George M. O'Brien Kidney Resource Alliance

George M. O'Brien Kidney Resource Alliance Opportunity Pool Program
Round 2 Funding Opportunity Announcement (Clinical trials not allowed)
(Distributed September 2024)

National Institute of Diabetes and Digestive and Kidney Diseases
and the University of Alabama at Birmingham

Background	The George M. O'Brien Kidney Resource Alliance (OKRA) is a consortium of National Resource Centers (NRCs) whose primary purpose is to provide kidney researchers with specialized resources, tools, technologies, services, and expertise beyond those typically available in an individual lab or institutional core. The consortium includes seven NRCs and a National Coordinating Center. The overarching goal of this OKRA Opportunity Pool Program (OPP) funding opportunity is to provide support for early-stage investigators or investigators new to kidney research who plan to utilize existing OKRA resources. A separate funding opportunity announcement for established kidney investigators will be released at a later date. Resubmissions of unfunded applications from the OKRA Opportunity Pool Round 1 are highly encouraged.
Overview	The program aims to provide one year of support to fund projects broadly related to kidney research. During this funding cycle, multiple projects will be awarded. Justified total costs (including direct and indirect costs) should not exceed a total of $50,000 for the one-year project period. Applications may address a broad array of topics, but **must** utilize an existing OKRA NRC resource to address basic and/or clinical questions in kidney research. A listing of all OKRA NRCs and their resources is attached as an Appendix to this funding announcement. Before submitting a letter of intent or full application, the applicant should contact the leadership that manages the NRC resource the applicant proposes to use to discuss resource appropriateness for the project. A Support Letter indicating that the NRC can provide the requested resource and the budget is appropriate is due with the full application. Contact information is listed at the end of this announcement. Clinical trials, as defined by the NIH (https://grants.nih.gov/policy/clinical-trials/definition.htm), are beyond the scope of this program. Awards are expected to prepare the applicant(s) to submit a future investigator-initiated project (e.g., NIH R01, K01, K08, K23).

Eligibility Criteria	• Only Early-Stage Investigators and established investigators new to kidney research (i.e., investigators who have not received any grants in kidney research and/or published primary research in the area of kidney research as first or senior author) are eligible to apply for this award. (Note: a separate OKRA Opportunity Pool notice of funding opportunity for established kidney investigators will be released at a later date.) • Applicants can only submit <u>one application</u> per round of the OKRA Opportunity Pool Program. • Current OKRA Opportunity Pool awardees are not eligible to apply. • Applicants must have a faculty position or a signed offer letter for a faculty position that starts on or before January 1, 2025 at the time the application is submitted. • Investigators at non-domestic (non-U.S.) entities (foreign institutions) are not eligible to apply. • NRC key personnel are not eligible to apply as PI. • Individuals from underrepresented racial and ethnic groups as well as individuals with disabilities are encouraged to apply. <u>Additional notes for ESIs regarding eligibility</u>: • Early-stage investigators (ESI), as defined by the NIH (https://grants.nih.gov/policy/early-investigators/index.htm) • ESIs are expected to identify at least one experienced kidney researcher to function as a mentor during the project. • The NCC will confirm the applicant's ESI status. Applications that are non-compliant will be administratively withdrawn.

Letter of Intent	A letter of intent is requested no later than **September 30, 2024** to assist in administration of the review process, but is optional and is not part of the review of applications. It should be a maximum of one page in length (no smaller than 11 pt Times New Roman font, single line-spacing) and include: 1. Title of the study. 2. PI name, degree(s), primary institution and early-stage investigator (ESI) or New to Kidney Research investigator status 3. NRC(s) from which resources will be requested 4. All members of the study team and their anticipated roles (e.g., mentor, co-investigator). 5. Specific instructions for ESIs: a. In addition, we would like to connect you with peers and near peers to help form a network of knowledge exchange (e.g., share scientific resources, ideas, career experiences). Please indicate in your letter of intent if you would like to participate in this peer networking opportunity regardless of the outcome of funding.

Full Application Instructions	Following submission of the letter of intent, all applicants are invited to submit a full application that will include the following elements: Abstract, Research Plan, Reference List, Protection of Human Subjects/IACUC protocol (as appropriate), NIH-style Biosketches, Budget and Justification, and a Support Letter from the participating OKRA NRC(s). The Support Letter is due at the time of the full application and should indicate that the NRC can provide the requested resource and the budget is appropriate (contact information is listed at the end of this announcement). The **Abstract** should be a succinct summary of the project that includes the significance of the proposed study, the hypothesis or aim, and the innovative potential of the study. The Abstract should not exceed 200 words in length. The **Research Plan** should not exceed three pages (no smaller than 11 pt Times New Roman font, single line-spacing, with 0.5" margins). Other aspects of the Full Application (i.e., Abstract, Reference List, Protection of Human Subjects/IACUC protocol, NIH-style Biosketches, and Budget and Justification) are not included within these page limitations. No appendices may be submitted. The research plan must include, within the three page limit: 1. (Optional for resubmissions): Brief response to the prior review 2. Specific Aim(s) 3. Research Strategy: a. Background and significance b. Innovation c. A detailed description of intended Methods/Study Design, a Statistical Analysis Plan including assessment of study power, Plans for Implementation, and Logistical Considerations d. Key Measurements and Data to be generated

