AWARD NUMBER: W81XWH-20-1-0666

TITLE: Modeling Lupus Nephritis in a Human Kidney Microphysiological System

PRINCIPAL INVESTIGATOR: Jonathan Himmelfarb, MD

CONTRACTING ORGANIZATION: University of Washington, Seattle, WA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The kidney is highly susceptible to injury from many systemic inflammatory and autoimmune diseases, including systemic lupus erythematosus (SLE), which is one of the FY19 CDMRP Topic Area. Among people with SLE, kidney involvement (lupus nephritis) affects 60%-80% of SLE patients and is recognized as a main contributor to morbidity and mortality. End stage renal disease occurs in ~25% of SLE patients within 15 years of onset, even with modern day immunosuppressive treatment. We have developed a three-dimensional flow directed "kidney-on-a-chip" populated with human kidney cells, with functional characterization of key component structures of the kidney cortical tubulo-interstitium. In Year 2, we continued our work from Year 1, and focused on major task 1 and major task 2, and have resolved the technical hurdles in the matrix variability to faithfully recapitulate kidney microvessel on a chip. Our overall goal is to use these pre-clinical studies to discover more effective strategies for treating and curing individual patients with SLE, so that clinical studies can then be conducted to improve their quality and quantity of life.

15. SUBJECT TERMS

Systemic Lupus Erythematosus (SLE), Lupus Nephritis, Kidney Disease, End-stage Renal Disease

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INTRODUCTION:

Background: Kidney disease, i.e. lupus nephritis, affects 60%-80% of SLE patients, and is a main contributor of morbidity and mortality. Development of lupus nephritis increases morbidity and mortality 26-fold compared with age and gender matched healthy people. End stage renal disease occurs in ~25% of patients within 15 years of onset, even with current treatment, and the extent of kidney tubulo-interstitial injury has been strongly correlated with the severity of lupus nephritis and demonstrated to be of great prognostic import in determining lupus nephritis outcomes. Unfortunately, animal models cannot disentangle whether abnormalities identified are cause or effect. Furthermore, they fail to account for the heterogeneity of clinical SLE nephritis because of human genetic variation that contributes both to immune dysfunction as well as end organ susceptibility.

Rationale: *In vitro* models that recapitulate critical aspects of kidney physiology, assess the mechanisms and response to injury, and test reparative mechanisms could substantially enhance therapeutic discovery. We have developed three-dimensional flow directed "kidney-on-a-chip" populated with human kidney cells, with functional characterization of key component structures of the proximal tubule and the peritubular microvascular network as an integrated unit. Strengths of the kidney on a chip include: 1) minimized contact of living cells with artificial materials; 2) living perfusable microvasculature and tubules; 3) interstitial compartments that permit flow and are comprised of modifiable extracellular matrix which can be populated with tissue-specific resident immune cells; 4) proven fidelity to multiple aspects of human renal physiology and pathophysiology. Use of this 'human kidney on a chip' has been successful in developing robust in vitro models of multiple kidney diseases that recapitulate critical aspects of kidney physiology, assess the mechanisms and response to injury, and test reparative mechanisms. We believe that this approach to understanding the pathobiology of lupus nephritis can substantially enhance progress towards disease understanding and ultimately lead to more precise, individual therapeutic approaches, and ultimately to cures.

Hypothesis: The extent of tubule-interstitial injury has been strongly correlated with the severity of lupus nephritis and demonstrated to be of great prognostic import in determining lupus nephritis outcomes (ref 42,77.) The overall hypothesis of this proposal is that the initiating mechanisms leading to kidney damage in SLE can be accurately identified in a kidney on a chip system with defined components that recapitulate in vivo biology.

Specific Aim #1: Modeling microvascular interactions with immune cells and humoral factors as initiating tubulo-interstitial disease in lupus nephritis. We will integrate immune cells and humoral factors into an existing flow directed, three-dimensional human kidney biomimetic microvascular network system in order to model the role of microvascular injury in initiating tubulo-interstitial disease in lupus nephritis.

Specific Aim #2: Use of a Novel Renal Vascular Tubular Unit to Create an Integrated MPS Model of Lupus Nephritis. We recently reported on the first fully tunable human kidney-on-a-chip platform, which allows the reconstruction of the native architecture of the renal cortical endothelial-epithelial exchange interface using entirely cell-remodelable matrix and patient-derived kidney cells. We will perfuse the tunable platform with the relevant cells, antibodies and / or soluble factors identified in Aim 1 and quantify tubulo-interstitial damage by immunologic, proteomic and RNA transcription profiles as well as biomarkers of kidney injury. We will determine the phenotypes and function of cell types attracted to tubules following injury.

