PMP22 Reduction by Enhanced Delivery Oligonucleotides Technology is a Promising Approach for Novel CMT1A Therapeutics

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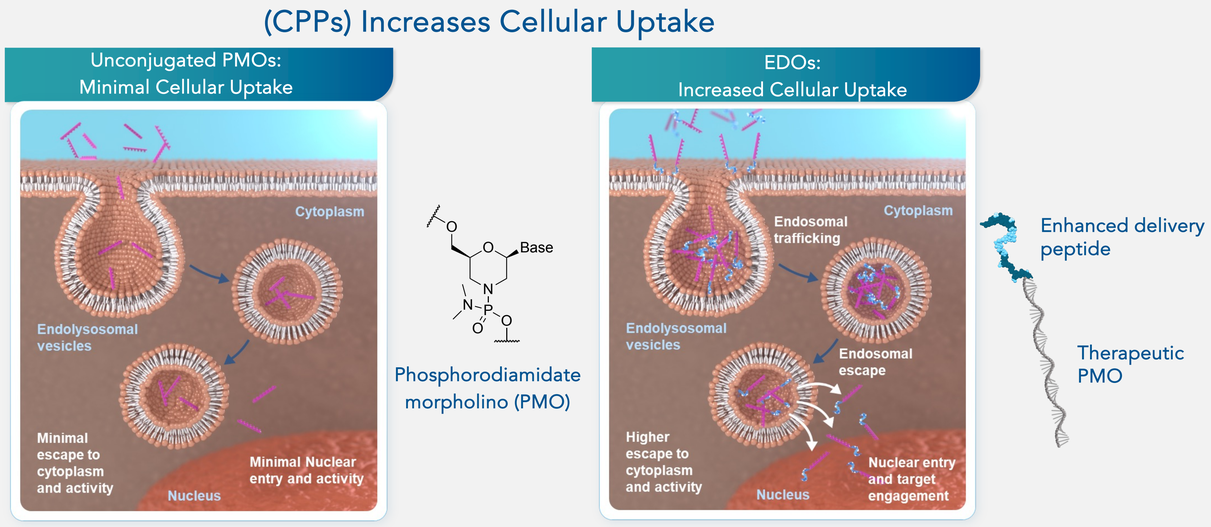
INTRODUCTION

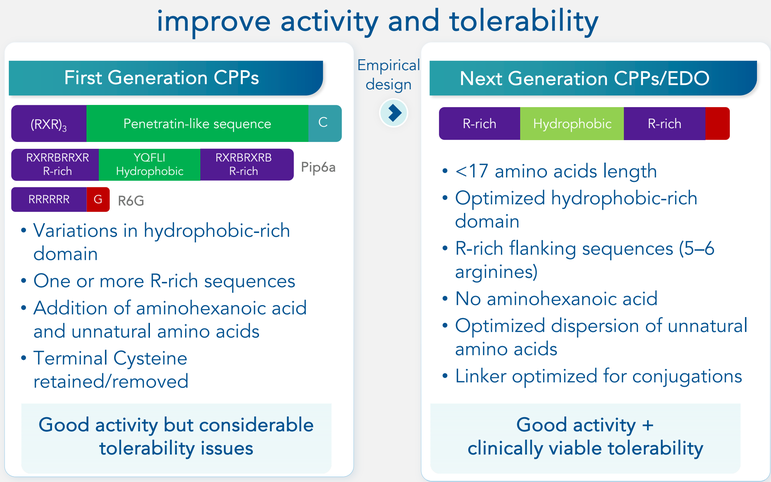
- Charcot-Marie Tooth 1A (CMT1A) accounts for 40-50% of genetically diagnosed CMT. The disease is characterized by demyelination and axonal loss, which leads to muscle weakness, atrophy, and sensory loss. The major genetic cause of CMT1A is a 1.4Mb duplication on chromosome 17, that includes the major myelin protein PMP22 gene. Experimental oligonucleotide therapies for the reduction of PMP22 have mitigated disease in rodent models; however, there is no approved disease modifying therapy for patients.
- We have developed a PMO-based strategy for PMP22 downregulation. Utilizing PepGen's Enhanced Delivery Oligonucleotide (EDO) technology, we show delivery to Schwann cells in the peripheral nerve, the key target cell type to treat CMT1A and present a novel and promising approach for treating CMT1A..

