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14. ABSTRACT

We have made substantial progress in obtaining skin biopsies with subsequent fibroblast cultures of a number of sporadic ALS, familial ALS, and control subjects in the last year. We have collected a variety of ALS phenotypes including slow progressing ALS, primary lateral sclerosis (PLS), lower motor neuron only ALS (LMN), ALS with frontotemporal dementia (ALS/FTD) and appropriate controls (Total of approximately 85 subjects collected to date). In addition, we have also obtained 28 samples from subjects with familial ALS (these include SOD1, ANG, FIG4, and FUS mutations). In collaboration with Johns Hopkins Investigators, we have developed 22 FALS iPSC lines, 8 sporadic ALS iPSC lines and 4 control iPSC lines. We have been using a method to allow for long-term differentiation of iPS cells into astroglia.

15. SUBJECT TERMS
Stem Cells, iPS cells, astrocytes, familial ALS

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Table of Contents

INTRODUCTION:
The overall objective is to examine whether human iPSC-GRPs (glial restricted precursors) derived from either sporadic ALS, familial (SOD1-mediated) ALS, or control subjects have the same capacity for engraftment, survival, and neuroprotective qualities following transplantation. It is not known whether iPSC-GRPs from ALS patients will in fact be normal (and thus possibly neuroprotective) or whether these iPSC-derived cells may in fact harbor ALS-specific abnormalities which may lack benefit or, potentially exacerbate disease. By comparing normal iPSC-GRPs with sALS iPSC-GRPs and fALS iPSC-GRPs we will also learn about inherent differences in astrocyte biology related to ALS which will provide potential insights into disease mechanisms.

BODY:

Aim #1. Generation of human induced pluripotent stem cell-derived glial restricted precursors (iPSC-GRPs) from ALS subject fibroblasts

Task 1. Skin biopsy of subjects with ALS to obtain fibroblasts (Months 1-18)
Total of approximately 85 subjects biopsied to date). These include subjects with familial ALS (these include SOD1, ANG, FIG4, and FUS mutations), as well as subjects with sporadic ALS and control subjects **(Table 1**).

Table 1

KNOWN FAMILIAL MUTATIONS		FIBROBLASTS	iPS
SOD1			
N139K	1	x	x
A4V	6	x	x
D90A	2	x	x
V148G	1	x	x
I113T	4	x	x
I112T	1	x	x
L144P	1	x	
C38G	1	x	x
D91A	1	x	x
E49K	1	x	x
E100G	1	x	x
G86R	1	x	x
FIG 4	1	x	x
FUS	3	x	x
TDP43	2		x
ANG	1	x	x
TOTAL	**28**		

OTHER	
SPORADIC	
SLOW PROGRESSING > 5 YRS	9
TYPICAL PROGRESSION < 5 YRS	29
ALS/FTD	2
UNKNOWN FAMILIAL	3
PLS	4
LMN ONLY	1
SBMA (KENNEDY'S)	1
CONTROLS	5
RELATED CONTROLS	3
TOTAL	**57**

iPS lines completed	
fALS	22
sporadic ALS	8
controls	4

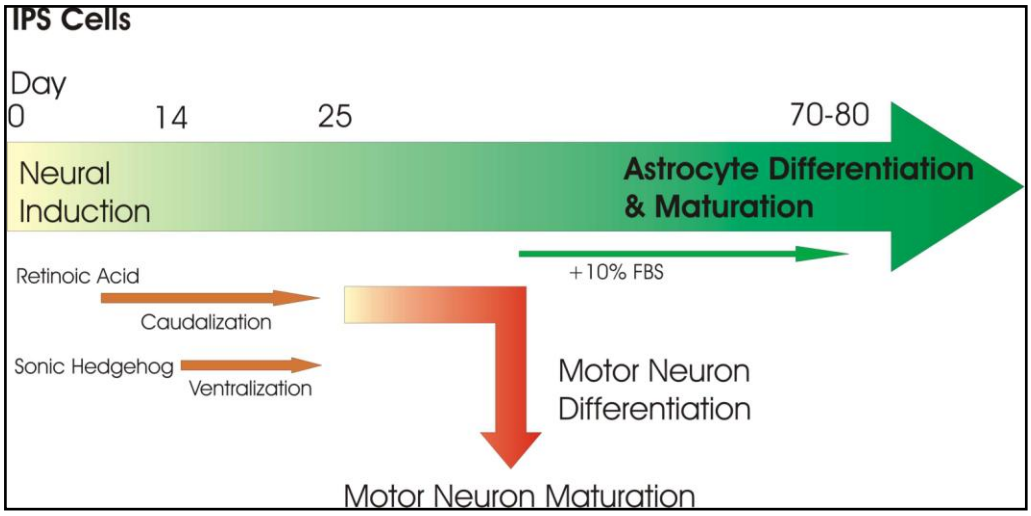
Task 2. Culture of human fibroblasts (Months 1-24)
Total of approximately 85 fibroblast lines cultured to date. Many of these lines have already been frozen down to allow for future use. **(Table 1)**