Short-term impact: A better understanding of the pathobiology of human lupus nephritis by (i) creating an integrated microphysiological model of lupus nephritis that is cross validated for clinical-pathological correlation with human clinical findings, and (ii) selectively accounting for the role of immune cells, autoantibodies, complement, immune complexes, chemokines, cytokines and growth factors in initiating kidney injury in SLE.
Long-term impact: Identification of individual pathways and mediators of tubulo-interstitial kidney injury that account for patient heterogeneity and allow rapid translation of the findings from bench to bedside for patients with lupus nephritis.
Relevance of project to FY19 CDMRP Topic Area: Lupus Research Program (LRP) is one of the target areas. The LRP mission is to fund research to understand, prevent, and diagnose lupus and to improve treatments and quality of life of patients, including Service members, Veterans, and beneficiaries. With the overall objective of identifying better treatment strategies, this proposal addresses a critical need in lupus research, exemplified by the focus areas aimed at "understanding disease mechanisms and determining the pathobiology of lupus disease in target human tissues".

1. KEYWORDS:

- Systemic Lupus Erythematosus (SLE)
- Lupus Nephritis
- Kidney Disease
- End-stage Renal Disease
- Human pluripotent stem cells (hPSCs)
- Microphysiological Systems (MPS)
- Neutrophils

2. ACCOMPLISHMENTS:

a. What were the major goals of the project?

Specific Aim #1: Modeling microvascular interactions with immune cells and humoral factors as initiating tubulo-interstitial disease in lupus nephritis. We will integrate immune cells and humoral factors into an existing flow directed, three-dimensional human kidney biomimetic microvascular network system in order to model the role of microvascular injury in initiating tubulo-interstitial disease in lupus nephritis.

Specific Aim #2: Use of a Novel Renal Vascular Tubular Unit to Create an Integrated MPS Model of Lupus Nephritis. We recently reported on the first fully tunable human kidney-on-a-chip platform, which allows the reconstruction of the native architecture of the renal cortical endothelial-epithelial exchange interface using entirely cell-remodelable matrix and patient-derived kidney cells. We will perfuse the tunable platform with the relevant cells, antibodies and / or soluble factors identified in Aim 1 and quantify tubulo-interstitial damage by immunologic, proteomic and RNA transcription profiles as well as biomarkers of kidney injury. We will determine the

phenotypes and function of cell types attracted to tubules following injury.

b. What was accomplished under these goals?

1) Major Activities:

Following the success in year 1, we evaluated the interactions between neutrophils and engineered kidney microvessels in the presence or absence of serum from normal or lupus nephritis patients. We have completed the studies for 11 serum samples from lupus nephritis patients and 3 healthy subjects perfused through the kidney microvessel systems. Compared to our original timeline, we encountered some delay due to two major reasons: 1) COVID - personnel sickness. As COVID-19 persisted, the personnel sickness has hindered our progress, and the average number of experiments we could run per unit time were much lower. Also, the COVID pandemic substantially reduced the numbers of patients in clinic making it much more difficult to obtain fresh samples from SLE patients. 2) Collagen issues: We had significant problems in collagen isolation from certain manufacturer's lots during the past year. This unexpectedly resulted in significant cell death in microvessels. After many months of debugging, we identified the heterogeneous charges on collagen as the likely reasons which was caused by partial gelation during lyophilization process. We have now overcome all of these problem, patient recruitment has improved allowing us to finish up the replicates in these conditions, and to work on Aim 2 with renal vascular-tubular unit.

2) Specific Objectives:

In Aim 1, our objective was to integrate immune cells and humoral factors into an existing flow directed, three-dimensional human kidney biomimetic microvascular network system, and model the role of microvascular injury in initiating tubulo-interstitial disease in lupus nephritis. In Aim 2, we utilize Renal Vascular Tubular Unit to evaluate tubulo-interstitial damage after lupus serum is perfused through the microvessels.

