Comprehensive Preclinical Specificity Profiling of MGX-001, a CRISPR-Based Factor VIII Gene Integration for Treatment of Hemophilia A

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Abstract

MGX-001 is a Factor VIII gene therapy leveraging the MG29-1 Type V CRISPR system and AAV-delivered donor template that enables precise integration of a promoterless Factor VIII transgene into the Albumin safe harbor locus. In preclinical studies, this approach achieves durable therapeutic expression at low integration frequencies and is a potentially curative, one-time treatment for patients with Hemophilia A. Optimization of on-target editing, achieved through chemically-modified guide RNA and optimized nuclease mRNA, ensures high editing efficiency. Early specificity profiling for off-target nomination incorporated in silico prediction tools (e.g., CRISPRme*) and both biochemical in vitro and in cellulo experimental assessments. These assays and methods informed the prioritization of guides with minimal off-target risks. To rigorously assess the lead guide, combined loci (624) from all methods were evaluated in primary human hepatocytes (PHH) at saturating to supersaturating doses, with no validated off-target editing events detected. In addition, no reproducible translocations were detected in PHH from 3 donors treated at supersaturating levels of editing.

Targeted enrichment approaches were used to assess AAV-mediated transgene integration in cellular (PHH) and animal (NHP liver) models. Consistent with traditional AAV gene therapies, the AAV profile in both models showed a semi-random integration profile with no overt clonal expansion. The addition of gene editor did not result in enrichment of integrations at potential off-target sites nominated for the lead guide. As observed by others, integration events showed enrichment near transcription start sites but no further enrichment near transcription start sites of cancer associated genes. Overall, our approach shows that our gene editing system had no major impact on the off-target AAV integration profile.

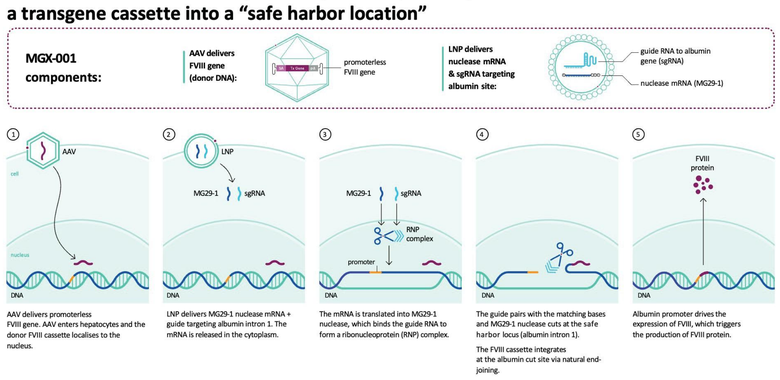
Finally, variant-aware methods were developed to assess the effect of genetic diversity on editing activity, both experimentally and at scale. GnomAD population variant data were used to guide variant selection. Candidate variants derived from gnomAD population data were evaluated using a tiered testing strategy, beginning with a sensitive in vitro biochemical assay and then followed up by an in cellulo variant-aware assay to characterize editing events while excluding biochemical false positives. For MGX-001, CRISPRme in conjunction with gnomAD nominated variant sequences plus their corresponding hg38 reference sites. Approximately 2% of the library oligos were nominated by the in vitro biochemical assay. None of the in vitro nominated variants had allele frequencies above $1.1x10^{-4}$.

The outcomes of our rigorous assessments demonstrate the high specificity of MGX-001 and its minimal impact to genome integrity. This evaluation provides confidence for safe clinical translation of MGX-001 to offer a potentially curative, one-time treatment for Hemophilia A patients.

Mechanism of action of the gene editing approach

Therapeutic Approach to Hemophilia A Genome Editing with MGX-001

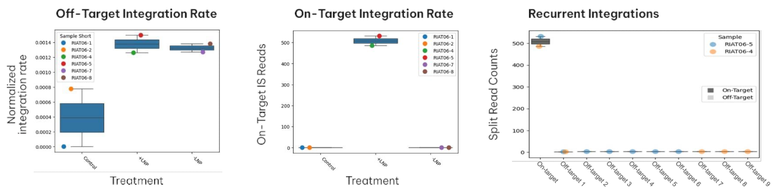
MGX-001 utilises a dual-component system to precisely insert a transgene cassette into a "safe harbor location"



- Therapeutic modality: In vivo genome editing therapy combining AAV and LNP platforms.
  - AAV vector: Delivers promoterless hFVIII donor DNA template.
  - LNP: Delivers mRNA for a CRISPR nuclease and a guide RNA.
- Mechanism:
  - Site-specific insertion of FVIII into the albumin locus in hepatocytes.
  - An engineered splice acceptor enables fusion of the endogenous albumin transcript to FVIII, allowing the native albumin promoter to drive durable expression.
- Built-in safety features:
  - Albumin "safe harbor": Ensures stable expression by hepatocytes.
  - Promoterless donor design: Eliminates risk of promoter-driven activation of endogenous genes following random AAV integration.
  - High specificity nuclease: MG29-1's restrictive PAM reduces off-target risk.
- Differs from conventional AAV therapies, which rely on episomal transgene expression that can be lost or silenced over time.

