NRTX-1001: First-in-class human inhibitory neuron cell therapy for phase I/II clinical investigation in chronic focal epilepsy

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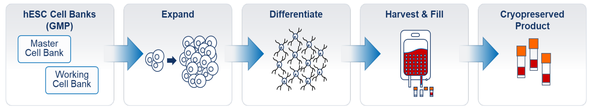
CIRM CALIFORNIA STEM CELL AGENCY (TRAN1-11611; CLIN2-13355)

INTRODUCTION

- Many people with mesial temporal lobe epilepsy continue to have seizures despite anti-seizure drug therapy
- Inhibitory interneuron cell therapy is a novel therapeutic strategy to provide targeted inhibition to hyperexcitable neural networks in the epileptic brain
- NTRX-1001 comprises GABAergic, post-mitotic interneurons of a specific pallial-type lineage derived from human pluripotent stem cells
- A multicenter, dose-escalation phase I/II clinical trial has been launched to evaluate NRTX-1001 in people with pharmacoresistant temporal lobe epilepsy (TLE)

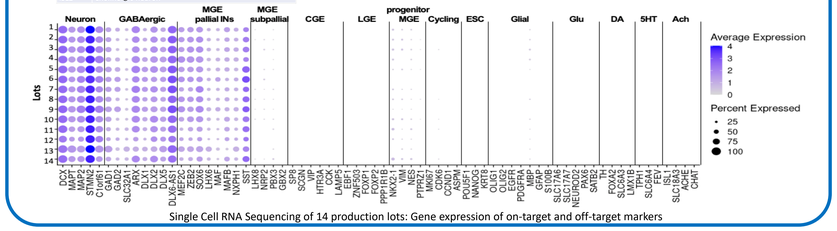
NRTX-1001 Manufacturing for Clinical Use



- Allogeneic, cryopreserved cellular therapeutic intended for single administration and long-term persistence in clinical population
- High purity: >98% medial ganglionic eminence (MGE) pallial-type GABAergic interneurons
- Post-mitotic and stage-specific: No proliferative or cycling cells detected
- Reproducible: 3 of 3 lots of cGMP clinical product passed release criteria (safety, identity, strength, purity, and composition) and support dosing of all subjects in Phase I/II epilepsy trial

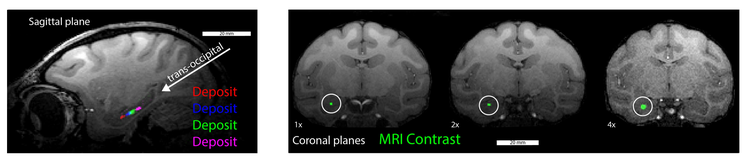
NRTX-1001 Composition: MGE Pallial-type Interneurons

Marker	Identity/Interpretation
SOX1	Neuroectoderm
FOXG1	Telencephalon
NKX2-1	MGE progenitor
OTX2	Rostroventral MGE progenitor
GAD1	GABAergic neuron
LHX6	MGE-derived GABAergic neuron
MAF	MGE-derived GABAergic pallial interneuron
MAFB	MGE-derived GABAergic pallial interneuron
NANOG	Pluripotent stem cell
KI67	Cycling progenitor
PAX6	Dorsal telencephalon progenitor
LHX8	MGE-derived GABAergic or cholinergic subpallial neuron
OLIG2	MGE progenitor or oligodendrocyte progenitor
SP8	CGE-derived interneuron or LGE-derived OB interneuron
NKX2-2	Hypothalamic neuron or oligodendrocyte progenitor
ISL1	Cholinergic neuron

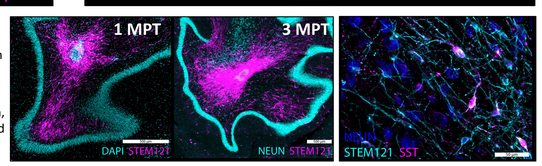


Single Cell RNA Sequencing of 14 production lots: Gene expression of on-target and off-target markers

