The RGC Repopulation, Stem Cell Transplantation, and Optic Nerve Regeneration Consortium

www.hopkinsmedicine.org/wilmer/rrestore

Discussion Agenda for the RReSTORe Workshop
Saturday April 30th, 2022
Embassy Suites Denver Downtown
1420 Stout Street
Denver, CO 80202

Table of Contents

RReSTORe Workshop Schedule

RReSTORe Workshop Schedule - Saturday, April 30th, 2022				
Start Time*	**End Time***	**Session Description**	**Location - Embassy Suites Denver Downtown**	**Recording**
7:00am	8:00am	Sign-in / Continental breakfast / informal discussion time	Crystal Foyer	None
8:00am	8:30am	Welcome and overview of RReSTORe	Crystal A, B	Video recording
8:45am	9:45am	Concurrent SDG Discussions: Topic #1	SDG #1: Aspen Room SDG #2: Cripple Creek 1 SDG #3: Cripple Creek 2 SDG #4: Crestone A SDG #5: Crestone B	Scribes
10:00am	11:00am	Concurrent SDG Discussions: Topic #2	SDG #1: Aspen Room SDG #2: Cripple Creek 1 SDG #3: Cripple Creek 2 SDG #4: Crestone A SDG #5: Crestone B	Scribes
11:15am	12:15pm	Concurrent SDG Discussions: Topic #3	SDG #1: Aspen Room SDG #2: Cripple Creek 1 SDG #3: Cripple Creek 2 SDG #4: Crestone A SDG #5: Crestone B	Scribes
12:15pm	1:15pm	Lunch / informal discussion time	Crestone Foyer	None
1:15pm	2:15pm	Concurrent SDG Discussions: Topic #4	SDG #1: Aspen Room SDG #2: Cripple Creek 1 SDG #3: Cripple Creek 2 SDG #4: Crestone A SDG #5: Crestone B	Scribes
2:30pm	3:30pm	Concurrent SDG Discussions: Topic #5	SDG #1: Aspen Room SDG #2: Cripple Creek 1 SDG #3: Cripple Creek 2 SDG #4: Crestone A SDG #5: Crestone B	Scribes
3:45pm	4:30pm	Summary of discussion sections	Crystal A, B	Video recording
4:30pm	5:00pm	Closing and information regarding RReSTORe Phase III	Crystal A, B	Video recording

***Please note that all times are in Mountain Daylight Time (MDT; UTC - 06:00), local to Denver**

Subtopic Discussion Groups (SDGs):

SDG #1: RGC Development and Differentiation
SDG #2: Transplantation Methods and Models
SDG #3: RGC Survival, Maturation, and Host Interactions
SDG #4: Inner Retinal Wirinng
SDG #5: Eye to Brain Connectivity

Concurrent SDG Discussions: Topic #1

8:45am – 9:45am

SDG #1: Aspen Room
SDG #2: Cripple Creek 1
SDG #3: Cripple Creek 2
SDG #4: Crestone A
SDG #5: Crestone B

Subtopic Discussion Group (SDG) #1: RGC Development and Differentiation

Topic #1: What is the current state of the RGC development and differentiation field in non-human model systems?

Moderators: Don Zack and Tom Reh
Introductory Comments: Jeff Mumm
Scribes: Casey Keuthan and Juliette Wohlschlegel

We currently know a good deal about the structure and function of RGCs in many animals, including humans; there is a wealth of information about the subtypes of RGCs and their role in vision. Although there is still much to be learned, particularly for RGC subtype development, there is a solid base of information to build on, and recent single cell transcriptomic characterization of RGCs is further building and extending this knowledge. Using scRNA-seq (Shekhar and Sanes; Blackshaw and Clark; Sridhar and Reh), it is becoming possible to infer the changes in transcriptome and epigenetics that lead to RGC subtype diversity through profiling of large numbers of cells across different ages of retinal development.

- Although many of the key transcription factors (TFs) for RGC specification have been defined in mice and other model organisms, we know much less about how RGC subtypes are specified during development (intrinsic and extrinsic factors) including specification of target, post-transcriptional mechanisms, and laterality projection.

- Although knockout studies have demonstrated the importance of several RGC TFs in their development, how are these TFs regulated to exert their function, i.e., gene expression or repression through mechanisms including chromatin structure, epigenetic changes, post-translational modifications, etc.?

- Recent advances in single cell genomics, etc have shown wide variations in the numbers of RGC subtypes in different species. Why do different species have a dramatically different number of RGC subtypes? Do the same RGC subsets carry out the same, or different, functions in different species?

Subtopic Discussion Group (SDG) #2: Transplantation Methods and Models

Topic #1: What preclinical models will be needed to adequately study the barriers to RGC axon growth into and through the optic nerve that pertain to human optic neuropathy?

Moderator: Brad Fortune
Introductory Comments: Brad Fortune
Scribes: Erika Aguzzi and Jonathan Soucy

- Glaucoma in human patients causes structural remodeling of the optic nerve head tissues, including the lamina cribrosa, peripapillary sclera and vasculature, that may pose a barrier to RGC axonal entry and are not adequately modeled in rodents. Which species are most amenable to preclinical studies of RGC transplantation for glaucoma (e.g., pigs, dogs, tree shrew, non-human primates)? What are the strengths and limitations of specific glaucoma models in those species (e.g., intracameral microbead injection, photocoagulation of the trabecular meshwork, naturally occurring ocular hypertension, optic nerve trauma, others)? How does the recipient microenvironment change with age and/or progressive stages of damage in such models and which ages of animals or stage(s) of damage should be prioritized for study? What steps should be taken to standardize the models being used as to produce the least variable and most comparable data?

