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14. ABSTRACT

This grant will provide critical data on tolerance and toxicity of cell dosing and numbers of permissible spinal cord injections. Rigorous experiments in Aim 1 of our grant have demonstrated that, even though the porcine spinal cord seems to tolerate escalating numbers and volumes of injections, the severity of acute transient morbidity should not be neglected. Moreover, escalating numbers and volumes of injections seem to be associated with lack of accuracy and reflux. Histological analysis will further assess morbidity by determining whether there is a reduction in the number of motor neurons or not in grafted areas in comparison with intact segments. Histological analysis will also determine whether reflux occurs with volume escalation as well as with fast (hand-held) injections.

At the same time, little is understood about the appropriate immunosuppressive therapy for spinal cord stem cell transplant recipients. In our ongoing human trial, aggressive immunosuppressant therapy has formed the single biggest source for adverse events. Aim 2 will help us to optimize immunosuppression, preventing needless complications.

15. SUBJECT TERMS
ALS, spinal cord, stem cell, swine, therapy, transplantation.

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INTRODUCTION:

"The present application will refine critical details required for successful cell transplantation.
Aim 1 (Optimal Surgical Technique) will provide critical data on tolerance and toxicity of cell dosing and numbers of permissible spinal cord injections. Aim 2 (Graft Rejection) will provide critical data on graft rejection and appropriate immunosuppression for human spinal cord stem cell transplantation."

Work in Year 2 has focused on both Aims 1 and 2.

The approved Statement of Work for such aims states the following:

- "**Aim 1: *Optimal Surgical Technique*** is required for both accuracy and safety.

The Svendsen Laboratory has extensive expertise in the production and propagation of neural progenitor lines. Ongoing preliminary studies have allowed the Boulis and Svendsen laboratories to develop Standard Operating Procedures (SOPs) for the transfer of healthy cells. Cells will be shipped overnight. On the next day, cell will be prepared for injections and viability counts will be performed in the Boulis Laboratory prior to transplantation. Surgeries will be performed in the Division of Animal Resources (DAR) at Emory University. Each pig surgery takes approximately 5 hours, making it possible to only perform a maximum of 2 surgeries in a day. Animals will be euthanized after 14 days. At the time of necropsy, animals will be sedated and perfused with heparin, followed by Paraformaldehyde. Fixed tissue will be sectioned. Tissue will be stored and analyzed for histological morbidity as well as for graft identification / migration."

- "**Aim 2: *Graft Rejection*** can be minimized by optimizing immunosuppression.

Cell production and shipment will occur in LA as described above. Surgery, Behavior, Immunosuppressant administration, Necropsy, and Tissue sectioning/analyses will occur at Emory in the Boulis laboratory as in Aim 1. All tissue sections will be analyzed for cell graft survival and migration in the Boulis laboratory. Alternating sections will be sent to the Transplantation Immunology Laboratory for analysis of cellular immune response and inflammatory mediators under the direct supervision of Dr. Allan Kirk."

- **Milestones**

Year 1:

"Complete analysis of reflux and transient morbidity with number and volume of injection of hNPCs (Boulis).
Create a cell bank of astrocyte restricted precursor IPS cells (Svendsen)."

Year 2:

"Tissue analysis (Aim 1). Surgeries / tissue analysis (Aims 2a and 2c)."
(Boulis)
Cell production for Aims 2a and 2c (Svendsen)."

BODY:

In Year 2 we have partially completed histological analyses pertaining to Aim 1. We have also completed surgical procedures pertaining to Aims 2a and 2c (Graft Rejection).
A total of 30 (5 pilot + 25 experimental) animals were used.
Because generation of porcine iPS-derived NPCs was not possible, we have dropped Aim 2b.

(Boulis Laboratory)

Aim 1. Histological Morbidity Analysis using Stereology

In Year 2 efforts were devoted towards tissue processing and histological analyses. Pilot studies were necessary early in the processing in order to identify the best strategy for stereological analysis of the tissue. **Stereological analysis of the volume escalation group from Aim 1 was completed and we are currently in the process of analyzing the number escalation group.** Preliminary statistical analysis performed to the volume escalation group show that there is a statistically significant (One-way ANOVA, p=0.004) relationship between increasing volumes and number of damaged injection sites (damage is considered when >50% of the graft area is missing). Additionally, our statistical analyses suggest the following trends:

- As volume increases the total number of stem cells that survive increases **(Figure 1)**