3) Significant Results/Key Outcomes:

Establishing kidney microvascular MPS for the study of their interactions with immune cells and humoral factors. Following the success in year 1, we have established methods to perfuse neutrophils into the microvascular network and monitor the cell adhesion and endothelial changes. We showed that normal neutrophils had mild adhesion and transmigration into the abluminal space (marked as asterisks) on endothelial luminal side after 30 mins of perfusion (Fig. 1A). Very significantly from the aims of the proposal, we demonstrated

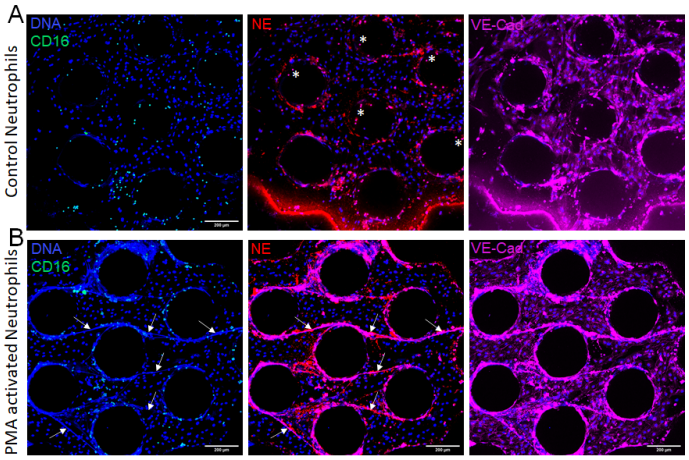

Figure 1. Neutrophil perfused through the kidney microvessel networks for control (A) and PMA treated (B) neutrophil conditions.

formation of neutrophil extracellular traps (NETs) in the 3D microvasculature, assessing formation of DNA fibers coated with neutrophil elastase by microscopy. Using PMA, a known inducer of NET formation, prominent DNA release was seen in the lumen of the microvasculature (marked as white arrows), and sometimes form transluminal structures that could extend to several millimeters (Figure 1B).

We have also evaluated the effect of human sera (11 SLE patients and 3 healthy subjects) on kidney microvessels and the consequent neutrophil adhesion and NET formation, Results were compared to the control, PMA treated neutrophil perfusion, as well

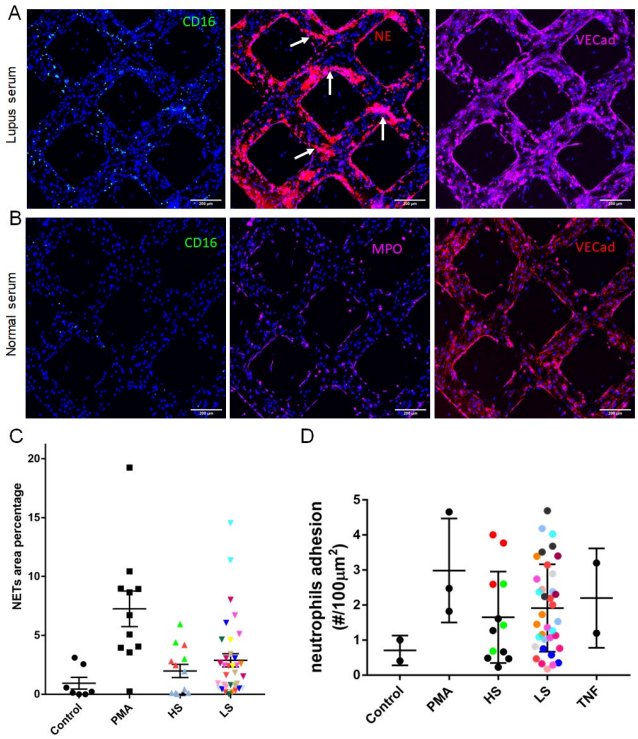

Figure 2. Representative images (A-B) of normal neutrophils perfused through kidney microvessels after perfused with lupus (A) and normal (B) serum. C-D. Quantification of NETs formation and neutrophil adhesion comparing all conditions. Color in each condition representing different patient samples.

as TNFα treated microvessels. In general, lupus serum led to increased neutrophil adhesion, at similar levels to TNFα treated microvessels, although there was variation of response for neutrophil adhesion and NET formation (Fig. 2). More replicates will be performed for perfusion of control and PMA treated neutrophils in the absence of sera to firmly establish the negative and positive cases for neutrophil adhesion. In addition, analysis of more healthy subjects are ongoing to determine statistical significance.

