Overview of Current Genomic Analytical Tools to Enable Advancement of Investigational *In Vivo* Genome Editing Products into Clinical Studies

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Agenda

- Overview of CRISPR/Cas9
- Comprehensive Genotoxicity Evaluation in Support of FIH Trial Applications
  - Case Study: NTLA-2002
- Proposed Testing Strategy to Characterize Off-Target Editing Risk Potential

Overview of CRISPR/Cas9

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Advancing a Full-Spectrum Genome Editing Company

CRISPR-Based Modular Platform

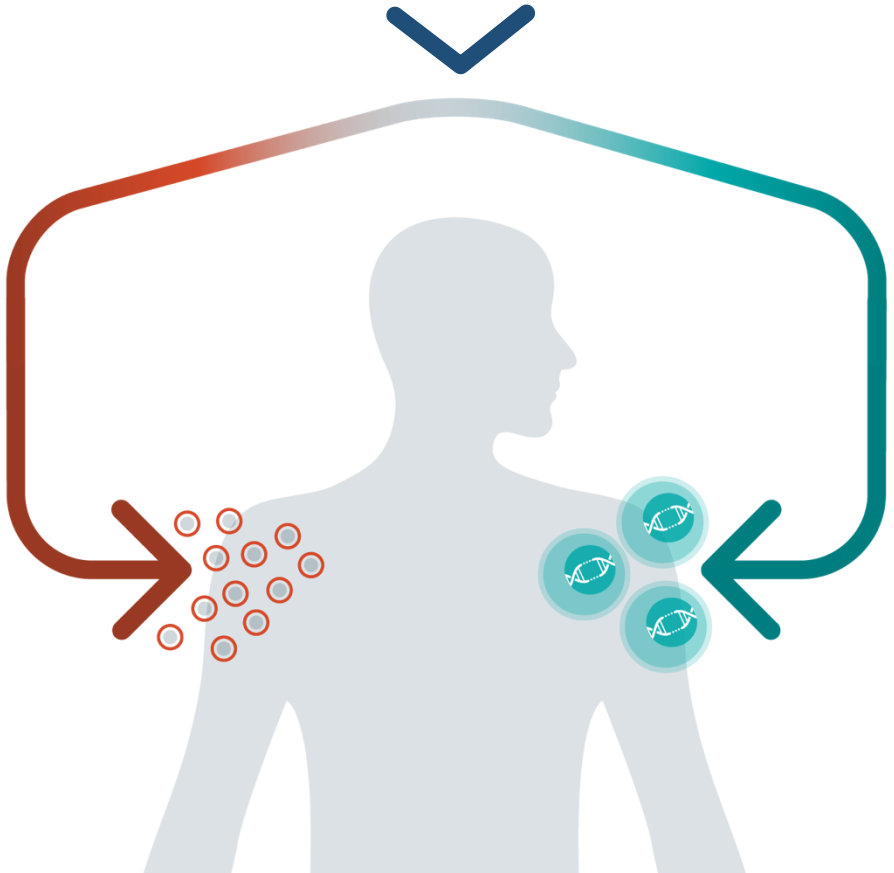
EMPLOY NOVEL EDITING AND DELIVERY TOOLS

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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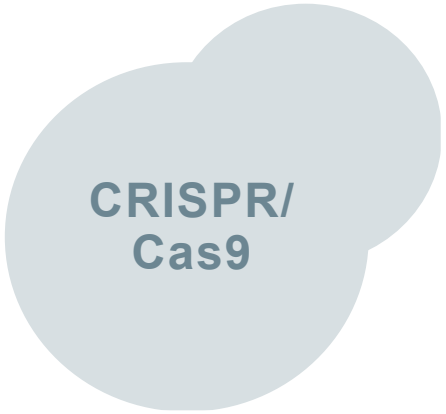
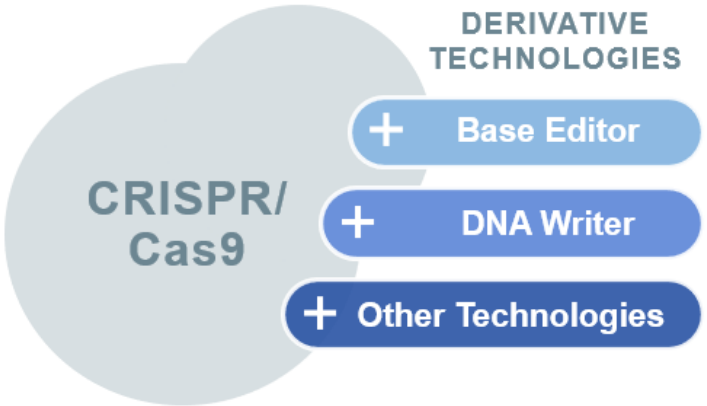
gRNA Selection Requires Comprehensive Specificity Assessment



Canonical CRISPR Cleavase

- gRNA sequence-dependent off-target editing only
- Cas9 adopts an auto-inhibited conformation until properly bound to target site; no random cutting



Derivative CRISPR Technologies

- Appropriate off target discovery and confirmation technologies need to be applied
- Bystander edits and gRNA sequence-independent off-target editing observed for base editors