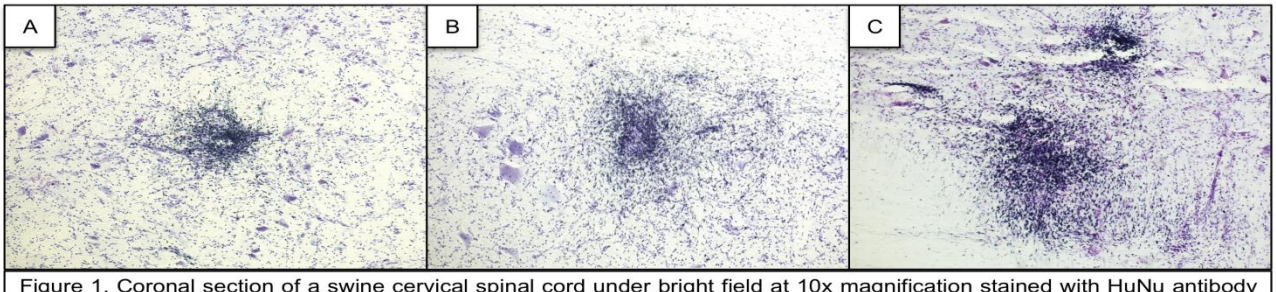

Figure 1. Coronal section of a swine cervical spinal cord under bright field at 10x magnification stained with HuNu antibody for human NPCs and Cresyl Violet for motor neurons. A) 10 microliter graft, B) 25 microliter graft and C) 50 microliter graft.

- As volume increases there is no significant decrease in neuronal density

- The engraftment percentage (remaining proportion of cells engrafted at the target site at a time point after transplantation) remains between 11 -17% depending on the injection volume

- The 25-microliter group showed the highest mean (12.8) for the total number of identified injection sites, as well as better integration of the graft to the tissue

The main struggles with tissue analysis were the within and in-between group variations. We found that all groups had different graft morphologies ranging from a relatively healthy graft to a graft with significant infiltration and damage. We also noted different degrees and types of infiltration, which were independent of the injected volume **(Figure 2)**. It remains outside the scope of Aim 1 to fully characterize the inflammatory and immune reaction to the transplanted cells in these animals, but we will be able to do so when analyzing tissue from Aim 2.

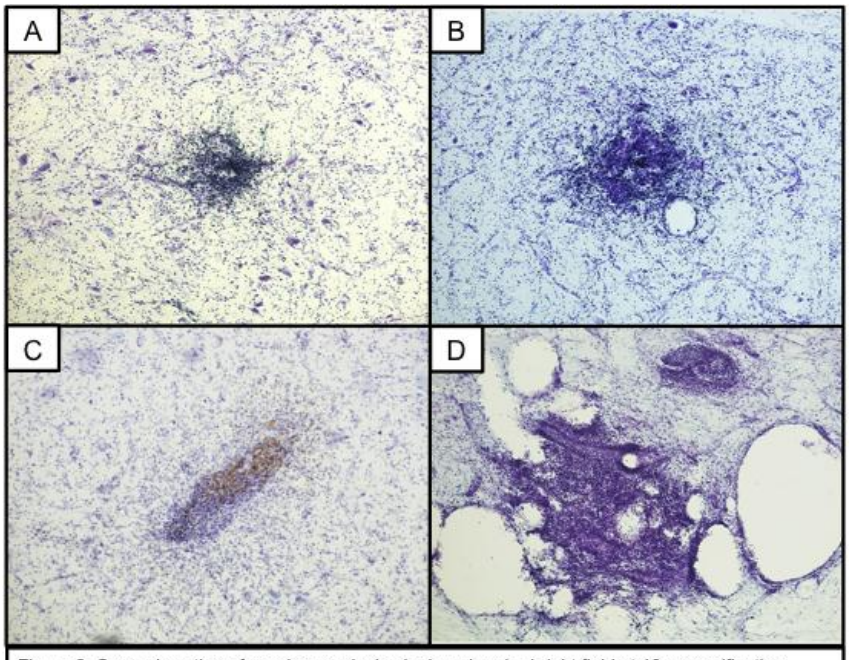

Figure 2. Coronal section of a swine cervical spinal cord under bright field at 10x magnification stained with HuNu antibody for human NPCs and Cresyl Violet for motor neurons. A) Shows a healthy graft, B) Shows a graft with some infiltration, C) Shows a graft with significant infiltration and D) Shows a graft with significant infiltration and damage.

Key learning points:

1. Our statistical analyses of volume escalation the data indicate that 25 microliters is the optimal volume to inject with the cell concentration used in these experiments **(Figure 3 and Table 1)**

2. The enormous variability in-between and within groups can explain the high variance in the data

3. The engraftment percentage achieved in the completed animals is slightly higher compared to what has been reported in the literature (Donnelly et al., 2012)

Figure 3. The one-way ANOVA shows a statistically significant difference (p=0.0042) in number of damaged injection sites across volume groups. In order to identify which groups had the significant difference, pair-wise comparison T-tests were done using the post-hoc Tukey's adjustment method. This analysis shows a statistically significant difference between the 10 and 50 microliter groups at a 0.05 significance level.

