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TITLE: Modeling Lupus Nephritis in a Human Kidney Microphysiological System

PRINCIPAL INVESTIGATOR: Jonathan Himmelfarb, MD

CONTRACTING ORGANIZATION: University of Washington

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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 1, we have focused on Aim 1, major task 1 and major task 2. 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:

We have focused on Aim 1 in the first year of this study. Briefly, we have established kidney specific vascular MPS and collected the collaborative effort to evaluate the interactions between neutrophils and engineered kidney microvessels in the presence or absence of serum from normal or lupus nephritis patients.

2) Specific Objectives:

In Aim 1, our objective is 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.

3) Significant Results/Key Outcomes:

Establishing kidney microvascular MPS for the study of their interactions with immune cells and humoral factors. We have utilized primary human kidney microvascular endothelial cells to recreate the kidney microvascular MPS as we described previously. 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). In addition, we have developed methods to assess 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). These phenomena are robust for over 5 runs of experiments with over 10 kidney microvessel replicates. HUVECs microvessel however, did not support NETs formation, and displayed EC detachment when PMA-treated neutrophils were perfused, more replicates are ongoing to confirm this phenomena.

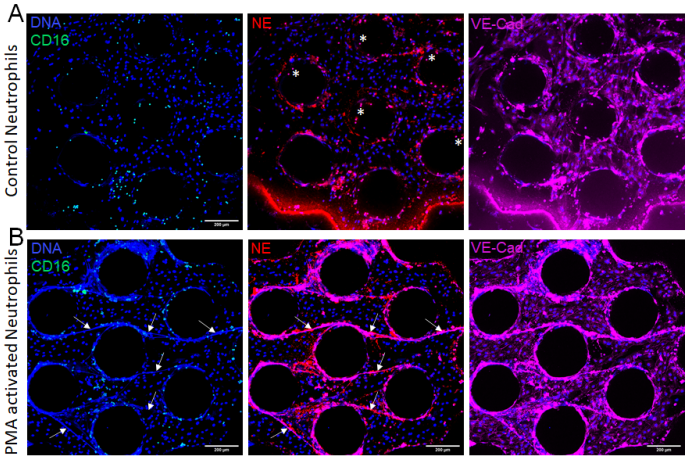

Figure 1. Neutrophil perfused through the kidney microvessel networks for control (A) and PMA treated (B) neutrophil conditions.

We next sought to evaluate the effect of human sera on kidney microvessels and the consequent neutrophil adhesion and NET formation. We found a large variation of response for neutrophil adhesion and NET formation (Fig. 2). Although categorization and quantification are still on going, we found roughly four categories of different neutrophil-kidney microvessel interactions – minimal adhesion and activation of neutrophils (SLE1); increased adhesion, and NETs formation (SLE2); vast amount of adhesion and mild NETs formation (SLE3); and strong NETs formation (SLE4). These phenomena are extracted from experiments with deidentified specimens of sera from 11 SLE patients. When the vessels were only treated with known inflammatory cytokines, TNF-alpha, we found consistently increased neutrophil adhesion without NETs formation.