	Include a **Reference List**. **Protection of Human Subjects** or an **IACUC protocol** should be addressed in a separate document, as appropriate. Indicate whether or not the project involves human subjects or animal research. If yes, please use the relevant attachment for research involving either human subjects or animals. Please include an **NIH Biosketch** for each investigator on the project. Please also include a detailed **Budget and Justification** using the NIH SF424 (R&R) Budget form. Include a signed **Support Letter** from the participating OKRA NRC(s) for the proposed study.
Key Dates	Letter of intent due date: **Monday, September 30, 2024** (by 5:00pm local time of the applicant's organization). Applicants who submit a letter of intent who are found to be ineligible for this funding opportunity will be notified prior to November 1, 2024. Full application due date: **Friday, November 1, 2024** (by 5:00pm local time of the applicant's organization). Notifications to awardees will be distributed early 2025.

Budget	Applicants may request justified total costs (direct + indirect costs) up to a maximum of $50,000. A narrative justification should be provided for key personnel and any major equipment (cost greater than $5,000) deemed to be necessary for the proposed project. This is a federal grant, so federally negotiated F&A rates apply. Budgets should be well-justified and account for all study costs, including costs to OKRA NRC(s), if applicable. Salary effort is an allowable expense and should be limited to the scope of the proposed project. Please contact the participating NRC(s) for resource budget and include that component in the budget section of the grant.
Application Submission Instructions	Letters of Intent and Full Applications must be submitted using the submission forms on the OKRA website (https://www.uab.edu/okra/funding/okra-opportunity-pool-program). If you have any issues with your submission, please email the OKRA National Coordinating Center (okrancc@uab.edu). Full Applications will be reviewed by peer reviewers using a standardized review form. Scientific merit will be assessed based on the programmatic priority areas and scientific merit, investigators and mentors, use of NRC resources, and the potential to contribute to the applicant's success in obtaining future independent funding. All applicants will be provided with a summary of comments from the reviewers.
Requirements of Awardees	Awardees are required to submit a progress report twelve months after receiving funding. Awardees will present their findings to the OKRA Steering Committee at the conclusion of the study. It is expected that each study will culminate in publication(s) or submission of applications for other independent awards such as K or R01 awards. Published work must cite OKRA as a funding source. Awardees will become members of the OKRA Consortium and will be expected to participate in reviews for subsequent OPP application rounds.
Contact Information	For questions, please contact: George M. O'Brien Kidney Resource Alliance National Coordinating Center The University of Alabama at Birmingham Okrancc@uab.edu

APPENDIX

OKRA National Resource Center (NRC) Resource Summaries

INDIANA UNIVERSITY
SCHOOL OF MEDICINE

Indiana O'Brien Center for Advanced Microscopic Analysis

Summary

The Indiana University George M. O'Brien Center for Advanced Microscopic Analysis has a unique, highly interactive, integrated and synergistic team of physicians, basic scientists, computer scientists, engineers and a highly trained and committed technical support team to provide local, national and international users cutting edge intravital optical microscopy and 3-dimensional quantitative digital image analysis of the kidney. They have two cores (Molecular Imaging and Intravital Microscopy) that offer a novel set of research methods, quantitative analysis tools and fluorescent biosensors or probes not available elsewhere to facilitate biomedical research, drug discovery and therapeutic approaches to kidney disease.

Molecular Imaging Resource Core

The Molecular Imaging Resource Core provides quantitative, large-scale microscopy of the kidney as a service to kidney and urologic investigators. Services include:

- 3-dimensional confocal tissue cytometry of kidney tissue
- CODEX multiplexed cytometry of mouse and human kidney tissue, other tissues/organs
- Spatial Transcriptomics of mouse and human kidney tissue (Visium SD, Visium FFPE)
- In situ sequencing of mouse and human kidney tissue (Xenium)
- Data cleaning, filtering, and mapping

Intravital Microscopy Resource Core

The Intravital Microscopy Resource Core provides quantitative intravital microscopy of rodent animal models customized to the specific needs of individual kidney and urologic investigators. Assays and techniques include:

- Glomerular Permeability
- Proximal tubule endocytosis
- Microvascular flow
- Microvascular permeability
- Mitochondrial function
- Cellular oxidative stress
- Apoptosis and necrosis
- Characterization of in vivo fluorescent probe delivery to the kidney
- Characterization of transgenic rodents expressing fluorescently tagged proteins in the kidney

- Fluorescence lifetime imaging of endogenous and exogenous fluorophores in the kidney
- Training in Intravital microscopy

Shared Core Resources

The following resources are shared between the Molecular Imaging Resource Core and the Intravital Microscopy Resource Core:

- Project consultation for imaging studies
- AKI study design and planning for imaging studies
- CKD study design and planning for imaging studies
- Rodent cisplatin kidney injury
- Rodent Kidney Ischemia reperfusion injury
- Rodent Nephrectomy
- Rodent sepsis models
- Training in Digital Image Analysis
- Methods development in microscopy and molecular imaging
- Manuscript development/publication support
- Animal transfer
- Data analysis and visualization
- Data transfer

Contact Information

If you would like more information regarding services provided by Indiana O'Brien Center for Advanced Microscopic Analysis, please contact:

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The Johns Hopkins O'Brien Center to Advance Kidney Health Equity

Summary

The overarching goal of the Johns Hopkins O'Brien Center to Advance Kidney Health Equity is to serve as a national resource for investigators conducting pre-clinical (basic), clinical or population health research addressing or related to kidney health disparities, and to make recommendations to inform strategies, interventions, and approaches aimed at achieving kidney health equity. The resources and services available from the Johns Hopkins O'Brien Center (JHOC) support this overarching goal.