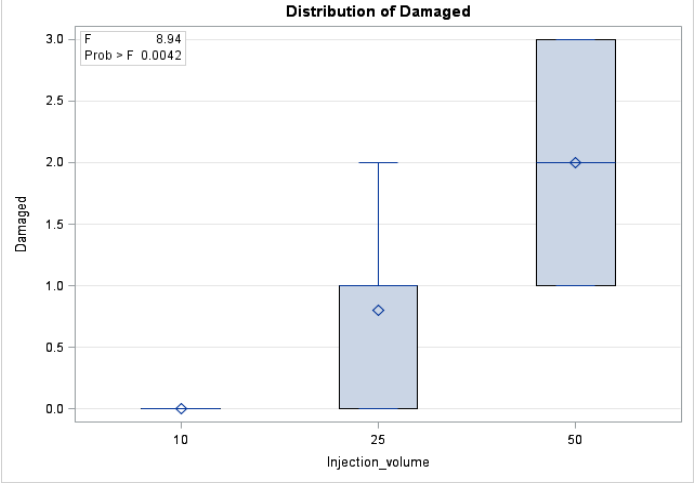

Table 1.

10 microliter Group (n=5)					
Variable	Mean	Std Dev	Variance	Minimum	Maximum
Neurons per mm^3	2276.1	1457.74	2124999.48	1309.15	4857.07
Total grafted cells	260647.8	360191.34	1.29738E+11	5047	879850
Number of grafts	9.4	7.8294317	61.3	1	20
Stem cells per graft	17754.62	16602.46	275641552	2601.67	43992.5
Engraftment %	17.754619	16.6024562	275.6415524	2.6016667	43.9925
On target injections	6.6	6.9137544	47.8	1	18
Off target injections	2.8	2.5884358	6.7	0	6
Damaged injection sites	0	0	0	0	0

25 microliter Group (n=5)					
Variable	Mean	Std Dev	Variance	Minimum	Maximum
Neurons per mm^3	2260.29	999.4256774	998851.68	1438.2	3944.96
Total grafted cells	393822.4	264216.41	69810310237	132795	803205
Number of grafts	12.8	2.7748874	7.7	9	16
Stem cells per graft	29339.02	14557.63	211924686	11066.25	50200.31
Engraftment %	11.7356063	5.8230533	33.9079498	4.4265	20.080125
On target injections	7.8	4.0249224	16.2	3	14
Off target injections	4.6	3.7815341	14.3	1	9
Damaged injection sites	0.8	0.83666	0.7	0	2

50 microliter Group (n=5)					
Variable	Mean	Std Dev	Variance	Minimum	Maximum
Neurons per mm^3	4101.14	1670.73	2791346.6	1805.15	5794.51
Total grafted cells	670832.4	581591.03	3.38248E+11	236229	1659912
Number of grafts	12	2.1213203	4.5	10	15
Stem cells per graft	60045.93	60358.18	3643109897	19685.75	165991.2
Engraftment %	12.0091853	12.071636	145.7243959	3.93715	33.19824
On target injections	8	4.3588989	19	3	13
Off target injections	4	2.5495098	6.5	1	7
Damaged injection sites	2	1	1	1	3

Aim 2a. We hypothesize that *allograft* survival (pig NPCs) will exceed that of *xenografts* (human NPCs) with and without immunosuppression. In this aim, animals were divided into four groups (n = 5/group) distinguished by the grafts (allo or xeno) and the immunosuppressive treatments (with or without). Animals underwent five unilateral *lumbar* injections with a 10µl cell suspension (10E4 cells/ul) containing either pig BrdU-labeled pig NPCs cells (groups 1 and 2) or human BrdU-labeled NPCs (groups 3 and 4). Animals in all groups were euthanized at 21 days. *(Although the original grant proposed cervical injections, we elected to perform lumbar injection, as the goal of Aim 2 did not include safety assessment of the procedures and lumbar injections are easier to perform than cervical ones. Also, because iPS cells could not be generated (as anticipated in Year 1 (pages 14-15), BrdU-labeled neural progenitor cells were used instead.)*

Aim 2c. We hypothesize that hNPCs are less immunogenic than differentiated cell lines in both in vitro and in vivo assays and, therefore, *triple immunosuppressant regimens* provide no added protection from graft rejection in comparison with better tolerated *monotherapy*. In this Aim, animals (n = 5) received five unilateral cervical injections with a 10µl cell suspension (10E4 cells/ul) containing pig NPCs. Animals received intravenous treatment with Basiliximab (two 10mg IV doses on post-op day 0 and day 4) + MMF (10 mg/kg, BID, IV) + Tacrolimus (0.025mg/kg, BID, IV), starting at the time of transplant. Animals were euthanized at 21 days.

As originally proposed, in addition to graft survival by stereological quantification (tissue is being currently processed for analysis), in these sub-aims we intend to compare the impact of different immunosuppression regimens on graft survival and the key components of the inflammatory response (with the guidance and collaboration of Dr. Allan Kirk and the Transplantation Immunology team). For such, we will:

1) Analyze the immunologic characteristics of human (hNPC) and pig neural progenitor cells (pNPC) with flow cytometry;

2) Collect peripheral blood from pig groups receiving intraspinal transplantation of hNPCs or pNPCs with different immunosuppression strategies;

3) Analyze the peripheral blood for the development of graft-specific pig antibodies with a Flow Cytometry Cross Match (FCXM);

4) Analyze the peripheral blood for graft-specific pig lymphocytes with a modified Mixed Lymphocyte Reaction (MLR).

Detection of peripheral biomarkers for the immune response to intraspinal cell grafts is essential to effective therapeutic monitoring (Dieterlen et al., 2011). Furthermore, analyzing the peripheral immune response to intraspinal cell grafts will further our understanding of the basic mechanisms underlying immune rejection in the CNS (Loewendorf et al., 2013).

Preliminary Results:
Flow Characterization: hNPCs were characterized for the expression of antigens specific to immunogenicity, differentiation, and function. The particular population of cells chosen for analysis of antigen expression was based on forward and side scatter patterns in the gating strategy **(Figures 4A and 5A).** The hNPCs expressed β2-microglobulin, a component of MHC Type 1 antigen **(4B)** and Human Leukocyte Antigen (HLA) – DR, a common MHC Type 2 antigen (**4C)**. However, the hNPCs do not express costimulatory molecules CD80 **(4D)** or CD86 **(4E)**. Of note, the hNPCs are positive for expression of neuronal and astrocytic markers of differentiation and various adhesion molecules. pNPCs were characterized for the expression of surface markers related to immunogenicity, adhesion, and differentiation.

See Appendices for a complete panel of antigens used for *flow characterization of* hNPCs and pNPCs.

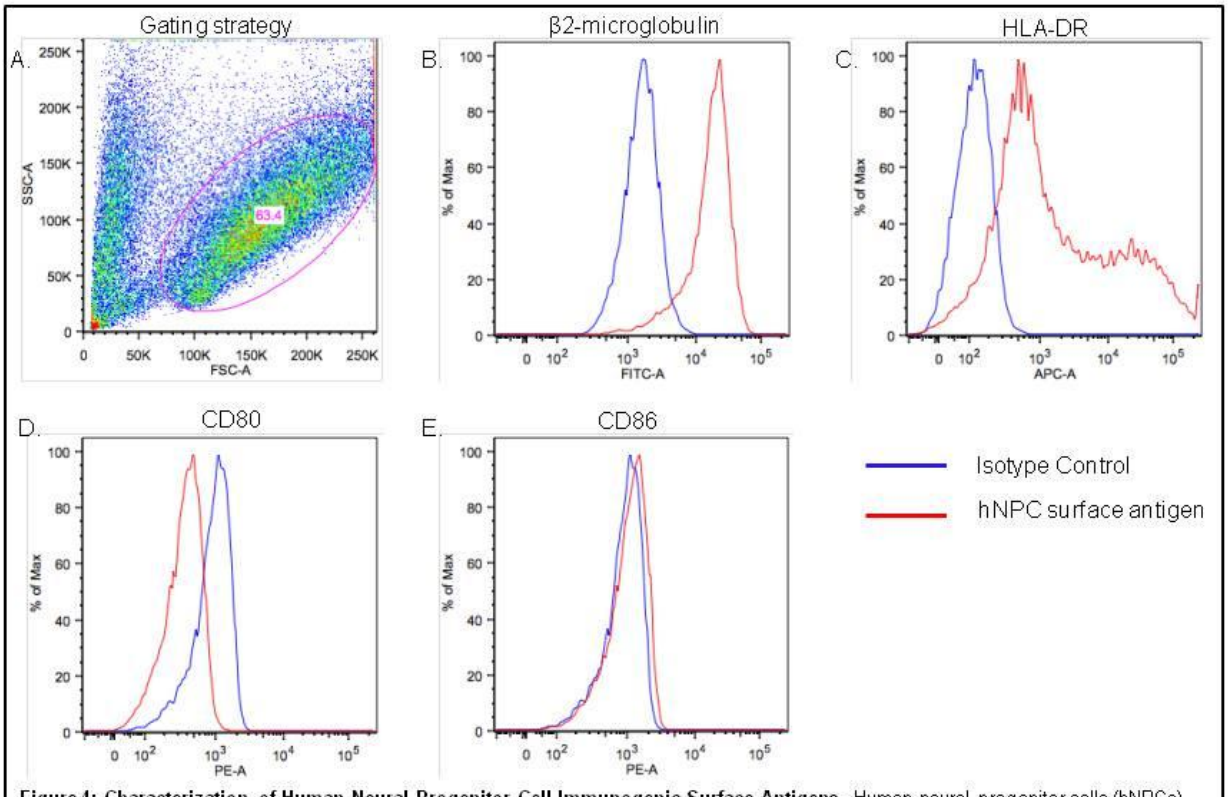

Figure 4: Characterization of Human Neural Progenitor Cell Immunogenic Surface Antigens. Human neural progenitor cells (hNPCs) were prepared for transplantation, incubated with fluorescent antibodies specific to Major Histocompatibility Complex (MHC) and costimulatory surface antigens, and analyzed with flow cytometry. Forward (FSC) and Side (SSC) scatter patterns **(A)** were used to gate populations of hNPCs for characterization of surface marker expression. Histograms of fluorescent signal intensity show positive hNPC expression of: **(B)** β2-microglobulin (FITC fluorescence), a component of MHC Type 1 antigen; and **(C)** Human Leukocyte Antigen (HLA) – DR (PERCP fluorescence), a common MHC Type 2 antigen, compared to isotype control. Histograms show hNPC do not express costimulatory antigens **(D)** CD80 and **(E)** CD86. Abbreviations: Fluorescein isothiocyanate (FITC); Allophycocyanin (APC); Phycoerythrin (PE); and Cluster of Differentation (CD).

FCXM: Graft specific pig antibodies were detected 7 days following transplantation **(Figure 6)**. The rise in pig antibodies specific to transplanted human cells was detected in all 5 animals and the mean value of the group was statistically significant ($p = 0.0124$). Furthermore, graft specific antibodies were detected 14 days following transplantation for certain animals. All animals returned to baseline at day 21. The pigs analyzed in this group did not receive immunosuppression. These experiments have been duplicated.

MLR: Preliminary experiments have been conducted to establish assays.

Key Learning Points and Future Directions:

Flow Characterization:

- Both human and pig NPCs express MHC Type 1 and 2 antigens. This allows them to directly present self and non-self antigens to the host immune system.

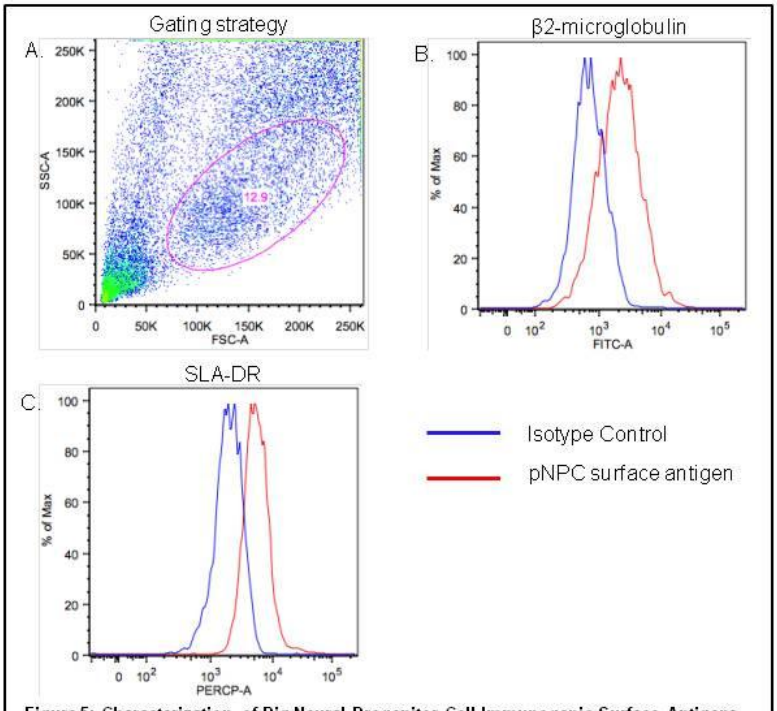

Figure 5: Characterization of Pig Neural Progenitor Cell Immunogenic Surface Antigens. Pig neural progenitor cells (pNPCs) were prepared for transplantation, incubated with fluorescent antibodies specific to Major Histocompatibility Complex (MHC) surface antigens, and analyzed with flow cytometry. Forward (FSC) and Side (SSC) scatter patterns **(A)** were used to gate populations of pNPCs for characterization of surface marker expression. Histograms of fluorescent signal intensity show positive pNPC expression of: **(B)** β2-microglobulin (FITC fluorescence), a component of MHC Type 1 antigen; and **(C)** Swine Leukocyte Antigen (SLA) – DR (PERCP fluorescence), a common MHC Type 2 antigen compared to isotype control. Abbreviations: Fluorescein isothiocyanate (FITC) and Peridinin Chlorophyll Protein Complex (PERCP).

- Flow characterization experiments are complete. Further analysis will be conducted to better characterize the cell populations.

Flow Cytometry Cross Match

- The detection of graft specific antibodies in the peripheral blood following intraspinal transplantation has not been described previously
- Systemic (peripheral blood) antibodies specific to the graft has implications on the mechanisms of graft-specific antigen presentation to the host immune system
- Experiments must be conducted for the remaining pig groups and the experiments in the first group must be done in triplicate

Mixed Lymphocyte Reaction: These experiments must be conducted. All PBMCs are cryopreserved and banked.

