Development of robust and personalized cell replacement therapy for Parkinson's Disease

Maria-Daniela Cirnaru[1], Matthew Goodus[1], Noelle Ying[1], Araceli Ramirez[1], Soo Ryeong Jung[1], Dana Ambruzs[1], Alistair Wilson[1], Robert Srijemac[1], Alexis Corl[1], Heidi Olivares[1], Steve Schreiber[1], Juan Pintor Cuellar[1], Anabel De La Garza[1], Young Lee[1], Su-Chun Zhang[1]

Affiliations: [1]BrainXell Therapeutics , 6370 Nancy Ridge Drive, Suite 110, San Diego, CA 92121

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, caused by the degeneration of the midbrain dopaminergic neurons. Current treatments for PD are limited to targeting the symptoms of the disease and do not modify or alter disease progression. Therefore, the development of restorative therapies to slow down, stop and even reverse PD is a high unmet need. Cell replacement therapy is an emerging potential treatment approach for PD. While disease modifying data are limited, the early safety results are encouraging for regenerative medicines. Different approaches present opportunities for optimal cell therapy, where we seek to find a personalized approach for PD.

Methods

Here we define and validate the patient-to-product path for an autologous iPSC-derived PD cell therapy product. For this, we established fibroblast cultures from PD patient skin biopsies that were reprogrammed to iPSCs via mRNA technology. The iPSC lines were banked and tested for PD mutations and determined to be idiopathic cases. Their genetic integrity was confirmed using Karyostat and aCGH testing. We generated a total of 28 iPSC lines from normal healthy volunteers and PD patients. Five PD lines were differentiated into highly enriched midbrain dopaminergic progenitors (mDAPs) via controlled innovative procedures(Fig. 1). The mDAPs from two of the lines were aggregated into spheroids, making BXT-110, which then was transplanted into the unilateral 6-OHDA-lesioned RNU rats for the efficacy study with a viability higher than 90% at transplantation (Fig. 2-4).

Results #1: Induction to highly enriched mDAPs of 5 PD patient donor iPSC lines

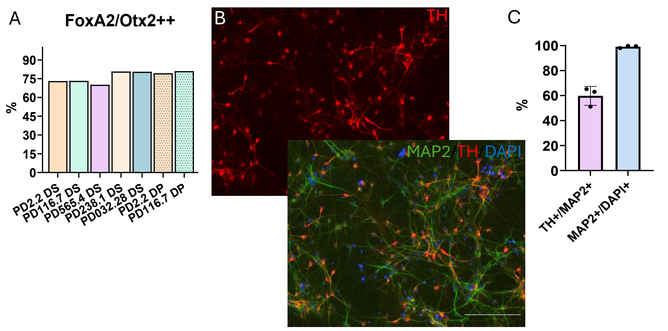

Figure 1. Analysis of expression profile of on target markers for mDAPs and differentiated Dopaminergic neurons derived from PD patient donor iPSCs lines. (A) Consistent expression of over 75% FOXA2/OTX2 expression analyzed by Flow Cytomtry. **(B)** Immunofluorescence analysis and quantification, scale bar = 200 µm **(C)** of TH and MAP2 expression of a representative mDAP indicating neuronal enrichment with over 95% MAP2+ cells and 60% TH+/MAP2 cells.

Results #2: Pharmacology efficacy studies of BXT-110 from two PD patient donor lines show consistent and significant recovery as early as 12 weeks post treatment