Jinek et al. Science 2014, 343, 1247997; Jiang et al. Science 2015, 348, 1477; Sternberg et al. Nature 2015, 527, 110; Lazzarotto et al. 2024 bioRxiv doi: 10.1101/2024.03.28.586621

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Comprehensive Genotoxicity Evaluation in Support of FIH Trial Applications

Case Study: NTLA-2002

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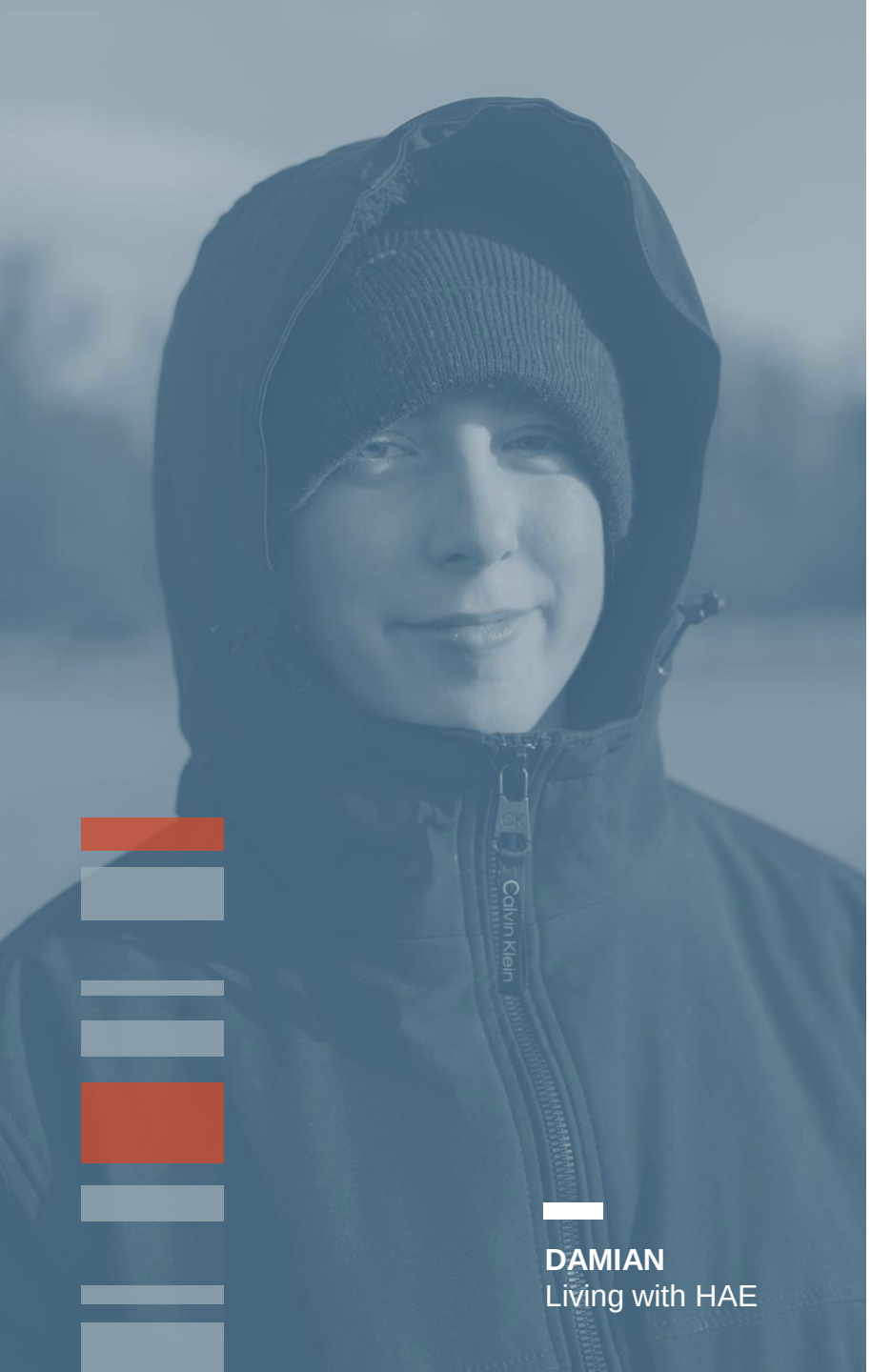

DAMIAN
Living with HAE

NTLA-2002 for Hereditary Angioedema (HAE)

About HAE

- Genetic disease characterized by recurring, severe and unpredictable swelling in various parts of the body
- Despite availability of existing therapies, significant unmet need persists
- Chronic dosing is required with current treatment options

Our Approach

Knock out *KLKB1* gene with a single-dose CRISPR-based treatment

- Reduce kallikrein activity to prevent attacks

Key Advantages Include Potential to:

- Be a single-dose treatment
- Provide extensive and continuous reduction in kallikrein activity
  - Intended to minimize the risk of breakthrough attacks
- Eliminate significant treatment burden