Task 3. Generation of human iPSC from ALS and control subjects (Months 1-24)
In collaboration with Dr. Song as well as a collaborative effort with Dr. Jeffrey Rothstein at Johns Hopkins University, we have now generated 22 FALS iPSC lines, 8 sporadic ALS iPSC lines and 4 control iPSC lines **(Table 1).**

Task 4. Characterization of iPS cells (Months 1-24)
iPSC have been generated from and verified that ALS and control iPSCs maintain their pluripotency and have normal karyotypes. All cell lines listed in **Table 1** have normal karyotype

Task 5. Differentiation and characterization of iPS cells from ALS subjects into glial restricted precursors and astrocytes (Months 1-24)
We have now generated multiple lines of iPSC-derived astrocytes using the protocol outlined in **Figure 1**. iPSC are maintained as we have previously described and then undergo a process of neural induction followed by approximately 60 or more days of maturation into astrocytes using 10% fetal bovine serum (FBS). As seen in **Figure 2** we have used a variety of control (C-iPS) and familial ALS (SOD1 N139K and SOD1 A4V) differentiated into astrocytes expressing the astrocyte marker GFAP. As noted in the inset, the percentage (%) of GFAP+ cells varies among the different cell lines. The significance of this variation is not yet known.
We have now initiated an in vitro evaluation of iPSC-derived astrocytes to ascertain whether they express appropriate astrocytic markers including GFAP, the astrocyte-specific glutamate transporter GLT1, connexin 43, aquaporin 4, the cell surface marker CD44 (a marker of astrocyte precursor identity), and the intermediate filament vimentin (**Figure 3**). To date, our data indicate that we have mixed populations of both immature and mature astrocytes.

Figure 1. Protocol for differentiation of iPSC into human iPSC-derived astrocytes



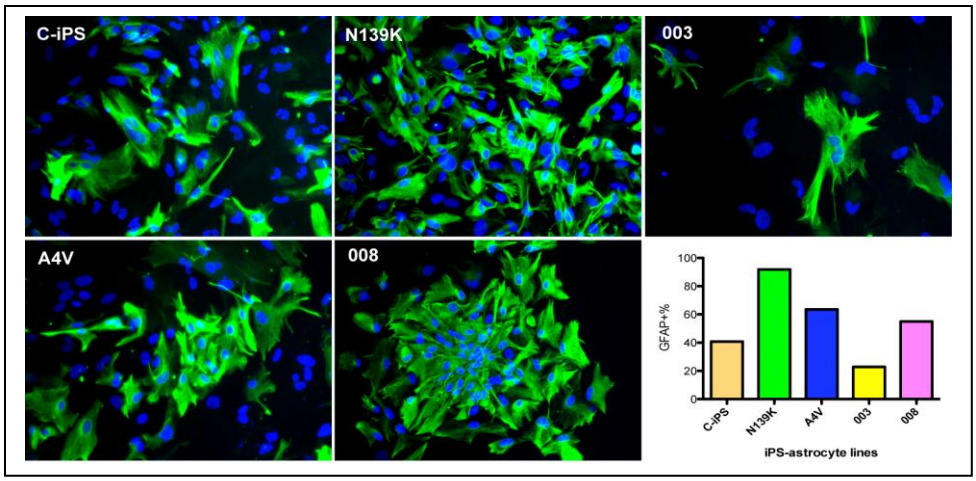
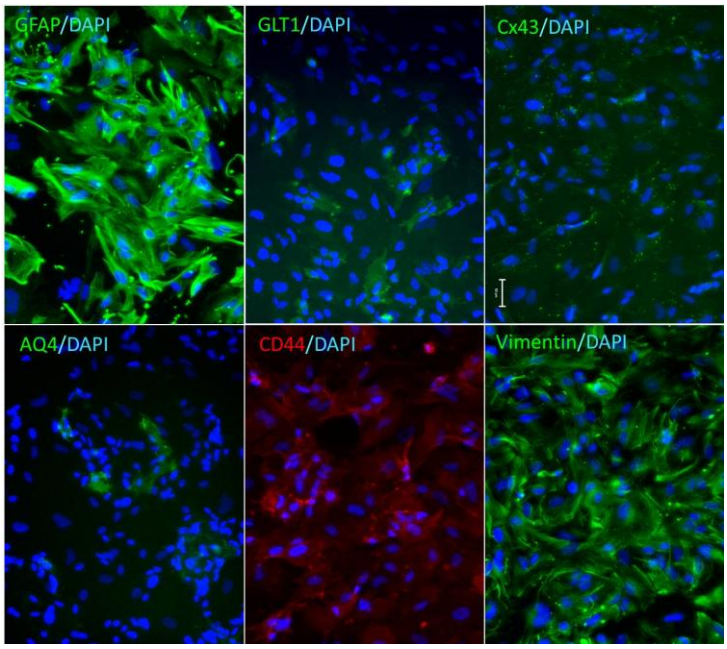


