PepGen™

Broadening the Understanding of Peptide Conjugated Oligonucleotide Platform to Expand Therapeutic Use

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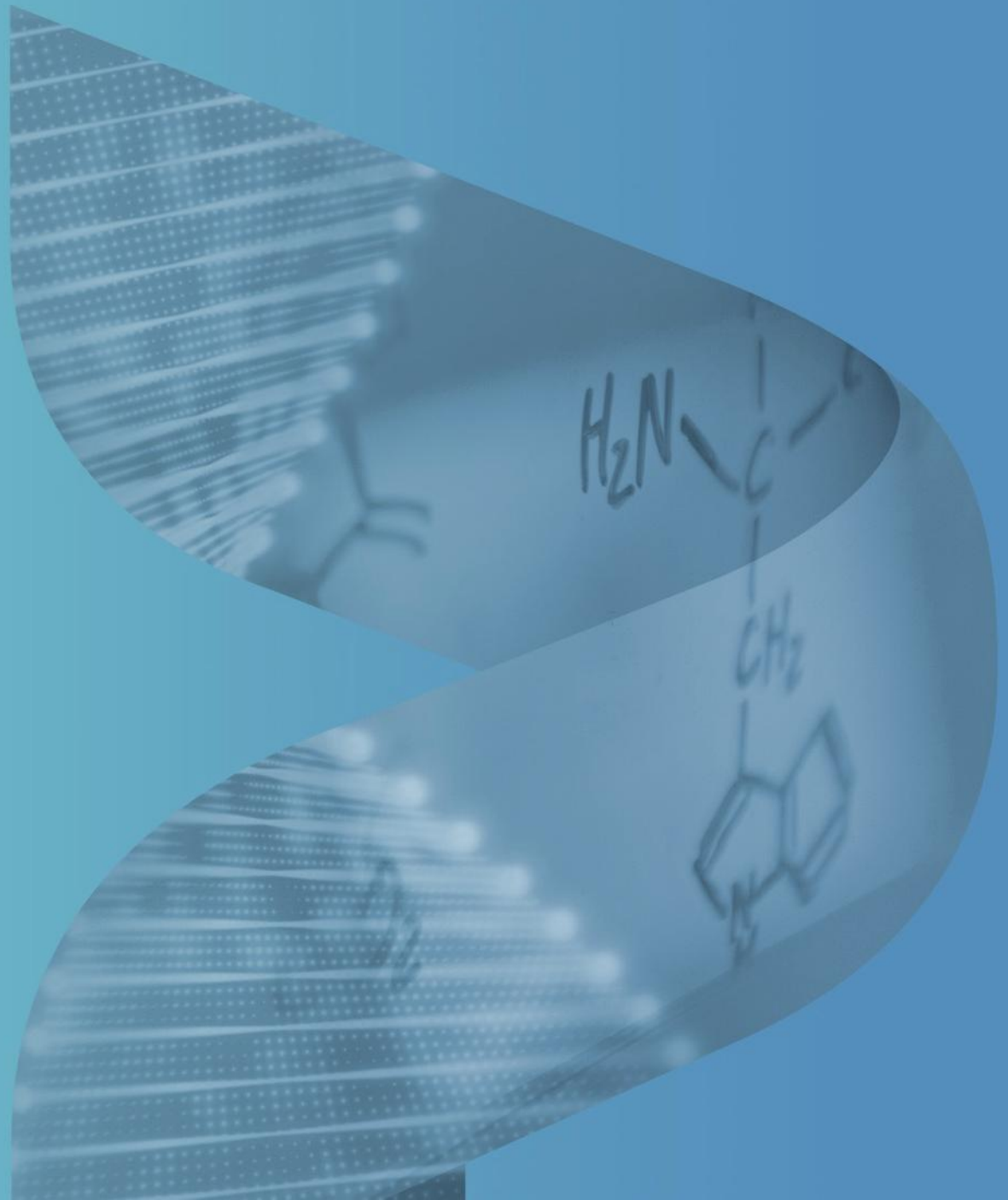
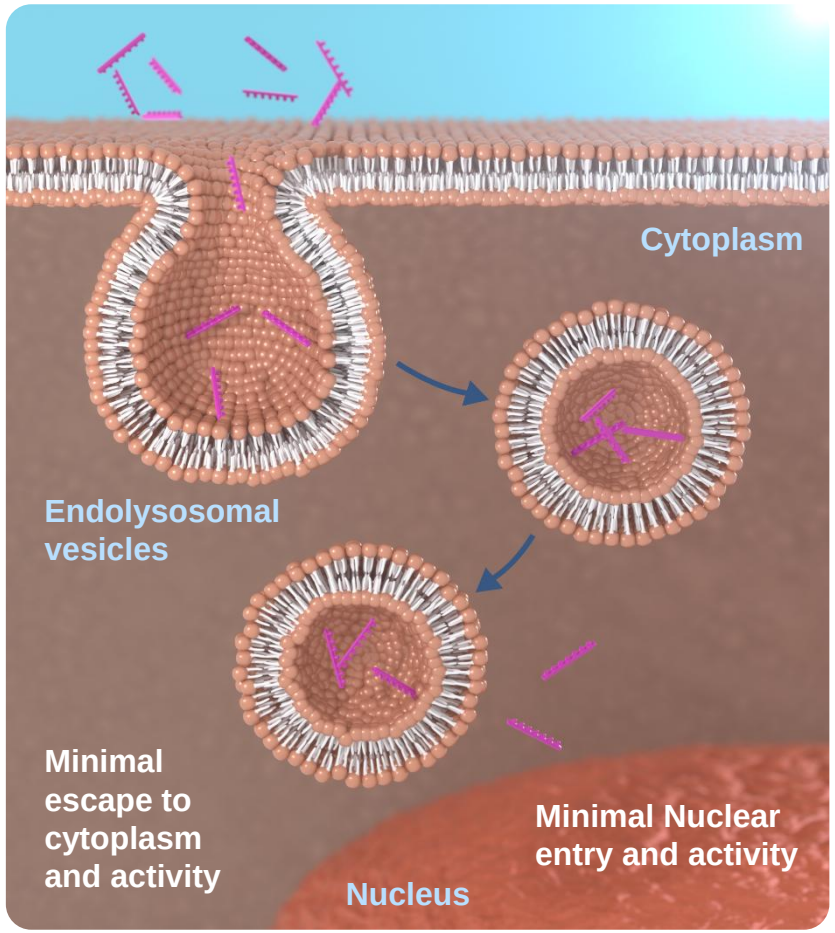


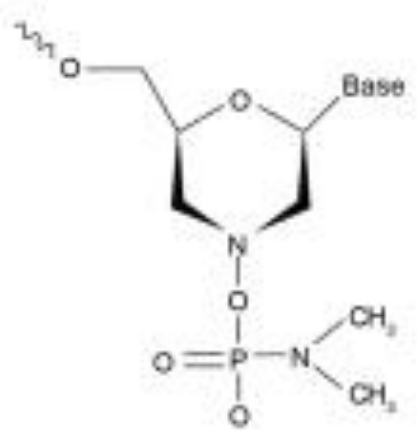
Plan

- Introduction to Enhanced Delivery Oligonucleotide (EDO) technology
- Attributes of EDOs that confer superior drug-like properties
- EDO enabled pipeline and future directions

