

Extracellular vesicles-based therapy for spinal cord injury



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INTRODUCTION

Patients with spinal cord injury (SCI) usually suffer from permanent neurological deficits, while spontaneous recovery and therapeutic efficacy are limited. Exosomes are natural membrane vesicles (50-150 nm) of endosomal origin, secreted by various cells including mesenchymal stem cells (MSCs). They have emerged as promising nanocarriers for drug delivery and targeted therapy, as alternatives to stem cell therapy. Phosphatase and tensin homolog (PTEN) is expressed in neurons and regenerating axons and plays a vital role in controlling the regeneration of corticospinal neurons via downregulating cytoplasmic mammalian target of rapamycin (mTOR) activity. This study presents intranasal administrations of MSC-derived exosomes loaded with PTEN-siRNA (ExoPTEN), which targeted the spinal cord lesion in rats with complete spinal cord injury and enabled significant functional recovery.

NurExone Biologic Inc. (TSXV:NRX)(FSE:J90) is a TSX Venture Exchange listed pharmaceutical company that is developing a platform for biologically-guided ExoTherapy to be delivered, non-invasively, to patients who suffered traumatic spinal cord injuries. ExoTherapy was conceptually demonstrated in animal studies at the Technion, Israel Institute of Technology. NurExone is translating the treatment to humans, and the company holds an exclusive worldwide license from the Technion and Tel Aviv University for the development and commercialization of the technology.

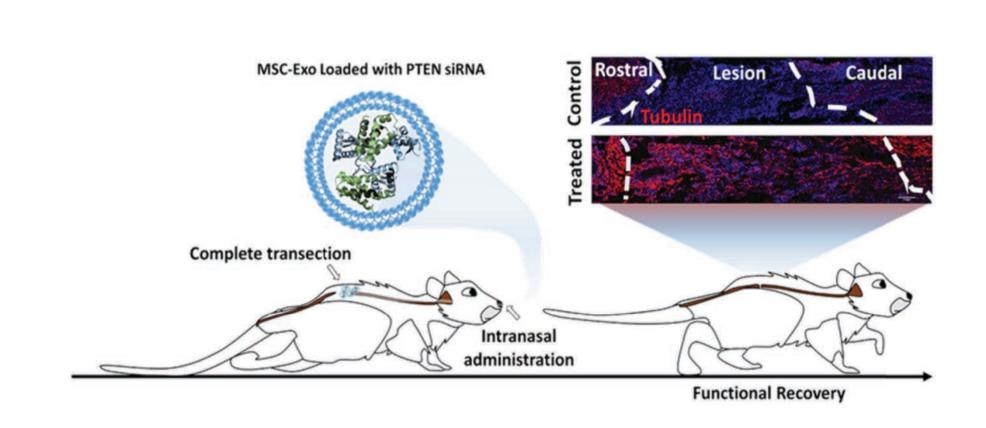
OBJECTIVES

Our goal is to develop a therapy for spinal cord injuries with the following features:

- Non- invasive
- Rapid
- Cell free
- Biologically-guided
- No immune response in patients
- Off the shelf administration