Biomedical Resource Core (BRC)

The Biomedical Resource Core (BRC) provides a portfolio of research services, resources, and tools to understand and ameliorate disparities in kidney disease, with a focus on dietary and social stressors that drive kidney health disparities. The BRC is comprised of two laboratories, detailed below, which provide services and resources for the conduct of pre-clinical, clinical and population research relevant to advancing kidney health equity,

- Clinical Science Dietary and Social Stressor Laboratory (C-DSSL) Services
    - Through a health equity lens, the C-DSSL will provide consultative services, validated protocols with well-established quality assurance/quality control procedures, and access to materials and equipment, with appropriate external resources. The C-DSSL will execute pilot studies or larger research projects and potential access to our community-based clinical research unit. Resources and services include the following:
        - Clinical study design
        - Dietary assessment and Dietary data
            - Feeding studies modeled on the DASH trials
        - Archived and Completed studies
            - Large longitudinal cohort study data from racially and socioeconomically diverse populations
            - Publicly available biospecimen repositories and datasets to support or conduct biomarker studies and secondary data analyses
            - EMR registries
        - Datasets with Deep phenotyping
            - Genomics
            - Proteomics
            - Metabolomics
            - Images
        - Samples
            - Stored biospecimens from completed studies
            - Healthy Volunteers for Reference Range
            - Prospective Remnant Sample collection
        - Behavioral intervention studies
            - Study design consultation and data and biobanked specimens from studies on behavioral interventions for lifestyle changes
        - Translational studies in the community
            - Design and features consultation
        - Biostatistical support (clinical trials, epidemiological cohorts, case-control studies, survey data, and -omics studies)

        - Biomarker measurement
            - Immunoassays
            - Clinical Chemistry
        - Biorepository support
            - Processing aliquoting, short- and long-term sample storage

- Basic Science Dietary and Social Stressor Laboratory (B-DSSL)
    - The B-DSSL will provide investigators with comprehensive services and resources for preclinical research to determine the mechanistic underpinnings of human health disparity stressors, using mice as a model system. Resources and services include the following:
        - Study design
        - Humanized mouse diet protocols
        - Comprehensive mouse kidney phenotyping for diet and stress mechanisms
            Metabolic Cage and Clearance Studies
            GFR measurements
            Telemetric Blood pressure Monitoring
            Optical Clearing
            Isolated Nephron segment Ex Vivo Analysis
            Small Sample RNAseq in Isolated Nephron Segments
            Bioinformatic Analysis
        - Indirect calorimetry and Lipid and carbohydrate metabolism phenotyping
            Oxymax and CLAMS metaboic cages to mointor food intake, water intake, physical activity, metabolic rate determination (VO2), respiratory exchange ratio (carbohydrate vs fat oxidation), and energy expenditure
        - Immunological manifestations of dietary and social stressors in the kidney
            protocols, training, and service to isolate and characterize kidney immune cells by flow cytometric analysis (FACS).
        - Microbiome; germ-free facility use and sample banks
        - Mouse biobank of dietary and social stress models
        - Manuscript development

Contact Information

If you would like more information regarding services provided by Johns Hopkins O'Brien Center to Advance Kidney Health Equity, please contact:

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The George M. O'Brien Michigan Kidney Translational Core Center (MKTC)

Summary

The George M. O'Brien Michigan Kidney Translational Core Center (MKTC) was established to assist investigators and clinicians worldwide in kidney disease research. Their core objective is to enable muti-omic multi-scalar data integration. MKTC consists of two Biomedical Cores: The Applied Systems Biology Core (ASBC) and Data Analytics Services Core (DASC). They provide specialized core services as well as user-friendly tools to enable kidney researchers and trainees to apply to their work.

Applied Systems Biology Core (ASBC)

The Applied Systems Biology Core (ASBC) primary role is to empower national kidney researchers to analyze, integrate and extract pertinent knowledge from multi-omic datasets (genetic, transcriptomic, epigenomic, metabolomic, proteomic data) from model systems and human studies in support of their individual research needs.

- Interactive, shared data mining services
  - ASBC will provide initial study design consultation and assist with developing customized workflow for integrative analysis for projects of individual investigators
  - Clinical and molecular interactions in investigators' data sets
  - Quantitative morphometric analyses of kidney biopsy samples
  - Model systems studies to identify pathways shared cross-species
  - Structured RNA-DNA sequence data integration

- Standardized workflows of recurrent service elements
  - Transcript driven data-mining workflow
    - Single cell RNA Sequencing analysis pipeline
    - Streamlined self-organizing map (SOM) to identify functional subgroups
    - Weighted Correlation Network Analysis (WGCNA) to identify co-expressed molecular elements within datasets
  - Metabolomics driven analysis workflow
    - Platform specific data processing, data normalization, identification of differentially expressed metabolites
  - Spatial metabolomics service (sample process and data analysis)
  - Multi-scalar data analysis and integration
    - Integration of different combinations of available clinical phenotype, morphology, genetic, epigenomic, transcriptomic, proteomic, metabolomic datasets.
    - Building models for classification or statistical association purposes
    - pathway mapping and enrichment analysis
    - clinical and molecular interactions in investigators' data sets
    - Multi-omic data integration to identify biomarkers and regulatory networks
    - Metscape applications
  - Manuscript development

Data Analytic Services Core (DASC)

The Data Analytic Services Core (DASC) provides access to unique sets of data through distinct data analysis platforms. The database is updated quarterly to provide investigators with quick and easy access to all the data

being generated as part of the Center and their web portals include sophisticated and user-friendly analytic tools that allow for mining of these datasets.

- Access, training, and support for data exploration with user-friendly web-based analysis tools
    - Nephroseq
        - A fully automated web-based systems biology search engine for context specific renal disease gene expression and data mining
    - TranSMART
        - An open-source platform that allows user-specified exploration of cohort study datasets to researchers
        - A new tranSMART custom configuration for the CPROBE and multiple other cohort studies, including NEPTUNE, H3Africa, M2C2, CPROBE, CRIC and CureGN networks
    - CELLxGENE and Vitessce
        - Single cell and spatial data mining instances for murine and human kidney disease data sets adapted by the DASC for the kidney investigative community

- Investigating biomarker association (gene, protein, metabolite level) in clinical existing cohorts such as C-PROBE through TranSMART instance (in collaboration with the primary study investigators)

- Developing generalized workflow for integrative web-based analysis

- Manuscript development

Contact Information

If you would like more information regarding services provided by MKTC, please contact:

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George M. O'Brien Michigan Kidney Translational Core Center

Northwestern University George M. O'Brien Kidney Resource Center (NUGoKidney)

Summary

The Northwestern University National Resource Center (NUGoKidney) aims to revolutionize kidney research by integrating physical science advances, such as nanotechnology, proteomics, and bioelectronics, into kidney disease study and treatment. Its core objectives include developing new methods for kidney research, providing widespread access to innovative physical science tools, and fostering community-wide innovation. The center comprises a Biomedical Resources Core for nanotechnology applications and a Resource Development Core for emerging physical science technologies. This integration of physical sciences and kidney research positions the NU-NRC as a leading institution in advancing kidney health and therapy.

Biomedical Resource Core (BRC)

Services provide precision targeting of desired payloads to specific cells or sub-regions within the kidney through:

- Guidance in Molecular and Cell Target Selection:
  - Our Core co-directors will assist in identifying specific molecular targets for precise drug and payload delivery in the kidney.
  - Application Supported: Therapy Delivery, Diagnostics/ Molecular Imagining

- Nanoparticle Carrier Selection Assistance
  - Co-directors will aid in choosing suitable nanocarriers that align with molecular targets for specific kidney sub-regions.
  - Cargos supported: siRNA, microRNA, Hydrophobic small molecule drug, Hydrophilic small molecule drug, Peptides.

- Synthesis and Attachment of Targeting Moieties
  - After selecting ligands and appropriate nanocarriers, the Core will oversee the synthesis of targeting moieties, leveraging the capabilities of the Peptide Synthesis Core for varied-scale production.
  - Kidney Cell Types or Structures for Nanoparticle-Enhanced Delivery: Proximal Tubule, Consultation with the Core Director (if unsure), Podocyte, Collecting Duct

- Gene Therapy and Drug Loading into Nanocarriers
  - The Core will facilitate the incorporation of therapeutic payloads into nanoparticles, utilizing various strategies based on the cargo and application.
  - Nanoparticle Types Supported: Liposomes, Lipid nanoparticles, gold nanoparticles, Polymeric nanoparticles, Peptide micelles.

- Evaluating Targeting Fidelity In Vitro
  - Core co-directors will provide guidance in assessing the targeting accuracy of nanotherapeutics in lab settings.