- RGC transplantation may benefit diverse forms of optic neuropathy. What non-glaucoma models of other optic nerves diseases (e.g., ischemic optic neuropathy, optic neuritis, inherited mitochondrial disease, etc.) should be prioritized for experimentation and which models should be employed?

- In vitro systems, while limited in their ability to model complex aspects of disease, are tightly controllable and high throughput. Organoids also provide a means to test RGC replacement in human tissues. How can human retinal organoids or retina-brain assembloids be leveraged to model RGC transplantation or transdifferentiation? How can other 2D in vitro systems, including microfluidics technology, organ-on-a-chip, or human retinal explants be leveraged to study aspects of RGC transplantation?

- Thinking ahead: which diseases and patient characteristics might be the best targets for first in-human trials? Which type of optic neuropathy is likely to be the most receptive to RGC replacement? Safety considerations for phase I trials may limit included patients to those with extreme vision loss, but such eyes may be the least receptive to RGC replacement strategies.

Subtopic Discussion Group (SDG) #3: RGC Survival, Maturation, and Host Interactions

Topic #1: What models and tools will be most useful for better understanding of the molecular mechanisms that contribute to death of endogenous and/or transplanted RGCs?

Moderator: Petr Baranov
Introductory Comments: Mandeep Singh and David Calkins
Scribes: Aboozar Monavarfeshani and Ziming Luo

- A major limitation to the study of cell signaling and elucidation of the molecular mechanisms contributing to RGC survival is their tremendous sensitivity to injury and degeneration. What drives RGC susceptibility and how can these be overcome to enhance RGC survival after transplantation? Mechanisms/characteristics of interest include age, metabolic stress, neuro-glial coupling, and anatomical limitations.

- It is becoming clear that not only are there multiple populations of RGCs, but that they "respond" to injury with varying levels of sensitivity and vulnerability. To what extent do different human RGC types differ in vulnerability to injury and how can each population be protected? Are we selectively differentiating different cell types in the pre- vs post-transplant process?

- Therapeutic restorative vision would involve transplantation in a disease state (neurodegeneration, elevated IOP, oxidative stress or ocular inflammation, etc). What are the primary threats to RGC survival in that context?

- Are there logistical considerations to enhancing RGC survival after transplantation? Can local translation of axonal mRNAs, enhanced axon regeneration, or other mechanisms also support RGC survival? Are there intrinsic vs non-cell autonomous mechanisms that play a role in improving transplantation outcomes?

- Are there current therapies in use that can be used to enhance RGC survival pre- and post-transplantation?

Subtopic Discussion Group (SDG) #4: Inner Retinal Wiring

Topic #1: RGC intrinsic Factors: What are the RGC-specific intrinsic signals required for dendritogenesis and synaptogenesis to permit successful integration and proper connectivity to the inner retinal circuitry during development and following transplantation?

Moderators: Alex Kolodkin
Introductory Comments: Greg Schwartz
Scribes: Julie Cho and Michael Gilhooley

- What is already known about the RGC intrinsic signaling pathways that govern establishment of inner retinal circuits during development, and what are the key areas where further research is needed? How can collaboration around single cell transcriptomic data sets comparing RGCs during development and in organoids (in multiple species) be leveraged to better understand the expression of relevant genes at varying stages of maturity?

- The success of RGC transplantation depends on the ability of transplanted RGC to extend dendrites and establish appropriate connections with their pre-synaptic targets in the retina. Can transplanted or regenerating RGCs integrate properly into the existing inner retinal circuitry? If so, can transplanted RGCs recapitulate subtype-specific connections? Can they establish sub-lamina or cell-specific synaptic connectivity? To what extent does this occur spontaneously?

- For successful integration, transplanted RGCs are likely to require intrinsic signals or genetic programs to recognize, navigate, and establish functional synapses in the IPL. What are the RGC-specific drivers for transplanted neurons to successfully wire with existing circuitry in the diseased IPL? What are the inhibitors (e.g., internal brakes) that will interfere with this process? What are the molecules required for proper dendritic lamination? Are guidance and recognition molecules or receptors expressed on the dendritic arbors of transplanted RGCs? Which key RGC-intrinsic pathways might control re-integration and how can we modulate these pathways?

- What role does visual experience and coordinated excitatory input play in refining inner retinal circuits during development and following RGC repopulation?

- How does the allocation of internal resources in RGCs contribute to their functional integration (e.g., energy, mitochondria, synaptic proteins)?

- How can we leverage organoid models to better understand these questions?

Subtopic Discussion Group (SDG) #5: Eye to Brain Connectivity

Topic #1. How can we get axons to extend or regenerate for long distances?