NurExone Biologic's Exotherapy holds a great promise for clinical healing

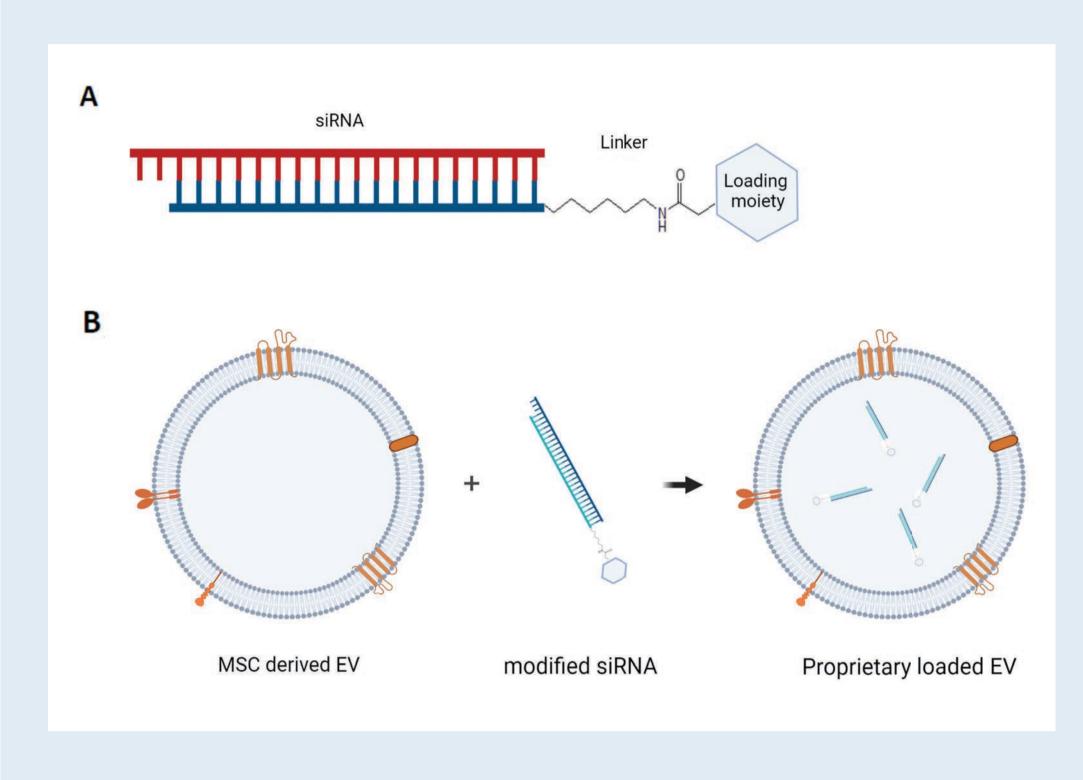
EXO-PTEN OVERVIEW



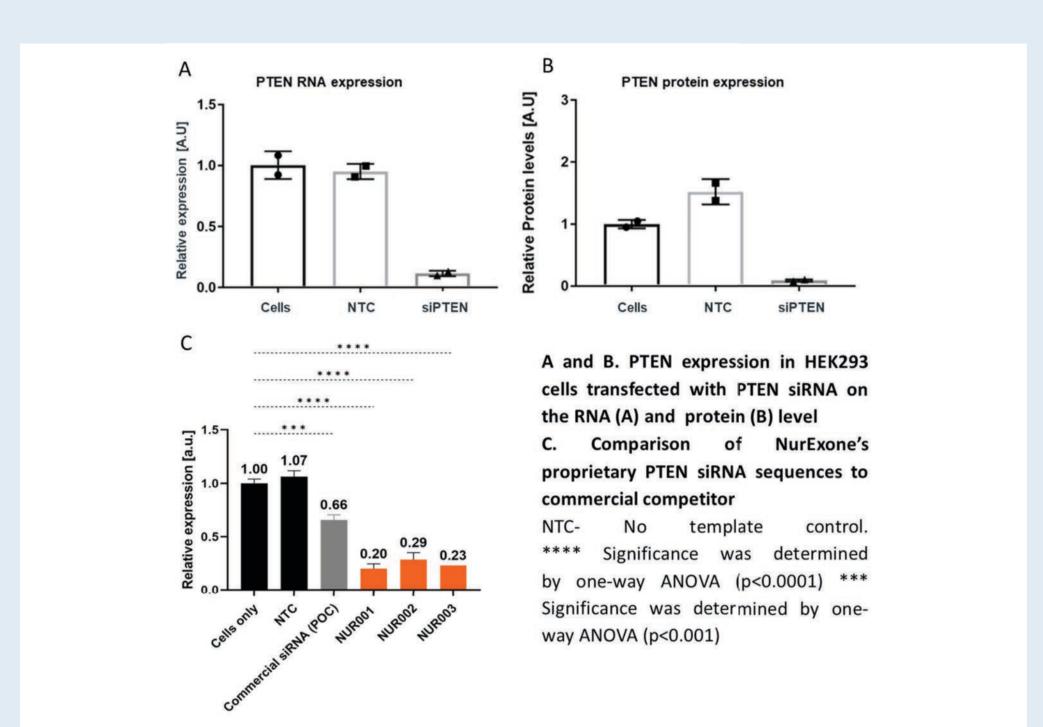
Intranasal ExoPTEN treatment induces locomotor, sensory, and bladder recovery.

