Notice of Award

EXPLORATORY/DEVELOPMENT GRANT
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/31/2019

NATIONAL EYE INSTITUTE

Grant Number: 1R21EY031120-01
FAIN: R21EY031120

Principal Investigator(s):
BRAD FORTUNE, OD
Jason Stephen Meyer (contact), PHD

Project Title: Targeting the diversity of retinal ganglion cells for replacement therapy

Martin, Steven Allen
Associate Vice President for Research Adminis
509 E. 3rd St.
Bloomington, IN 474013654

Award e-mailed to: spon2@iupui.edu

Period Of Performance:
Budget Period: 08/01/2019 – 07/31/2020
Project Period: 08/01/2019 – 07/31/2020

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $345,976 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to Trustees of Indiana University in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Eye Institute of the National Institutes of Health under Award Number R21EY031120. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Karen Robinson-Smith
Grants Management Officer
NATIONAL EYE INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 1R21EY031120-01

Award Calculation (U.S. Dollars)

Federal Direct Costs	$308,716
Federal F&A Costs	$37,260
Approved Budget	$345,976
Total Amount of Federal Funds Obligated (Federal Share)	$345,976
TOTAL FEDERAL AWARD AMOUNT	$345,976
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$345,976

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$345,976	$345,976

Fiscal Information:

CFDA Name:	Vision Research
CFDA Number:	93.867
EIN:	1356001673A1
Document Number:	REY031120A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019
EY	8472439	$345,976

NIH Administrative Data:
PCC: 4E /ESL / **OC**: 414A / **Released**: [redacted]07/25/2019
Award Processed: 07/31/2019 12:11:31 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R21EY031120-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R21EY031120-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal

Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R21EY031120. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the

expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.*

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – EY Special Terms and Conditions – 1R21EY031120-01

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

MODULAR GRANTS

This is a Modular Grant Award without direct cost categorical breakdowns issued in accordance with the guidelines published in the NIH Grants Policy Statement at: http://grants.nih.gov/grants/policy/nihgps/nihgps.pdf. Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

CONSORTIUM:
This award includes funds awarded for consortium activity with Legacy Emanuel Hospital and Health Center. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps/nihgps.pdf.

SALARY CAP:
None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Therefore, this award and/or future years are adjusted accordingly, if applicable. Current salary cap levels can be found at the following URL: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-19-099.html

REMINDER - CLOSEOUT:
Recipients must submit a final FFR, Final RPPR, and Final Invention Statement and Certification within 120 calendar days of the end of the period of performance (project period). The reports become overdue the day after the 120 calendar day period ends. Learn more about closeout: https://grants.nih.gov/grants/policy/nihgps/HTML5/section_8/8.6_closeout.htm.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

A Final Research Performance Progress Report (Final RPPR) must be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf.

PRIOR APPROVAL:
Requests which require the prior approval of the NEI must be submitted in writing to the Grants Management Specialist. All requests should reference the complete grant number and must be signed by the authorized official of the business office of the grantee organization and by the principal investigator.

ROLES & RESPONSIBILITIES:
If you need assistance from the National Eye Institute (NEI) during the course of this grant, please contact the grants management and program staff listed on the Notice of Grant Award (NGA). The telephone numbers of these individuals, as well as other extramural staff members of the NEI, can be located on the NEI web site, http://www.nei.nih.gov. ;;;; The grants management and program staff members work closely with one another through all phases of the project to facilitate the award and the administration of the grant. Their functions are defined as follows:

GRANTS MANAGEMENT CONTACT: The Grants Management Specialist is responsible for all business management matters associated with the review, negotiation, award, and administration of grants. Grants Management Specialists serve as the focal point for receiving and responding to all questions and correspondence related to business management and policy matters, such as correspondence giving or denying any prior approval required by Public Health Service (PHS) policy or special Terms and Conditions of Award, transfer of the grant to another institution, a change in the period of support, or any action which commits, or may result in committing the NEI to a change in the amount of funding.

PROGRAM CONTACT: The Program Director is responsible for all scientific and technical matters associated with the grant. The program official reviews and monitors scientific progress of the project and provides advice and assistance relative to all technical problems to ensure that

the scientific objectives of the research program can be pursued effectively and successfully. All questions or correspondence dealing with research progress, changes in research direction, unique scientific opportunities, or any other scientific needs should be addressed to the Program Director.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Chantell Stevenson-brown
Email: Chantell.Stevenson-brown@nih.gov **Phone**: 301-451-2020

Program Official: Ellen S Liberman
Email: esl@nei.nih.gov **Phone**: (301) 451-2020 **Fax**: 301-402-0528

SPREADSHEET SUMMARY
GRANT NUMBER: 1R21EY031120-01

INSTITUTION: Trustees of Indiana University

Budget	Year 1
TOTAL FEDERAL DC	$308,716
TOTAL FEDERAL F&A	$37,260
TOTAL COST	$345,976

Facilities and Administrative Costs	Year 1
F&A Cost Rate 1	58.5%
F&A Cost Base 1	$63,693
F&A Costs 1	$37,260

PI: **Meyer, Jason Stephen**	Title: Targeting the diversity of retinal ganglion cells for replacement therapy	
Received: 03/08/2019	FOA: EY19-001 Clinical Trial:Not Allowed	Council: 08/2019
Competition ID: FORMS-E	FOA Title: NEI Audacious Goals Initiative: Preliminary Studies for Translation-Enabling Models of the Visual System (R21 Clinical Trial Not Allowed)	
1 R21 EY031120-01	Dual:	Accession Number: 4282965
IPF: 577806	Organization: INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS	
Former Number:	Department:	
IRG/SRG: ZEY1 VSN (08)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 200,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
[redacted]	Legacy Emanuel Hospital and Health Center	MPI
Jason Meyer	Trustees of Indiana University	PD/PI

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 6030079020000

Legal Name*: Trustees of Indiana University
Department: Office of Research Administrat
Division:
Street1*: 980 Indiana Avenue
Street2: Lockefield Village, RM 2232
City*: INDIANAPOLIS
County:
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 462022915

Person to be contacted on matters involving this application

Prefix: First Name*: Steven Middle Name: Allen Last Name*: Martin Suffix:
Position/Title: Associate Vice President for Research Adminis
Street1*: 509 E. 3rd St.
Street2:
City*: Bloomington
County:
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 474013654
Phone Number*: 3172783473 Fax Number: 3172745932 Email: spon2@iupui.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-356001673-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Targeting the diversity of retinal ganglion cells for replacement therapy

12. PROPOSED PROJECT

Start Date* 08/01/2019
Ending Date* 07/31/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
IN-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Jason Middle Name: Stephen Last Name*: Meyer Suffix:
Position/Title: Associate Professor
Organization Name*: Trustees of Indiana University
Department:
Division:
Street1*:
Street2:
City*: Indianapolis
County:
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 462025191
Phone Number*: (317) 274-1040 Fax Number: Email*: meyerjas@iupui.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$345,339.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$345,339.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Steven Middle Name: Allen Last Name*: Martin Suffix:
Position/Title*: Associate Vice President for Research Adminis
Organization Name*: Trustees of Indiana University
Department: Office of Research Administrat
Division:
Street1*: 509 E 3rd St
Street2:
City*: Bloomington
County:
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 474013654
Phone Number*: 3172783473 Fax Number: 3172745932 Email*: spon2@iupui.edu

Signature of Authorized Representative*
Heike Marino

Date Signed*
03/08/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Trustees of Indiana University
Duns Number: 6030079020000
Street1*: [redacted]
Street2:
City*: INDIANAPOLIS
County: IN
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 462025191
Project/Performance Site Congressional District*: IN-007

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Legacy Emanuel Hospital and Health Center
DUNS Number: 0509730980000
Street1*: [redacted]
Street2:
City*: Portland
County:
State*: OR: Oregon
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 97232-2003
Project/Performance Site Congressional District*: OR-003

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ● No

IACUC Approval Date: 06-16-2017

Animal Welfare Assurance Number D16-00585

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract.pdf
8. Project Narrative*	Narrative.pdf
9. Bibliography & References Cited	References.pdf
10.Facilities & Other Resources	Facilities.pdf
11.Equipment	Equipment.pdf

ABSTRACT

Numerous retinal degenerative disorders are characterized by the loss of retinal ganglion cells (RGCs). As RGCs serve as the connection between the eye and the brain, the loss of these cells is often associated with a loss of vision. While neuroprotective strategies may help rescue vision at early stages of a disease, the subsequent loss of RGCs necessitates the development of strategies to functionally replaces those cells that have been lost. As the projection neurons of the visual system, however, efforts to replace RGCs have encountered numerous obstacles owing to their long-distance projections and need to establish precise synaptic contacts. As such, limited success has been achieved to date in efforts to either regenerate endogenous RGCs or to replace these cells with stem cell-derived RGCs. Although previous approaches to replace RGCs have largely focused upon the use of rodent models, numerous differences exist between rodent and primate retinas. Notably, the number and types of RGCs present within the retinas of the two species vary, with rodents having approximately twice the diversity of RGC subtypes. Importantly, these subtypes have been shown to exhibit differential susceptibility to RGC damage in degenerative disorders such as glaucoma. Also unlike the mouse or rat eye, the primate optic nerve head (ONH) has an elaborate collagenous lamina cribosa spanning the scleral canal, which is thought to be the primary site of RGC injury in glaucoma. Thus, novel approaches to cellular replacement need to account for these differences to better model the environment in the human retina and ONH. The macaque monkey non-human primate (NHP) glaucoma model results in highly reproducible damage to RGC axons and subsequent RGC loss. As such, the opportunity exists to further develop this system as a novel and powerful model to explore RGC replacement, including strategies that take into consideration the diversity of RGC subtypes. Human pluripotent stem cells (hPSCs) represent a virtually unlimited source of cells for the generation of RGCs for replacement purposes, with the recent demonstration of a variety of RGC subtypes derived from hPSCs. As such, the development of hPSC-based RGC cell replacement strategies can be customized to target those RGC subtypes preferentially affected by the degenerative process, resulting in a more robust engraftment of transplanted cells with greater axonal outgrowth and connectivity, with the goal of effectively restoring visual function.

Narrative

Damage and loss of retinal ganglion cells (RGCs) is characteristic of many disorders of the visual system, with loss of vision resulting from loss of RGC connectivity to the brain. Among the model systems utilized for the development of cellular replacement strategies to date, most studies have taken advantage of rodent systems despite significant differences with the primate retina including the number and types of RGCs as well as the presence of an elaborate collagenous lamina cribosa spanning the scleral canal in primates. As such, the development of RGC cell replacement strategies should include the development of animal models that more closely recapitulate the anatomy of the human retina, while also focusing upon the diversity of RGCs utilized for replacement purposes.

Facilities
Jason S. Meyer

The performance site of the proposal will be the School of Science of Purdue University located in Indianapolis, IN. In addition to the educational mission, the campus has a strong commitment to basic and translational research. All of the physical resources used by the PI and his research team are located [redacted] The Indianapolis campus of IU currently holds more than $300 million in research grants and contracts, including $133 million from the National Institutes of Health. In regards to intellectual resources, the campus is home to over 500 principal investigators and 49 different research centers/institutes. In summary, Indiana University provides an outstanding environment for the proposed research and will allow project outcomes to be met. Specific details of the environment are provided below.

Institutional Commitment to the PI

Dr. Meyer is a tenured Associate Professor in the Department of Biology, with adjunct appointments in the Department of Medical and Molecular Genetics, the Department of Ophthalmology, as well as the Stark Neuroscience Research Institute. Approximately 750 sq. ft. of laboratory space was assigned to Dr. Meyer by the Department of Biology, including distinct rooms for cell culture room and microscopy. His laboratory is well equipped for the studies proposed within the proposal, including supplies and equipment for cell culture. He has successfully obtained significant external funds to support his research, and has access to research cores and several pieces of shared departmental equipment (see *Equipment* section). Administrative and computing support is also provided by the department.

Resources:

- Flow Cytometry Core: This facility is located [redacted] Access is provided by appointment on a fee-for-service basis. Equipment includes several multichannel flow cytometers, two laminar-flow biocontainment cabinets for sample preparation, and four dedicated Apple computer workstations with flow cytometry analysis software (FlowJo and Cellquest). The facility is staffed by at least two technicians during normal business hours to assist investigators. Flow cytometer access is supplied on a fee-for-use basis.
- Preclinical Histology Core: This facility is located [redacted] They can provide fee-for-service histology support to the proposed research by providing paraffin embedding of tissues, preparation of slides for immunohistochemistry and H&E staining, and pathology consultation. The core employs a histotechnologist, project manager, and is overseen by a senior veterinary pathologist.
- *Indiana Center for Biological Microscopy:* This facility is located [redacted] laboratory. It houses several confocal microscopes in individual rooms optimized for microscope work. In addition to the scope systems, the facility has a 5.4 terabyte IBM Fileserver and two public workstations. Interactive 3D (voxel-based) image processing is performed using PCs equipped with 3.2 GHz Xeon processors, FireGL and Radeon-based video boards, and 6 to 8GB of memory.
- *Indiana University Center for Computational Biology and Bioinformatics:* This facility is located on campus in Indianapolis and provides services including microarray, next-gen sequencing, RNA-seq, etc. The expertise provided by the center can be instrumental for bioinformatics experiments designed to identify transcriptional similarities/differences in healthy and diseased retinal ganglion cells.

Facilities & Other Resources

Devers Eye Institute Research Laboratories,

is a full service state-of-the-art research facility within the contains 65,000 square feet of basic science laboratories as well as research facilities for outpatient and inpatient research. In addition, square foot area devoted to support of research studies. as a committed team of administrators to support all scientists with regulatory compliance oversight including IRB and IACUC, legal review coordination, IP-consultation, pre- and post-award management of grants, contracts and sponsored studies, and facilities support and management. Security staff assigned to are in the building and on the premises 24/7, and access to laboratory areas and the animal facility is by agenda is structured to bring the most advanced health care treatment modalities and cutting-edge technologies quickly on-line, where they can be applied to the benefit of our patients and community.

The esearch Laboratories, the research arm of Devers Eye Institute (DEI), which contains square feet of research space. This includes laboratory spaces for animal imaging, surgery, and basic science research, a necropsy suite, a cell culture area, a histology suite, as well as scientist and personnel offices. Currently, DEI employs a total of 7 active principal investigators with NIH- or CDC-supported grants, 3 research associates, 3 post-doctoral research fellows, research assistants and technicians all integrated into a highly productive team.