Unconjugated PMOs Have Minimal Cellular Uptake



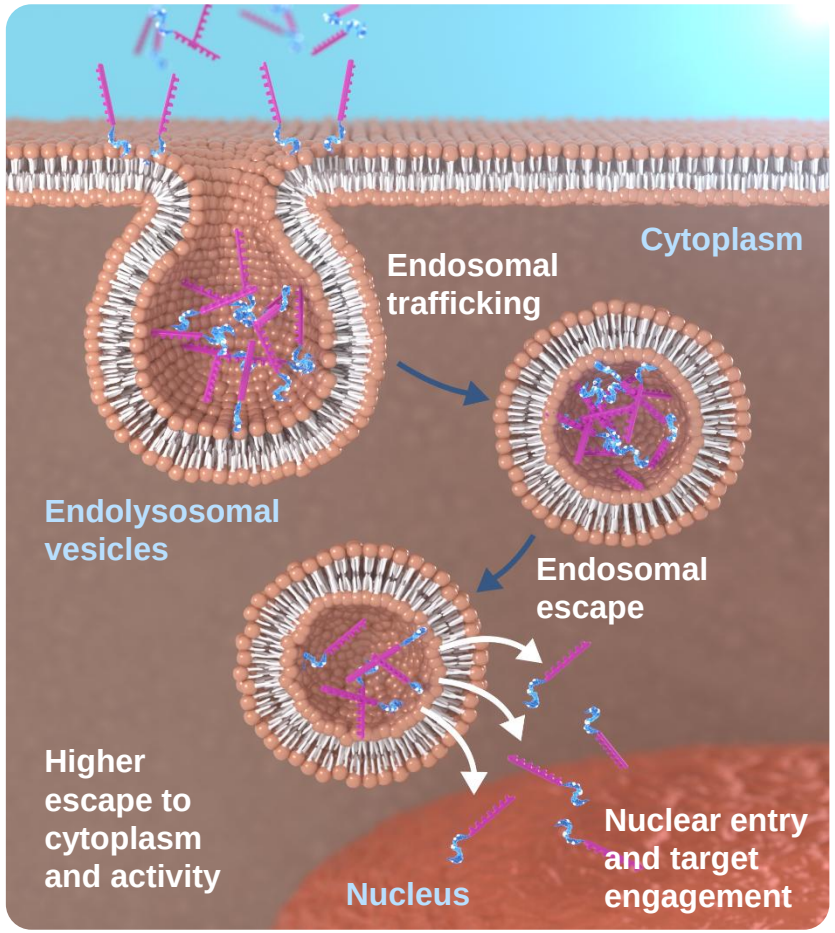
- Neutral
- Steric binding oligonucleotide
- Nuclease resistant
- Improved binding affinity
- Non-immunogenic



Phosphorodiamidate morpholino (PMO)

Tsoumpra MK et al., 2019, EBioMedicine, Gait M et al., 2019, Nucleic acid Therapeutics

EDOs are Therapeutic PMOs Conjugated to Delivery-Enhancing Peptides that Increase Cellular Uptake



Enhanced delivery peptide

Therapeutic PMO

Enhanced Delivery Oligonucleotide

Tsoumpra MK et al., 2019, EBioMedicine, Gait M et al., 2019, Nucleic acid Therapeutics

Evolution of Cell Penetrating Peptides (CPPs)

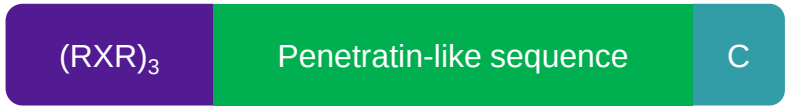
Empirical design

CPP Motifs

Arginine-rich sequence (RXR motif)

Penetratin sequence RQIKIWFQNRRMKWKK

First Generation CPPs



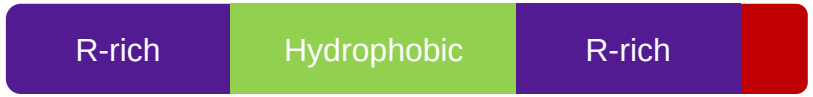
Pip6a

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

Next Generation CPPs/EDO



- <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

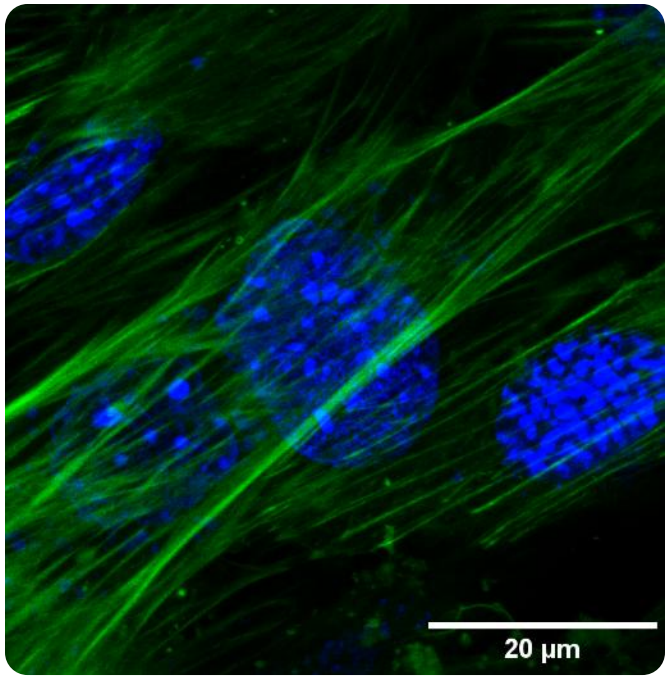
Good activity + clinically viable tolerability

How Do EDO's Drug-Like Properties Compare with First Generation CPPs?

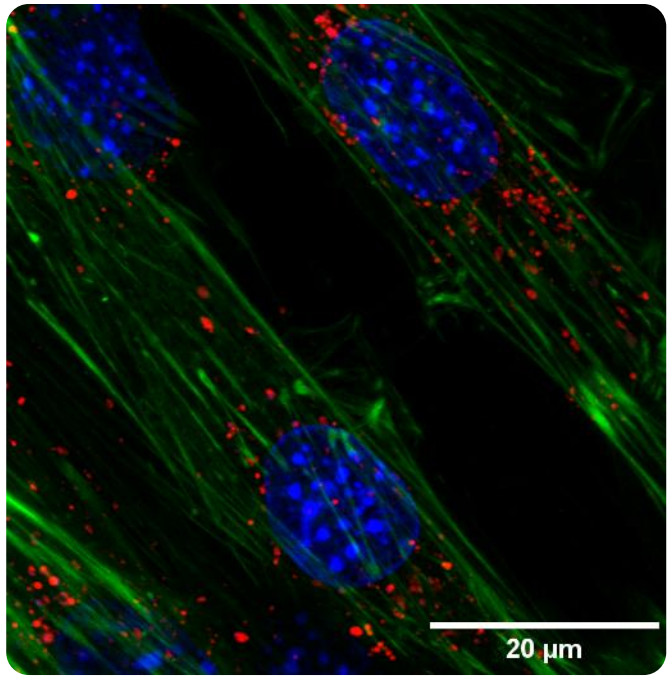
Cellular uptake	?
Endosomal escape	?
Stability	?
Activity	?