Overall, we have established methods to evaluate the interactions of neutrophils and kidney specific microvessels, and developed quantitative methods to measure NETs formation in these microvessels. Molecular studies will provide more mechanisms of kidney vascular injuries in this processes. In the next stage we will use our established renal vascular-tubule unit to evaluate the consequent tubular and interstitial changes after NETs formation in microvessels.

4) Other Achievements:

Nothing to report

c. What opportunities for training and professional development has the project provided?

Nothing to report.

d. How were the results disseminated to communities of interest?

A manuscript describing the initial findings from this work is currently in preparation.

e. What do you plan to do during the next reporting period to accomplish the goals?

In the NCE, we will finish the study reported above and submit the manuscript, and then focus on Aim 2. In Aim 2, we will compare the effects of serum and neutrophils from SLE patients with and without nephritis to damage the vasculature and also the proximal tubule cells. We will quantify damage using kidney injury biomarkers as outlined in the proposal.

3. IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

We have developed the conditions to analyze the effects of serum on the renal vasculature in the MPS system. We have also shown, for the first time, that we can observe both neutrophil adhesion and NET formation in the MPS system. These

landmark developments will allow us to compare the effects of serum and cells from patients with and without kidney injury as outlined in the proposal. These findings could have a major impact on our understanding of the mechanisms responsible for tubulointerstitial nephritis.

b. What was the impact on other disciplines?

Nothing to report.

c. What was the impact on technology transfer?

Nothing to report.

d. What was the impact on society beyond science and technology?

Nothing to report.

4. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

We have not made major changes to the approach outlined in the approved SOW updated in December 2020.

b. Actual or anticipated problems or delays and actions or plans to resolve them

As explained above, we encountered some delays in making progress due to two major reasons: 1) COVID - personnel sickness. As COVID-19 persisted, University restrictions coupled with personnel sickness hindered our progress. The average number of experiments we could run per unit time was much lower then what we could do without COVID. Also, the COVID pandemic considerably reduced the numbers of patients in clinic making it more difficult to obtain fresh samples from SLE patients. 2) Collagen issues: We had significant problems in collagen isolation during the past year which resulted in significant cell death in microvessels. After approximately 6 months debugging, we finally identified the heterogeneous charges on collagen was the likely reason and we observed partial gelation during lyophilization process. All of these problems have now been overcome. We are finishing up more replicates in these conditions, and proceeding with Aim 2 with renal vascular-tubular unit.

c. Changes that had a significant impact on expenditures

Nothing to report.

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

As requested by Allison McClean, Human Subjects Protection Scientist with the Human Research Protection Office, we obtained UW IRB concurrence that the use of residual samples from Dr. Elkon's existing study, STUDY00001145, does not meet the definition of human subjects activity. This IRB determination letter was provided to Allison and uploaded to eBRAP.

e. Significant changes in use or care of human subjects

The change in the IRB approved study that will serve as the source of human subjects, from Keith Elkon's study to Benjamin Freedman's study, has already been communicated and approved through the updated SOW dated December 2020.

f. Significant changes in use or care of vertebrate animals

Nothing to report.

g. Significant changes in use of biohazards and/or select agents

Nothing to report.

5. PRODUCTS:

a. Publications, conference papers, and presentations

A manuscript describing the model and initial findings is currently in preparation.

***b.* Website(s) or other Internet site(s)**

Nothing to report.

***c.* Technologies or techniques**

Nothing to report.

***d.* Inventions, patent applications, and/or licenses**

Nothing to report.

***e.* Other Products**

Nothing to report.

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name: Jonathan Himmelfarb, MD
Project Role: PI
Research Identifier (eRA Commons): JHIMMELFARB
Nearest Person Months Worked: 1
Contribution to the Project: Dr. Himmelfarb is the PI at the University of Washington. He has coordinated the research, planned experiments, and interpreted data.
Funding Support: this project

Name: Keith Elkon, MD
Project Role: Co-Investigator
Nearest Person Months Worked: 1
Contribution to the Project: Dr. Elkon oversees all the immunological experiments. He has collaborated with all investigators on the project, planned experiments, and interpreted data.
Funding Support: this project