    - Main Priorities for NanoCore Assistance: In vitro binding, In vitro biocompatibility, in vitro therapeutic efficacy; design of In vivo pharmacokinetics, design of in vivo targeting, design of in vivo therapeutic efficacy studies
- Identifying pathway for therapy
- NW publication assistance, grant proposal assistance, manuscript assistance, and background search
- Feasibility assessment
- Identifying target peptides
- Designing in vitro and in vivo studies
- Polymer synthesis for nanoparticles formation
- Peptide purification
    - High-Performance Liquid Chromatography (HPLC)
    - Matrix-Assisted Laser Desorption/Ionization (MALDI)
- Purified peptide conjugation with DSPE-PEG for micelles synthesis
    - High-Performance Liquid Chromatography (HPLC)
    - Matrix-Assisted Laser Desorption/Ionization (MALDI)
- Fluorophore conjugation and siRNA conjugation with DSPE-PEG
- Micelles, vesicles, LNP, liposome formation
- Incorporation of conjugated siRNA into micelles
- Nanocarrier/micelles characterization
    - Particles size—Dynamic Light Scattering (DLS)
        - Polydispersity Index (PDI)
        - Zeta potential
    - RNA incorporation—Gel Electrophoresis
        - RNA protection against nucleases
    - Morphology—Transmission Electron Microscopy (TEM)
- Quantification of drug encapsulation in nanocarriers by HPLC
- In vitro studies
    - Micelles with Proximal Tubule cells
    - Micelles with Collecting Duct cells
        - Cortical collecting duct (Principal cells)
        - Cortical collecting duct (M1)
        - Inner medullary
- In vitro evaluations
    - In vitro cell viability evaluation—MTS assay/plate reader
    - In Vitro Micelles Binding Evaluation
        - Flow Cytrometry
        - Confocal Microscopy
    - In Vitro Micelles Efficacy Evaluation
        - Quantification of proteins related—ELISA
        - Quantification of proteins related—Western blot
        - Quantification of markers related—qPCR

- In Vivo Therapeutic Efficacy
    - In Vivo Therapeutic Efficacy guidance
    - Immunohistochemistry (IHC) Imaging
    - Hematoxylin and Eosin (H&E) Imaging
- LEAPFROG Platform
    - Applications of immunomagnetic cell sorting

Resource Development Core (RDC)

The Resource Development Core (RDC) offers a range of services including:

- Development of New Research Resources
    - The RDC is committed to creating and improving research tools and technologies that can be utilized by the Biomedical Resource Core. This is done through 3 main projects:
        - **Advancing RNA Delivery to Kidney Using Nanotechnology:** This project focuses on the development and optimization of methods for delivering short RNAs (siRNAs and miRNAs) to the kidney.
        - **High Throughput Single-Cell Profiling for Drug Target Identification:** Leveraging the LeaPFroG (Large-Scale Phenotypic Functional Genomics at Scale) platform developed by Shana Kelley in the Chemistry department at Northwestern University, this project aims to identify new drug targets and modifiers of kidney disease progression. A proof-of-concept study will be conducted to find modifiers of IgA binding/interaction with mesangial cells, a key mechanism in the renal injury associated with IgA nephropathy.
        - **Proteoform Imaging Mass Spectrometry (PiMS) Optimization:** Proteoform Imaging Mass Spectrometry (PiMS) technology will be optimized to identify and map proteoforms in kidney thin sections. This project represents a significant step forward in understanding the complexity of kidney protein structures and functions at the molecular level. (In years 3-5)
- Innovative Technology Incubation:
    - The RDC is dedicated to fostering the development and validation of emerging physical science technologies in kidney research, ensuring their applicability, quality, and potential impact through meticulous evaluation.
        - **Community Engagement:** Engages with leading scientists to incubate and integrate new technologies, nurturing a collaborative atmosphere for the exchange of ideas and technological improvement.
        - **Validation and Quality Control:** Implements standardized evaluation protocols to assess and ensure the performance and reliability of new technologies within research settings.
        - **Impact Assessment**: Dedicates efforts to gauge the potential contributions of innovative technologies to kidney research and their role in enhancing established research methodologies.
        - **Technology Integration Support**: Offers robust infrastructure and support for seamlessly incorporating new technologies into current research, aiding researchers in effective technology adoption and utilization.
        - **Educational Outreach**: Conducts workshops and seminars aimed at educating the kidney research community on emerging technologies, ensuring the widespread dissemination of knowledge regarding the latest advances.
- Interdisciplinary Collaboration
    - The RDC leverages Northwestern University's strong base of physical scientists in

nanomedicine, bioengineering, and chemistry, all committed to kidney research, fostering a robust interdisciplinary approach.

- Use of Cell Surfaceome lentiviral CRISPR gRNA library
- Cell-type specific antigen/target selection for magnetic ranking cytometry
- Rare-cell isolation/enrichment using the LEAPFROG Platform
- Pooled CRISPR editing and phenotypic screening experimental designs
- LEAPFROG Platform
    - Cell surfaceome target detection optimization and characterization
    - Injection moulding based microfluidic chip fabriacation
    - Use of injection moulded microfluidic chips for cell sorting
    - Applications and use of magnetic ranking cytometry
    - Use of microfluidic chips for cell sorting
- Target-antibody-magnetic nanoparticles coupling/optimization
- Cell surface target-specific phenotypic screening
- Cell surfaceome library plasmid prep and lentiviral prep
- Cell-type specific target expression and distribution assessment
- Cell surfaceome lentiviral CRISPR gRNA library
- NGS data analysis (CRISPR gRNA enrichment assessment)

Contact Information

If you would like more information regarding services provided by NUGoKidney, please contact:

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Northwestern University George M. O'Brien Kidney Resource Center

Pittsburgh Center for Kidney Research

Summary

The O'Brien Kidney Research Center at the University of Pittsburgh and Icahn School of Medicine at Mount Sinai seeks to enhance the efficiency and productivity of a large number of kidney-related research projects currently in progress; facilitate acquisition of data for new projects; promote collaborations among center investigators; and provide tools to facilitate translational research. These functions depend on the Center's Biomedical Cores: Physiology Core, Model Systems Core, and KIDNIT (Kidney Imaging) Resource Development Core. Our Cores provide physiological, cell biological, genetic, analytical, molecular biological, and drug discovery tools, as well as model organisms.

