Mechanistic characterization of enhanced delivery oligonucleotide (EDO) platform

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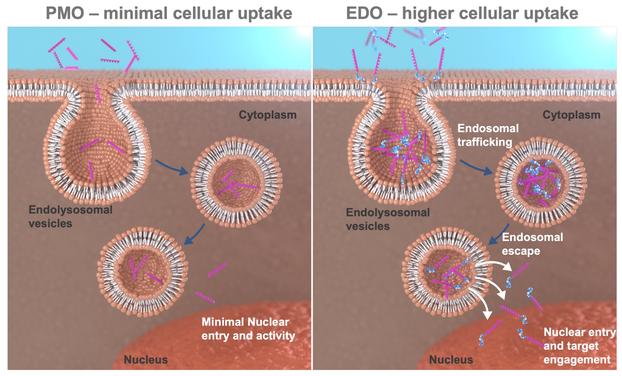
INTRODUCTION

- Oligonucleotide drugs have limited ability to cross the cell membrane and reach their targets.
- PepGen's enhanced delivery oligonucleotide (EDO) technology consists of extensively evolved next-generation cell penetrating peptides (CPPs) empirically designed to improve drug delivery to target tissues.
- Here we show that EDOs have better drug-like properties compared to unconjugated phosphorodiamidate morpholino oligomers (PMO) and first generation R6G-PMOs.



CELL PENETRATING PEPTIDE EVOLUTION

CPP Motifs → **First Generation CPPs** → **Next Generation CPPs/ EDO**

Arginine-rich sequence (RXR motif)

Penetratin sequence RQIKIWFQNRRMKWKK

$(RXR)_3$ | Penetratin-like sequence | C

R-rich | Hydrophobic | R-rich | Pip

RRRRRR | G | R6G*

- Variations in hydrophobic-rich domain
- One or more R-rich sequences
- Addition of aminohexanoic acid and unnatural amino acids
- Terminal Cysteine removed

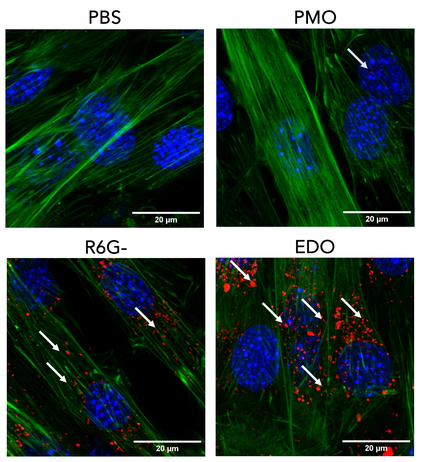
R-rich | Hydrophobic | R-rich | EDO*

- Optimized hydrophobic-rich domain
- R-rich flanking sequences
- No aminohexanoic acid
- Optimized dispersion of unnatural amino acids
- Linker optimized for conjugations

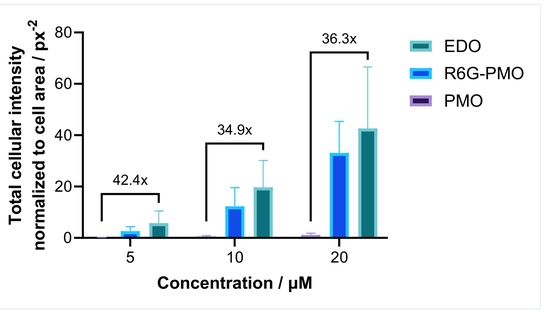
*Used in this study
R6G is believed to be structurally equivalent to the peptide component of vesleteplirsen

CELLULAR UPTAKE

EDOs SHOW HIGHER CELLULAR UPTAKE IN C2C12 MYOTUBES



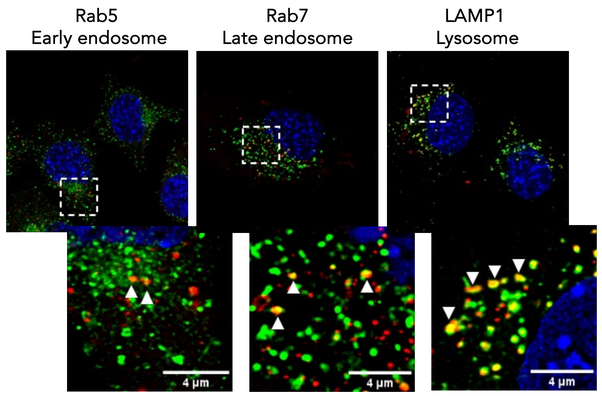
Green = actin stain; Red = TAMRA labelled conjugate; Blue = nucleus