Name: Ying Zheng, PhD
Project Role: Co-Investigator
Nearest Person Months Worked: 1
Contribution to the Project: Dr. Zheng oversees experiments intended to study the interaction of SLE serum and cellular components with kidney endothelium in 2D monolayer and 3D microvessels under perfusion.
Funding Support: this project

Name: Christian Lood, PhD
Project Role: Co-Investigator
Nearest Person Months Worked: 1
Contribution to the Project: Dr. Lood assists with experiment design, isolation and characterization of immune cell subsets.
Funding Support: this project

Name: Benjamin Freedman, PhD
Project Role: Co-Investigator
Nearest Person Months Worked: 1
Contribution to the Project: Dr. Freedman is a leading expert in generating kidney organoids as well as directed cell differentiation, in each case from human pluripotent stem cells (hPSCs). His lab will supply hPSC derived kidney cells for the experiments of this project.
Funding Support: this project

Name: Jie An, PhD
Project Role: Senior Staff Scientist

Nearest Person Months Worked: 2
Contribution to the Project: Dr. An assists with QPCR, flow cytometry, and performs most of the immunofluorescence staining of immune cells in Keith Elkon's lab.
Funding Support: this project

Name: Xizhang Sun, PhD
Project Role: Research Scientist
Nearest Person Months Worked: 1
Contribution to the Project: Dr. Sun assists Dr. An with QPCR, flow cytometry, and assists with the immunofluorescence staining of immune cells in Keith Elkon's lab.
Funding Support: this project

Name: Ping Luo, PhD
Project Role: Research Scientist
Nearest Person Months Worked: 4
Contribution to the Project: Dr. Luo performs the experiments in studying SLE serum and cellular components interacting with kidney endothelium under flow in Dr. Zheng's lab.
Funding Support: this project

Name: Andreas Farny
Project Role: Project Research Scientist
Nearest Person Months Worked: 1
Contribution to the Project: Mr. Farny assists Dr. Luo with experiments in studying SLE serum and cellular components interacting with kidney endothelium under flow in Dr. Zheng's lab.
Funding Support: this project

b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

New Grants:

Himmelfarb, Jonathan:
Title: Safety and Efficacy of Human Clinical Trials Using Kidney-on-a-Chip Microphysiological Systems
Major Goals: We hypothesize that kidney-on-a-chip microphysiological systems (MPS) will manifest patient-specific phenotypic responses in vitro commensurate with clinical trial outcomes in vivo, establishing a robust molecular and cellular basis for kidney precision medicine approaches.
*Status of Support: Active
Project Number: 4UH3TR003288-03
Name of PD/PI: MPI: Himmelfarb & Kretzler
*Source of Support: NIH
*Primary Place of Performance: University of Washington, WA

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/01/2022 – 06/30/2025
* Total Award Amount (including Indirect Costs): total cost years 3-5

Title: Therapeutic bubble tea: Preventing the formation of uremic toxins with hydrogel immobilized microbes
Major Goals: The target of this work is to lower indole and p-cresol in the intestines to prevent the formation of indoxyl sulfate and p-cresol sulfate in the liver by enriching for indole/precresol degraders and then apply them as oral therapeutic.
*Status of Support: Active
Project Number: R01DK130815
Name of PD/PI: MPI: Mari Winkler
*Source of Support: NIH
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2022-12/2026
*Total Award Amount (including Indirect Costs): total cost all years
Title: Rat and Canine Microphysiological Systems of the Kidney Proximal Tubule for Chemical Toxicity Screening
Major Goals: This project is a collaboration between Nortis Inc. and the University of Washington School of Pharmacy. The goal of this project is to develop commercially viable kidney proximal tubule microphysiologic systems (KPT-MPS) using rat and canine proximal tubule epithelial cells (PTECs) that can be used in pre-clinical drug development to identify potential nephrotoxicity.
*Status of Support: Active
Project Number: R44ES032393
Name of PD/PI: T. Neumann, SubK Co-PIs: Yeung/Kelly
*Source of Support: NIH/NIEHS
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/14/2021 –04/30/2023
*Total Award Amount (including Indirect Costs): total cost

