Nonclinical in vitro and in vivo data demonstrated low immunogenicity risk and favorable profiles of PGN-EDO51 for the treatment of DMD

Brijesh Garg, Ashling Holland, Jeffrey Foy, Shaoxia Yu, Jason Flavin, Pallavi Lonkar
PepGen Inc., Boston, MA, USA

Introduction

PGN-EDO51 is PepGen's Phase 2 clinical-stage candidate Enhanced Delivery Oligonucleotide (EDO) for the treatment of people with Duchenne muscular dystrophy amenable to an exon 51 skipping approach

Our EDO platform is engineered to **optimize the tissue penetration, cellular uptake and nuclear delivery** of oligonucleotide therapeutics as unconjugated oligonucleotides are not readily distributed to muscle and are not efficiently taken up into cells and the nucleus

Immunogenicity potential of PGN-EDO51 was evaluated in vitro (EpiScreen™ time course T-cell assay) & anti-drug antibody (ADA) response was evaluated from a chronic study in non-humFan primates

- **PGN-EDO51 was non-cytotoxic and resulted in low stimulation in T-cells in vitro and did not elicit any ADA response following 11 monthly doses**

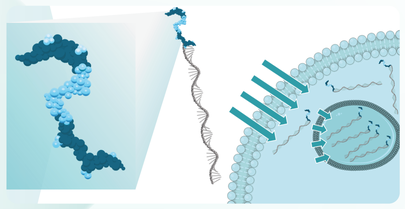
Nonclinical data suggest PGN-EDO51 has no/low potential of eliciting immunogenicity responses in repeat – dose clinical studies and support continued clinical development of PGN-EDO51

Enhanced Delivery Oligonucleotides

PepGen's EDO Peptides
Designed for enhanced uptake and improved tolerability

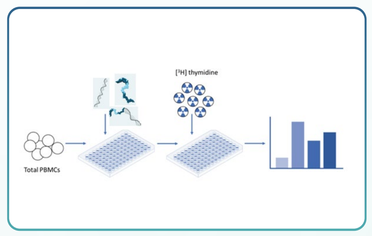
- Two poly-Arg domains where the number of arginines have been minimized are interspersed with non-natural amino acids to confer greater peptide stability
- Hydrophobic core coupled with poly-Arg domains contributes to endosomal escape
- Peptide sequence is linear with a length of less than 20 amino acids and is designed to be non-immunogenic

PepGen's EDO
Designed to increase nuclear uptake of oligos in muscle and other target tissues



Episcreen™ Immunogenicity Analysis Of PGN-EDO51

Study Design

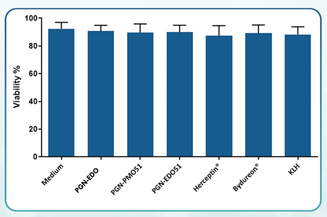


Cells. 2023 Jan 20;12(3):386

01 Test compound 1: Unconjugated peptidef
02 Test compound 2: Unconjugated oligonucleotide (PGN-PMO51)
03 Test compound 3: Oligonucleotide conjugate (PGN-EDO51)
04 Neo-antigen keyhole limpet hemocyanin, KLH: Positive control
05 Bydureon® used as a clinical benchmark control
06 Herceptin® was used as a low immunogenicity control
07 Media only control

- PBMCs from healthy donors were treated (± test or control compounds)
- On days 5, 6, 7, 8, cells were incubated with [³H] thymidine and cpm measured
- Cell viability and proliferation assessments were performed

PBMC viability from ten healthy donors with the test or control compounds



Peptide, PMO and PPMO components of PGN-EDO51 did not affect cell viability

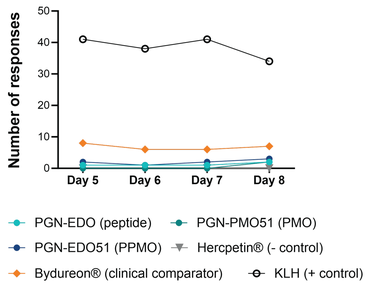
T cell proliferation assay

Test Item ID	Mean SI	% Response
PGN-EDO (peptide)	2.82	4
PGN-PM051	2.02	4
PGN-EDO51	2.37	8
Herceptin®	N/A	0
Bydureon®	4.66	20
KLH	8.45	94