EDO Peptides Enable Higher Delivery of PMO Oligonucleotides into Cells

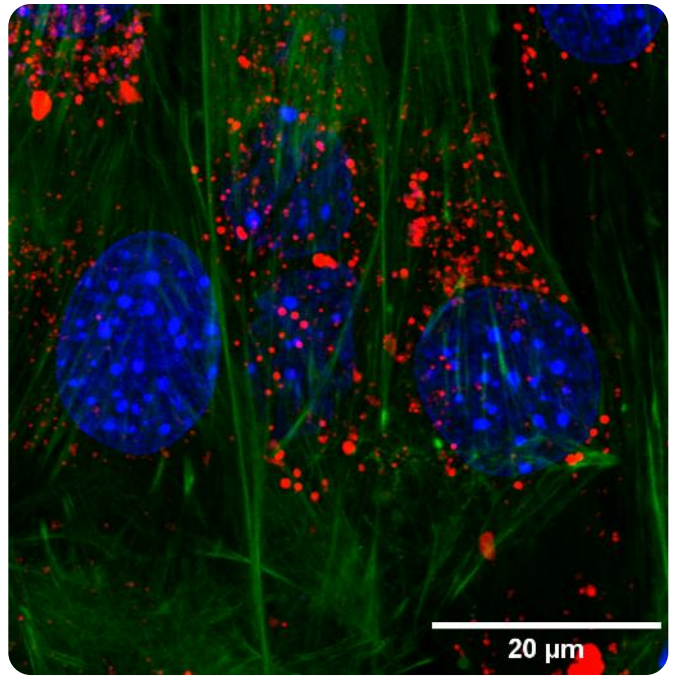
Unconjugated PMO



First generation CPP



PepGen's EDO Next generation CPP



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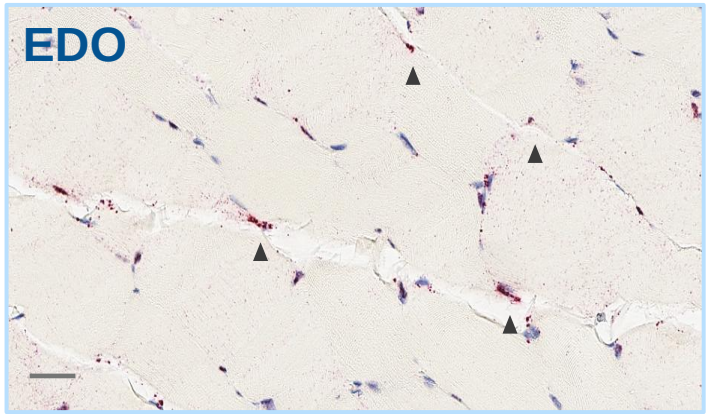
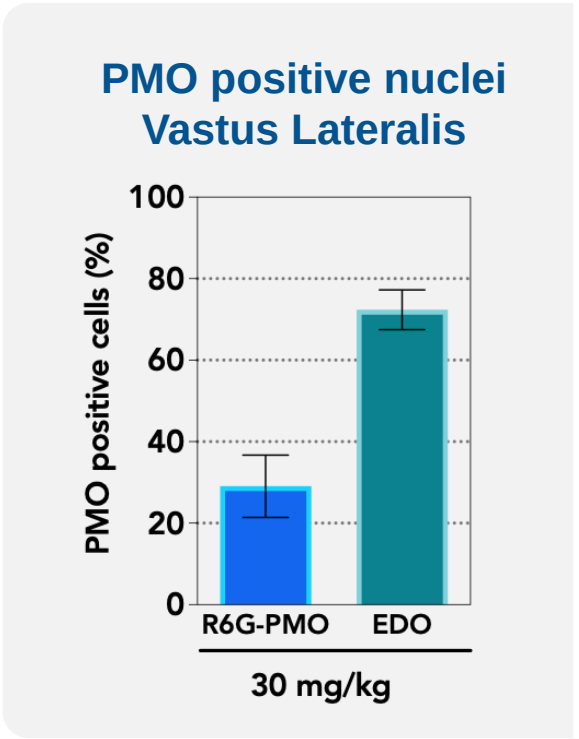
C2C12 myotubes, treated for 24h , mean± s.d., n=3

EDO Peptides Enable Substantial Intracellular Uptake in Non-Human Primate Muscle

Translation of improved uptake to NHP



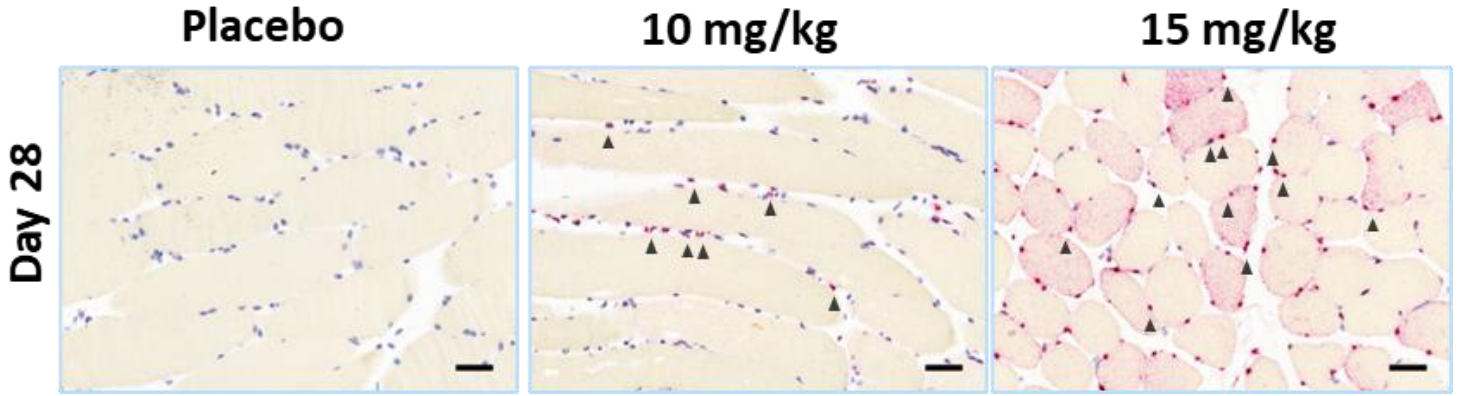


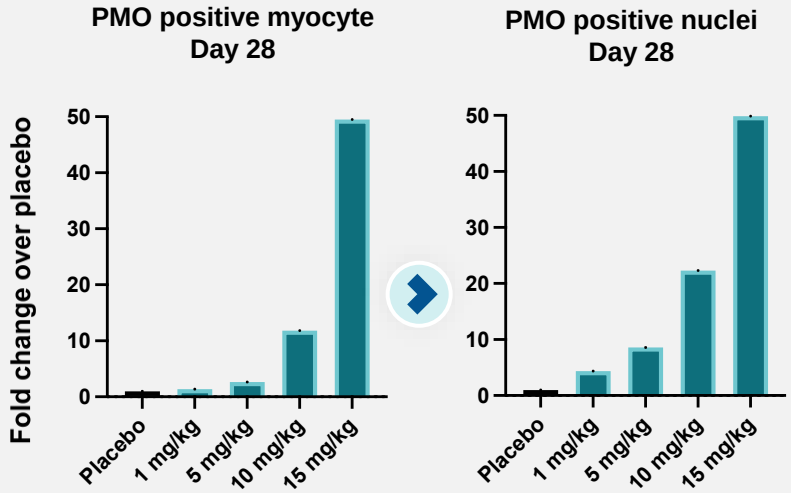
NHPs were IV dosed at 30 mg/kg twice on Day 1 and Day 15 with a PMO conjugated to either R6G or tool PepGen peptide. Tissues were collected 7 days later and assessed for PPMO levels using a probe targeting the PMO sequence. Image analysis and quantification was done using Halo imaging software. Scale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

