Retinal Regeneration Through Reversion of Müller Glia Into Photoreceptors: A Novel Strategy for Diabetic Retinopathy

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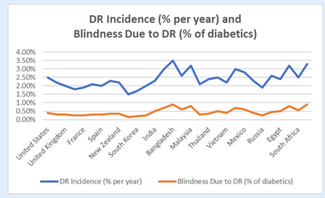
Introduction

Diabetic retinopathy causes irreversible blindness by destroying retinal neurons that the adult human retina cannot naturally regenerate. Unlike regenerative species, human Müller glia (MG) remain locked in a non-neurogenic state due to restricted chromatin, inflammation, and lack of proneural factors such as ASCL1. We propose a regenerative strategy using **mRNA-delivered ASCL1** with epigenetic enhancers to **unlock MG plasticity**. ASCL1, a pioneer transcription factor, opens chromatin, **represses glial identity**, and activates neurogenic programs. This approach aims to reprogram MG into neuron-producing cells to **restore vision lost** in diabetic retinopathy.

Prevalence

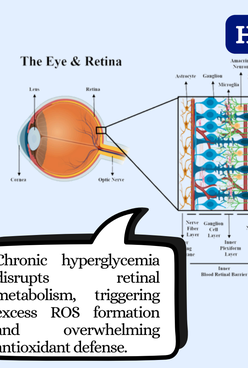


Diabetic retinopathy (DR) has become a global vision crisis, with prevalence rates soaring to **20–34%** in high-burden regions. Each year, **2–4%** of individuals with diabetes advance to sight-threatening DR, and a growing fraction enter the irreversible trajectory toward blindness. As diabetes surges worldwide, DR now stands among the **most rapidly escalating** causes of permanent vision loss. This rising epidemiological burden underscores an urgent scientific imperative: to move beyond disease management toward **regenerative strategies** capable of replacing the retinal neurons destroyed by diabetic neurodegeneration.

Müller Cell Reversion

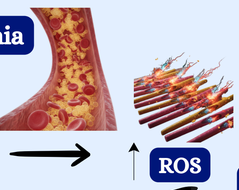
Müller glia, the retina's central support cells, typically enter **reactive gliosis** in diabetic retinopathy, showing **GFAP elevation, STAT3 activation**, and **tightly closed chromatin** at neurogenic loci. This rigid glial state blocks their ability to re-enter a progenitor phase or regenerate lost neurons. Reversion-based therapy aims to overcome these barriers using **ASCL1**, a master proneural transcription factor, combined with epigenetic priming through HDAC inhibitors or TET activators. This combination opens chromatin, suppresses glial identity, and r**estores progenitor markers** like **SOX2, Pax6, and Lhx2.** Once de-differentiated, Müller glia transform into **retinal progenitor-like cells** capable of adopting **photoreceptor fate** when guided by key regulators such as **CRX, OTX2,** and **NRL.** This strategy enables the diabetic retina to **regenerate functional photoreceptors**, offering a powerful and promising regenerative therapy for **vision restoration.**

Mechanistic Pathway of Müller Glia Reprogramming for Photoreceptor Regeneration in Diabetic Retinopathy



Hyperglycemia

Chronic hyperglycemia disrupts retinal metabolism, triggering excess ROS formation and overwhelming antioxidant defense.



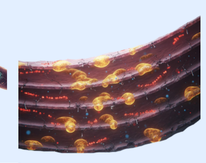
ROS

ROS overload impairs mitochondrial function, causing energy failure, lipid peroxidation, and cellular oxidative damage

Mitochondrial Dysfuction

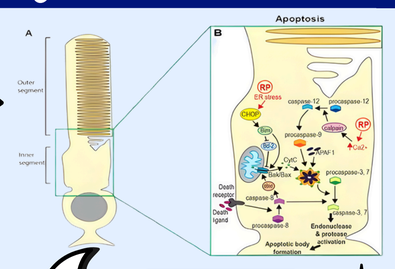
Lipid peroxidation

Excess ROS trigger mitochondrial dysfunction and lipid peroxidation, overwhelming retinal defenses and accelerating photoreceptor injury.



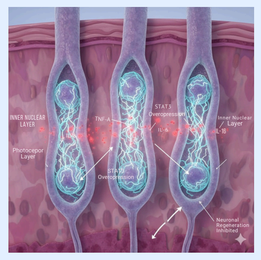
Oxidative Injury to Rods & Cones

Rods and cones sustain oxidative injury, impairing their structural and functional stability.