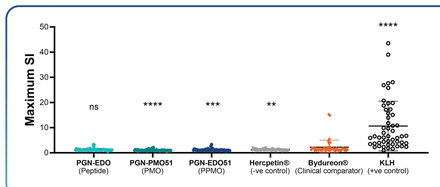
PGN-EDO51 showed low positive responses

Stimulatory Index (SI): PGN-EDO51 induced a low/minimal immune response (SI <3)

Episcreen™ Time Course Assay Demonstrated Low Immunogenicity Risk For PGN-EDO51



The number of positive CD4⁺ T cell proliferation responses for PGN-EDO51 was lower than the clinical comparator indicating no/low immunogenicity potential



Healthy donor CD4⁺ T cell responses/proliferation (SI) for PGN-EDO51 were lower than the clinical comparator over the time course indicating no/low immunogenicity potential

Chronic 39-week Repeat Dosing Of PGN-EDO51 In NHP (Study Design)

Test Material	Dose Level (mg/kg)	Main Study	Recovery Study
		No. of Animals	
		Males	Males
Control item	**0**	8	4
PGN-EDO51	15	8	4
PGN-EDO51	30	8	4
PGN-EDO51	**45**	8	4

- **ADA collection timepoints:** Baseline, 1M, 2M, 3M, 6M, 9M timepoint samples (all pre-dose), and 4 week & 8 week post last dose recovery group samples; M = month
- **ADA testing:** Only Control and 45 mg/kg PGN-EDO51 group samples were tested

PGN-EDO51 Antibody Production

01 Rabbit immunization with PGN-EDO51

02 Serum Isolation

03 Serum tested by ELISA for binding to PGN-EDO51

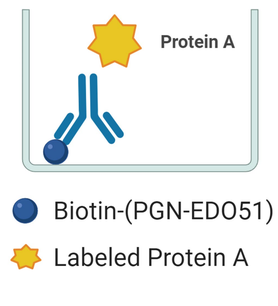
04 Affinity-purified EDO51 polyclonal antibody

05 SDS-PAGE analysis

- Anti-PGN-EDO51 antibody was generated in the rabbit against the entire PGN-EDO51 molecule

Anti-drug Antibody (ADA) Method Validation

ELISA Assay Design (Direct)



ADA Validation Parameters
Assay cut-point(s)
Titer Precision
Sensitivity
Drug tolerance
Specificity
Selectivity
Precision (intra- & inter-assay)
Stability of critical reagents (F/T, RT)
Prozone (hook effect)
Hemolysis

PGN-EDO51 ADA assay was validated using the 2019 FDA Guidance*

PGN-EDO51 did not demonstrate any ADA response in NHP following 11 monthly doses

Timepoint	No. of Animals	Tested for screening: Total (positive)	Tested for Confirmatory: Total (positive)	Titer range
Baseline	12	12(8)	8(5)	15 (MRD) to 240
1-month	8	8(1)	1(0)	NA
2-month	8	8(0)	NA	NA
3-month	8	8(0)	NA	NA
6-month	8	8(0)	NA	NA
9-month	8	8(0)	NA	NA
Recovery; 4-wk	4	4(0)	NA	NA
Recovery; 8-wk	4	4(0)	NA	NA

Highest dose samples were analyzed using a validated assay (direct ELISA)

- No PGN-EDO51 related ADA was observed in NHP following 11 monthly doses at 45 mg/kg via intravenous infusion.

Conclusion

Assays	Readouts
EpiScreen™ Immunogenicity Analysis of PGN-EDO51	**PGN-EDO51 was non-cytotoxic** and considered to have a low risk of immunogenicity in the clinic
ADA assesment in NHP	**No PGN-EDO51 related ADA** observed following chronic dosing

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

*2019 FDA Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection