-target list
- No universally agreed upon % editing threshold for calling bona fide off-targets
- Further clarity needed on the types of off-targets that warrant routine testing
- Link between off-target edits and product safety largely unknown

** Image adapted from Cromer et al. 2023*

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CMC Recap & ASGCT Committee Recommendations to FDA

CMC Recap

- ➢ Changes in editing reagent quality or production process are common-place during product development.
- ➢ When an editing reagent change occurs, it remains unclear whether the full off-target analysis pipeline is warranted.
- ➢ Off-target analysis methods and data interpretation vary widely among programs.
- ➢ The potential relationship between off-target findings and product safety remains largely unknown.

ASGCT Committee CMC Recommendations to FDA

- ❑ Further guidance should be provided to sponsors regarding proper characterization of editing reagents and how this information may be used to reduce comparability burden downstream of quality/process changes.
- ❑ FDA should consider additional ways to share information with sponsors (e.g., townhalls, workshops, companion guidances to the finalized 2024 Human Genome Editing guidance) to facilitate further standardization of off-target editing analysis and data interpretation.
- ❑ FDA should continue to evaluate the relevance of low frequency, non-disease related off-target events to product safety and encourage sponsors to adopt a risk-based approach to addressing off-target findings.
    - A key goal will be providing further guidance to the industry on translation of off-target data to routine drug product testing, taking into consideration donor-to-donor variability, assay sensitivity and biological relevance of bona fide off-target events.

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Acknowledgements

Presentation Work Group

- Daniel Bauer, MD, PhD, Harvard Medical School
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- Brent Morse, Dark Horse Consulting
- Mark Osborn, PhD, University of Minnesota
- Jessica Seitzer, Intellia Tx
- Aron Stein, PhD, Intellia Tx
- Kristin Van Goor, PhD, Takeda
- Lauren Woodard, PhD, Vanderbilt University Medical Center

Thank you

Reactions and Questions from FDA Attendees

2:15 – 2:25 pm ET

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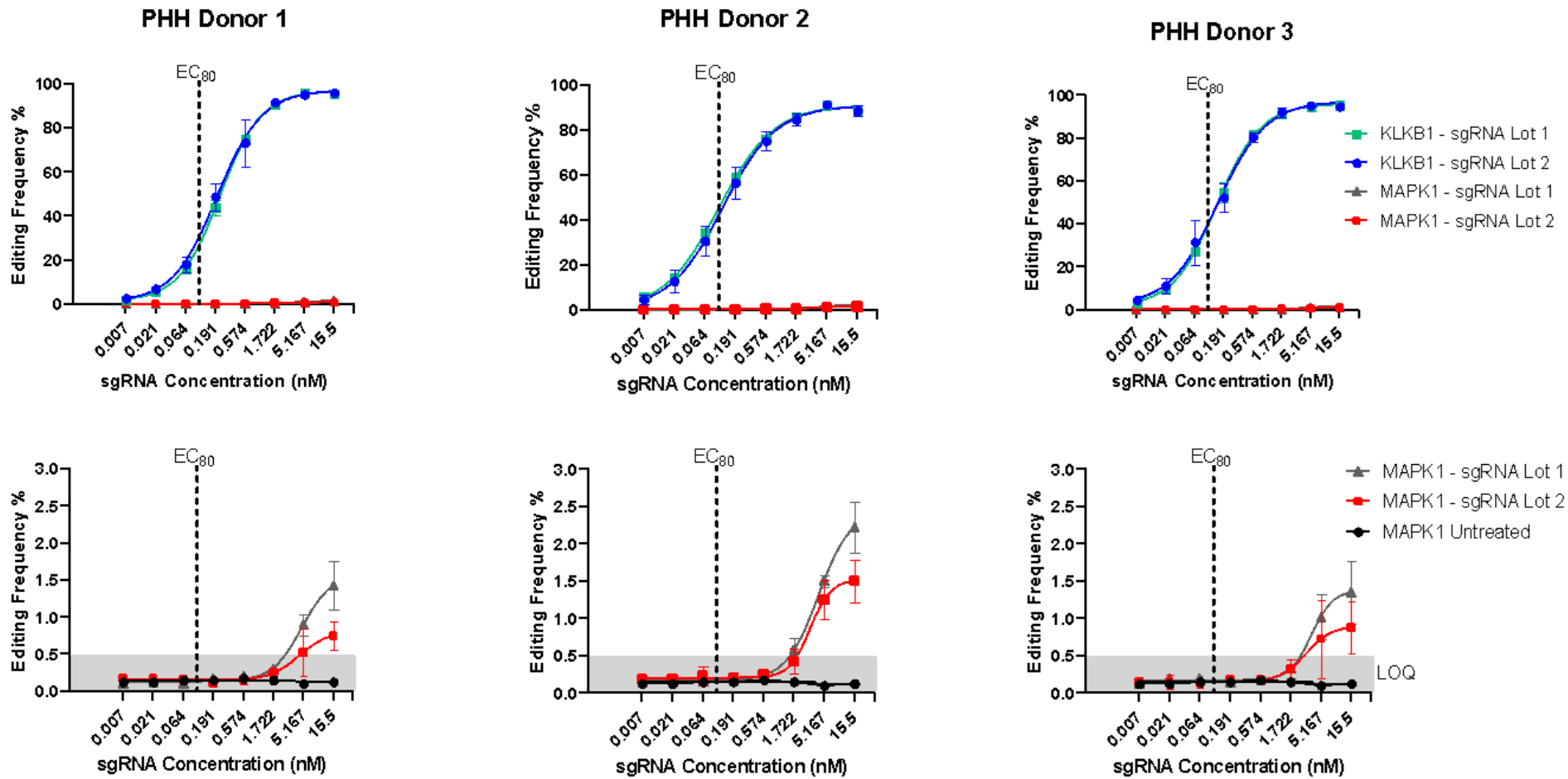
Backup slides

