Novel triplet chemistries informed by structural biology expand OPERA® platform capabilities

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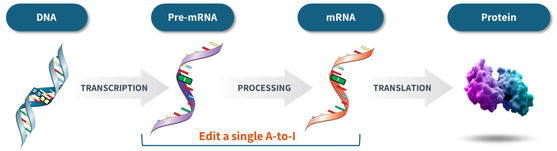
Introduction and Aims



ADAR-mediated A-to-I RNA editing offers a **precise, reversible approach to correct pathogenic transcripts without permanent genomic modification**. Korro's OPERA® (Oligonucleotide Promoted Editing of RNA) platform enables rational design and optimization of guide oligonucleotides that recruit endogenous ADAR enzymes, delivering titratable and re-dosable genetic medicines[1]. A key determinant of editing efficiency lies in the chemistry of the oligonucleotide, and specifically within the triplet region flanking the target adenosine, which influences ADAR recognition and catalysis. Therefore, the introduction of new chemistries in this triplet region can have significant impacts on RNA editing efficiency.

In this work, **we aimed to evaluate a range of chemically modified sugars in the -1 position of the oligonucleotide** (5' to the orphan) and assess editing against multiple targets. Specifically, we tested chemical modifications that would be predicted to impart either a duplex distortion, as seen previously for the RNA duplex bound to ADAR in co-crystal structures, or those that allow the possibility of additional contacts with the ADAR protein. These -1 modifications would be evaluated in the context of two different orphan nucleotides – Deoxyhexose C and DNA X (where X is a currently undisclosed nucleobase).

Lead -1 modifications were taken forward and evaluated *in-vivo* to assess editing, half-life, and clinical pathology safety parameters.

Chemical Modifications used and Structural Hypotheses

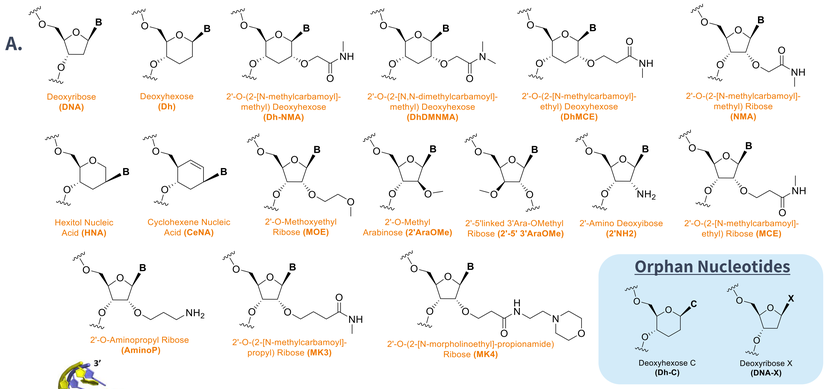
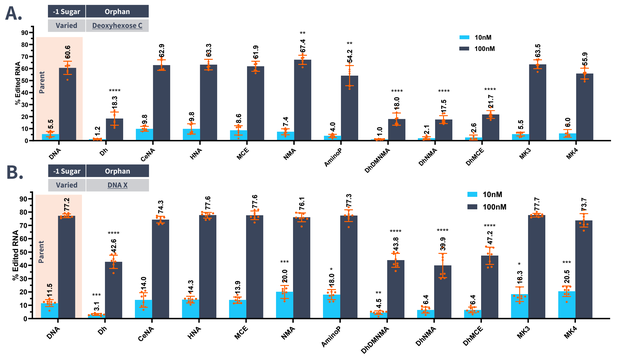


Figure 1. Chemical structures of modified sugars and structural hypotheses probed. **A)** Chemical structures of 15 sugars containing ring and/or 2'-modifications compared to DNA. Nucleobase is represented by B and varies per target assessed. Blue Inset: Structures of Orphan Deoxyhexose-C and DNA-X used in this work.

Two distinct predicted structural hypotheses for impacting ADAR-triplet interactions: **B)** Beal and co-workers[2,3] have shown in a co-crystal structure of ADAR2 bound to an RNA:oligonucleotide duplex that a significant structural deviation is seen around the triplet of the oligo. **C)** Extended 2'-modifications at the -1 position of the oligo could reach across and H-bond with Serine486, as modelled based on PDBID: 5HP3[2].