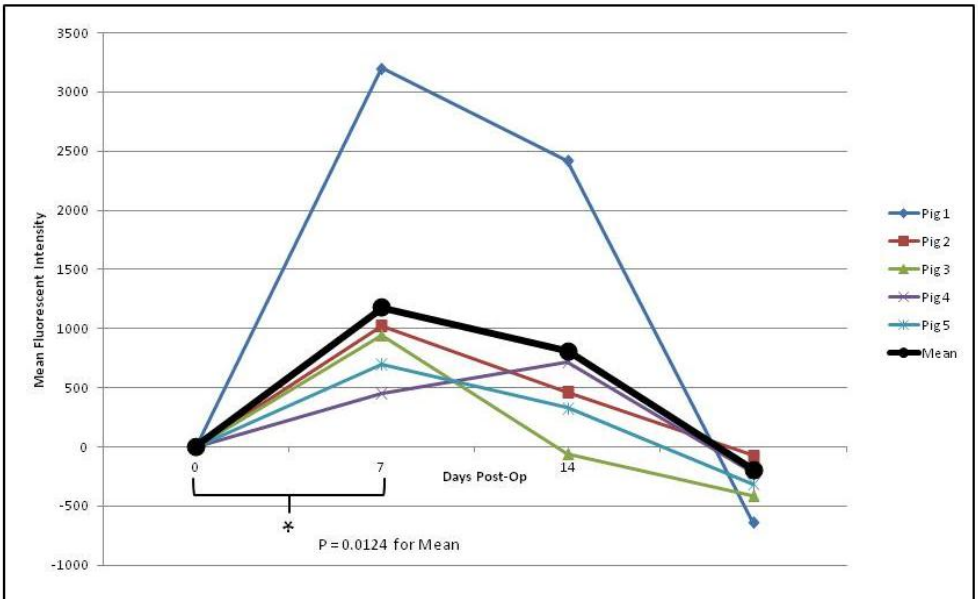

Figure 6: Detection of Pig Antibodies Specific to Transplanted Human Neural Progenitor Cell Grafts. A group of pigs (n=5) received intraspinal transplantation of human Neural Progenitor Cells (hNPCs) and were not treated with immunosuppression. Pig plasma was collected pre-operatively and 7, 14, and 21 days post-operatively. A separate aliquot of hNPCs from the same lot of cells was prepared for transplantation and incubated with the collected pig plasma containing pig antibodies. The cells were washed and incubated with a secondary fluorescent antibody specific to pig antibody. The cells were washed and mean fluorescent intensity (amount of pig antibody bound to hNPCs) was quantified with flow cytometry. A statistically significant ($p < 0.05$) increase in pig antibody specific to transplanted hNPCs was detected for the group (mean).

(Svendsen Laboratory)

<u>Generation of fetal cortical-derived pig neural progenitors and BrdU labeling</u>: As previously reported in Year 1, we have encountered some hurdles in the generation of porcine iPS-derived NPCs. In order to pursue Aim 2 of the grant proposal we have circumvented this issue by isolating and expanding cortical and spinal neural progenitors from embryonic minipigs.

Two initial attempts were performed to generate a sufficient amount of fetal pig NPCs:

1. Isolation of E50 embryos from an aborted SOW (Lot #1)
2. Isolation of E25 embryos isolated via a C-section (Lot #2)

Unfortunately cell from Lot #1 did not expand as expected and senesced at low passage. In our second attempt, (Lot #2) cells were isolated and cortical-derived pig neural progenitors were expanded as spheres in media supplemented with epidermal growth factor (EGF) and fibroblast growth factor-1 (FGF-1), passaged by mechanical chopping and vials were frozen down for future experiment at various passages.

Following transplantation, human cells can easily be identified with the use of human specific antibodies. However, as allogeneic grafts, the transplanted pNPCs can only be identified following transplantation by means of an exogenous marker. In this instance, we selected to label proliferating cell *in vitro* via pulsing with 5-bromo-2'-deoxyuridine (BrdU). Although toxic at high concentrations (Caldwell et al., 2005) we have found that pulsing cells with BrdU at a concentration of 0.2µM or lower is non-toxic and a reliable labeling strategy for allogeneic transplant or assessment of cell proliferation (Suzuki et al., 2006; Ebert et al., 2010). For this study, as the pNPCs are slow growing cells, we found that we obtained the best results by pulsing for 3 days with 0.05µM BrdU **(Figure 7A-C)**. This paradigm resulted in the labeling of 79% and up to 45% of human and pig NPCs respectively **(Figure 7C)**. Using these parameters we provided an initial 9 vials of BrdU pulsed hNPCs (passage 29) and 13 vials pNPCs (passage 12) to the Boulis group that have successfully been used for transplantation in Aims 2a and 2c. Other attempts (2 attempts) at thawing and expanding pNPCs to provide a greater number of cells for the Boulis group have not been successful. At this point in time, we are unsure of the factors causing the rapid senescence of the pNPCs. However, low expansion rates and viability of cells post-thaw have resulted in these cells not being suitable for transplantation.