NRTX-1001 Delivery into the Primate Hippocampus

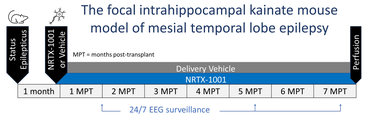


- Rhesus macaques received NRTX-1001 using the clinical delivery system, and a trans-occipital approach to the hippocampus, which is similar to that used for Laser Interstitial Thermal Therapy (LITT)
- With clinically-analogous immunosuppression, NRTX-1001 persisted and were well-integrated in the NHP hippocampus at 1 month and 3 months following transplantation

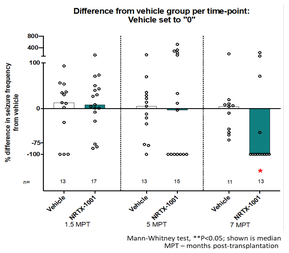


STEM121 = human cells; SST = somatostatin; DAPI = nuclei

NRTX-1001 Transplantation Reduces Focal Electrographic Seizure Frequency in Epileptic Mice



Seizure Frequency and Responder Rate



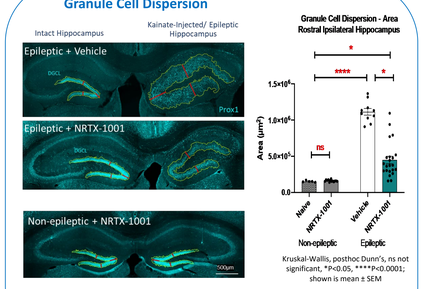
Mann-Whitney test, **P<0.05; shown is median
MPT = months post-transplantation

Treatment	Electrographic Seizure Frequency at 7 MPT (Median)	# Animals with >95% seizure reduction at 5 MPT Fraction (Percent)	# Animals with >95% seizure reduction at 7 MPT Fraction (Percent)
Vehicle	5.7	1/13 (8%)	0/11 (0%)
NRTX-1001	0	7/15 (47%) *	9/13 (69%)***

Fisher's exact test *P<0.05, ***P<0.001

At 7 months post-transplant, seizure frequency is significantly reduced in epileptic animals that received NRTX-1001

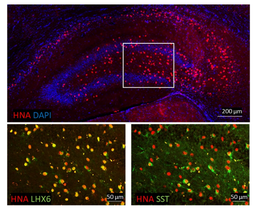
Granule Cell Dispersion



Kruskal-Wallis, posthoc Dunn's, ns not significant, *P<0.05, ***P<0.0001; shown is mean ± SEM

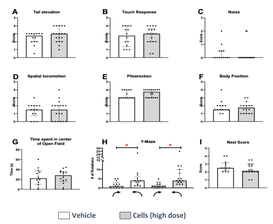
At 7 months post-transplant, granule cell layer dispersion is significantly reduced in epileptic animals that received NRTX-1001

NRTX-1001 Cell Fate and Persistence



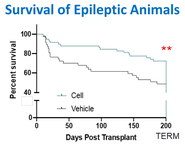
At 7 months post-transplant, NRTX-1001 persist in the epileptic hippocampus (Human Nuclear Antigen, red), and express the MGE-type GABAergic neuronal marker LHX6 (green) and interneuron subtype marker somatostatin (SST; green)

Animal Health and Behavioral Responsiveness



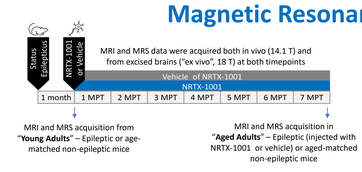
NRTX-1001 at 25x scaled clinical dose does not produce behavioral deficits or decreased activity in epileptic animals

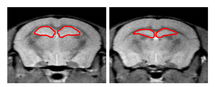
Survival of Epileptic Animals



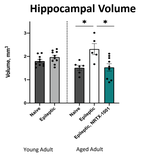
Transplantation of NRTX-1001 improves survival of mice in the intrahippocampal kainate model of chronic focal epilepsy

Magnetic Resonance Imaging & Spectroscopy

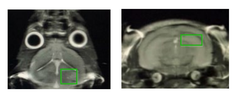




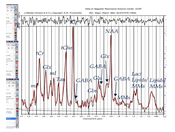
- Volume of the hippocampus was quantified using T1-weighted images.
- Manual ROIs (red outline) were drawn in two consecutive sections and volume was computed as the average for each hemisphere separately.