Title: Effect of voclosporin on renal vessels, immune cell adherence and proximal convoluted tubules in microphysiological systems
Major Goals: To compare the injurious effects of Voclosporin (Voclo) versus Tacrolimus (TAC) on renal vessels in Microphysiological Systems (MPS) under flow conditions. To compare the effects of Voclo vs TAC on adherence of activated neutrophils and macrophages to kidney vessels in MPS under flow conditions. To compare Voclo vs TAC on proximal convoluted tubular (PCT) injury under flow conditions.
*Status of Support: Active
Project Number: N/A
Name of PD/PI: T. Himmelfarb and Elkon
*Source of Support: Aurinia Pharmaceuticals
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/16/2022-09/15/2023

*Total Award Amount (including Indirect Costs): total cost

Title: Identification and Validation of Biological Sub-phenotypes of Sepsis-induced Acute Kidney Injury: A Precision Medicine Approach to Improve Clinical Outcomes
Major Goals: This application will determine what AKI sub-phenotypes are present in the emergency room, whether AKI sub-phenotypes respond differently to volume of intravenous fluids, and whether differences in kidney histopathology and urinary proteomics exist between sepsis-induced AKI sub-phenotypes.
*Status of Support: Active
Project Number: R01 number TBD
Name of PD/PI: Bhatraju, Pavan
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY): 07/15/2022-06/30/2027
*Total Award Amount (including Indirect Costs):

Title: Extended Longevity of 3D Tissues and Microphysiological Systems for Modeling of Acute and Chronic Exposures to Stressors
Major Goals: This project will improve the understanding of transporter-mediated renal tubular secretion and the impact of uremic solutes on transporter function, drug exposure, and drug-induced kidney damage. The team will investigate the uremic solute-drug interaction at the renal epithelial barrier using transfected cell systems, a novel microphysiologic “kidney on a chip”, and in a human study of 40 patients with chronic kidney disease and 20 healthy people.
*Status of Support: Active
Project Number: 21-3DTMPS_2-0001 (proposal number)
Name of PD/PI: Cathy Yeung
*Source of Support: National Aeronautics and Space Administration
*Primary Place of Performance: University of Washington
Project/Proposal Start and End Date: (MM/YYYY) (if available):
7/2022-6/2026
*Total Award Amount (including Indirect Costs):

Elkon, Keith:

Title: cGAMP as an immunotransmitter of the interferon response to UV light
Major Goals: The Major goals of this project is to determine the role of the cGAS-STING pathway and the messenger cyclic dinucleotide cGAMP, as a local and systemic immunotransmitter of the IFN response following skin exposure to UVB light.
*Status of Support: Active
Project Number: R21 AR077842-01
Name of PD/PI: Elkon, Keith
*Source of Support: NIH
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2021 – 08/2023
*Total Award Amount (including Indirect Costs):

Title: cGAMP in SLE
Major Goals: To confirm whether patients with high cGAMP have a different RNA-seq signature, PBMC from 50 SLE patients with a SLEDAI of 4 or more will be tested for cGAMP concentrations and RNA as well as serum stored.
*Status of Support: Active
Project Number: N/A
Name of PD/PI: Elkon, Keith
*Source of Support: Celgene
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/2021 – 09/2022
*Total Award Amount (including Indirect Costs):
Title: Discovery of cGAS Inhibitors for Interferon-Driven Autoimmune Diseases.
Major Goals: Major goal of this project is to design and interpret interventions relating to interferon and inflammation in the mouse skin following UW light exposure.
*Status of Support: Active
Project Number: 2R44AI141281-02
Name of PD/PI: Elkon, Keith
*Source of Support: BellBrook Labs LLC
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2022 – 03/2023
*Total Award Amount (including Indirect Costs):

Title: Role of mitochondria in SLE and its cardiovascular complications
Major Goals: The major goals of this project are to identify novel anti-mitochondrial antibodies in SLE, determine their clinical utility in predicting thrombosis, investigating their pro-thrombotic properties in vitro and in vivo, as well as explore mechanisms involved in clearance of extracellular mitochondria to limit development of anti-mitochondrial antibodies.
*Status of Support: Active
Project Number: 1 R01 HL 158606
Name of PD/PI: Lood, J.C.
*Source of Support: NHLB
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 7/1/2021-06/30/2026
*Total Award Amount (including Indirect Costs):
Title: Effect of voclosporin on renal vessels, immune cell adherence and proximal convoluted tubules in microphysiological systems
Major Goals: To compare the injurious effects of Voclosporin (Voclo) versus Tacrolimus (TAC) on renal vessels in Microphysiological Systems (MPS) under flow conditions. To compare the effects of Voclo vs TAC on adherence of activated neutrophils and macrophages to kidney vessels in MPS under flow conditions. To compare Voclo vs TAC on proximal convoluted tubular (PCT) injury under flow conditions.
*Status of Support: Active
Project Number: N/A