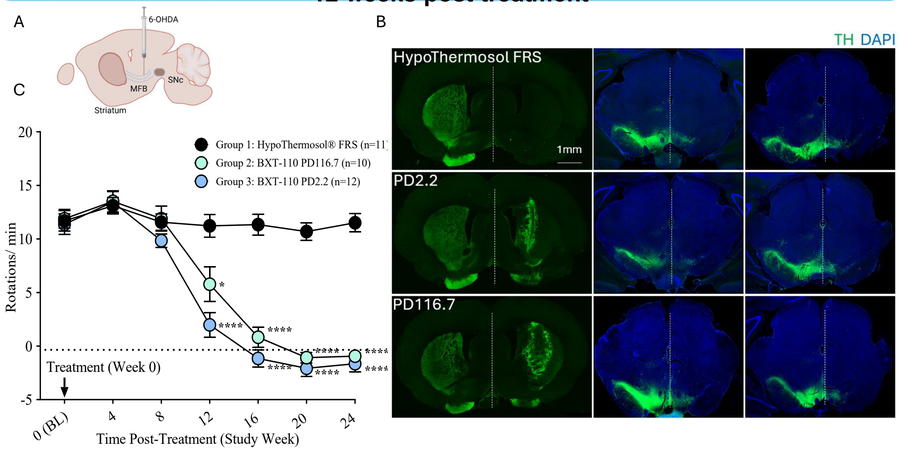

Figure 2. Reproducible efficacy of BXT-110 derived from two PD patient donor lines *in vivo*. (A) Schematic of the 6-OHDA lesioning paradigm in the medial forebrain bundle (MFB) of Rowett nude rat (RNU) **(B)** Validation of the 6-OHDA animal model by immunostaining with TH antibody (green), of coronal cryosections displayed rostral to caudal. Scale bar = 1 mm **(C)** Intrastriatal brain administration of BXT-110 Test Article from cell lines PD116.7 (Group 2) and PD2.2 (Group 3) robustly reverts the amphetamine-induced rotational asymmetry compared to HypoThermosol® FRS Vehicle Control Article (Group 1) in female 6-OHDA-lesioned RNU rats. Data are graphed as mean ± SEM net ipsiversive rotations during the 0-90 min testing period beginning 10-27 minutes following amphetamine administration. Statistical significance represents *p<0.05, ****p<0.0001 BXT-110 Drug Product Test Article Group 2 and Group 3 vs. HypoThermosol® FRS Vehicle Control Article (Group 1) at each time point (two-way repeated measures ANOVA with Tukey's multiple comparisons post hoc test). BL, baseline; SEM, standard error of the mean.

Results #3: BXT-110 intrastriatal delivery established robust cell survival and minimal expression of off targets at 26 weeks post transplantation

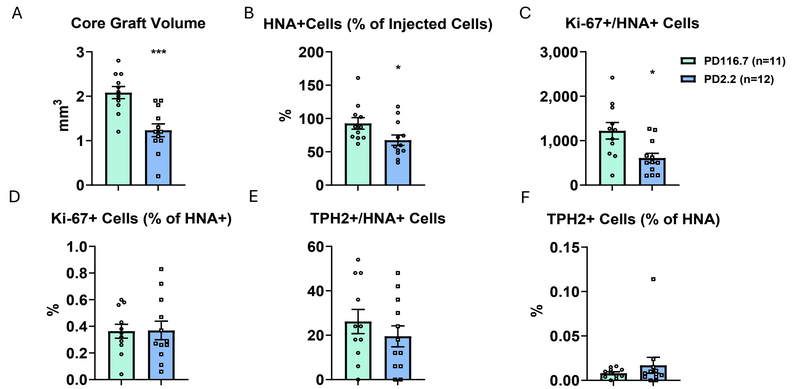

Figure 3. Quantification of HNA+ graft volume and Off targets at 26 weeks post transplantation. (A) Individual and groups mean core HNA+ graft volume (mm3) and percentage of HNA+ cells that survived the transplant **(B)** within the striatum for each treatment group (***p<0.001 unpaired t-test) indicating over 60% cell survival in both PD patient donor lines analyzed. **(C, D)** Quantification of Ki67+/HNA+ cells **(E, F**)TPH2+/HNA in BXT-110 Test Article grafts from PD116.7 and PD 2.2. Error bars = mean ± SEM

Results #4: BXT-110 shows reproducible dopaminergic differentiation and host integration at 26 weeks post transplantation