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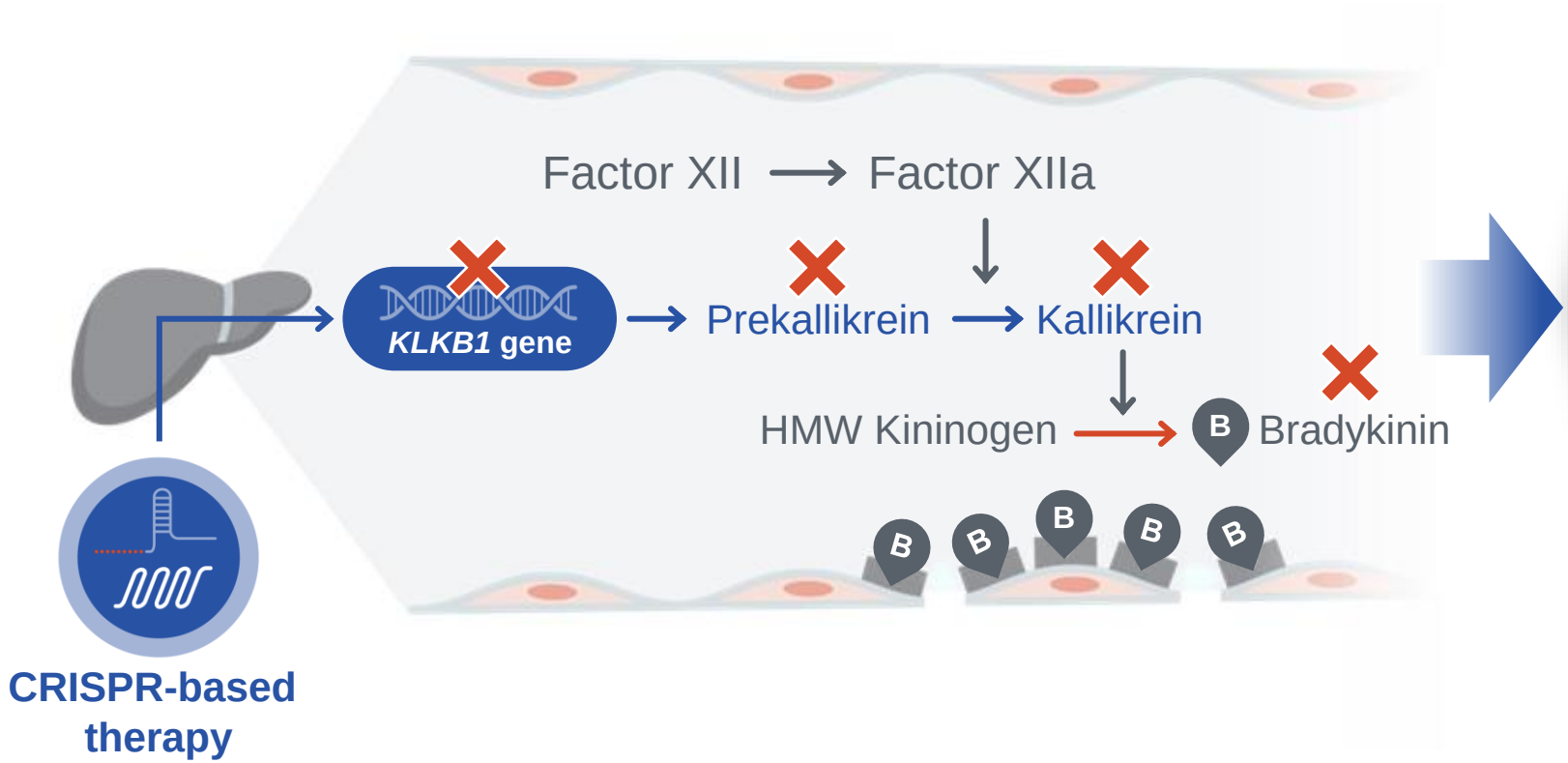
Knocking Out KLKB1 Gene Expression for Long-term Prophylaxis of Hereditary Angioedema (HAE) Attacks



1. Knocking out *KLKB1* gene is intended to prevent production of kallikrein
2. Decreasing kallikrein to rebalance the pathway by reducing bradykinin production
3. Reducing bradykinin to prevent HAE attacks

HAE: Hereditary Angioedema; **HMW**: High-molecular weight
Adapted from Zuraw BL. Hereditary angioedema. *N Engl J Med*. 2008;359:1027-1036

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Orthogonal Techniques used to Characterize Mutagenicity and Large-Scale Chromosomal Integrity

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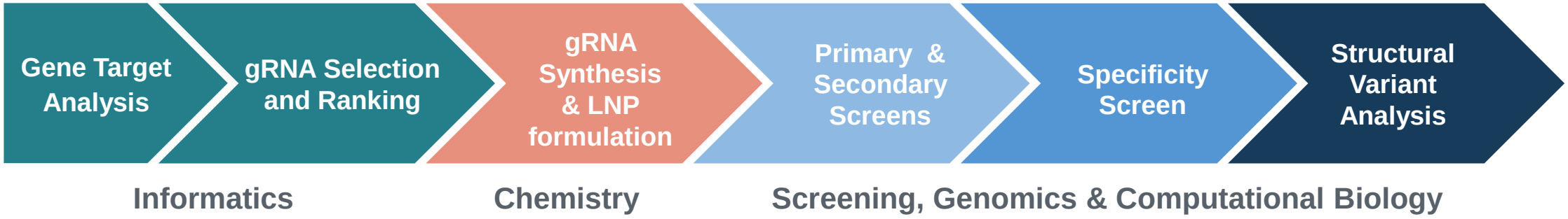
Intellia's gRNA Selection and Qualification Platform



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

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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.