EDO Peptides Enable Substantial Intracellular Uptake in Human Healthy Volunteer Muscle

Translation of uptake to human

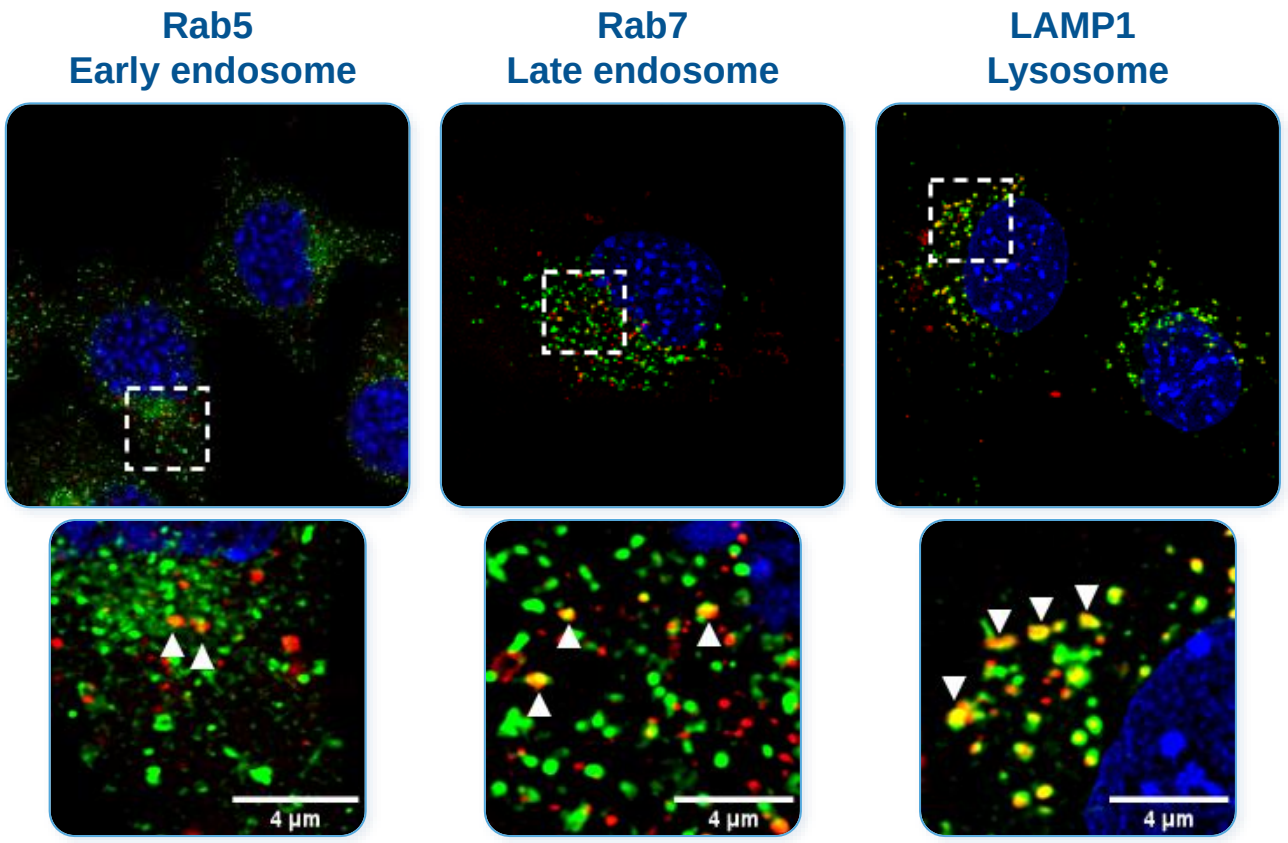
- Study population: Healthy adult males (n = 32; 8 per cohort, 3:1 PGN-EDO51:placebo)
- Dosing: Single dose, IV administration
- Bicep biopsies conducted on Day 10 and Day 28



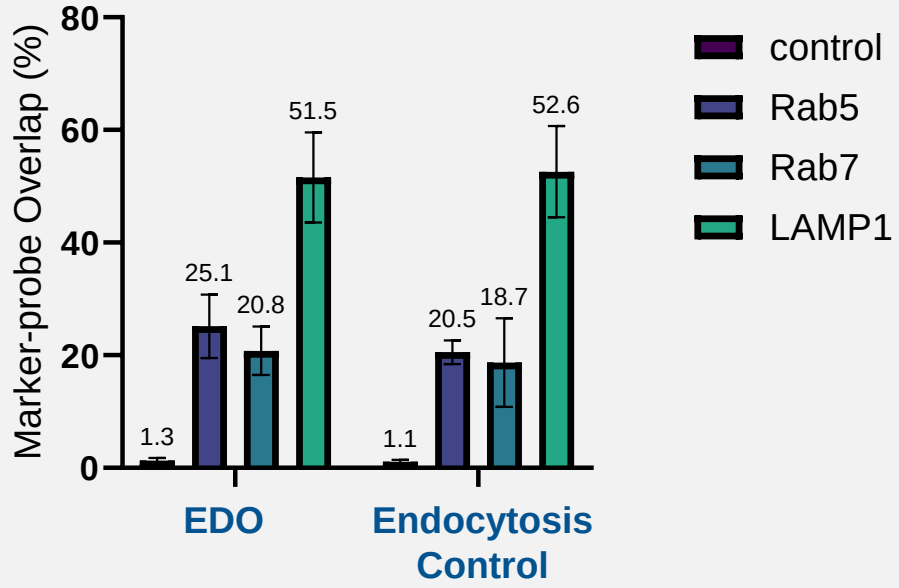


Healthy volunteers were dosed with placebo or 1, 5, 10, 15 mg/kg PGN-EDO51 via iv infusion. Biceps samples were collected at day 10 and day 28, assessed for PGN-EDO51 levels in post hoc in situ hybridization analysis using a probe targeting the PMO sequence. Image analysis and quantification was done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

