## A Human Chimeric Retinal Organoid Model for Studying Donor-Host Cell Interactions in Cell Therapies for USH2A-Associated Retinitis Pigmentosa

Cassandra D'Amata

**Introduction:** There are currently no human models of retinal cell engraftment that enable the study of allogenic donor-host cell interactions between healthy and disease cells in a degenerating retina. Our lab has generated chimeric retinal organoids ("chimeroids") from a mixture of pluripotent stem cell lineages. We hypothesize that our chimeroids represent a novel in vitro human model of retinal cell engraftment in a common USH2A mutant causing autosomal recessive retinitis pigmentosa, and will provide a new way to study cell-cell interactions between healthy and diseased human retinal cells.

**Methods:** Chimeroids were generated using patient-derived USH2A mutant iPSCs, which we edited with CRISPR/Cas9 to express nuclear H2B promoter-driven GFP (USH2A-GFP), and H9 ESCs that are lentiviral-transduced to express nuclear H2B promoter-driven RFP (H9-RFP). We combined cultures of undifferentiated H9-RFP and USH2A-GFP cells in various ratios in 2D, alongside single lineage controls. We then differentiated 3D retinal organoids following established protocols. USH2A has a role in photoreceptor (PR) outer segment (OS) maintenance, which is critical to PR cell survival. Chimeroids and control organoids were evaluated for markers of retinal cell fate specification, gene expression, and PR morphology at different stages of organoid development.

**Results:** USH2A diseased organoids show markedly shorter presumptive OS, lower Rhodopsin (Rho) expression, and lower Arrestin-3 (Arr3) expression in mature organoids (Week 24) compared with healthy H9 controls. Decreased Rho and Arr3 gene expression in USH2A organoids was confirmed by qRT-PCR. In contrast, chimeroids display longer presumptive OS as well as improved Rho and Arr3 protein expression compared to diseased controls. Restored Rho- and Arr3-expressing USH2A-derived cells within chimeroids can be observed by immunohistochemistry in regions of high mosaicism as well as USH2A-GFP dominated regions.

**Conclusion:** We have generated retinal chimeroids using a patient-derived diseased iPSC line. Ongoing small molecule testing and FAC sorting will allow us to examine the modified properties of healthy and diseased cells in our chimeroids. This may reveal new therapeutic targets. Our modular chimeroid platform may, in principle, be applied to any inherited retinal disease genotype for which human iPSCs and an organoid phenotype exist.