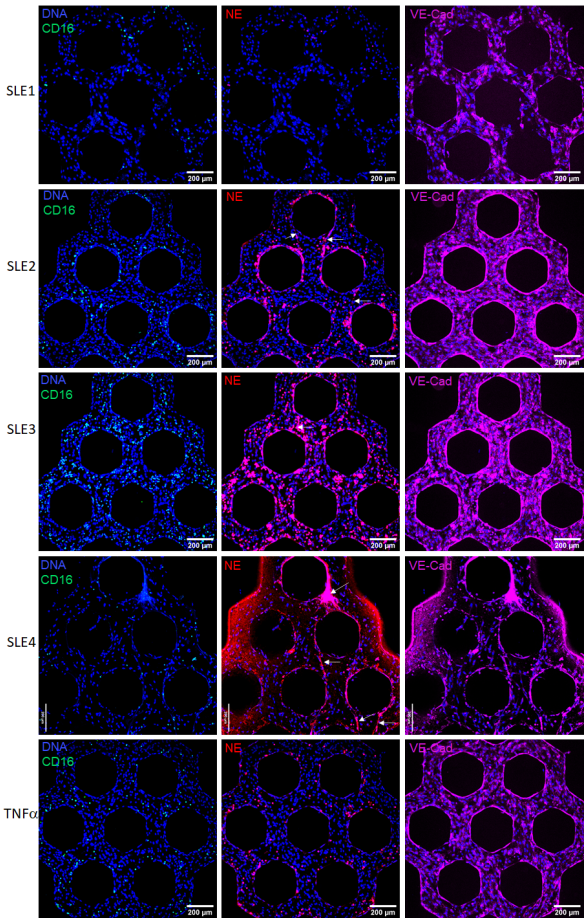

Figure 2. Normal neutrophil perfused through the kidney microvessel networks that were treated with patient serum (SLE1 – 4) or inflammatory cytokines TNFα. Green: CD16, red: NE, blue: DNA, magenta: VE-Cad. White arrows: NETS formation.

Overall, we have established methods to evaluate the interactions of neutrophils and kidney specific microvessels, and demonstrated NETs formation in these microvessels when neutrophils are activated. The presence of lupus serum can also activate neutrophils and form NETs in these kidney microvessels. Further quantification and molecular studies would provide more mechanisms of kidney vascular injuries in these processes.

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?

As outlined in the approved SOW dated December 2020, in the next reporting period we will complete Aim 1, including neutrophil adhesion and NET formation. and focus on Aim 2. Specifically, 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

The restrictions imposed by COVID-19 has somewhat slowed our progress, for example the number of experiments that be performed per unit time due to limits on personnel in the laboratory. We are hoping that with some removal of restrictions, this will not impair progress in year 2 but the situation is fluid considering the delta variant.

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: 3
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: 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

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: Improving the Safety of Genome Editing with Human Kidney Organoids Major Goals: The major goal of this project 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.
*Status of Support: Active
Project Number: 8 U01 DK127553-02
Name of PD/PI: Freedman
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/16/2019 – 05/31/2023
*Total Award Amount (including Indirect Costs): total cost all years

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: 1UG3TR003288-01
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): 07/15/2020 – 06/30/2025
*Total Award Amount (including Indirect Costs): total cost all years

Title: Cellular and Molecular Mechanisms of COVID-19 Mediated Kidney Injury
Major Goals: Using our 'human kidney-on-a-chip' microphysiological systems (MPS), this project aims to better understand the mechanisms of kidney involvement in COVID-19 infection to foster development of effective therapies beyond supportive care. We aim to 1) Characterize SARS-CoV-2 (COVID-19) receptor expression, binding, engagement and modulation in kidney proximal tubular epithelial cells, podocytes, and microvascular endothelial cells within human kidney and vascular MPS, and 2) Assess SARS-CoV-2 (COVID-19) candidate therapeutics including recombinant ACE 2, DPP-4 inhibitors, and angiopoietin 1 hyper-signaling constructs for ability to block kidney specific cellular uptake of SARS-CoV-2 (COVID-19) and/or alter downstream signaling and injury phenotypes within human kidney and vascular MPS.
*Status of Support: Active
Project Number: 3 UG3/UH3TR002158-04S1
Name of PD/PI: Himmelfarb
*Source of Support: NIH/NCATS
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/01/2020 –06/30/2021
*Total Award Amount (including Indirect Costs): total cost

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: 1R44ES032393-01
Name of PD/PI: T. Neumann, SubK Co-PIs: Yeung/Kelly
*Source of Support: NIH/Nortis
*Primary Place of Performance: University of Washington, WA

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/20/2020 – 08/31/2021
* Total Award Amount (including Indirect Costs): total cost

Elkon, Keith:
1 R56 AR073848 (Elkon) 09/09/2019-08/31/2021 2.4 Cal Months
Mechanisms of end organ damage in novel polygenic lupus models NCE
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

Lupus Research Alliance (LRA) (Elkon) 12/30/2020-11/30/2022 .96 Cal months
cGAMP as an immune transmitter of the interferon response
The major goals of this project are to understand how metabolism of cGAMP affects spreading of the interferon response following UV exposure in the skin.