Animal Care: The building includes excellent facilities for the housing and maintenance of small animals including non-human primates. The and includes fully-equipped surgical suites, and holding and procedure rooms. The facility is fully staffed with a veterinarian and technicians, who provide housing, care, and feeding year round. Furthermore, the facility has full AAALAC accreditation and is in full compliance with both USPHS and USDA standards and practices. All facilities and care are designed to comply with Federal guidelines for the care and use of laboratory animals (DHEW publications #78-32 and updates, PHS #99-158 and updates, and assurance of Office for Protection from Research Risk – A-3094-01). A facility for housing and breeding of transgenic animals has recently been added. Finally, benefits from many ongoing interactions with the . With more than 6000 monkeys on site it is the supplier of the majority of monkeys studied at DIS and has been an excellent and reliable source of young and old animals.

Multi-Display Conference Facility: A 24-person conference room is available with three projectors, four remote-controlled video cameras and audio equipment to conduct remote conferences utilizing Citrix's GoTo Meeting software for up to 25 attendees simultaneously (25 different sites, 6 for video).

Core Facilities at

has a fully outfitted core imaging suite with the following equipment available to all investigators:

1) Leica TCS SPE-II confocal system on an inverted DMi8 research microscope with 10x, 20x, 40x, and 63x objectives. The system features four solid-state lasers (405nm/25mW, 488nm/10mW, 561nm/20mW, 635nm/18mW), a freely tunable spectrometer-detector with 5nm resolution, automated DIC prisms with transmission light detection, four filter cubes (DAPI, FITC, RHOD, Y5) and a Leica DFC365 FX 1.4mp CCD monochrome digital camera with temporal resolution of up to 21 fps. The motorized and software controlled stage contains inserts for glass slides, petri dishes up to 35mm or microtiter plates and is equipped with an adaptive focus control, an active focus control loop based on back reflection from cover glass. Leica's LAS X software with the advanced 3D visualization module is run on a dedicated high power HPZ420 workstation with 3.6GHz quad-core CPU, 1GB GPU, 8GB of RAM and is running windows 7 professional (64 bit) OS.

2) Leica DM RXE/TCS SPL confocal with 5x, 10x, 20x, 40x, and 63x objectives. The microscope and motorized x-y stage (ASI MS-2000) are driven by a dedicated workstation and stage-controller. The workstation is connected to the gigabit network for remote access of captured images/stacks. A separate

workstation outfitted with an NTSC color camera is also available to capture single frame images from the scope.

3) Leica DMIRBE (brightfield and fluorescence) inverted microscope with 10x, 20x, and 100x objectives. The microscope is outfitted with a 2750x2200, 14-bit CCD monochrome digital camera (QImaging Retiga 6000, QImaging Inc., Surrey, BC) and a motorized x-y-z stage (ASI MS-2000, Applied Scientific Instrumentation, Eugene, OR). Custom software has been developed to control the motorized stage and to acquire images from the digital camera. The software includes a custom autofocus routine which allows the system to acquire focused images at every pixel location, thus providing Enhanced Depth of Field capabilities to the imaging system. With this setup, large (>1200 images) composites can be captured unattended.

4) Leica DMLB upright Epifluorescence microscope with 2.5x, 5x, 10x, 20x, and 40x objectives and motorized x-y stage controlled by a dedicated imaging workstation.

Equipment
Jason S. Meyer

PI Laboratory: Approximately 750 sq. ft. of laboratory space was assigned to Dr. Meyer by the Department of Biology. His laboratory is well equipped for the studies proposed within the proposal, including supplies and equipment for cell culture including:

Dedicated 150 sq ft cell culture room containing:
- Two 6 ft. Baker laminar flow workstations
- Four 37 degree CO_2 incubators
- Two TS-100F cell culture microscopes with QImaging digital camera and Nikon Intensilight fluorescence

For cell and molecular biology studies for analysis of our experiments, the lab is equipped with:
- Two thermocyclers
- One PCR workstation
- Centrifuges
- Agarose gel electrophoresis systems
- Gel imaging station
- Incubator/Shaker for bacterial cultures
- Western blotting systems

For microscopy, a dedicated 70 sq ft dark room containing:
- Leica DM550B upright microscope system
- Hamamatsu Digital Camera
- Fluorescence filter cubes for DAPI, GFP, TxRed, and Cy5 excitation/emission
- PC computer running the LAS-Advance Fluorescence software with deconvolution.

For computer work, there are eight PCs in Dr. Meyer's laboratory for general laboratory purposes, including:
- Sequence analysis
- Microscope image acquisition and processing
- Database searching

Shared equipment nearby includes:
- Warm room (37 degrees)
- Cold room (4 degrees)
- Nanodrop 2000 spectrophotometer
- Autoclaves
- Liquid Nitrogen
- Optima Max-XP Ultracentrifuge
- Optima XPN-90 Ultracentrifuge
- Licor Odyssey CLx Scanner
- Leica Cryostat Microtome CM3050
- Roche Light Cycler Real Time PCR system
- Storm Phosphoimager
- Milli-Q ultrapure water system
- GE Typhoon FLA 7000 IP
- GE ImageQuant LAS 4000

The laboratory also has access to core facilities for confocal microscopy, flow cytometry, and bioinformatics.

Equipment – [redacted]

Devers Eye Institute, [redacted]: Laboratory equipment for in vivo imaging and functional measures includes: Spectral Domain OCT with fluorescence-capable confocal scanning laser ophthalmoscope (Spectralis™ HRA+OCT, Heidelberg Engineering, GmbH, Heidelberg, Germany); Spectralis™ HRA+OCT2, runs at >2x faster A-line rate than OCT1 (85kHz vs 40 kHz) and includes OCT-angiography mode; Scanning Laser Polarimeter (GDxVcc, Carl Zeiss Meditec, Inc., Dublin, CA); Heidelberg Retinal Tomograph II (Heidelberg Engineering, GmbH); Simultaneous-stereographic ocular fundus camera (3-Dx, NIDEK Co., Ltd.); Zeiss fundus camera with an OIS 2048x2048 CCD digital back; Topcon fundus camera; portable slit lamp biomicoscope (900, Haag Streit AG, Köniz, Switzerland) and laser slit lamp adapter for argon laser (for trabecular meshwork photocoagulation); LKC UTAS-E3000 (LKC Technologies, Gaithersburg, MD) used for all customized PERG and full-field flash ERG protocols; VERIS™ system (version 6; EDI, San Mateo, CA), used for all customized multifocal ERG protocols; Reichert Ocular Response Analyzer and calibrated Tonopen tonometers; Laser Speckle Blood Flowgraphy device; Anesthesia machines, vital signs monitors, infusion pumps; Corning medical blood gas analyzer; Hoppl Fibermatic ophthalmic surgical microscope with a 150 mm objective lens located in a dedicated surgery suite (250 square feet).

Laboratory equipment for in histopathology includes: sonicating baths; an IEC clinical tabletop centrifuge; Sakura Tissue-Tek VIP Tissue Processor and Tissue-Tek Tissue Embedding Console System (Sakura Finetek U.S.A., Inc., Torrance, CA); Microtomes (our lab) and Cryo-microtome (Legacy Research Core); The histology suite has a vented hood, solvent storage space, refridgerators and freezers (our lab) and -80°C freezer (Legacy Research Core).

Computer:

Shared Resources

The Devers Eye Institute [redacted] share central computational resources, which include:

1) A Dell PowerVault NX3100 server which shares over 65TB of online storage from five RAID-5 disc arrays connected through a dedicated gigabit Storage Area Network. This server is used as the central lab data repository where all images, data, and laboratory documents are stored for general lab access.
2) Dell PowerEdge 2950 database and intranet server running ColdFusion MX with MySQL database backend. This server stores and manages data collected in individual experiments and procedures. Data is made available to investigators in the form of an internal website.
3) A computational Linux server with of eight 1.5 GHz dual-core, 64-bit Itanium2 microprocessors and 32 GB of RAM used to process and serve three-dimensional data and to run computational finite element models.
4) A second Linux server with two dual-core 2.0 GHz AMD Opteron 64-bit processors and 16 GB RAM also used for processing data using Matlab and finite element software.
5) Two HP MSL4048 and two Tandberg Magnum 224 tape backup libraries with a total of 160TB storage capacity to perform nightly, weekly, and monthly data backups.
6) Gigabit Ethernet network throughout the main facilities to connect workstations and servers and to facilitate the exchange of large datasets.

The administrative computational capabilities of the lab include one graphics workstation outfitted with flatbed, film, and slide scanners, a network-enabled document scanner, and both color and monochrome workgroup laser printers. All acquired images and lab-related documents and data are stored on the shared 65 TB storage pool. Development tools such as Microsoft Visual Studio and Qt cross-platform development libraries are also available. A version control system is available and used by all software developers. All custom-developed software is distributed though an intranet website and deployed with internal version control features so that users automatically run the latest version of all custom software.

Software:

1) ONHLabAccess (custom application). This is a ColdFusion web application used to store all information about each experimental subject (e.g. demographics such as species, birthdate, age, weight, history), experimental details and running notes, as well as to track several data metrics in real time such

as intraocular pressure, optic nerve head topography information measured by confocal scanning laser tomography using the HRT-II (e.g. parameters such as MPD and TCA maps), RNFL thickness measured by SDOCT, SLP retardance measured by SLP and numerous ERG parameters (including the raw recordings).
2) Raindrop Geomagic (commercial application). This package is used to work with the delineated geometries to digitally "dissect" the surfaces to produce masks (contours) used to separate all delineated classes from the RGB images.
3) MathWorks Matlab 7 (commercial application). Used for custom application development.

Office:
The Principal Investigator, [redacted] and all key personnel have offices within the [redacted] Secretarial and administrative support is shared.

[redacted]**office** has an Apple MacPro (Early 2009) with two 2.25 GHz Quad-Core Intel Xeon processors, 16GB of RAM, 2TB of storage capacity, and a 30" display with software including: Microsoft Office 2011, Endnote.X5, Prism.v5, Matlab, ImageJ, GIMP, and custom software for 3D volumetric data set (e.g. SDOCT and confocal microscopy) visualization, segmentation and measurement.

[redacted]**Research Laboratory Office Space** has two desks and workstations, each outfitted with an Apple 21.5-inch iMac computer with 2.8 GHz Quad-Core Intel Corel7 processors, 32GB RAM and software including: Microsoft Office 2011, ImageJ, GIMP, and custom software for 3D volumetric data set (e.g. SDOCT and confocal microscopy) visualization, segmentation and measurement.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Jason Middle Name Stephen Last Name*: Meyer Suffix:

Position/Title*: Associate Professor
Organization Name*: Trustees of Indiana University
Department:
Division:
Street1*:
Street2:
City*: Indianapolis
County:
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 462025191

Phone Number*: (317) 274-1040 Fax Number:

E-Mail*: meyerjas@iupui.edu

Credential, e.g., agency login:

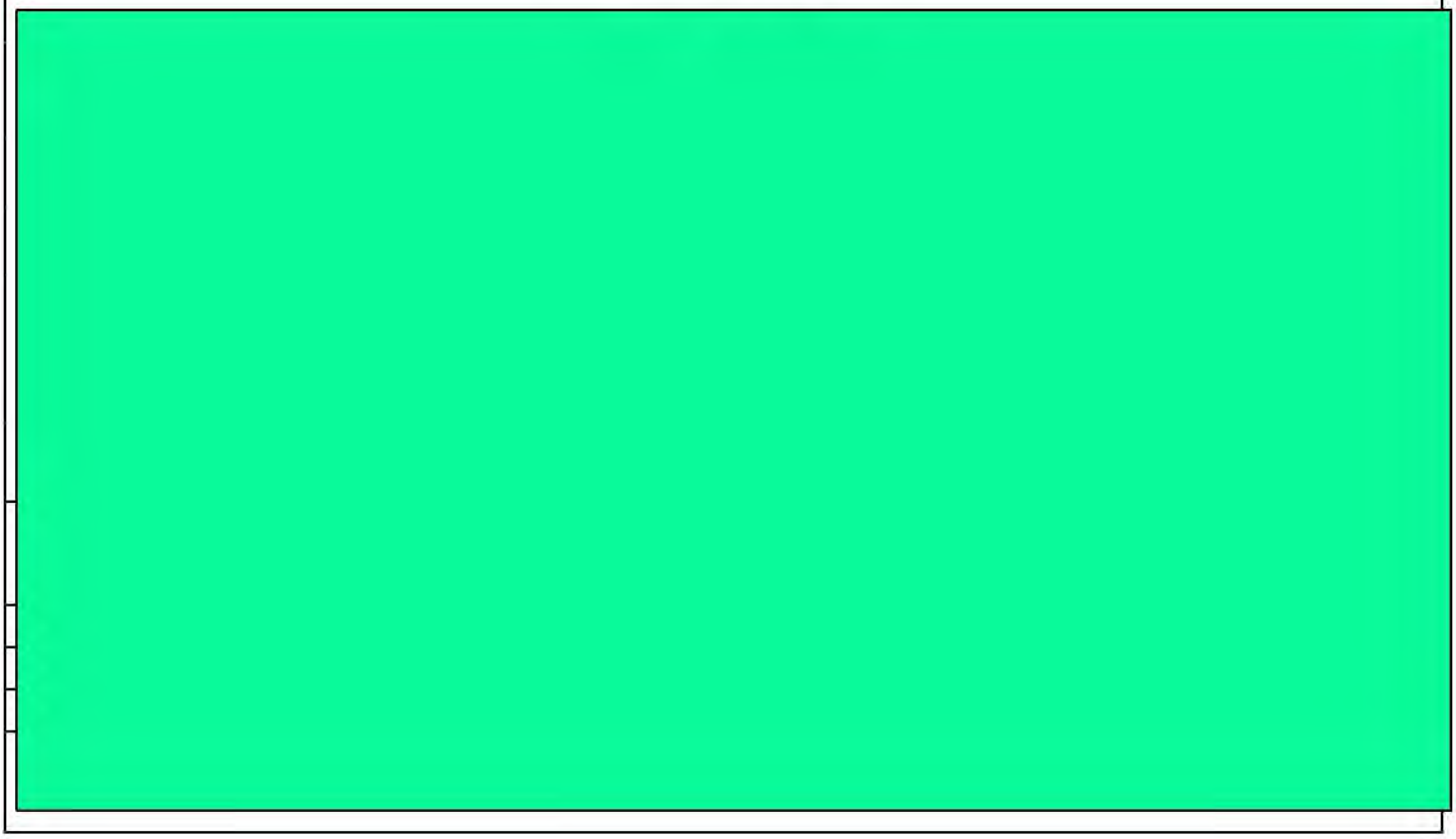
Project Role*: PD/PI Other Project Role Category:

Degree Type: PHD,BA Degree Year: 2004,1998

Attach Biographical Sketch*: File Name: Biosketch_Meyer.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person



BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jason S. Meyer

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Associate Professor of Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Colgate University, Hamilton NY	B.A.	05/1998	Biology
University of Missouri, Columbia MO	PhD	12/2004	Biological Sciences
University of Wisconsin, Madison WI	Postdoc	08/2010	Stem Cell Biology

A. Personal Statement

My research goals involve the ability of human pluripotent stem cells to faithfully recapitulate the development of the neural retina, as well as disorders affecting the visual system. My lab has considerable expertise in both maintaining and differentiating human induced pluripotent stem cells to retinal cells, including the organization of these cells into three-dimensional retinal organoid structures, with the identification and isolation of retinal ganglion cells as a natural application of these organoids. Beyond the differentiation of retinal ganglion cells, we have also demonstrated the great diversity of retinal ganglion cells that can be derived from human pluripotent stem cells, similar to the diversity previously observed in animal models. Furthermore, we have been among the first to successfully demonstrate the ability to model optic neuropathies with patient-derived induced pluripotent stem cells, along with the potential for drug development using these cells as a platform. These studies have resulted in numerous highly cited publications in prominent journals, as well as the funding of subsequent grant proposals to further our research goals. Given our track record of successful and impactful research projects, along with the expertise of the Fortune lab using a NHP model of glaucoma, the experiments proposed have a high likelihood for success.

