INTRODUCTION

Neuronal Ceroid Lipofuscinosis, or Batten Disease, is a group of fatal, neurodegenerative disorders primarily affecting children. Of the 13 subtypes, CLN3 Batten Disease is the most common with an incidence of one in 100,000. A common first presenting symptom is vision loss which progresses to blindness, rapid developmental and cognitive delay, seizures, loss of movement, and death. Our group has developed several intrathecal AAV9 based gene replacement therapeutic constructs for different forms of Batten Disease 1,2. While preliminary results from preclinical studies and potentially clinical trials are promising, the visual component of the disease might not be fully addressed. Recent studies in mice suggest that certain retinal cell types must be rescued to prevent vision loss 3,4.

**Goal:** Develop an AAV9-based retinal gene therapy for Batten Disease that can be safely and effectively combined with CSF based therapy that is already in clinic.

RATIONAL

Despite photoreceptor cells being the primary affected cell type in Batten Disease related vision loss (Figure 1), recent studies suggest cells of the inner nuclear layer, such as bipolar cells, need to be rescued to prevent photoreceptor degeneration 1,2.

**One step further:** We performed 3DX Single-Cell RNA Sequencing on Wild-Type Non-Human Primate (Macaque) retinas to identify CLN expressing cell types (Figure 2).

METHODS

**Intravitreal Injection** with AAV9 seminar results in transduction of a limited number of cell types, mainly ganglion cells and Mueller glia with limited targeting of the inner nuclear layer.

**Potential Solution:** Core deliver AAV9 with Neuraminidase enzyme, previously used by Wilson et al. to target targeting of other organs, to enhance retinal transduction of additional cell types.

RESULTS

**Neuraminidase Treatment Doubles the Number of AAV9 Transduced Amacrine Cells**

AAV9 alone transduces, on average, less than 10% of amacrine cells. Transduction increased to almost 20% with neuraminidase treatment. (Confocal, [50,000nm scale bar]).

**Neuraminidase Treatment Doubles the Number of AAV9 Transduced Ganglion Cells**

AAV9 alone transduces, on average, less than 1% of ganglion cells. Transduction increased to almost 20% with neuraminidase treatment. (Confocal, [50,000nm scale bar]).

CONCLUSIONS

• The addition of neuraminidase to intravitreal injections of AAV9-GFP drastically increased transduction to all layers of the retina.
• With the exception of ganglion cells, neuraminidase nearly doubled transduction of AAV9 in all retinal cell types. Ganglion cells are normally and efficiently transduced with AAV9 alone.
• Neuraminidase visibly increases transduction of AAV9 to photoreceptor cells, suggesting this treatment might be a safe and effective alternative to photoreceptor targeting with subretinal injection.
• Histology of Neuraminidase only injected retinas indicate a transient immune response with no detectable damage to the retina.

**Future Directions:** Test this proof-of-concept therapy in large mammals and further determine efficacy and safety in Batten Disease mouse models prior to potential transition to clinic.