Physiology Core

The overall goal of the Physiology Core is to elucidate, at a molecular and cellular level, the function and regulation of key proteins involved in kidney health and disease. The Core provides in vitro and in vivo technologies to explore the function and regulation of membrane transporters, channels, and other resident proteins using single molecule approaches, model organisms, and native epithelia. Services provided are listed below:

- Available Resources
    - Isolation of single nephron segments for immunofluorescence, enzyme assays, RNA and protein isolation
    - In vitro microperfusion of isolated tubules for measurement of transepithelial ion and solute transport
    - In vitro microperfusion of isolated tubules for functional fluorescence assays of single cell function
    - Isolation of Xenopus laevis oocytes
    - Oocyte two-electrode voltage clamp
    - Quantification of surface protein expression
    - Patch-clamp analyses
    - Epithelial transport in Ussing chambers
    - Functional imaging analyses
    - Other procedures

- Hands-on Training
    - Xenopus laevis technologies
    - use of microinjectors
    - use of micropipette pullers and microforges to craft micropipettes for patch-clamp and in vitro microperfusion
    - Two-electrode voltage clamp
    - Ussing chamber systems
        - Patch-clamp
        - Nephron segment microdissection/isolation and microperfusion

- Data analysis
    - Cellular and whole animal physiological studies, or imaging studies

- Manuscript development

Model Systems Core

The overall goal of the Model Systems Core is to provide a diverse array of innovative model systems ranging from yeast, to cultured kidney cells and organoids, to whole animal rodent models to understand and explore kidney development, function, and systemic physiology in normal and disease states. Core directors will facilitate integration between these model systems, enabling investigators to test hypotheses at multiple resolutions from molecules to cells to tubules to whole animals. Services provided are listed below:

- Yeast Models Subcore
  - Protein quality control (degradation, ubiquitylation) assays
  - Protein trafficking assays (immunofluorescence, density centrifugation)
  - Positive genetic selection assays
  - Computational identification of functional variants
  - Bioinformatic analysis of genotype-phenotype correlations (TopMed, Clinvar, UK BioBank)

- Epithelial Models Subcore
  - Mammalian cell culture models (proximal tubule, collecting duct, polarized epithelia)
  - Organoid models to study nephron development
  - Organoid models to study kidney physiology
  - Organoid models to study kidney injury

- Animal Models Subcore
  - Measurement of GFR and RBF
  - Metabolic cage studies, urine metabolites
  - Blood pressure telemetry
  - Real-time super-resolution ultrasound of kidney microvasculature
  - Purine metabolomics
  - Mitochondrial / Bioenergetics (Seahorse)
  - Injury biomarker assessment
  - Disease Models: AKI (ischemia-reperfusion injury)
  - Disease Models: AKI (cisplatin nephrotoxicity)
  - Disease Models: AKI (gentamicin nephrotoxicity)
  - Disease Models: AKI (cecal ligation and puncture)
  - Disease Models: AKI (sickle cell crisis)
  - Disease Models: CKD (genetic models)
  - Disease Models: CKD (FSGS)
  - Disease Models: CKD (unilateral ureteral obstruction)
  - Disease Models: CKD (two kidney one clip hypertension)
  - Disease Models: CKD (chronic angiotensin II infusion)
  - Special Rodent Diets (low and high Na^+ and K^+)

Kidney Imaging: Developing Novel and Innovative Tools (KIDNIT) Development Core

The KIDNIT Resource Development Core provides a national resource for investigators who require detailed and quantitative morphological analysis of kidney-associated cells and tissues, particularly those that are epithelial in nature, as well as whole organs. Services include:

- Consultation and Training opportunities
  - Consultation for development of new approaches to study the kidney and lower urinary tract using morphological tools.
  - Training in sample preparation of kidney and bladder tissues for light and electron microscopic analysis.
  - Training in specialized sample preparation to perform labeling and analysis of cleared organs and tissues.
  - Training in quantitative image analysis.
  - Training in live-cell and intravital approaches to study the biology/pathobiology of kidney and bladder tissues.