1. Ohlemacher SK, Sridhar A, Xiao Y, Hochstetler AE, Sarfarazi M, Cummins TR, and **Meyer JS** (2016) Stepwise Differentiation of Retinal Ganglion Cells from Human Pluripotent Stem Cells Enables Analysis of Glaucomatous Neurodegeneration. *Stem Cells* 34(6), 1553-62.
2. Langer KB, Ohlemacher SK, Phillips MJ, Fligor CM, Jiang P, Gamm DM, **Meyer JS** (2018) Retinal Ganglion Cell Diversity and Subtype Specification from Human Pluripotent Stem Cells, *Stem Cell Reports* 10(4), 1282-1293. PMID 29576537.
3. Fligor CM, Langer KB, Sridhar A, Ren Y, Shields PK, Edler MC, Ohlemacher SK, Sluch VM, Zack DJ, Zhang C, Suter DM, **Meyer JS** (2018) Three-Dimensional Retinal Organoids Facilitate the Investigation of Retinal Ganglion Cell Development, Organization, and Neurite Outgrowth from Human Pluripotent Stem Cells, *Scientific Reports*, 8(1), 14520. PMID 30266927.
4. VanderWall KB, Vij R, Ohlemacher SK, Sridhar A, Fligor CM, Feder EM, Edler MC, Baucum AJ, Cummins TR, **Meyer JS** (2019), Astrocytes Regulate the Development and Maturation of Retinal Ganglion Cells Derived from Human Pluripotent Stem Cells, *Stem Cell Reports*, 12(2), 201-212. PMID 30639213.

B. Positions and Honors

Positions and Employment

2005-2007 Postdoctoral Research Associate, Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison WI. Sponsor: Su-Chun Zhang
2007-2008 Postdoctoral Research Associate, Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison WI. Sponsor: David Gamm
2008-2010 Assistant Scientist, Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison WI. Sponsor: David Gamm
2010-2016 Assistant Professor, Department of Biology, Indiana University Purdue University Indianapolis
2011-2016 Adjunct Assistant Professor, Department of Medical and Molecular Genetics, Indiana University
2011-present Primary Investigator, Stark Neurosciences Research Institute, Indiana University
2016-present Associate Professor, Department of Biology, Indiana University Purdue University Indianapolis
2016-present Adjunct Associate Professor, Department of Medical and Molecular Genetics, Indiana University
2018-present Adjunct Associate Professor, Department of Ophthalmology, Indiana University

Other Experience and Professional Memberships

2001-present Society for Neuroscience
2005-present International Society for Stem Cell Research
2007-present Association for Research in Vision and Ophthalmology
2014-present International Society for Eye Research
2012 Ad-hoc reviewer, Department of Defense Vision Research Panel
2012, 2016 Ad-hoc reviewer, New York State Stem Cell Initiative
2013 Ad-hoc reviewer, National Science Foundation, Integrative Organismal System section
2016 Participant, National Eye Institute Retina Organoid Challenge Competition Technical Planning Meeting
2016 Ad-hoc reviewer, National Eye Institute/NIH, Audacious Goals Initiative
2016 Participant, National Eye Institute Audacious Goals Initiative Workshop

Honors

2001-2002 Preparing Future Faculty fellow, University of Missouri
2001-2004 Graduate Assistance in Areas of National Need (GAANN) fellow, University of Missouri
2002 Society for Neuroscience/Eli Lilly & Co. Graduate Student Travel Award
2004 Keystone Symposia Travel Award
2011 ARVO-AFER/Merck Innovative Ophthalmology Award Winner
2012 Thomas R. Lee Award, American Health Assistance Foundation
2012 Prestigious External Award Recognition, Indiana University
2018 Trustee's Teaching Award, Indiana University
2018 Outstanding Graduate Mentor Award, Indiana University Purdue University Indianapolis

C. Contribution to Science

1. Pluripotent Stem Cells As An In Vitro Model Of Retinal Development

In recent years, I have made significant contributions to our understanding of how human pluripotent stem cells, including both human embryonic stem cells (hESCs) as well as human induced pluripotent stem cells (hiPSCs) can be effectively utilized as novel *in vitro* models of human retinogenesis. In particular, the differentiation of these cells to a retinal lineage occurs in a manner which closely recapitulates the known stages of retinal development, proceeding through stages analagous to the eye field, optic vesicle, optic cup, and eventually the primitive retina itself. Subsequent efforts have also demonstrated the capacity of these cells to self-organized into three-dimensional structures reminiscent of the developing retina.

1. **Meyer JS**, Shearer RL, Capowski EE, Wright LS, Wallace KA, McMillan EL, Zhang SC, and Gamm DM (2009) Modeling Early Retinal Development With Human Embryonic and Induced Pluripotent Stem Cells, *Proc Nat Acad Sci.* 106(39), 16698-703. PMID: 19706890.

2. Zhong X, Gutierrez C, Xue T, Hampton C, Vergara MN, Cao LH, Peters A, Park TS, Zambidis ET, **Meyer JS**, Gamm DM, Yau KW, and Canto-Soler MV (2014) Generation of Human iPSC-Derived 3-Dimensional Retinal Cups With Functional Photoreceptors. *Nature Communications*. 5, 4047. PMID: 24915161

3. Langer KB, Ohlemacher SK, Phillips MJ, Fligor CM, Jiang P, Gamm DM, **Meyer JS** (2018) Retinal Ganglion Cell Diversity and Subtype Specification from Human Pluripotent Stem Cells, *Stem Cell Reports* 10(4), 1282-1293. PMID 29576537.

4. Fligor CM, Langer KB, Sridhar A, Ren Y, Shields PK, Edler MC, Ohlemacher SK, Sluch VM, Zack DJ, Zhang C, Suter DM, **Meyer JS** (2018) Three-Dimensional Retinal Organoids Facilitate the Investigation of Retinal Ganglion Cell Development, Organization, and Neurite Outgrowth from Human Pluripotent Stem Cells, *Scientific Reports*, 8(1). 8(1), 14520. PMID 30266927.

2. Applications of Pluripotent Stem Cells for Modeling of Retinal Degenerative Disease

In additional studies, efforts have been focused on the ability of pluripotent stem cells to be utilized as a tool for the in vitro modeling of retinal degenerative diseases, as well as applications of these patient-derived stem cells for pharmacological screening. I have been among the first to successfully demonstrate the ability to utilized pluripotent stem cells for these purposes, including the ability to study mechanisms underlying degenerative disease and also utilize these cells as a platform for *in vitro* pharmacological screening of novel compounds.

1. **Meyer JS**, Howden SE, Wallace KA, Verhoeven AD, Wright LS, Capowski EE, Pinilla I, Martin JM, Tian S, Stewart R, Pattnaik B, Thomson JA, and Gamm DM (2011), Optic Vesicle-Like Structures Derived from Human Pluripotent Stem Cells Facilitate a Customized Approach to Retinal Disease Treatment. *Stem Cells* 29(8), 1206-18. PMID 21678528.

2. Ohlemacher SK, Sridhar A, Xiao Y, Hochstetler AE, Sarfarazi M, Cummins TR, and **Meyer JS** (2016) Stepwise Differentiation of Retinal Ganglion Cells from Human Pluripotent Stem Cells Enables Analysis of Glaucomatous Neurodegeneration. *Stem Cells* 34(6), 1553-62. PMID 26996528.

3. Development of Methods for Cellular Replacement in the Injured Retina

In my previous efforts, we have also focused upon the use of relevant animal models to serve as a platform to test the ability of differentiated pluripotent stem cells to readily incorporate into the degenerating retina. In such conditions, these cells have demonstrated the ability to adopt retinal-like phenotypes. Furthermore, I was among the first to demonstrate that the presence of these cells in the host retina confers a neuroprotective effect upon host neurons, thereby offering a means of rescuing the host retina from degeneration.

1. **Meyer JS**, Katz ML, Maruniak JA, Kirk MD (2004), Neural Differentiation of Mouse Embryonic Stem Cells In Vitro and After Transplantation Into Eyes of Mutant Mice With Rapid Retinal Degeneration. *Brain Research* 1014(1-2), 131-44. PMID 15212999.

2. **Meyer JS**, Katz ML, Kirk MD (2005), Stem Cells for Retinal Degenerative Disorders. *Ann N Y Acad Sci* 1049, 135-45. PMID 15965113.

3. **Meyer JS**, Katz ML, Maruniak JA, Kirk MD (2006), Embryonic Stem Cell-Derived Neural Progenitors Incorporate Into Degenerating Retina and Enhance Survival of Host Photoreceptors. *Stem Cells* 24(2), 274-83. PMID 16123383.

4. VanderWall KB, Wang S, and **Meyer JS** (2018), Differential Susceptibility of Retinal Ganglion Cell Subtypes Following Optic Nerve Crush. BioRxiv XXXXXX [Preprint] September 28, 2018. Available from https://www.biorxiv.org/content/early/2018/10/04/429282.

Complete List of Published Articles in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47644552/?sort=date&direction=descending

D. Research Support

Ongoing Rsearch Support

1R01EY024984 **Meyer** (PI) 12/1/2014-11/30/2019
NIH/NEI
Derivation and Disease Modeling of Human Stem Cell-Derived Retinal Ganglion Cells.

Role: Primary Investigator
Major Goals: To identify mechanisms underlying RGC differentiation from iPS cells and to explore their use for disease modeling of glaucoma.

Spinal Cord and Brain Injury Research Fund **Meyer** (PI) 07/01/2018-06/30/2020
Indiana Department of Health
Stem cell-mediated repair of the optic nerve following traumatic brain injury
Role: Primary Investigator
Major goals: To test the ability of hPSC-derived RGCs to functionally integrate into the retinal circuitry.

R01NS098772 Brustovetsky (PI), **Meyer** (Co-I) 04/01/2017-02/28/2022
NINDS/NIH
CRMP2, Mitochondria, and Huntington's Disease
Role: Co-Investigator (Nickolay Brustovetsky, PI)
Major goals: To establish CRMP2-mediated mechanisms contributing to defects of mitochondrial dynamics and cell death in human iPSC-derived neurons expressing mHtt

U24AG021886 Foroud (PI), **Meyer** (Co-I) 06/01/2018-05/31/2021
NIA/NIH
National Cell Repository for Alzheimer's Disease
Role: Co-Investigator (Tatiana Foroud, PI)
Major goals: To establish a centralized repository for the banking and distribution of induced pluripotent stem cells derived from Alzheimer's Disease patients

Completed Research Support

Spinal Cord and Brain Injury Fund **Meyer** (PI) 07/01/2016-06/30/2018
Indiana Department of Health
Overcoming barriers to optic nerve regeneration with human induced pluripotent stem cells

IU Collaborative Research Grant **Meyer**, Grant (Co-PIs) 06/1/16-05/31/17
Overcoming barriers to optic nerve regeneration

Page 106 of 195 to Page 110 of 195

Personnel

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Budget Period: 1

Start Date: 08/01/2019 End Date: 07/31/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	200,000.00
Consortium Indirect (F&A)	108,716.00
Total Direct Costs*	308,716.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	57.50	63,693.00	36,623.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, 214-767-3261

Indirect (F&A) Rate Agreement Date: 06/24/2015

Total Indirect (F&A) Costs: 36,623.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 345,339.00

Funding Opportunity Number: RFA-EY-19-001. Received Date: 2019-03-08T15:47:03.000-05:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	200,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	108,716.00
Section A, Total Direct Costs for Entire Project Period ($)	308,716.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	36,623.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	345,339.00

2. Budget Justifications

Personnel Justification	PersonnelJustification.pdf
Consortium Justification	ConsortiumJustification.pdf
Additional Narrative Justification	AdditionalJustification.pdf

Funding Opportunity Number: RFA-EY-19-001. Received Date: 2019-03-08T15:47:03.000-05:00

Personnel Justification

Jason S. Meyer, Ph.D., Co-Principal Investigator, will devote [redacted] academic months effort to the project. Dr. Meyer will be responsible for oversight of the project, experimental design, and communication between the Meyer and [redacted] labs.

[redacted] Graduate Student, will devote [redacted] months effort to the project. [redacted] will be responsible for the differentiation of RGCs and will coordinate the delivery of cells to the [redacted] lab. If needed, [redacted] will transport the cells to the [redacted] lab to ensure proper handling of cells for transplant. [redacted] will also oversee the histological examination of tissues post-transplantation.

Budget Justification
KEY PERSONNEL – LEGACY DEVERS EYE INSTITUTE, [redacted] LABORATORIES

[redacted]**D., Ph.D., Principal Investigator** (effort = [redacted] calendar months): [redacted] area of expertise is clinical and experimental electrophysiology of the visual system, as well as clinical imaging of the retina, retinal nerve fiber layer (RNFL) and optic nerve head by spectral domain optical coherence tomography (SDOCT), scanning laser polarimetry (SLP), confocal scanning laser tomography (CSLT) and other techniques. [redacted] particular emphasis for the past 20 years has been on human and experimental glaucoma, including pathophysiology and development of diagnostics. [redacted] has an integral role in many of the ongoing studies at the Devers Eye Institute, including development of clinical diagnostic tools in patient based research, electrophysiological assessment of vision function in animal models, and investigation of pathophysiological mechanisms in glaucoma. As Co-Principal Investigator for this project, [redacted] will hold primary responsibility for the overall conduct of in vivo data collection, generating experimental glaucoma in the nonhuman primates for Aim 2, animal welfare, all in vivo testing, data integrity, analysis and reporting. Although he will oversee the conduct of in vivo data collection, he will actively coordinate with Dr. Meyer and his team for the post mortem tissue preservation, allocation and evaluation. [redacted] will devote [redacted] of his time to this project. Therefore [redacted] of his salary is requested from this grant.

