

## Abstract of proposed student project

**Proposal title:** *Decoding Vascular Irregularities in Canine Hemangiosarcoma Leveraging AI*

### Proposal

**i. Brief Introduction:** Hemangiosarcoma (HSA) is a malignant tumor that forms irregular blood vessels in dogs. Irregular, tortuous blood channels are key hallmarks for histological diagnostics of HSA. The pathogenic significance of the complex vascular tissue architecture remains unknown.

**ii. Problem Description and Significance:** HSA is aggressive and life-threatening, with the rupture of visceral tumors causing medical emergencies and sudden death, even without metastatic disease. Due to its pathological nature and aggressive clinical progression, the overall median survival time is generally 3 - 4 months, and only 10% of dogs with this disease can live longer than 1 year. Artificial intelligence (AI) can be deployed for spatial biology, providing a large-scale, automated, versatile, scalable analytic platform. Yet, its utilization in spatial pathobiology remains in its infancy within veterinary oncology. For the proposed project, we will decode the complexity of irregular malignant vascular formation, ultimately leading to a paradigm shift in canine HSA therapeutics.

**iii. Hypothesis and Objectives:** Our *central hypothesis* is that canine HSAs create malignant blood vessels that contribute to the spatially distinct tumor organization.

Objective 1. To establish *in vitro* platform recapitulating vascular growth patterns of HSA cells.

Objective 2. To decode the complex patterns of malignant blood vessels in HSA tissues and characterize the spatial orientation of tumor growth.

### iv. Study Design and Methods:

Objective 1. We will conduct tube-formation assays using two canine HSA cell lines, COSB and DHS-1426. Distinct fluorescent protein tags (GFP, RFP, and BFP) will be introduced into HSA cells, normal endothelial cells, and fibroblasts to enable co-culture experiments and time-dependent monitoring of *in vitro* tube-forming capacity. Phase-contrast and fluorescence microscopy will be used to image cells cultured on Matrigel, allowing quantitative analysis of tube-formation dynamics. We will evaluate the TIE-2-targeting kinase inhibitor Rebastinib for its ability to suppress tube formation and to assess its anti-vasculogenic effects as a potential therapeutic approach for HSA. In this objective, we will also apply AI models to digitally characterize and quantify *in vitro* tube-formation patterns of HSA cells.

Objective 2 (brief). We will utilize deep learning AI models to digitalize HSA tissue images and characterize the spatial orientation of malignant blood vessels. We will incorporate tissue images with data obtained from *in vitro* tube-formation to develop AI models to predict malignant vessel formation and tumor growth of HSA.

**Role of the Veterinary Student:** The student will assist in maintaining canine HSA cell lines under standard culture conditions and in performing Matrigel-based tube-formation assays with and without Rebastinib treatment, as described in the faculty mentor, Dr. Kim's previous work [1, 2]. Through these activities, the student will learn fundamental cell biology techniques, including mammalian cell culture, morphological analysis, microscopic image acquisition, and basic statistical analysis. The student will recover frozen cell lines from liquid nitrogen storage, expand them for experiments, and cryopreserve them for long-term storage. The student will also quantify tube formation on Matrigel and characterize the shape and spatial organization of vascular structures using ImageJ plug-in modules. These digital image datasets will be used for AI model development. In addition, the student will conduct literature searches and reviews and gain foundational knowledge in cancer biology, with a primary focus on canine HSA. The FVSP student will work closely with our research team and will be directly mentored by postdoctoral fellows Drs. Don Lee and Md Abdullah during the summer research period.

**Citations for methods cited** (first author name *et al.*, DOI; only relevant to the student's work)

1. Kim JH, et al., Genomically complex human angiosarcoma and canine hemangiosarcoma establish convergent angiogenic transcriptional programs driven by novel gene fusions. *Mol Cancer Res.* 2021;19(5):847-61. DOI: 10.1158/1541-7786.MCR-20-0937.
2. Lee D, et al., PIK3CA mutation fortifies molecular determinants for immune signaling in vascular cancers. *Cancer Gene Ther.* 2025;32(2):254-67. DOI: 10.1038/s41417-024-00867-4.