

## Retinopathy of Prematurity Lab

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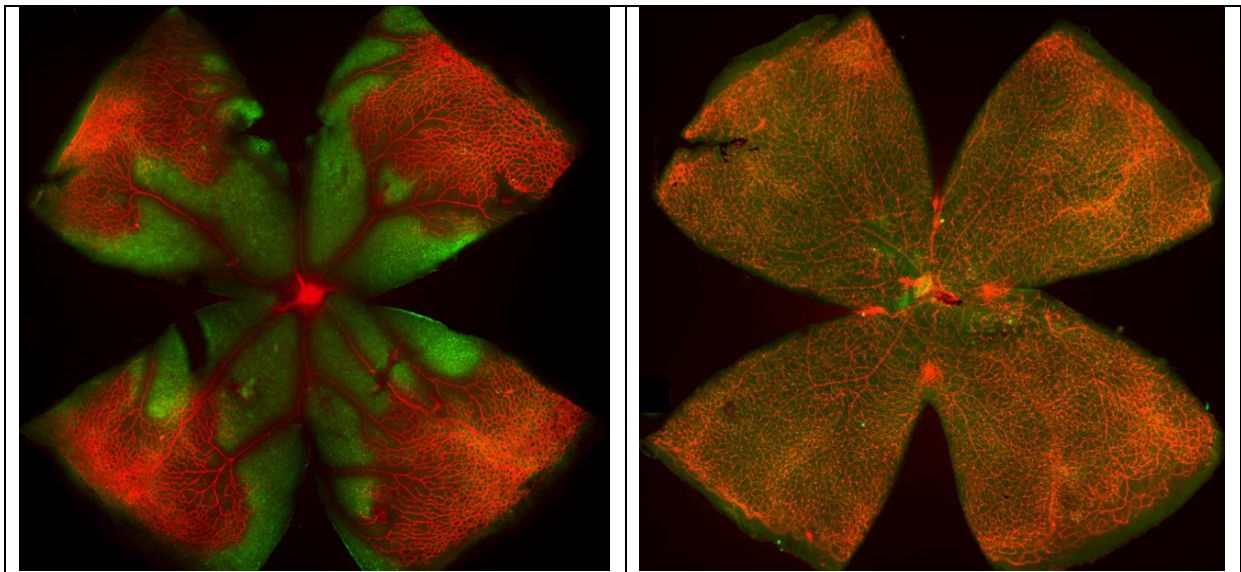
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### Laboratory goals/projects:

- 1) Eradicate retinopathy of prematurity by using a proangiogenic strategy during phase one to direct the normal sequential growth of blood vessels.
- 2) Define the metabolic basis of liver induced retinovascular plasticity.
- 3) Understand the molecular mechanism of oxygen-induced retinopathy.

**The long term goal** of this laboratory is to eradicate retinopathy of prematurity (ROP) - the most common form of infant blindness worldwide, accounting for 150,000 blind children annually. Survival after premature birth requires oxygen supplementation that is paradoxically associated with toxicity to premature developing tissues, such as the lung alveoli, nephrons of the kidney, cerebral cortex, and retinal capillaries. The direct relationship of oxygen saturation to disease severity in clinical trials as well as in preclinical investigations has placed the oxygen sensitive transcription factor hypoxia inducible factor (HIF) as a central mediator of retinovascular growth and development. We have definitively demonstrated the safety and efficacy of HIF stabilization in the prevention of oxygen-induced retinopathy (OIR) via HIF prolylhydroxylase inhibition (PHi) in preclinical models in two different species, achieving a protected phenotype for both retina and lung simultaneously by systemic PHi.



**Figure 1:** Hypoxia inducible factor stabilization prevents oxygen induced vasoobliteration and retinovascular growth attenuation. Panel left is a P12 mouse pup cycled through the oxygen induced retinopathy model, panel right is a littermate that received intraperitoneal small molecule carboxamide injection during hyperoxia. Green marks hypoxia using pimidazole stain, red marks isolectin-B4 conjugated to fluorophore.