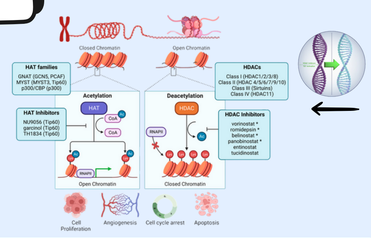


Photoreceptor apoptosis

Photoreceptors undergo early apoptosis in DR, even before microvascular injury becomes clinically apparent.



Müller glia enter a reactive gliosis state

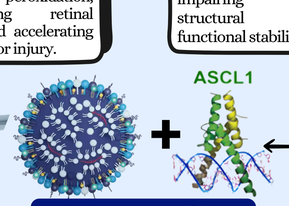


Unlocking the DNA via Epigenetic Priming

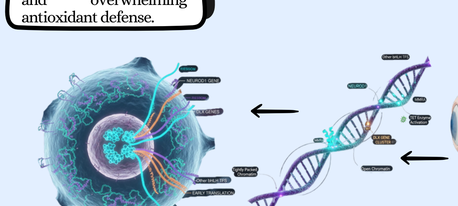
ASCL1 binds neuronal promoters and represses glial genes, initiating reprogramming when chromatin is accessible.

Neurogenic loci remain epigenetically silenced in MG, requiring chromatin opening for regenerative reprogramming.

MG hypertrophy, cytokine release, and STAT3 activation create a pro-inflammatory, regeneration-resistant retinal environment.



ASCL1 Gene Delivery mediated by nanoparticle delivery system

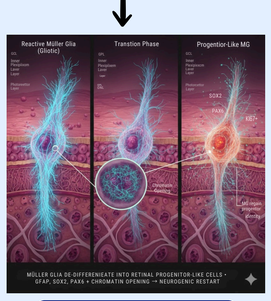


CRX, OTX2, NRL and NEUROD1 drive MG-derived progenitors toward committed photoreceptor identity.

Reduced GFAP/CRALBP with rising SOX2, Pax6 and Lhx2 drives MG toward a retinal progenitor state

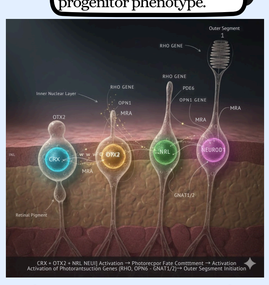
Reduction of Glial Identity

HDAC inhibition and DNA demethylation create an ASCL1-permissive chromatin statedrive Müller-glia toward a progenitor phenotype.



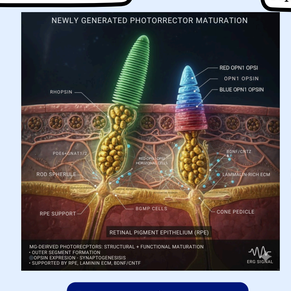
Activation of Stem Markers (SOX2, PAX6 ↑)

Glial markers fall as progenitor genes rise, shifting MG into a stem-like regenerative state.



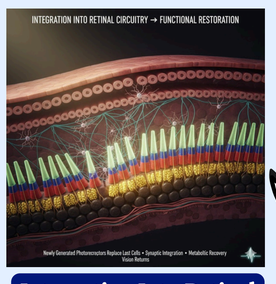
Photoreceptor Fate Specification

Reprogram-med MG-derived progenitors commit toward rod and cone photoreceptor lineages.



Maturation Into Functional Photoreceptors

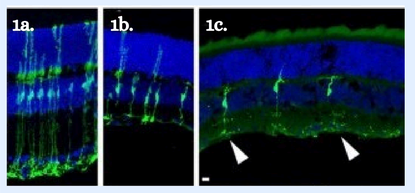
Newborn photoreceptors undergo structural and functional maturation into light-sensing cells.



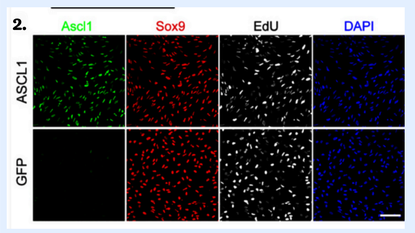
Integration Into Retinal Circuitry

Regenerated neurons integrate into existing retinal circuits, restoring signal transmission.