Results

Target 1 – *in-vitro* screening



Target 1: Triplet and Edit Site

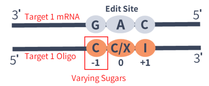
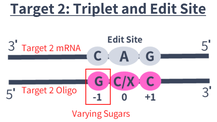


Figure 2. *In-vitro* screening results for ADAR recruiting oligonucleotides against Target 1 containing varying sugar modifications in the -1 position of the triplet. **A)** Oligonucleotides containing a Deoxyhexose Cytosine Orphan, **B)** Oligonucleotides containing a DNA Orphan (nucleobase X). All oligonucleotides are dosed by gymnotic delivery at 10 and 100 nM, for 48h in Primary Mouse Hepatocytes. N=8 for all samples.

Target 2 – *in-vitro* screening

Target 2: Triplet and Edit Site



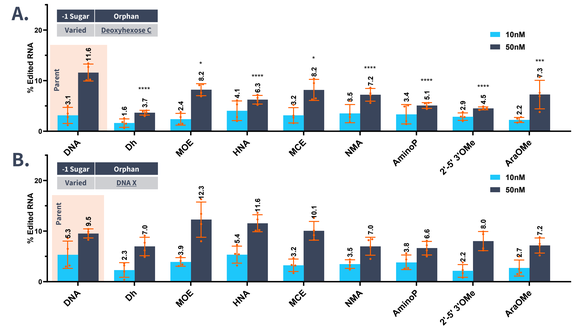
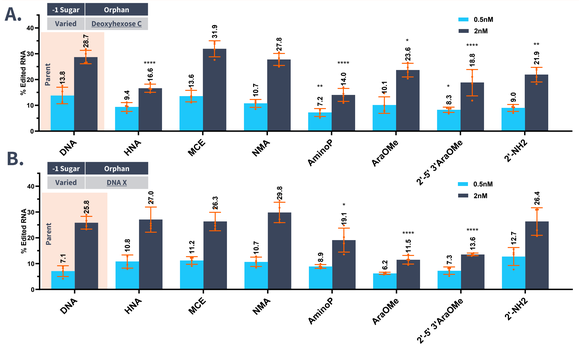


Figure 3. *In-vitro* screening results for ADAR recruiting oligonucleotides against Target 2 containing varying sugar modifications in the -1 position of the triplet. **A)** Oligonucleotides containing a Deoxyhexose Cytosine Orphan, **B)** Oligonucleotides containing a DNA Orphan (nucleobase X). All oligonucleotides are dosed by gymnotic delivery at 10 and 50 nM, for 48h in Primary Human Hepatocytes. N=4 for all samples.

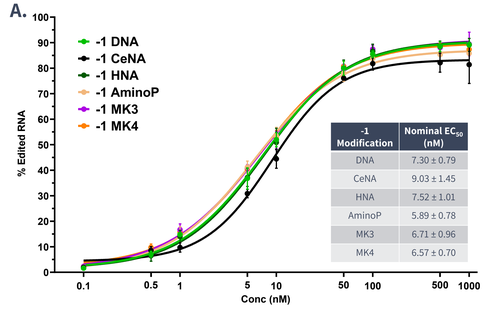
Target 3 – *in-vitro* screening



Target 3: Triplet and Edit Site

Figure 4. *In-vitro* screening results for ADAR recruiting oligonucleotides against Target 3 containing varying sugar modifications in the -1 position of the triplet. **A)** Oligonucleotides containing a Deoxyhexose Cytosine Orphan, **B)** Oligonucleotides containing a DNA Orphan (nucleobase X). All oligonucleotides are dosed by gymnotic delivery at 0.5 and 2 nM, for 48h in C57BL/6-PiZ mouse hepatocytes. N=4 for all samples.