Moderators: Kimberly Gokoffski and William Guido
Introductory Comments: Kimberly Gokoffski and William Guido
Scribes: Micalla Peng and Meher Saleem

- What are the cell intrinsic versus cell extrinsic barriers to axon extension?
- What are the most effective current strategies to promote axon regeneration?
- What is needed to promote the emergence of growth cones on regenerating or injured axons?
- What are the steps and time course for axo-genesis?
- How do we get past the lamina cribrosa and optic chiasm?
- Is axon regeneration simply a recapitulation of development?
- Should we consider regeneration from an adult framework?
- How does axonal degeneration impede axon guidance and regeneration?
- What role does activity play in axon regeneration?
- What are the lessons learned from spinal cord injury?
- We will also discuss future ideas and combinatorial approaches

Concurrent SDG Discussions: Topic #2

10:00am – 11:00am

SDG #1: Aspen Room
SDG #2: Cripple Creek 1
SDG #3: Cripple Creek 2
SDG #4: Crestone A
SDG #5: Crestone B

Subtopic Discussion Group (SDG) #1: RGC Development and Differentiation

Topic #2: Are there unique features of human RGCs that are particularly important for RGC repopulation and function, and how are human lineages regulated?

Moderators: Don Zack and Tom Reh
Introductory Comments: Bob Johnston
Scribes: Casey Keuthan and Juliette Wohlschlegel

The previous discussion focused on model systems, but importantly, do these same principles apply to primate retinas, which have many distinct features? Characterization of human fetal development indicates that many of the same TFs are expressed in the human retina, although most of these RGC TFs characterized in mouse and other model systems have not been experimentally validated in human development.

- How do RGC subtypes develop in humans? While new data in mice show the emergence of diversity within the RGC population in fetal stages, this is less clear in fetal human retina, likely because of the relatively restricted time window where human fetal samples are available (FW7- FW20). How can we fill this gap?

- What are the extrinsic (non-cell autonomous) signaling factors that regulate RGC development in humans? Will target derived factors be needed to reach subtype differentiation? What about activity? Presynaptic inputs? Since organoids are not perfect copies of developing retina, what other features of organoid development need to be considered? Other possible sources may derive from glial cells, vasculature, or extracellular matrix. Understanding such signaling could aid driving RGC type development toward desired paths.

- How is the fovea specified during development? The fovea is a unique specialization in primates. The midget RGCs are more highly represented in the fovea, and may be an important subtype to generate for transplantation, but we do not understand what factors lead to the specification of the fovea and how to mimic this in vitro. What determines the higher density of RGCs in the macular region? How can we model this?

Subtopic Discussion Group (SDG) #2: Transplantation Methods and Models

Topic #2 What are the optimal sources and characteristics of RGCs (or RGC progenitors) for transplantation?

Moderator: Brad Fortune
Introductory Comments: Jason Meyer
Scribes: Erika Aguzzi and Jonathan Soucy

- What stage of maturity is most likely to lead to survival and functional integration? Work in photoreceptors suggests that immature photoreceptor progenitors may exhibit greater engraftment than mature rods and cones. If immature RGCs or progenitors are transplanted, to what extent will maturation occur in vivo and how might this be best studied and controlled? What are the most relevant markers or assays to identify specific stages of maturation? How will we determine whether and how transplanted neurons mature in the retina over time?

- Multiple methods for obtaining RGCs from stem cells or endogenous sources exist - directed 2D differentiation by small molecules or transcription factor delivery, via retinal organoid differentiation, or via endogenous transdifferentiation. How do the outcomes of transplanting the various RGCs compare?

- What are the options for purifying RGCs for transplantation, and how can this be done in a GMP-compatible manner? Negative selection techniques may be preferable to positive selection, since antibodies or biological reagents needed for positive selection would be subject to regulatory control and approval. What are the relevant strengths and weaknesses of autologous vs allogenic cell sources and how can these be overcome most effectively?

- Are certain RGC subtypes more or less amenable to integration and can they be specified prior to transplantation, or will specification take place post-transplantation? How can we consider the differences in RGC subtypes between rodents and primates? What are the most efficient methods for studying subtype specification of repopulated RGCs?

- Are there cofactors that can/should be included in the transplant to enhance survival/maturation/engraftment (e.g., exosomes, neurotrophic factors, small molecules)? Can neurons themselves be genetically modified in some way prior to transplantation to enhance these outcomes?

Subtopic Discussion Group (SDG) #3: RGC Survival, Maturation, and Host Interactions

Topic #2: What are the factors to consider beyond survival of endogenous RGCs, that extend to enhancing survival of transplanted RGCs?

Moderator: Petr Baranov
Introductory Comments: Dong Feng Chen
Scribes: Aboozar Monavarfeshani and Ziming Luo

- How can we provide for a neuroprotective retinal environment for the transplanted RGCs? Potential strategies include:
    - Neurotrophic factors (how to deliver them in post transplanted cells)
    - Restoring balance of injury/glaucomatous retina to a normal milieu to optimizing transplantation efficiency
    - Potential for transplanting or enhancing signaling in "host" cells pre vs post transplantation
    - Understanding how waste products in RGCs and neighboring cells (e.g. microglia) impact the health of function of transplanted RGCs.

- How much is understood about the role that transplanted RGCs play at improving cell survival of RGCs in disease models? Studies have suggested that transplanted RGCs and certain types of stem cells improve survival of endogenous RGC in the face of injury (potentially from trophic support vs signals for maturation, or development of an ideal microenvironment). Could co-transplants be leveraged to enhance donor RGC survival?

