**2023 Multidisciplinary Research Program in Medicine Project:** Neuronal Degeneration in the Retina: Validation by In Vivo Imaging and Ex Vivo Cellular and Molecular Analysis in Mouse Models of Age-related Macular Degeneration (AMD)

**Hypothesis or Research Question(s):** Our hypothesis is that Granzyme B (GzmB), a serine protease enzyme recently identified in human and mouse outer retina, cleaves key extracellular matrix proteins and thereby contributes to AMD development by triggering RPE cell degeneration and subsequent breakdown of the blood-retinal barrier. This project will develop novel in vivo and ex vivo methods to identify the ophthalmic signature of RPE atrophy and blood-retinal barrier abnormalities.

**PROJECT BACKGROUND & SUMMARY**

Rationale: Age-related macular degeneration (AMD) is a common eye condition that can cause loss of vision among people 50 and older. It causes damage to the back of the eye, in an area called the macula, which is needed for sharp focus and central vision. In some people, the 'dry' form of AMD advances so slowly that vision loss does not occur for years. However, in patients with the 'wet' form of AMD, vision deteriorates quickly and may lead to permanent visual loss in the macular region of the eye. There are no treatments for the dry form, and drugs for the wet form have come under recent scrutiny by scientists as the drugs may harm the retina over the many years of use that is required to keep 'wet' AMD from reoccurring. Also, some 'wet' AMD patients do not respond or become resistant to the existing drugs. We will study Granzyme B (GzmB), an enzyme recently identified by us to be present in the human and mouse retina. GzmB activity causes a slow deterioration of the proteins in the extracellular matrix of the outer retina, features that have been linked to dry AMD. Extracellular GzmB activity also causes blood vessels to grow, a process that has been linked to dry AMD. Extracellular GzmB activity also causes blood vessels to grow, a process that has been linked to dry AMD.

Proposed Multidisciplinary Research Approach: A key feature of dry AMD is the degeneration, or atrophy, of the retinal pigment epithelial (RPE) cell. We will study the cellular and molecular signature of the RPE cell as it degenerates in a mouse model of dry AMD. We will pair this cell and molecular approach with a bioengineering approach to image the RPE cell in the live, anesthetised mouse, which will provide an in vivo imaging signature of RPE atrophy. Given that the RPE is an important component of the outer blood-retinal barrier, this project will also focus on imaging the outer blood-retinal barrier function. This multidisciplinary approach is valuable for future translational studies on all AMD patients, as the methods for ophthalmic imaging developed in mice will be translated to the clinic to allow physicians to track key changes in the outer retina in dry AMD patients. This will provide an important quantification method to assess whether future drugs developed for AMD can slow the degeneration or atrophy of the RPE cell. The eye images acquired from microscopy will provide a dataset for developing deep neural networks to discern key image features that will become the "retinal signatures" that represent RPE atrophy and other markers of AMD progression.

Expected Outcomes: The expected outcomes of the proposed study include 1) data collection of cellular and molecular changes that characterize RPE atrophy in eye tissue; 2) development of hardware and software to support bioengineering methods of sensorless adaptive optics and polarization diversity/contrast as it relates to ophthalmic imaging of mouse models and 3) characterization of key in vivo features of RPE atrophy and validation of these in vivo features by ex vivo measurements of RPE atrophy by tissue processing and confocal/super-resolution microscopy.

**BENEFIT TO THE STUDENTS**
2023 Multidisciplinary Research Program in Medicine Project: Neuronal Degeneration in the Retina: Validation by In Vivo Imaging and Ex Vivo Cellular and Molecular Analysis in Mouse Models of Age-related Macular Degeneration (AMD)

GAIN UNDERSTANDING OF CONDUCTING HIGH QUALITY RESEARCH- TS will learn how to conduct a high quality research project by several methods: TS will read selected, relevant scientific papers and discuss/critique experimental design with both supervisors. TS will learn the ethical considerations of using animals in research and safety methods for using biohazard by taking online UBC courses. TS will learn basic statistics to identify the value of a power calculation to estimate sample size in animal studies, how to develop a testable hypothesis, and appropriate statistical tests for studies. TS will learn to problem-solve and trouble-shoot experimental methods and data collection as the situation requires. TS will zoom with at least one, most likely both supervisors, once every week to provide ample time to discuss and ask questions towards research project.

DEVELOP NEW SKILLS- In this multidisciplinary project TS will develop skills in cellular/molecular methods as well as in optics, retinal structure and function and in vivo retinal imaging. Cellular/molecular skills will include confocal microscopy, advanced image analysis, protein assays (immunohistochemistry, ELISA and western blots). Bioengineering/optics skills will include understanding the basics of optics of the eye and lens, adaptive optics technology, optical coherence tomography, principles of confocal microscopy and super-resolution microscopy.

INTERACTIONS WITH OTHER RESEARCHERS- TS will collaborate and work with both supervisors and their respective lab members, which will include postdoctoral fellows, graduate students, medical students, undergraduate students, research associates and technical staff. TS will attend weekly lab meetings to discuss and network with other lab members, participate in journal club presentations. TS will give progress reports on their data every two weeks.

AVAILABLE RESOURCES- TS will work in an established basic science laboratory fully equipped for proposed studies using protein assays, tissue culture, paraffin and cryostat microtomes, bright and dark-field microscopes, confocal and super-resolution microscopes. TS will work with team members in the in vivo imaging lab situated in the Jack Bell animal facility, equipped with both commercial ophthalmic imaging and custom-designed imaging devices for rodents. TS will have opportunities to present their research results at departmental research days, and in virtual or in person scientific meetings held in Canada and USA.

TIMELINE- In order to meet the 16 week timeline, TS will work together and obtain assistance when needed from the lab members of the two faculty supervisors. In Months 1 and 2, TS will work together to complete protein assays (WB and Immunofluorescence) using previously collected eye tissue samples. In Month 3, TS will continue assays and begin to learn and analyze in vivo retinal images of mouse models obtained from Micron IV and custom hardware systems. In Month 4 TS will correlate data from ex vivo eye tissue assays and in vivo imaging towards defining the in vivo "signature" of RPE degeneration and blood-retinal barrier dysfunction.