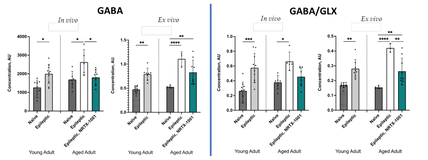


- In young adult mice, hippocampal volume is similar in epileptic and non-epileptic animals
- In aged adult mice, hippocampal volume in epileptic animals is significantly increased; animals that received NRTX-1001 7 months earlier have hippocampal volume that is similar to that of age-matched, non-epileptic mice



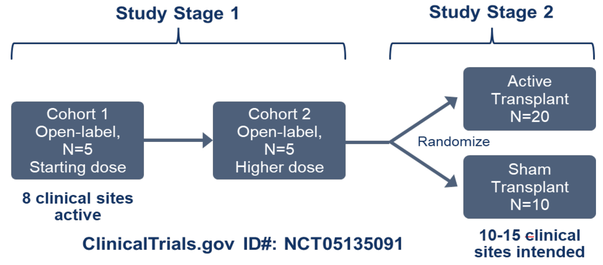
- In vivo MRS: bilateral with voxel size = 1.4 x 2.7 x 2.2 mm (green ROI), capturing the dorsal hippocampus; at 14.1 T
- Ex vivo MRS: individual homogenized hippocampi; at 18 T





- In both young adult and aged adult mice, hippocampal GABA concentration and ratio of GABA/GLX concentrations are elevated in epileptic animals
- In aged adult epileptic mice, the hippocampal GABA concentration and ratio of GABA/GLX concentrations in animals that received NRTX-1001 are returned toward levels measured in non-epileptic mice
- Ex vivo results are similar to in vivo results at both time points

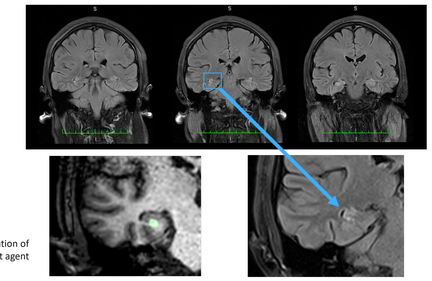
Clinical Administration of NRTX-1001



- **Design**: Multicenter dose escalation, subjects receive a single administration of NRTX-1001 into the hippocampal seizure focus with stereotactic MRI-guided delivery
- **Primary Endpoint**: Safety. Frequency of adverse events
- **Secondary Endpoint**: Efficacy
- Reduction in seizure frequency and severity, responder rate, Quality of Life measures, neuropsychological outcomes, MRS imaging biomarkers, anti-seizure drug dose reduction

First human subject treated with NRTX-1001 in June 2022

- Averaged 30 seizures/month in the 6 months prior to screening; had failed to achieve seizure control with the use of 4 different anti-seizure medications
- Intraoperative MRI: contrast dye-containing NRTX-1001 cell suspension was delivered on-target into the affected hippocampus
- Subject returned home from hospital the next day, as planned
- 3 Month Follow-Up visit: No serious adverse events; normal visual field assessment, a total of 4 reported seizures since administration of NRTX-1001



CONCLUSIONS

- NRTX-1001 is a highly-pure, clinical-grade, allogeneic cellular therapeutic comprising post-mitotic MGE-type GABAergic interneurons
- NRTX-1001 can be delivered on-target in the primate hippocampus, where cells persist, distribute and integrate within the tissue, without neuroinflammation
- NRTX-1001 transplantation consistently reduces focal electrographic seizures and hippocampal pathology in the intrahippocampal kainate mouse model of chronic mesial temporal lobe epilepsy; delivery of NRTX-1001 does not alter behavior and extends the life of the epileptic animals
- NRTX-1001 do not produce toxicities in preclinical rodent or NHP models
- NRTX-1001 transplantation rebalances GABA and glutamate in the kainate mouse model of chronic focal epilepsy; may act as a biomarker of cell persistence
- A phase I/II clinical trial is underway to explore the clinical safety and preliminary efficacy of NRTX-1001 in people with chronic temporal lobe epilepsy (NCT05135091)

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