Student Summer Scholar Program Application Examples

A4. Project Feasibility

Example 2

The goal of this project is to study whether blocking ERK1/2 enzymatic activity could restore partial or full heart function in mice impaired by diabetes. In these 12 weeks, [redacted] will work with Prof. [redacted] to induce type I diabetes in mice during the first two weeks (aim 1), then feed half of the diabetic mice with chow containing the ERK1/2 inhibitor U0126 for 4 weeks. At the end of this project, mouse hearts will be harvested to further compare histological and functional differences between these three groups of mice (aim 2). This project doesn't involve special equipment and complicated experimental procedures. Prof. [redacted] also had extensive research experience using mice. After initial training with Prof. [redacted], [redacted] should be able to perform the drug administration and glucose measurement. [redacted] will take animal usage training in February which should provide sufficient knowledge for using mice and collecting tissues from animals.

3a. Specific aims:

Specific aim 1: to induce type I diabetes in mice by streptozotocin injection

Materials: 8-week-old C57BL/6 male mice (n=25) will be purchased from Charles River Laboratory and housed in the vivarium of Kindschi Hall of Science with free access to food and water. Streptozotocin will be purchased from Sigma, and dissolved in 0.1M sodium citrate buffer (pH 4.5) to research a final concentration of 7.5 mg/ml.

Methods: One week after their arrival, mice will be split into two groups-control group (n=5) and a diabetic group (n=20). All mice will be weighed to determine their initial body weight. For the diabetic group, mice will be fasted for 4 hours to measure their blood glucose level, then injected with STZ at the dose of 75 mg/kg body weight for 5 consecutive days. For the control group, only the sodium citrate buffer is injected. For these injection experiments, Prof. [redacted] will perform the injection on the first day to show [redacted] the procedures, and [redacted] will do the rest injections with some supervision from Dr. [redacted]. One week after STZ injection, mice will be restrained to collect one drop of tail blood (~ 5 µl) for glucose measurement using a glucometer. Mice with fasting glucose level higher than 300 mg/dL will be chosen for the diabetic group. Based on our preliminary data from last two years, more than 80% of mice will become diabetic after STZ injection.

Specific aim 2: test the effect of U0126 on the development of diabetic cardiomyopathy

Materials: Diabetic mice established in aim 1 will be split into two groups: diabetic group and U0126 treatment group (Fig. 2). Normal chow containing the ERK1/2 inhibitor U0126 will be formulated by Research Diets.

Methods: 1) *induction of heart disease for 4 weeks*: the diabetic group will be fed a normal chow, and the U0126 group will be on a chow containing U0126. [redacted] will change the mouse chow

every two days to maintain the stability and efficiency of U0126. 2) *compare anatomical and physiological differences between three groups of mice*: At the end of the study, all 25 mice will be first euthanized to extract the whole heart to perform the following experiments. Dr[redacted] and [redacted] will perform the euthanization together.

Heart weight and histology: heart weight will be measured to compare across all three groups. Then half of the heart tissue will be fixed for 48 hours in 10% neutral buffered formalin solution, then stored in 70% ethanol. Heart tissues will be further processed at the histology core at [redacted] Institute for HE and trichrome staining to visualize the disease marker. Dr. [redacted] will work together with Caroline to visualize the images using the light microscope.

Gene expression analysis: [redacted] will purify messenger RNA samples from mouse hearts of three groups (n=4 for each group). Complementary DNA (cDNA) will be performed using a first strand DNA synthesis kit, followed by real-time PCR analysis using SYBR dye on the Applied Biosystems PCR machine. Caroline will watch a Youtube video and learn how to analyze the realtime PCR data using the 2(-Delta Delta C(T)) method.

Protein level analysis: Heart samples will be solubilized in 1% triton lysis buffer to harvest proteins through high-speed centrifugation. 40 μg of proteins will be used to perform SDSPAGE and Western blot to compare level of signaling proteins such as Glut4 and AKT phosphorylation.

3b. Relevance to mentor's area of expertise: Before joining GVSU in the Fall of 2016, Dr. [redacted] was trained for 5 years as a postdoctoral research associate in the lab of Dr. [redacted]'s lab at [redacted] Hospital Medical Center. In Dr[redacted]'s lab, Dr. [redacted] conducted independent research and published 4 first-author papers including 3 research articles on the role of protein phosphatases in mouse hearts. For this proposed study on the effect of ERK1/2 in the development of diabetic cardiomyopathy, [redacted] will learn how to take care of mice daily, and the methods for drug administration and blood glucose measurement. All these techniques are within Dr. [redacted]'s area of expertise. Dr. [redacted] has both the laboratory space, equipment, and more importantly the undergraduate mentoring experience over the last 6 years. The experimental techniques and scientific training Dr. [redacted] has will help [redacted] complete this project on time.

3c. Timeline for the research project

Week 1: [redacted] will visit the mouse room and get training from Dr. [redacted] on mouse care. [redacted] will study the literature on STZ and its injection method. Later this week, [redacted] will measure mouse weight to determine the STZ amount to be injected.

Week 2: Under Dr. [redacted]'s supervision, [redacted] will perform daily STZ injections on these 20 mice.

Week 3: [redacted] will measure fasting glucose level in both control mice and STZ injected mice. At the same time, [redacted] will use control mice to determine their daily chow consumption amount.

Week 4-7: [redacted] will feed half of the diabetic mice every day with a fresh U0126 diet with a calculated amount from week 3. The other diabetic mice will be still on normal chow. During the

waiting period, [redacted] will learn from Dr. [redacted] on how to extract RNA and protein samples from animal tissues.

Week 8: With the training from Dr. [redacted], [redacted] will euthanize mice, measure body and heart weight, and collect blood. Heart tissues will be collected for following biochemical and histological analysis.

Week 9: [redacted] will process the heart tissues for histological staining at [redacted] Institute. Later this week, [redacted] will purify RNA from heart tissues to analyze the gene expression difference between these three groups of mice.

Week 10: [redacted] will perform a biochemical analysis of Glut4 level among all three groups.

Week 11: [redacted] will analyze the heart histology using microscopes from 3rd floor of Kindschi Hall of Sciences.

Week 12: Statistical analysis and image data processing/finalization

3d. Appendix 1-Budget Worksheet

Title of Project: Pharmacological inhibition of ERK1/2 proteins to study their role in diabetic heart disease	
Student name: [redacted]	
Faculty mentor(s) [redacted]	

STIPENDS	
Student stipend[1]	$5000
Faculty stipend	$3000

PROJECT COSTS (please list items/services and estimated costs)[2]

1. normal chow containing U0126	$565
2. glucose test stripes	$185
TOTAL	$750

FUNDING FROM OTHER SOURCES (list amount and source)[3]

BMS research grant	$2000

1 The entire student stipend is expected to be used as summer pay for the student researcher.

Additional funds needed beyond the S^3 budget for travel or equipment should be obtained through other sources.

2 Justify the purchase of any services, materials, and/or supplies necessary to the project.
1: this is to purchase customized diet containing the ERK1/2 inhibitor U0126 to feed the diabetic mice
2. This is to measure blood glucose level one week after STZ injection.

3 Indicate other sources of funding for this project applied for and/or obtained, and describe how those funds support this proposed project.

We plan to use the BMS research grant($2000) to purchase 25 mice used for this study, as well as the STZ chemical, the cost for final analysis (heart histology, disease marker comparison).