- Many short-term neuroprotection studies have been performed that identify pro-survival pathways for RGCs. How can these pathways be modulated in donor RGCs to enhance long term survival? Post transplantation considerations regarding un-checked pro-survival signaling should be considered in terms of risk of oncogenic potential.
    - For example: To what extent can apoptotic and regenerative signaling be uncoupled safely (targeting c-jun or DLK)?
    - Are additional signals for enhancing axon regeneration needed?

- Do we have a way of establishing that once transplanted RGCs have survived, that they are mature and functioning? Should endpoints of function and maturity be established, and if so, what are the candidate endpoints? Does functional integration enhance RGC survival?

Subtopic Discussion Group (SDG) #4: Inner Retinal Wiring

Topic #2: RGC extrinsic factors: What are the local retinal microenvironmental (RGC extrinsic) signals that effect dendritogenesis and synaptogenesis to permit successful integration and proper connectivity to the inner retinal circuitry during development and following transplantation?

Moderator: Yvonne Ou
Introductory Comments: Tom Johnson
Scribes: Julie Cho and Michael Gilhooley

- What is already known about the role of non-RGC signals (e.g., including bipolar and amacrine cell surface receptors, extracellular matrix molecules, secreted factors) that govern establishment of inner retinal circuits during development, and what are the key areas where further research is needed?

- What is the role of the internal limiting membrane in establishing RGC somal lamination and polarization? What implications does this have for considerations of ILM disruption or removal for enhancing RGC engraftment following intravitreal or epiretinal transplantation?

- How do the expression and localization of relevant RGC-extrinsic factors that control IPL circuit specification change during development, and are the necessary factors still expressed and similarly localized in the mature retina?

- What environmental factors inhibit dendrite sprouting and promote synapse stability in maturity, and how might these impact integration of repopulated RGCs? How can these inhibitory signals be modified?

- How can we leverage organoid models to better understand these questions?

Subtopic Discussion Group (SDG) #5: Eye to Brain Connectivity

Topic #2. What is the role of glia in axonal regeneration?

Moderator: Kimberly Gokoffski
Introductory Comments: Kimberly Gokoffski
Scribes: Micalla Peng and Meher Saleem

- What role do glia play in the inhibition/promotion of axon regeneration?
- What are the cell type and subtypes specific responses to glial derived signals?
- What are the lessons learned from spinal cord injury?
- How important is timing for the suppression or promotion of glial activity?
- How is myelination specified along regenerating axons?

Concurrent SDG Discussions: Topic #3

11:15am – 12:15pm

SDG #1: Aspen Room
SDG #2: Cripple Creek 1
SDG #3: Cripple Creek 2
SDG #4: Crestone A
SDG #5: Crestone B

Subtopic Discussion Group (SDG) #1: RGC Development and Differentiation

Topic #3: What are the relative strengths and weaknesses of various approaches to producing new RGCs, how can we improve upon current methods with an eventual goal of RGC replacement, and how can we target cells to better effect RGC differentiation and correct axonal guidance?

Moderators: Don Zack and Tom Reh
Introductory Comments: Jason Meyer (Arup Das)
Scribes: Casey Keuthan and Juliette Wohlschlegel

RGCs can be generated from ESCs and iPSCs through dissociated cell culture protocols and by making retinal organoids. CRISPR-generated cell lines that have fluorescent reporters that can be used to monitor the production of RGCs are available.

- Guidelines are needed for the molecular and functional characterization of ex vivo generated human RGCs. What are appropriate quality controls metrics for RGC generation?

- Is it possible to generate differentiated RGCs, or push them further than the precursor state, in a 2D culture? What are the markers (TF, protein, morphological, functional) of fully differentiated RGCs in vitro? How do RGCs generated from 2D culture differ from RGCs generated from 3D organoids?

- As we learn more about the factors that control RGC subtype, can we use this knowledge to generate RGC subtypes from pluripotent stem cells? Reporter iPSCs for subtypes of RGCs may help to develop protocols for generating or selecting subtypes of RGCs. Are there any currently?

- Can we generate RGCs more efficiently in vitro via organoids? A protocol is needed to make RGC-rich organoids (with cells preferentially adopting RGC cell fate), that survive durably, and that are derived from a cell type that won't alarm host immunity. What is the role of bio-reactors or scaffolds?

- What are the critical factors that support the survival and maturation of RGCs in retinal organoids? In most retinal organoid preparations, RGCs start to die by about 2-3 months of age. Identifying the causes and mechanisms of this RGC disorganization cell death and the critical factors that could support the survival of RGCs and their final maturation will help guide strategies to achieve efficient RGC production.
    - Can we improve on retinal organoids by co-culturing target cells/tissues, in assembloids?
    - What is the role of neuroprotective strategies, such as DLK inhibition, in the context of 2D cultures and organoids?
    - Are non-neural cells required for RGC development in vitro? Some cells, such as microglia cells are important for the development of the retina, because they regulate neuronal survival and synaptic pruning. However, microglia have a hematopoietic lineage. For creating proper cell-cell interactions within the organoids, is it important to encourage the development of multiple lineages?

Subtopic Discussion Group (SDG) #2: Transplantation Methods and Models

Topic #3: To what extent do the host immune and glial responses affect donor RGC survival and engraftment after transplantation?