EDOs are Trafficked Intracellularly via the Endolysosomal Pathway



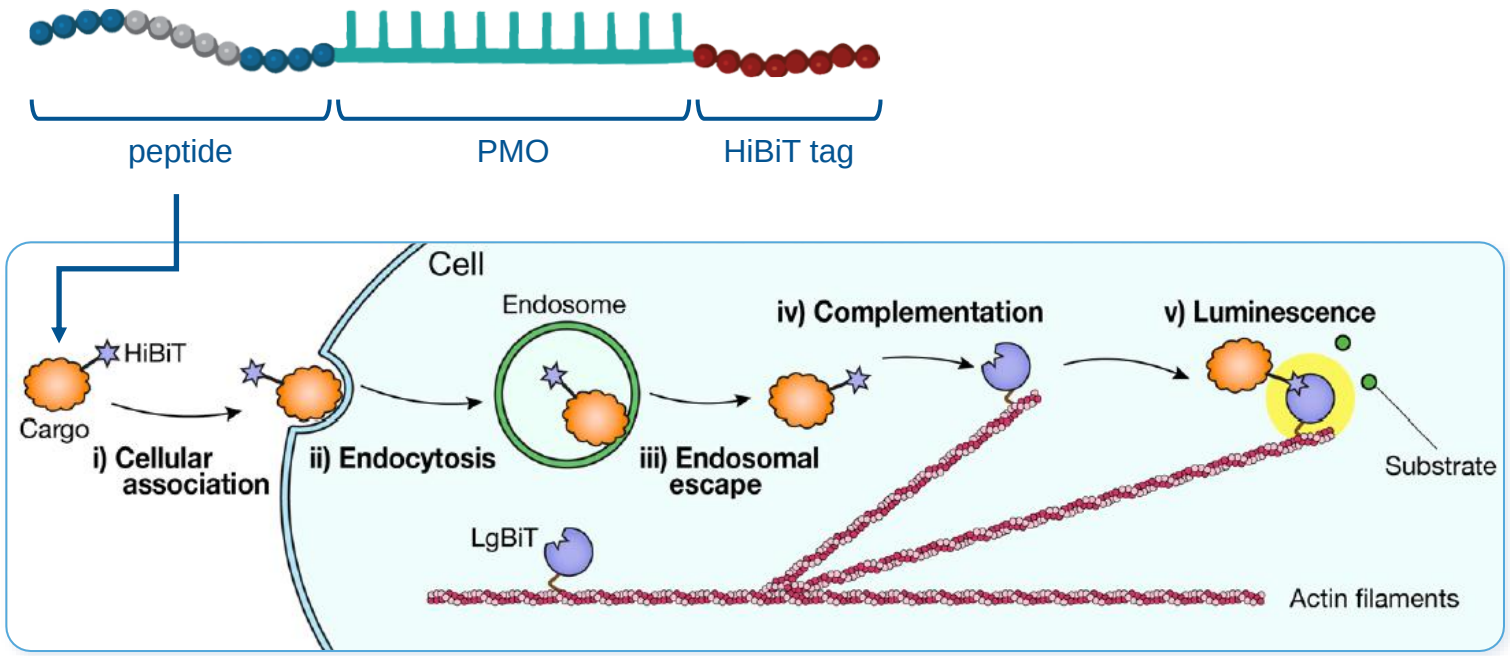
Green = endocytic marker; Red = TAMRA labelled conjugate; Blue = nucleus



C2C12 myoblasts, 5–10 µM treatment for 24h, n=2, ± s.d..

Split Luciferase Assay to Measure the Endosomal Escape of EDOs

EDO with HiBiT protein tag

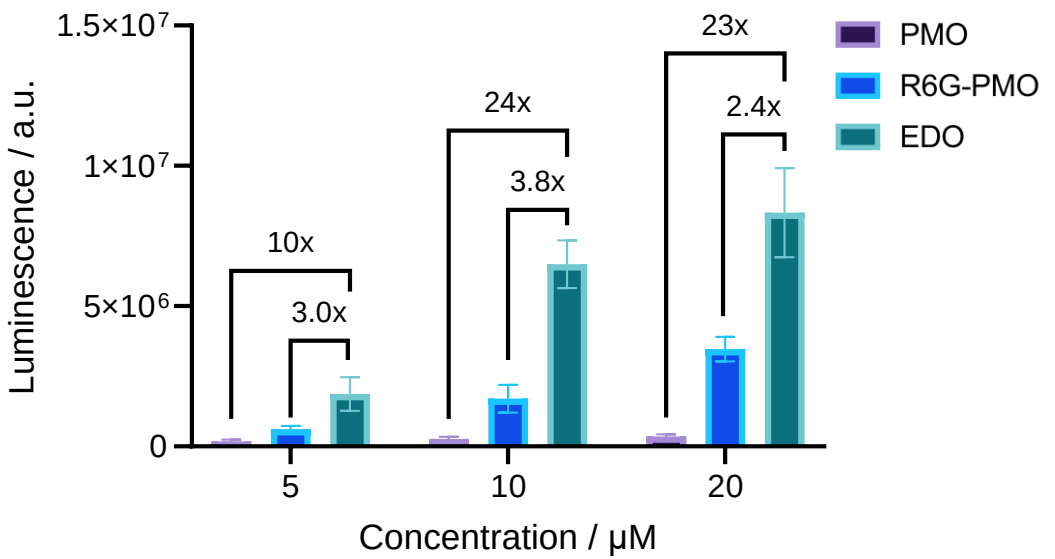


LgBiT protein expressed as a fusion to actin to tether to cytoskeleton

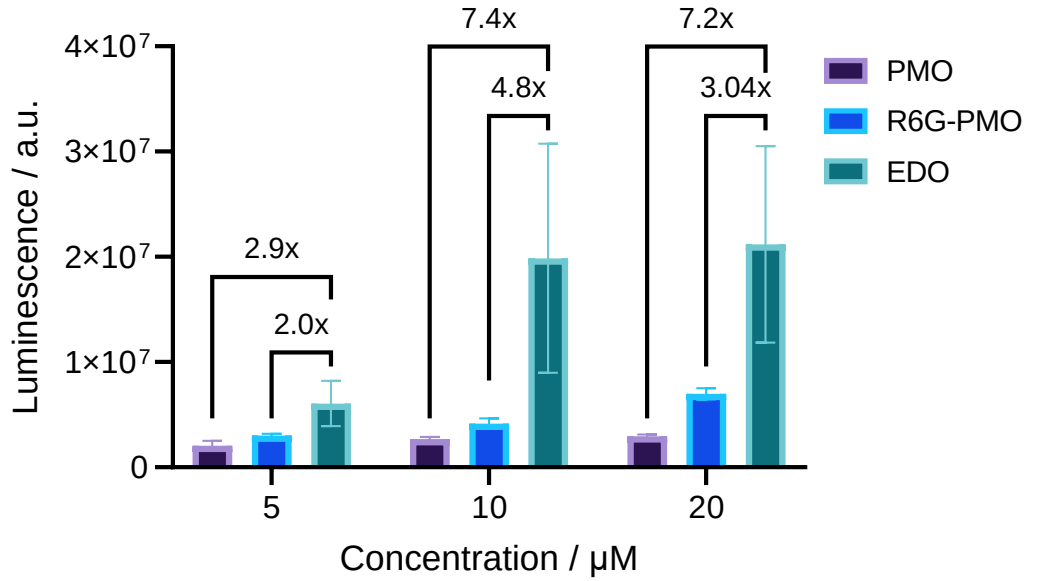
- Complementation between HiBiT-PPMO and LgBiT-Actin forms a functional luciferase enzyme complex, which gives off bright luminescence in the presence of a substrate.
- This allows for the sensitive and quantitative measurement of the endosomal escape and cytosolic delivery of EDOs.