PEPGEN'S ENHANCED DELIVERY OLIGONUCLEOTIDE (EDO) PLATFORM IS DESIGNED TO ENHANCE UPTAKE OF OLIGONUCLEOTIDES

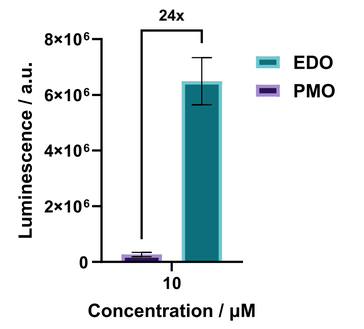
PMO Conjugation to Next Generation Cell Penetrating Peptides (CPPs) Increases Cellular Uptake



PepGen's CPPs are empirically derived to improve activity and tolerability



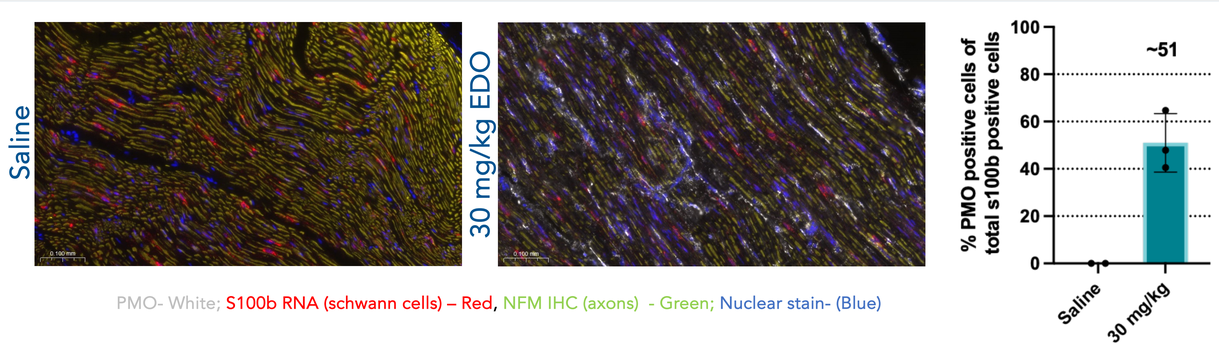
EDOs Efficiently Escape the Endosome



HeLa Cells stably expressing LgBit fused to actin were treated with 10 µM HiBiT-EDO for 24 h. Complementation between HiBiT and LgBiT forms a functional luciferase enzyme complex, which emits bright luminescence. This allows for the sensitive and quantitative measurement of the endosomal escape and cytosolic delivery of EDOs. n=3, Mean ± SD.

EDO TECHNOLOGY EFFICIENTLY DELIVERS OLIGO TO SCHWANN CELLS IN NON-HUMAN PRIMATES

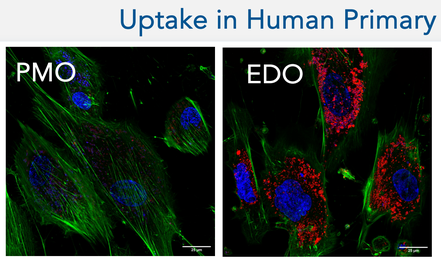
Uptake in NHP Sciatic Nerve



PMO- White; S100b RNA (schwann cells) – Red, NFM IHC (axons) - Green; Nuclear stain- (Blue)

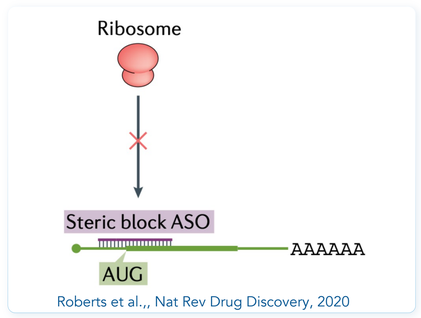
NHPs dosed with 30mg/kg EDO on Day 1, 29 and 57, tissues collected 7 days later and processed with RNAScope to detect EDO , Schwann cells (s100b RNA) and IHC to to detect axons (Neurofilament medium stain); * PMO Positive Cells = >4 dots per cell. Mean ± SD.

Uptake in Human Primary Schwann Cells



Green=actin; Red=TAMRA labelled conjugate;
Blue=nucleus,
25µM, 24 h treatment.

