Student Checklist (1A)

This form is required for ALL projects.

1. a. Student/Team Leader: Asma Rasheed Grade: 12

   Email: asma309@gmail.com Phone: 516-519-4785

   b. Team Member: _____ c. Team Member: _____

2. Title of Project:

   An Omics Approach to Identify Model-agnostic Disease-driving Nodes in AKI: Implicatic

3. School: George W. Hewlett High School School Phone: 516-792-8001

   School Address: 60 Everit Ave.
   Hewlett, NY 11557

4. Adult Sponsor: Terrence Bissoondial Phone/Email: 516-792-4043

5. Does this project need SRC/IRB/IACUC or other pre-approval? ☐ Yes ☑ No Tentative start date: 06/25/2019

6. Is this a continuation/progression from a previous year? ☐ Yes ☑ No
   If Yes:
   a. Attach the previous year's ☐ Abstract **and** ☐ Research Plan/Project Summary
   b. Explain how this project is new and different from previous years on
   ☐☐ Continuation/Research Progression Form (7)

7. This year's laboratory experiment/data collection:

   06/25/2019 (Actual Start Date: (mm/dd/yy)) 11/10/2019 (End Date: (mm/dd/yy))

8. Where will you conduct your experimentation? (check all that apply)
   ☑ Research Institution ☐ School ☐ Field ☐ Home ☐ Other: _____

9. List name and address of all non-home and non-school work site(s):

   Name: Angion Biomedica

   Address: 51 Charles Lindbergh Blvd
   Uniondale, NY 11553

   Phone/email: 516-326-1200/ pnarayan@angion.

10. **Complete a Research Plan/Project Summary following the Research Plan/Project Summary instructions and attach to this form.**

11. **An abstract is required for all projects after experimentation.**

Research Plan/Project Summary Instructions

A complete Research Plan/Project Summary is required for ALL projects and must accompany Student Checklist (1A).

1. All projects must have a Research Plan/Project Summary
   a. Written prior to experimentation following the instructions below to detail the rationale, research question(s), methodology, and risk assessment of the proposed research.
   b. If changes are made during the research, such changes can be added to the original research plan as an addendum, recognizing that some changes may require returning to the IRB or SRC for appropriate review and approvals. If no additional approvals are required, this addendum serves as a project summary to explain research that was conducted.
   c. If no changes are made from the original research plan, no project summary is required.

2. Some studies, such as an engineering design or mathematics projects, will be less detailed in the initial project plan and will change through the course of research. If such changes occur, a project summary that explains what was done is required and can be appended to the original research plan.

3. The Research Plan/Project Summary should include the following:
   a. **RATIONALE:** Include a brief synopsis of the background that supports your research problem and explain why this research is important and if applicable, explain any societal impact of your research.
   b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:** How is this based on the rationale described above?
   c. Describe the following in detail:
      - **Procedures:** Detail all procedures and experimental design including methods for data collection. Describe only your project. Do not include work done by mentor or others.
      - **Risk and Safety:** Identify any potential risks and safety precautions needed.
      - **Data Analysis:** Describe the procedures you will use to analyze the data/results.
   d. **BIBLIOGRAPHY:** List major references (e.g. science journal articles, books, internet sites) from your literature review. If you plan to use vertebrate animals, one of these references must be an animal care reference.

Items 1–4 below are subject-specific guidelines for additional items to be included in your research plan/project summary as applicable.

1. **Human participants research:**
   a. **Participants:** Describe age range, gender, racial/ethnic composition of participants. Identify vulnerable populations (minors, pregnant women, prisoners, mentally disabled or economically disadvantaged).
   b. **Recruitment:** Where will you find your participants? How will they be invited to participate?
   c. **Methods:** What will participants be asked to do? Will you use any surveys, questionnaires or tests? If yes and not your own, how did you obtain? Did it require permissions? If so, explain. What is the frequency and length of time involved for each subject?
   d. **Risk Assessment:** What are the risks or potential discomforts (physical, psychological, time involved, social, legal, etc.) to participants? How will you minimize risks? List any benefits to society or participants.
   e. **Protection of Privacy:** Will identifiable information (e.g., names, telephone numbers, birth dates, email addresses) be collected? Will data be confidential/anonymous? If anonymous, describe how the data will be collected. If not anonymous, what procedures are in place for safeguarding confidentiality? Where will data be stored? Who will have access to the data? What will you do with the data after the study?
   f. **Informed Consent Process:** Describe how you will inform participants about the purpose of the study, what they will be asked to do, that their participation is voluntary and they have the right to stop at any time.