OTHER PERSONNEL – LEGACY DEVERS EYE INSTITUTE, [redacted] LABORATORIES

[redacted]

Legacy is requesting an estimated $269,000 total costs for the work associated with this project.

Additional Justification

Tuition costs and all subcontract dollars above $25,000 is excluded from indirect cost calculations.

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SpecificAims.pdf
3. Research Strategy*	ResearchPlan.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals2.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	MultiplePIPlan.pdf
8. Consortium/Contractual Arrangements	ConsortiumAgreement.pdf
9. Letters of Support	
10. Resource Sharing Plan(s)	ResourceSharingPlan.pdf
11. Authentication of Key Biological and/or Chemical Resources	AuthenticationofKeyBiologicalResources.pdf
Appendix	
12. Appendix	

SPECIFIC AIMS

Damage and loss of retinal ganglion cells (RGCs) is characteristic of many disorders of the visual system, with loss of vision resulting from loss of RGC connectivity to the brain[1-5]. Following the loss of endogenous RGCs, options for repair of the visual system and restoration of vision necessitate the development of cellular replacement strategies. Among the many considerations for cellular replacement is that a great diversity of RGC subtypes exists in the primate retina, with these subtypes being morphologically, phenotypically, and functionally unique[6-19]. Most RGCs belong to either the midget RGC, parasol RGC, or intrinsically photosensitive RGC (ip-RGC) classes, and these subtypes have been shown to exhibit differential susceptibility to RGC damage[20-25]. Thus, a need exists to develop a comprehensive strategy to not only replace RGCs, but also develop a customized approach that takes into account RGC diversity.

Among the model systems utilized for the development of cellular replacement strategies, most studies have taken advantage of rodent systems due to accessibility, the use of transgenic models, and the ability to obtain replicates in a relatively cost-effective manner. However, while there are similarities between rodent and human retinas, significant differences do exist[26-31]. Notably, the number and types of RGCs present within the retinas of the two species vary, with rodents having approximately twice the diversity of RGC subtypes. Also unlike the mouse or rat eye, the primate optic nerve head (ONH) has an elaborate collagenous lamina cribrosa spanning the scleral canal, which is thought to be the primary site of RGC injury in glaucoma. Thus, novel approaches to cellular replacement need to account for these differences to better model the environment in the human retina and ONH. The macaque monkey non-human primate (NHP) glaucoma model applied extensively by the Fortune lab results in highly reproducible damage to RGC axons and subsequent RGC loss[32-39]. As such, the opportunity exists to further develop this novel system as a model to explore RGC replacement.

Human pluripotent stem cells (hPSCs) have the potential to serve as a tool for cellular replacement, with previous studies demonstrating effective differentiation of hPSCs toward a retinal fate[40-55], including the detailed differentiation and enrichment of RGCs from hPSCs[49,56-69]. Studies from the Meyer lab have recently demonstrated the differentiation of a diverse number of RGCs from hPSCs[60], including those exhibiting features of midget, parasol, and ip-RGCs. As such, the development of hPSC-based RGC replacement strategies can now be customized to target those RGC subtypes that most readily survive and integrate into the retina, leading to strategies to target those subtypes with greater susceptibility to degeneration.

The replacement of RGCs following injury or disease is indeed an audacious goal, with numerous obstacles potentially hindering the success of RGC replacement efforts. Thus, the pursuit of RGC replacement strategies should benefit from the approach we take here to exploit a novel and audacious strategy to enhance RGC transplantation, survival, and integration. As detailed in this proposal, our *specific aims intend to focus upon the diversity of RGCs present within the retina for the development of targeted replacement strategies for specific RGC subtypes in a non-human primate model of glaucoma.* In so doing, these aims will establish the feasibility of hPSC-derived RGCs as an effective tool for the replacement of RGCs following damage and loss due to optic neuropathies, establishing the strong justification for the future replacement of those RGC subtypes that preferentially integrate in normal and glaucomatous retina.

Aim 1. To establish the feasibility of transplantation of hPSC-derived RGCs into the NHP retina. RGCs will be differentiated from hPSCs, purified and transplanted into the vitreous of healthy macaque eyes. Survival and integration of hPSC-derived RGCs will be assessed along with safety through comprehensive electroretinography (ERG), Optical Coherence Tomography (OCT) and Confocal Scanning Laser Ophthalmoscopy (CSLO) imaging. Macaques will be sacrificed 4 months after transplant, eyes enucleated, retinas analyzed to determine survival and integration of transplanted RGCs as well as which subtype(s) had most efficiently integrated.

Aim 2. To identify hPSC-derived RGC subtypes that more efficiently integrate in the glaucomatous retina. The unilateral experimental glaucoma (EG) model in the macaque will be induced and monitored as previously described[32-39]. After confirmation of RGC loss by OCT (~2 months after induction), hPSC-derived RGCs will be transplanted into the EG eye of each NHP. Longitudinal assessment in vivo by ERG, OCT and SLO will continue for 4 months, followed by comprehensive post mortem analysis of RGC integration by subtype.

This proposal is significant because to date, advances in RGC replacement have been limited. Feasibility will be powerfully supported by evidence of successful integration in a larger animal disease model. This proposal brings together experts in complementary fields of research essential for the success of this audacious project. Through these aims, powerful new approaches will be developed to identify those RGC subtypes that can most efficiently survive and integrate, with the potential to enhance vision restoration for optic neuropathies.

SIGNIFICANCE: Numerous degenerative disorders adversely affect the ganglion cells of the retina, with injury leading to cell death, axon degeneration and consequential vision impairment or blindness[1-5]. While early intervention strategies are often focused upon the neuroprotection of these cells, a critical need exists for cellular replacement strategies at later stages of these degenerative diseases once a large number of RGCs have been lost[69-72]. However, these efforts have been largely restricted to date due to the difficulty in deriving sufficient numbers of RGCs for replacement, as well as limitations with existing models for optic neuropathies that do not accurately represent the environment found within the degenerative human retina. These previous studies have often made use of rodent models which do not reflect the diversity of RGCs found within the primate retina, while the rodent retina also lacks an elaborate collagenous lamina cribrosa spanning the scleral canal, which is thought to be the primary site of RGC injury in glaucoma. Thus, efforts to develop strategies for the replacement of RGCs should take into consideration these significant differences. The macaque monkey retina closely mirrors the architecture and composition of the human retina, and the Fortune lab has extensive experience using a powerful glaucoma model the rhesus macaque that more closely recapitulates the loss of RGCs in human patients[32-39]. As such, opportunities now exist to leverage this NHP glaucoma model as a system to develop RGC replacement therapies that are more applicable to human patients. Additionally, this proposal takes advantage of the ability to derive unlimited numbers of RGCs from human pluripotent stem cells (hPSCs) as described by the Meyer lab and others[49,56-69], particularly the ability to derive a diverse set of RGCs as the Meyer lab has recently demonstrated[60]. ***The significance of this proposal derives from its synergistic combination of using hPSC-derived RGCs for cell replacement and a physiologically relevant NHP glaucoma model, bolstered by the ability to exploit a diversity of RGC subtypes to determine which will more efficiently replace degenerated RGCs***. Through a series of transplantation experiments into healthy and glaucomatous eyes, we will assess the ability of specific subtypes of hPSC-derived RGCs to preferentially survive and integrate into the macaque retina. The successful completion of these aims will lead to the development of novel strategies for cellular replacement targeting those RGC subtypes that can more readily repopulate and repair the retina following RGC loss in glaucoma.

INNOVATION: The replacement of RGCs necessitates innovative approaches to both the derivation of replacement cells as well as the application of these cells to a physiologically relevant degeneration model. In this proposal, we describe ***several innovative approaches*** that will greatly advance the field of RGC cellular replacement and bring us closer to visual system repair using stem cell-derived RGCs.

1. **Our study focuses upon the diversity of RGC subtypes in which some classes may more readily survive and integrate into the degenerative retina.** Previous studies have focused upon the attempts to replace RGCs particularly in rodent models[69-72], yet no replacement strategies to date have targeted the diversity of RGCs for the establishment of cellular replacement therapies. The Meyer lab has pioneered the derivation of RGCs from hPSCs[49,56,60,68], including the recent demonstration of a myriad of diverse RGC phenotypes derived from hPSCs[60]. The transplantation of these diverse RGC populations will elucidate which subtypes of RGCs most readily survive and integrate into the retina, allowing for the future development of targeted approaches to retinal repair in which cellular replacement is focused upon those subtypes that will more readily replace those cells that have been lost.
2. **We apply a powerful non-human primate glaucoma model for prospective replacement of RGCs.** Numerous significant differences exist between rodent models and the glaucomatous human retina[26-31], underscoring the need for a more physiologically relevant model for the development of RGC replacement approaches. The Fortune lab has pioneered the development of a macaque non-human primate model of glaucoma that closely recapitulates the phenotypes observed in human patients[32-39]. As such, the experiments proposed represent the most physiologically-relevant attempt at RGC replacement.
3. **Our approaches involve a comprehensive analysis of visual restoration following transplantation and integration of hPSC-derived RGCs.** A multitude of functional analyses will be employed to test for the functional integration of transplanted hPSC-derived RGCs, including electroretinography (ERG), OCT and SLO imaging. Dr. Fortune has extensive experience with these structural and functional analyses in the non-human primate macaque model[32-39].
4. **Our experienced, interdisciplinary team represents a dynamic combination of motivated scientists with complementary fields of expertise necessary to achieve the Aims of this audacious project.** The successful differentiation, transplantation, integration, and functional assessment of hPSC-derived RGCs is indeed an audacious goal, with tremendous implications for the development of strategies to treat or cure optic neuropathies in the future. We expect to learn which RGCs subtypes exhibit preferential survival and integration in healthy and glaucomatous retina and to provide critical evidence of feasibility for the approach that will inform subsequent proposals aiming to refine and extend the approach.

Aim 1. To establish the feasibility of transplantation of hPSC-derived RGCs into the NHP retina.

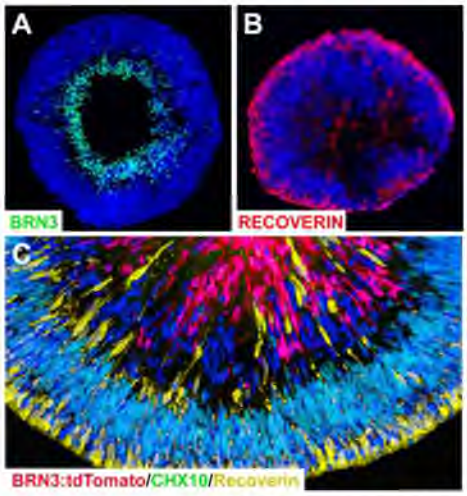


Figure 1. Retinal organoids recapitulate the temporal and spatial patterning of the retina. Retinal ganglion cells are organized within inner layers, while photoreceptors are found within outer layers of retinal organoids.

Hypothesis: The transplantation of hPSC-derived RGCs into the macaque retina allows for the analysis of RGC survival and integration in a physiologically relevant NHP model.

Background and Rationale: The replacement of RGCs is an audacious goal, with many factors influencing the survival and integration of cells following transplantation. To date, a number of models have been used to test the ability of transplanted cells to engraft, with most of these studies focused upon the use of rodent models for RGC replacement. While rodent models offer a number of advantages, significant differences exist between the rodent retina and that of primates, including humans. In particular, the study of glaucomatous neurodegeneration, as well as efforts to replace RGCs subsequent to this degeneration, is complicated by many of the structural differences between species, notably the presence of an elaborate lamina cribrosa in primates that is thought to be the primary site of glaucomatous damage. Thus, in order to accurately reflect the challenges associated with RGC replacement for human patients, and critically extend evidence of feasibility, the replacement approach should also be proven in an animal model that more closely resembles the human eye and recapitulates the human disease. The rhesus macaque (*Macaca mulatta*) represents a non-human primate (NHP) whose retina and optic nerve head are highly similar to that of humans, including a collagenous lamina cribrosa through which RGC axons extend to connect with postsynaptic targets in the brain. The Fortune lab has extensive experience with macaque NHP models of RGC injury, including the unilateral experimental glaucoma (EG) model and the array of tools applied in this proposal to test the feasibility of RGC replacement.

Among the differences between rodents and primates, the diversity of RGCs within the retina represents another significant difference, with rodents possessing approximately twice as many subtypes of RGCs compared to the primate retina. Additionally, for many of the subtypes found within the primate retina (e.g. midget RGCs, parasol RGCs, etc), an analogous subtype does not exist in rodent models. Thus, the use of the macaque model would also allow for the consideration of RGC subtype variability in a model that more closely represents the human retina. The diversity of RGCs for cellular replacement can be experimentally manifested in multiple ways, including the study of those RGC subtypes that preferentially survive and integrate into the retina upon transplantation. The experimental identification of those subtypes that lead to greater engraftment into the retina than others would facilitate and refine approaches to RGC replacement, with a focus upon the enrichment of RGCs for those subtypes that are more likely to contribute to the host retina. While this is a novel concept for RGC replacement, a strong precedent for this type of approach exists within the retina research community as previous studies have demonstrated preferential engraftment of certain photoreceptor populations into the retina following transplantation[73,74]. In the future, the targeted replacement of individual RGC subtypes can also be expanded to more accurately replace those subtypes that are lost in the disease state, as previous studies have indicated that unique subtypes may have different responses to glaucomatous injury with some subtypes more susceptible to damage and loss than others[20-25].

hPSCs represent an attractive option for the derivation of RGCs for replacement approaches, as these cells can be derived in unlimited quantities and have the potential to be derived from autologous sources[75-79]. Numerous previous studies have demonstrated the ability to differentiate hPSCs into retinal cells[40-55], and the Meyer lab has significant expertise in the derivation of RGCs from hPSCs[49,56,60,68]. In this process, RGCs are derived through the initial differentiation of hPSCs into retinal organoids[40,45,47,54-56], which closely approximate the spatial and temporal patterning of the human retina (Figure 1). Subsequent to the differentiation of retinal organoids, efforts can be made to isolate and enrich for RGCs (Figure 2). Upon isolation, these cells exhibit numerous morphological features of RGCs including the extension of numerous and complex neurites and the expression of characteristic markers. As such, the goals of this first aim are to examine the ability of hPSC-derived RGCs to survive and integrate into the healthy macaque retina, with these results establishing the optimal parameters to explore RGC replacement in glaucomatous conditions in Aim 2. Additionally, given the short timeframe associated with this award, these strategies also allow for the optimization of transplantation approaches while glaucoma is experimentally induced in another cohort of macaques in Aim 2.

