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.

Noah Kava

Osteopontin and Lupus Nephritis

Biomedical and Health Sciences (BMED)

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

Systemic lupus erythematosus (SLE) is an autoimmune disease with over 60 genetic risk markers (Shipman, 2016). Symptoms include prolonged fatigue, butterfly rash, and joint pain (Mayo Clinic, 2017), and there is currently no known cure. Lupus predominantly affects the kidneys – an organ that works to preserve homeostasis by maintaining appropriate mineral balance in the blood and excretes excess mineral waste as urine (Thompson, 1900). The human kidney is comprised of functional units known as nephrons. Nephrons contain tubules (known as proximal and distal tubules) and collecting ducts for urine filtration (Hill, 2019). In lupus, immune complexes deposit in the kidney causing inflammation and tubular injury. This condition called lupus nephritis (LN), causes deterioration of kidney function (Stewart, 1999). Osteopontin (OPN) is one of the prominent cytokines upregulated in SLE patients (Niewold 2015). OPN is a protein (encoded for by the Spp1 gene) known for stimulating activity in bones (Sodek, 2000) and mediating migration, adhesion, and activates type I interferon (Shinohara, 2006). Type-1 interferons (IFN-α) are cytokines that inhibit viral proliferation (Norfray, 2009) and are upregulated and overexpressed in Lupus patients, contributing to the onset and worsening condition of the autoimmune disease (Ronnblom, 2011; Elkon, 2012). High IFN

activity is a heritable trait and a primary factor for Lupus pathogenesis (Niewold, 2007). OPN has a heightened expression in kidney tubules and glomeruli in LN patients (Xie, 2001). Additionally, OPN and IFN-α have a positive correlation with one another (Li, 2004), which represents a potential cycle for disease intensification and inhibition, but this cycle has remained unproven. OPN is expressed by many immune cells, and macrophages are the cells believed to upregulate OPN in diseases such as brain cancer (Chen, 2019) Macrophages are tissue-sentinel immune cells (Italiani, 2014) that carry out phagocytosis (engulfing) of parasites and microbes (Elhelu,1983). Macrophages are mainly classified into two categories, first the tissue-resident or embryonic-derived and second, monocyte-derived macrophages. The monocyte-derived macrophages originate from circulating monocytes while resident macrophages are produced in the embryonic stage of human development (Perdiguero, 2016). It was observed that the kidney-resident macrophages upregulate osteopontin when phagocytosing the dying cells (Puranik, 2018). The efficiency of phagocytosis is decreased in SLE patients (Herrmann, 2004), and this causes the immune system to recruit monocyte-derived macrophages to infiltrate the diseased kidneys (Maria, 2017).

Systemic Lupus Erythematosus (SLE) is an autoimmune disease with over sixty genetic risk alleles (Shipman, 2016) and chronic symptoms of fatigue, joint pain, and butterfly rash (Mayo Clinic Staff, 2017). It is considered by many to be overlooked by the scientific community. Lupus Nephritis (LN) is a type of SLE that causes intense inflammation to the kidneys (Stewart, 1999). Previous studies have concluded that interferon (IFN-1) is upregulated in LN patients, resulting in an overexpressed immunological response which causes the immune

system to attack itself (Ronnblom, 2011 ; Elkon, 2012). Osteopontin (OPN) is a protein that is encoded by the Spp1 gene. OPN has been proven to be upregulated in LN patients (Xie, 2001). Whether or not this is causal or correlative to LN is unknown to this point. There are active hypotheses which propose that OPN inhibits IFN-1 production in the kidneys of LN patients.

Macrophages are cells that serve the immune system by engulfing cellular waste and debris (Elhelu, 1983). In LN patients, many macrophages infiltrate the kidneys in order to carry out their immunological role (Puranik, 2018). The next step for research in lupus nephritis is to determine where OPN and IFN is being produced from and to determine whether or not macrophages are significantly involved in the process.

b) Research Question(s)/Hypothesis(es)/Engineering Goal(s)/Expected Outcome(s): How is this based on the rationale described above?

The research hypothesis is that the quantity of macrophages in high-intensity lupus nephritis patients will correlate with the quantity of osteopontin-positive tubular cells.

c) Detailed description of methods or procedures, Risk and Safety Analysis, Data Analysis. See Subject-Specific Guidelines in bottom 4 items for further guidance

- Methods/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.

As the conductor of this experiment, I will be given seven LP scan images of de-identified human lupus nephritis kidneys that have already been stained to indicate

osteopontin, macrophages, and nuclei. I will divide the images into smaller subset images for a total of around 300 composite images.

- Risk and Safety Analysis- Identify any potential risks and safety precautions needed.

Since I will not be handling any physical tissue, there are no extra safety precautions or risks that need to be adhered to.

- Data Analysis- Describe the procedures you will use to analyze the data/results.

Using the Inform image analysis software, I will create algorithms that segment images by tissue type, phenotype cells, and score for the percentage of cells expressing the experimental phenotype. I will apply the algorithm to every composite image I have and create a data sheet. I will perform standard t-tests and calculate p-values to determine if data values correlate for OPN and macrophage quantities. Lastly, I will compare my results with clinical data to determine if the intensity of the disease (obtained from the SLEDAI scale) impacted the findings.

d) Bibliography – List major references, no minimum required. Vertebrate projects require at least one animal care reference.

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Addendum

NO ADDENDUMS EXIST