Human Astrocytes (AstroRx®) Derived from Pluripotent Stem Cells for the Treatment of ALS

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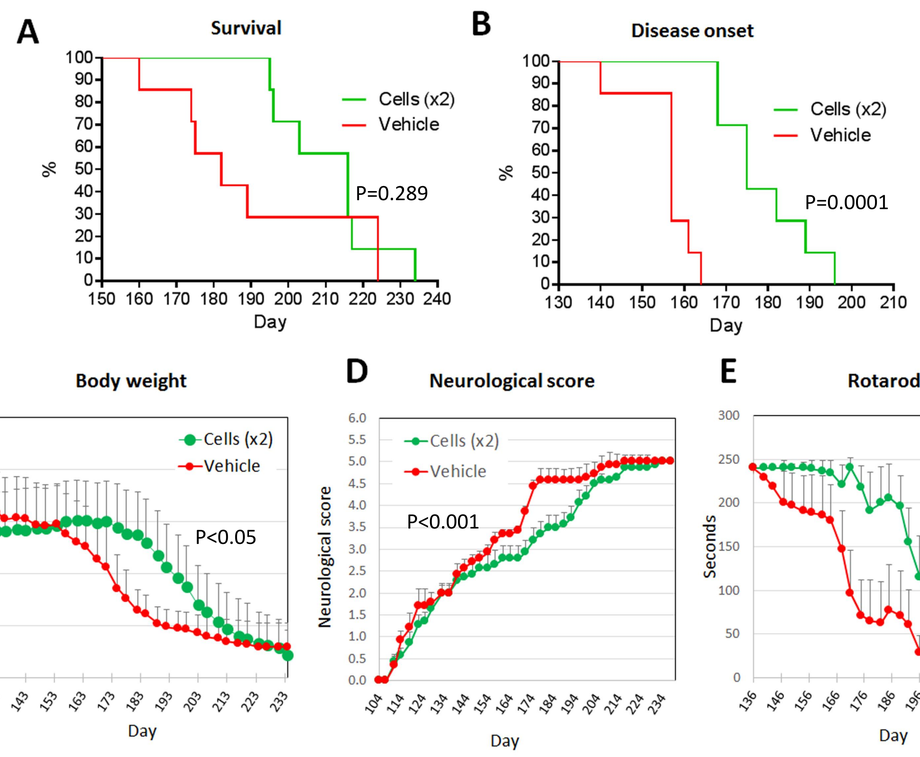
AstroRx© - Functional Human Astrocytes for Treating ALS Disease

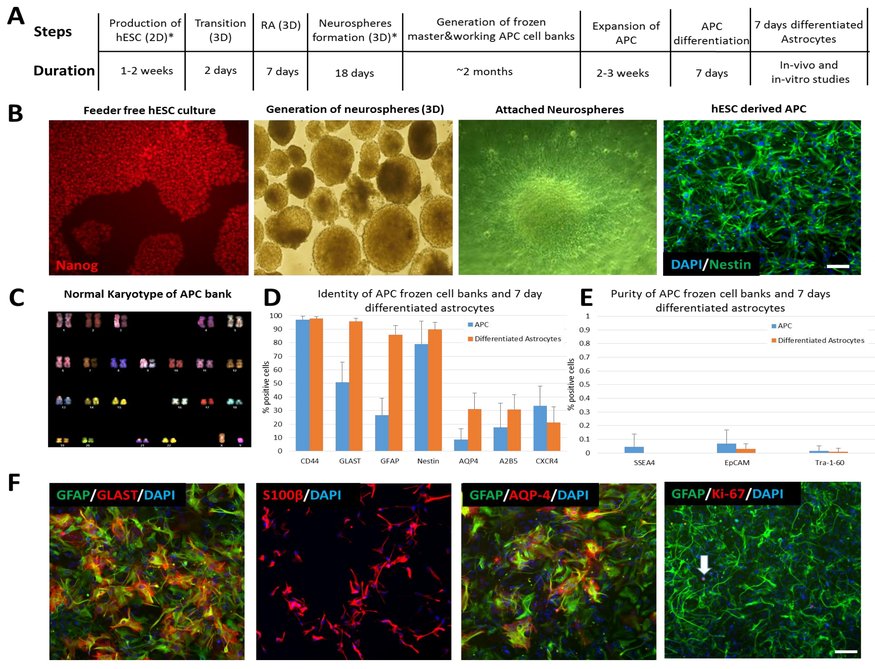
Despite the selective MN cell death in ALS, there is growing evidence that malfunctional astrocytes play a crucial role in ALS disease progression. Thus, transplantation of healthy astrocytes may compensate for the diseased astrocytes in ALS, offering a new approach to protect dying MNs. Here we describe a new cellular therapy, based on transplantation of clinical-grade human astrocytes (AstroRx®) derived from embryonic stem cells. *In vitro*, AstroRx® cells promote neurite and axon outgrowth, protect from oxidative stress and uptake glutamate. A secretome analysis showed that these astrocytes secrete many pro-neuronal factors. Intrathecal injection of AstroRx into the CSF of hSOD1^{G93A} transgenic mice and rats significantly delayed disease onset and improve motor performance compared to sham injected animals. A nine-month safety study in immunodeficient NSG mice showed that AstroRx cells survive in the CSF, efficiently distribute along the CNS, do not proliferate and do not form tumors.