- Standard light microscopy
  - Brightfield, darkfield, epifluorescence, DIC
  - Macro (stereo) imaging microscopy

- Confocal microscopy
  - Laser scanning confocal microscopy
  - Spinning disk confocal microscopy
  - Ribbon scanning confocal microscopy for cleared organs/tissues
  - Fluorescent lifetime imaging
  - Fluorescent recovery after photobleaching

- Multiphoton microscopy
  - Multiphoton confocal microscopy
  - Fluorescent lifetime imaging
  - Fluorescent recovery after photobleaching
  - Intravital imaging

- TIRF microscopy
  - TIRF imaging acquisition and analysis

- Super-resolution microscopy
  - STED
  - CREST
  - STORM
  - AX/NSPARC

- Live-cell microscopy
  - Spinning disk confocal microscopy
  - Fluorescent lifetime imaging
  - Fluorescent recovery after photobleaching
  - Lattice light sheet microscopy
  - Incubator microscopy
  - Microinjection

- Large Volume Light-Sheet microscopy
    - Meso-Spim light sheet microscopy

- Additional imaging modalities
    - Whole slide scanning - brightfield and epifluorescence
    - iPOL imaging

- Electron microscopy
    - Scanning electron microscopy (field-emission)
    - Transmission electron microscopy
    - Freeze fracture microscopy
    - Platinum replica electron microscopy
    - Negative staining
    - Freeze substitution
    - Ultramicrotomy
    - Ultrathin cryo EM
    - CLEM
    - Immuno-Electron microscopy

- Image analysis
    - Photoshop
    - Imaris
    - Metamorph
    - NIS elements
    - Leica LASX
    - NIH image/FIJI

- Data storage
    - Imaging files

- Data analysis
    - Cellular and whole animal physiological studies, or imaging studies

- Manuscript development

Contact Information

If you would like more information regarding services provided by Pittsburgh Center for Kidney Research, please contact:

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Pittsburgh Center for Kidney Research

UAB-UCSD O'Brien Center for Acute Kidney Injury Research

Summary

The University of Alabama at Birmingham – University of California at San Diego (UAB-UCSD) O'Brien Center for Acute Kidney Injury (AKI) Research is an interdisciplinary center of excellence in AKI-related research. The overall mission of the UAB-UCSD O'Brien Center for AKI Research is to improve the health of patients by fostering research specifically targeted to the prevention and treatment of AKI and its complications. The two Biomedical Research Cores (a Clinical Core and a Pre-clinical Core) and Resource Development Core integrate existing intellectual and technological resources to provide the Consortium a set of services/resources for innovative investigation in AKI-related research.

Clinical Core

The Clinical Core catalyzes the translation of bench discoveries to applications that impact human AKI. They achieve this through providing access to curated clinical data from prior clinical studies and new harmonized real world multimodal data from electronic health records (EHR) to support epidemiological studies of AKI and clinical research, including AKI risk-classification, sub-phenotyping, and simulated trials. They also have access to biospecimens from prior studies hosted by the core. Below is a list of detailed services provided by this core:

- AKI Study Planning and Design
- AKI Clinical Study Initiation and Conduct
- AKI Epidemiological Study Initiation and Conduct
- AKI Data Analysis and Interpretation
- AKI Biological Sample Repository
- Cloud-based multimodal databases for AKI and critical care nephrology research studies
- Predictive Modeling of Clinical and Laboratory Data
- AI Model Assessment for Trustworthy, Fairness and Implementation
- Clinical Trial Design and Enrichment
- Quality Improvement Study: Design, Conduct and Analysis
- Implementation Science Study: Design, Conduct and Analysis
- Biostatistics and Bioinformatics Support Services
    - Analysis of pilot data
    - Design of epidemiological, clinical studies or clinical trials
    - Sample size and/or power analysis
    - Analyses of study results
    - Database development and maintenance
    - Forms, manual of operations, and/or instrument development
    - Data Safety and Monitoring
    - Big data transformation & pipeline development
    - Methodologies and Applications of Artificial Intelligence and Digital Health
- Assistance with manuscript development or publication support

Pre-Clinical Core

The Pre-Clinical Core provides the facilities and requisite skills (Animal Models Resource) to generate and study murine models of AKI and provides unique facilities and requisite skills (Renal Physiology Resource) to determine renal physiological changes in AKI. Pre-Clinical s Core services include:

- Kidney ischemia/reperfusion (IRI)
- Unilateral Ureteral Obstruction
- Orthotopic kidney transplantation
- Tail vein injection or venipuncture (per animal)
- GFR (transcutaneous FITC-sinistrin) in rodents
- Cannulation (indwelling with injection port)
- Use of a microsurgical workstation (per hour)
- Small Animal Microsurgical Core Training (per hour/per day)
- Misc. surgical services involving body wall penetration (per hour)
- 5/6th nephrectomy (both steps)
- Radio-telemetry determination of blood pressure
- LC-MS/MS Creatinine determination (per test)
- Functional renal imaging (tracer dependent, per hour)
- Structural and metabolic imaging (US, MRI, microCT) (per hour)
- Nuclear imaging (Gamma camera, SPECT/CT, PET/CT) (per hour)
- Optical Imaging (Bioluminescence, Fluorescence) (per hour)
- Image Analysis
- Whole kidney clearance/oxygen consumption studies
- Tissue oxygen partial pressure determination in kidney
- Determination of GFR in awake rodents
- Micropuncture for nephron function and TG feedback
- Metabolic cage experiments in mice (per animal and week)
- Automated tail-cuff blood pressure determination (each, 6 days)
- Laser Capture Microdissection from frozen tissue (~70 pieces individually collected per sample; sample quality and amount appropriate for RNA-Seq or potentially proteomics)
- Bulk RNA Seq
    - From Fastq to analysis
    - Sample to analysis
    - Training (per day)
- Assistance with manuscript development or publication support

Resource Development Core

With both the clinical and pre-clinical cores, the Resource Development Core will provide a dynamic resource and platform to develop, test and refine innovations that will accelerate pre-clinical and clinical research. In the pre-clinical area, new analytical approaches in metabolomics as well as functional in vivo imaging will be incubated to probe unique biological characteristics of disease development. In the clinical area, tools for federated machine learning using big EHR data will be developed.