A new lot of cells (Lot #3) was recently generated from E25 embryos. At passage 3, following differentiation via growth factor withdrawal and similar to hNPCs, a majority of the cells expressed the astrocyte marker GFAP **(Figure 7E, G)** and a lower proportion of Tuj1 positive cells **(Figure 7F)**. Although the number of GFAP+ cells is significantly higher in pNPC than for hNPCs (GFAP: *P<0.05) the higher astrocyte to neuron ratio in the pNPCs is representative of the human cortical-derived NPCs population and thus would serve as a good population for the studies proposed by the Boulis group.

We have pulsed 5 confluent T175 of pNPCs at passage 8 but the cell viability was suboptimal (24.8% for the pulsed cells and 21% for the non-pulsed cells). As an alternate plan, lower passage cells have been thawed and will be pulsed with BrdU at passage 5 or 6 (depending on growth rate) in order to ensure a sufficient quantity of cells for future aims associated with the proposal. These experiments are expected to be completed by the end of October 2013.

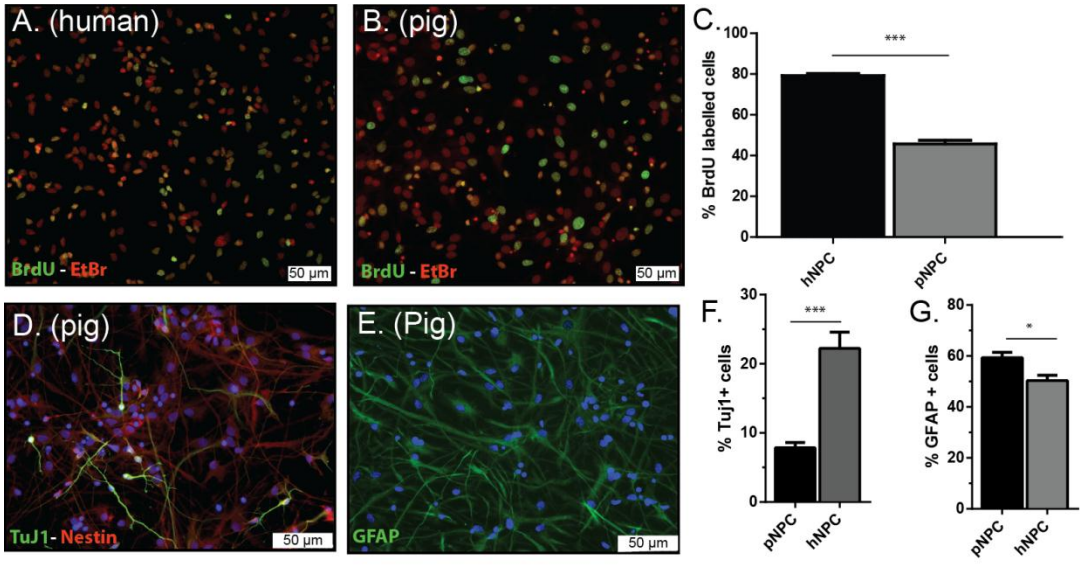

Figure 7: BrdU labeling and characterization of fetal cortical-derived pig neural progenitors cells. (A-B) Human and pig NPCs were pulsed with 0.05µM BrdU for 3 days. An EtBr stain (red) is used to identify the nucleus of cells stained with an anti-BrdU antibody (green). A significantly higher proportion of human cells are labeled with BrdU (***P<0.001) is likely due to their higher rate of proliferation *in vitro* compared to pNPCs. Panel D-E represents lot #3 of pNPCs (p3) following a 5 day differentiation via growth factor withdrawal. (F-G) Quantification of (F) Tuj1 (neurons) and (G) GFAP (astrocytes) positive cells indicated similar proportions of astrocytes in pig fetal cortical cultures at passage 3 compared to human fetal cortical cultures at passage 28.

KEY RESEARCH ACCOMPLISHMENTS:

(Boulis Laboratory)

- **Aim 1** (Histological morbidity analysis using Stereology for Aim 1a has been completed. Histological morbidity analysis using Stereology for Aim 1b is ongoing. Statistical analyses are ongoing.)

- **Aim 2** (Surgical procedures and initial immunological analysis have been completed for Aims 2a and 2c. Tissue processing for histological analysis has been initiated. Immunological analysis has been initiated.)