Zheng, Ying:
Title: 3D Models of the Blood-Brain Barrier for Studying Trauma-Induced Cerebral and Systemic Injuries
Major Goals: The goal of this project is to reconstruct human BBB, and exploit roles of blood derived factors in maintaining and disrupting the BBB integrity and function, to identify intracellular signal pathways that contribute to BBB breakdown in TBI.
Status of Support: Active
Project Number: 1R61/33HL154250
Name of PD/PI: Zheng/Dong MPI
Source of Support: NHLBI
Primary Place of Performance: University of Washington Seattle
Project/Proposal Start and End date: 09/15/2020 – 08/31/2022
Total Award Amount (including Indirect Cost): to my lab /year

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: UG3 TR003288-01
Name of PD/PI: Himmelfarb/Kretzler MPI
Source of Support: NCATS
Primary Place of Performance: University of Washington Seattle
Project/Proposal Start and End date: 09/01/2020 – 06/30/2025
Total Award Amount (including Indirect Cost): to my lab /year

Lood, Christian:
*Title: Mitochondrial calcification in juvenile dermatomyositis
Major Goals: The goal of this study is to determine mechanisms of mitochondrial calcification, and their contribution to calcinosis in JDM.
*Status of Support: Active
Project Number: 1 R21 AR 077565-01
Name of PD/PI: Lood, J.C.
*Source of Support: NIAMS
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 9/1/2020-08/31/2022
*Total Award Amount (including Indirect Costs):

*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: Active
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: Active
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):

*Title: Investigating how platelet-derived growth factor receptors direct synovial fibroblast-mediated pathology in inflammatory arthritis.
Major Goals: The goal of this study is to characterize PDGFR expression on synovial fibroblast as well as their role in inflammatory arthritis.
*Status of Support: Active
Project Number: 1 R56 AR 073844-01
Name of PD/PI: Lood, J.C.
*Source of Support: NIAMS
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 9/10/2020-08/31/2022
*Total Award Amount (including Indirect Costs):

*Title: Mitochondrial DNA-mediated inflammation in SLE pathogenesis
Major Goals: The goal of this study is to investigate mechanisms of mitochondrial DNA extrusion by activated neutrophils.
*Status of Support: Active
Project Number: N/A
Name of PD/PI: Lood, J.C.
*Source of Support: Gilead
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 6/1/2021-12/31/2023
*Total Award Amount (including Indirect Costs):

Freedman, Benjamin:
*Title: Rapidly test a novel therapeutic strategy for COVID-19 in human organoids and blood vessel structures
Major Goals: The goal of this project is to utilize 21st century technologies – artificial organs and artificial intelligence – to establish an 'antidote lab' that is capable of rapidly developing therapies for COVID-19 and related diseases.
*Status of Support: Active
Project Number: W81XWH2110007
Name of PD/PI: Ruohola-Baker & Freedman (MPI)
*Source of Support: Department of Defense
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/15/2021 – 01/14/2025
*Total Award Amount (including Indirect Costs):

*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: Active
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, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/01/2020 – 11/30/2021
*Total Award Amount (including Indirect Costs): otal cost

*Title: Improving the Safety of Genome Editing with Human Kidney Organoids Major Goals: The goal is to perform genome editing in human kidney organoid cultures in order to establish a tractable model in which to identify and characterize potentially deleterious, acute, and chronic consequences in human kidney cell types.
*Status of Support: Active
Project Number: 8U01DK127553-02

Name of PD/PI: Freedman (PI)
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/16/2019 – 05/31/2023
*Total Award Amount (including Indirect Costs): total cost all years

*Title: ADPKD nonsense mutation read-through in human kidney organoids
Major Goals: The specific aims of this project are to (1) generate nonsense-bearing PKD iPS cells; (2) derive organoids from these and demonstrate cystogenesis; and (3) screen compounds provided by Eloxx for their capacity to ameliorate the disease phenotype. This will establish a functional, human-specific framework in organoids for the pre-clinical validation of therapeutic compounds that target read-through.
*Status of Support: Active
Project Number: Eloxx SRA-MTA
Name of PD/PI: Freedman (PI)
*Source of Support: Eloxx Pharmaceuticals, Inc.
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/30/2020 – 01/29/2022
*Total Award Amount (including Indirect Costs): total cost

*Title: Modeling Diabetic Nephropathy with Human Kidney Organoids
Major Goals: The goal of this project is to establish a human cellular model of diabetic nephropathy (DN) in vitro, with which to analyze mechanisms driving disease progression and screen therapeutic interventions
*Status of Support: Active
Project Number: Agreement CMS-7154
Name of PD/PI: Freedman (PI)
*Source of Support: Novo Nordisk A/S
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/13/2019 – 12/12/2021
*Total Award Amount (including Indirect Costs): total cost