Assessing AAV integrations in PHH edited with the gene editor

No Increase in Recurrent and Off-Target AAV Integration Burden with the Addition of LNP



- Overall off-target AAV integration rate is unchanged with addition of LNP (control, AAV alone, AAV+LNP).
- On-target FVIII integration occurs only with addition of Albumin-targeting LNP.
- Off-target integrations remain non-recurrent with LNP addition.

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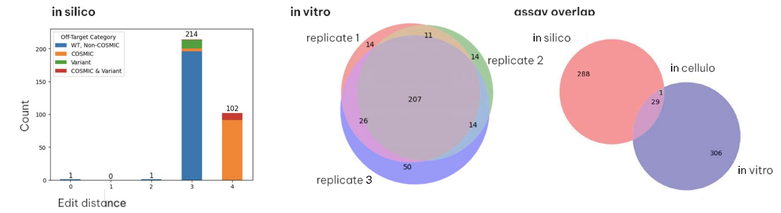
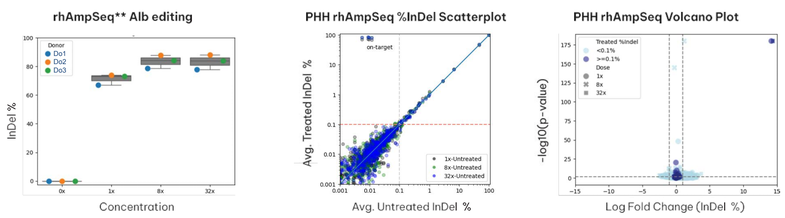
Assessing off-target editing in primary human hepatocytes and in extrahepatic tissue representative cells

623 Potential Off-target Sites Nominated Through Orthogonal Discovery Methods for MG29-1 ALB gRNA



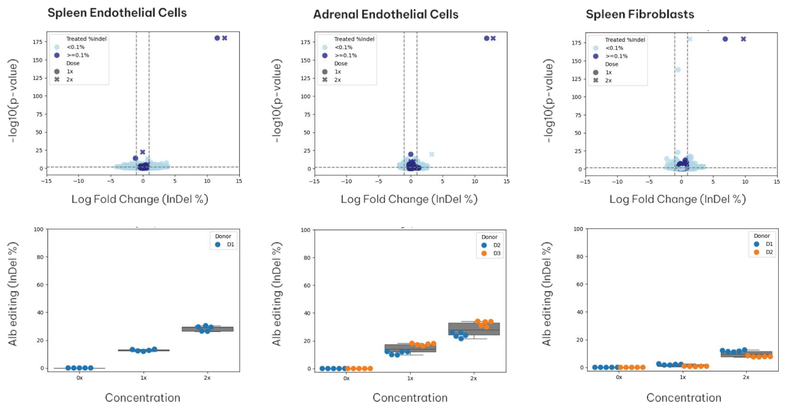
- CRISPRme-nominated sites grouped by edit distance from the ALB gRNA. COSMIC genes (orange), variants (MAF <1%, green), and COSMIC + variant (red). Only COSMIC genes included at edit distance 4.
- Overlap of in vitro nominated sites across three assay replicates.
- Overlap of sites nominated by all three orthogonal assays (in silico, in vitro, in cellulo).

No confirmed off-targets for MG29-1 ALB gRNA in PHH



- rhAmpSeq on-target InDels in PHH at 1x, 8x, and 32x EC90 in 3 donors confirm robust editing.
- InDel frequencies at 1x (grey), 8x (green), and 32x (blue) EC90 vs. untreated controls across all analyzed sites in 3 PHH donors with only the on-target significantly deviating from the diagonal.
- GLMM analysis (log10 p-value vs. log2 fold change) showing only on-target site meet significance criteria (LFC > 1, $p \leq 0.05$).

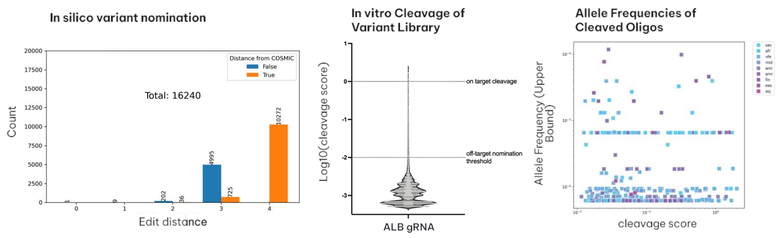
No confirmed off-targets in extrahepatic tissue representative human cells



- Across three extrahepatic tissue representative human cell types analyzed, only the on-target site is significantly edited.