2. **Vertebrate animal research:**
   a. Discuss potential ALTERNATIVES to vertebrate animal use and present justification for use of vertebrates.
   b. Explain potential impact or contribution of this research.
   c. Detail all procedures to be used, including methods used to minimize potential discomfort, distress, pain and injury to the animals and detailed chemical concentrations and drug dosages.
   d. Detail animal numbers, species, strain, sex, age, source, etc., include justification of the numbers planned.
   e. Describe housing and oversight of daily care
   f. Discuss disposition of the animals at the termination of the study.

3. **Potentially hazardous biological agents research:**
   a. Give source of the organism and describe BSL assessment process and BSL determination.
   b. Detail safety precautions and discuss methods of disposal.

4. **Hazardous chemicals, activities & devices:**
   - Describe Risk Assessment process, supervision, safety precautions and methods of disposal.
   - Material Safety Data Sheets are not necessary to submit with paperwork.

An Omics Approach to Identify Model-agnostic Disease-driving Nodes in AKI: Implications for Drug Development

Asma Rasheed

RESEARCH PLAN

A.RATIONALE

Acute Kidney Injury (AKI) is defined as the abrupt loss in kidney function caused by either injury or impairment. AKI can be caused by decreased blood flow, which can be caused by overuse of pain medication, heart failure, severe allergy or major surgery. AKI can be induced by mercury chloride, folic acid and domoic acid. Mercury is a toxic heavy metal which comes in both organic and inorganic forms. Mercury toxicity significantly affects the kidneys and can be caused by fish consumption or dental amalgam (Dhanapriya et al, 2016). Many times within Asian communities, mercury chloride is seen as an at home remedy. Folic Acid is a synthetic dietary supplement. It is often present in artificially enriched foods and pharmaceutical vitamins. Many times, vitamins containing folic acid are recommended to pregnant women (Greenberg et al, 2011). Domoic acid is naturally produced in phytoplankton and accumulates in seafood during harmful algae blooms (Ferriss et al, 2017).

There have been lots of disparities surrounding AKI and its diagnosis. In order to identify the severity of cases of AKI, Acute Tubular Necrosis (ATN) scoring is utilized. AKI could either be categorized into pre-renal AKI or ATN. Clinical trials in AKI are often punctuated with failures. Lack of robust translational success can at least in part be explained by the fact model systems might not fully recapitulate human AKI. ATN, the hallmark pathological feature of AKI, might not necessarily be governed by the same pathway in animal models in comparison to human subjects. This study examines if ROCK2 expression correlates with ATN scoring in induced AKI in mice.

B. RESEARCH QUESTION

How does ROCK2 expression correlates with ATN scoring?

- *Hypothesis*

  As the severity of AKI increases (higher ATN score), the level of ROCK2 expression will increase.

- *Expected Outcome*

  There will be higher level of ROCK2 transcripts in tissues with high ATN scores

C. PROCEDURE

Prepared hematoxylin-eosin-stained kidney slides will be obtained from mice treated $HgCl_2$, folic acid or domoic acid (IACUC #2016-004). These slides were already prepared by mentor. These slides will be examined using a compound light microscope and will be into sorted five categories based on the size of their urinary casts, seen as hyaline and granular casts in AKI. The size of the cast will be measured by Image J. The largest-sized casts will receive a score of 5 and the smallest received a score of 0 or 1. These scores should mirror Acute Tubular Necrosis (ATN) Scores (Perezella et al, 2008). A *t*-test was used to determine significance between measured values of the Sham (Control) and AKI groups.

Upon determining the presence of AKI, RT-PCR will be performed on RNA isolated from frozen tissue (IACUC #). RNA was already isolated by mentor and stored in freezer. RNA was used for other purposes than this study. RNA will be reversed transcribed and the ROCK2 gene will be amplified using qPCR.