Figure 2. Differentiation of both control and familial (SOD1) iPSC into GFAP+ astrocytes in vitro

Figure 3. iPSC-derived astrocytes express astrocyte-specific proteins.

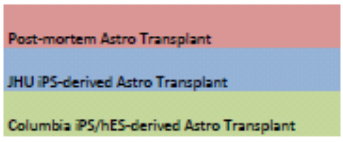


Aim #2. In vivo comparison of iPS cell-derived glial restricted precursors (iPSC-GRPs) from control, sporadic ALS, and familial ALS (SOD1) following transplantation into wildtype spinal cord

Task 1. Transplantation of iPSC -GRPs into wildtype rats

a. Characterization of iPSC -GRPs cell survival, differentiation (Months 1-24)

We have transplanted approximately 50 animals with a variety of human cell subtypes including astrocytes derived from NSC's isolated from postmortem brain (Pink), iPSC-derived astrocytes cultured and maintained at Johns Hopkins University (Blue), and iPS or human embryonic stem cell (ESC) derived astrocytes transplanted in collaboration with investigators (Chris Henderson, PhD) at Columbia University (Yellow).



Our preliminary data from **Table 2** demonstrate that we have used a variety of different hosts (wildtype rats, mice, nude mice) and a variety of different strategies for immunosuppression. To date, we have only seen limited survival of cells for a period of days to weeks but without significant long-term survival.

To address the relatively poor long term survival, we are transplanting greater numbers of cells. We also hypothesize that the state of differentiation may affect cell survival. Therefore, we are planning to transplant cells at varying stages of differentiation to establish the most effective differentiation state.