Experimental Plan:

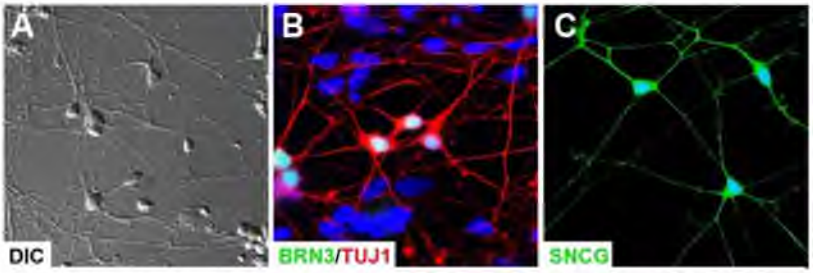


Figure 2. Derivation of RGCs from hPSCs. (A) RGCs exhibit elaborate morphologies including the extension of lengthy neurites. **(B-C)** hPSC-derived RGCs express characteristic RGC markers including BRN3 and SNCG.

RGC differentiation from hPSCs: For these studies, hPSCs from both male and female sources (miPS2[52] and IMR90-4[77], respectively), with both cell lines previously engineered to express a BRN3:tdTomato:Thy1.2 RGC reporter, as previously described[63]. Male cells will be used for transplant into female donors, and female cells transplanted into male donors to aid in identification of donor cells after transplant. Differentiation will be accomplished following previously established protocols which proceed through an optic cup-like retinal organoid stage in order to derive RGCs[48]. Initially, cultures of hPSCs are induced to differentiate via the formation of embryoid bodies and transitioned into a Neural Induction Medium (NIM) consisting of DMEM/F12 (1:1) with 1% N2 supplement and heparin (2 μg/ml). Following 7 days of differentiation in NIM, embryoid bodies are plated onto a laminin-coated substrate and maintained in NIM for an additional 9 days. At this point, retinal organoids are mechanically lifted and grown in suspension culture in Retinal Differentiation Medium (RDM), consisting of DMEM/F12 (3:1) with 2% B27 supplement and 1x PSA. Organoids will then be maintained until a total of 50 days of differentiation is reached, at which point organoids will be shipped to the Fortune lab for RGC isolation and transplantation. RGC isolation will be accomplished in consultation with the Meyer lab, and retinal organoids will be enzymatically dissociated using Accutase. The resulting single cell suspension will be sorted using Magnetic Activated Cell Sorting (MACS) techniques to isolate RGCs based on the expression of the Thy1.2 antigen, with these cells also identifiable by the expression of tdTomato, as previously described[63,68]. Following MACS enrichment, cells will be resuspended in saline at a concentration of 50,000 cells/ul for subsequent transplantation as outlined below.

Intravitreal injection of hPSC-derived RGCs: A total 3 rhesus monkeys will be used in this aim, including both sexes, with age ranging from 4-18 years. Intravitreal injection of hPSC-derived RGCs will be performed using sterile surgical procedure. Anesthesia will be induced with ketamine (10-40 mg/kg M) and xylazine (0.2-0.5mg/kg IM) and maintained with isoflurane (0.75-5% ET inhalation). After sedation, the animal will be placed prone with surgical drape exposing only the area around the eye. Topical anesthetic will be applied to the ocular surface (0.5% proparacaine), then a 5% solution of povidone-iodine will be applied to the lid margins and lashes using a cotton tip applicator and drops applied to the ocular surface. A sterile lid speculum will be inserted and additional povidone-iodine 5% drops are applied over the injection site (to pass through the superior temporal pars plana, 3.5 mm posterior to the limbus). The injection will be made using a 30-gauge needle into the middle of the vitreous cavity. 50 μl of cell suspension prepared as described above at a concentration of 50,000 cells/μl will be injected over one minute, with the needle held in position for an additional minute to avoid reflux. The needle will then be slowly extracted and a sterile cotton swab is immediately placed over the injection site to prevent reflux. The eye will then be dressed with a broad-spectrum antibiotic ointment and the animal allowed to recover from sedation. Subconjunctival injections of 125mg/1ml cefuroxime and 10mg/1ml dexamethasone will be delivered to each eye, and erythromycin ophthalmic ointment will be applied topically. Animals will receive topical steroid (prednisolone1%) and antibiotic (ofloxacin0.3%) eyedrops in both eyes twice daily for 5 days starting on postoperative day1. Animal will receive oral cyclosporine at 35mg/kg for three days preoperatively and then continuously for two weeks.

Evaluation: The engraftment of cells will be analyzed every two weeks for 4 months after transplantation of hPSC-derived RGCs. Longitudinal retinal imaging in vivo will include SD-OCT and CSLO, as previously described in detail[35,39,80,81] (see also Vertebrate Animals section for detailed description of protocols). In brief, a comprehensive array of SD-OCT anatomical measures of RGC integrity will be monitored in transplanted and fellow control eyes[33,35]. Also during each bi-weekly session, CSLO imaging will be used to detect the presence and distribution of transplanted cells, with previous studies showing that individual RGCs can be resolved and counted over time in vivo using this method[80] (unpublished data also in NHP). In addition to imaging of the retina post-transplantation, multifocal ERG (mfERG), pattern ERG (PERG) and full-field ERG recordings (as previously described in detail[34,38,39,82]) will be obtained at 1, 2, and 4 months post-transplant to determine if the integration of transplanted cells leads to any changes in visual function. This battery of imaging and ERG testing also serves to assess overall retinal safety, layer-by-layer, longitudinally in vivo.

Histology:
Four months after RGC transplantation, macaques will be euthanized by sodium pentobarbital overdose, followed by perfusion fixation using cold 4% paraformaldehyde. Eyes will be enucleated, and a cut will be made in the cornea to facilitate additional fixative penetration. The optic nerves and brain will be collected and further post-fixed in 4% paraformaldehyde in phosphate buffer for 2 hours, while the brain tissue will be fixed overnight at 4°C. Eyes, brain and optic nerves will be infiltrated with 10%, 20% and 30% sucrose for 2 hours at each concentration, then kept in 30% sucrose overnight at 4°C. The next day, tissue will be embedded in OCT for cryostat sections, and tissue blocks will be shipped to the Meyer lab for further analysis. Cryostat sections of retinal and optic nerve tissue will be cut at 10µm for histology and immunofluorescence. Both retinal and optic nerve sections will be analyzed to check overall survival and graft distribution based on expression of the tdTomato fluorescent reporter, with an analysis focused upon the degree of RGC integration into the retina. Integration of grafted hPSC-derived RGCs within the host retina will be analyzed by double-staining with retinal cell markers for bipolar cells (PKCα), amacrine cells (calretinin, parvalbumin) and synaptophysin (for presumptive synaptic contacts in the inner plexiform layer). If grafted donor cells are found in the optic nerve, measurements will be taken to determine the length and relative abundance of these hPSC-derived RGC axons. Furthermore, brain sections will be cut and stained with human specific antibodies on sections containing optic nerve tract, dorsal lateral geniculate nucleus and superior colliculus to examine whether grafted RGCs extend its axons to its target area based on the expression of the tdTomato reporter.

Expectations/Alternatives/Pitfalls:
The experiments outlined in this aim are all based upon the strengths of the collaborating labs, so no significant procedural hurdles are expected. Initially, cells differentiated in the Meyer lab will be transported to the Fortune lab by [redacted] and she will be responsible for cell preparation for transplant. It is expected that the Fortune lab members will learn how to prepare cells for future experiments so that cells may be shipped. However, if needed, [redacted] will transport cells and perform cell preparation as needed. After transplant, it is possible that issues with graft survival may occur and if so, subsequent experiments can be supplemented with the addition of RGC survival factors such as BDNF in the transplantation. It is also possible that transplanted cells may survive but not integrate into the retina, especially because the inner limiting membrane would still be intact. If a lack of integration occurs, analysis would focus upon the degree of survival of transplanted cells along the retinal surface. Finally, if integration does appear to occur, the expression of the tdTomato reporter may indicate cytoplasmic transfer rather than integration of donor cells. To explore this possibility, tdTomato cells will be analyzed for the expression of X and Y chromosome markers; as an example, male cells transplanted into female hosts should express tdTomato as well as both X and Y chromosomes. An XX result would indicate transfer of the tdTomato reporter to host cells.

Aim 2. To identify those hPSC-derived RGC subtypes that more efficiently integrate in the glaucomatous retina.

Hypothesis: Survival and integration of hPSC-derived RGCs will occur in a subtype-dependent manner, with some subtypes more readily integrating into the host than others.

Background and Rationale: As the macaque model offers numerous similarities to the human retina[26,27,30], it can also serve as an effective model for glaucomatous neurodegeneration in response to elevated intraocular pressure. Given the structural and anatomical similarities to the human retina, studies of glaucoma in this model are more physiologically relevant and similar to the course and character of damage in human glaucoma patients. The Fortune lab has extensive experience in the induction and assessment of experimental glaucoma in the macaque, achieved through the laser photocoagulation of the trabecular meshwork resulting in the gradual and moderate elevation of intraocular pressure[32-39]. This approach results in a slow damage to RGCs, including a thinning of the nerve fiber layer, loss of RGC cell bodies and loss of axons from the optic nerve (Figure 3).

The diversity of RGCs within the primate retina allows for unique opportunities to craft customized therapeutic approaches to cellular replacement. Among the most promising of these opportunities is the ability to identify those subtypes of RGCs that are most amenable to transplantation and integration within the host retina. Initial efforts have focused upon the diversity of RGCs derived from hPSCs, with the expression of several markers indicating the presence of cells resembling three of the most prominent subtypes in the primate retina, including midget RGCs, parasol RGCs, and ipRGCs (Figure 4). Thus, the use of hPSC-derived RGCs for transplantation can not only focus upon the generic ability of these cells to replace those that have been lost in the host retina, but these approaches can also be refined to identify those subtypes that demonstrate an increased ability to survive and integrate into the glaucomatous host retina. In future studies, this diversity can

also be leveraged to target the replacement of specific subtypes that are more susceptible to degeneration with hPSC-derived replacements of a similar phenotype.

Experimental Plan:

Induction of glaucoma in macaque eyes: Induction of glaucoma will be initiated as previously described by Dr. Fortune[32-39]. Three macaques will be used for this aim, including both male and female subjects. Prior to the initiation of unilateral experimental glaucoma (EG), each animal will undergo baseline testing by spectral domain OCT (SD-OCT) and by three modes of ERG 3-5 times each. One eye of each animal will then be randomly assigned to have induction of EG, which is initiated by application of laser photocoagulation to the trabecular meshwork to induce chronic, mild-to-moderate elevation of intraocular pressure (IOP). Initially, 180° of the trabecular meshwork will be treated in one session, then the remaining 180° will be treated in a second session approximately two weeks later. If necessary, laser treatments will be repeated in subsequent weeks (limited to a 90° sector) until an IOP elevation is noted or if the initial post-laser IOP increase has returned to normal levels. The average number of laser treatments (± SD) for the Fortune lab is 5.2 ± 2.6. For each animal, the contralateral eye will serve as a control. IOP will be measured in both eyes at the start of every session using a Tonopen XL (Reichert Technologies, Inc., Depew, NY). The value recorded for each eye will be the average of three successive measurements. Testing by SD-OCT and ERG will continue on alternating weeks for each animal until its pre-defined study target has been reached; for these experiments, that will be 10% loss of baseline retinal nerve fiber thickness, corresponding to 20-25% loss of retinal ganglion cell axons from the anterior orbital optic nerve, which lasts approximately 6 months, on average, from the start of laser procedures to induce EG.

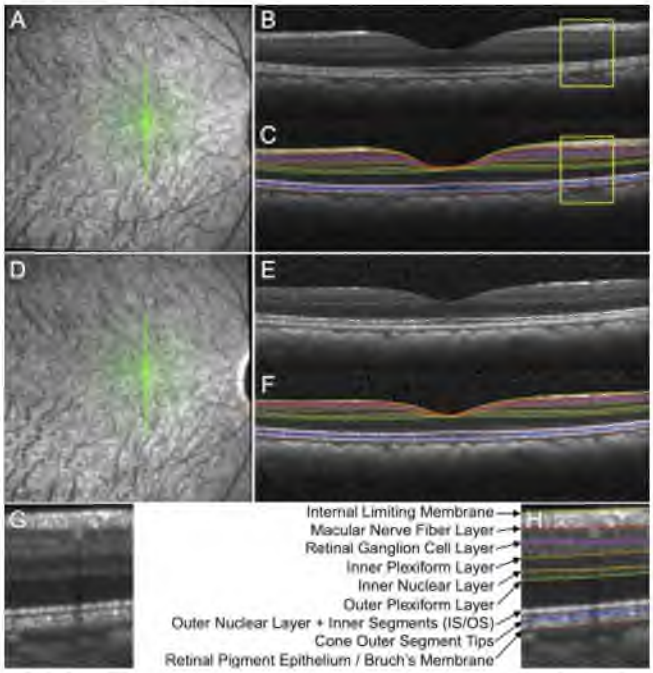


Figure 3. Spectral domain OCT imaging from IOP elevated macaque glaucoma model. Baseline measurements were made before the induction of glaucoma in A-C. Following induction of glaucoma (D-F), obvious thinning of retinal layers is observed.

RGC differentiation from hPSCs: hPSCs (cell lines miPS2[52] and IMR90-4[77]) will be differentiated to a retinal fate and RGCs isolated as described above. For transplantation purposes, cells will be concentrated to 50,000 cells/ul resuspended in saline. To establish a baseline for the diversity of RGCs used in transplantation experiments, any cells leftover after transplantation experiments will be plated onto Poly-D-Ornithine and Laminin-coated dishes at a concentration of 50,000 cells/ml and grown in BrainPhys medium (StemCell Technologies) for an additional 2 weeks to allow for further maturation of these cells. At this point, cells will be fixed with 4% paraformaldehyde and immunostained with antibodies against markers identifying each of the major subtypes of the primate retina, including OPN4 for ipRGCs, SPP1 for parasol RGCs, and MEIS2 for midget RGCs. OPN4 has long been associated with ipRGCs as the photopigment underlying the ability of these cells to transduce light information. Recent studies from the Sanes lab in primate models has identified SPP1 as a marker for primate parasol RGCs, and MEIS2 is expressed by OFF-RGCs. Given that our preliminary data indicates widespread expression of MEIS2 in hPSC-derived RGCs that is apparently expressed in populations distinct from SPP1, those MEIS2-positive, SPP1-negative cells are likely to represent a set of midget RGCs. From these experiments, the percentage of each of these presumptive subtypes in hPSC-derived RGC populations will be quantified and subsequently compared to similarly quantified results from cells surviving transplantation to identify those presumptive subtypes capable of enhanced survival and integration into the retina. Quantified data will be analyzed for significant differences using a student's t-test with significance determined at $p < 0.05$.