Moderator: Tom Johnson
Introductory Comments: Shane Liddelow and Petr Baranov
Scribes: Erika Aguzzi and Jonathan Soucy

- What is the role of adaptive immunity and graft rejection in limiting RGC survival or engraftment? How does the immune system respond to xenogeneic, allogeneic, or autologous transplants? What are the strengths and weaknesses of various options for overcoming these adaptive immune responses? Potential strategies include:
    - Local or systemic immune suppression
    - Autologous transplantation from iPSCs
    - Donor cell HLA banking and recipient-matching
    - Engineering donor RGCs for immune evasion

- What is the role of induced innate immunity and inflammation (including the response of microglia) in limiting or enhancing RGC survival, engraftment, and synaptic maintenance? To what extent does the transplantation procedure itself induce these processes in comparison to the donor RGCs themselves? How are these responses similar or different in the retina, optic nerve head, optic nerve, and brain? How can inflammatory responses be controlled experimentally and clinically?

- How does age or various optic neuropathy disease states prime the immune system to interact with donor RGCs? How might therapeutic delivery methods alter the eye's immune response and interactions with host RGCs?

- What is the role of reactive gliosis in limiting (or encouraging) RGC survival and engraftment? How can this be controlled experimentally and clinically?

- What models are necessary to study the immune and glial responses to neuronal transplantation? Can rodents provide sufficient information given inherent differences compared to the primate and human immune system?

Subtopic Discussion Group (SDG) #3: RGC Survival, Maturation, and Host Interactions

Topic #3: What is the best way to assess appropriate functional integration of transplanted RGCs with host cells?

Moderator: Ahmara Ross
Introductory Comments:
Scribes: Aboozar Monavarfeshani and Ziming Luo

- Is there a way to encourage functional connectivity of donor RGCs with inner retinal neurons (bipolar and amacrine cells)? What molecular signaling pathways are most important in driving IPL synaptogenesis during development? Can modulation of those pathways following RGC transplantation encourage synaptogenesis in an organized way?

- What are important models or demonstrations of establishing meaningful functional integration of transplanted RGCs on the single-cell or cell population level?

- Is there a role for transsynaptic labeling techniques? Can modified transsynaptic viruses be used in a way that minimizes neurotoxicity to infected cells?

- What do we understand about the process of synaptic pruning during development and during RGC degeneration from glaucoma or other optic neuropathies? To what extent do those processes relate to functional integration of repopulated RGCs?

- Are certain RGC subtypes more or less amenable to integration and can they be specified prior to transplantation, or will specification take place post-transplantation? What are the most efficient methods for studying subtype specification of repopulated RGCs?

Subtopic Discussion Group (SDG) #4: Inner Retinal Wiring

Topic #3: The diseased RGC: How do optic nerve injuries alter endogenous RGC structure and connectivity in the IPL, and are the implications for RGC repopulation?

Moderator: Adriana Di Polo
Introductory Comments: Bart Borghuis and Lieve Moons
Scribes: Julie Cho and Michael Gilhooley

- Damage to the optic nerve alters RGC dendritic morphology and synaptic connectivity. However, a full picture of how RGC structure and connections in the IPL are altered in optic neuropathies is still lacking. Recent studies suggest that some RGC subtypes are more vulnerable to dendritic damage than others (i.e., OFF RGCs). What are the most vulnerable RGC subtypes that undergo loss of synaptic connections in the IPL in human optic neuropathies? What are the specific structural and functional changes that occur in distinct RGC subtypes following optic nerve injury? How is this similar or different for different forms of optic nerve injury and among different species?

- RGC transplantation is a promising strategy for cell replacement in optic neuropathies. Should specific RGC subtypes be favored for transplantation to maximize re-establishment of intraretinal circuits? Which RGC subtypes and inner retinal circuits are most readily regenerated by transplantation or transdifferentiation? Can we use new knowledge of RGC vulnerability versus resilience to select the “best” RGC subtypes for transplantation to enhance integration in the IPL? Can we reprogram RGCs based on the most resilient types to improve integration and connectivity?

- What are the minimal RGC subtypes required for vision restoration? Which RGC subtypes and inner retinal circuits are most crucial to regenerate in humas? Will excitatory synapse development be enough, or will functional vision require the re-establishment of more complex patterns of synaptic inputs (inhibitory, sublamina-specific)? What aspects of inner retinal circuits can be modeled in mice, and which will require models that are more similar to humans?

Subtopic Discussion Group (SDG) #5: Eye to Brain Connectivity

Topic #3. How do axons navigate to their appropriate targets?

Moderator: William Guido
Introductory Comments: William Guido
Scribes: Micalla Peng and Meher Saleem

- How do we study axon target interactions in the absence of long-range guidance navigation (i.e., development of distal injury models)?
- How do we assess the nature of re-innervation from a structural, functional, and behavioral perspective?
- What are the behavioral assessment tools needed to assess successful re-innervation?
- How important is it to rebuild topography?
- How do we "reprogram RGCs" to reinnervate original targets?
- How should we prioritize axon-target interactions (by cell type, retinorecipient targets)?
- How do axon-axon vs axon target cues contribute to re-innervation?
- What is the interplay of extrinsic (activity) and intrinsic (molecules) factors in promoting axon target interactions?
- How do we promote synapse formation after re-innervation?
- How do we consider circuit integration?
- What are the consequences of aberrant targeting and synapse formation?
- What are the steps needed for the induction and maintenance of targeting and innervation?