A clinical trial to evaluate the safety and efficacy of AstroRx® transplantation in ALS patients was initiated in April 2018 (ClinicalTrials.gov Identifier: NCT03482050).

AstroRx® Delays Disease Onset, Improves Motor Performance and Prolongs Survival of hSOD1^{G93A} Rats



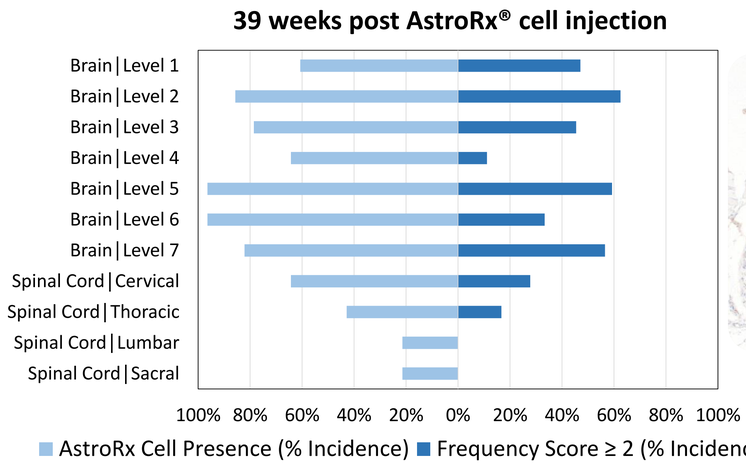
Production of Clinical-Grade Human Astrocytes (AstroRx®)

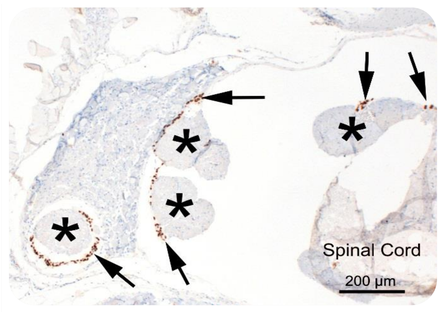


Differentiation of hESCs towards highly-enriched population of functional astrocytes (AstroRx®) is carried out using Kadimastem's stepwise proprietary protocol. Manufacturing of clinical-grade AstroRx® cells is done under GMP conditions using xeno-free materials.

The first stage of the protocol is generation of committed astrocyte progenitor cells (APC) that are kept frozen as cell banks. *In vitro* maturation of APCs for 1 week upon thawing yields a population (>95%) of post-mitotic astrocytes expressing multiple astrocytic markers (i.e. GFAP, AQP-4, GLAST, GLT-1 and CD-44).

Distribution within the Brain and Spinal Cord of AstroRx® Cells after Intrathecal Injection to the Cisterna Magna



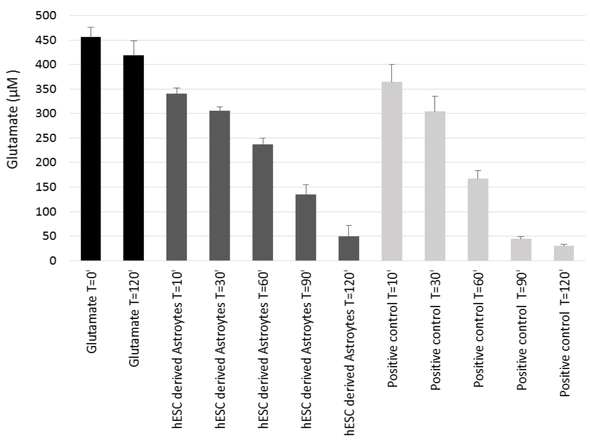


AstroRx® cells (*Alu*+, arrows) attached to nerve bundles in the sacral spinal cord