Functional analysis of hPSC-derived RGC transplants: To study the ability of transplanted hPSC-derived RGCs to contribute to the host retina and potentially lead to functional improvements, several assays will be performed. Cellular contribution to the host retina will be assessed via OCT and CSLO imaging to assess changes in the structure of the retina resulting from cell integration. Any changes in visual responses will similarly be assessed by multifocal ERG as described above, with contralateral eyes serving as controls as well as historic data from prior untreated glaucomatous eyes. Assays will be performed weekly for 4 months at which point animals will be sacrificed for histological analysis.

Euthanasia and Histological Analysis: At four months after cell injection, macaques will be sacrificed as described above and tissue will be harvested, fixed in 4% paraformaldehyde, embedded in OCT and shipped to the Meyer lab for histological analysis. Retinas, optic nerves, and brain tissue will be analyzed for the integration of hPSC-derived RGCs. Donor cells will be visualized by tdTomato expression in all three tissues, and the presumptive RGC subtypes will be assessed in retinal sections with antibodies against OPN4, SPP1, and MEIS2. The percentage of cells surviving transplant and integrating into the retina will be quantified and compared to the total number of cells that were initially transplanted. Subsequently, the relative distribution of presumptive RGC subtypes surviving and integrating into the retina will be quantified and compared to the percentages of these presumptive subtypes in those cells grown in vitro. Differences in the percentage of these subtypes between in vitro and in vivo conditions will be assessed by student's t-test with significant differences determined at $p < 0.05$. Additionally, the ability of donor RGCs to extend axons into the optic nerve and form presumptive contacts with target tissue will be assessed through an analysis of these tissues for the expression of the tdTomato reporter. The length of axons extending into the optic nerve will be compared to similar experiments in healthy animals described in Aim 1, and the potential for enhanced axonal extension into the optic nerve determined by student's t-test of axonal lengths, with $p < 0.05$ indicating significant differences.

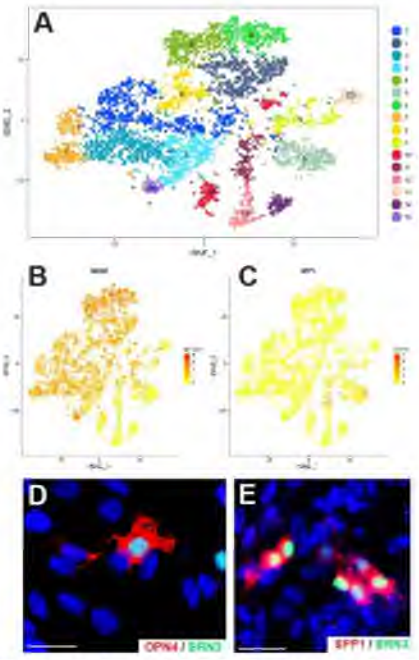


Figure 4. hPSC-derived RGC cellular diversity. (A) t-SNE plot indicates 17 distinct cellular populations. **(B-C)** MEIS2 and SPP1, indicative of midget and parasol RGCs, respectively, are expressed in distinct populations. **(D-E)** Immuno-staining for RGC subtype-associated markers.

Expectations/Alternatives/Pitfalls: Success of this aim will provide the proof of principle that the diversity of RGCs can be leveraged to enhance RGC integration and replacement based on those subtypes that are more capable of survival and integration. The experiments presented within this aim are all built upon the complementary expertise of the two PIs, and as such there is a strong potential for the success of these approaches, with important implications for future success in RGC replacement in this model as part of subsequent Audacious Goals Initiatives. However, there is a possibility that some approaches will lead to results that were not necessarily expected. For example, the use of OCT and CSLO imaging may indicate a reduced thinning of the nerve fiber layer that due to integrated donor cells. The possibility exists that part of this effect could also be due to some degree of neuroprotection by the donor cells. This possibility will be assessed by quantification of the area of the nerve fiber layer excluding the tdTomato-expressing cells, and compared to controls. Additionally, in the analysis of axon growth into the optic nerve toward postsynaptic targets, these axons will not likely be associated with presumptive subtypes as SPP1 and MEIS2 are localized within the cell body and nucleus, respectively. In these studies, growth into the optic nerve will assess the potential for enhanced integration due to glaucomatous injury. Although beyond the scope of this proposal, the possibility exists in the future to assess subtype connectivity through the use of retrograde tracers coupled with identification of the subtypes in the retina. Finally, in future experiments, success of these aims will allow for a more targeted replacement of those cells that are both more capable of integration, as well as those subtypes preferentially lost in glaucoma.

Scientific Rigor

For experiments throughout the proposal, the experimenter performing the analysis will be masked to the treatment and/or phenotype of the cells studied. For animal studies, the number of animals proposed is based on a detailed, conservative calculation of the number of animals needed to achieve statistically significant results and assure reproducibility.

Consideration of sex as a biological variable

For all animal experiments proposed, roughly equal numbers of males and females will be used for studies. Likewise, for hPSCs to be utilized in these studies, we have selected lines from both male and female sources.

		Months					
	Task	0-2	2-4	4-6	6-8	8-10	10-12
Specific Aim #1	Procure macaques and quarantine	X					
	Establish baseline functional values		X				
	Differentiate RGCs from hPSCs	X	X				
	Transplantation of RGCs into control macaques		X	X	X		
	Analysis of transplants - imaging and ERG		X	X	X		
	Analysis of transplants - histology				X	X	
Specific Aim #2	Procure macaques and quarantine	X					
	Induction of experimental glaucoma		X	X	X		
	Establish baseline functional values from glaucomatous macaques				X		
	Differentiate RGCs from hPSCs				X	X	
	Transplantation of RGCs into glaucomatous macaques					X	
	Analysis of transplants - imaging and ERG					X	X
	Analysis of transplants - histology						X

Figure 5. Timeline of experiments to be completed within these aims.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

VERTEBRATE ANIMALS

1) Animal Description:

Species: Rhesus Macaque monkey (*Macaca mulatta*)
Ages: Adult, 4-18 years
Sex: Male and Female (approximately 3:3)
Number: 6 animals total

Description of Procedures: Animals will undergo weekly non-invasive testing that includes bilateral ophthalmic imaging (scanning laser ophthalmoscopy, SLO in fluorescence and reflectance modes; spectral domain optical coherence tomography, SDOCT); and OCT-angiography (OCT-A); retinal function testing by electroretinography (ERG) and intraocular pressure (IOP) measurements, all under appropriate general and local topical anesthesia (see below). After a period of baseline testing (5 imaging and ERG sessions for each animal), one eye of each of the Aim-2 animals is selected at random to have laser photocoagulation applied to the trabecular meshwork to reduce aqueous humor outflow and induce chronic, mild-to-moderate IOP elevation. Weekly follow-up testing continues on both eyes (the contralateral eye serves as an internal control) until the study endpoint is reached as described in the Research Strategy. At that point, the animal is euthanized for histopathological evaluation of tissues (described in detail in the Research Strategy).

2) Justification: The major goal of this proposal is to establish feasibility of RGC transplantation and to extend the translational impact of Dr. Meyers ongoing work by applying that work in a species whose ocular anatomy and physiology are very similar to human, and to systematically gather additional pre-clinical evidence from a larger animal model. Therefore, we are proposing to include rhesus monkeys (*Macaca mulatta*) for these studies. Rhesus monkeys have remarkably similar anatomical and physiological characteristics to humans, particularly with regard to the retinal nerve fiber layer (RNFL) and anterior optic nerve/optic nerve head anatomy. This will strengthen considerably the clinical applicability of our findings and their relevance to human disease. We have extensive experience with the experimental glaucoma model in rhesus macaque, which we use here to inform our sample size requirements. We have tried to minimize the number of non-human primates (NHP) used to establish feasibility, the primary goal of this limited R21 project. Animal costs are budgeted for 6 adult monkeys, including per diem housing and care costs.

3) Minimization of Pain and Distress: Animals will be handled for purposes of restraint and administration of anesthetic. Monkeys are restrained using a squeeze cage for induction of general anesthesia. Anesthesia will be used for all non-terminal and terminal procedures. General anesthesia is induced initially by intramuscular injection of ketamine (12-15 mg/kg) in combination with either xylazine (0.8-1.5 mg/kg IM) or midazolam (0.2 mg/kg IM), along with a single subcutaneous injection of atropine sulphate (0.05 mg/kg). Animals are then intubated and breathe 100% oxygen for retinal function testing by electroretinography (ERG), during which anesthesia is maintained using a combination of ketamine (5 mg/kg/hr IV) and xylazine (0.8 mg/kg/hr IM). Anesthesia for structural imaging sessions is maintained using isoflurane gas (1-2%; typically 1.25%) via endotracheal tube or pentobarbital constant rate infusion, depending on the experiment. During all procedures, heart rate and arterial oxyhemoglobin saturation are monitored continuously and maintained above 75 beats per min and 95%, respectively. Body temperature is maintained at 37°C.

Humane Endpoints: Any animal losing 15% of its body weight would be removed from the study and euthanized, however, this kind of complication has never been encountered previously. Blindness due to unexpected complications, as monitored behaviorally by our staff on a daily basis and by our alternating weekly examinations of ocular structure and function, will also be considered a humane endpoint.

4) Experimental Procedures:

Macaque surgeries: A total 6 rhesus monkeys will be used in this project, including both sexes, with age ranging from 4-18 years. All experimental procedures will begin with induction of general anesthesia using ketamine (10-25 mg/kg IM) in combination with either xylazine (0.8-1.5 mg/kg IM) or midazolam (0.2 mg/kg IM), along with a single injection of atropine sulphate (0.05 mg/kg IM). Animals will then be intubated with an endotracheal tube to breathe a mixture of 100% oxygen and air for maintaining oxyhemoglobin saturation ≥95%, as close to 100% as possible. During ERG testing, anesthesia will be maintained using a combination of ketamine (5 mg/kg/hr IV) and xylazine (0.8 mg/kg/hr IM). In some cases, a constant rate infusion (1.2-3.0 mg/kg/hr) is used for IV ketamine delivery after a loading dose of 3.0-8.0 mg/kg. For all SDOCT imaging sessions, anesthesia after initial induction is maintained using isoflurane gas (1-2%; typically 1.25%) mixed with 100% oxygen and delivered via endotracheal tube. For SD-OCT imaging, a clear, rigid gas permeable contact lens filled with 0.5%

carboxymethylcellulose solution will be placed over the apex of each cornea. In all sessions, IV fluids (lactated Ringer's solution, 10-20 mL/kg/h) will be administered via the saphenous vein, and vital signs will be monitored throughout and recorded every 10-15 mins, including, heart rate, blood pressure, arterial oxyhemoglobin saturation, endtidal CO_2 and body temperature. Body temperature will be maintained at 37°C with a heart rate above 75 beats per min and oxygen saturation above 95%.

In vivo SD-OCT imaging. SD-OCT will be conducted every two weeks for the first month, then monthly. SD-OCT is used to measure the average peripapillary RNFL thickness from a single circular, 12° diameter B-scan consisting of 1536 A-scans (the primary outcome measure defining the "stage" of glaucomatous damage[8,10] and thus the point of intervention by injection of hPSC-RGCs). Also obtained are dense raster and/or radial star patterned scans centered over the fovea for macular layer-by-layer analysis[14]. Nine to sixteen individual sweeps are averaged in real time to comprise the final stored B-scan at each session. The position of the scan is centered on the optic nerve head (or fovea) at the first imaging session and all follow-up scans are acquired at this same location using the instrument's automatic active eye tracking software. A trained technician masked to the specific purpose of this study manually corrects the accuracy of the instrument's native automated layer segmentations.

CSLO Imaging *In Vivo.* After SD-OCT, confocal scanning laser ophthalmoscopy (CSLO) will be performed using the same multimodal instrument (Spectralis OCT+HRA). CSLO images are acquired in reflectance mode using the infrared source simultaneously with the OCT imaging, in fact, it is the CSLO channel that guides the real-time eye tracking for OCT sweep averaging and alignment of follow-up scans to the same location as baseline. In this experiment, CSLO imaging will additionally include fluorescence mode to detect the tdTomato reporter of the hPSC-derived cells. We have shown previously (as have others) that individual RGCs can be resolved and counted over time in vivo using this method to track loss of RGCs after experimental optic nerve injuries including optic nerve transection and both acute and chronic experimental glaucoma models.

Multifocal ERG. Multifocal ERG (mfERG) recordings will be obtained at 1, 2, and 4 months post-transplant using VERIS™ (Electro-Diagnostic Imaging, Inc). The stimulus will consist of 103 un-scaled hexagonal elements subtending a total field size of ~55° at the test distance of 30 cm. The luminance of each hexagon is independently modulated between dark (1 cd/m^2) and light (200 cd/m^2) according to a pseudorandom, binary m-sequence. The temporal stimulation rate is slowed by insertion of 7 dark frames into each m-sequence step ("7F"). The m-sequence exponent is set to 12, thus the total duration of each recording is 7 min 17 sec. Signals are amplified (gain = 100,000), band-pass filtered (10-300 Hz; with an additional 60 Hz line filter), sampled at 1.2 kHz (i.e. sampling interval = 0.83 ms), and digitally stored for subsequent off-line analyses. Two such recordings will be obtained for each eye at each time point and averaged. Alignment of the stimulus on the visual axis is achieved via adjustment during a series of brief, standard m-sequence ("0F") multifocal ERG recordings prior to the pair of 7F recordings. From the average of the two 7F recordings at each time point, a subset of local responses from the full array, limited to the central element and the three rings surrounding it (37 local responses in total,), is processed to derive summary outcome parameters, available the same day of testing: a high-pass filter (5-pole, >75 Hz) will be applied to each local mfERG response to extract the high frequency components (HFC), the specific measure of retinal ganglion cell function[7-9,13,14]. The low frequency component (LFC) of each response is represented as the raw response minus the HFC. The amplitude of the HFC is calculated as the root mean square (RMS) for the epoch between 0-80 ms of each filtered record. Peak amplitudes for LFC features are quantified as follows: the first negative feature (N1) is calculated as the maximum negative excursion from baseline in the epoch up to 30 ms; the amplitude of the first positivity (P1) is calculated as the voltage difference between the maximum peak and the N1 trough; and the second negativity (N2) is calculated as the difference between baseline and the minima from 30-80 ms.