Concurrent SDG Discussions: Topic #4

1:15pm – 2:15pm

SDG #1: Aspen Room
SDG #2: Cripple Creek 1
SDG #3: Cripple Creek 2
SDG #4: Crestone A
SDG #5: Crestone B

Subtopic Discussion Group (SDG) #1: RGC Development and Differentiation

Topic #4: How can we create and engineer RGCs for improved survival and synapse formation?

Moderators: Don Zack and Tom Reh
Introductory Comments: Melanie Samuel
Scribes: Casey Keuthan and Juliette Wohlschlegel

As noted in the previous discussion, several protocols now exist for producing RGCs from human pluripotent stem cells. However, it is not known whether the cells derived from these protocols will survive transplantation and will integrate with the host in a functionally meaningful way.

- RGCs can be produced in organoids or by 2D protocols. These are typically of various stages of maturation. What is the optimal stage of development for transplanting RGCs (or precursors) for survival, integration, migration, axon growth and dendrites and synapses to form with the host?

- RGC subtypes appear to vary in their susceptibility to degeneration in disease. Are some RGC subtypes more resilient? How can we determine whether certain subtypes have an advantage after transplantation? Which RGC subtypes are better able to survive in glaucoma pathogenesis? Can we develop methods for selectively generating these subtypes for transplantation strategies? Can we transfer the relative increased survival ability of some RGC subclasses to other more vulnerable subclasses?

- What are the caspase-independent pathways that lead to RGC death, in vivo and in vitro? The mechanisms of RGC death in disease and in normal development are still not completely understood. Caspase inhibitors have been shown to rescue up to 34 percent of RGCs in rats in a previous study. RGC death might also be linked to caspase-independent reactions. Inhibiting the caspase-dependent mechanisms may allow RGC survival, but will they be functional?

Subtopic Discussion Group (SDG) #2: Transplantation Methods and Models

Topic #4: What should be the tissue- or animal-level outcomes or metrics by which success of RGC repopulation is evaluated? (Note that cell-level assays will be addressed by SDG #3, Topic #3)

Moderator: Tom Johnson
Introductory Comments: Brad Fortune
Scribes: Erika Aguzzi and Jonathan Soucy

- What are the most promising state-of-the-art methods for tracking and evaluating repopulated RGCs in vivo? How can we utilize OCT, including the possibility of contrast methods, to permit identification of individual donor RGCs? How can scanning laser ophthalmoscopy be adapted in vivo for visualization of fluorescent markers, adaptive optics for high resolution, and volumetric depth discrimination. What other in vivo imaging techniques might be particularly useful, including fluorescence lifetime imaging of endogenous fluorophores, multiphoton imaging, single-cell calcium imaging, and transsynaptic circuit tracing?

- Which functional outcomes will provide the greatest sensitivity and specificity for identifying visual improvements related to RGC repopulation? How do we parse functional contributions of donor RGCs from surviving endogenous RGCs? Multiple ERG methods for detecting RGC activity exist, including the scotopic threshold response, the photopic negative response, and aspects of the pattern ERG. What are the strengths and weaknesses of each? How might multifocal ERG be leveraged to provide functional information on RGCs with some spatial resolution? What is the role for VEP or optic nerve action potential recording? How can we leverage optical electrophysiology (in the retina or visual cortex) for in vivo assessment of neuronal function? The ISCEV provides standards on visual electrophysiological outcomes, but to what extent is greater standardization or information needed for optimal RGC-specific assays?

- What behavioral assays are feasible and would provide adequate sensitivity and specificity to discern improved visual function in rodents and large animal models?

- What methods should be employed to adequately assess for graft-to-host material transfer to ensure evaluation of true RGC replacement and rule out neuroprotective effects?

- We will discuss approaches to standardizing outcomes to facilitate comparisons across multiple approaches.

Subtopic Discussion Group (SDG) #3: RGC Survival, Maturation, and Host Interactions

Topic #4: How do non-RGCs in the retinal or optic nerve environment affect RGC transplant efficacy?

Opening: Claire Mitchell
Moderator: Jeff Goldberg
Scribes: Aboozar Monavarfeshani and Ziming Luo

- What is the possibility of developing a human *in vitro/ex vivo* retinal model to better study the process of RGC integration that reflects the true complexity of the environmental variables? Will such a model adequately mimic the "hostile" environment transplanted RGCs will face in human neurogenerative disease (glaucoma, ischemia, compression, post-trauma)?

- What is the role of glial and neuro-inflammation in RGC survival? What mechanisms are involved in the process of gliosis? Can this process be dampened, for example to facilitate more successful RGC transplantation?
    - What factors determine the neuroprotective vs neuro-destructive relationship of glial cells and RGCs?
    - How are mechanosensitive pathways linked to RGC and microglial interactions?

- What do we understand about neuro-vascular communication in the retina?
    - Is the process of functional hyperemia important for driving our understanding of neurovascular coupling?
    - What is the significance of transplanted RGCs localizing along retinal blood vessels?