Aim	Brief Explanation	Number Pigs	Number Injections	Cell type	Immunosuppression	Survival
2a	Allograft vs. Xenograft	5	5	Pig NPCs	None	21d
		5			Tacrolimus (0.025 mg/kg, BID, IV)	
		5		Human NPCs	None	
		5			Tacrolimus (0.025 mg/kg, BID, IV)	
2c	Mono vs. Triple Immunotherapy	5	5	Pig NPCs	Basiliximab (two 10mg IV doses on post-op day 0 and day 4) + MMF (10 mg/kg, BID, IV) + Tacrolimus (0.025mg/kg, BID, IV)	21d

(Svendsen Laboratory)

- Generated, labeled, and shipped cells for procedures in **Aims 2a and 2c.**

REPORTABLE OUTCOMES:

(Boulis Laboratory)

Abstracts submissions and preparation of manuscripts on the following:

1. Miller JH, Hurtig CV, Grin N, Lamanna JJ, Federici T, Boulis NM. Porcine behavior and functional status following large volume cervical spinal cord injections. Abstract for AANS/CNS Spine Section meeting: Phoenix, AZ March 6-9, 2013.

2. Gutierrez J, Lamanna JJ, Grin N, Gary M, Hurtig CV, Miller JH, Riley J, Urquia L, Federici T, Boulis NM. Preclinical Validation of Multilevel Intraspinal Stem Cell Therapy — Dose escalation assessment in Gottingen minipigs. Abstract for American Association of Neurological Surgeons Annual Meeting: San Francisco, CA April 5-9, 2014.

3. Lamanna JJ and Espinosa J, Gutierrez J, Urquia L, Grin N, Federici T, Kirk AD, Boulis NM. Peripheral blood detection of a graft-specific immune response to intraspinal stem cell therapy in Gottingen minipigs. Abstract for American Association of Neurological Surgeons Annual Meeting: San Francisco, CA April 5-9, 2014.

CONCLUSIONS:

From Aim 1 histological analysis:

- Our statistical analyses of volume escalation the data indicate that 25 microliters is the optimal volume to inject with the cell concentration used in these experiments.

- The enormous variability in-between and with-in groups can explain high variance in the data.

- The engraftment percentage achieved in the completed animals is slightly higher compared to what has been reported in the literature.

From Aim 2 immunological analysis:

Flow Characterization:

- Both human and pig NPCs express MHC Type 1 and 2 antigens. This allows them to directly present self and non-self antigens to the host immune system.

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APPENDICES:

Tables. Complete panel of antigens used for *flow characterization of* hNPCs and pNPCs.

Antibody	Justification	Expression
B2-microglobulin	MHC I component: antigen presentation	Yes
HLA-DR	MHC II: antigen presentation	Yes
CD80	Costimulatory antigen	Yes
CD86	Costimulatory antigen	Yes
GLAST	Astrocytic differentiation	Yes
PSA-NCAM	Neuronal differentiation	Yes
CD29	Adhesion, signaling	Yes
CD44	Leukocyte rolling, homing, aggregation	Yes
CD56	Adhesion	Yes
CD57	Adhesion	Yes
CD9	Adhesion, migration	Yes
CD90	Adhesion, signaling	Yes
GATA3	Transcription factor	Yes
CD105	Erythroid precursors, angiogenesis	No
CD11b	Microglia	No
CD133	HSC subsets, neural precursors	No
CD166	Adhesion, T cell activation	No
CD184	Homing, HIV entry	No
CD25	IL-2Rα	No
CD271	Oligodendricites	No
CD54	Extravasation, t cell activation	No
Ki67	Proliferation	No

Table 1: Characterization of Human Neural Progenitor Cell Surface Antigens. Human neural progenitor cells (hNPCs) were prepared for transplantation and incubated with fluorescent antibodies specific to cellular antigens. Expression of hNPC antigens was quantified with flow cytometry. Abbreviations: Major Histocompatibility Complex (MHC); Human Leukocyte Antigen (HLA); Cluster of Differentation (CD); Glutamate Aspartate Transporter (GLAST); Polysialyated-Neural Cell Adhesion Molecule (PSA-NCAM); Human Stem Cell (HSC); and Interleukin (IL).

Antibody	Justification	Expression
B2-microglobulin	MHC I component: antigen presentation	Yes
SLA-DR	MHC II: antigen presentation	Yes
CD105	Erythroid precursors, angiogenesis	Yes
CD25	IL-2Rα	Yes
CD29	Adhesion, signaling	Yes
CD44	Leukocyte rolling, homing, aggregation	Yes
CD56	Adhesion	Yes
CD9	Adhesion, migration	Yes
GFAP	Astrocytic differentiation	No
CD90	Adhesion, signaling	No
CD11b	Microglia	No
CD54	Extravasation, t cell activation	No
CD106	Adhesion	No
Ki67	Proliferation	No

Table 2: Characterization of Pig Neural Progenitor Cell Surface Antigens. Pig neural progenitor cells (pNPCs) were prepared for transplantation and incubated with fluorescent antibodies specific to cellular antigens. Expression of pNPC antigens was quantified with flow cytometry. Abbreviations: Major Histocompatibility Complex (MHC); Swine Leukocyte Antigen (SLA); Cluster of Differentation (CD); Glial Fibrillary Acidic Protein (GFAP); and Interleukin (IL).