HIGH CONTENT IMAGING: C2C12 myotubes were differentiated for 5 days and treated with TAMRA tagged PPMO or PMO molecules at 20, 10, and 5 µM for 24 h. Fixed cells were imaged on a Phenix Opera HC imager. TAMRA signal per cell was quantified using a custom analysis pipeline in Harmony analysis software. Representative images are shown of live cells treated with 10 µM TAMRA labelled compound for 24 h and stained with CellMask Green actin and Hoechst 33342 dyes. Images were obtained using a Leica Stellaris 5 confocal microscope and 63X oil-immersion objective.
IF: C2C12 myoblasts were treated with 5 µM EDO23-TAMRA for 24 prior to fixation, permeabilization, and immunofluorescent staining with antibodies towards Rab5, Rab7, and LAMP1. Images were obtained using a Leica Stellaris 5 confocal microscope and 63x oil-immersion objective.
ENDOSOMAL ESCAPE: HeLa LgBiT-SNAP-actin cells were treated with 20, 10, or 5 µM HiBiT-conjugate for 24 h. NanoGlo live cell assay reagent was added to measure cytosolic signal. Next, cell were incubated with 0.01% digitonin and total cellular signal was measured. Mean luminescence is shown (± s.d., n=3).

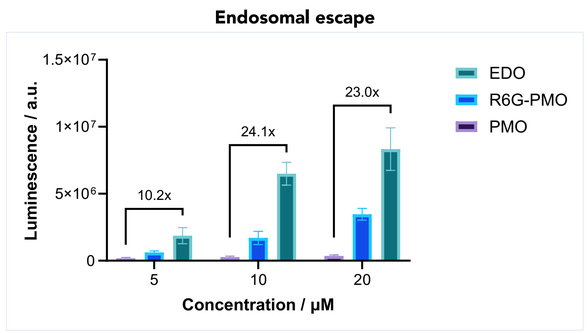
ENDOSOMAL TRAFFICKING AND ESCAPE

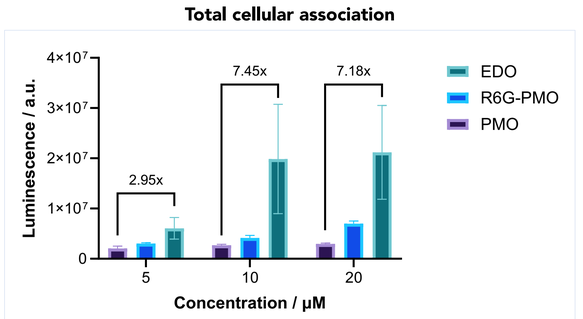
EDOs ARE TRAFFICKED VIA THE ENDOLYSOSOMAL PATHWAY