- Astrocytes play a critical role in development and neuroprotection of RGCs. How well do transplanted RGCs establish functional contact with existing astrocytes? What mechanisms should be considered and potentially enhanced during the transplantation process?

Subtopic Discussion Group (SDG) #4: Inner Retinal Wiring

Topic #4: The Diseased IPL: How does the IPL environment change in the context of optic neuropathies? Will the IPL in a degenerating retina be amenable to functional integration of transplanted or regenerating RGCs?

Moderator: Yvonne Ou
Introductory Comments: Matt Van Hook
Scribes: Julie Cho and Michael Gilhooley

- Although loss of vision in optic neuropathies has been largely attributed to RGC death, recent evidence suggests that other retinal neurons are also affected. How do bipolar and amacrine cells respond to optic nerve damage? Are there structural and functional changes in bipolar and amacrine cells during disease progression? How does the structure of the inner retinal circuit and synaptic connectivity change between RGC and bipolar or amacrine cells? How does this vary among types of optic nerve injury and among species (including humans)?

- If a transplanted RGC now replaces a dead RGC, what molecules need to be expressed by pre- and post-synaptic neurons to re-establish connectivity? Are there attractive and repulsive cues that influence pre- and post-synaptic interactions?

- How do glial cells in the inner retina respond to optic nerve damage and how do they change the IPL environment? Is the change conducive or detrimental to the integration of transplanted RGCs? Are retinal microglia involved in synaptic plasticity and/or pruning of inappropriate connections of transplanted RGCs?

- How is the extracellular matrix (ECM) affected by optic nerve injury and what is the resultant impact on recreating the ordered lamination of the IPL during RGC regeneration or following RGC transplantation?

Subtopic Discussion Group (SDG) #5: Eye to Brain Connectivity

Topic #4. What is the best model system to study RGC axon regeneration?

Moderator: Kimberly Gokoffski
Introductory Comments: Kimberly Gokoffski
Scribes: Micalla Peng and Meher Saleem

- What are the best in vitro and in vivo models for studying axonal extension, regeneration, or pathfinding in different disease states, and what novel models would be useful?

- Can we apply one regenerative strategy for all optic neuropathies? If not, what disease-specific characteristics need to be considered in regenerating the optic nerve?

- What are the best in vivo models that recapitulate human anatomy?

- Should we consider standardizing models, and if so, what are the most important characteristics?

- Should we consider developmental, adult, or aged models?

Concurrent SDG Discussions: Topic #5

2:30pm – 3:30pm

SDG #1: Aspen Room
SDG #2: Cripple Creek 1
SDG #3: Cripple Creek 2
SDG #4: Crestone A
SDG #5: Crestone B

Subtopic Discussion Group (SDG) #1: RGC Development and Differentiation

Topic #5: How can we leverage Muller glial transdifferentiation for RGC replacement?

Moderators: Don Zack and Tom Reh
Introductory Comments: Tom Reh
Scribes: Casey Keuthan and Juliette Wohlschlegel

Muller Glia are able to generate new neurons in vivo in the adult mouse retina after the overexpression of the pro-neuronal transcription factor Ascl1. However, with expression of only Ascl1, the new neurons adopt a bipolar or amacrine fate. Finding the right combination of transcription factors to generate mature RGCs from Muller glia derived could lead to regenerative therapy directly to patients suffering from optic neuropathies such as Glaucoma.

- What is the optimal combination of transcription factors that could allow reprograming of Muller glia derived into to mature RGCs? How can we find this? Will this be the same in human? Will this work for subtypes?

- Does the microenvironment of the mature retina constrain the types of neurons that can be generated from Muller glia? Does the type of injury affect which types of neurons can be regenerated with this approach? Retinal degeneration causes the Muller glia to become reactive; will these changes in the cells inhibit reprogramming with TFs?

- New neurons generated from reprogrammed Muller glia will form functional synapses with existing neurons in the adult mouse retina, suggesting that mature neurons possess the ability to form new synapses; however, are the connections as specific as those that form during development, or do inappropriate synapses form?

- Functional analysis of Muller glial derived neurons has so far been demonstrated with single cell electrophysiology. What tests would provide the best evidence for functional restoration (or improved vision) in cases where neurons have been derived from Muller glia?

- RGCs derived from Muller glia, like those from transplants, will need to send axons to the brain targets to provide any benefit. What additional factors will be needed to stimulate long distance axon growth of reprogrammed Muller glia-RGCs, through the mature visual pathways to find the correct targets and make the correct synapses?

- How can we be sure that vector-mediated cell reprogramming is actually generating retinal ganglion cells? Recently, several studies have made spectacular claims about the ability to rapidly and efficiently reprogram Muller glia into retinal ganglion cells using simple AAV-based constructs (e.g. Fu, et al. Cell 2020; Xiao, et al. Front Cell Dev Bio 2021). Other studies, however, have claimed this in fact reflects technical artifacts resulting from leaky expression of glial-specific GFAP promoters (Wang, et al. Cell 2021; Chen, et al. bioRxiv 2021), and support these claims using genetic lineage analysis (Lyu, et al. bioRxiv 2021). How can we avoid similar pitfalls when applying vector-based reprogramming to species where genetic lineage analysis studies are practical, such as primates?