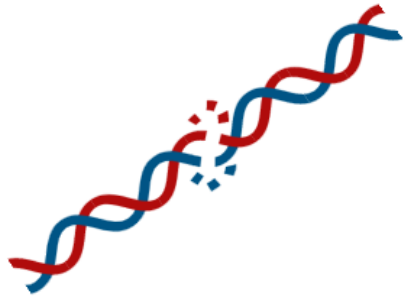
Novel off-targets overlapping with exonic regions are inspected manually considering positions of remaining mismatches in the target region, position of off-target in gene, and expression profile of the mRNA.

[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

Two Classes of Potential Unintended Genome Editing with CRISPR/Cas9

Off-target DNA Editing (mutagenesis) - Safety

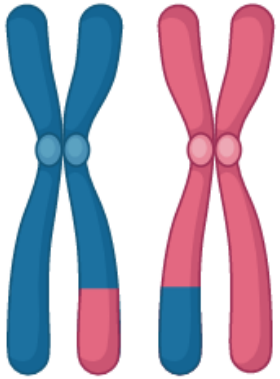
Indel formation at unintended loci in the human genome



DNA Structural Variants (SV) (chromosomal integrity) - Safety

Imperfect restoration of chromosome structure

1. Inter-chromosomal translocations
2. Intra-chromosomal
   - DNA inversions
   - DNA duplications
   - large deletions



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Comprehensive gRNA Specificity Assessment: An Off-Target Workflow

1: Discovery of Potential Off-Target Edits

Computational prediction

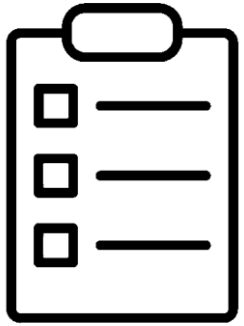
+

SITE-Seq Genomic DNA digest

+

GUIDE-seq Cell-based DNA repair

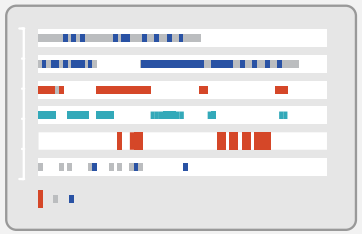
Aggregate **ALL** potential off-target genomic loci



2: Cell-based Validation of True Off-Target Edits by Deep Sequencing

Multiplex panel for NGS

Targeted Amp-Seq NGS follow-up



Ex Vivo

- Cell drug product

In Vivo

- Edit cells *in vitro* using drug product formulation
- Therapeutically relevant human primary cell type(s) (multiple donors)
- Dose range to exceed projected therapeutic exposure (≥10X)

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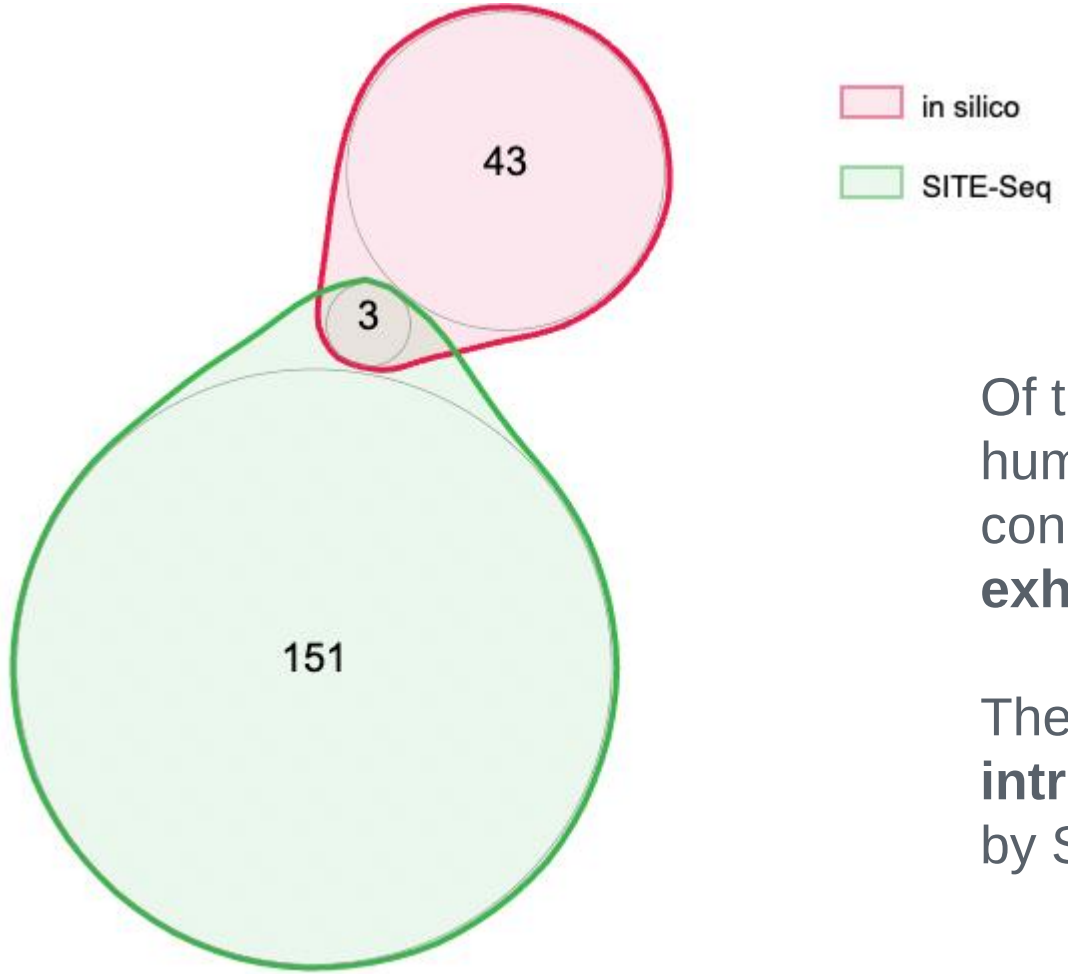
NTLA-2002 Potential Off-target Sites Discovered by Cas-OFFinder and SITE-Seq Exhibit Minimum Overlap