The procedure followed will be according to manufacturer's instruction in the high capacity cDNA Reverse Transcription Kit (Qiagen, Cat #: 204054). 500 ng of RNA will be reverse transcribed and amplified using the Power-Up SYBR Green Master Mix. The primers for PCR: Forward: TGGCCCAGTTTGCATCTTTC and Reverse: AGCAAGTTGTGTTCCCAACC

RISK AND SAFETY

All chemicals will be handled carefully using tools which will be thoroughly cleaned. Hands will be washed before and after experimentation. Proper PPE (gloves, safety goggles, lab coats, fume hood) will be used. All waste will be collected by Angion Biomedica and be properly disposed of.

Chemicals in the cDNA Reverse Transcription Kit (Qiagen, Cat #: 204054)

- Buffer RLT: Harmful is swallowed, causes severe skin burns and eye damage and harmful to aquatic life with long lasting effects. Gloves, goggles and body protection.
- Buffer RPE: May cause skin irritation. Gloves, goggles and body protection .
- Buffer RW1: Causes serious eye damage, may cause skin irritation in susceptible persons. Gloves, goggles and body protection.
- Multiscribe Reverse Transcriptase: Not Hazardous. Gloves, goggles and body protection.
- 10x RT Buffer: Not Hazardous. Gloves, goggles and body protection.
- dNTP Mix: Not Hazardous. Gloves, goggles and body protection.
- 10x RT Random Primers: Not Hazardous. Gloves, goggles and body protection.
- PowerUp SYBR Green Master Mix: Not Hazardous. Gloves, goggles and body protection.

List of Sources of Safety Information:

- SDS from ThermoFisher Scientific
- SDS from Qiagen

DATA ANALYSIS

Image J will be used to measure the size of the casts from the prepared slides. Prism Graphpad software will be used to determine significance between the size of the casts in various treatment groups. A t-test will also be used to determine significance between measured values of the Sham and AKI groups. A Pearson Product Moment Correlation was used to determine the strength of the association between the ROCK2 gene queried and the increase in Bowman's Space. A p-value ≤ 0.05 will be considered to be statistically significant

D. BIBLIOGRAPHY

Amano, M., Nakayama, M., & Kaibuchi, K. (2010). Rho-kinase/ROCK: A key regulator of the cytoskeleton and cell polarity. Cytoskeleton (Hoboken, N.J.), 67(9), 545–554. doi:10.1002/cm.20472

Barré-Sinoussi, F., & Montagutelli, X. (2015). Animal models are essential to biological research: issues and perspectives. Future science OA, 1(4), FSO63. doi:10.4155/fso.15.63

Biesen, W. V., Vanholder, R., & Lameire, N. (2006). Defining Acute Renal Failure: RIFLE and Beyond: Table 1. Clinical Journal of the American Society of Nephrology, 1(6), 1314–1319. doi: 10.2215/cjn.02070606

Dhanapriya, J., Gopalakrishnan, N., Arun, V., Dineshkumar, T., Sakthirajan, R., Balasubramaniyan, T., & Haris, M. (2016). Acute kidney injury and disseminated intravascular coagulation due to mercuric chloride poisoning. Indian Journal of Nephrology, 26(3), 206. doi: 10.4103/0971-4065.164230

Ferriss, B. E., Marcinek, D. J., Ayres, D., Borchert, J., & Lefebvre, K. A. (2017). Acute and chronic dietary exposure to domoic acid in recreational harvesters: A survey of shellfish consumption behavior. Environment International, 101, 70–79. doi:

10.1016/j.envint.2017.01.006

Funk, J. A., Janech, M. G., Dillon, J. C., Bissler, J. J., Siroky, B. J., & Bell, P. D. (2014). Characterization of renal toxicity in mice administered the marine biotoxin domoic Acid. Journal of the American Society of Nephrology : JASN, 25(6), 1187–1197. doi:10.1681/ASN.2013080836

Gameiro, J., Agapito Fonseca, J., Jorge, S., & Lopes, J. A. (2018). Acute Kidney Injury Definition and Diagnosis: A Narrative Review. Journal of clinical medicine, 7(10), 307. doi:10.3390/jcm7100307

Grams, M. E., & Rabb, H. (2012). The distant organ effects of acute kidney injury. Kidney International, 81(10), 942–948. doi: 10.1038/ki.2011.241

Greenberg, J. A., Bell, S. J., Guan, Y., & Yu, Y. H. (2011). Folic Acid supplementation and pregnancy: more than just neural tube defect prevention. Reviews in obstetrics & gynecology, 4(2), 52–59.