*Title: Cellular and Molecular Mechanisms of COVID-19 Mediated Kidney Injury Major Goals: Using our ‘human kidney-on-a-chip’ microphysiological systems (MPS), this project aims to better understand of the mechanisms of kidney involvement in COVID-19 infection to foster development of effective therapies beyond supportive care. We aim to 1) Characterize SARS-CoV-2 (COVID-19) receptor expression, binding, engagement and modulation in kidney proximal tubular epithelial cells, podocytes, and microvascular endothelial cells within human kidney and vascular MPS, and 2) Assess SARS-CoV-2 (COVID-19) candidate therapeutics including recombinant ACE 2, DPP-4 inhibitors, and angiopoietin 1 hyper-signaling constructs for ability to block kidney specific cellular uptake of SARS-CoV-2 (COVID-19) and/or alter downstream signaling and injury phenotypes within human kidney and vascular MPS.

Project Number: 3 UG3/UH3TR002158-04S1
Name of PD/PI: Himmelfarb
*Source of Support: NIH/NCATS
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/15/2020 – 06/30/2021
*Total Award Amount (including Indirect Costs): total cost

*Title: Cell specific delivery of novel therapies to enhance and repair glomerular regeneration
Major Goals: The primary goal of this study is to change the treatment paradigm for diseases affecting the podocytes, cells that physically filter the blood to form the urine, by combing therapeutics development with cell-specific delivery to enhance the natural ability of the body to repair and regenerate these highly specialized cells.
Project Number: 1 UC2 DK126006-01
Name of PD/PI: Shankland
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/01/2020 – 05/31/2025
*Total Award Amount (including Indirect Costs): total cost all years

*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.
Project Number: 1 UG3 TR003288-01
Name of PD/PI: Himmelfarb & Kretzler MPI
*Source of Support: NIH/NCATS
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/15/2020 – 06/30/2022
*Total Award Amount (including Indirect Costs): total cost, years 1-2 (UG3)

*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: Active
Project Number: Contract pending – Inv Initiated Project
Name of PD/PI: Freedman
*Source of Support: Aeovian Pharmaceuticals, Inc.
*Primary Place of Performance:

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/01/2021 – 04/30/2022
* Total Award Amount (including Indirect Costs): total cost

* 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: Awarded, pending NOA
Project Number: 1R01DK130386-01
Name of PD/PI: Akilesh, Freedman, Smith (MPI)
* Source of Support:
* Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/01/2021 – 08/31/2024
* Total Award Amount (including Indirect Costs): total cost

Recently Completed Support:

Himmelfarb, Jonathan:
Title: Rebuilding the Glomerular Filtration Barrier by Regenerating Adult Podocytes
Major Goals: Podocytes are cells in the kidney's glomerular filtering units that limit the passage of proteins from the blood in to the urine. As adults, they cannot proliferate to replace themselves, and therefore they are reliant on other stem cells for their regeneration. In this grant, we will study such stem cells in podocyte repair to rebuild a kidney.
*Status of Support: Completed
Project Number: UH3DK107343
Name of PD/PI: MPI: Shankland, Zheng
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/23/15-08/31/20

*Title: Anti-Inflammatory Interventions in Maintenance Hemodialysis Patients
Major Goals: The goal of this project is to test anti-inflammatory agents in pilot clinical trials in patients undergoing hemodialysis.
*Status of Support: Completed
Project Number: U01DK099923
Name of PD/PI: MPI: Ikizler, Himmelfarb
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/15/13-06/30/20 (NCE)

*Title: Biological Determinants of Peritoneal Dialysis Outcomes (Bio-PD)
Major Goals: This is a proposal for an ancillary study of an international multi-center prospective cohort study of peritoneal dialysis patients, the Peritoneal Dialysis Outcomes and Practice Patterns (P-DOPPS). The ancillary study will undertake a genome-wide association study to identify genetic determinants of peritoneal membrane solute transport rate and its change over time.
*Status of Support: Completed
Project Number: R01DK099165
Name of PD/PI: Mehrotra
*Source of Support: NIH/NIDDK
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/23/14-04/30/21 (NCE)