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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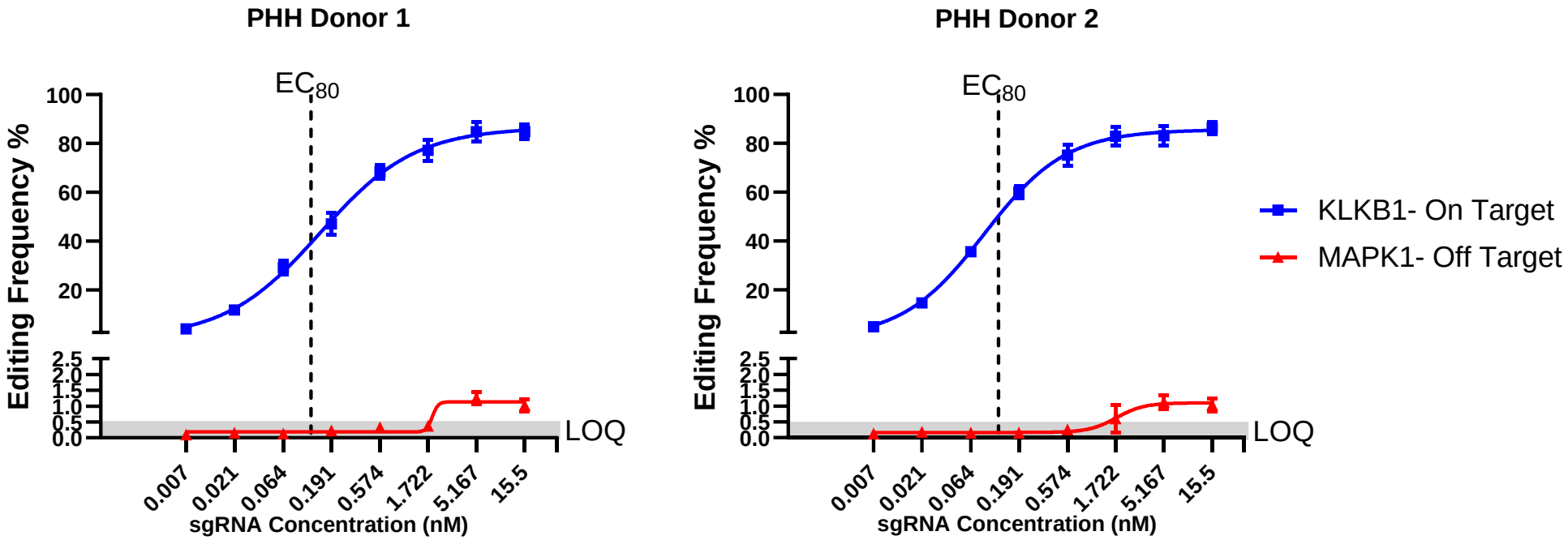
No Detectable Confirmed Off-Targets at Multiples of the Intended Human Dose in Primary Human Hepatocytes