EDO Technology Increases Endosomal Escape of PMOs

Endosomal escape

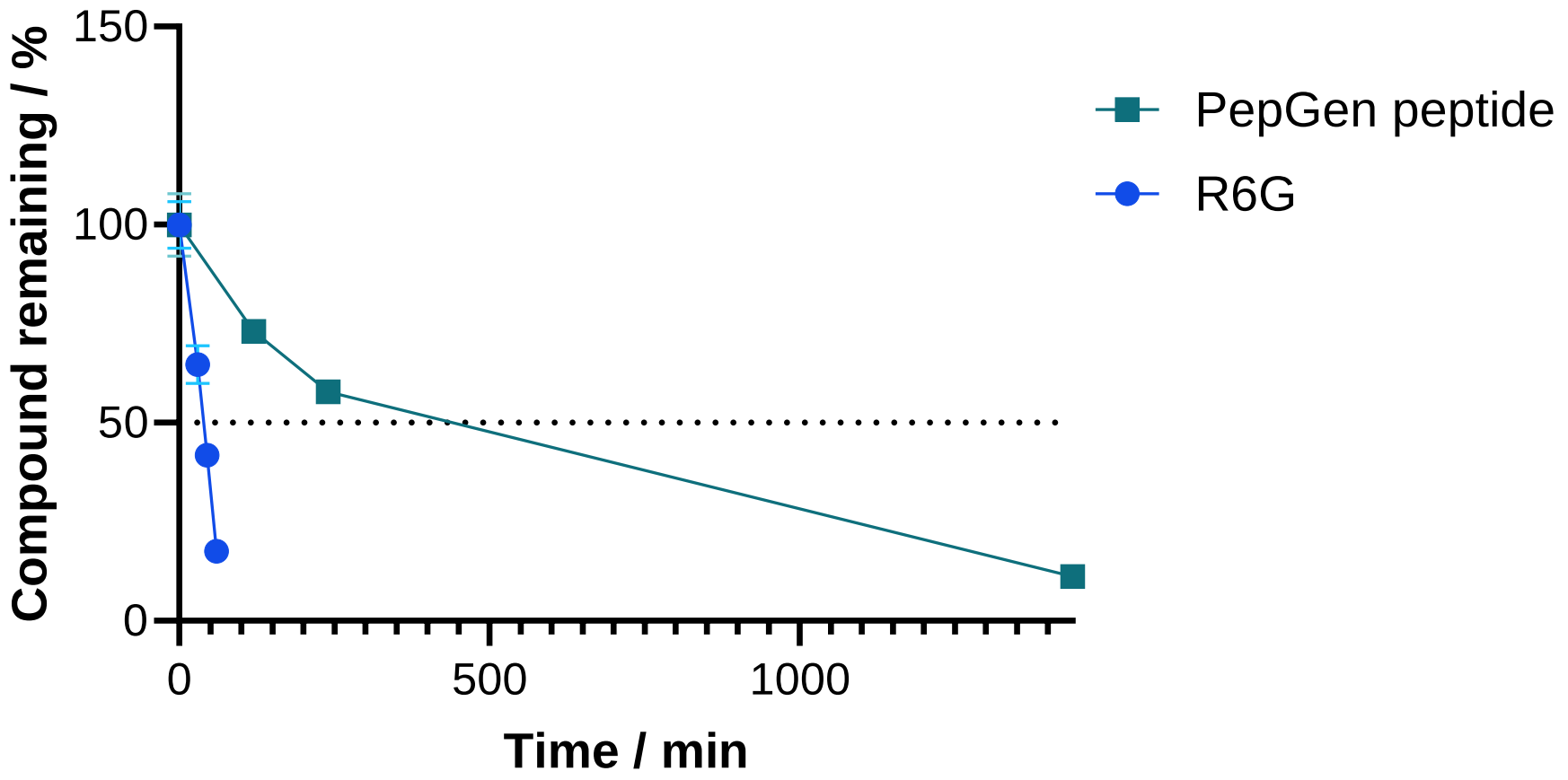


Total cellular association



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

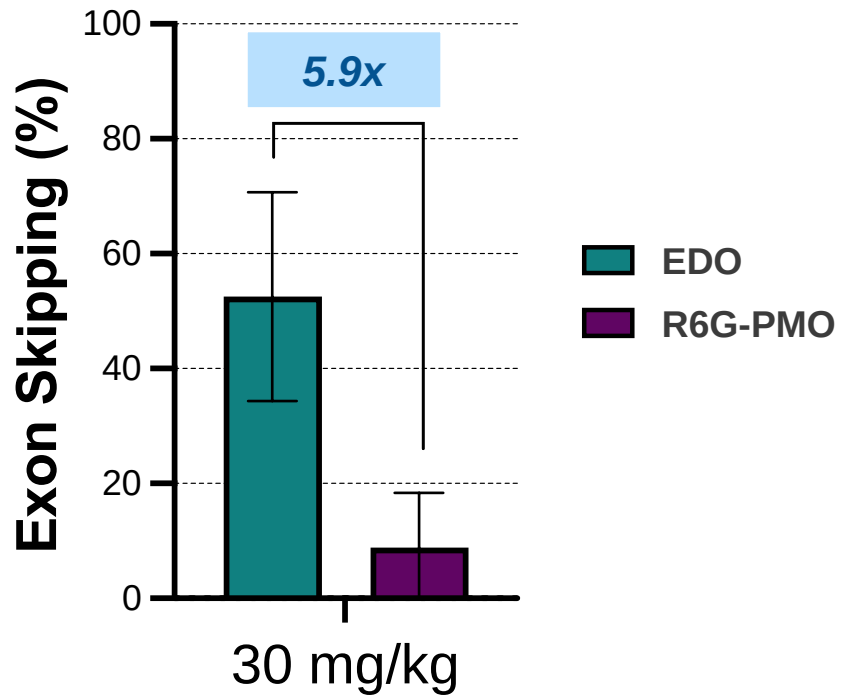
EDO Peptides Show Better Stability in Human Plasma (*in vitro*) vs Previous Generation CPPs