4) Euthanasia: Animals will be euthanized according to methods approved by the Panel on Euthanasia of the Veterinary Medical Association. For euthanasia due to any unplanned complication (see Humane Endpoints above), the animal would be placed under deep ketamine/midazolam anesthesia, then sacrificed with an IV overdose of pentobarbital. After confirmation of lack of pulse and respiration, a scalpel will be used to inflict a double pneumothorax to ensure death. For all planned sacrifices occurring at the pre-determined study endpoint, the procedure begins with induction of deep anesthesia using ketamine (10-25 mg/kg IM) and midazolam (0.2 mg/kg IM), followed by an overdose of IV pentobarbital. Tissues are preserved by perfusion fixation via bilateral carotid arteries using a mixture of ice cold paraformaldehyde and glutaraldehyde in cacydylate buffer (~1 to 1.5 liters).

MULTIPLE PD/PI LEADERSHIP PLAN

This is a dual-site, Co-PI study and as such requires significant shared leadership. We have jointly shared in the development of this project and proposal, and the PIs have extensive complementary expertise and proven track records of research excellence in their respective fields.

At Indiana University, this project will be led by Dr. Jason Meyer (contact PI), an Associate Professor in the Department of Biology with adjunct appointments in the Department of Medical and Molecular Genetics and the Department of Ophthalmology, as well as an affiliation at the Stark Neurosciences Research Institute. Dr. Meyer is a stem cell biologist with an extensive track record of high impact publications in the field of retinal stem cell research. He has pioneered the field of retinal differentiation from hPSCs and his research has paved the way for the development of retinal organoid technologies. He will take on the role of contact PI for this project, facilitating communications with NIH and with the [redacted] lab. Furthermore, his lab will be responsible for the generation of RGCs for all of the experiments outlined in this application, and will coordinate the shipment of cells for transplantation to the Fortune lab. His lab will also be responsible for the histological analysis of retinal tissue following the transplantation of hPSC-derived RGCs.

[redacted] is an Associate Scientist and the Director of Electrodiagnostic Services at the Devers Eye Institute. He is a leading expert in the use of non-human primate models of eye diseases, and has considerable expertise in the surgical induction of glaucoma in the rhesus model. [redacted] will be responsible for the generation of the glaucoma model in rhesus monkeys described in this project, as well as the surgical engraftment of hPSC-derived RGCs. He will also oversee the behavioral and functional tests associated with cell engraftment. At the end of experiments [redacted] will also oversee the euthanasia of animals as well as the harvesting of retinal tissue, which will then be sent to the Meyer lab for histological analysis.

Collaborative team meetings. PIs on this proposal will schedule monthly teleconference calls to update all team members on experimental status, and to brainstorm and/or troubleshoot ideas. Additional conversations will occur as needed in addition to these regularly scheduled monthly conversations.

Budget allocation and scientific responsibilities have been outlined in the budget justification, and all PIs have agreed upon this budget.

Conflict resolution. Study results will be submitted for publication. Authorship will be based on the relative scientific contributions of study staff. Should there be disagreement among the PIs, a careful discussion will ensue and consultation with independent researchers as agreed upon by the PIs will help to resolve the disagreement.

Change in PD/PI location. If a PI moves to a new institution, the PIs will work to ensure the transfer of the related portion of grant to the new institution.

REFERENCES:

1 Benowitz, L. I., He, Z. & Goldberg, J. L. Reaching the brain: Advances in optic nerve regeneration. *Experimental neurology* **287**, 365-373, doi:10.1016/j.expneurol.2015.12.015 (2017).

2 Benowitz, L. I. & Yin, Y. Optic nerve regeneration. *Archives of ophthalmology (Chicago, Ill. : 1960)* **128**, 1059-1064, doi:10.1001/archophthalmol.2010.152 (2010).

3 Crair, M. C. & Mason, C. A. Reconnecting Eye to Brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **36**, 10707-10722, doi:10.1523/jneurosci.1711-16.2016 (2016).

4 Erskine, L. & Herrera, E. Connecting the retina to the brain. *ASN neuro* **6**, doi:10.1177/1759091414562107 (2014).

5 Laha, B., Stafford, B. K. & Huberman, A. D. Regenerating optic pathways from the eye to the brain. *Science (New York, N.Y.)* **356**, 1031-1034, doi:10.1126/science.aal5060 (2017).

6 Badea, T. C. & Nathans, J. Morphologies of mouse retinal ganglion cells expressing transcription factors Brn3a, Brn3b, and Brn3c: analysis of wild type and mutant cells using genetically-directed sparse labeling. *Vision research* **51**, 269-279, doi:10.1016/j.visres.2010.08.039 (2011).

7 Berson, D. M. in *The Senses: A Comprehensive Reference* (eds R. H. Masland & T.D. Albright) 491-519 (Elsevier, 2008).

8 Callaway, E. M. Structure and function of parallel pathways in the primate early visual system. *The Journal of physiology* **566**, 13-19, doi:10.1113/jphysiol.2005.088047 (2005).

9 Daniszewski, M. *et al.* Single cell RNA sequencing of stem cell-derived retinal ganglion cells. *Scientific data* **5**, 180013, doi:10.1038/sdata.2018.13 (2018).

10 Dhande, O. S., Stafford, B. K., Lim, J. A. & Huberman, A. D. Contributions of Retinal Ganglion Cells to Subcortical Visual Processing and Behaviors. *Annual review of vision science* **1**, 291-328, doi:10.1146/annurev-vision-082114-035502 (2015).

11 Huberman, A. D. *et al.* Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron* **62**, 327-334, doi:10.1016/j.neuron.2009.04.014 (2009).

12 Lee, S. K. & Schmidt, T. M. Morphological Identification of Melanopsin-Expressing Retinal Ganglion Cell Subtypes in Mice. *Methods in molecular biology (Clifton, N.J.)* **1753**, 275-287, doi:10.1007/978-1-4939-7720-8_19 (2018).

13 Muzyka, V. V., Brooks, M. & Badea, T. C. Postnatal developmental dynamics of cell type specification genes in Brn3a/Pou4f1 Retinal Ganglion Cells. *Neural development* **13**, 15, doi:10.1186/s13064-018-0110-0 (2018).

14 Rheaume, B. A. *et al.* Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nature communications* **9**, 2759, doi:10.1038/s41467-018-05134-3 (2018).

15 Sanes, J. R. & Masland, R. H. The types of retinal ganglion cells: current status and implications for neuronal classification. *Annual review of neuroscience* **38**, 221-246, doi:10.1146/annurev-neuro-071714-034120 (2015).

16 Schmidt, T. M., Chen, S. K. & Hattar, S. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends in neurosciences* **34**, 572-580, doi:10.1016/j.tins.2011.07.001 (2011).

17 Siegert, S. *et al.* Transcriptional code and disease map for adult retinal cell types. *Nature neuroscience* **15**, 487-495, s481-482, doi:10.1038/nn.3032 (2012).

18 Struebing, F. L., Lee, R. K., Williams, R. W. & Geisert, E. E. Genetic Networks in Mouse Retinal Ganglion Cells. *Frontiers in genetics* **7**, 169, doi:10.3389/fgene.2016.00169 (2016).

19 Sumbul, U. *et al.* A genetic and computational approach to structurally classify neuronal types. *Nature communications* **5**, 3512, doi:10.1038/ncomms4512 (2014).

20 Della Santina, L. & Ou, Y. Who's lost first? Susceptibility of retinal ganglion cell types in experimental glaucoma. *Experimental eye research* **158**, 43-50, doi:10.1016/j.exer.2016.06.006 (2017).

21 Duan, X. *et al.* Subtype-specific regeneration of retinal ganglion cells following axotomy: effects of osteopontin and mTOR signaling. *Neuron* **85**, 1244-1256, doi:10.1016/j.neuron.2015.02.017 (2015).

22 El-Danaf, R. N. & Huberman, A. D. Characteristic patterns of dendritic remodeling in early-stage glaucoma: evidence from genetically identified retinal ganglion cell types. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **35**, 2329-2343, doi:10.1523/jneurosci.1419-14.2015 (2015).

23 Majander, A. *et al.* The Pattern of Retinal Ganglion Cell Loss in OPA1-Related Autosomal Dominant Optic Atrophy Inferred From Temporal, Spatial, and Chromatic Sensitivity Losses. *Investigative ophthalmology & visual science* **58**, 502-516, doi:10.1167/iovs.16-20309 (2017).

24 Ou, Y., Jo, R. E., Ullian, E. M., Wong, R. O. & Della Santina, L. Selective Vulnerability of Specific Retinal Ganglion Cell Types and Synapses after Transient Ocular Hypertension. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **36**, 9240-9252, doi:10.1523/jneurosci.0940-16.2016 (2016).

25 Puyang, Z. *et al.* Different functional susceptibilities of mouse retinal ganglion cell subtypes to optic nerve crush injury. *Experimental eye research* **162**, 97-103, doi:10.1016/j.exer.2017.06.014 (2017).

26 Anderson, D. R. Ultrastructure of human and monkey lamina cribrosa and optic nerve head. *Archives of ophthalmology (Chicago, Ill. : 1960)* **82**, 800-814 (1969).

27 Burgoyne, C. F., Downs, J. C., Bellezza, A. J. & Hart, R. T. Three-dimensional reconstruction of normal and early glaucoma monkey optic nerve head connective tissues. *Investigative ophthalmology & visual science* **45**, 4388-4399, doi:10.1167/iovs.04-0022 (2004).

28 May, C. A. & Lutjen-Drecoll, E. Morphology of the murine optic nerve. *Investigative ophthalmology & visual science* **43**, 2206-2212 (2002).

29 Morrison, J. *et al.* Structure and composition of the rodent lamina cribrosa. *Experimental eye research* **60**, 127-135 (1995).

30 Quigley, H. A. & Addicks, E. M. Chronic experimental glaucoma in primates. II. Effect of extended intraocular pressure elevation on optic nerve head and axonal transport. *Investigative ophthalmology & visual science* **19**, 137-152 (1980).

31 Weinreb, R. N. & Lindsey, J. D. The importance of models in glaucoma research. *Journal of glaucoma* **14**, 302-304 (2005).

32 Fortune, B., Burgoyne, C. F., Cull, G. A., Reynaud, J. & Wang, L. Structural and functional abnormalities of retinal ganglion cells measured in vivo at the onset of optic nerve head surface change in experimental glaucoma. *Investigative ophthalmology & visual science* **53**, 3939-3950, doi:10.1167/iovs.12-9979 (2012).

33 Fortune, B., Cull, G., Reynaud, J., Wang, L. & Burgoyne, C. F. Relating Retinal Ganglion Cell Function and Retinal Nerve Fiber Layer (RNFL) Retardance to Progressive Loss of RNFL Thickness and Optic Nerve Axons in Experimental Glaucoma. *Investigative ophthalmology & visual science* **56**, 3936-3944, doi:10.1167/iovs.15-16548 (2015).

34 Fortune, B., Cull, G. A. & Burgoyne, C. F. Relative course of retinal nerve fiber layer birefringence and thickness and retinal function changes after optic nerve transection. *Investigative ophthalmology & visual science* **49**, 4444-4452, doi:10.1167/iovs.08-2255 (2008).

35 Fortune, B. *et al.* Comparing Optic Nerve Head Rim Width, Rim Area, and Peripapillary Retinal Nerve Fiber Layer Thickness to Axon Count in Experimental Glaucoma. *Investigative ophthalmology & visual science* **57**, Oct404-412, doi:10.1167/iovs.15-18667 (2016).

36 Fortune, B. *et al.* Experimental Glaucoma Causes Optic Nerve Head Neural Rim Tissue Compression: A Potentially Important Mechanism of Axon Injury. *Investigative ophthalmology & visual science* **57**, 4403-4411, doi:10.1167/iovs.16-20000 (2016).

37 Fortune, B., Reynaud, J., Wang, L. & Burgoyne, C. F. Does optic nerve head surface topography change prior to loss of retinal nerve fiber layer thickness: a test of the site of injury hypothesis in experimental glaucoma. *PloS one* **8**, e77831, doi:10.1371/journal.pone.0077831 (2013).

38 Wilsey, L. *et al.* Comparing three different modes of electroretinography in experimental glaucoma: diagnostic performance and correlation to structure. *Documenta ophthalmologica. Advances in ophthalmology* **134**, 111-128, doi:10.1007/s10633-017-9578-x (2017).

39 Wilsey, L. J., Reynaud, J., Cull, G., Burgoyne, C. F. & Fortune, B. Macular Structure and Function in Nonhuman Primate Experimental Glaucoma. *Investigative ophthalmology & visual science* **57**, 1892-1900, doi:10.1167/iovs.15-18119 (2016).

40 Capowski, E. E. *et al.* Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines. *Development (Cambridge, England)* **146**, doi:10.1242/dev.171686 (2019).

41 Gonzalez-Cordero, A. *et al.* Recapitulation of Human Retinal Development from Human Pluripotent Stem Cells Generates Transplantable Populations of Cone Photoreceptors. *Stem Cell Reports* **9**, 820-837, doi:10.1016/j.stemcr.2017.07.022 (2017).

42 Lamba, D. A., Karl, M. O., Ware, C. B. & Reh, T. A. Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 12769-12774, doi:10.1073/pnas.0601990103 (2006).

43 Lamba, D. A. *et al.* Generation, purification and transplantation of photoreceptors derived from human induced pluripotent stem cells. *PloS one* **5**, e8763, doi:10.1371/journal.pone.0008763 (2010).

44 Mellough, C. B., Sernagor, E., Moreno-Gimeno, I., Steel, D. H. & Lako, M. Efficient stage-specific differentiation of human pluripotent stem cells toward retinal photoreceptor cells. *Stem cells (Dayton, Ohio)* **30**, 673-686, doi:10.1002/stem.1037 (2012).

45 Meyer, J. S. *et al.* Optic vesicle-like structures derived from human pluripotent stem cells facilitate a customized approach to retinal disease treatment. *Stem cells (Dayton, Ohio)* **29**, 1206-1218, doi:10.1002/stem.674 (2011).

46 Meyer, J. S. *et al.* Modeling early retinal development with human embryonic and induced pluripotent stem cells. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 16698-16703, doi:10.1073/pnas.0905245106 (2009).

47 Nakano, T. *et al.* Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell stem cell* **10**, 771-785, doi:10.1016/j.stem.2012.05.009 (2012).