Dose responsive on-target editing with off-target editing at the *MAPK1* intronic locus was only detectable at supratherapeutic 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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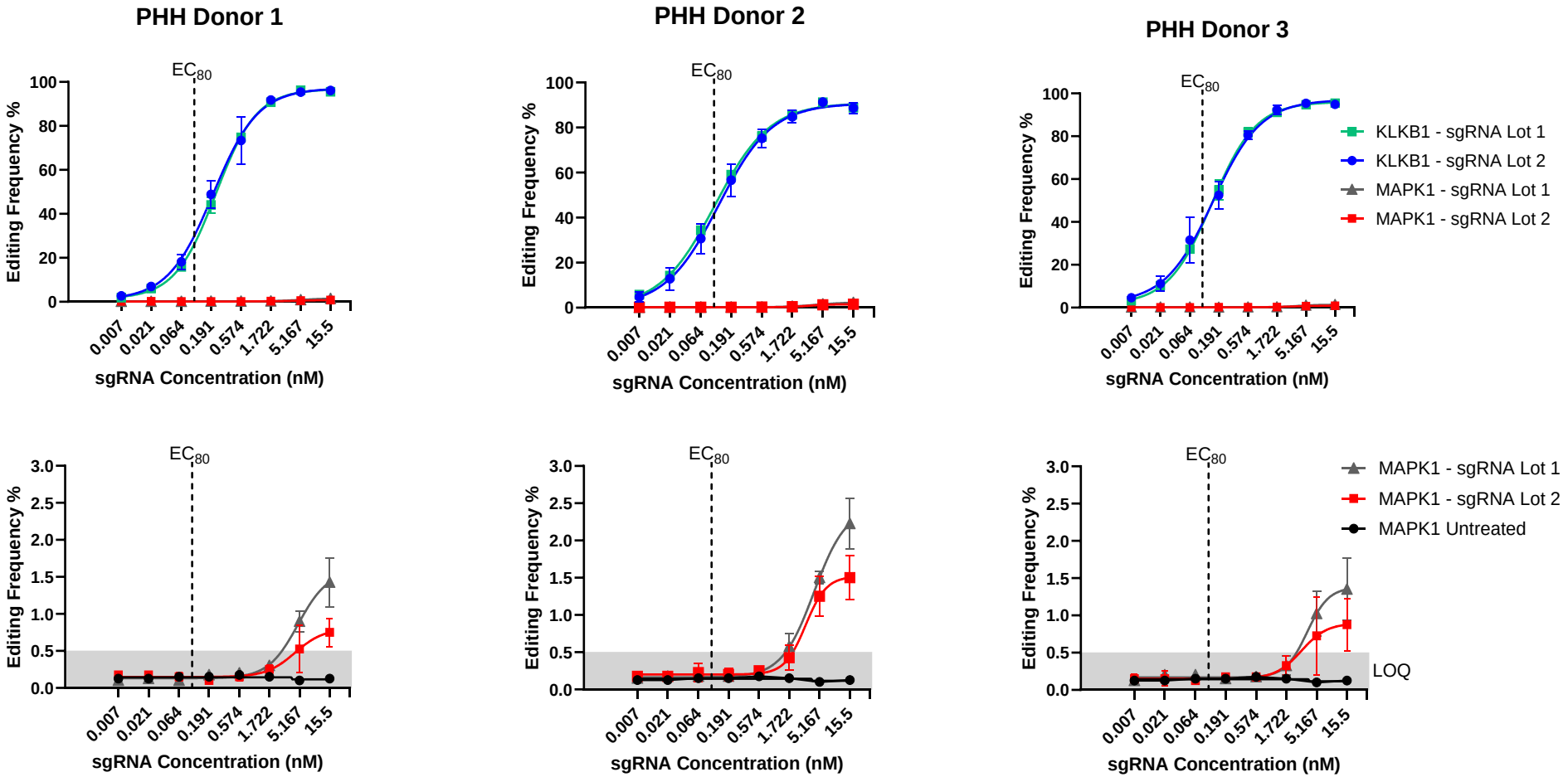
Consistent On-Target and Off-Target Profiles Observed Across Multiple sgRNA Lots and Donors of Primary Human Hepatocytes



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%).

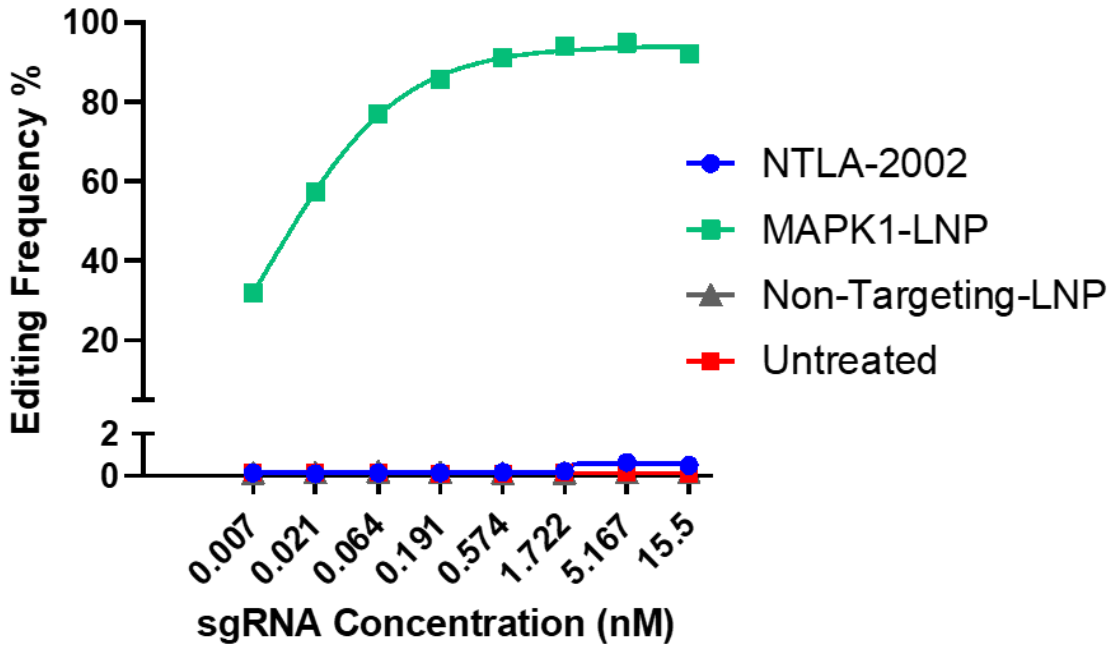
Additional Derisking Performed To Evaluate Any Potential Biological Risk From Off-Target Editing at the MAPK1 Intronic Locus