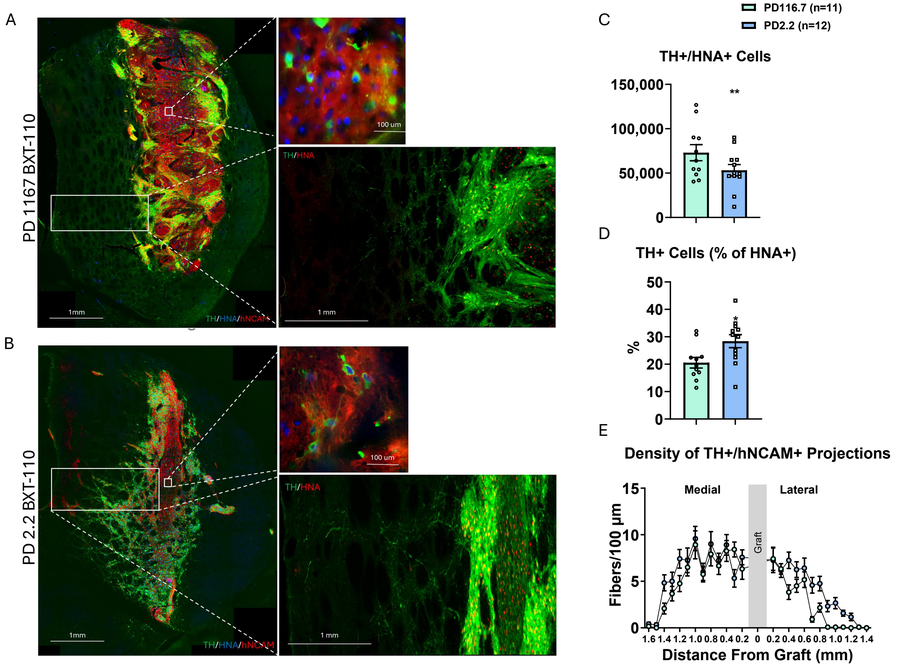

Figure 4: Quantification of HNA+/TH+ dopamine neurons in BXT-110 ~~Drug Product~~ Test Article grafts. (A) Left panel: representative image of a coronal section from a BXT-110 Test Article PD116.7 graft at 26 weeks post-transplant showing robust HNA+ (blue) cell survival, TH+ (green) dopaminergic graft cells and processes, and hNCAM+ (red) human-specific neuronal processes (20x magnification; scale bar = 1 mm). Boxed panels to the right represent magnified images showing HNA+/TH+/hNCAM+ transplanted cells within the graft core (Top right panel; scale bar = 100 µm) and TH+ processes (green) extending out from the grafted cells and innervating the surrounding striatum (Bottom right panel; scale bar = 1 mm). **(B)** same as (A) for BXT-110 Test Article PD 2.2 graft . **(C)** Stereological quantification of TH+/HNA+ cells identified in the transplanted hemisphere in BXT-110 ~~Drug Product~~ Test Article grafts from both PD lines. (p>0 unpaired t-test; error .05 bars = mean ± SEM). **(D)** Stereological quantification of TH+/HNA+ cells expressed as percentage of total HNA+ cells showed that the BXT-110 Drug Product Test Article from both cell lines generated more than 20% TH+ cells *in vivo* at 26 weeks post-transplant (*p<0.05 unpaired t-test; error bars = mean ± SEM). **(E)** Fiber outgrowth analysis shows comparable medial and lateral fiber projections of BXT-110 graft-derived TH+/hNCAM+ dopaminergic neurons into the striatum. Error bars = mean ± SEM

Conclusion

Our in vitro results indicate a robust cell production process with greater than 60% TH+ dopaminergic neurons and consistent on-target/off-target marker expression. The efficacy of BXT-110 was evidenced by reduction of amphetamine-induced rotations in grafted animals as early as 12 weeks and sustained efficacy up to six months for two cell lines. Thus, in vitro and in vivo data demonstrates sustained and reproducible disease-modifying activity as a potential Best-In-Class personalized approach for patients with PD.

This research was made possible by funding from the California Institute for Regenerative Medicine (CIRM), a state of California Agency that funds regenerative medicine, stem cell, and gene therapy research. ***(Grant # 14671).***

brainxell.com