1. **Title: The regulation of mouse coronary collateral growth**

   Principal Investigator: Liya Yin, Associate Professor, Integrative Medical Sciences
   Location: NEOMED

2. **Abstract.** Ischemic heart disease continues to be a leading cause of death, and ill-health in the United States. The presence of coronary collateral vessels—the naturally occurring vessels that supply flow to an area of the heart to bypass a blocked vessel—confers a significant benefit to patients. The incidence of death decreases. The ability to survive a heart attack is better. And the amount of tissue that dies following a heart attack is less. However, the presence of such collateral vessels occurs in only 10-15% of all patients, so that the vast majority suffer the full consequences of death and ill-health in the event of a blockage in a vessel supplying the heart muscle. Currently, our understanding of coronary collateral growth (also termed coronary arteriogenesis) is based on studies in live animals, in which certain inhibitors are administered to reduce the vascular growth. A limitation of such "loss of function" studies is the cellular "target" of the inhibitor is unknown. The inhibitor could be acting on endothelial cells, smooth muscle cells, cardiac myocytes, inflammatory cells, and/or fibroblasts. Currently there is no way to decipher the cell-based mechanisms of coronary blood vessel growth. Moreover pharmacological inhibitors suffer from the problem on non-specificity. To overcome these deficiencies, we use the transgenic mouse model to interrogate many questions regarding regulation of process of coronary arteriogenesis in normal or diseased model (obesity and diabetes) and which cell types may be involved in this adaptive vascular growth. We hope that these studies will eventually lead to new therapies designed to help patients with ischemic heart disease grow new blood vessels in their hearts.

3. **The significance of the overall research**
   The patients with coronary collaterals have nature bypass during ischemia and have better prognosis after heart attack. If we understand the mechanism of regulation of coronary collateral growth, we can stimulate coronary collateral growth and amplify the effect of coronary collateral growth, especially for the patients who have impaired coronary collateral growth such as patients with metabolic syndrome.

4. **Goals and objectives**. The goal of this summer research is to study the mechanism of coronary collateral growth and how to stimulate and amplify the effect of CCG.

5. **Research Methods.** Mice will be anesthetized, intubated, and prepared for sterile surgery (involved areas will be shaved and scrubbed with betadine). In all animals an incision will be made through the sternum, and a special occluder will be situated on the surface of the heart around the left anterior descending artery. The wounds will be repaired and the chest evacuated and closed. Post-operative pain will be treated by injection of an analgesic for the first day post-op, and then as needed (we will defer to the attending veterinarian's advice). At several points after implantation of occluder, we will perform non-invasive echocardiography to evaluate cardiac function. In some animals, terminal experiments will be made at intervals (days 3, 7, and 14) up to 21 days after implantation of the occluder using contrast echocardiography to measure flow. These measurements will be made while the animals are anesthetized using gas anesthesia. Final measurements blood flow to the heart and blood pressure will be made in anesthetized mice 21 days following implantation of the occluder using contrast

echocardiography. This is done in anesthetized mice (isoflurane) in which arterial pressure is measured from a femoral catheter, and contrast microbubbles and drug infusions are done via a tail vein catheter or a catheter inserted in a jugular vein. After completion of the measurements, the mouse will be euthanized and the hearts will be removed for various in vitro and imaging studies. We will digest the heart and isolate cells for single cell RNA-sequecing. We also use the microfil to perfuse the heart for micro-CT to analyze the coronary collateral growth. We also use immunohistology and molecular biology techniques to study the genes/proteins involved in CCG.

6. **Proposed method of data analysis.** The analysis will involve only unpaired t-tests as we will compare shams to animals instrumented with the occluder. $P<0.05$ will be accepted for statistical significance.The bioinformatic data analysis will be used for single cell -RNA -sequecing for temporal difference at different stage of CCG.

7. **Significance of anticipated findings.** If the study is successful, it will accelerate our understanding of mechanisms underlying coronary collateral growth as we will be able to use better link particular genes with this adaptive process. This will lead to the novel therapeutic approach to patients with ischemic heart diseases.

Appendix: **Summer Research Fellow Training/Mentoring Plan.** The plan we have devised is arranged in a hierarchical manner.

a. First, the student will interact in a 1:1 manner with Dr. Liya Yin for experimental design, the protocols, data collection and reviewing and the interpretations.
Second, the student will interact with other lab personnel including graduate students, postdoc, research assistant for surgery, transgenic mouse breeding, phenotyping, contrast echo calculation and image analysis of micro-CT.
Third, the student will present in our lab meetings and the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Bratz, Raman, Thodeti, Meszaros, Yin, Ohanyan, Dong, Mayorga, and Yun) and will present her results in this weekly meeting.
Fourth, the student will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate.
Fifth, the student will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.

b. All the necessary resources (echocardiographs, anesthesia machines, computer for measuring evaluating echo images, mice, surgical instruments, surgical supplies, ultrasonic contrast, micro-CT, Fluorescent imaging system including multiphoton and confocal scope) and financial resources for completing the research are available.

c. The research will be completed at NEOMED.