Subtopic Discussion Group #2: Transplantation Methods and Models

Topic #5: What surgical transplantation strategies will maximize RGC survival and engraftment?

Moderator: Tom Johnson
Introductory Comments: Kapil Bharti
Scribes: Erika Aguzzi and Jonathan Soucy

- The vitreous may limit migration of intravitreally injected RGCs towards the retina and the vitreous cavity may not be supportive of sustained RGC survival (due to hypoxia, lack of metabolic support, and lack of a growth substrate). What approaches might help overcome this, and what is feasible in rodents versus larger animal models? Potential approaches include:
    - Vitreous digestion or permeabilization
    - Vitrectomy
    - Chemokine gradients to encourage mobilization of donor RGCs to the retina
    - Biocompatible scaffolds
    - Slow release neuroprotective or immunomodulating drugs or growth factors

- The ILM limits engraftment of donor RGCs. What are the strengths and weaknesses of various approaches to circumventing the ILM barrier? How might these affect Muller glia? Potential strategies include:
    - Enzymatic digestion
    - Mechanical peeling
    - Sub-ILM injection

- How do the vitreous, ILM, and retinal extracellular matrix change with age or in disease states and what implications does this have for RGC engraftment?

- How should cells be delivered into the eye? What are the strengths and weaknesses of intravitreal versus subretinal transplantation? Scaffolds have been shown to improve RPE and photoreceptor transplants. How might scaffolds be leveraged to augment RGC survival and engraftment? What are potentially beneficial design characteristics?

- Some published data suggest that transplanting fewer RGCs yields greater cell survival (on a percentage basis). Why might this be? Considering this finding, how many RGCs should be transplanted and how should this be scaled according to the size of the eye / animal model being studied?

- What extent of topographic retinal coverage by donor RGCs is necessary to yield meaningful functional improvements in vision? What local RGC density is necessary? How can spatial localization and density be controlled with RGC delivery techniques?

Subtopic Discussion Group #3: RGC Survival, Maturation, and Host Interactions

Topic #5: What are the clinical and translational considerations of successful RGC transplantation, integration, and function?

Moderator: Ahmara Ross
Opening: Juliette McGregor and Jeff Goldberg
Scribes: Aboozar Monavarfeshani and Ziming Luo

- How can transplanted RGCs be imaged and followed safely in living animal models (including humans) over long periods of time? What are the best markers of function to track over time that can give an appropriate assessment of the biological, chemical, and electrical processes?

- Methods to identify and track donor, newborn and rescued cells in vivo need to be established and rigorously characterized and compared. Tracers, cell lines and other molecular tools can be used to visualize cell structure and health and parse donor from host cells. What are the limitations of dye tracers, genetically encoded tracers, and AAV mediated tracers? How do we deal with intercellular material transfer, including cell fusion, cytoplasm exchange and other forms of material transfer as confounding factors to study RGC survival?

- What are the major considerations and limitations for establishing meaningful neuro-protection and/or restoration of vision? How many, what subtypes, and what retinal locations of functional RGCs are required to support useful vision?

- How can we best measure neuroprotection or vision restoration in the clinic? Are ultra-low vision psychometric tests needed? Should QoL be a potential endpoint?

Subtopic Discussion Group #4: Inner Retinal Wiring

Topic #5: Studying and modulating the host retina: Can we modify the inner retinal environment to enhance functional integration of transplanted or regenerating RGCs, and how do we most effectively study this process?

Moderator: Adriana Di Polo
Introductory Comments: Melanie Samuel
Scribes: Julie Cho and Michael Gilhooley

- Can gene expression be manipulated in bipolar and/or amacrine cells to facilitate the integration and connection of transplanted RGCs? If so, what types of molecules are desirable for this purpose (e.g., guidance molecules, enhancers of dendritogenesis, synaptogenesis)?

- Can Muller cells be genetically modified to provide support to transplanted or regenerating RGCs during neurodegeneration and neuroregeneration?

- What neurotrophic or growth factors can be provided to facilitate and support RGC integration?

- Can the ECM be modified to enhance the functional integration of transplanted RGCs?

- What other methods or strategies can we envision to manipulate the inner retina to facilitate RGC integration and functional recovery?

- How might organoids be leveraged to model RGC transplantation or repopulation in a high-throughput and highly controllable manner?

- How should the successful integration and proper connectivity of transplanted RGCs be functionally characterized? Should there be a standard set of functional assessments, and if so, how do we define successful functional integration? What are the strengths and weaknesses of optical versus whole cell electrophysiology? How can transsynaptic circuit tracing methods be leveraged to study functional inner retinal circuits over time, given the inherent neurotoxicity of commonly-used viral tracers (i.e., rabies virus and HSV)?

Subtopic Discussion Group #5: Eye to Brain Connectivity

Topic #5. Is there a critical period for regeneration?

Moderator: William Guido
Introductory Comments: William Guido
Scribes: Micalla Peng and Meher Saleem

- Is regeneration simply a recapitulation of development?
- Does regeneration require the activation of developmental pro-growth signaling pathways?
- How does regeneration proceed?
- Is there a critical window (after insult) for regeneration?
- What is the interaction between axonal degeneration and axonal regeneration?
- Does the delayed clearing of degenerative debris impede regeneration?
- How important is age? Can adult RGCs support the length and speed of axon extension needed for regeneration?