Green = endolysosome marker; Red = TAMRA labelled EDO; Blue = nucleus

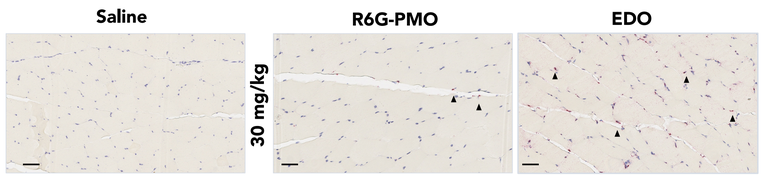
EDOs EFFICIENTLY ESCAPE THE ENDOSOME



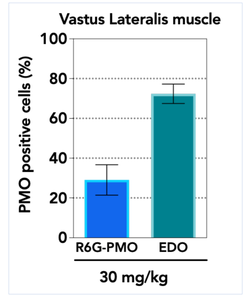


INTRACELLULAR UPTAKE IN NHP AND HUMAN MUSCLE

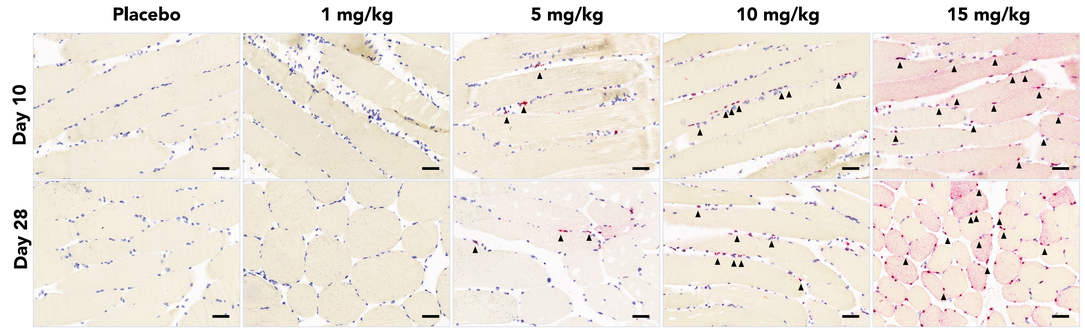
EDO SHOWS SUBSTANTIAL INTRACELLULAR UPTAKE IN NON-HUMAN PRIMATE MUSCLE



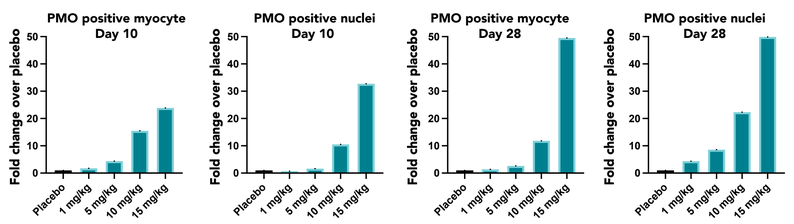
NHPs were IV dosed at 30 mg/kg twice on Day 1 and Day 15 with a PMO conjugated to either R6G or tool PepGen peptide. Tissues were collected 7 days later and assessed for PPMO levels using a probe targeting the PMO sequence. Image analysis and quantification was done using Halo imaging software.
Scale = 50µm, Red-PMO, Blue-Nuclei, n=3; mean± SD



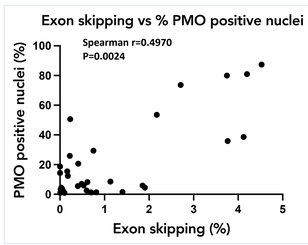
EDO SHOWS DOSE DEPENDENT UPTAKE INTO HUMAN MUSCLE



DOSE DEPENDENT EDO UPTAKE IS DETECTED IN BOTH CYTOPLASMIC AND NUCLEAR COMPARTMENTS

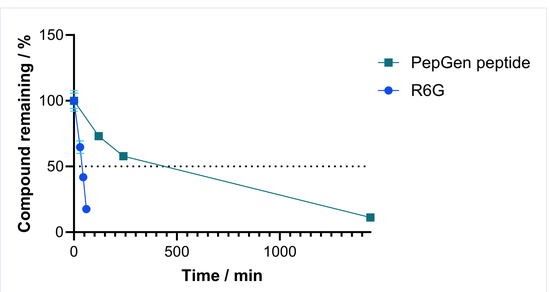


NUCLEAR UPTAKE SIGNIFICANTLY CORRELATES TO EXON SKIPPING ACTIVITY



Healthy volunteers were dosed with placebo or 1, 5, 10, 15 mg/kg PGN-EDO51 via iv infusion. Biceps samples were collected at day 10 and day 28, assessed for PGN-EDO51 levels in post hoc in situ hybridization analysis using a probe targeting the PMO sequence. Image analysis and quantification was done using Halo imaging software.
Myocyte signal = nuclear signal + cytoplasmic signal. Scale = 50µm, Red – PMO, Blue-nuclei

PEPGEN PEPTIDE HAS INCREASED PLASMA STABILITY vs R6G PEPTIDES



PLASMA STABILITY: Plasma samples were incubated with 10µM of PepGen's proprietary peptide for 0, 120, 240, 1440 min (n=3 for each timepoint). 10µM R6G was incubated in plasma at 0, 30, 45, 60 min (n=3 for each timepoint) as rapid clearance was observed and peptide was undetectable in later timepoints. At the specific timepoints, samples are protein precipitated with three volumes of 1:1 H2O:MeCN+2% formic acid containing 100ng/mL internal standard. Samples were centrifuged at 14000xg to pellet precipitated proteins and 100uL of supernatant was diluted with 100uL of H2O and analyzed via UHPLC/MS.

CONCLUSIONS

- PepGen's empirically engineered enhanced delivery oligonucleotide (EDO) technology shows better plasma stability, higher intracellular uptake and endosomal escape compared to unconjugated PMOs and R6G-PMOs.
- EDO's superior attributes translates to NHP and human muscle tissue.
- Clinical trials evaluating the safety and exploring the potential efficacy of the EDO technology, including PGN-EDO51 (exon 51 skipping) for Duchenne muscular dystrophy and PGN-EDODM1 for myotonic dystrophy type 1 (liberation of MBNL1) are ongoing.

PepGen Disclosures: All authors are employees of PepGen Inc. and hold PepGen equity.