- *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.

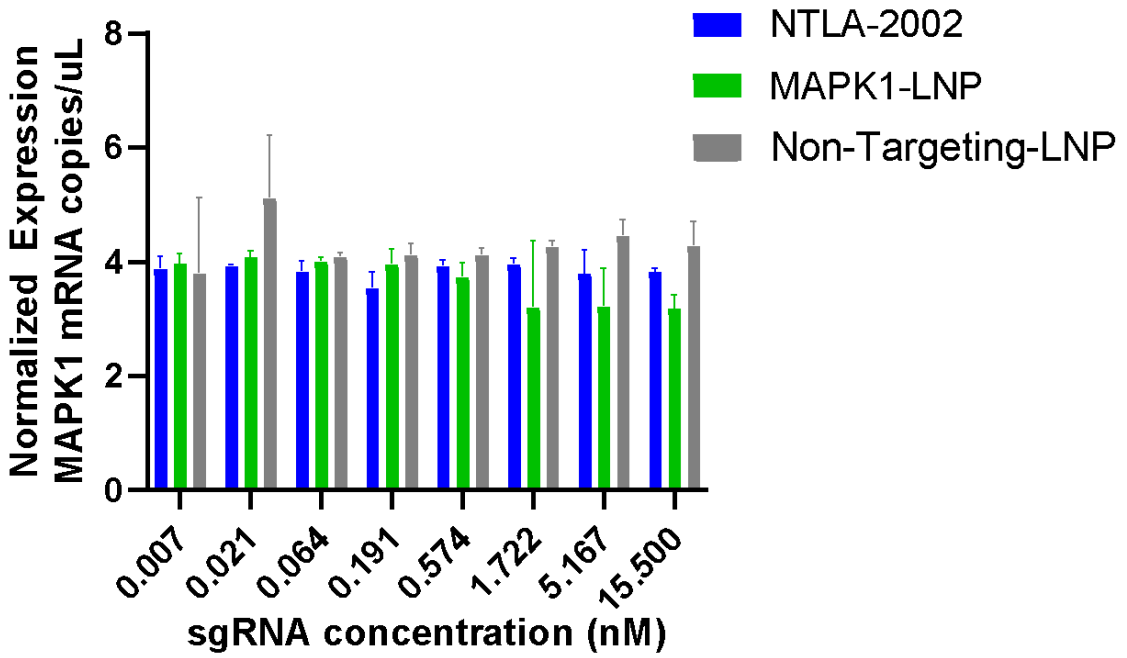
https://www.ncbi.nlm.nih.gov/gene/5594

Full Editing of MAPK1 Intronic Locus with Tool sgRNA Had 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

Step 1: Discovery of potential off-target editing sites
- In silico prediction
- Biochemical and/or cell-based discovery assays: SITE-Seq, GUIDE-Seq, ONE-Seq etc.

Step 2: Validation of the potential off-target editing sites in primary cell type
- Single high dose (>10x Therapeutic Dose)
- Dose-response curve analysis of sites identified in single dose experiment

No editing detected

Editing detected

Step 2A – Supplemental off-target site analyses for additional relevant cell types*
- UMI analysis from Discovery assay
- Chromatin accessibility analysis in BD tissue

Low UMI count
Heterochromatin

Low risk

No off-target editing confirmed at potential site

Step 2B – Validation in additional relevant primary cell types (see Step 2)