Name of PD/PI: T. Himmelfarb and Elkon
*Source of Support: Aurinia Pharmaceuticals
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available):
03/16/2022-09/15/2023
*Total Award Amount (including Indirect Costs): total cost

Zheng, Ying:
Title: Engineering Human Brain Neurovascular Niche for Modeling Brain Diseases
Major Goals: to develop and understand the structure and function of the neurovascular unit for modeling neurodegenerative diseases.
*Status of Support: Active
Project Number: R21AG074373
Name of PD/PI: Zheng/Young
*Source of Support: NIH/NHAID
*Primary Place of Performance: UW SLU
Project/Proposal Start and End Date: 09/1/2021 – 08/31/2023
*Total Award Amount (including Indirect Costs):

Title: Safety and Efficacy of Human Clinical Trials Using Kidney-on-a-Chip Microphysiological Systems
Major Goals: We hypothesize that kidney-on-a-chip microphysiological systems (MPS) will manifest patient-specific phenotypic responses in vitro commensurate with clinical trial outcomes in vivo, establishing a robust molecular and cellular basis for kidney precision medicine approaches.
*Status of Support: Active
Project Number: 4UH3TR003288-03
Name of PD/PI: MPI: Himmelfarb & Kretzler
*Source of Support: NIH
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/01/2022 – 06/30/2025
*Total Award Amount (including Indirect Costs): total cost years 3-5

Lood, Christian:
None.

Freedman, Benjamin:
Title: Developing a therapeutic strategy for Nephropathic Cystinosis with iPS cells
Major Goals: The main goal of this project is to develop pluripotent stem cells with cystinosis mutations and study the phenotypes in organoids.
*Status of Support: Active
Project Number: CRF-2021-003
Name of PD/PI: Freedman
*Source of Support: Cystinosis Research Foundation
*Primary Place of Performance: University of Washington, Seattle, WA
Project/Proposal Start and End Date: 02/01/2022-1/31/2024

*Total Award Amount (including Indirect Costs): total cost
Title: Editing the Kidney Epigenome (SCGE COF Supplement)
Major Goals: The aims of this supplement for the SCGE consortium are to 1) optimize novel epigenome editors at the genome-wide level, 2) determine the efficacy and safety of epigenome editors in human kidney organoids, and 3) demonstrate epigenome editing in mouse kidneys in vivo.
*Status of Support: Active
Project Number: Active
Name of PD/PI: Freedman & Ruohola Baker (MPI), Gersbach (collaborating PI)
*Source of Support: NIH
*Primary Place of Performance: University of Washington, Seattle, WA, and Duke University, Durham, NC
Project/Proposal Start and End Date: 12/01/2021 – 11/30/2022
*Total Award Amount (including Indirect Costs): total cost (total cost Freedman lab)

Title: Mechanisms of Kidney Injury in COVID-19
Major Goals: The goal of this project is to use primary human tissue specimens, in vitro human kidney model systems and a new mouse model of COVID-19 to define direct and indirect mechanisms of SARS-CoV-2 associated kidney injury in three specific aims.
*Status of Support: Active
Project Number: 1R01DK130386-01
Name of PD/PI: Akilesh, Freedman, Smith (MPI)
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, Seattle, WA Project/ Proposal Start and End Date: 09/07/2021 – 08/31/2024
*Total Award Amount (including Indirect Costs): total cost (Freedman lab total costs)

Title: Ultrasound-programmable gene editing in kidneys
Major Goals: The primary goal of this R21 is to provide the foundation for a clinically-relevant, imaging-guided and quantitative gene-editing tool for kidney diseases that leverage the portable and non-invasive nature of diagnostic ultrasound.
*Status of Support: Active
Project Number: R21DK128638-01A1
Name of PD/PI: Medina
*Source of Support: NIH
*Primary Place of Performance: University of Pennsylvania, State College, PA (parent grant) and University of Seattle, Seattle, WA (subaward)
Project/Proposal Start and End Date: 9/1/2021-8/31/2023
*Total Award Amount (including Indirect Costs): total costs Freedman lab subaward