Elkon, Keith:
Title: Innate Immune Response to Hepatocyte Death
Supporting Agency: NIH
Performance Period: 02/10/2015-01/31/2020
Funding Amount
Project Goals: The delivery of the Diphtheria Toxin Receptor to hepatocytes using an AAV8-based vector, followed by the administration of Diphtheria Toxin, provokes acute liver injury. Thus, we can directly study the innate immune response to hepatocyte death. Our Preliminary Data implicate TLR3 as the critical DAMP receptor
Specific Aims: Aim 1: Positive Identification of the TLR3 DAMP responsible for mediating Liver inflammation. Aim 2. Test whether hepatocytes are DAMP sensors. Aim 3. Determine whether the TRIFF pathway is involved in inflammation

Title: HTS Assays for Targeting the cGAS-STING Pathway in Autoimmune Diseases and Cancer
Supporting Agency: BellBrook Labs, LLC
Performance Period: 05/01/2018-04/30/2020
Funding Amount
Project Goals: To develop a small molecule inhibitor of cGAS
Specific Aims: Aim 1. To screen libraries for cGAS inhibitors. Aim 2. To develop sensitive immunoassays to detect cGAMP

Title: cGAS-STING and Mitochondrial DNA in Lupus
Supporting Agency: Rheumatology Research Foundation
Performance Period: 07/01/2018-06/30/2020
Funding Amount
Project Goals: To identify surrogate markers of cGAS activation in human autoimmune diseases
Specific Aims: Aim 1A. Develop surrogate markers of cGAS-STING activation in SLE peripheral blood. Aim 1B. Determine whether Ox-mt-DNA is the ligand for stimulation of cGAS-STING in SLE patients. Aim 1C. Determine whether STING downstream pathways can be induced in a target tissue by an environmental stimulus, UVB.

Zheng, Ying:
None.

Lood, Christian:
*Title: Functional significance of NETosis in intraocular inflammation
Major Goals: The major goals of this project are to define the role of neutrophil extracellular traps in uveitis pathogenesis and inflammation.
*Status of Support: Active
Project Number: 1 R21 EY 029391-01A1
Name of PD/PI: Pepple, K.
*Source of Support: DHHS
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 7/1/2019-06/30/2021
*Title: NET-mediated inflammation in gout
Major Goals: The major goals of this project was to measure levels of neutrophil activation markers in gout patients in relation to cardiovascular disease.
*Status of Support: Completed
Project Number: N/A
Name of PD/PI: Lood, J.C.
*Source of Support: Horizon Diagnostic
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 6/1/2019-05/31/2020

*Title: Impaired mitochondrial clearance in SLE
Major Goals: The major goals of this project was to determine mechanisms of mitochondrial extrusion and clearance by complement and their role in SLE pathogenesis.
*Status of Support: Completed
Project Number: N/A
Name of PD/PI: Lood, J.C.
*Source of Support: Lupus Research Institute
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 1/1/2017-12/31/2020

*Title: Mitochondrial-mediated inflammation and autoimmunity in RA
Major Goals: The major goals of this project was to measure levels of circulating mitochondrial components and determine their clinical utility in RA patients.
*Status of Support: Completed
Project Number: N/A
Name of PD/PI: Lood, J.C.
*Source of Support: Arthritis National Research Foundation
*Primary Place of Performance: University of Washington, Seattle
Project/Proposal Start and End Date: (MM/YYYY) (if available): 6/1/2019-12/31/2020

Freedman, Benjamin:
*Title: High Throughput Nephrotoxicity Screening with Fluorescence-Reporter Kidney Organoids
Major Goals: The goal is to develop kidney organoids with fluorescence reporters of nephrotoxicity in high throughput formats capable with automated manufacture and analysis.
Project Number: Translational Science Grant
Name of PD/PI: Freedman
*Source of Support: Allen Institute
*Primary Place of Performance: University of Washington, WA
Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/01/2018-06/30/2021 (NCE)

*Title: Understanding the role of non-muscle myosin in polycystic kidney disease and kidney fibrosis
Major Goals: To apply pharmacological and transcriptomic tools to kidney organoids with mutations relevant to polycystic kidney disease, to dissect the role of non-muscle myosin in cyst formation and fibrosis and develop a therapeutic lead compound
Project Number: ITHS TL1 Fellowship
Name of PD/PI: Helms
*Source of Support: ITHS
*Primary Place of Performance:
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/01/2020-06/31/2021
* Person Months Per Budget Period: Freedman Mentor (no measurable effort or salary support)

c. What other organizations were involved as partners?

None.