Table 2

Animals	N	Immuno-suppresion	Cell Type	Cell Characteristics	# of Cells Transplanted	Time prior to sacrifice	Results
Rats	n=2	Cyclosporine (20 mg/kg)	Post-mortem fALS SOD1 A4V astrocytes	p10, GFAP+	25,000 (C4), 75,000 (C5), 125,000 (C6)	1 week	Technical difficulties with huNA Ab localizing inj. site
Rats	n=2	Cyclosporine (20 mg/kg)	Post-mortem fALS SOD1 A4V astrocytes	p10, GFAP+	100,000 (C4), 150,000 (C5), 200,000 (C6)	4 hours	Cells are there and alive
Rats	n=2	Cyclosporine (20 mg/kg)	Post-mortem fALS SOD1 A4V astrocytes	p10, GFAP+	75,000 (C4), 100,000 (C5), 150,000 (C6)	1 week	Cells alive, GFAP neg. in vivo, hazy stain
Rats	n=1	Cyclosporine (20 mg/kg)	Post-mortem fALS SOD1 A4V astrocytes	p10, GFAP+	75,000 (C4), 100,000 (C5), 150,000 (C6)	1 month	Cells alive?, GFAP neg., CD44+, hazy stain
Rats	n=2	Cyclosporine (20 mg/kg)	Post-mortem fALS SOD1 A4V astrocytes	p11, GFAP+	25,000 (C5), 75,000 (C6)	1 week	hazy stain
Rats	n=1	Cyclosporine (20 mg/kg)	Post-mortem fALS SOD1 A4V astrocytes	p11, GFAP+	25,000 (C5), 75,000 (C6)	3 months	Cells alive?, still CD44+, GFAP neg., hazy stain
Rats	n=6	Cyclosporine (20 mg/kg)	Control iPS-derived astros (Ying)	p10, LV-GFP, GFAP neg	25,000 (C5 & C6)	1 week	Poor cell survival
Mice	n=1	Rapamycin/ FK506 (1 mg/kg)	Control iPS-derived astros (Ying)	p10, LV-GFP, GFAP neg	25,000 (C5 & C6)	2 weeks	Poor cell survival
Mice	n=4	Rapamycin/ FK506 (1 mg/kg)	Control iPS-derived astros (Ying)	p10, LV-GFP, GFAP neg	25,000 (C5 & C6)	5-6 weeks	Poor cell survival
Rats	n=1	Cyclosporine (20 mg/kg)	Human ES-derived astrocytes (Laurent)	Day 104, LV-GFP, GFAP neg, R1 line	15,000 (C5)	2 weeks	Poor cell survival
Rats	n=1	Cyclosporine (20 mg/kg)	Human ES-derived astrocytes (Laurent)	Day 104, LV-GFP, GFAP neg, R1 line	15,000 (C5)	4 weeks	Poor cell survival
Mice	n=1	Rapamycin/ FK506 (1 mg/kg)	Human ES-derived astrocytes (Laurent)	Day 95, GFAP+, R1 line	25,000 (C5-bilateral inj)	2 weeks	Poor cell survival
Mice	n=3	Rapamycin/ FK506 (1 mg/kg)	Human ES-derived astrocytes (Laurent)	Day 95, GFAP+, R1 line	25,000 (C5-bilateral inj)	4-5 weeks	Poor cell survival
Nude Mice	n=2	None	Human ES-derived astrocytes (Laurent) *non-transduced*	Day 112, GFAP+, R1 line	50,000 (C5-bilateral inj)	1 week	Poor cell survival
Mice	n=2	Rapamycin/ FK506 (1 mg/kg)	N139K.2 iPS-derived astros (Ying) p7	p7, GFAP+	25,000 (C5-bilateral inj)	1 week	Cells survived, ~25% GFAP+
Mice	n=1	Rapamycin/ FK506 (1 mg/kg)	N139K.2 iPS-derived astros (Ying) p7	p7, GFAP+	25,000 (C5-bilateral inj)	1 month	Poor cell survival
Mice	n=2	Rapamycin/ FK506 (1 mg/kg)	4944MA post-mortem astros (SOD1 A4V)	p11, GFAP+	25,000 (C5-bilateral inj)	1 week	Poor cell survival
Mice	n=2	Rapamycin/ FK506 (1 mg/kg)	4944MA post-mortem astros (SOD1 A4V)	p11, GFAP+	25,000 (C5-bilateral inj)	1 month	Poor cell survival
Mice	n=2	Rapamycin/ FK506 (1 mg/kg)	Human ES-derived astros (Laurent)	Day 25, GFAP neg, H13 line, CNTF treated 4d.	25,000 (C5-bilateral inj)	1 week	Poor cell survival
Mice	n=1	Rapamycin/ FK506 (1 mg/kg)	Human ES-derived astros (Laurent)	Day 25, GFAP neg, H13 line, CNTF treated 4d.	25,000 (C5-bilateral inj)	1 month	Poor cell survival
Nude Mice	n=2	None	4944MA post-mortem astros (SOD1 A4V)	p11, GFAP+	150,000 (C5-bilateral inj)	1 week	Poor cell survival
Nude Mice	n=2	None	4944MA post-mortem astros (SOD1 A4V)	p11, GFAP+	150,000 (C5-bilateral inj)	1 month	Poor cell survival
Rats	n=2	Cyclosporine (20 mg/kg)	Human ES-derived astros (Laurent)	Day 53, H13 line	150,000 (C5-bilateral inj)	2 weeks	In progress
Rats	n=2	Cyclosporine (20 mg/kg)	Human ES-derived astros (Laurent)	Day 53, H13 line	150,000 (C5-bilateral inj)	7 weeks	In progress
Rats	n=2	Cyclosporine (20 mg/kg)	Human iPS-derived astros (Laurent)	Day 53, 11a line	150,000 (C5-bilateral inj)	2 weeks	In progress
Rats	n=2	Cyclosporine (20 mg/kg)	Human iS-derived astros (Laurent)	Day 53, 11a line	150,000 (C5-bilateral inj)	7 weeks	In progress