RESULTS

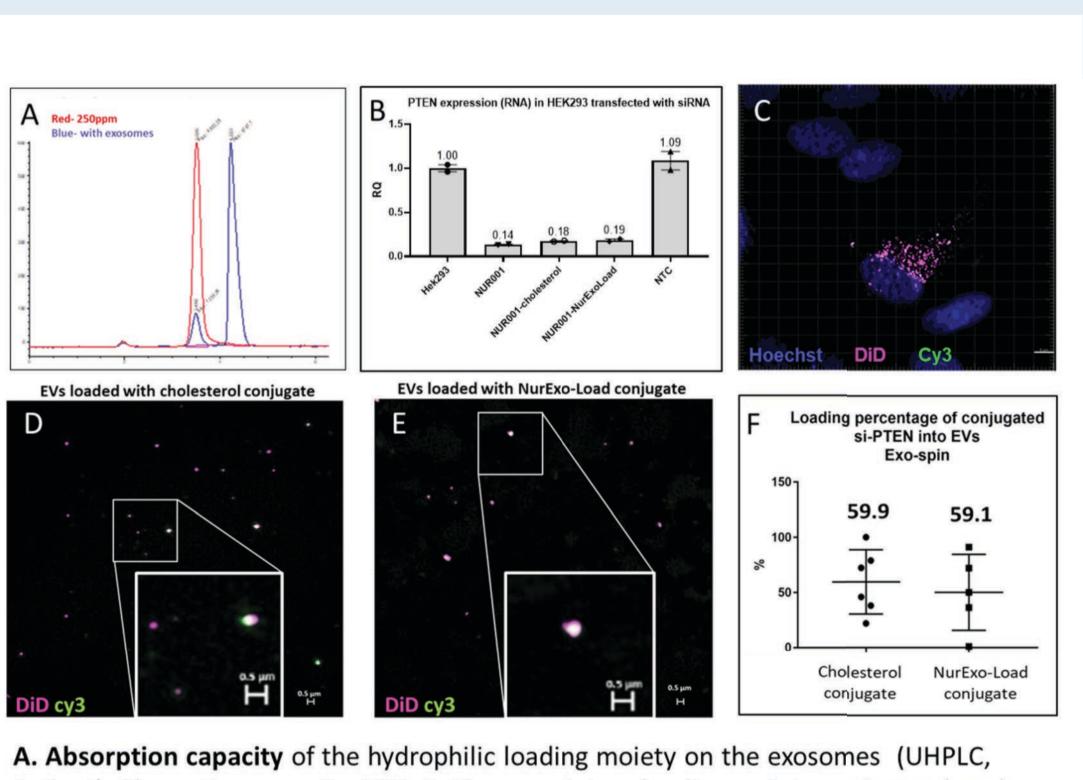
NurExone Biologic's ExoTherapy for spinal cord Injury



Development of a new proprietary siRNA against PTEN

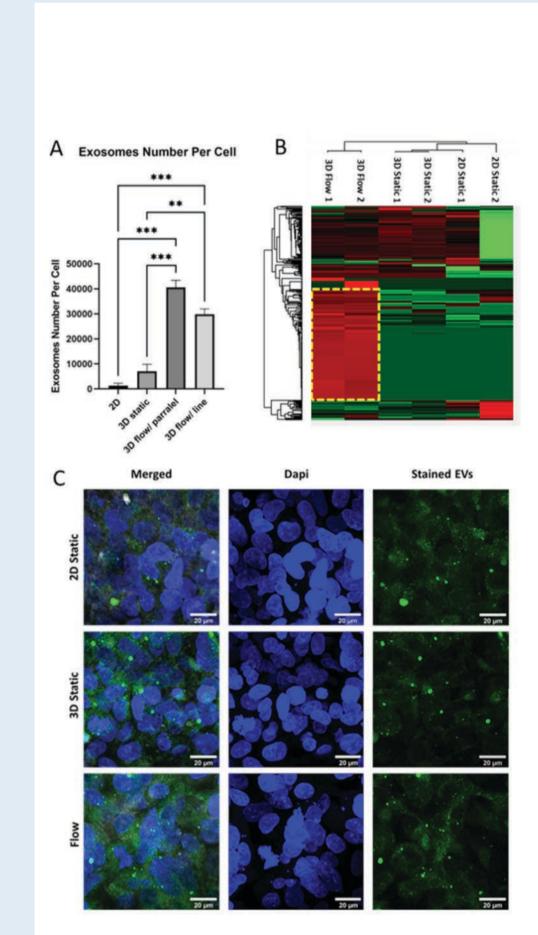


Development of a new proprietary loading moiety



A. Absorption capacity of the hydrophilic loading moiety on the exosomes (UHPLC, Agilent). Absorption capacity 40%. B. The proprietary loading moiety conjugated to the siRNA does not affect the efficiency of the NurEx001 siRNA lead molecule to knock down PTEN expression (qPCR) C. Cellular uptake of EVs loaded w/ NurExo-Load conjugate in HEK293 cells. D, E and F. Extracellular vesicles isolated from bone marrow derived MSCs (in violet) loaded with Cy3-labeled siRNA (in green) using cholesterol and NurExone's proprietary loading moiety observed by super resolution microscopy (Light Sheet, Zeiss Z7) showing ~59% loading efficiency (colocalization in white)