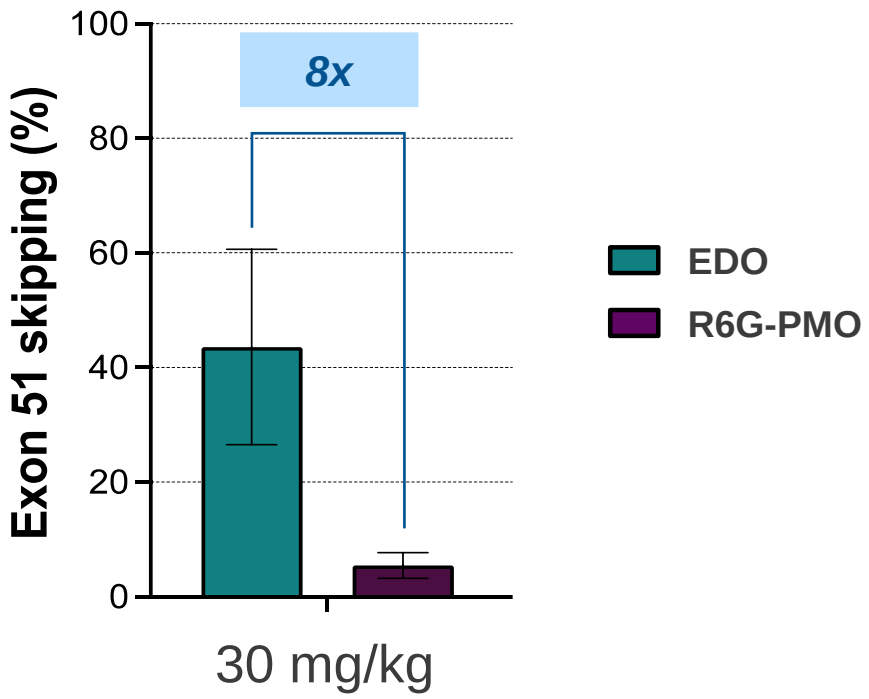
Plasma samples were incubated with 10µM of peptides. Analyzed via UHPLC/MS).

EDO Driven Uptake and Endosomal Escape Translates to Enhanced Exon Skipping Activity In Vivo

Improved Exon Skipping observed in mouse biceps



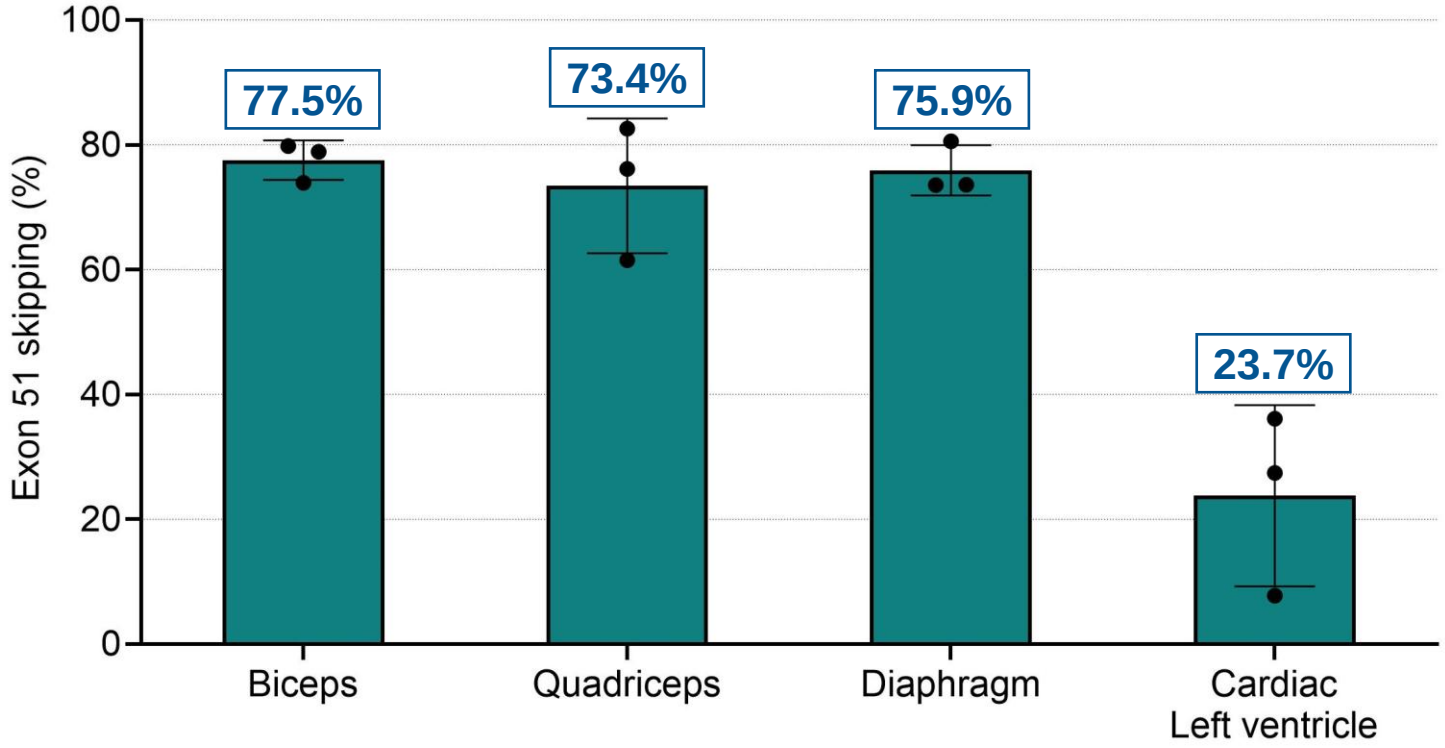
Improved Exon Skipping observed in NHP biceps



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

EDO Platform: Activity Across Broad Muscle Groups Impacted in Neuromuscular Diseases