b. Assessment of iPSC-GRP induced ALS pathologies (**Figure 4**)
Preliminary data to date suggest that iPSC-derived astrocytes isolated from subject with an SOD1 N139K mutation can be transplanted into the ventral horn of the spinal cord of wildtype mice and be identified by a human mitochondrial-specific antibody, and express GFAP (38%).

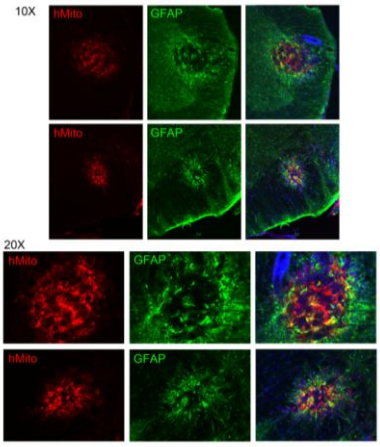


Figure 4

Planned experiments Years #2-3

c. Behavioral assessment of iPSC-GRP transplanted animals (Months 12-36)
Animals: Wildtype Sprague Dawley Rats: Approximately 40 (10 with control iPSC-GRP, 10 sALS iPSC-GRP, 10 fALS iPSC-GRPs, 10 media control injections)

Aim #3. Determine the capacity for neuroprotection of iPSC-derived glial restricted precursors (iPSC-GRPs) following transplantation into the SOD1^{G93A} rat model of ALS.

Task 1. Transplantation of iPSC-GRPs into SOD1^{G93A} rats
Dr. Nicholas Maragakis, Johns Hopkins University

a. Behavioral assessment of (iPSC-GRP transplanted GRPs in the SOD1^{G93A} rat (Months 12-36)

1. forelimb and hindlimb grip strength
2. Survival studies
3. Electrophysiological studies

Animals: SOD1^{G93A} Sprague Dawley Rats: Approximately 40 (10 with control iPSC-GRP, 10 sALS iPSC-GRP, 10 fALS iPSC-GRPs, 10 media control injections)

b. Pathological assessment of iPSC-GRP in the SOD1^{G93A} rat (Months 12-36)
Animals: SOD1^{G93A} Sprague Dawley Rats: Approximately 40 (10 with control iPSC-GRP, 10 sALS iPSC-GRP, 10 fALS iPSC-GRPs, 10 media control injections)

KEY RESEARCH ACCOMPLISHMENTS:

--Induced Pluripotent Stem Cell lines have been created from subjects with familial ALS, Sporadic ALS, and controls
--IPS Cell-derived astrocytes have been successfully cultured from subjects with familial ALS, Sporadic ALS, and controls
--Initial transplantation experiments of IPS Cell-derived astrocytes are underway in a variety of animal models.

REPORTABLE OUTCOMES: NONE

CONCLUSION:
We have made substantial progress in obtaining skin biopsies with subsequent fibroblast cultures of a number of sporadic ALS, familial ALS, and control subjects in the last year. We have collected a variety of ALS phenotypes including slow progressing ALS, primary lateral sclerosis (PLS), lower motor neuron only ALS (LMN), ALS with frontotemporal dementia (ALS/FTD) and appropriate controls. In addition, we have also obtained 28 samples from subjects with familial ALS (these include SOD1, ANG, FIG4, and FUS mutations). In collaboration with Johns Hopkins Investigators, we have developed 22 FALS iPSC lines, 8 sporadic ALS iPSC, and 5 control iPSC lines. Using these cells we have been developing a method to allow for long-term differentiation of iPS cells into astroglia. These cell lines have been developed along with an additional collaboration with Dr. Rothstein and are in the process of being made available to the research community.

REFERENCES: NONE
APPENDICES: NONE