### Studies:

### 1) Synergy between the liver and the retina

We have extensively tested dozens of small molecules with varying efficacy in stabilizing HIF. Among these, we have determined that carbonyl glycines, hydrazones, and benzolamides are three classes of drugs capable of initiating protection against hyperoxia, but each has varied specificities for liver versus eye versus other organs. For example, dimethylloxaloylglycine is able to target only the liver yet still protects retinovascular tissue whereas other compounds such as Roxadustat work synergistically with both the liver and the retina to create protection against oxygen toxicity. A western blot using both dose and time response did not see changes in retinal HIF concentrations after intraperitoneal injection of DMOG. Instead we saw clear upregulation of HIF-1 in the liver. Using a luciferase-ODD *in vivo* reporter gene, we clearly found that DMOG targeted only the liver. Surprised by this result, we next conditionally ablated hepatic *HIF1A* to demonstrate that hepatic HIF-1 $\alpha$ , with DMOG as a drug, was necessary and sufficient to transduce protection. We further proved this result by comparing two carbonyl amides- DMOG and Roxadustat using RNA seq. Transcriptional profile was nearly identical between the two drugs in the liver, but they were vastly different in the retina where DMOG had little transcriptional effect that had no overlap with Roxadustat. Finally, using LC-MS 1 and MS 2 we definitively demonstrated that DMOG was so labile as to not survive the first pass in the liver but rather never entered the blood. Roxadustat on the other hand not only entered blood but also entered retina. Therefore Roxadustat could override the hepatic HIF-1 $\alpha$  KO and worked synergistically to provide near total protection to the retina. In summary, western blot, luc-ODD reporter gene analysis, RNA-seq, conditional KO experiments, and LC-MS confirmed that indeed a visceral organ could protect a distal capillary bed and provided the notion that low and intermittent dosage of HIF stabilizers might be safe and effective in fragile premature infants in hyperoxia.

### 2) Metabolic basis of liver induced retinovascular plasticity

Transcriptional and knockout studies of PHi animals described above have determined a unique liver-eye axis that directs “remote” protection against oxygen-induced retinopathy and cardiac ischemia, respectively. We are using targeted and untargeted metabolite profiling to determine how the liver might contribute to protection of a distal capillary bed such as in the retina. After uncovering no protein based/hepatokines induced protection from the liver we used untargeted metabolite profiling to link 1) retinal serine/glycine levels and 2) activation of both the hepatic urea cycle and retinal serine/1-carbon metabolism to hepatic HIF-1 dependent, providing a metabolic phenotype of mice protected by pharmaceutical HIF stabilization against oxygen toxicity.

### 3) Metabolic basis of oxygen induced retinopathy

Using both cells in culture and animal models, we have determined that hyperoxia induces a pronounced shift in metabolism of retinal Müller glia and retina in general. Despite the well known fact that HIF stabilization induces anaerobic glycolysis, we were stunned to find that hyperoxia also restricts flow of glycolytic carbon into the TCA cycle. Instead, Müller cells use glutamine-fueled anaplerosis to generate energy. For every mole of glutamine deamidated to glutamate and further deaminated to  $\alpha$ -ketoglutarate, 2 moles of ammonia are released providing the hypothesis that oxygen toxicity to the retina may involve ammonia toxicity and therefore links nitrogen balance through transamination of 2-oxoacids and upregulation of the urea cycle as two metabolic, liver dependent pathways that might explain the synergy of liver/retina by certain classes of HIF stabilizers.

**Innovations:** The concept of pro-angiogenic strategy preventing pathologic angiogenesis is novel. This concept is translational and applies to all forms of ischemic disease, offering the potential to prevent

vascular loss before it happens even in the setting of stimuli that creates ischemia, such as in ROP or diabetes.

**Lab staff members:**



**Lab Members:** Back row: Jonathan E. Sears, MD; Charandeep Singh PhD, Research Fellow; George Hoppe, PhD, Project Staff; Vincent Tran, BS, Research Technician; Front row: Youstina Bolok, MS, Research Technician; Leah McCollum, BS, Research Technician.

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- Jonathan E. Sears, MD, Principal Investigator
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