Comprehensive Genotoxicity Evaluation in Support of CRISPR/Cas9 FIH Trial Applications

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



EC80, concentration inducing 80% of maximal effect; sgRNA, single guide RNA
The gray boxes indicate values that fall below the level of quantitation (0.5%).

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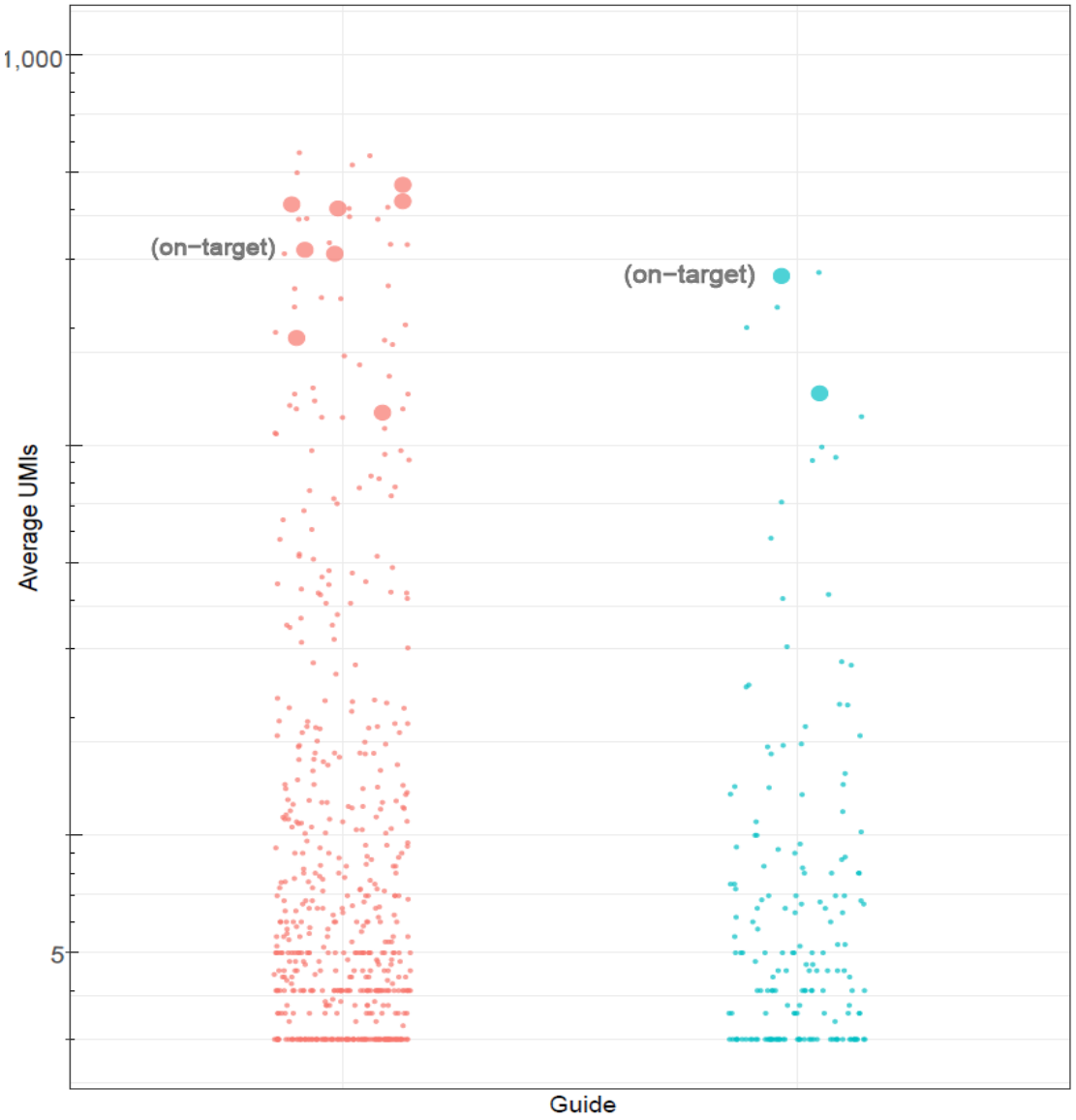
NTLA-2002 Off-Target De-risking – Editing *MAPK1* Intronic locus Did Not Affect *MAPK1* mRNA Expression