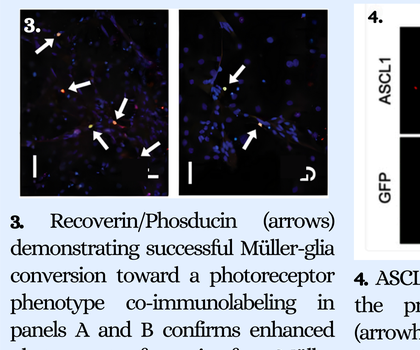
Key Research Studies Supporting This Therapeutic Approach



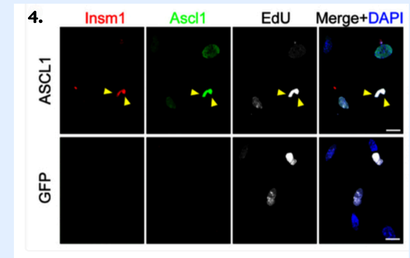
1a.- Control Müller glia showing normal radial glial morphology
1b.- ASCL1-expressing Müller glia retain predominantly glial structure with minimal change.
1c.- ASCL1 + injury + TSA–treated Müller glia acquire neuron-like morphology with extended processes **[1]**. ● -ASCL1 expressing cells



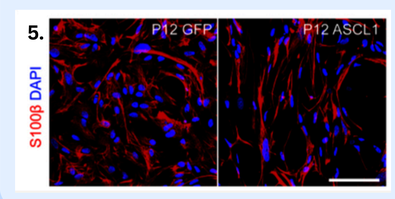
Graph 2 -
ASCL1-infected P12 MG express Sox9 (red), incorporate EdU (white) and express Ascl1 protein (green)
(4 dpi). GFP-infected MG do not express Ascl1. Scale bar: 100 µm **[2]**.



3. Recoverin/Phosducin (arrows) demonstrating successful Müller-glia conversion toward a photoreceptor phenotype co-immunolabeling in panels A and B confirms enhanced photoreceptor formation from Müller glia under reprogramming conditions compared with control [2].



4. ASCL1-infected ASCL1+EdU+ MG express the progenitor marker Insm1 (4 dpi) (arrowheads indicate triple-labeled cells)
(Data are mean±s.e.m. *P<0.05, **P<0.01; Student's t-test. Scale bars: in A, 100 µm; in C, 20 µm.) **[3]**.



Graph 5. showing reduced glial protein S100β after ASCL1 injection in right image.
Left image showing GFP(control) **[3]**.
● -S100β (glial marker)
● -DAPI (nucleus)

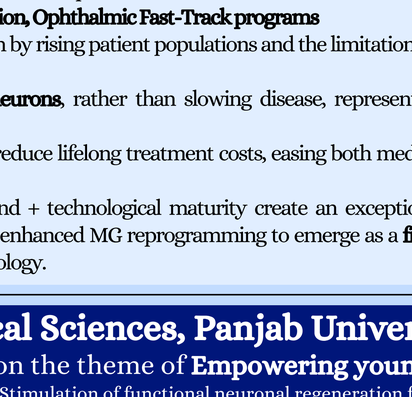
Mechanistic Pathway of Müller Glia Reprogramming for Photoreceptor Regeneration in Diabetic Retinopathy