48 Ohlemacher, S. K., Iglesias, C. L., Sridhar, A., Gamm, D. M. & Meyer, J. S. Generation of highly enriched populations of optic vesicle-like retinal cells from human pluripotent stem cells. *Current protocols in stem cell biology* **32**, 1h.8.1-1h.8.20, doi:10.1002/9780470151808.sc01h08s32 (2015).

49 Ohlemacher, S. K. *et al.* Stepwise Differentiation of Retinal Ganglion Cells from Human Pluripotent Stem Cells Enables Analysis of Glaucomatous Neurodegeneration. *Stem cells (Dayton, Ohio)* **34**, 1553-1562, doi:10.1002/stem.2356 (2016).

50 Osakada, F. *et al.* Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nature biotechnology* **26**, 215-224, doi:10.1038/nbt1384 (2008).

51 Phillips, M. J. *et al.* Blood-derived human iPS cells generate optic vesicle-like structures with the capacity to form retinal laminae and develop synapses. *Investigative ophthalmology & visual science* **53**, 2007-2019, doi:10.1167/iovs.11-9313 (2012).

52 Sridhar, A., Ohlemacher, S. K., Langer, K. B. & Meyer, J. S. Robust Differentiation of mRNA-Reprogrammed Human Induced Pluripotent Stem Cells Toward a Retinal Lineage. *Stem cells translational medicine* (2016).

53 Sridhar, A., Steward, M. M. & Meyer, J. S. Nonxenogeneic growth and retinal differentiation of human induced pluripotent stem cells. *Stem cells translational medicine* **2**, 255-264, doi:10.5966/sctm.2012-0101 (2013).

54 Wahlin, K. J. *et al.* Photoreceptor Outer Segment-like Structures in Long-Term 3D Retinas from Human Pluripotent Stem Cells. *Scientific reports* **7**, 766, doi:10.1038/s41598-017-00774-9 (2017).

55 Zhong, X. *et al.* Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nature communications* **5**, 4047, doi:10.1038/ncomms5047 (2014).

56 Fligor, C. M. *et al.* Three-Dimensional Retinal Organoids Facilitate the Investigation of Retinal Ganglion Cell Development, Organization and Neurite Outgrowth from Human Pluripotent Stem Cells. *Scientific reports* **8**, 14520, doi:10.1038/s41598-018-32871-8 (2018).

57 Gill, K. P. *et al.* Enriched retinal ganglion cells derived from human embryonic stem cells. *Scientific reports* **6**, 30552, doi:10.1038/srep30552 (2016).

58 Huang, L. *et al.* Retinoid acid and taurine promote NeuroD1-induced differentiation of induced pluripotent stem cells into retinal ganglion cells. *Molecular and cellular biochemistry* **438**, 67-76, doi:10.1007/s11010-017-3114-x (2018).

59 Inagaki, S. *et al.* Effect of Timolol on Optineurin Aggregation in Transformed Induced Pluripotent Stem Cells Derived From Patient With Familial Glaucoma. *Investigative ophthalmology & visual science* **59**, 2293-2304, doi:10.1167/iovs.17-22975 (2018).

60 Langer, K. B. *et al.* Retinal Ganglion Cell Diversity and Subtype Specification from Human Pluripotent Stem Cells. *Stem Cell Reports* **10** (2018).

61 Maekawa, Y. *et al.* Optimized Culture System to Induce Neurite Outgrowth From Retinal Ganglion Cells in Three-Dimensional Retinal Aggregates Differentiated From Mouse and Human Embryonic Stem Cells. *Current eye research*, 1-11, doi:10.3109/02713683.2015.1038359 (2015).

62 Riazifar, H., Jia, Y., Chen, J., Lynch, G. & Huang, T. Chemically induced specification of retinal ganglion cells from human embryonic and induced pluripotent stem cells. *Stem cells translational medicine* **3**, 424-432, doi:10.5966/sctm.2013-0147 (2014).

63 Sluch, V. M. *et al.* Enhanced Stem Cell Differentiation and Immunopurification of Genome Engineered Human Retinal Ganglion Cells. *Stem cells translational medicine*, doi:10.1002/sctm.17-0059 (2017).

64 Sluch, V. M. *et al.* Differentiation of human ESCs to retinal ganglion cells using a CRISPR engineered reporter cell line. *Scientific reports* **5**, 16595, doi:10.1038/srep16595 (2015).

65 Tanaka, T. *et al.* Generation of retinal ganglion cells with functional axons from human induced pluripotent stem cells. *Scientific reports* **5**, 8344, doi:10.1038/srep08344 (2015).

66 Teotia, P. *et al.* Generation of Functional Human Retinal Ganglion Cells with Target Specificity from Pluripotent Stem Cells by Chemically Defined Recapitulation of Developmental Mechanism. *Stem cells (Dayton, Ohio)* **35**, 572-585, doi:10.1002/stem.2513 (2017).

67 Teotia, P. *et al.* Modeling Glaucoma: Retinal Ganglion Cells Generated from Induced Pluripotent Stem Cells of Patients with SIX6 Risk Allele Show Developmental Abnormalities. *Stem cells (Dayton, Ohio)* **35**, 2239-2252, doi:10.1002/stem.2675 (2017).

68 VanderWall, K. B. *et al.* Astrocytes Regulate the Development and Maturation of Retinal Ganglion Cells Derived from Human Pluripotent Stem Cells. *Stem Cell Reports*, doi:10.1016/j.stemcr.2018.12.010 (2018).

69 Wang, S. T. *et al.* Transplantation of Retinal Progenitor Cells from Optic Cup-Like Structures Differentiated from Human Embryonic Stem Cells In Vitro and In Vivo Generation of Retinal Ganglion-Like Cells. *Stem cells and development* **28**, 258-267, doi:10.1089/scd.2018.0076 (2019).

70 Al-Shamekh, S. & Goldberg, J. L. Retinal repair with induced pluripotent stem cells. *Translational research : the journal of laboratory and clinical medicine* **163**, 377-386, doi:10.1016/j.trsl.2013.11.002 (2014).

71 Khan, S., Hung, S. S. & Wong, R. C. The use of induced pluripotent stem cells for studying and treating optic neuropathies. *Current opinion in organ transplantation* **21**, 484-489, doi:10.1097/mot.0000000000000348 (2016).

72 Parameswaran, S. *et al.* Continuous non-cell autonomous reprogramming to generate retinal ganglion cells for glaucomatous neuropathy. *Stem cells (Dayton, Ohio)* **33**, 1743-1758, doi:10.1002/stem.1987 (2015).

73 MacLaren, R. E. *et al.* Retinal repair by transplantation of photoreceptor precursors. *Nature* **444**, 203-207, doi:10.1038/nature05161 (2006).

74 Pearson, R. A. *et al.* Restoration of vision after transplantation of photoreceptors. *Nature* **485**, 99-103, doi:10.1038/nature10997 (2012).

75 Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-872, doi:10.1016/j.cell.2007.11.019 (2007).

76 Yu, J. *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. *Science (New York, N.Y.)* **324**, 797-801, doi:10.1126/science.1172482 (2009).

77 Yu, J. *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science (New York, N.Y.)* **318**, 1917-1920, doi:10.1126/science.1151526 (2007).

78 Park, I. H. *et al.* Disease-specific induced pluripotent stem cells. *Cell* **134**, 877-886, doi:10.1016/j.cell.2008.07.041 (2008).

79 Park, I. H. *et al.* Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141-146, doi:10.1038/nature06534 (2008).

80 Choe, T. E., Abbott, C. J., Piper, C., Wang, L. & Fortune, B. Comparison of longitudinal in vivo measurements of retinal nerve fiber layer thickness and retinal ganglion cell density after optic nerve transection in rat. *PloS one* **9**, e113011, doi:10.1371/journal.pone.0113011 (2014).

81 Fortune, B. In vivo imaging methods to assess glaucomatous optic neuropathy. *Experimental eye research* **141**, 139-153, doi:10.1016/j.exer.2015.06.001 (2015).

82 Fortune, B. *et al.* Local ganglion cell contributions to the macaque electroretinogram revealed by experimental nerve fiber layer bundle defect. *Investigative ophthalmology & visual science* **44**, 4567-4579 (2003).

Indiana University | Office of Research Administration

SUBRECIPIENT COMMITMENT FORM

If your institution is participating in the FDP Clearinghouse Pilot, complete sections A and C only. If your institution is not participating in the FDP Clearinghouse Pilot, complete all sections. This form must be approved and signed by your organization's Authorized Organizational Representative (AOR). **Please ensure all applicable documents (Statement of Work, Budget, etc) are included with the request.**

SECTION A: Project Information

Subrecipient Legal Name: LEGACY EMANUEL HOSPITAL AND HEALTH CENTER Subrecipient PI: [redacted]

Subrecipient Central Email: jcouchman@lhs.org Subrecipient Admin. Contact Email: jcouchman@lhs.org

Direct Costs: $ 159,877.00 Indirect Costs: $ 108,716.00 Total Costs: $ 268,593.00

Project Title: Targeting the diversity of retinal ganglion cells for replacement therapy

Period of Performance: 9/1/2019 to 8/30/2020

Subrecipient's Research Includes (check as applicable): ☐ Human Subjects ☑ Animals ☐ Biosafety ☐ None

If applicable, does your organization certify that it will follow the NIH single IRB plan developed for this project? ☐ Yes ☐ No

Perfomance Address: Legacy Research Institute [redacted] Portland, Oregon 97232-2003

Subaward Type: ☑ Cost Reimbursement ☐ Fixed Price

SECTION B: Subrecipient's Institutional Information

DUNS #: 0509730980000 EIN: 930386823 Congressional District: OR-003

1. ☐ Yes ☑ No ☐ N/A Is your organization or PI and/or employees on this project presently debarred, suspended, proposed for debarment, declared ineligible or voluntarily excluded from participation in any federal department or agency or delinquent on repayment of any federal debt including direct and guaranteed loans and other debt as defined in Uniform Guidance?

2. ☐ Yes ☑ No ☐ N/A If application is to a federal or federal pass-through sponsor, have any lobbying activities been or will any be conducted regarding this proposal?

3. ☑ Yes ☐ No ☐ N/A If applicable, does your organization certify that it currently has a PHS-compliant Financial Conflict of Interest (FCOI) policy and a PHS Financial Disclosure for each of the Subrecipient's key personnel?

4. ☑ Yes ☐ No Does your organization have a federally negotiated F&A rate? If yes, please provide a copy of your F&A rate agreement.

5. ☑ Yes ☐ No Does your organization receive a single audit in accordance with Uniform Guidance §200.514 (formerly A-133)? If no, please provide a contact and email address below.
Name: _____ Email: _____

SECTION C: Subrecipient's Authorized Official Representative (AOR) Approval

I certify that the information provided is true and correct. I am the authorized official representative (AOR) of the Subrecipient named herein, and I have the authority to legally bind my organization in grants administration matters. I understand that: (a) any work we begin and/or expenses we incur related to our proposal prior to full execution of a subaward agreement will be at my organization's own risk. The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the agency's consortium agreement policy and are prepared to establish the necessary inter-organizational agreement(s) consistent with that policy.

_____ 03/06/2019

Subrecipient's Authorized Official Date:

Name: Joe Frascella, PhD

December 2017

Resource Sharing Plan

We will readily share any and all results associated with the successful completion of these specific aims. Data generated from these experiments will be presented at scientific conferences and published in scientific journals.

The projects described in this application also involve the use of modified lines of existing hPSCs to express the tdTomato and Thy1.2 antigens specifically in RGCs, which could serve as a resource for the scientific community. Upon request, we will readily share these reporter lines upon the receipt of permission from Dr. [redacted] whose lab has originally developed this reporter. In the case of the IMR90-4 cell line, these cells were initially established by [redacted] at the University of Wisconsin – in these cases we will ask the requesting institution to get approval from the Wisconsin Alumni Research Foundation (WARF) before the materials may be shipped.

Authentication of Key Biological and/or Chemical Resources

Several specific mechanisms will authenticate key reagents in this research program.

Good Laboratory Practice Laboratory: In view of the overall goal of delivering novel treatments to human patients (which requires high quality, reproducible, standardized preclinical data), my laboratory follows Good Laboratory Practice (GLP) procedures, which includes generating standard operating procedures, validating protocols and equipment, adopting standardized inventory and document control systems and developing a quality assurance group who oversees study document design, data collection, quality testing, employee training etc. The studies outlined in this application will be performed under GLP conditions.

Validation of hPSC lines
hPSC lines to be used in this study have already been developed and previously characterized. However, as changes may occur to cell lines over time, or lines may become cross-contaminated, several steps will be taken to ensure to purity and pluripotent nature of these cells. First, hPSC lines will be routinely screened for the maintenance of a normal karyotype through services provided by the Cytogenetic Services within the Indiana University Department of Medical and Molecular Genetics. Lines will be screened every 10-15 passages, and cells will not be used past passage number 40. Cross-contamination of cell lines will also be screened for through the use of DNA fingerprinting techniques using a Fluidigm DNA fingerprinting platform, which identifies cell lines according to 96 different SNPs. The maintenance of pluripotency will also be analyzed periodically at times when karyotyping is performed via the TaqMan Human Pluripotent Stem Cell Scorecard Panel. After real-time PCR, gene expression data are uploaded to the web-based hPSC Scorecard Analysis Software.

Validation of macaque glaucoma model
The generation of the macaque glaucoma model will be performed following procedures established within the [redacted]lab. Elevation of IOP will be routinely measured and thinning of the retina will be accomplished via OCT imaging to confirm thinning of peripapillary retinal nerve fiber layer. Any animal losing 15% of its body weight would be removed from the study and euthanized. Blindess due to unexpected complications (as monitored behaviorally by DCM staff on a daily basis and by our alternating weekly examinations of ocular structure and function) would also be considered a humane endpoint resulting in euthanasia.

Validation of Antibodies
Antibodies will be used to help identify transplanted cells, including some of the subtypes of RGCs that survive and integrate into the retina. To ensure reliability of these antibodies, several precautions will be taken. First, antibodies described in the proposal have already been tested and confirmed to work for the indications proposed. Antibodies will also be used only for purposes for which the manufacturer has indicated. Additionally, upon receipt of new antibodies as needed, they will be tested on samples that have screened either positive or negative for expression of the gene based on RT-PCR analyses. Those antibodies that meet these criteria will then be tested by western blot to confirm the specificity of the antibody to the molecular weight of the protein of interest.

Masking of the investigator
For semi-quantitative analyses such as evaluation of post-transplant cellular survival, the investigator will be masked to any potential known variables, including both the phenotype of the cells being evaluated as well as the condition of the macaque retina (e.g. glaucomatous vs. control).