Mechanistic Characterization of Enhanced Delivery Oligonucleotide (EDO) Platform

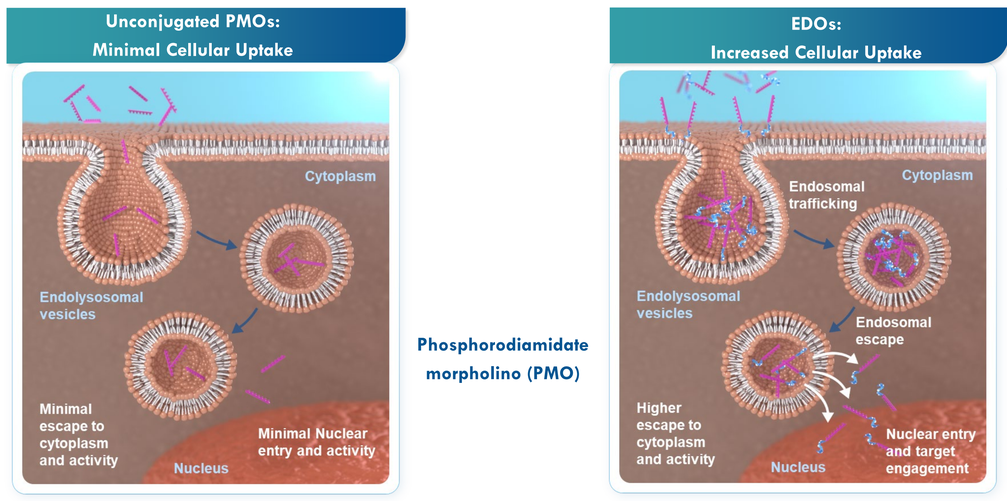
Cole Emanuelson, Katherine Williams, Jane Larkindale, Pallavi Lonkar, and Mangala M. Soundarapandian

PepGen Inc., Boston, MA, USA

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.



EVOLUTION OF CELL PENETRATING PEPTIDES (CPPs)

CPP Motifs

Arginine-rich sequence (RXR motif)

Penetratin sequence RQIKIWFQNRRMKWKK

First Generation CPPs

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

RXRRBRRXR R-rich | YQFLI Hydrophobic | RXRBRXRB R-rich — Pip6a

RRRRRR | G — R6G

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

Good activity but considerable tolerability issues

Empirical design

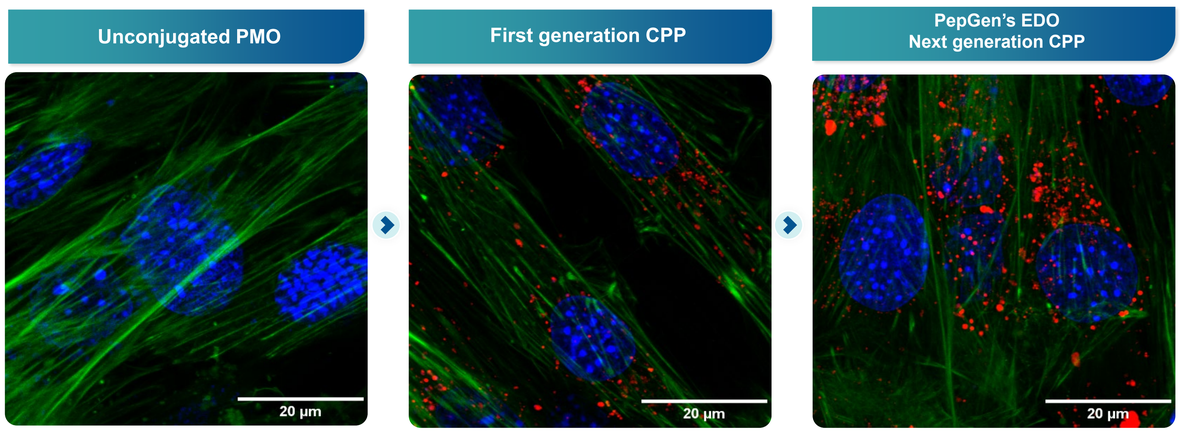
Next Generation CPPs/EDO

R-rich | Hydrophobic | R-rich

- <17 amino acids length
- Optimized hydrophobic-rich domain
- R-rich flanking sequences (5–6 arg)
- No aminohexanoic acid
- Optimized dispersion of unnatural amino acids
- Linker optimized for conjugations