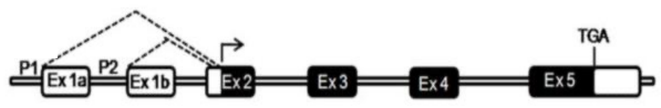
NOVEL STRATEGY IDENTIFIED TO DOWNREGULATE PMP22 IN SCHWANN CELLS



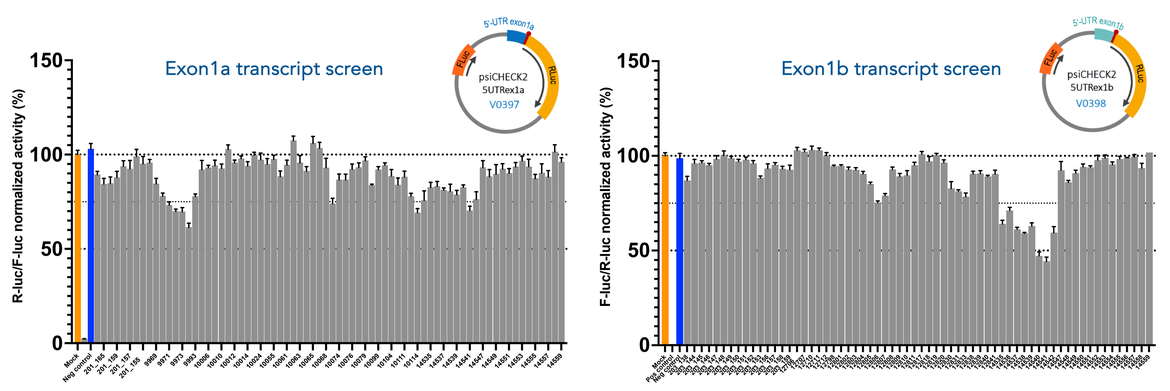
Roberts et al.,, Nat Rev Drug Discovery, 2020

Steric blocking PMOs were strategically targeted to the 5'UTR region of PMP22 mRNA to reduce PMP22 levels

Multiple hotspots amenable for downregulation of PMP22 protein expression identified in primary screen



Pantera H et al., Brain Research, 2020, 1726
Li J et al., Molecular Neurobiology, 2012, 47(2)

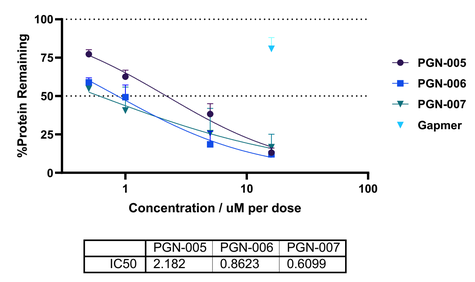


Hepa1-6 cells were co-transfected with reporter plasmid and PMO (5 µM). 24h treatment. Relative luciferase activity plotted. n = 4 technical replicates, Mean ± SD.

COMPARISON OF OLIGONUCLEOTIDE TECHNOLOGIES FOR PMP22 DOWN REGULATION

Company	Oligonucleotide chemistry	Mechanism of action
PepGen	Enhanced delivery peptide conjugated PMO	Reduce protein translation
IONIS	Antisense ASO Gapmer	RNase H-dependent mRNA degradation
Shift Pharmaceuticals	Naked PMO	Exon skipping of pre-mRNA to decrease functional mRNA
DTx Pharma	Fatty acid ligand conjugated siRNA	RISC mediated mRNA degradation

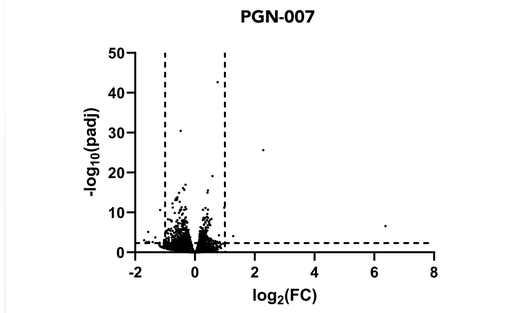
Lead candidates show dose-dependent protein knockdown



	PGN-005	PGN-006	PGN-007
IC50	2.182	0.8623	0.6099

Primary human Schwann cells, indicated EDO treatment at 0 and 30h, collection at 72h, JESS simple western assay , % protein remaining relative to mock normalized to ACTB plotted. n=2, Mean ± SD.

Lead EDO shows limited sequence based off-target DEGs



Primary human Schwann cells, 5 µM EDO treatment for 24h in technical triplicate, RNASeq, DEG- Differential gene Expression

CONCLUSIONS

- We have identified a novel strategy to downregulate protein expression using EDOs targeted to the PMP22 5'UTR region.
- *In vitro* data in human primary Schwann cells shows significant potency and on-target specificity. Follow up studies in rodent disease models and NHP are being planned to further characterize lead molecules.
- PepGen's EDO platform effectively delivers PMOs to Schwann cells in NHP sciatic nerve, the key cell type with PMP22 expression and hence critical to reach for developing CMT1A therapies. As such, we believe PepGen's EDOs are a promising therapeutic opportunity for CMT1A.

Disclosures: All authors are employees of PepGen Inc. and hold PepGen equity; * Former employee

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