Treatment	PHH Donor	Guide (nM)	*MAPK1* Intronic Editing %	*MAPK1* mRNA Relative Expression	p-value	Rank (i)	Total Tests (m)	(i/m)Q	Significance P<(i/m)Q
MAPK1 Targeting LNP	1	1.722	**94.13**	1.05	**0.454**	4	6	0.033	FALSE
		0.191	**86.77**	0.83	**0.780**	10	6	0.083	FALSE
	2	1.722	**94.10**	0.75	**0.394**	13	6	0.108	FALSE
		0.191	**85.67**	0.96	**0.283**	10	6	0.083	FALSE
NTLA-2002	1	1.722	0.23	0.91	**0.437**	3	6	0.025	FALSE
		0.191	0.23	0.95	**0.814**	11	6	0.092	FALSE
	2	1.722	0.23	0.93	**0.020**	2	6	0.017	FALSE
		0.191	0.13	0.86	**0.162**	6	6	0.050	FALSE

- No statistical difference in *MAPK1* mRNA expression across multiple lots of primary human hepatocytes at 10 and 14 days post treatment.
- No impact on *MAPK1* mRNA expression coupled with no detectable editing at therapeutically relevant doses supports low biological safety risk.

MAPK1 mRNA transcript copy was quantified and normalized to the copy concentration of reference gene RPP30 in each sample, controlling for sample input differences. Statistical analyses were conducted using paired 2-tailed t-test between treated cells and control (non-targeting LNP), and false discovery rate was adjusted using Benjamini-Hochberg procedure

SITE-Seq Average UMIs Across Potential Off-Target Sites Support Leveraging UMI Counts to Identify Loci With Higher Probability to Confirm



Confirmation
- Confirmed
- Not Confirmed

Program
- NTLA-2001
- NTLA-2002

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CMC Considerations for Off-target Genome Editing Analysis