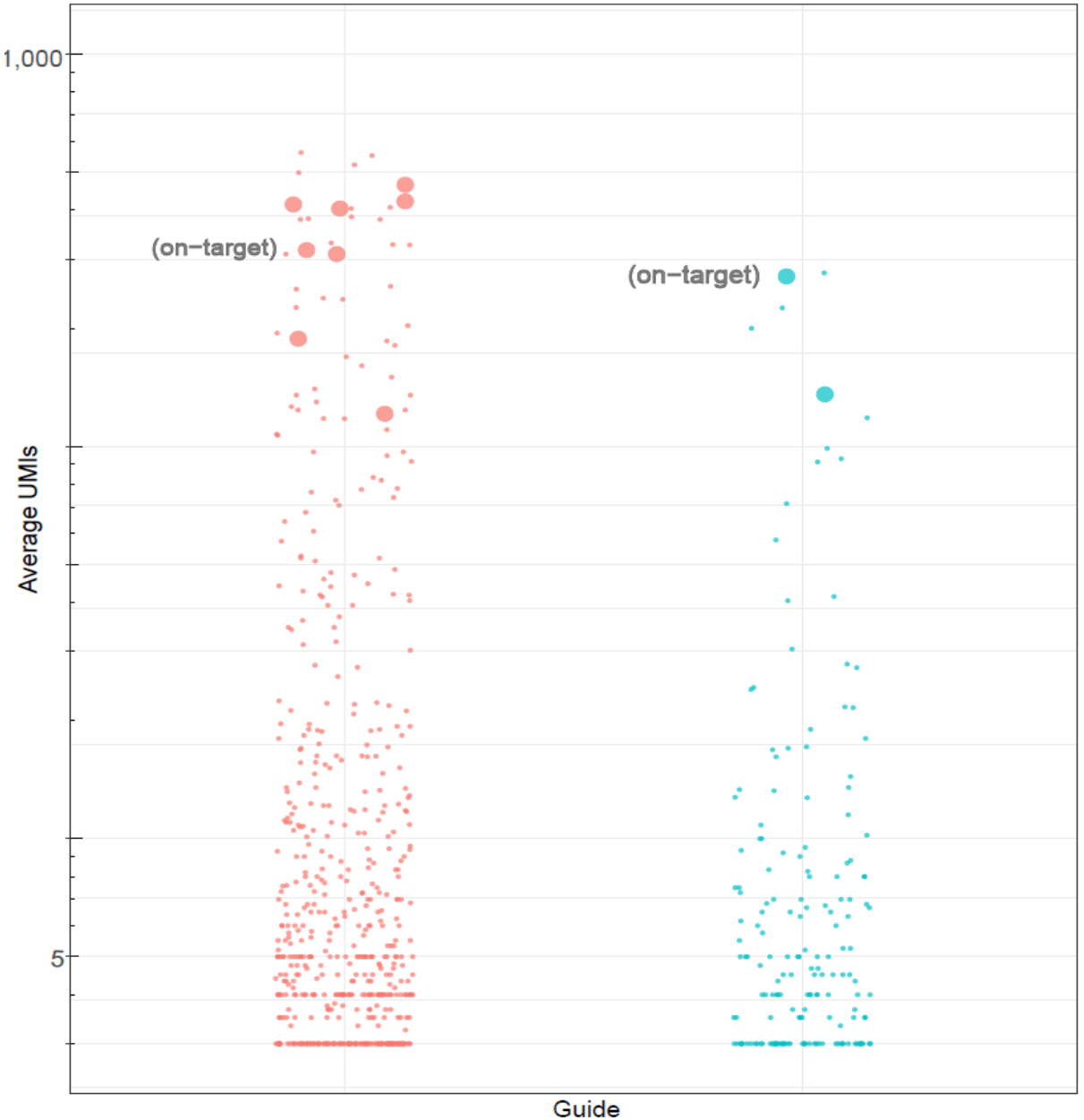
No editing detected

Potential high risk

Step 3: Biological impact assessment
- Location (**exonic**, intronic, intergenic)
- **Biological role in cell/tissue type**
- Cross check with COSMIC database v96 for **cancer tier annotation**
- **Derisking studies** depending on site location and potential for biological impact (exonic, cancer tier annotation, overlap with cis-regulatory elements, or potential for novel splicing)

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

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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Off-Target Assay Development: Leverage WGS to Establish a Minimum Ground Truth for Testing New Off-Target Discovery Assays

- Many new genome wide off-target discovery assays are being developed especially pertaining to new editing technologies that may have nuances where current discovery methods are not applicable (Ex. Base Editing, DNA writing, etc.)

- Each assay claims to be more sensitive and comprehensive than another, but no minimum ground truth exists to test these claims

- **Recommendation:** Leverage WGS (>100x coverage) of a tool gRNAs in the relevant cell type with the applicable gene editing technology to establish a control set of known confirmed off-target sites as the minimum set of sites that need to be identified by the discovery assay to qualify as a relevant assay.

Key Takeaways

- Evaluation of potential mutagenicity is achieved with multiple technologies
  - Comprehensive genome-wide candidate site identification employs a Discovery phase using in silico, biochemical and cellular methods
  - Deep next-generation sequencing (NGS) is employed as a Validation phase to characterize all candidate sites
- Off-target risk potential can be evaluated, and de-risking strategies applied to assess biological risk potential
  - NTLA-2002 Case Study (*in vitro*):
    - *MAPK1* intronic off-target was confirmed at supratherapeutic concentrations in primary human hepatocytes
    - Derisking studies leveraging a *MAPK1* intronic specific guide confirmed that there was no impact to *MAPK1* mRNA expression at saturating editing levels
- Testing strategy can be leveraged to characterize off-target editing risk potential
  - Off-target genome-wide discovery and validation workflow in conjunction with leveraging UMI count from the genome-wide discovery assay and chromatin accessibility can be implemented to assess risk of off-target editing in relevant cell types

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