1 ASCL1-modRNA With Epigenetic Design

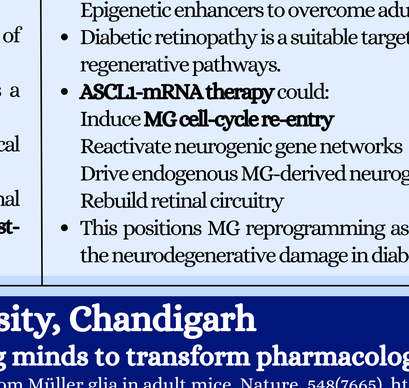
2 Engineered LNPs for Targeted Delivery

3 Intravitreal Administration of LNPs

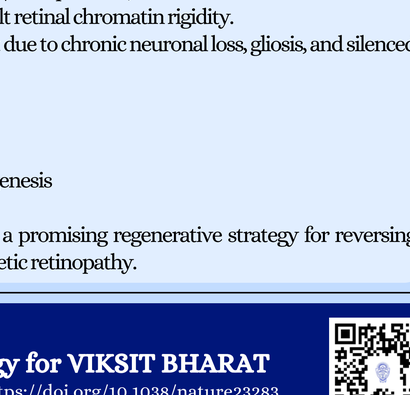
4 LNP Diffusion Across Retinal Barriers



5 ASCL1 Translation Inside Müller Glia



6 Photoreceptor Regeneration Begins



Results & Discussions

- We propose a **regenerative strategy** capable of **restoring vision lost** to diabetic retinopathy.
- **mRNA-delivered ASCL1** enables precise, transient expression without genomic integration.
- ASCL1 functions as a potent pioneer transcription factor, binding previously inaccessible E-box motifs in closed chromatin.
- **Reactivates dormant neurogenic programs** in Müller glia (MG).
- **Induces key retinal progenitor regulators**: Hes5, Insm1, HES6.
- Triggers **controlled MG proliferation,** restoring developmental plasticity.
- Initiates neuronal differentiation pathways required for rebuilding degenerated retinal layers.
- **Epigenetic enhancers** loosen compacted chromatin and amplify ASCL1's transcriptional access.
- Enables transcriptional reprogramming of MG even in the restrictive environment of the diseased adult retina.
- MG transition from **static glial cells → neurogenic progenitors** capable of generating multiple retinal neuronal subtypes.
- Induced neurons can potentially:
  - Re-establish synaptic connectivity
  - Rebuild damaged retinal circuitry
  - Restore phototransduction and visual signaling
- This strategy surpasses conventional disease-slowing treatments, offering **true retinal reconstruction.**

Regulatory & Market

- DR is now one of the fastest-increasing causes of irreversible blindness, creating a major unmet clinical need.
- Current standard-of-care treatments (laser, corticosteroids, anti-VEGF) → slow progression but do not repair neurodegeneration.
- This therapeutic gap creates a high-value market opportunity for MG-based regenerative therapies.
- mRNA-delivered transcription factors, such as ASCL1, align strongly with modern regulatory infrastructures established for:
  mRNA vaccines, Gene-modulating biologics , Non-integrating advanced therapeutics
- mRNA platforms offer **scalability, manufacturability, transient expression**, and strong safety profiles.
- Regulatory pathways supportive of advanced therapies include:
  RMAT designation, Orphan drug acceleration, Ophthalmic Fast-Track programs
- The global DR market is expanding, driven by rising patient populations and the limitations of long-term, maintenance-only treatments.
- A therapy capable of **restoring retinal neurons**, rather than slowing disease, represents a disruptive category shift.
- Regenerative MG reprogramming could reduce lifelong treatment costs, easing both medical and economic burdens.
- Regulatory momentum + market demand + technological maturity create an exceptional opening for mRNA-ASCL1, epigenetically enhanced MG reprogramming to emerge as a **first-in-class regenerative therapy** in ophthalmology.

Future Prospects

- ASCL1-mRNA therapy is grounded in strong mechanistic evidence from **rodent studies** and **human in-vitro systems**.
- In murine retinas, ASCL1:
  Opens closed chromatin
  Activates neurogenic transcriptional programs
  Reprograms Müller glia (MG) into retinal progenitor–like cells
- In human **retinal organoids** and **fetal MG**, ASCL1 activates conserved regulators (HES6, INSM1, DLL1) and initiates **neuronal differentiation.**
- These conserved effects provide a robust foundation for clinical translation.
- Future **therapeutic development** requires:
  Stabilized mRNA constructs
  Targeted delivery systems (ocular LNPs/nanoparticles)
  Epigenetic enhancers to overcome adult retinal chromatin rigidity.
- Diabetic retinopathy is a suitable target due to chronic neuronal loss, gliosis, and silenced regenerative pathways.
- **ASCL1-mRNA therapy** could:
  Induce **MG cell-cycle re-entry**
  Reactivate neurogenic gene networks
  Drive endogenous MG-derived neurogenesis
  Rebuild retinal circuitry
- This positions MG reprogramming as a promising regenerative strategy for reversing the neurodegenerative damage in diabetic retinopathy.

22nd November, 2025
Affiliation: University Institue of Pharmaceutical Sciences, Panjab University, Chandigarh
Presented at the 55th ANNUAL CONFERENCE OF **INDIAN PHARMACOLOGICAL SOCIETY** on the theme of **Empowering young minds to transform pharmacology for VIKSIT BHARAT**

[1] Jorstad, N. L., Wilken, M. S., Grimes, W. N., Wohl, S. G., Vandenbosch, L. S., Yoshimatsu, T., Wong, R. O., Rieke, F., & Reh, T. A. (2017). Stimulation of functional neuronal regeneration from Müller glia in adult mice. Nature, 548(7665). https://doi.org/10.1038/nature23283
[2] Giannelli, S. G., Demontis, G. C., Pertile, G., Rama, P., & Broccoli, V. (2011). Adult human Müller glia cells are a highly efficient source of rod photoreceptors. Stem Cells, 29(2). https://doi.org/10.1002/stem.579
[3] Pollak, J., Wilken, M. S., Ueki, Y., Cox, K. E., Sullivan, J. M., Taylor, R. J., Levine, E. M., & Reh, T. A. (2013). ASCL1 reprograms mouse Müller glia into neurogenic retinal progenitors. Development (Cambridge), 140(12). https://doi.org/10.1242/dev.091355