Assessing off-target editing of genomic variants

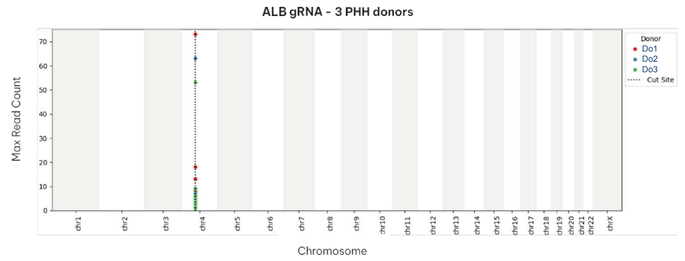
None of the in vitro cleaved variants for ALB gRNA had allele frequencies above $1.1x10^{-4}$



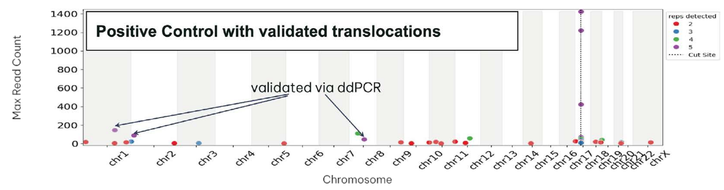
- In silico OT nomination with gnomAD without a MAF cut off identified 16,240 variants for testing.
- 496 oligos exceeded the 0.01 cleavage cutoff; vast majority of which were genomic variants.
- None of the in vitro cleaved variants had allele frequencies above $1.1x10^{-4}$.
- As follow up, the in vitro cleaved variants were nominated for follow up using our in cellulo variant-aware assay.

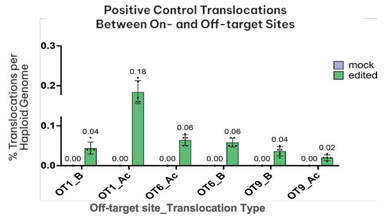
Assessing on-target translocations in primary human hepatocytes

No Detected Translocations in PHH edited with MG29-1 ALB gRNA



- 3 PHH donors were edited with MG29-1 ALB gRNA LNP (4x EC90) to assess genome-wide translocations. Split-read junctions detected by a molecular translocation assay were exclusively cut-site proximal on chromosome 4 across all donors.

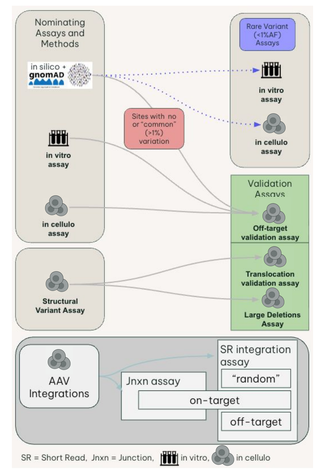




- Genome-wide split-read analysis with a positive control MG29-1 gRNA shows reproducible translocations between the on-target site and off-target sites.
- Events identified with positive control were validated by ddPCR at frequencies of 0.02–0.18% per haploid genome (B: Balanced, Ac: Accentric).

Specificity Characterization Overview

Multi-tiered strategy for assaying potential off-target sites, on-target editing, and AAV integrations in the genome



- Potential off-target sites are nominated using three orthogonal assays (in silico, in vitro, in cellulo), then followed by in-cellulo validation in PHH or in consisting of rare population variants, variant-aware in vitro and in cellulo assays.
- Beyond standard on-target editing characterization via short read amplicon sequencing, translocations and large deletions are detected by Structural Variant detection assay and, if present, validated using ddPCR translocation assays and Long Read sequencing based Large Deletions assay in PHH.
- Finally, AAV integration at on-target, off-target, or background sites are characterized in PHH and human cell models representative of extrahepatic tissues using a combination of Short Read (SR) integration assay and Junction PCR.

Conclusions

- No off-target editing was detected in PHH and human cells representative of tissues where LNP distributes and on-target editing occurs.
- None of the in vitro nominated variants had allele frequencies above $1.1x10^{-4}$. We plan to follow up with all cleaved variant candidates using a variant-aware, cell based assay with a lower FDR.
- We detected no translocations between the on-target site and any off-target site genome-wide.
- We found no clonal or recurring sites for AAV integrations genome wide. AAV integrations exhibited no change in integration rate and profile with the addition of gene editor with the exception of the desired ALB integration.
- Overall, the safety profile of MGX-001 shows high specificity across orthogonal assays, supporting safe clinical translation.

References

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