- Development of novel microfluidic methods aimed at high resolution molecular cartography in kidney tissue and molecular and functional kidney-specific imaging approaches in pre-clinical AKI.
- Development of a federated learning platform using a collaborative digital workspace to support big EHR data analyses across multiple institutions.
- Assistance with manuscript development or publication support

Contact Information

If you would like more information regarding services provided by UAB-UCSD O'Brien Center for AKI Research, please contact:

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Washington University Kidney O'Brien Center for Chronic Kidney Disease Research

Summary

The Washington University Kidney O'Brien Center for Chronic Kidney Disease Research (WUCKD-NRC) primary goals are to develop and disseminate tools and new technologies for the investigation of chronic kidney disease and fibrosis. The WUCKD-NRC achieves these goals through two Biomedical Cores – the Metabolism Core (Meta-Core) and the Variant Validation Core.

Metabolism Core (Meta-Core)

The aim of the Metabolism Core (Meta-Core) is to facilitate the use of metabolic assays in pre-clinical models of chronic kidney disease (CKD) for the kidney community by providing the following services following consultation with core faculty:

- Seahorse bioflux analyzer (XF24/XF96)
  - Measures metabolism and define metabolic substrate preferences ideally on adherent cells but also on freshly isolated tubules.
  - Measure glycolysis (i.e. extracellular acidification rate) in primary proximal tubule cells
  - Measure mitochondrial oxidative respiration in primary proximal tubule cells
  - Measure glucose oxidation in primary proximal tubule cells
  - Measure glutamine oxidation in primary proximal tubule cells
  - Measure palmitate (fatty acid) oxidation in primary proximal tubule cells

- High resolution respirometry (Oroboros)
  - Measures mitochondrial function in tissues ex vivo, and this is facilitated through the Nutrition Obesity Research Center (NORC).

- Substrate oxidation assays
  - Utilizes radioactive-labeled substrates (e.g. 3H-palmitate) to measure oxidation in tissue ex vivo
  - Fatty acid oxidation in tissue ex vivo using 3H-palmitate
  - Glucose oxidation in tissue ex vivo using 14C-pyruvate
  - Branched chain amino acid oxidation in tissue ex vivo using 14C-KIVA

- Untargeted metabolomics
  - Performed on cells or tissues using the mass spectrometry resources of the Metabolomics Core through the lab of Gary Patti.
  - Lipids
  - Water Soluble

- Stable isotope flux studies
  - Used to look at specific pathway activity and flux in both cell culture systems and whole animals using mass spectrometry.
  - Metabolomics and stable isotope flux study
  - Stable isotope tracing studies in vivo and using proximal tubules in vitro (13C-glucose, 13C-palmitate/oleate, and other isotopes upon request):
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- Choosing the right metabolic assay

- Bioinformatics and biostatistical support

- Assistance with manuscript development or publication support

Variant Validation Core

The widespread and increasing use of whole exome sequencing (WES) and whole genome sequencing (WGS) to investigate the genetic bases for CKD and other kidney disorders has led to a much better understanding of the etiology of monogenic diseases. The aim of the Variant Validation Core (VVC) is to investigate variants of uncertain significance (VUS) discovered in patients with kidney disease or a kidney developmental disorder for potential pathogenicity. The VVC will use experimental approaches in cultured cells and in mice. The experimental approach for each VUS will be tailored for 1) the particular gene and protein that are potentially involved; and 2) the particular disease or disorder that would likely develop if the VUS is indeed pathogenic. Immediate services and resources that are being offered include:

- Choosing an assay for COL4A3/A4/A5 VUS analysis
- COL4A3/A4/A5 VUS (missense) analysis by N-terminal split nano-Luciferase collagen IV assembly and secretion assays using transfected cells
- COL4A3/A4/A5 VUS (missense) analysis by split C-terminal nano-Luciferase collagen IV assembly and secretion assays using transfected cells
- COL4A3/A4/A5 VUS (intronic) analysis by minigene RNA splicing assays using transfected cells
- COL4A3/A4/A5 VUS analysis by high resolution quantitative immunofluorescence imaging in paraffin sections of kidney biopsies
- COL4A3/A4/A5 VUS analysis in vivo by mouse gene editing
- Analysis of VUS in other genes through design and implementation of tailored laboratory assays
- Analysis of VUS in non-COL4A genes through generation and analysis of gene edited models
- Training in the performance of collagen IV heterotrimerization assays using split nanlLuciferase tagged COL4A chains
- Analysis of variant UMOD protein trafficking in cultured cells
- Design of assays for other protein trafficking in cultured cells

Contact Information

If you would like more information regarding services provided by WUCKD-NRC, please contact:

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