Development of a new proprietary 3D bioprocess

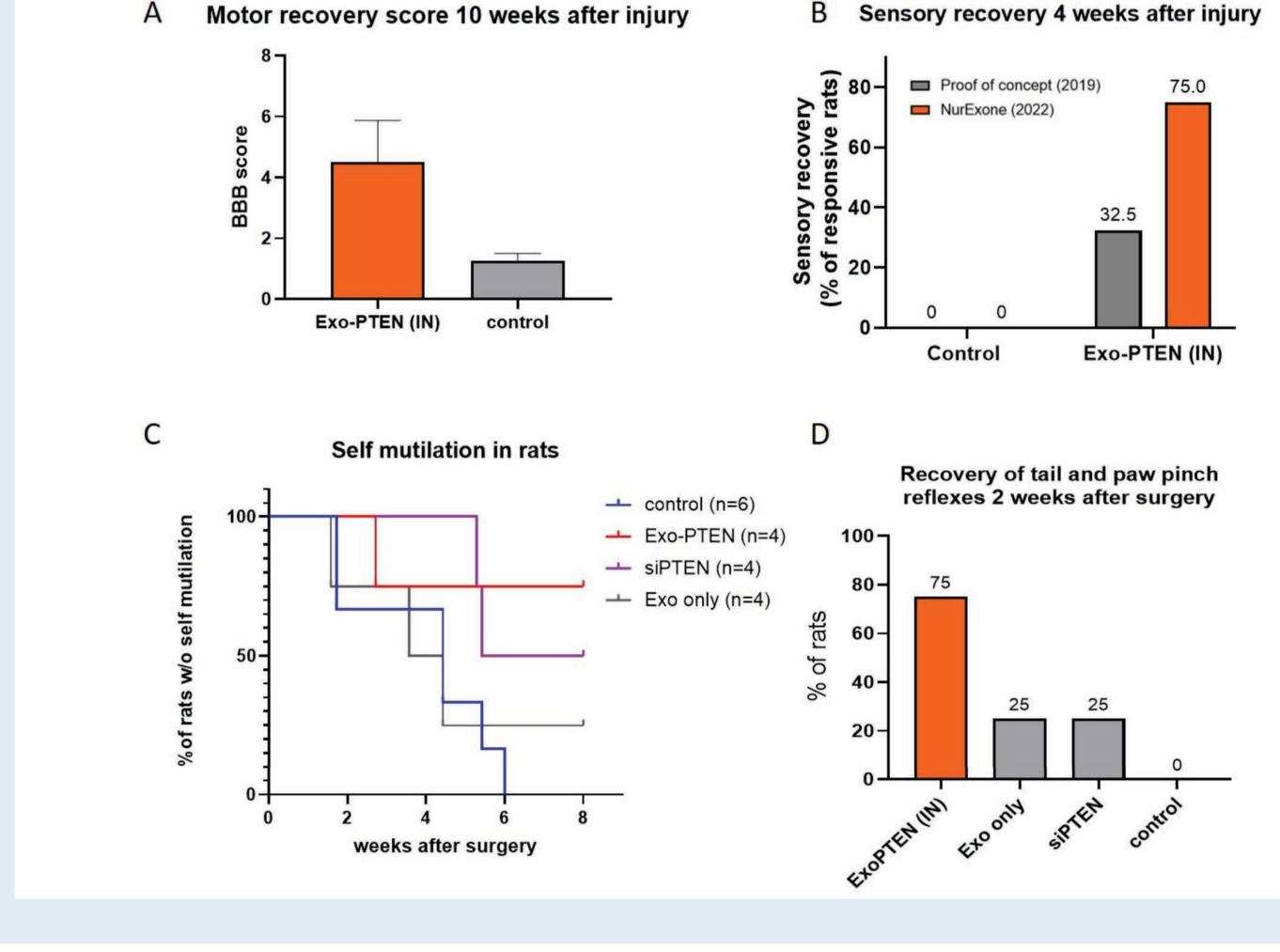


A. Exosomes secretion rates under various cultivation conditions. One-way ANOVA with multiple comparisons. **p<0.001.

B. Proteomics analysis for exosomes produced under various cultivation methods. Heatmap comparison for protein expression for exosomes produced under 2D static, 3D static or 3D flow induced cultivation methods.

C. Cellular exosomes uptake assay. Uptake assay performed for stained exosomes from various conditions including 2D static, 3D static and flow. . Images were taken by confocal microscope with X63 magnification

Preclinical studies in rats with NurExone's proprietary Exo-PTEN for the treatment of spinal cord injury



EXOPTEN Intranasal treatment improves locomotor, sensory, reflexes recovery and well being in injured rats. Results of in vivo studies of spinal cord injury (full following rats transection) **EXOPTEN** intranasal proprietary treatment

A. Motor rehabilitation assessed by the evaluation of the BBB score

- B. Improvement of the **sensory** recovery evaluated with Von Frey filaments
- C. Decrease of self-eating tendency
 D. Recovery of tail and paw pinch

reflexes

Our product includes a complete bioprocess, starting with isolation from 3D cultured BM-derived mesenchymal stem cells with an increased yield, followed by loading of the exosomes with PTEN-siRNA) and intranasal administration in rats for in vivo studies. These results have significant clinical therapeutic application for SCI and other neurological diseases with neuroinflammation.