Target 1 – EC_{50} and *in-vivo* editing and safety evaluation



-1 Modification	Nominal EC_{50} (nM)
DNA	7.30 ± 0.79
CeNA	9.03 ± 1.45
HNA	7.52 ± 1.01
AminoP	5.89 ± 0.78
MK3	6.71 ± 0.96
MK4	6.57 ± 0.70

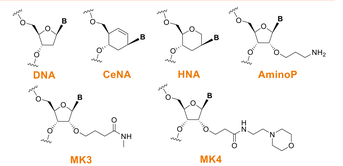
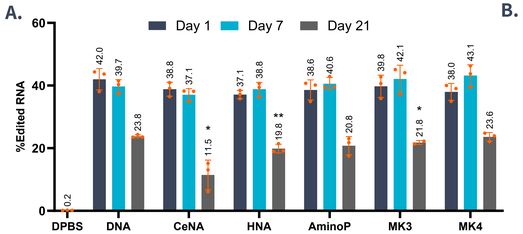


Figure 5. ***In-vitro* dose response and calculated nominal EC_{50} values for selected top performing -1 modified oligonucleotides against Target 1.** All oligonucleotides contain the more permissive DNA-X Orphan. **A)** 9-point concentration gradient ranging from 0.1 to 1000 nM was used to calculate EC50. All oligonucleotides are dosed by gymnotic delivery at 10 and 50 nM, for 48h in Primary Mouse Hepatocytes. N=3-4 for all samples. **B)** Chemical structures of the lead -1 sugars in combination with Orphan DNA X from screening in Target 1.



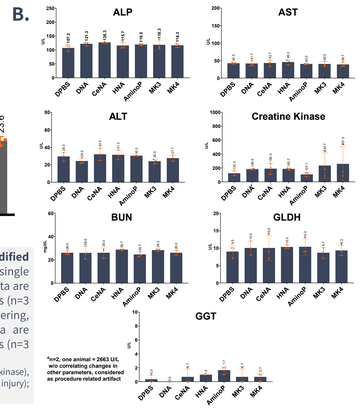


Figure 6. ***In-vivo* RNA editing and clinical chemistry safety endpoints for selected -1 modified oligonucleotides. A)** RNA editing from livers of male C57BL6 mice (8-10 weeks old) following a single subcutaneous administration of 20 mg/kg of designated oligonucleotides. Individual animal data are represented by orange circles, with mean ± SD confidence intervals and annotated mean values (n=3 mice/group). **B)** Single timepoint (5 days post dose) clinical pathology safety parameters covering, hepatocellular injury, cholestasis, myotoxicity, and renal function. Individual animal data are represented by orange circles, with mean ± SD confidence intervals and annotated mean values (n=3 mice/group).
Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK (creatine kinase), marker of muscle injury; BUN, blood urea nitrogen (renal function); GLDH, glutamate dehydrogenase (mitochondrial liver injury); GGT, gamma-glutamyl transferase.

Conclusion

- 15 diverse -1 sugar modifications were successfully incorporated into ADAR-recruiting oligonucleotides, with several chemistries (e.g., HNA, MCE, NMA, CeNA, MK4) demonstrating good tolerance and maintaining/improving RNA editing efficiency across targets.
- *In-vitro* screening highlighted that editing outcomes are highly context dependent, with both triplet composition and orphan nucleotide identity influencing performance.
- Lead -1 sugar modifications showed comparable potency (nominal EC50 values) and consistent translation from *in-vitro* to *in-vivo* systems, supporting the predictive value of the screening cascade.
- *In-vivo* studies demonstrated sustained liver RNA editing for over 3-weeks following a single dose, with similar activity across selected chemistries. No treatment related changes were observed in standard clinical chemistry markers, indicating a favorable safety profile. Half-life and stability analysis is ongoing.
- Together, these results expand the chemical design space of the OPERA® platform and support continued optimization of triplet chemistries.

Statistical Analysis:
All data were analyzed by two-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Comparisons without annotation are not statistically significant.

References:
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2. M. M. Matthews, J. M. Thomas, Y. Zheng, K. Tran, K. J. Phelps, A. I. Scott, J. Havel, A. J. Fisher and P. A. Beal, *Nat. Struct. Mol. Biol.*, 2016, **23**, 426–433
3. H. F. Brinkman, V. J. Matos, H. G. Mendoza, E. E. Doherty, and P. A. Beal, *RSC Chem. Biol.*, 2023, **4**, 74-83