Hartmann, S., Ridley, A. J., & Lutz, S. (2015). The Function of Rho-Associated Kinases ROCK1 and ROCK2 in the Pathogenesis of Cardiovascular Disease. Frontiers in pharmacology, 6, 276. doi:10.3389/fphar.2015.00276

Higuchi, S., Kabeya, Y., Matsushita, K., Yamasaki, S., Ohnishi, H., & Yoshino, H. (2019). Urinary cast is a useful predictor of acute kidney injury in acute heart failure. Scientific reports, 9(1), 4352. doi:10.1038/s41598-019-39470-1

Kentrup, D., Reuter, S., Schnöckel, U., Grabner, A., Edemir, B., Pavenstädt, H., … Büssemaker, E. (2011). Hydroxyfasudil-mediated inhibition of ROCK1 and ROCK2 improves kidney function in rat renal acute ischemia-reperfusion injury. PloS one, 6(10), e26419. doi:10.1371/journal.pone.0026419

Lopes, J. A., & Jorge, S. (2013). The RIFLE and AKIN classifications for acute kidney injury: a critical and comprehensive review. Clinical Kidney Journal, 6(1), 8–14. doi: 10.1093/ckj/sfs160

Luo, K., Fu, S., Fang, W., & Xu, G. (2017). The optimal time of initiation of renal replacement therapy in acute kidney injury: A meta-analysis. Oncotarget, 8(40), 68795–68808. doi:10.18632/oncotarget.17946

Makris, K., & Spanou, L. (2016). Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes. The Clinical biochemist. Reviews, 37(2), 85–98.

Ostermann, M., & Joannidis, M. (2016). Acute kidney injury 2016: diagnosis and diagnostic workup. Critical care (London, England), 20(1), 299. doi:10.1186/s13054-016-1478-z

Perazella, M. A., Coca, S. G., Kanbay, M., Brewster, U. C., & Parikh, C. R. (2008). Diagnostic

value of urine microscopy for differential diagnosis of acute kidney injury in hospitalized patients. Clinical journal of the American Society of Nephrology : CJASN, 3(6), 1615–1619. doi:10.2215/CJN.02860608

Perlman R. L. (2016). Mouse models of human disease: An evolutionary perspective. Evolution, medicine, and public health, 2016(1), 170–176. doi:10.1093/emph/eow014

Prakash, J., de Borst, M. H., Lacombe, M., Opdam, F., Klok, P. A., van Goor, H., … Kok, R. J. (2008). Inhibition of renal rho kinase attenuates ischemia/reperfusion-induced injury. Journal of the American Society of Nephrology : JASN, 19(11), 2086–2097. doi:10.1681/ASN.2007070794

Sawhney, S., & Fraser, S. D. (2017). Epidemiology of AKI: Utilizing Large Databases to Determine the Burden of AKI. Advances in Chronic Kidney Disease, 24(4), 194–204. doi: 10.1053/j.ackd.2017.05.001

Swart, M. J., Bekker, A. M., Malan, J. J., Meiring, A., Swart, Z., & Joubert, G. (2010). The simplified modification of diet in renal disease equation as a predictor of renal function after coronary artery bypass graft surgery. Cardiovascular journal of Africa, 21(1), 9–12.

Wang, H. W., Liu, P. Y., Oyama, N., Rikitake, Y., Kitamoto, S., Gitlin, J., Boisvert, W. A. (2008). Deficiency of ROCK1 in bone marrow-derived cells protects against atherosclerosis in LDLR-/- mice. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 22(10), 3561–3570. doi:10.1096/fj.08-108829

Wang, Y., Zhang, H., Yang, Z., Miao, D., & Zhang, D. (2017). Rho Kinase Inhibitor, Fasudil, Attenuates Contrast-induced Acute Kidney Injury. Basic & Clinical Pharmacology & Toxicology, 122(2), 278–287. doi: 10.1111/bcpt.12895

ADDENDUM

No changes were made to the Research Plan