Exon Skipping activity in NHP muscle

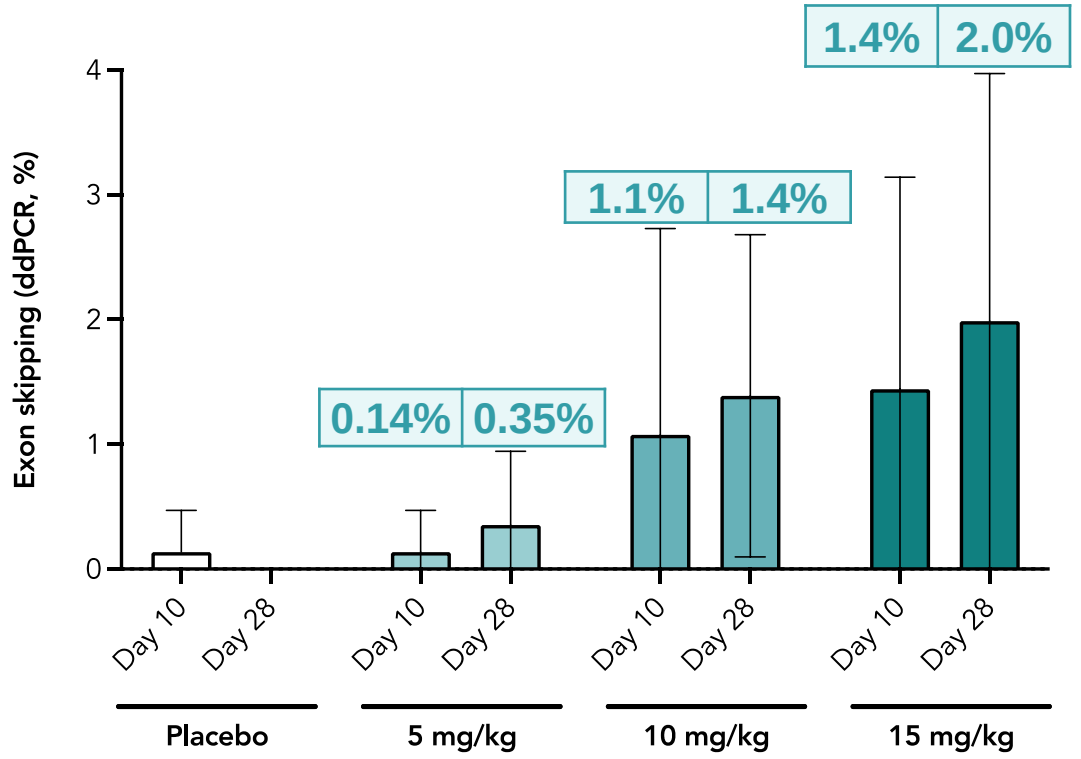


30 mg/kg PGN-EDO51,
3 doses every 2 weeks,
biopsy 7d after last dose

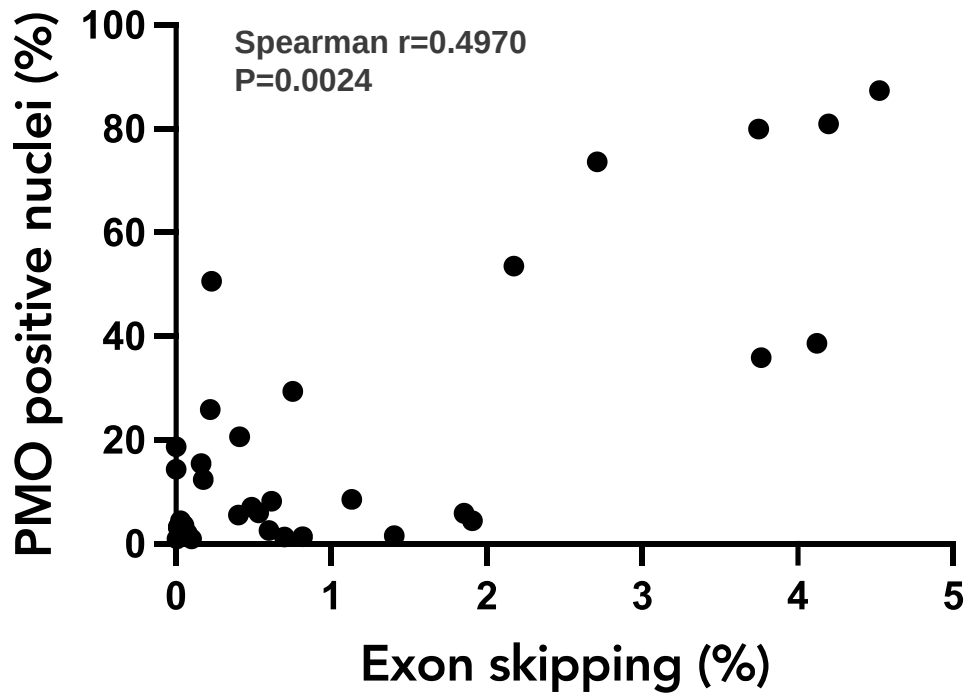
Mean ± SD; n = 3 per group. Study was not powered for statistical significance.

Exon Skipping Activity in HV Muscle Correlates with Nuclear Uptake

Exon Skipping (Biceps, healthy volunteer)



Exon Skipping vs PMO positive nuclei



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).

Summary

PepGen's empirically engineered Enhanced Delivery Oligonucleotide (EDO) technology possess superior attributes suitable for therapeutic investigation

Cellular uptake – *in vitro*, NHP, Humans	✓
Endosomal escape	✓
Stability	✓
Activity – Mouse, NHP, Humans	✓

PepGen's Pipeline Enabled by EDO Technology

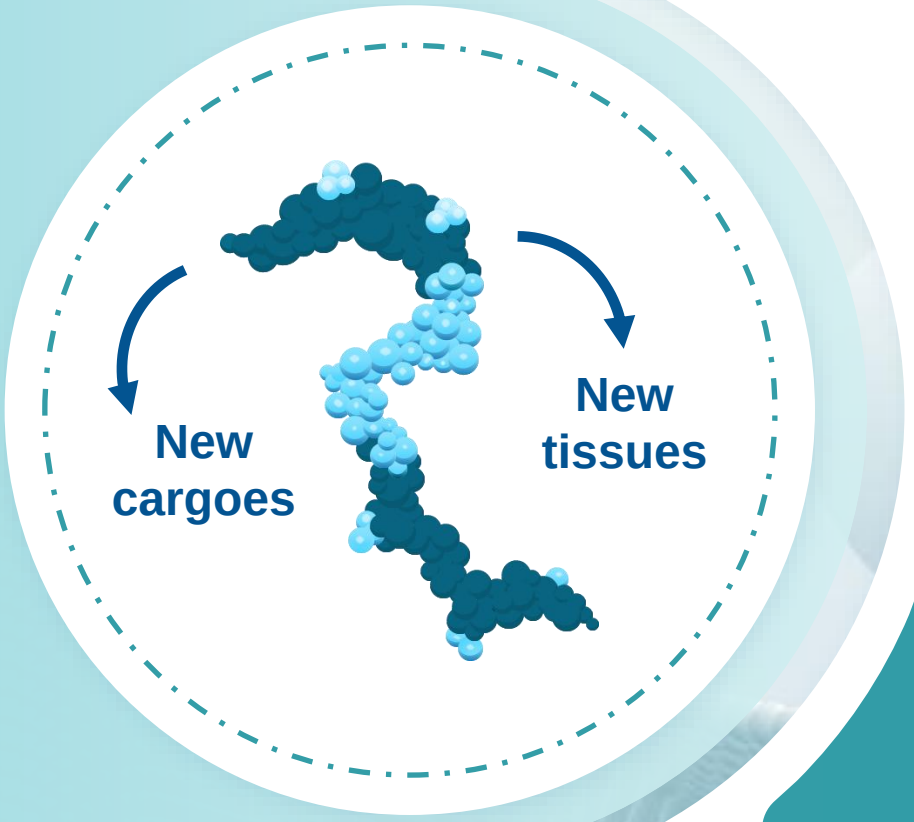
INVESTIGATIONAL CANDIDATES	CLINICAL PROGRAMS	INDICATIONS	PRECLINICAL	PHASE 1	PHASE 2	PIVOTAL
PGN-EDO51	Connect	DMD – *Exon 51*				
PGN-EDODM1	Freedom	DM1 – *DMPK*				
PGN-EDO53		DMD – *Exon 53*				

Research

- DMD *Exon 45, Exon 44*
- Additional neuromuscular diseases
- Neurological diseases

Note: Clinical plans are subject to alignment with regulatory authorities.

What is Next for EDO Discovery?



Reach new tissues

- Explore potential of platform across multiple tissues/cell types

Deliver new cargoes

- Utilize modular nature of EDO platform to evaluate new cargo technologies
- Explore potential for non-PMO modalities

Harness the power of EDO platform to develop new therapies for rare diseases

Thank You!

Preclinical collaborators

Community and clinical advisors

Clinical site staff and investigators

Clinical study participants and their families