Good activity + clinically viable tolerability

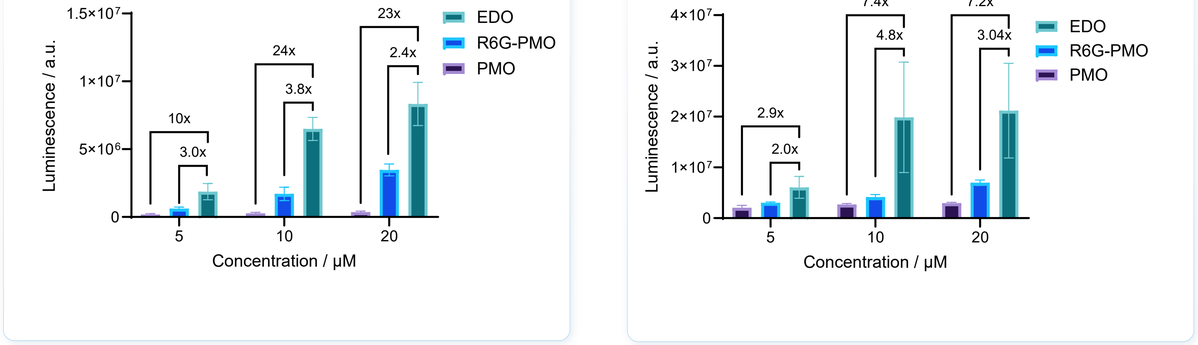
EDO PEPTIDES ENABLE HIGHER DELIVERY OF PMO OLIGONUCLEOTIDES INTO CELLS



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

C2C12 myotubes, treated for 24 h , mean ± s.d., n=3.

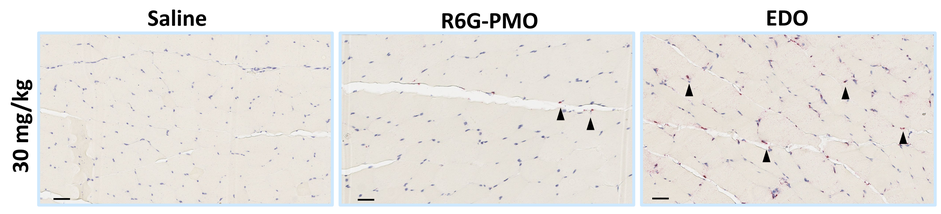
EDO TECHNOLOGY INCREASES ENDOSOMAL ESCAPE OF PMOs

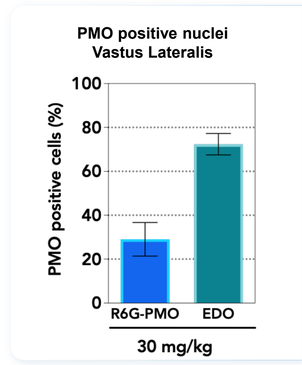


HeLa Cells stably expressing LgBit fused to actin were treated with HiBiT-conjugate for 24 h. Mean luminescence is shown (± s.d., n=3).

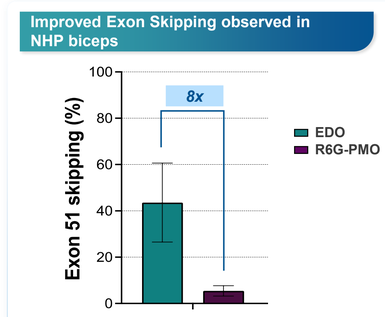
EDO PEPTIDES ENABLE SUBSTANTIAL DELIVERY AND ACTIVITY IN NON-HUMAN PRIMATE MUSCLE

EDO SHOWS IMPROVED INTRACELLULAR UPTAKE AND ENHANCED EXON SKIPPING



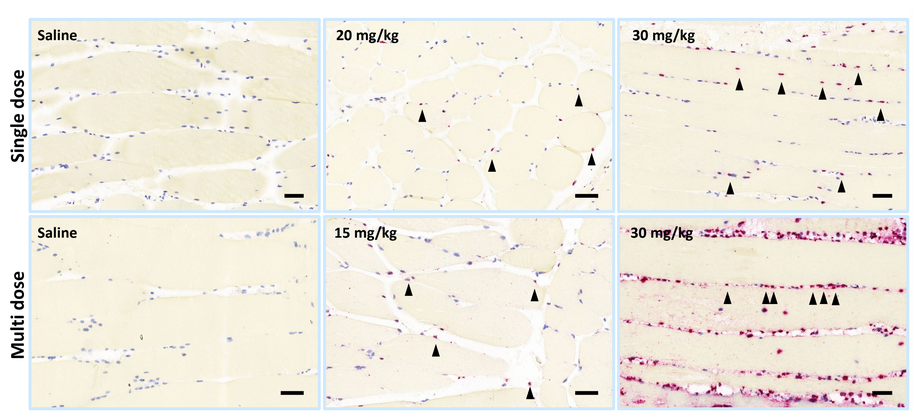


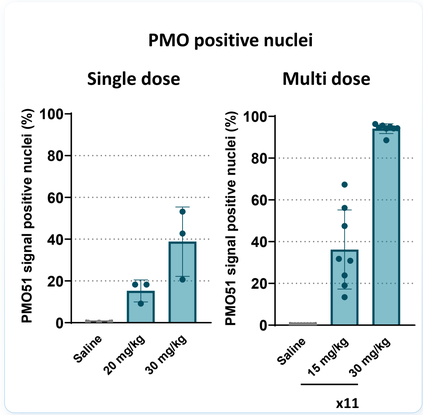
NHPs were IV dosed at 30 mg/kg twice on Day 1 and Day 15 with a PMO conjugated to either R6G or tool PepGen peptide. Tissues were collected 7 days later and assessed for PPMO levels using a probe targeting the PMO sequence. Image analysis and quantification were done using Halo imaging software. Scale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