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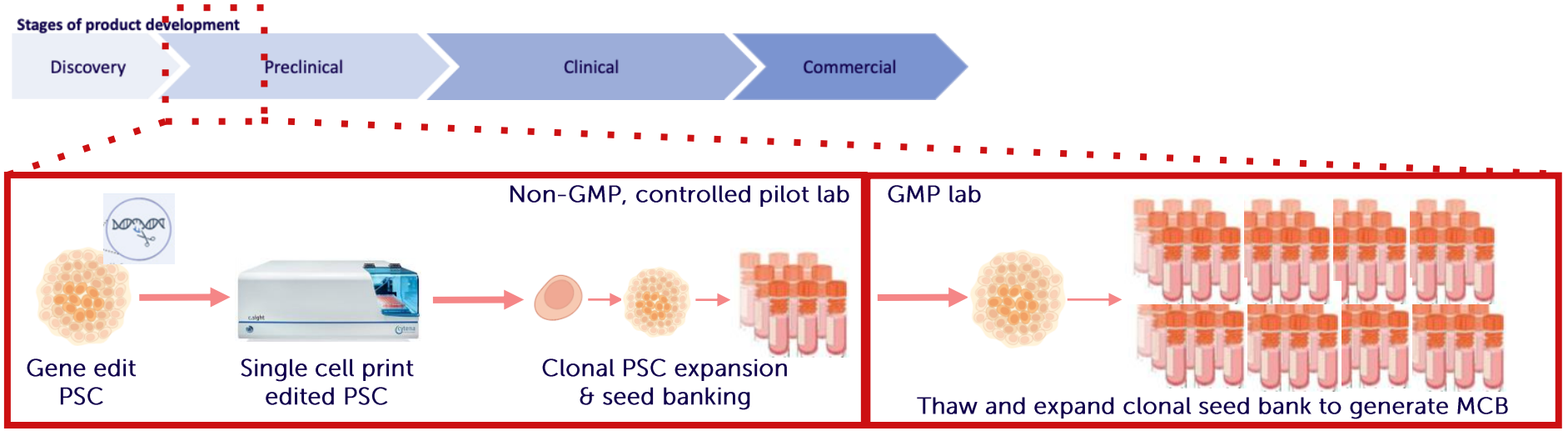
CMC Consideration 1: Sourcing of Editing Reagents

Use of non-GMP editing reagents for pluripotent stem cell (PSC) seed bank production



Scenario 3

1. Gene editing and clonal seed bank production performed on PSC line using non-GMP editing reagents in a controlled pilot lab.
2. Clonal seed banks assessed for off-target editing, translocation events, and overall genome integrity.
3. Clonal seed bank used as starting material for GMP production of an iPSC master cell bank (MCB).
4. MCB assessed for off-target editing, translocation events, and overall genome integrity (plus safety per ICH Q5D).

Consideration: Providing that

✓ Editing reagents are appropriately characterized

✓ The downstream GMP MCB is comprehensively tested for off-target editing and overall genome integrity

It should be acceptable to use non-GMP editing reagents for PSC seed bank production within a controlled pilot lab.

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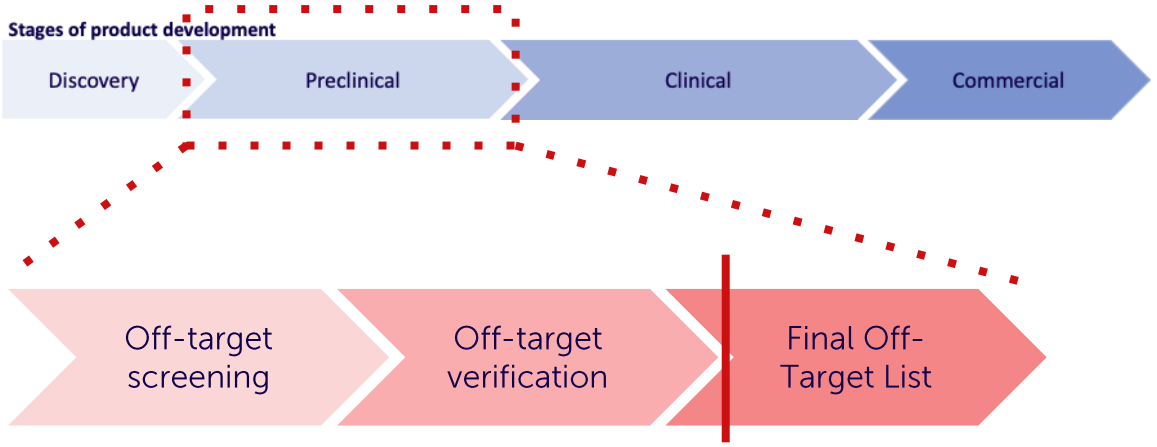
** Image created using BioRender.com*

CMC Consideration 2: Off-target Analysis Pipeline & Results

Off-target editing analyses vary widely among programs; interpretation lacking standardization

Scenario 4



1. Off-target verification by amplicon sequencing identifies several off-target sites in 2 or more donors at or near the assay's LLOQ (e.g., ~0.1-0.3%).
2. All off-target sites occur in non-coding regions or coding regions with no known correlation to human disease or function of the drug product.

Question for the Agency: Providing that the Sponsor

- ✓ Evaluates drug product harboring the off-target events with available in vitro/in vivo safety assays
- ✓ Confirms that the off-target events are consistently low frequency across multiple MFG runs (e.g., n ≥ 3)

Is it acceptable to exclude these off-target sites from routine drug product testing?