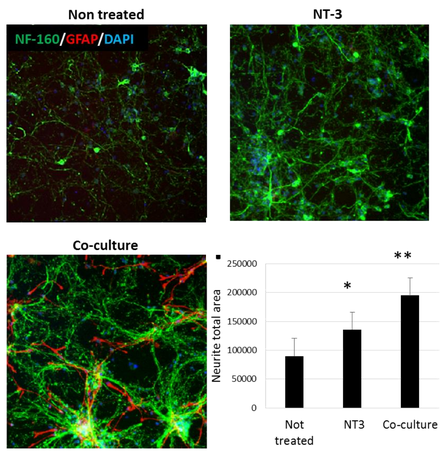
Analysis of AstroRx® distribution along the brain and spinal cord following intrathecal injection was performed by quantifying *Alu*+ cells in representative sections of the CNS. Analysis was done 4, 17 and 39 weeks post cell injection. Frequency score of ≥ 2 indicates 1 to 3 foci of 10-20 cells per foci.

AstroRx® cells uptake glutamate , secrete a variety of pro-neuronal proteins and promote neurite outgrowth

Astrorx® cells uptake glutamate *in vitro*



AstroRx® cells promote neurite outgrowth of E19 rat cortical neurons in co-culture



Secretome analysis of AstroRx® conditioned medium reveals a variety of factors with effects on neurons, or with antiprotease activity

Secreted factors with effects on neurons (ng/10^6 AstroRx® cells)	
Osteopontin (OPN)	53.1 ± 29
Dickkopf-3 (DKK-3)	43.1 ± 14.2
Thrombospondin (TSP-1)	22.7 ± 11.5
Secreted Frizzled Prot, (sFRP3)	20.8 ± 10.9
Brevican proteoglycan	15.6 ± 4.9
Tripeptidyl peptidase (CLN2)	11.5 ± 4.2
Clusterin	9.5 ± 3.2
Midkine	8.4 ± 3.0
NSE	3.5 + 1.8
MIF chemokine	1.8 ± 0.6
CXCL16	1.5 ± 0.8
Thrombospondin-2	0.85 ± 0.4
GRF alpha-1	0.45 ± 0.2
VEGF	0.05 ± 0.02
Antiprotease activity (ng/10^6 AstroRx® cells)	
Fetuin A	1,816.0 ± 677
Tissue inhibitor of metalloprotease TIMP-2	16.6 ± 6.8
PAI-1 Serpine 1 protease inhibitor	7.2 ± 6.2
Tissue inhibitor of metalloprotease TIMP-1	7.0 ± 3.8
Serpin A4	4.3 ± 2.5

A Phase I/IIa, Open Label, Dose-Escalating Clinical Study to Evaluate Transplantation of AstroRx® in ALS Patients

ClinicalTrials.gov ID	**NCT03482050**
Number of patients	**21**
Primary endpoints	**Safety and tolerability**
Secondary endpoints	**Efficacy (ALSFRS-R, SVC, JAMAR, HHD, ALSAQ-40)**
Disease stage	**ALSFRS-R≥30, two years or less from ALS diagnosis**
Administration method	**Intrathecal (by lumbar puncture)**
Study's arms (AstroRx® dose)	**A. 100x10^6 cells B. 250x10^6 cells C. 2X250x10^6 cells (2 months apart) D. 500x10^6 cells**
Follow-up duration post AstroRx® injection	**6 months**
Clinical site	**Hadassah Ein-Karem, Jerusalem, Israel**
Patient Enrollment	**April 2018**

Summary

AstroRx® is a cell-based product composed of clinical-grade functional human astrocytes. AstroRx® ameliorates neurological symptoms in ALS animal models. The safety and biodistribution of AstrotRx® was demonstrated in a 9-month preclinical study. The safety and efficacy of AstroRx® are currently evaluated in a PI/IIa clinical trial in ALS patients.

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