Title: SCGE Comparative Studies Supplement
Major Goals: The goal of the U01 is to apply genome editors in organoid cultures to establish a predictive model for adverse events in human kidney cell types, including

both acute and chronic disorders with life- threatening consequences. Genome editing platforms enable the efficient manipulation of specific DNA sequences in the human genome, and therefore have enormous potential as therapeutics
*Status of Support: Active
Project Number: 3U01DK127553-03S1
Name of PD/PI: Freedman
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, Seattle, WA
Project/Proposal Start and End Date: 09/01/2021 – 05/31/2022
*Total Award Amount (including Indirect Costs): total cost

Title: Understanding and controlling the cellular fate of fluorinated biologics
Major Goals: The goal of this project is to advance knowledge on how organofluorine compounds interact with proteins and DNA, and its effects on cells, to help guide the rational design of new perfluorinated compound (PFC) enabled technologies with desirable functional properties for drug discovery and nanomedicine applications.
*Status of Support: Active
Project Number: R35GM142902
Name of PD/PI: Medina
*Source of Support: NIH/NIGMS
*Primary Place of Performance: Pennsylvania State University, State College, PA (parent grant) and University of Washington, Seattle, WA (subaward)
Project/Proposal Start and End Date: 07/01/2021 – 06/30/2023
*Total Award Amount (including Indirect Costs): total cost (total cost Freedman subaward)

Recently Completed Support:

Himmelfarb, Jonathan:
None.

Elkon, Keith:
Title: Mechanisms of end organ damage in novel polygenic lupus models
Major Goals: The long-term goals of this project are: to understand the protective role of the AC opsonin, MFG-E8, in the kidney; to explore the protective roles of complement in the kidney under conditions of nephritogenic antibodies and impaired AC clearance; to identify the effector mechanisms of kidney injury in TM mice and the mechanisms responsible for UV mediated kidney disease
*Status of Support: Completed
Project Number: R56 AR073848
Name of PD/PI: Elkon
*Source of Support: NIH
*Primary Place of Performance: University of Washington, Seattle, WA
Project/Proposal Start and End Date: 09/09/2019-08/31/2021 NCE
*Total Award Amount (including Indirect Costs):

Zheng, Ying:
None.

Lood, Christian:
Title: Clinical utility of neutrophil biomarkers in SLE
Major Goals: The goal of this study is to determine neutrophil activation markers in SLE patients as relates to disease activity stratification.
*Status of Support: Completed
Project Number: N/A
Name of PD/PI: Lood, J.C.
*Source of Support: Exagen Diagnostic
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available):
7/1/2020-07/15/2021
*Total Award Amount (including Indirect Costs):
Title: Neutrophil activation and cell death markers in monitoring of treatment responses in RA
Major Goals: The goal of this study is to measure neutrophil activation markers in RA patients to determine whether they can predict treatment responses.
*Status of Support: Completed
Project Number: N/A
Name of PD/PI: Lood, J.C.
*Source of Support: Eli Lilly
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available):
4/23/2020-04/22/2022
*Total Award Amount (including Indirect Costs):
Freedman, Benjamin:
Title: Cas9 ribonucleoprotein delivery targeted to kidney epithelium
Major Goals: This project aims to use MTA-tethered Cas9 RNP for targeted editing of kidney epithelial cells in vivo. This is a collaboration between the Wilson Lab (University of California, Berkeley) and the Freedman Lab (University of Washington).
*Status of Support: Completed
Project Number: Collaboration Opportunity Fund
Name of PD/PI: Wilson, Freedman (MPI)
*Source of Support: NIH/Somatic Cell Gene Editing Consortium/Medical College of Wisconsin
*Primary Place of Performance: University of Washington, Seattle, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/01/2020 – 11/30/2021
*Total Award Amount (including Indirect Costs): total cost

Title: Targeting of mTOR complex I in experimental models of polycystic kidney disease
Major Goals: The goal of this work is to establish proof of concept for the use of selective mTOR complex I inhibitors (mTORC1i) to treat polycystic kidney disease.
*Status of Support: Completed

Project Number: Inv Initiated Project
Name of PD/PI: Freedman
*Source of Support: Aeovian Pharmaceuticals, Inc.
*Primary Place of Performance: University of Washington, Seattle, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/01/2021 –04/30/2022
*Total Award Amount (including Indirect Costs): total cost

c. What other organizations were involved as partners?

None.