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Clinical Considerations

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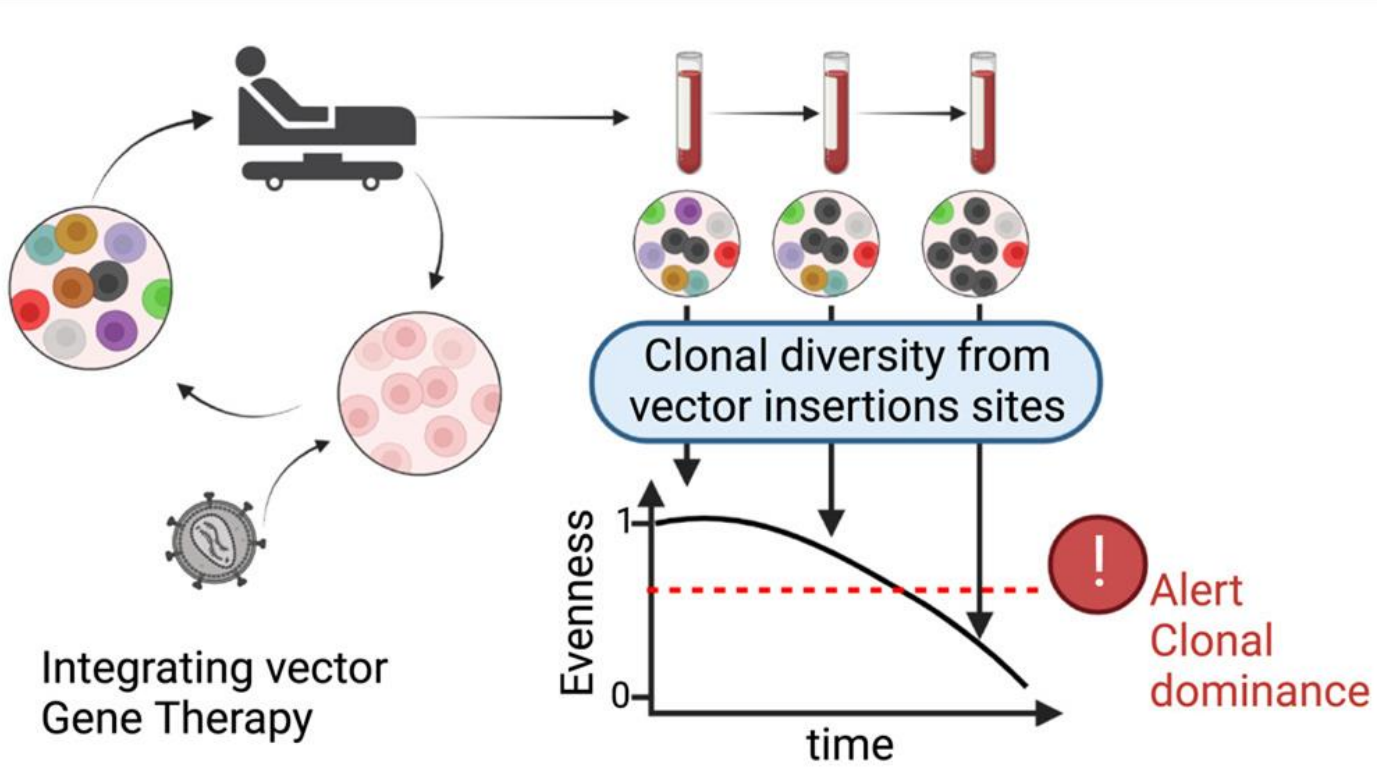
Clinical Monitoring

- *Challenge*: If TEAEs were to occur, the onset time would be uncertain and might have long latency.
  - 15 year clinical follow-up is the current requirement for therapeutic gene editing.

- *Challenge*: If any TEAEs were to occur, their etiology would be initially uncertain.
  - Suggesting sample banking to be fit-for-purpose to investigate possible future TEAEs. [This is mainly for *ex vivo* where the edited cells are accessible post-treatment.]

Edit Distribution Reflects Clonal Composition of Hematopoietic Graft



- Monitoring clonal composition may inform safety (clonal dominance) and efficacy (therapeutic edits).
  - Analogy to integrating vector gene therapy, although gene edits may not be as diverse as vector integrations (different clones may share same edits).

Corre and Galy. Mol Ther Methods Clin Dev (2023) 29:418.

American Society of Gene + Cell Therapy

Thank you