Single dose PD, Activity assessed by DMD Exon Skipping using RT-PCR, n=3–4

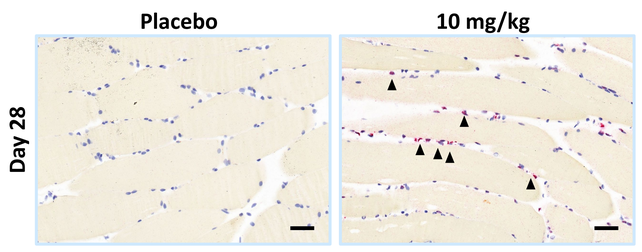
EDO INTRACELLULAR UPTAKE INCREASES WITH REPEAT DOSING

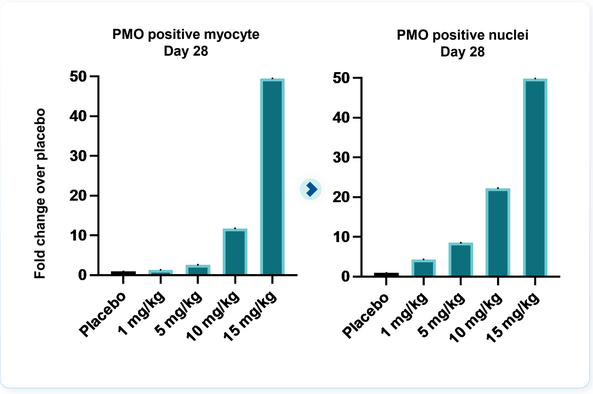




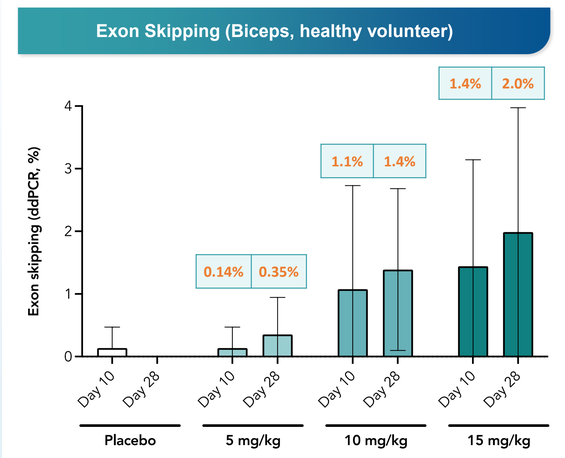
Multi dose- Repeat dosing every 4 weeks for total of 11 doses (39 weeks), Tissues were collected 7 days later and assessed for PPMO levels using a probe targeting the PMO sequence. Image analysis and quantification was done using Halo imaging software. Scale = 50µm, Red-PMO, Blue-Nuclei, mean± SD

EDO PEPTIDES ENABLE SUBSTANTIAL INTRACELLULAR UPTAKE AND ENHANCED EXON SKIPPING IN HEALTHY VOLUNTEER MUSCLE





Healthy volunteers were dosed with placebo or 1, 5, 10, 15 mg/kg PGN-EDO51 via IV infusion. Biceps samples were collected on 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-nucleus



Protocol PGN-EDO51-101: Phase 1, first in human, randomized double blind, placebo controlled, single ascending dose study in healthy adult volunteers. Single dose of either PGN-EDO51 or Placebo were administered by IV infusion at doses indicated. Participants were followed for 28-day period following dose administration to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD). Needle biopsies of biceps muscle were taken on Day 10 and Day 28. Exon Skipping measured by ddPCR. Shown as mean ± SD; n = 6 PGN-EDO51: 2 Placebo per cohort (n = 5 for D10 at 15 mg/kg).

CONCLUSIONS

- We show here that PepGen's next generation CPPs have superior drug-like attributes compared to naked PMOs and PMOs conjugated to first generation CPPs.
- This was reflected in their higher cellular uptake and endosomal escape in cells. *In vivo*, this translated into higher nuclear delivery in non-human primates and healthy volunteers (HV).
- In a post-hoc analysis of PGN-EDO51 Phase 1 HV single ascending dose (SAD) study of bicep biopsies, we observed approximately 50% of muscle cell nuclei positive with PGN-EDO51 oligonucleotide. The nuclear delivery translated to up to 2% exon skipping in human HV biceps.
- Based on these and other studies, the EDO technology is being employed in investigational treatments for Duchenne muscular dystrophy (DMD) targeting exon 51 skippable mutations (PGN-EDO51) and myotonic dystrophy type 1 (PGN-EDODM1).

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